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Ecology of yeasts in forest soils

Ekologie kvasinek v lesních půdách

Ph.D. Thesis

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Declaration

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

Prague, 18.09.2017 Tereza Mašínová

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Abstract

Microbial communities inhabiting upper soil horizons represent an important component of forest ecosystems. However, despite the evidence that yeasts represent an integral part of topsoil fungal communities, their role in forest ecosystems received so far little attention. The aims of my PhD thesis were to describe yeast communities in soil and litter of a temperate forest using high-throughput sequencing of environmental DNA, identify dominant yeast species and explore how the composition of yeast communities reflects the biotic and abiotic factors of the environment. I also aimed to isolate yeasts from forest topsoil, describe novel yeast taxa abundant according to the environmental DNA survey and screen representative isolates for the traits relevant to their involvement in organic matter transformation.

I have demonstrated that in forest topsoil, yeasts represent a substantial proportion of fungal communities with higher relative abundance in soil than in litter. In litter, yeast communities differ significantly among beech, oak and spruce-dominated stands. Drivers of community assembly are probably more complex in soils and comprise the effects of soil chemistry and vegetation. Even though there are similarities in the response of the communities of yeasts and filamentous fungi to environmental conditions, many differences are also evident. Despite taxonomic heterogeneity, yeasts represent a fungal group with a specific nutritional strategy dissimilar to other soil fungi. While the efficient decomposition of hemicellulose, cellulose or chitin appears to be restricted to only a few taxa, results of the carbon sources utilization assays indicate that most yeasts can efficiently act as mutualists that utilize products of decomposition, provided by other microbes. Importantly, large fraction of enzymes produced by yeasts is associated with their cell surfaces. This adaptation should ensure that the decomposition takes place at the cell surface of the unicellular microorganisms and the resulting compounds are readily available to the producers of the enzymes. Based on the results of this thesis, forest soil yeasts seem to have unique ecology which may reflect their unicellular growth form. Three novel yeast species were described, all belonging to the subphylum Pucciniomycotina, class Microbotryomycetes: *Leucosporidium krtinense* f.a. sp. nov., *Yurkovia mendeliana* sp. nov., and *Libkindia masarykiana* sp. nov. Based on the analysis of environmental DNA, these new species are common and abundant in the studied environment which indicates their high environmental relevance in the temperate mixed forest.

Abstrakt

Společenstva mikroorganismů ve svrchních horizontech půdy představují významnou složku lesních ekosystémů. Navzdory tomu, že kvasinky jsou nedílnou součást společenstev půdních hub, jejich roli v lesních ekosystémech dosud nebyla věnována dostatečná pozornost. Cílem této dizertační práce bylo popsat složení společenstva kvasinek v půdě a opadu temperátního lesa pomocí sekvenace environmentální DNA, identifikovat dominantní druhy kvasinek a popsat, jak složení jejich společenstva obráží biotické a abiotické podmínky prostředí. Dalším cílem bylo izolovat kvasinky z lesní půdy a opadu a popsat nové druhy, které jsou významně zastoupené v environmentální DNA. Reprezentativní izoláty byly charakterizovány z hlediska jejich schopnosti využívat organické látky a podílet se na rozkladu mrtvé organické hmoty.

Výsledky této práce ukázaly, že kvasinky představují podstatnou část společenstev hub v půdě a opadu temperátního lesa s vyšším relativním zastoupením v půdě. V opadu se společenstva kvasinek liší mezi porosty buku, dubu a smrku. Faktory, ovlivňující složení společenstva v půdě, jsou pravděpodobně složitější a zahrnují vliv abiotických podmínek a vegetace. Přestože faktory prostředí ovlivňují společenstva kvasinek a vláknitých hub podobně, existuje mezi nimi rovněž mnoho rozdílů. Navzdory taxonomické heterogenitě představují kvasinky houbovou skupinu se specifickou nutriční strategií odlišnou od jiných půdních hub. Zatímco účinný rozklad hemicelulózy, celulózy nebo chitinu se zdá být omezen pouze na několik taxonů, schopnost využívat široké spektrum zdrojů uhlíku naznačuje, že kvasineky mohou působit jako mutualisté, využívající produkty rozkladu, poskytované jinými mikroorganismy. Za pozornost stojí, že velká část enzymové aktivity je u kvasinek vázána na povrch buněk. Tato adaptace pravděpodobně slouží k snadnějšímu příjmu produktů rozkladu do buněk kvasinek, které enzymy produkují. Na základě výsledků této práce lze konstatovat, že kvasinky v lesních ekosystémech obsazují unikátní niku, která pravděpodobně odráží jejich jednobuněčnou růstovou formu. Byly popsány tři nové druhy kvasinek, které patří do podkmene Pucciniomycotina, třídy Microbotryomycetes: *Leucosporidium krtinense* f.a. sp. nov., *Yurkovia mendeliana* sp. nov. a *Libkindia masarykiana* sp. nov. Analýza environmentální DNA naznačuje, že tyto druhy jsou ve studovaném prostředí hojné, a tedy pravděpodobně významné pro půdu temperátního lesa.

List of abbreviations

AM	arbuscular mycorrhiza
C	carbon
DNA	deoxyribonucleic acid
ECM	ectomycorrhiza
HTS	high-throughput sequencing
ITS	internal transcribed spacer
N	nitrogen
OTU	operational taxonomic unit
PCR	polymerase chain reaction

1 Aims of the thesis

Forests play an important role in the global carbon cycling and fungi are considered to be the key players in decomposition of dead organic matter. However, despite the evidence that yeasts represent an integral part of soil fungal communities, their role in forest ecosystems has received little attention so far. Yeasts are a taxonomically heterogeneous group of fungi containing members of various orders within the Ascomycota and Basidiomycota that are able to live in a unicellular form. Due to unicellularity, we can expect that yeast communities will be affected by different factors than filamentous fungi whose hyphae allow them to bridge sites with unfavourable conditions, to colonize efficiently bulky substrates and to translocate heterogeneously distributed nutrients.

Our current knowledge about soil-inhabiting yeasts is based mainly on cultivation experiments that might give a biased picture of the yeast community. Unfortunately, despite the existence of high-throughput sequencing (HTS) datasets, this approach has not yet been used for specific analyses of yeasts in forest topsoils. The aim of this thesis was to expand the current knowledge about the diversity and ecological role of yeasts in the litter and soil of temperate forests. To reach this aim, I have combined HTS with strain isolation and characterization.

The aims of my PhD thesis were:

1. To describe yeast communities in soil and litter of a temperate forest using HTS of environmental DNA, identify dominant yeast species and explore how the community of yeasts changes across a range of abiotic and biotic factors including variable ground vegetation and the composition of the tree layer (Paper I).

2. To compare the effect of dominant vegetation and site properties on the community composition of yeasts and filamentous fungi using HTS of environmental DNA (Paper II).

3. To isolate yeasts from forest topsoil and describe novel taxa among the yeasts most abundant in environmental DNA surveys (Paper III).

4. To screen representative isolates of abundant yeasts for the traits relevant to their involvement in organic matter transformation and nutrition and to compare their decomposition potential with other groups of fungi (Paper IV).

To answer these questions, I have conducted a series of field sampling experiments in the area of the Training Forest Enterprise Masaryk Forest Křtiny of the Mendel University in Brno (Křtiny forest). Křtiny forest has a total area of 103 km^2 of mixed temperate forest (latitude 16°15' E, longitude 49°15 N) and is located north of Brno, Czech Republic. The area has an altitude range of 210-575 m, the mean annual temperature is 7.5 °C and mean annual precipitation 610 mm, and is characterised by a variety of natural geomorphological conditions. The forest stands are composed of approximately 54% broadleaf and 46% coniferous trees, with the five most dominant species being *Fagus sylvatica, Quercus petraea* agg., *Picea abies, Pinus sylvestris,* and *Carpinus betulus*. Due to the variation of environmental conditions and stand composition, the area is suitable for the characterization of fungal communities in the temperate forest zone. Two field experiments were conducted with the following designs.

(1) Whole Area Experiment (performed in September 2013)

Soil and litter for HTS analysis was collected at 64 forested sites covering an approximately 8 × 8 km grid with a distance of approximately one km between the closest sampling sites. The sampling sites across the study area exhibited high diversity in vegetation cover (Fig. 1).

(2) Dominant Tree Experiment (performed in January, April, June, and October 2013)

For this experiment, six blocks of of *Picea abies*, *Fagus sylvatica*, and *Quercus petraea* monoculture stands were selected in six areas within the Křtiny forest to cover the variation of other environmental factors. Soil and litter collected at these sites were used for HTS analysis of environmental DNA and for isolation of yeast strains (Fig. 1; Fig. 2).

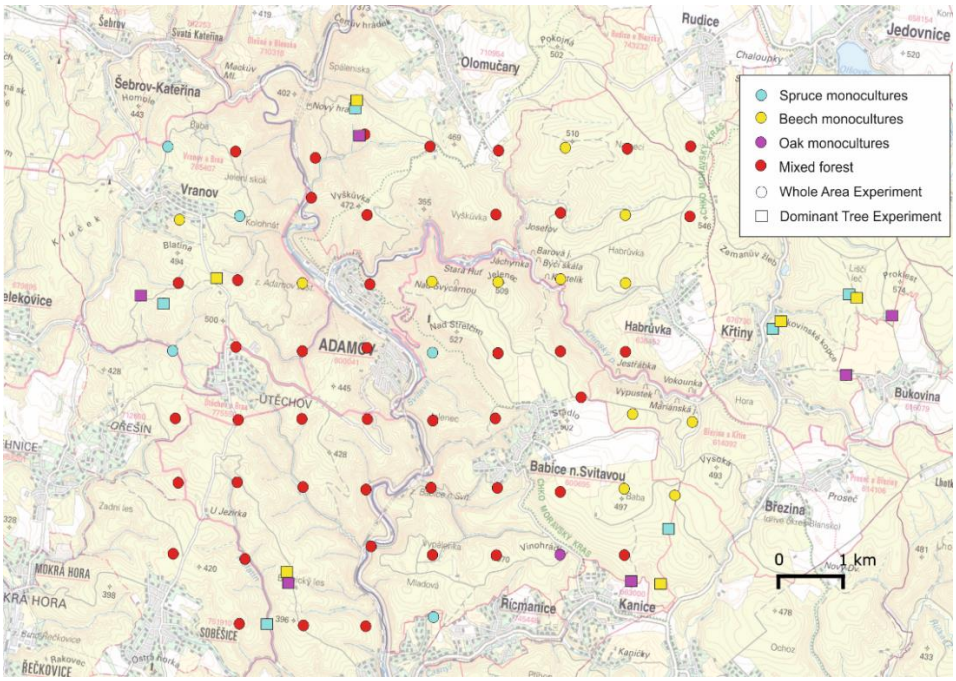

Figure 1: Localization of sampling sites in the Křtiny forest.

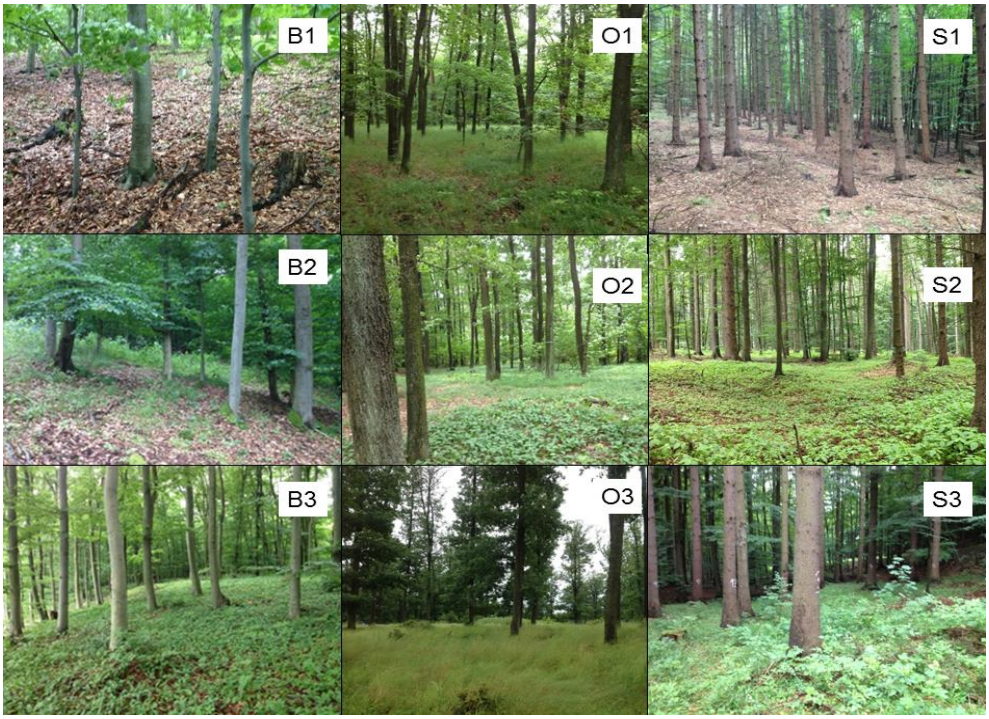

Figure 2: Examples of sites where Dominant Tree Experiment sampling occurred. Beech dominated sites: B1, B2, B3; oak dominated sites: O1, O2, O3; spruce dominated sites: S1, S2, S3.

2 Introduction

2.1 Forest ecosystems and the importance of microbial communities

Forests are major terrestrial ecosystems that are distributed across the globe covering more than 40 million km^2 and representing 30% of the global land area (Keenan et al., 2015). Temperate forests cover the area of 570 milion ha (FAO and JRC, 2012) between 25° and 55° north and south of equator and, thus, belong to important biomes on Earth. They are characterized by temperature ranges between -30 and 30°C, with hot summers and cold winters and with 750 to 1,300 mm of precipitation per year.

One of the most important features of forests is their role as a globally important pool of organic carbon (C). Almost half of the total terrestrial C is concentrated in forests, with the majority shared between soils and live biomass (44% and 42%, respectively) followed by deadwood (8%) and litter (5%) (Pan et al., 2011). C enters the soil environment from aboveground as deadwood or litter that accumulates on the forest floor and also belowground either as root litter or through rhizodeposition. Dead microbial biomass also represents an important C source (Brabcová et al., 2016). Geographically, about half of C pool (55%) is stored in tropical forests, whereas boreal and temperate forests contribute with 32% and 14%, respectively. While tropical forests store C mainly in live biomass (56%), boreal and temperate forests contain larger C stocks in soils and dead plant material (Pan et al., 2011). It has been estimated that up to ninety percent of the annual plant biomass is not consumed by herbivores and enters the dead organic matter pool (Gessner et al., 2010).

Microbial communities inhabiting upper soil horizons represent an important component of forest ecosystems. These communities are complex assemblages of prokaryotic and eukaryotic organisms, including archaea, bacteria, algae, protists and fungi that mediate a wide range of biogeochemical processes. They play an important role as decomposers, plant symbionts or pathogens, influencing the C turnover and retention and the availability of other nutrients (Trivedi et al., 2013; Uroz et al., 2016; Baldrian, 2017). Therefore, understanding their role in these processes is essential for understanding forest ecosystems.

As a consequence of decomposition of litter-derived organic matter and weathering of the mineral matrix, it is possible to recognize three main compartments of temperate forest topsoil profile characterized by decreasing organic matter content, microbial biomass, respiration and activity of extracellular enzymes. The top compartment is referred as litter and is composed of relatively fresh organic residues and identifiable plant material, such as leaves, wood or twigs deposited on the soil surface. Some discoloration or other signs of early decomposition may be visible, but the origins of plant residues are still easy to discern. The uppermost soil horizon, the organic soil horizon, develops where decomposing organic matter, sometimes transformed into humic materials, accumulates and mixes with mineral material. The deeper part of soil profile is referred to as mineral horizon(s) and is characterized by a low content of organic C.

2.1.1 Nutrient sources of soil-inhabiting microorganisms in forest ecosystems

2.1.1.1 Plant litter composition

Plant litter is a complex mixture of organic components. It contains residues of storage materials of plants that are easily degradable and thus easily accessible for soil-inhabiting microorganisms. The major components of plant litter are, however, the constituents of the plant cell wall such as cellulose, hemicellulose or lignin that are less easily decomposable. Cellulose and hemicellulose are polysaccharides, whereas lignin is an aromatic polymer. The composition and proportions of these compounds vary between plants (Malherbe and Cloete, 2002; Perez et al., 2002).

Cellulose is considered to be the most abundant biopolymer on Earth. It is a linear chain of glucose units (>10 000) which are linked by β-1,4-glycosidic bonds. The regular arrangement of the hydroxyl groups along the cellulose chain leads to the formation of H-bridges and therefore to the fibrous structure with crystalline properties. Approximately 15% of cellulose molecules have amorphous structure (Kögel-Knabner, 2002) which is more susceptible to enzymatic degradation (Perez et al., 2002). A set of hydrolytic enzymes needed for cellulose degradation consists of endo-cleaving endo-1,4-β-glucanase (endocellulase), exo-cleaving cellobiohydrolase (exocellulase) and β-glucosidase that cleaves cellobiose or cello-oligosaccharides.

Hemicelluloses differ from celluloses in their composition of sugar units including D-xylose, D-glucose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acid that are linked together by β-1,4, β-1,3 or other glycosidic bonds, are more or less strongly branched and have a lower degree of polymerization. The most important hemicelluloses are xylans, mannans and galactans (Kögel-Knabner, 2002; Sánchez, 2009). Hemicelluloses are enzymatically degraded to monomeric sugars and acetic acid. Although similar enzymes are involved in cellulose and hemicellulose degradation, more enzymes are required for complete decomposition of hemicelluloses because of its chemical heterogeneity (Malherbe and Cloete, 2002).

Lignin is the second most abundant biopolymer in nature and also the most recalcitrant polymer of the plant cell wall which is linked to both cellulose and hemicellulose. It is a three-dimensional macromolecule consisting of phenyl propane units. The primary building units of lignin are the coniferyl alcohol, sinapyl alcohol and coumaryl alcohol. The monomers react through the so-called dehydrogenative polymerization to a three-dimensional macromolecule, which contains a multitude of C-C and ether-linked compounds (Kögel-Knabner, 2002; Sánchez, 2009). The key enzymes involved in lignin degradation are oxidative enzymes such as lignin peroxidases, manganese peroxidases and laccases.

2.1.1.2 Dead fungal biomass

In addition to plant biomass, fungal mycelia also represent an important pool of organic matter in forest litter and soil (Baldrian et al., 2013; Brabcová et al., 2016). Ectomycorrhizal fungi (ECMF) represent the bulk of soil fungal biomass reaching up to 600 kg h^{-1} (Cairney, 2012; Hendricks et al., 2016), and the annual production of fungal mycelia in spruce forest ranges from 100 to 300 kg ha^{-1} (Ekblad et al., 2013). The amount of fungal biomass in litter per g substrate dry mass can be even 10-fold higher than in soil (Baldrian et al., 2013)

Fungal mycelia typically contain mostly cell wall materials such as polysaccharides (e.g. glucans, glucomannans, chitin) representing 80-90% of the total cell wall mass, lipids and proteins (Baldrian et al., 2013) which makes them an important source of both C and nitrogen (N)

(Colpaert et al., 1996). Especially the relatively high content of N makes mycelia an attractive target for decomposers in forest litter and soil, which are often N limited (Lindahl et al., 2007). Fungal biomass represents a more readily decomposable substrate than lignocellulose.

2.1.1.3 Root exudates

Rhizosphere, the soil that surrounds plant roots and is influenced by their activity is just a few millimeters thick, but it is a place where complex biological and ecological processes occur because roots continuously produce and secrete compounds to their surrounding (Gleba et al., 1999; Bais et al., 2001). These may be either low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics and other secondary metabolites or high-molecular weight exudates, such as polysaccharides and proteins (Uren, 2007). Root exudates may represent between 25 and 63% of the gross plant primary production (Litton et al., 2007). Exudates may be involved in several processes - they serve as phytotoxins or play an important roles in resource availability by altering soil chemistry, soil processes, and microbial populations.

2.2 Soil fungal communities

Fungi are the most studied microbes in temperate forests soils. They exhibit a variety of growth forms and trophic strategies including saprotrophs obtaining organic compounds from dead organic matter or various symbionts. Fungi mediate key ecosystem processes such as facilitation of nutrient cycling through their ability to decompose recalcitrant plant biomass or translocation of nutrients and moisture via their extensive hyphal networks. They are also involved in the formation of soil aggregates and thus soil structure via their hyphal growth form.

Mycorrhizal symbiosis is based on reciprocal exchange of resources where fungi provide mineral nutrients to plants in return for plant assimilates. In natural ecosystems, plants obtain up to 80% of their requirement for N and up to 90% of phosphorus from mycorrhizal fungi (van der Heijden et al., 2008). Moreover, mycorrhizal fungi can also provide resistance to stress, drought and in some cases to soil pathogens (Augé, 2001; Sikes et al., 2009). We can identify several types of

mycorrhiza that differ in anatomy and physiology. Endomycorrhizae are characterized by penetration of the fungus into the root cells and include arbuscular, orchid and ericoid mycorrhizas. ECMF typically do not penetrate into the plant host cells.

ECM is predominantly formed between members of the fungal phyla Basidiomycota and Ascomycota and certain perennial plants, mainly forest trees in temperate and boreal ecosystems. It is estimated that ECM forms between fungi and the roots of around 2% of plant species (Tedersoo et al., 2010). ECMF form an entirely intercellular interface, consisting of highly branched hyphae forming a latticework between epidermal and cortical root cells, known as the Hartig net.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in terrestrial ecosystems. They could potentially have played an important role in terrestrial ecosystems for more than 460 million years, as evidenced by fossilized fungal structures (Redecker et al., 2000). Nowadays, AM is found in 80% of vascular plant families (Schüβler et al., 2001) and plants are the only source of C for the obligatorily biotrophic AMF. Nutrients are exchanged between fungi and plants through specific structures called arbuscules.

Saprotrophic fungi are considered to be the most efficient decomposers found in forest ecosystem due to their ability to produce a variety of extracellular lignocellulolytic enzymes and the capability to readily colonize new substrates. They have two types of extracellular enzymatic systems: (1) hydrolytic systems, which produces hydrolases that are responsible for polysaccharide degradation. These enzymes are produced by many microorganisms. (2) Oxidative and extracellular ligninolytic system which degrades lignin and open phenyl rings. Only a small group of fungi known as white-rot fungi has evolved with the ability to efficiently produce these enzymes and break down lignin to CO_2 (Sánchez, 2009).

Saprotrophic fungi may occur in two growth forms (regardless of their taxonomical relatedness) – as filamentous organisms or yeasts that typically exist as single cells. Yeasts are polyphyletic heterogeneous group of fungi that includes both the members of Ascomycota (some members of subphylum Taphrinomycotina: classes Neolectomycetes, Pneumocystidiomycetes, Schizosaccharomycetes, Taphrinomycetes; subphylum Saccharomycotina: class Saccharomycetes) and Basidiomycota (some members of subphylum Pucciniomycotina: classes

Agaricostilbomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes; subphylum Agaricomycotina: class Tremellomycetes; subphylum Ustilaginomycotina: classes Exobasidiomycetes, Ustilaginomycetes) (Fig. 3). Functionally, yeasts can be conceived as special adaptation to live in liquid or semi liquid mediums with a high concentration of easy-to-use nutrients (Starmer and Lachance, 2011). However, most yeasts, even those that grow as single-cell organisms during most of their lifecycle, are able to switch to filamentous growth as a response to changing environmental conditions. Some of them produce only pseudohyphae, cylindrical cells that are more similar to elongated yeast cells than to real hyphae. However, many species can form typical hyphae too. Thus, most yeasts are not exclusively unicellular in the traditional sense (Dickinson, 2005). For the purpose of this work, with regard to the fact that no clear definition of yeasts has been provided so far, fungal species were considered as yeasts, when they were described as such in the book "The yeasts: A taxonomic study" by Kurtzman et al., (2011). In addition, newly described relatives of listed species were considered as well as isolates of novel taxa that form colonies composed of single cells or grow as single cells in culture.

The predominant growth form (filamentous or yeasts) affects the realized niche of the fungus. It is assumed that fungal hyphae allow it to bridge sites with unfavorable conditions and cross nutrient-poor spots in searching for the heterogeneously distributed nutrient resources. This enables them to easily colonize new substrates and virtually dominate certain decomposition niches (de Boer et al., 2005) Filamentous growth also allows the fungus to transport scarce nutrients such as N and iron, to a distant nutrient poor-lignocellulosic substrate that constitutes its C source (Cadisch and Giller, 1997). Yeasts have to rely on local resources and are, therefore, highly affected by the patchiness of soil properties. Dispersal abilities of these two growth forms may also differ. Yeast cells are easily transmitted with air and water currents, while hyphal chains attached to a substrate have a lower probability of distribution. An ability of some basidiomycetous yeasts to produce forcibly ejected spores may also increase their ability to spread effectively.

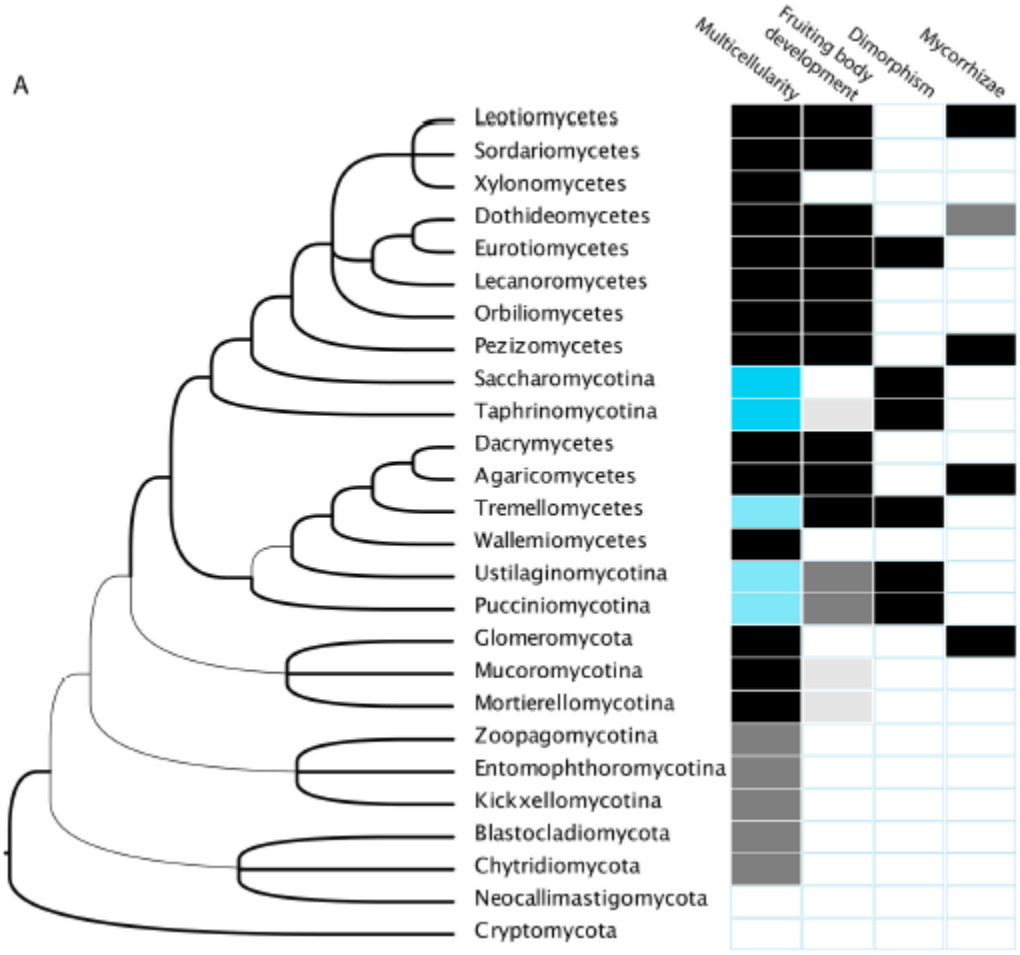

Figure 3: Phylogenetic distribution of selected traits among fungi. Thick branches denote well-known relationships, while thin branches mark uncertainties in our understanding of fungal relationships. Presence of absence of a given trait is highlighted by black or white shading, respectively. Grey shading denotes rare or not fully developed states of the given trait, while blue represents the secondary partial loss of multicellular growth in yeasts (modified from Nagy et al., 2017).

2.2.1 Factors shaping distribution of fungi in space and time

Observations from diverse forest soils suggests that many environmental factors, such as nutrient content, vegetation cover, temperature, water availability, pH or dominant tree species may be important factors affecting fungal community composition in space and time (e.g. Aponte et al., 2010; Kaiser et al., 2010; Landesman and Dighton, 2011). Dominant tree species is shaping communities in both litter and soil, however, it seems that communities in litter are affected more

(Urbanová et al., 2015). Litter communities are influenced by pre-existing endophytic community present in the leaf before leaf drop (Peršoh, 2013) as well as the differences in nutrient availability or specific litter chemistry (Bray et al., 2012). In soil, dominant tree species mainly affect communities of ECMF as these have often species-specific relationships with their host plants (Tedersoo et al., 2012; Tedersoo et al., 2014). AMF mainly establish associations with herbaceous plants and thus they are highly affected by ground vegetation (Opik et al., 2009).

Abiotic conditions seems to be less important for shaping fungal communities at larger spatial scales (Rousk et al., 2010; Crowther et al., 2014) than factors that are highly influenced by dominant tree species such as nutrient availability. Fungal diversity often decreases with soil depth as a consequence of a decreasing amount of organic matter (Voříšková et al., 2014) and for the same reason the distribution of functional guilds in soil horizon is not uniform either. Saprotrophic taxa are more abundant close to the surface of the forest floor whereas mycorrhizal fungi that have limited decomposition abilities increase in abundance with soil depth (Lindahl et al., 2007; Voříšková and Baldrian, 2013; Voříšková et al., 2014; Sterkenburg et al., 2015).

Another factor strongly affecting community composition and diversity of fungi areseasonal changes in resource availability including photosynthate allocation below ground by roots during the growth period and seasonal inputs of fresh litter. Litter quality changes during the course of its transformation and so does the activity of litter-associated microorganisms (Dilly et al., 2001). Fungi involved in the decomposition of litter have been divided into early, intermediate and late decomposers (Frankland, 1998; Tang et al., 2005) based on their ability to decompose substrates of different recalcitrance. In general, ascomycetous fungi dominate the initial stages of litter decay, but they are gradually replaced by basidiomycetous fungi, especially the saprotrophic cord formers (Frankland, 1998; Osono, 2007; Voříšková et al., 2014). Litter community exhibits higher seasonal changes than the community in the deeper horizons (Voříšková et al., 2014).

2.3 Soil yeast communities

2.3.1 How to study soil yeast communities: High throughput sequencing versus cultivations

Two methodological approaches were combined in this thesis – cultivations and HTS. During cultivation experiments which used to be the main approach to study soil-inhabiting microorganisms in previous decades, filamentous fungi usually highly outnumber yeasts (Botha, 2011) that were thus regarded as a minor group. The most common approach nowadays is HTS which is able to capture unicellular and filamentous organisms equally well and shows that high proportion of fungal community may be composed of yeasts. For example, Bueé et al. (2009) sequenced six different soils in France and showed that *Saitozyma podzolica* and *Solicoccozyma terricola* were among the species with the highest sequence abundance at the studied sites and Yarwood et al. (2010) identified these two species among the most abundant taxa in the forest soils of Oregon, USA. Furthermore, sequences with their closest matches to yeasts represented between 0.2-16% and 3-27% of all fungal sequences in litter and soil, respectively, of forests developed under different trees in postmining sites (Urbanová et al., 2015) and studies focused on decomposition of plant-derived materials also showed high proportions of yeasts (Voříšková and Baldrian, 2013; Voříšková et al., 2014).

Despite the existence of HTS datasets, this method has not yet been used for specific analyses of yeasts in soil and litter due to the presence of various methodological constrains. One of them is that reference databases used for identification of environmental sequencing libraries lack many yeast species or contain incorrect identifications, very often classified to the level of large former polyphyletic genera e.g. *Cryptococcus* sp., *Rhodotorula* sp., or *Trichosporon* sp. This situation is accentuated by the fact that the most common marker used for identification of yeasts species is D1/D2 region of the 26S rDNA gene (Scorzetti et al., 2002; Kurtzman et al., 2011) while the most frequent marker used in environmental sequencing libraries is ITS2 region.

The current knowledge of soil yeast communities is still based mainly on cultivation experiments (e.g Slavikova and Vadkertiova, 2000; Yurkov et al., 2012a; Yurkov et al., 2012b; Mestre et al., 2014), which only allow it to describe cultivable taxa or can be affected by the selection of cultivation media and, thus, might give a biased picture of yeast community composition. Only

less than 1% of the estimated microbial diversity is thought to be cultivable in laboratory (Amann et al., 1995) but it remains unknown how this number translates to yeasts. However, compared to most microorganisms, yeasts seems to be easy to culture as they often do not require special media and selective conditions of temperature, acidity or gas phase. One of the positive aspect of cultivations is obtaining of pure cultures for phenotypic analysis or description of new species and consequent description of yeast diversity. Although approximately 1,500 yeast species have been described up to 2011 (Kurtzman et al., 2011), the estimates of the total numbers of species suggest a number ten times higher (Lachance, 2006) and thus, this direction of research should not be underestimated.

2.3.2 Distribution of yeasts in forest soil and litter

Even though our knowledge of soil yeasts is biased towards temperate and boreal forests that have been studied most extensively, we know that yeast are more abundant in these zones because of the slow organic matter decomposition rates (Chernov, 2005; Botha, 2006). Litter usually hosts highly abundant and variable yeast communities with high proportion of transient species brought to this habitat from phyllosphere that contain abundant tree species-specific yeast populations reaching up to millions of cells per gram (Yurkov et al., 2008). Yeast communities in soils seem to be less numerous and diverse (Yurkov et al., 2012b). The number of yeasts that mostly occur in the top 10 cm of soil usually ranges between hundreds and thousands (Botha, 2006, 2011), however, the relative proportion of yeast sequences compared to those of the filamentous fungi seems to be higher in soil. Urbanová et al. (2015) studied fungal communities on sites dominated by 7 different dominant tree species and showed that relative abundance of yeasts was always higher in soil than in litter reaching tens of percent in the soil horizon.

Approximately 130 yeast species have been reported to be associated with soils worldwide (reviewed in Yurkov et al., 2011) which indicates that soil is a primary habitat of these species. Several adaptations might facilitate yeast capability for surviving in this environment. For example, species frequently found in soil have been shown to grow in media with low concentrations of nutrients (Kimura et al., 1998; Botha, 2006). Oligotrophy is thought to provide a competitive advantage over other soil microbes. Sugar turnover rates in soils are very high, so

the uptake of monosaccharides by microorganisms takes place in seconds to minutes (Gunina and Kuzyakov, 2015). Yeasts have developed active mechanisms allowing them to outcompete other species involving e.g. killer activity, substrate depletion, acidification or ethanol production (e.g. Golubev, 2006; Starmer and Lachance, 2011).

In most cases, yeast numbers and species composition are distributed rather unevenly in the topsoil as this habitat is highly heterogeneous and contains microenvironments characterized by different properties such as acidity, water activity or the availability of N and C sources (Fig. 4). Because yeasts inhabit small niches, the properties of their immediate environment rather than the average soil properties affect the local yeast community.

Culture-based studies on yeast communities from upper forest soil horizons have yielded mainly isolates belonging to the Basidiomycota (e.g. Mestre et al., 2011; Yurkov et al., 2012a; Yurkov et al., 2012b). It has been reported that saprotrophic basidiomycetous fungi are able to break down plant litter and wood more rapidly than Ascomycetes (e.g. Osono and Takeda, 2002, 2006). A similar trend was observed in the studied soil yeasts where higher and broader enzymatic activities were detected in basidiomycetous yeasts. Although some exceptions exist, most ascomycetous yeasts tested so far efficiently utilized simple sugars, while their ability to degrade complex polysaccharides was reported as absent or low. Therefore, they are often considered to be primary inhabitants of fresh litter or rhizosphere (Middelhoven, 2006; Mestre et al., 2011). In contrast, basidiomycetes are expected to be more abundant in the nutrient-limited bulk soil. Besides metabolic adaptations, yeast communities in the bulk soil tend to aggregate and form biofilms surrounded by polysaccharide capsules that facilitate their survival in this environment.

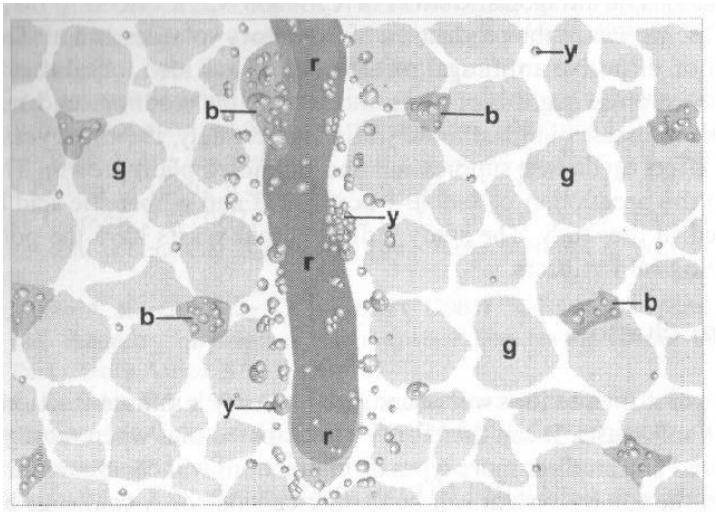

Figure 4: A simplified illustration demonstrating the distribution of soil yeasts (y). More yeasts are found in the rhizosphere close to the root (r) than further away from plant in the bulk soil. Yeasts in the nutrient poor region are usually able to form biofilms (b) enabling them to sequester nutrients. The polysaccharide capsules producing yeasts play a role in soil aggregate formation by binding soil particles (g) together (Botha, 2006).

2.3.3 The importance of yeasts in soil

2.3.3.1 Involvement of yeasts in decomposition of dead plant biomass

The decomposition and C utilization by various groups of filamentous fungi that are considered as primary degraders of organic matter in temperate forest soil and litter have been frequently addressed (e.g. Martinez et al., 2005; Baldrian et al., 2011; Eichlerová et al., 2015). However, the role of yeasts in these processes received less attention. The majority of soil yeasts were regarded as saprothrophs contributing to C mineralization processes in the environment by utilizing various C compounds. In his review on soil yeasts, Botha (2006) noted that most of them are able to utilize the products of the enzymatic hydrolysis of lignocellulosic plant materials such as L-arabinose, D-xylose and cellobiose and intermediates of lignin degradation, i.e. ferulic acid, 4-hydroxybenzoic acid and vanilic acid (e.g Henderson, 1961; Sampaio, 1999; Slavikova and Vadkertiova, 2000; Mestre et al., 2011). On average, basidiomycetous yeasts were reported to utilize a wider spectrum of C sources, including low-molecular-mass aromatic compounds, than ascomycetous yeasts (Fonseca, 1992; Sampaio, 1999; Botha, 2006; Middelhoven, 2006).

Although yeasts were repeatedly isolated from decaying plant material indicating that they might be involved in decomposition of such materials, those studies mainly focused on the description of the novel yeast species (e.g. Péter et al., 2003; Middelhoven, 2006; Middelhoven and Kurtzman, 2007; Kurtzman et al., 2011). Our understanding of functional relationships of yeasts in decomposition processes is thus limited to a few studies (e.g. Sampaio, 1999; Middelhoven, 2006; Yurkov et al., 2012a). The initial phase of fresh litter decomposition starts with the rapid depletion of simple sugars by fast-growing and often fermenting yeasts which may not experience much competition during this phase and are often brought to the litter horizon with freshly fallen litter as a part of the phylosphere. During the litter decomposition, these species are replaced by other yeasts (often basidiomycetous) that can utilize a wider spectrum of C sources, including cellulose, hemicellulos, phenol and products of the enzymatic hydrolysis of lignocellulosic plant materials (e.g. Margesin et al., 2005; DeRito and Madsen, 2009).

Culture-independent studies that targeted the involvement of fungal communities during the decomposition showed that some operational taxonomic units (OTUs) identified as yeast species tend to rapidly increase with time indicating their involvement in this process (e.g. Bueé et al., 2009; Voříšková and Baldrian, 2013). The study of the species involved in decomposition of cellulose showed that OTUs assigned to polyphyletic genera *Cryptococcus* and *Trichosporon* were among the most abundant basidiomycetous genera involved in this process and that their relative abundance increased with time of decomposition (Štursová et al., 2012). Voříšková and Baldrian (2013) studied the development of the fungal community over 24 months of litter decomposition in a forest with dominant *Quercus petraea* and showed that the abundance of yeast increased with time and that *Trichosporon* was among the most abundant fungi. Interestingly, in the study on seasonality of fungal community composition in a *Quercus petraea* forest, yeast taxa did not show seasonal dynamics in soil. In litter, however, their relative abundances were significantly higher in the autumn and winter (Voříšková et al., 2014).

2.3.3.2 Other functions of yeasts in forest soil environment

Many yeasts are able to grow on rock surfaces (Sterflinger and Prillinger, 2001; Burford et al., 2003) where they contribute to the weathering thus contributing the phosphorus and sulphur

cycles within soil. In addition, yeasts have also been observed to solubilize insoluble phosphates making this nutrient more readily available to plants (Burford et al., 2003; Botha, 2006, 2011). Weathering is a process of great importance in soils, where minerals represent and important pool of inorganic nutrients. Yeasts also play an important role in formation of soil aggregates due to the ability of many species to produce extracellular polymeric capsules that bind soil particles (Vishniac, 1995; Botha, 2006, 2011).

Yeasts also interact with other organisms in soil and litter. Many different types of interactions with animals such as mutualism or antagonistic interactions have been observed, but most of them have been so far studied mainly *in vitro*. Yeasts may also serve as a food source for soil inhabiting bacteria, invertebrates and protists (Botha, 2006; Yurkov et al., 2008; Botha, 2011). A growing number of studies indicate that plant growth may be directly or indirectly enhanced by yeasts in the rhizosphere (Medina et al., 2004; Nassar et al., 2005; Cloete et al., 2010). Presence of yeasts has been observed to increase nodulation as well as AMF colonization and hyphal growth (Ravnskov et al., 1999; Fracchia et al., 2003). Some yeasts produce plant growth regulators (El-Tarabily and Sivasithamparam, 2006) while others may act antagonistically to the growth of fungal root pathogens (El-Tarabily, 2004).

2.3.4 Composition of yeast communities in forest topsoil

Cultivation studies showed that yeast communities on a single plot consist of a few species only and thus exhibit low α-diversity (Yurkov et al., 2011). Variability in a community composition on a larger scale (β-diversity) is usually very high (e.g., Slavikova and Vadkertiova, 2000; Vishniac, 2006; Starmer and Lachance, 2011; Yurkov et al., 2012b) indicating that yeast distribution in soils is often fragmented with a few species only shared between sampling sites. It is assumed that yeast community composition is not limited by dispersal constrains as yeasts are easily transported by air currents (Starmer and Lachance, 2011), organismal vectors and their also translocate with plant material as epiphytes or endophytes (e.g. Fonseca and Inácio, 2006; Zhang et al., 2010; Francesca et al., 2014). We can therefore expect that yeast communities might be affected by local environmental properties also on a larger scale than just at the level of patchiness of soil properties.

2.3.4.1 Effect of abiotic conditions on topsoil yeast communities

It has been reported that community composition and abundance of yeasts reflect abiotic conditions such as pH, water activity, temperature and nutrient availability. One of the most important factors affecting yeast communities is moisture. The structure of yeast communities along vast latitudinal gradients showed that species composition changed significantly with rainfall intensity (Vishniac, 2006). Similarly, changes in the yeast community of forest soils correlated with soil moisture and thus followed seasonal changes (Slavikova and Vadkertiova, 2000). Yurkov et al., (2016) studied yeasts in Mediterranean forests and reported that communities significantly differed between three forests differing in precipitation level. However, it seems that water content does not affect yeast population size as Vreulink et al. (2007) studied low nutrient sandy soil and found no correlation between soil moisture content and soil yeast population size

Birkhofer et al. (2012) and Yurkov et al. (2012b) showed that neither soil yeast community composition nor abundance within the same type of forest in Germany were significantly related to soil properties such as pH, N content and C/N ratio. However, yeasts were, in contrast to other fungi, highly abundant in forest soils of Schorfheide region with low pH (3.2) and average annual precipitation (520-600 mm). Similarly, Yarwood et al. (2010) showed that basidiomycetous yeasts were abundant in nutrient rich and well drained soils with low pH. Another study showed that a positive correlation exists between soil yeast population size and both organic C and organic nitrogen content of the soil (Moawad et al., 1986; Botha, 2006).

2.3.4.2 Effect of biotic conditions on topsoil yeast communities

Upper soil horizons always harbour substantial proportion of litter species-specific transient species originating from leaf material (e.g. Maksimova and Chernov, 2004; Yurkov et al., 2008; Yurkov et al., 2012b) and it is thus difficult to estimate the extent in which litter quality (e.g. composition of secondary metabolites, recalcitrance) affect yeast community composition. However, such properties are important to some extent as Yurkov et al. (2012b) showed that soil

collected underneath litter hosted more variable communities than soils underneath logs where selective and stable conditions promote growth of few highly specialised soil-borne yeasts.

The effect of dominant trees on the composition of yeast communities in temperate forest soils has been addressed and significant effect has been shown several times (e.g. Wuczkowski and Prillinger, 2004; Yurkov et al., 2004; Yurkov et al., 2011; Mestre et al., 2014). For example, Maksimova and Chernov (2004) studied yeast communities of boreal forests and discovered that yeast communities differed among spruce, alder and birch forests. Moreover, yeast communities in birch forests with similar climatic conditions, soil type and vegetation of two geographically separated regions of Russia were notably similar (Yurkov et al., 2004). The effect of the dominant tree can be explained by the fact that different trees produce different profiles of root exudates and form soils of different chemistry (Fan et al., 2001) and these factors affect yeast species composition. It has been shown that yeast quantity, diversity, and community composition also reflects other forest properties, such as age and management history (Yurkov et al., 2012b).

However, despite all of the above information, drivers of yeast communities need to be confirmed in culture-independent studies.

3 List of publications

This thesis consist of the following papers:

Paper I

Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K & Baldrian P (2017): Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiology Ecology* **93**: fiw223.

Paper II

Bahnmann B, Mašínová T, Halvorsen R, Davey M, Sedlák P, Tomšovský M, Baldrian P: Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest. Submitted for publication.

Paper III

Mašínová T, Pontes A, Carvalho C, Sampaio JP & Baldrian P (2017): *Libkindia masarykiana* gen. et sp. nov., *Yurkovia mendeliana* gen. et sp. nov. and *Leucosporidium krtinense* f.a. sp. nov., isolated from temperate forest soils. *International Journal of Systematic and Evolutionary Microbiology* **67**: 902-908

Paper IV

Mašínová T, Yurkov A, Baldrian P: Forest soil yeasts: Decomposition potential and the utilization of carbon sources. Submitted for publication

Paper I

Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K & Baldrian P (2017): Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiology Ecology* **93**: fiw223.

Fungi represent a group of soil microorganisms fulfilling important ecological functions. Although several studies have shown that yeasts represent a significant proportion of fungal communities, our current knowledge is based mainly on cultivation experiments. In this study, we used amplicon sequencing of environmental DNA to describe the composition of yeast communities in European temperate forest and to identify the potential biotic and abiotic drivers of community assembly. Based on the analysis of ITS2 PCR amplicons, yeasts represented a substantial proportion of fungal communities ranging from 0.4-14.3% of fungal sequences in soil and 0.2-9.9% in litter. The species richness at individual sites was 28 ± 9 in soil and 31 ± 11 in litter. The basidiomycetous yeasts dominated over ascomycetous ones. In litter, yeast communities differed significantly among beech, oak and spruce-dominated stands. Drivers of community assembly are probably more complex in soils and are comprise the effects of environmental conditions and vegetation.

Paper II

Bahnmann B, Mašínová T, Halvorsen R, Davey M, Sedlák P, Tomšovský M, Baldrian P: Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest. Submitted for publication.

Despite the progress in the past few years, the drivers of the composition of fungal communities in temperate forest soils are not fully identified. Here we have explored the factors driving the variation in natural-occurring fungal communities in litter and soils by sampling sites dominated by either spruce, beech or oak across a temperate forest. Randomized complete-block design with six replicates blocks spanning a $100km^2$ forested area was used and sampling was performe four times over a one-year period to account for temporal variability. Fungal communities were characterized using amplicon sequencing. We found that fungal community composition differed between litter and soil and among stand types but community structure (richness, functional-guilds) was similar. Litter community composition was strongly coupled to dominant tree species. In soil communities, both dominant tree and abiotic variables were important with each variable explaining a unique parts of the variation in the community composition. Analyses of the community by functional-group subsets of showed some variation to these overall community patterns. Both the litter and soil communities of given stand type were well-characterized by a set of low-abundance indicator species with consistent presence, regardless of location, suggesting stand type is an important local filter. The marked difference in annual growth patterns between coniferous and deciduous stands were not found to correlate with changes in fungal community composition, however during our sampling period, a common time-dependent trend was found across all soil communities and among all soil functional-group subsets regardless of the dominant tree type.

Paper III

Mašínová T, Pontes A, Carvalho C, Sampaio JP & Baldrian P (2017): *Libkindia masarykiana* gen. et sp. nov., *Yurkovia mendeliana* gen. et sp. nov. and *Leucosporidium krtinense* f.a. sp. nov., isolated from temperate forest soils. *International Journal of Systematic and Evolutionary Microbiology* **67**: 902-908

One hundred and ninety-eight isolates of soil yeasts were isolated from mixed temperate forests in the Czech Republic, and their abundance and distribution in the litter and soil were evaluated using amplicon sequencing of soil fungal communities. Abundant taxa with no close identified hits were selected for further characterization as potential novel species of yeasts. Phylogenetic analyses using sequences of the D1/D2 domains, the ITS region and RPB1 and TEF1 genes support the recognition of the following three new species belonging to the subphylum Pucciniomycotina, class Microbotryomycetes: *Leucosporidium krtinense* f.a. sp. nov. (type strain CBS 14304^T = PYCC 6879^T = DSM 101892^T), *Yurkovia mendeliana* sp. nov. (type strain CBS 14273^T = PYCC 6884^T = DSM 101889^T), and *Libkindia masarykiana* sp. nov. (type strain CBS 14275^T = PYCC 6886^T = DSM 101891^T). Since the later two novel taxa cannot be assigned to existing genera, two new genera, *Libkindia* gen. nov. and *Yurkovia* gen. nov. are also described.

Paper IV

Mašínová T, Yurkov A, Baldrian P: Forest soil yeasts: Decomposition potential and the utilization of carbon sources. Submitted for publication

Fungi that inhabit upper forest soil horizons are important decomposers of dead plant organic matter. Fungi living in soils can be divided into two functional groups: filamentous, multicellular fungi and predominantly unicellular yeasts. Due to an inability to efficiently translocate nutrients, the nutritional mode and realized niche of yeasts in the soil is expected to differ from that of filamentous fungi. Soil yeasts comprise a systematically artificial group of fungi, some of which are able to switch between filamentous and unicellular growth. In this study, we explored the decomposition potential and carbon utilization profiles of twenty-five dominant yeasts from the topsoil of a temperate forest. The results indicated that despite taxonomic heterogeneity, yeasts represent a fungal group with a specific nutritional strategy that is dissimilar from other tested soil fungi. Yeast isolates frequently produced enzymes involved in the degradation of hemicellulose: β-xylosidase, α-galactosidase, β-galactosidase, β-mannosidase, β-glucuronidase, and arabinosidase activity was observed in 44-92% of strains. The ability to utilize cellulose was relatively common, with 84% of strains producing exocellulase, and all of the tested yeast strains exhibited high β-glucosidase activity. The activity of laccase, an enzyme that potentially contributes to the transformation of lignin and other phenolics, was rarely observed, and only in association with yeast cell walls. Chitinase activity was present in 72% of yeast strains, although it was typically low. While the efficient decomposition of hemicellulose, cellulose or chitin appeared to be restricted to only a few taxa, the results of carbon source utilization assays indicated that most yeasts could efficiently act as mutualists, utilizing the decomposition products generated by other microbes. Importantly, a large fraction of total enzyme activity was associated with yeast cell surfaces. This adaptation likely ensures that the decomposition products are produced at the cell surface of the unicellular microorganisms and are readily available to the organisms producing the enzymes.

4 Methods

Bioinformatic analyses of amplicon sequencing data (Paper I, II, III, IV)

Cultivation of yeasts (Paper III, IV)

Description of new yeast species (Paper III)

DNA extraction from cultures (Paper III, IV)

DNA extraction from soil (Paper I, II)

Enzyme assays (Paper III, IV)

Library preparation for DNA high-throughput sequencing (Paper I, II, III, IV)

Molecular taxonomical identification of isolated yeast strains (Paper III, IV)

Polymerase chain reaction (Paper I, II, III, IV)

Quantification of fungal biomass (Paper II)

Soil sampling (Paper I, II, III)

Statistical and diversity analyses (Paper I, II, III, IV)

5 Discussion

This thesis consists of four papers that contribute to the understanding of the ecology of yeasts in temperate forest topsoil. For the first time, HTS on the Illumina MiSeq platform was used in order to identify dominant yeast species and explore how yeast community reflects the abiotic and biotic factors including variable ground vegetation and the composition of the tree layer. The aim was also to compare the factors that are shaping yeast community composition with those that are important for filamentous fungi. The combination of HTS and isolations was used to select those isolates that either represented abundant but not yet described species or were representative for the yeast communities that were further used for physiological characterization in order to elucidate the involvement of yeast in C cycling and decomposition of dead organic matter. Furthermore, three novel yeast species with high relevance for the temperate forest environment were described together with soil yeast diversity.

5.1 Diversity and community composition of yeasts

Eighteen sites in the Křtiny forest dominated by spruce, beech and oak were repeatedly sampled in October 2013 and April 2014 to isolate yeast strains. In total, 198 yeast strains were isolated and tentatively identified using BLASTn against the UNITE and GenBank (http://www.ncbi.nlm.nih.gov) databases. To ensure a clear identification, only the hits with a coverage > 90% and a similarity > 97% were retained. This criterion was fulfilled for 128 isolates that belonged to 24 genera: *Candida, Cryptococcus, Cutaneotrichosporon, Cystofilobasidium, Dioszegia, Fellozyma, Filobasidium, Heterocephalacria, Holtermaniella, Itersonilia, Kwoniella, Lachancea, Leucosporidium, Naganishia, Oberwinkleozyma, Pichia, Piskurozyma, Rhodosporidiobolus, Saitozyma, Sporobolomyces, Solicoccozyma, Trichosporon, Vishniacozyma, Yamadamyces*. The rest of the isolated strains probably belong to more than 20 yet undescribed yeast species. This is in accordance with previous studies that indicated that more than 30% of yeast species inhabiting temperate forests have not been described previously (Mestre et al., 2011; Yurkov et al., 2012b; Yurkov et al., 2016). Therefore, further studies dealing with the description of yeast diversity and formal description of new yeast species are necessary.

Three new yeast species belonging to the subphylum Pucciniomycotina, class Microbotriomycetes were described. These species were named as *Leucosporidium krtinense* f.a. sp. nov. (type strain CBS 14304^T = PYCC 6879^T = DSM 101892^T), *Yurkovia mendeliana* sp. nov. (type strain CBS 14273^T = PYCC 6884^T = DSM 101889^T), and *Libkindia masarykiana* sp. nov. (type strain CBS 14275^T = PYCC 6886^T = DSM 101891^T). Since the latter two novel taxa cannot be assigned to existing genera, two new genera, *Libkindia* gen. nov. and *Yurkovia* gen. nov. were also described. Based on the analysis of environmental DNA, the new species constitute common and abundant taxa in the studied environment which indicates their high environmental relevance in the temperate mixed forest ecosystems.

Eighty-two sites in the Křtiny forest were explored with HTS, 18 of them across four seasons. The species richness of yeasts at individual sites was 28 ± 9 in soil and 31 ± 11 in litter. These numbers are comparable to the number of species obtained with cultivation approaches (e.g., Mestre et al., 2011; Yurkov et al., 2011; Yurkov et al., 2012a). Basidiomycetous yeasts acounted for more than 90% of sequence reads in our study and also dominated among isolated strains. This is in line with the previous reports concerning forest soils that aslo yielded mainly isolates belonging to this group (e.g. Maksimova and Chernov, 2004; Yurkov et al., 2012b; Yurkov et al., 2016). The same was also shown in other culture-independent surveys (e.g. Bueé et al., 2009; Yarwood et al., 2010).

Relative abundance of yeast sequences within the total fungal community ranged from 0.4-14.3% in soil and 0.2-9.9% in litter (Fig. 5). Higher mean relative abundances of yeasts in soil than in litter has also been indicated by the analyses of amplicon sequencing data by Urbanová et al. (2015) who studied temperate forests that were dominated by 7 different dominant tree species. The traditional view based on cultivation studies expects, however, higher abundances of yeasts in litter than in soil as litter represents more suitable habitat for survival of yeast species than soil (Yurkov et al., 2012a). Considering the fact that the amount of fungal biomass in litter per g substrate dry mass can be 10-fold higher than in soil (Baldrian et al., 2013), we can conclude that some yeasts are well adapted to the nutrient-poor soil environment and occupy specific niches that allow them to reach high relative abundances.

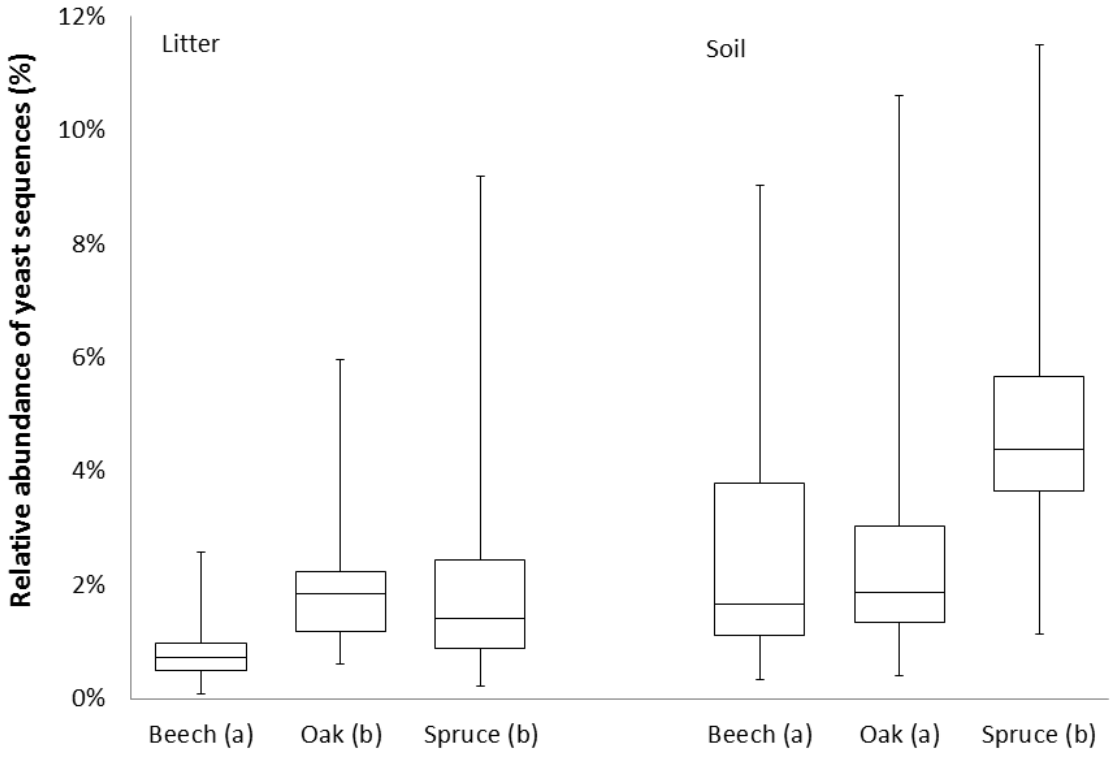

Figure 5: Relative abundance of yeasts in the temperate forest litter and soils in stands with different dominant trees. The results represent maxima, minima, upper and lower quartiles and medians from six sites per tree. Different letters indicate significant differences (ANOSIM, $p < 0.05$).

Yeast communities differed significantly between litter and soil. Soil yeast community in the studied temperate forest was highly uneven, represented by a few highly abundant OTUs and many rare taxa, while the yeast community in litter was more even. The most abundant OTUs identified in metagenomics study were *Solicoccozyma terricola (Fillobasidiales)*, *Saitozyma podzolica (Tremellales)*, *Apiotrichum porosum (Trichosporonales*), *Apiotrichum dulcitum (Trichosporonales)*, *Cutaneotrichosporon moniliiforme (Trichosporonales)* and *Fellozyma inositophila (Sporidiobolales)*(Fig. 6). Unfortunately, comparison of relative abundances of yeast species obtained with cultivations and HTS is not possible as the cultivations were focused on capturing the yeast diversity and thus number of strains that were isolated from individual sites does not necessarily correspond to their abundance on plates. However, our results showed a

good overlap between cultivated and detected species suggesting that soil yeasts can be fairly well cultured.

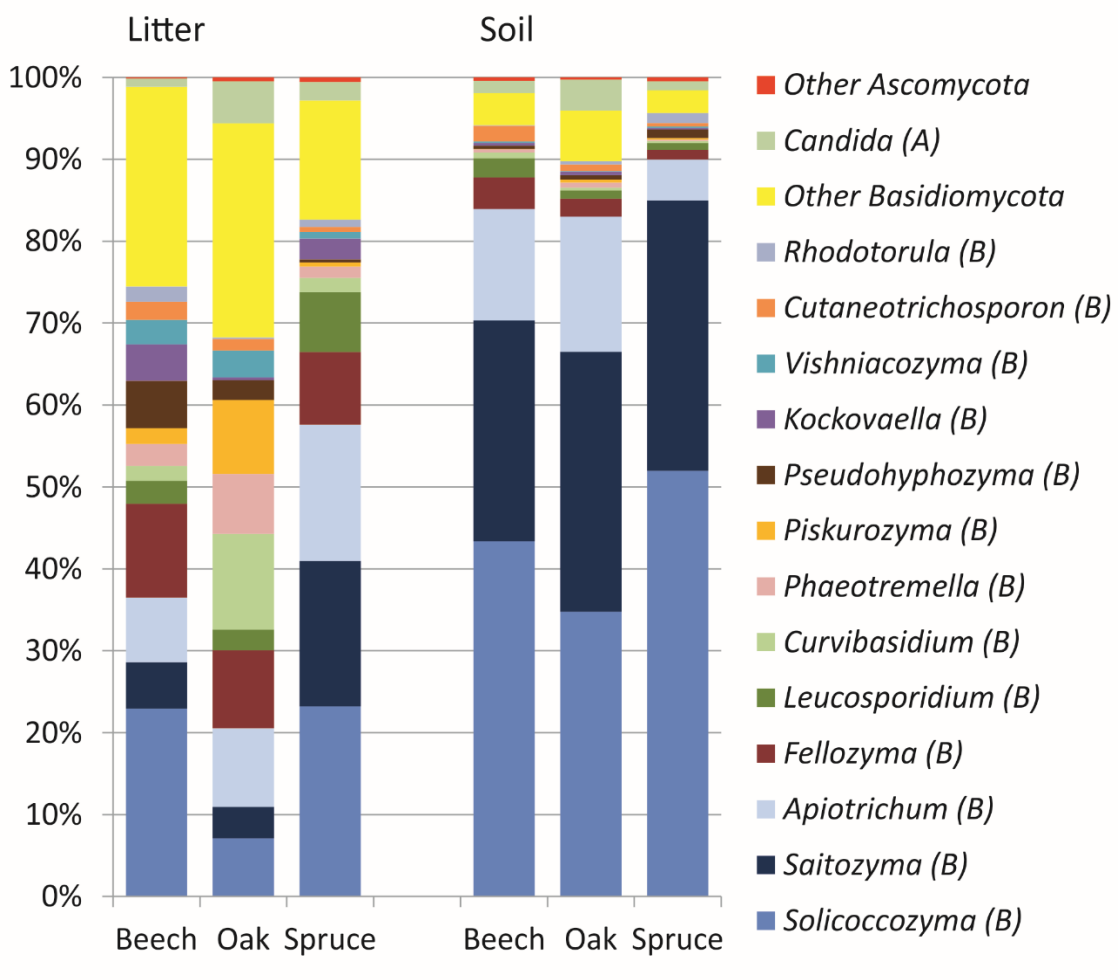

Figure 6: Communities of yeasts from the litter and soil of temperate beech, oak and spruce forests. OTU identifications are based on the taxonomy of the most closely related yeast taxon. The data represent mean values from six sites per tree. Abbreviations: A – *Ascomycota*, B - *Basidiomycota*

5.2 Biotic and abiotic drivers of yeast community composition

Biotic and abiotic drivers affecting yeast community composition were studied using amplicon sequencing of environmental DNA. Communities in litter were significantly different among tree

species while the effect of chemistry was lower. It is known that a high proportion of litter-associated yeast communities are composed of taxa that are not restricted to this habitat and may passively enter litter (e.g. Vishniac, 2006; Yurkov et al., 2012a). Litter input may contribute substantially to the composition of yeast communities because phylloplane may contain abundant tree species-specific yeast populations reaching up to millions of cells per gram (Yurkov et al., 2008). It is also likely that yeasts are selected by the composition of secondary metabolites in litter rather than the content of macronutrients, indicating that these secondary metabolites are the source of their nutrition. Indeed, litters with similar nutrient content but of different origin were found to host different microbial communities (Urbanová et al., 2015). Also Yurkov et al. (2012a) showed that soil collected underneath litter hosted more variable communities than soils underneath logs where selective and stable conditions promote growth of few highly specialized soil-borne yeasts. This hypothesis may be also supported by the fact that the relative abundance of yeasts was significantly different between dominant tree species (Fig. 5).

While the effect of tree layer vegetation was also significant in the Whole Area Experiment soil samples, yeast communities under beech, oak and spruce stands (Dominant Tree Experiment) were similar. These results suggest that relationships between dominant trees and yeast communities in soil may be more complex, and perhaps result from the strong effect of some tree species while others may share similar taxa. This would not be surprising, as stands of different trees exhibit different levels of specificity to their soil fungal communities (Urbanová et al., 2015). On the other hand, soil communities seemed to be highly affected by abiotic factors, especially by pH and moisture. This is in agreement with previous studies that showed the effect of these two factors on community composition of yeasts (Vishniac, 2006; Yarwood et al., 2010; Yurkov et al., 2016). Indicator species analysis was used to assess the level of specificity of yeast for the three studied trees (Dominant Tree Experiment). Only a few yeast taxa were identified as indicator species for litter samples. Generally, there was little overlap between dominant and indicator taxa, and the abundance of most identified indicator taxa was low.

An important step towards the understanding of the role of yeasts in temperate forest soils and the drivers of their occurrence and distribution in the environment is to understand their nutritional traits. Yeasts exhibit rapid growth and a larger number of offspring can be produced asexually

instead of following a sexual cycle. The resulting yeast populations may constitute distinct biotypes subject to unusual forms of selection. Therefore, we picked up the yeast isolates that were obtained in the Křtiny forest, compared them with HTS data and selected those that well-represented the local yeast community.

5.3 Decomposition potential and the utilization of carbon sources by yeasts

Saprotrophic fungi inhabiting forest topsoil are the most important decomposers of dead plant material converting recalcitrant lignocellulosic compounds into smaller molecules that are available for other organisms. Even though yeasts represent a substantial part of these communities (Bueé et al., 2009; Yarwood et al., 2010; Voříšková and Baldrian, 2013), their involvement in decomposition processes remained largely unknown.

Therefore, 25 yeast species were tested for their potential involvement in decomposition of dead organic matter and ability to utilize different C sources. The studied isolates represented OTUs that together accounted for 42.3% and 29.4% of the sequence counts of yeasts in soil and litter, respectively. They belonged to three lineages of fungi, Saccharomycotina, class Saccharomycetes (four species), Agaricomycotina, class Tremellomycetes (16 species) and Pucciniomycotyna, class Microbotryomycetes (five species).

It has been repeatedly demonstrated that saprotrophic basidiomycetous fungi are able to break down plant litter and wood more rapidly and efficiently than Ascomycetes (e.g. Osono and Takeda, 2002, 2006). Although some exceptions exist, most ascomycetous yeasts tested so far efficiently utilized simple sugars, while their ability to degrade complex polysaccharides was reported as absent or low. Therefore, they are often considered to be primary inhabitants of fresh litter or rhizosphere (e.g. Middelhoven, 2006; Mestre et al., 2011). Nevertheless, a few ascomycetous yeasts have been isolated from forest soils, including *Candida railenensis* and *Lachansea thermotolerans*. In our study, the activity of four ascomycetous yeasts did not differ substantially from basidiomycetous yeasts, some of which also displayed a limited decomposition potential.

Studied yeasts frequently produced enzymes involved in the degradation of hemicellulose; β-xylosidase, α-galactosidase, β-galactosidase, β-mannosidase, β-glucuronidase and arabinosidase were produced by 44-92% of strains. This observation is in line with earlier reports of the ability of some yeasts isolated from forests to assimilate different types of hemicelluloses, such as xylan or galactomannan (Jiménez et al., 1991; Middelhoven, 2006). Although the activity of hemicellulases was low in most cases, yeasts showed a strong ability to utilize hemicellulose-derived monosaccharides such as arabinose and xylose (Kurtzman et al., 2011; Mestre et al., 2011).

The ability to utilize cellulose was rather common among the studied species as 84% of strains produced exocellulases, although their activity was often low. Cellulolytic activity was only rarely reported in yeasts isolated from rotten wood (Jiménez et al., 1991; Middelhoven, 2006), but Štursová et al., (2012) showed that tremelloid yeasts assigned to the previously polyphyletic genera *Cryptococcus* and *Trichosporon* acquired C from ^{13}C-cellulose added to soil. In our study, all yeasts displayed high activity of β-glucosidase. This enzyme can hydrolyse cellobiose (a product of cellulose hydrolysis) into two glucose molecules. The ability to utilize this disaccharide seems to be relatively common among yeasts (reviewed by Botha, 2006; Kurtzman et al., 2011) and most of the tested strains oxidized and assimilated this compound. These observations may indicate that even though cellulose decomposition by yeasts is probably rare, they can efficiently use products of cellulose degradation and act as potential commensalists of cellulolytic filamentous fungi.

The activity of laccase, an enzyme potentially contributing to the transformation of lignin and other aromatic compounds, is rather widespread among filamentous fungi (Eichlerová et al., 2015). Even though only a limited number of yeasts was tested for this enzyme, laccase was detected in some of them (Petter et al., 2001; Ikeda et al., 2002; Bovers et al., 2008; Pajot et al., 2011). In our study laccase activity was detected in a few species, always in association with yeast cell walls. Although lignin decomposition is not a frequently reported trait in yeasts, some indications on the utilization of this compound exist (Jiménez et al., 1991) and several yeasts have previously been reported to utilize phenolic compounds that arise during lignin degradation (e.g. Henderson, 1961; Sampaio, 1999; Middelhoven, 2006). However, whether laccases or other

hydrolytic enzymes are involved in the degradation of aromatic compounds by yeasts requires additional studies.

Even though the ability to produce chitinase (N-acetylglucosaminidase) was present in 72% of tested yeast strains, the activity of this enzyme was typically low with just a few exceptions. Similarly, yeasts from the rotten wood, which is rich in fungal biomass, were unable to grow on colloidal chitin (Middelhoven, 2006). It has been previously reported that production of chitinolytic enzymes is widespread among both ascomycetous and basidiomycetous filamentous fungi isolated from the forest environment (Lindahl and Finlay, 2006; Baldrian et al., 2011) and, therefore, the studied soil yeasts seems to be an exception in this respect. However, chitinases were reported to be expressed by several soil-related yeasts (Buzzini and Martini, 2002; Bar-Shimon et al., 2004; Yu et al., 2008). Screening of isolates showed that many yeasts were able to use the final product of chitin degradation – N-acetylglucosamine suggesting that the importance of chitinases for soil yeasts, either as enzyme producers or commensalists, cannot be ruled out. It is important to document that multiple yeasts, identified as members of the polyphyletic genera *Candida*, *Cryptococcus*, *Trichosporon* and *Asterotremella* were found enriched on fungal mycelia that decomposed in the soil (Brabcová et al., 2016). The utilization of chitin offers not only the access to C but also to N which makes it highly attractive in the nutrient-limited forest environment (Date, 1973; Reich et al., 2006). Compared to saprotrophic soil micromycetes and litter decomposing Basidiomycota, yeasts exhibited higher activities of leucine and valine aminopeptidases and it seems that proteins belong to important C and N sources for them.

While the efficient decomposition of cellulose, chitin or hemicelluloses is probably restricted to only a few yeast taxa, most yeasts can efficiently act as mutualists that utilize products of decomposition, provided by other microbes. This view is supported by the observation of yeasts associated with decomposing litter and fungal mycelia (Voříšková and Baldrian, 2013; Brabcová et al., 2016) as well as the accumulation of C from cellulose (Štursová et al., 2012). The HTS results of yeast abundance, however, seem to be contradictory, showing that the relative share of yeasts is higher in soil than in litter despite decomposition rates are higher in the latter environment. The unicellularity of yeasts is probably also the reason for the association of their enzymes with their cell walls. This should ensure that decomposition products are produced at the cell surface and can be readily taken up.

Both culture-dependent and culture-independent studies suggest that soil yeasts are more prominent in temperate and boreal soils, where decomposition rates are slow. Although the C sources most frequently utilized by the screened yeasts were mono- and oligosaccharides, many soil yeasts are polytrophic. There is a growing body of evidence that basidiomycetous yeasts possess a diverse enzymatic machinery that can be activated in a certain stage of their life cycle. It is important to recall that many of basidiomycetous yeasts (including relatives of some soil-related lineages) are dimorphic mycoparasites switching between filamentous and unicellular stages (Oberwinkler, 2017). Accordingly, their interactions with wood decomposers might change from commensalism to parasitism. This would explain the polytrophy and diverse enzymatic capabilities observed in this study. It further implies that the role of yeasts in soils is more than those of copiotrophs relying on nutrients from filamentous fungi and soil bacteria. Capabilities and functions of soil yeasts are far more diverse than those routinely measured in the lab. Thus, future studies that address decomposition in forest soils using meso- and microcosms, metatranscriptomics and stable isotopes probing should include fungi commonly considered as yeasts.

5.4 Difference between yeasts and filamentous fungi

The predominant growth form of fungi (filamentous or yeasts) affects its realized niche. Therefore, enzyme production patterns of yeasts and filamentous saprotrophs were compared as well as the factors shaping community composition of yeasts and three ecophysiological guilds of filamentous fungi (saprotrophs, ECMF, AMF).

5.4.1 Enzyme production patterns

Yeasts are typically able to produce multiple enzymes that allow them to utilize carbohydate biopolymers, however, they produce most of these enzymes in lower extent and thus their involvement in decomposition processes is probably lower, similar to bacteria. To prove this hypothesis, API ZYM^{TM} (Biomerieux, France), a laboratory kit for semi-quantitative analysis of the production of the selected hydrolytic enzymes by microorganisms was used to compare yeasts with other groups of saprotrophic filamentous fungi: wood-associated Ascomycota, saprotrophic

micromycetes, white-rot fungi, brown-rot fungi and litter decomposing Basidiomycota (Eichlerová et al., 2015). The NMDS analysis showed that composition and activity of enzymes produced by soil yeasts is different from litter decomposing Basidiomycota, white-rot fungi, and brown-rot fungi. Some wood-associated ascomycetes and saprotrophic micromycetes showed enzymatic capabilities similar to those of the yeasts. However, a quantitative approach showed that the analyzed properties of yeast enzymatic machineries were significantly different from all other functional groups of fungi. Overall, enzyme activities measured in yeast cultures were lower than in the other groups of fungi (Fig. 7).

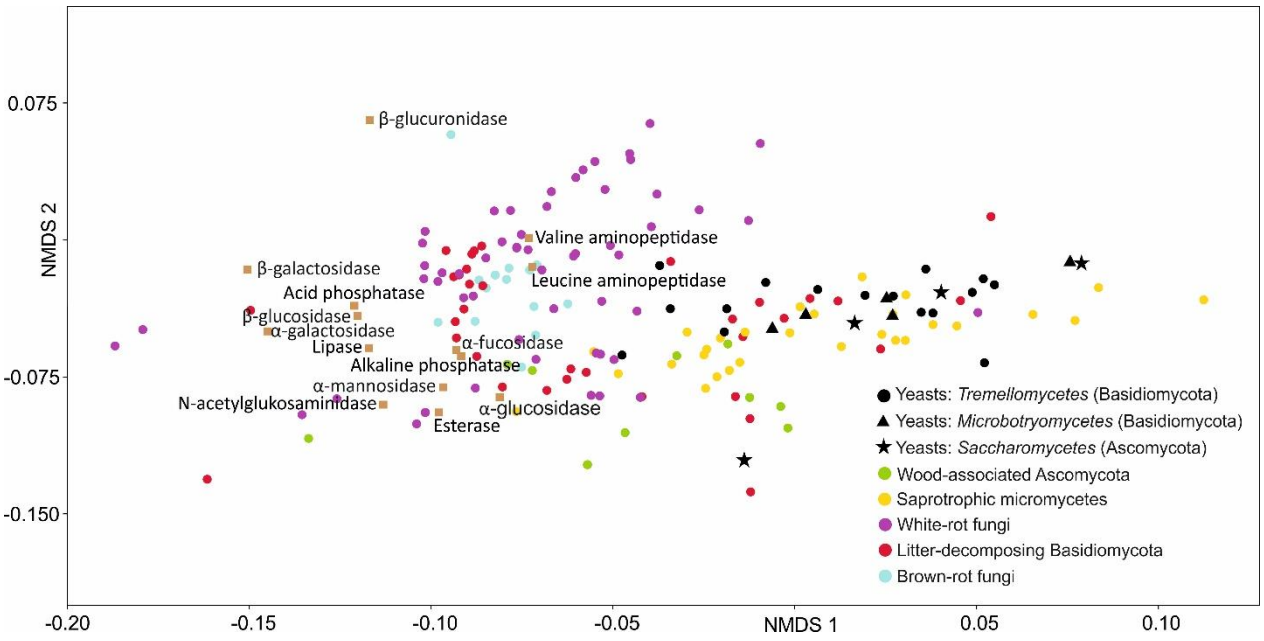

Figure 7: Three dimensional non-metric multidimensional scaling (NMDS, stress = 0.29) of enzyme production by fungi belonging to six ecophysiological groups. Each point represents one fungal species. Brown squares represent individual enzymes. Data for non-yeast fungi are from (Eichlerová et al., 2015)

5.4.2 Factors shaping community composition of yeasts, filamentous saprotrophs, ECMF, and AMF

To compare factors shaping community composition of yeasts and filamentous fungi, 18 sites, 6 of them dominated by spruce, beech and oak were sampled in 4 seasons (January, April, June, October).

The NMDS ordinations of soil samples by functional guild OTU subsets found only ECMF communities clearly differentiated according to stand type. The influence of dominant tree was also apparent for soil yeasts and (nonyeast) saprotrophs, although communities under the three dominant tree types were not significantly different. Yeasts, saprotrophs and ECMF also showed association to the understory vegetation. pH affected communities of saprotrophs and yeasts. None of the measured variables was found to significantly affect soil AMF communities. Litter samples showed significant segregation by stand types for yeasts, saprotrophs and ECMF communities, while the effect on AMF was less strong (Fig. 8). Post-hoc tests indicated that saprotroph and yeast communities are significantly different in each stand type. Saprotrophs and yeast communities were also affected by understory vegetation. AMF communities were affected by moisture and understory vegetation. ECMF communities in litter significantly responded to pH.

In litter, both saprotroph and yeast communities were distinct among all three stand types. Specificity of the litter decomposer community to a particular dominant tree species has been previously reported and attributed to the pre-existing endophytic community present in the leaf before leaf drop (Peršoh, 2013) as well as differences in nutrient availability or specific litter chemistry (Bray et al., 2012). The primary axes of the ordinations for these guilds suggest that the major variation in the saprotroph community is associated with the differences between broad-leaf and coniferous litter while the major variation in the yeasts community is associated with the differences among each litter type. This may be evidence of the generalist nature of saprotroph ecology where species are known to be accessing resources under a broad range of conditions by releasing extracellular oxidative and hydrolytic enzymes. These enzymes act compounds widely present (for example, cellulose). The specificity of yeast communities supports the hypothesis of the importance of secondary metabolites for their nutrition or survival.

Differences in turnover patterns between time points were found for all functional guilds in soil, where a significant loss of species was observed between January and April and significant gain of species between July and September. This pattern is similar among all stand types. In litter, differences between time points was only found for the yeast community, where a greater gain of yeast OTUs between July and September sampling periods was found. This can be due to the ability of yeasts to respond quickly to suitable conditions with rapid growth (Botha, 2006, 2011;

Kurtzman et al., 2011). Interestingly, this study did not find other temporal patterns that have been previously reported for deciduous litter (Voříšková et al., 2014).

Yeasts were found to be in many respects similar to other functional groups of filamentous fungi (ECMF, AMF, saphrotrops). Non-contiguous forest stands dominated by mature beech, oak or spruce harbored distinct filamentous fungal and yeast communities. Litter communities of filamentous fungi as well as yeasts had significantly higher evenness than soil communities. Both litter and soil communities of yeasts and filamentous fungi in each stand type were characterized by a subset of indicator species with high fidelity but relatively low abundance. In litter, the main factor driving community composition of both groups was the dominant tree species. In soils both the dominant tree species and local abiotic variables were important drivers. On the other hand, several dissimilarities indicating the specificity of yeast communities were found as well. We showed that yeasts have lower decomposition abilities than saprotrophic filamentous fungi and most yeasts can efficiently act as mutualists that utilize products of decomposition, provided by other microbes. We showed that yeast communities are to some extent shaped by other environmental properties, however, they seems to be most closely related to saprotrophs. Therefore, this group may have unique ecology in forest ecosystems which may be related to their unicellular growth form and, therefore, we should see them as a unique group of fungi with many specifics.

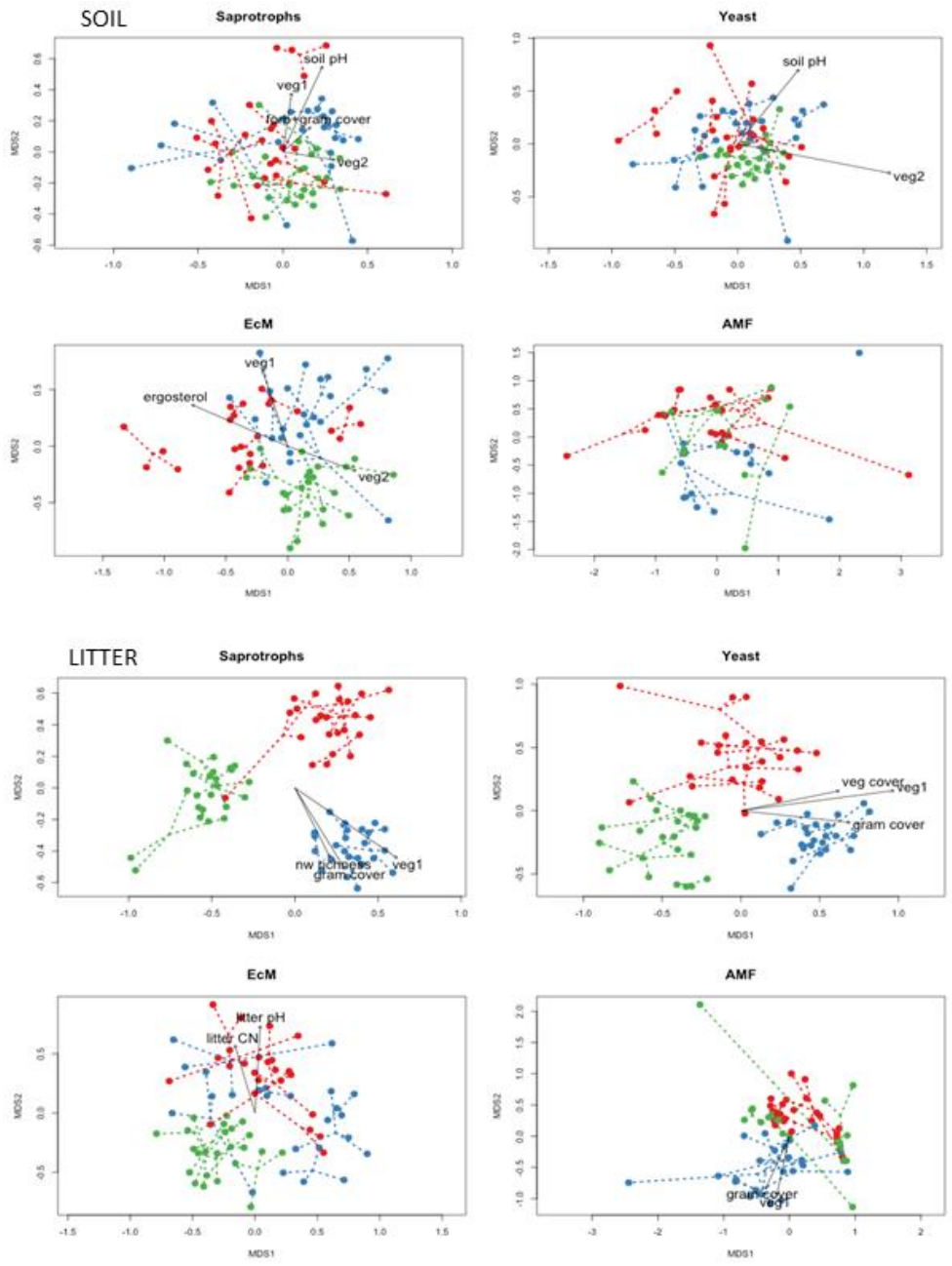

Figure 8: Non-metric multidimensional scaling ordination of fungal communities by functional group in soils and litter under natural stands of beech, oak and spruce; n=18, sampling time=4, k=2. Spruce=green, Beech=red, Oak=blue. Repeated samples are connected to the site centroids by dotted lines; centroids represent the site score averages. Environmental variables with significant correlations are shown as vectors in black. Soil samples: saprotrophs: samples=72, total spp=1917 stress= 0.2422; yeasts: samples =72 total spp=194 stress=0.2460; EcM: samples =72, total spp =495, stress=0.2471; AMF samples =57, total spp=23, stress=0.0914. Litter: saprotrophs: samples=72, total spp=2079 stress= 0.1535; yeasts: samples =72 total spp=320 stress=0.2048; EcM: samples =72, total spp =351, stress=0.2815; AMF samples =66, total spp=18, stress=0.1565.

Conclusions

This thesis consists of 4 papers that contribute to the understanding of ecology and role of yeasts in temperate forest topsoil. In the first paper, amplicon sequencing of environmental DNA was used to describe the composition of yeast communities and identify the potential biotic and abiotic drivers of community assembly. Based on the analysis of ITS2 PCR amplicons, yeasts represented a substantial proportion of fungal communities with higher relative abundance in soil. In litter, yeast communities differed significantly among beech, oak and spruce-dominated stands. Drivers of community assembly are probably more complex in soils and comprise the effects of environmental conditions and vegetation.

In the second paper, effects of dominant vegetation and local site properties on community composition of yeasts and filamentous fungi were compared using amplicon sequencing of metagenomic DNA. Even though yeasts´ and filamentous fungal communities were in many respects similar to each other, many differences are also evident. Therefore, it can be concluded that yeasts have unique ecology in forest ecosystems which may be related to their unicellular growth form.

In the third paper, the decomposition potential and C utilization profiles of twenty five dominant yeasts from a temperate forest topsoil was explored. The results indicate that despite taxonomic heterogeneity, yeasts represent a fungal group with a specific nutritional strategy dissimilar from other soil fungi. While the efficient decomposition of hemicellulose, cellulose or chitin appears to be restricted to only a few taxa, results of utilization of C sources indicate that most yeasts can efficiently act as mutualists that utilize products of decomposition, provided by other microbes. Importantly, large fraction of total enzyme activity was associated with yeast cell surfaces. This adaptation should ensure that the decomposition products are produced at the cell surface of the unicellular microorganisms and are readily available to the producers of the enzymes.

In the fourth paper three novel yeast species were described, all belonging to the subphylum Pucciniomycotina, class Microbotryomycetes: *Leucosporidium krtinense* f.a. sp. nov. (type strain CBS 14304^T = PYCC 6879^T = DSM 101892^T), *Yurkovia mendeliana* sp. nov. (type strain CBS 14273^T = PYCC 6884^T = DSM 101889^T), and *Libkindia masarykiana* sp. nov. (type strain CBS 14275^T = PYCC 6886^T = DSM 101891^T). Based on the analysis of environmental DNA, the new

species constitute common and abundant taxa in the studied environment which indicates their high environmental relevance in the temperate mixed forests ecosystems.

Even though I believe that that this work has contributed to the understanding of soil yeast communities in temperate forests and broadened the methodological approaches that can be used to address question dealing specifically with soil yeasts, many important questions still remain unaddressed and others emerge. For example, it is clear that yeast diversity has not yet been fully explored and many important yeast taxa are still waiting for description. In addition, the questions regarding the nutritional traits of yeasts in soil remain partly open. The results of this thesis seem to support the view that the contribution of yeasts to decomposition in soils is limited, but this conclusion still needs to be validated using alternative approaches such as metatranscriptomics or the use of substrates labelled with stable isotopes. I also believe that whole-genome analyses will shed more light on the diversity of enzymes in soil yeasts.

6 Refrences

Amann, R.I., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews 59, 143-169.

Aponte, C., García, L.V., Marañón, T., Gardes, M., 2010. Indirect host effect on ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks. Soil Biology and Biochemistry 42, 788-796.

Augé, R.M., 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11, 3-42.

Bais, H.P., Loyola-Vargas, V.M., Flores, H.E., Vivanco, J.M., 2001. Root-specific metabolism: The biology and biochemistry of underground organs. In Vitro Cellular & Developmental Biology - Plant 37, 730-741.

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109-130.

Baldrian, P., Větrovský, T., Cajthaml, T., Dobiášová, P., Petránková, M., Šnajdr, J., Eichlerová, I., 2013. Estimation of fungal biomass in forest litter and soil. Fungal Ecology 6, 1-11.

Baldrian, P., Voříšková, J., Dobiášová, P., Merhautová, V., Lisá, L., Valášková, V., 2011. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. Plant and Soil 338, 111-125.

Bar-Shimon, M., Yehuda, H., Cohen, L., Weiss, B., Kobeshnikov, A., Daus, A., Goldway, M., Wisniewski, M., Droby, S., 2004. Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent Candida oleophila. Current Genetics 45, 140-148.

Birkhofer, K., Schoning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., Marhan, S., Oelmann, Y., Wubet, T., Yurkov, A., Begerow, D., Berner, D., Buscot, F., Daniel, R., Diekotter, T., Ehnes, R.B., Erdmann, G., Fischer, C., Foesel, B., Groh, J., Gutknecht, J., Kandeler, E., Lang, C., Lohaus, G., Meyer, A., Nacke, H., Nather, A., Overmann, J., Polle, A., Pollierer, M.M., Scheu, S., Schloter, M., Schulze, E.D., Schulze, W., Weinert, J., Weisser, W.W., Wolters, V., Schrumpf, M., 2012. General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. PLoS One 7, e43292.

Botha, A., 2006. Yeasts in Soil, In: Péter, G., Rosa, C. (Eds.), Biodiversity and Ecophysiology of Yeasts. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 221-240.

Botha, A., 2011. The importance and ecology of yeasts in soil. Soil Biology and Biochemistry 43, 1-8.

Bovers, M., Hagen, F., Kuramae, E.E., Boekhout, T., 2008. Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. Fungal Genetics and Biology 45, 400-421.

Brabcová, V., Novaková, M., Davidová, A., Baldrian, P., 2016. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytologist 210, 1369-1381.

Bray, S.R., Kitajima, K., Mack, M.C., 2012. Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. Soil Biology and Biochemistry 49, 30-37.

Bueé, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184, 449-456.

Burford, E.P., Fomina, M., Gadd, G.M., 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals, Mineralogical Magazine, p. 1127.

Buzzini, P., Martini, A., 2002. Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. Journal of Applied Microbiology 93, 1020-1025.

Cadisch, G., Giller, K.E., 1997. Driven by Nature: Plant Litter Quality and Decomposition. CAB International.

Cairney, J.W.G., 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. Soil Biology and Biochemistry 47, 198-208.

Cloete, K.J., Valentine, A.J., Botha, A., 2010. Effect of the soil yeast *Cryptococcus laurentii* on the photosynthetic water and nutrient-use efficiency and respiratory carbon costs of a Mediterranean sclerophyll, *Agathosma betulina* (Berg.) Pillans. Symbiosis 51, 245-248.

Colpaert, J.V., Van Laere, A., Van Assche, J.A., 1996. Carbon and nitrogen allocation in ectomycorrhizal and non-mycorrhizal Pinus sylvestris L. seedlings. Tree Physiology 16, 787-793.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., Bradford, M.A., 2014. Untangling the fungal niche: the trait-based approach. Frontiers in Microbiology 5, 579.

Date, R.A., 1973. Nitrogen, a major limitation in the productivity of natural communities, crops and pastures in the pacific area. Soil Biology and Biochemistry 5, 5-18.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795-811.

DeRito, C.M., Madsen, E.L., 2009. Stable isotope probing reveals *Trichosporon* yeast to be active in situ in soil phenol metabolism. ISME Journal 3, 477-485.

Dickinson, J.R., 2005. Are yeasts free-living unicellular eukaryotes? Letters in Applied Microbiology 41, 445-447.

Dilly, O., Bartsch, S., Rosenbrock, P., Buscot, F., Munch, J.C., 2001. Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest. Soil Biology and Biochemistry 33, 921-930.

Eichlerová, I., Homolka, L., Žifčáková, L., Lisá, L., Dobiášová, P., Baldrian, P., 2015. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. Fungal Ecology 13, 10-22.

Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J., Plassard, C., 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant and Soil 366, 1-27.

El-Tarabily, K.A., 2004. Suppression of Rhizoctonia solani diseases of sugar beet by antagonistic and plant growth-promoting yeasts. Journal of Applied Microbiology 96, 69-75.

El-Tarabily, K.A., Sivasithamparam, K., 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Mycoscience 47, 25-35.

Fan, T.W.-M., Lane, A.N., Shenker, M., Bartley, J.P., Crowley, D., Higashi, R.M., 2001. Comprehensive chemical profiling of gramineous plant root exudates using high-resolution NMR and MS. Phytochemistry 57, 209-221.

FAO, JRC, 2012. Global Forest land-usechange 1990-2005, by E.J. Lindquist, R. Annuzio, A. Gerrand, K. MacDicken, F. Achard, R. Beuchle, A. Brink, H.D. Eva, P. Mayaux, J. San-Miguel-Ayanz, H-J Stibig. FAO forestry paper No. 169. Food and Agriculture Organization of the United Nations and European Comission Joint Research Centre. Rome, FAO.

Fonseca, A., 1992. Utilization of tartaric acid and related compounds by yeasts: taxonomic implications. Canadian Journal of Microbiology 38, 1242-1251.

Fonseca, Á., Inácio, J., 2006. Phylloplane Yeasts, In: Péter, G., Rosa, C. (Eds.), Biodiversity and Ecophysiology of Yeasts. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 263-301.

Fracchia, S., Godeas, A., Scervino, J.M., Sampedro, I., Ocampo, J.A., García-Romera, I., 2003. Interaction between the soil yeast *Rhodotorula mucilaginosa* and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Soil Biology and Biochemistry 35, 701-707.

Francesca, N., Carvalho, C., Sannino, C., Guerreiro, M.A., Almeida, P.M., Settanni, L., Massa, B., Sampaio, J.P., Moschetti, G., 2014. Yeasts vectored by migratory birds collected in the Mediterranean island of Ustica and description of *Phaffomyces usticensis* f.a. sp. nov., a new species related to the cactus ecoclade. FEMS Yeast Research 14, 910-921.

Frankland, J.C., 1998. Fungal succession – unravelling the unpredictable. Mycological Research 102, 1-15.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hattenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology & Evolution 25, 372-380.

Gleba, D., Borisjuk, N.V., Borisjuk, L.G., Kneer, R., Poulev, A., Skarzhinskaya, M., Dushenkov, S., Logendra, S., Gleba, Y.Y., Raskin, I., 1999. Use of plant roots for phytoremediation and molecular farming. Proceedings of the National Academy of Sciences of the United States of America 96, 5973-5977.

Golubev, W.I., 2006. Antagonistic Interactions Among Yeasts, In: Péter, G., Rosa, C. (Eds.), Biodiversity and Ecophysiology of Yeasts. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 197-219.

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. Soil Biology and Biochemistry 90, 87-100.

Henderson, M.E., 1961. The metabolism of aromatic compounds related to lignin by some hyphomycetes and yeast-like fungi of soil. Journal of General Microbiology 26, 155-165.

Hendricks, J.J., Mitchell, R.J., Kuehn, K.A., Pecot, S.D., 2016. Ectomycorrhizal fungal mycelia turnover in a longleaf pine forest. New Phytologist 209, 1693-1704.

Chernov, I., 2005. [The latitude-zonal and spatial-successional trends in the distribution of yeasts]. Zhurnal Obshchei Biologii 66, 123-135.

Ikeda, R., Sugita, T., Jacobson, E.S., Shinoda, T., 2002. Laccase and melanization in clinically important *Cryptococcus* species other than *Cryptococcus neoformans*. Journal of Clinical Microbiology 40, 1214-1218.

Jiménez, M., González, A.E., Martínez, M.J., Martínez, A.T., Dale, B.E., 1991. Screening of yeasts isolated from decayed wood for lignocellulose-degrading enzyme activities. Mycological Research 95, 1299-1302.

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist 187, 843-858.

Keenan, R.J., Reams, G.A., Achard, F., de Freitas, J.V., Grainger, A., Lindquist, E., 2015. Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. Forest Ecology and Management 352, 9-20.

Kimura, Y., Nakano, Y., Fujita, K., Miyabe, S., Imasaka, S., Ishikawa, Y., Sato, M., 1998. Isolation and characteristics of yeasts able to grow at low concentrations of nutrients. Yeast 14, 233-238.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139-162.

Kurtzman, C., Fell, J.W., Boekhout, T., 2011. The Yeasts: A Taxonomic Study. Elsevier Science.

Landesman, W.J., Dighton, J., 2011. Shifts in microbial biomass and the bacteria: fungi ratio occur under field conditions within 3 h after rainfall. Microbial Ecology 62, 228-236.

Lindahl, B.D., Finlay, R.D., 2006. Activities of chitinolytic enzymes during primary and secondary colonization of wood by basidiomycetous fungi. New Phytologist 169, 389-397.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Hogberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611-620.

Litton, C.M., Raich, J.W., Ryan, M.G., 2007. Carbon allocation in forest ecosystems. Global Change Biology 13, 2089-2109.

Maksimova, I.A., Chernov, I.Y., 2004. Community Structure of Yeast Fungi in Forest Biogeocenoses. Microbiology 73, 474-481.

Malherbe, S., Cloete, T.E., 2002. Lignocellulose biodegradation: Fundamentals and applications. Reviews in Environmental Science and Biotechnology 1, 105-114.

Margesin, R., Fonteyne, P.A., Redl, B., 2005. Low-temperature biodegradation of high amounts of phenol by *Rhodococcus* spp. and basidiomycetous yeasts. Research in Microbiology 156, 68-75.

Martinez, A.T., Speranza, M., Ruiz-Duenas, F.J., Ferreira, P., Camarero, S., Guillen, F., Martinez, M.J., Gutierrez, A., del Rio, J.C., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. International Microbiology 8, 195-204.

Medina, A., Vassileva, M., Caravaca, F., Roldan, A., Azcon, R., 2004. Improvement of soil characteristics and growth of *Dorycnium pentaphyllum* by amendment with agrowastes and inoculation with AM fungi and/or the yeast *Yarowia lipolytica*. Chemosphere 56, 449-456.

Mestre, M.C., Fontenla, S., Rosa, C.A., 2014. Ecology of cultivable yeasts in pristine forests in northern Patagonia (Argentina) influenced by different environmental factors. Canadian Journal of Microbiology 60, 371-382.

Mestre, M.C., Rosa, C.A., Safar, S.V., Libkind, D., Fontenla, S.B., 2011. Yeast communities associated with the bulk-soil, rhizosphere and ectomycorrhizosphere of a *Nothofagus pumilio* forest in northwestern Patagonia, Argentina. FEMS Microbiology Ecology 78, 531-541.

Middelhoven, W.J., 2006. Polysaccharides and phenolic compounds as substrate for yeasts isolated from rotten wood and description of *Cryptococcus fagi* sp.nov. Antonie Van Leeuwenhoek 90, 57-67.

Middelhoven, W.J., Kurtzman, C.P., 2007. Four novel yeasts from decaying organic matter: *Blastobotrys robertii* sp. nov., *Candida cretensis* sp. nov., *Candida scorzettiae* sp. nov. and *Candida vadensis* sp. nov. Antonie Van Leeuwenhoek 92, 233-244.

Moawad, H., Salem, S.H., Badr El-Din, S.M.S., Khater, T., Iskandar, M., 1986. Yeasts in soils of Egypt. Zentralblatt für Mikrobiologie 141, 431-435.

Nagy, L.G., Toth, R., Kiss, E., Slot, J., Gacser, A., Kovacs, G.M., 2017. Six Key Traits of Fungi: Their Evolutionary Origins and Genetic Bases. Microbiology Spectrum 5.

Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K., 2005. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. Biology and Fertility of Soils 42, 97-108.

Oberwinkler, F., 2017. Yeasts in Pucciniomycotina. Mycological Progress 16, 831-856.

Opik, M., Metsis, M., Daniell, T.J., Zobel, M., Moora, M., 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytologist 184, 424-437.

Osono, T., 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecological Research 22, 955-974.

Osono, T., Takeda, H., 2002. Comparison of litter decomposing ability among diverse fungi in a cool temperate deciduous forest in Japan. Mycologia 94, 421-427.

Osono, T., Takeda, H., 2006. Fungal decomposition of *Abies needle* and *Betula* leaf litter. Mycologia 98, 172-179.

Pajot, H.F., Fariña, J.I., de Figueroa, L.I.C., 2011. Evidence on manganese peroxidase and tyrosinase expression during decolorization of textile industry dyes by *Trichosporon akiyoshidainum*. International Biodeterioration & Biodegradation 65, 1199-1207.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333, 988-993.

Perez, J., Munoz-Dorado, J., de la Rubia, T., Martinez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. International Microbiology 5, 53-63.

Peršoh, D., 2013. Factors shaping community structure of endophytic fungi–evidence from the *Pinus*-*Viscum*-system. Fungal Diversity 60, 55-69.

Péter, G., Tornai-Lehoczki, J., Fülöp, L., Dlauchy, D., 2003. Six new methanol assimilating yeast species from wood material. Antonie Van Leeuwenhoek 84, 147-159.

Petter, R., Kang, B.S., Boekhout, T., Davis, B.J., Kwon-Chung, K.J., 2001. A survey of heterobasidiomycetous yeasts for the presence of the genes homologous to virulence factors of *Filobasidiella neoformans*, CNLAC1 and CAP59. Microbiology 147, 2029-2036.

Ravnskov, S., Larsen, J., Olsson, P.A., Jakobsen, I., 1999. Effects of various organic compounds on growth and phosphorus uptake of an arbuscular mycorrhizal fungus. New Phytologist 141, 517-524.

Redecker, D., Morton, J.B., Bruns, T.D., 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Molecular Phylogenetics and Evolution 14, 276-284.

Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M., Naeem, S., Trost, J., 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440, 922-925.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 4, 1340-1351.

Sampaio, J.P., 1999. Utilization of low molecular weight aromatic compounds by heterobasidiomycetous yeasts: taxonomic implications. Canadian Journal of Microbiology 45, 491-512.

Sánchez, C., 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advances 27, 185-194.

Scorzetti, G., Fell, J.W., Fonseca, A., Statzell-Tallman, A., 2002. Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Research 2, 495-517.

Schüβler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution Mycological Research 105, 1413-1421.

Sikes, B.A., Cottenie, K., Klironomos, J.N., 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97, 1274-1280.

Slavikova, E., Vadkertiova, R., 2000. The occurrence of yeasts in the forest soils. Journal of Basic Microbiology 40, 207-212.

Starmer, W.T., Lachance, M.-A., 2011. Chapter 6 - Yeast Ecology A2 - Kurtzman, Cletus P, In: Fell, J.W., Boekhout, T. (Eds.), The Yeasts (Fifth Edition). Elsevier, London, pp. 65-83.

Sterflinger, K., Prillinger, H., 2001. Molecular taxonomy and biodiversity of rock fungal communities in an urban environment (Vienna, Austria). Antonie Van Leeuwenhoek 80, 275-286.

Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytologist 207, 1145-1158.

Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80, 735-746.

Tang, A.M., Jeewon, R., Hyde, K.D., 2005. Succession of microfungal communities on decaying leaves of *Castanopsis fissa*. Canadian Journal of Microbiology 51, 967-974.

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N.S., Wijesundera, R., Villarreal Ruiz, L., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Poldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Partel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L.D., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Fungal biogeography. Global diversity and geography of soil fungi. Science 346, 1256688.

Tedersoo, L., Bahram, M., Toots, M., Diedhiou, A.G., Henkel, T.W., Kjoller, R., Morris, M.H., Nara, K., Nouhra, E., Peay, K.G., Polme, S., Ryberg, M., Smith, M.E., Koljalg, U., 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21, 4160-4170.

Tedersoo, L., May, T.W., Smith, M.E., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217-263.

Trivedi, P., Anderson, I.C., Singh, B.K., 2013. Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. Trends in Microbiology 21, 641-651.

Urbanová, M., Šnajdr, J., Baldrian, P., 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biology and Biochemistry 84, 53-64.

Uren, N., 2007. Types, Amounts, and Possible Functions of Compounds Released into the Rhizosphere by Soil-Grown Plants, The Rhizosphere. CRC Press, pp. 1-21.

Uroz, S., Buée, M., Deveau, A., Mieszkin, S., Martin, F., 2016. Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. Soil Biology and Biochemistry 103, 471-488.

van der Heijden, M.G., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296-310.

Vishniac, H.S., 1995. Simulated in situ competitive ability and survival of a representative soil yeast, *Cryptococcus albidus*. Microbial Ecology 30, 309-320.

Vishniac, H.S., 2006. A multivariate analysis of soil yeasts isolated from a latitudinal gradient. Microbial Ecology 52, 90-103.

Voříšková, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME Journal 7, 477-486.

Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201, 269-278.

Vreulink, J.M., Esterhuyse, A., Jacobs, K., Botha, A., 2007. Soil properties that impact yeast and actinomycete numbers in sandy low nutrient soils. Canadian Journal of Microbiology 53, 1369-1374.

Wuczkowski, M., Prillinger, H., 2004. Molecular identification of yeasts from soils of the alluvial forest national park along the river Danube downstream of Vienna, Austria ("Nationalpark Donauauen"). Microbiological Research 159, 263-275.

Yarwood, S.A., Bottomley, P.J., Myrold, D.D., 2010. Soil microbial communities associated with Douglas-fir and red alder stands at high- and low-productivity forest sites in Oregon, USA. Microbial Ecology 60, 606-617.

Yu, T., Wang, L., Yin, Y., Wang, Y., Zheng, X., 2008. Effect of chitin on the antagonistic activity of *Cryptococcus laurentii* against *Penicillium expansum* in pear fruit. International Journal of Food Microbiology 122, 44-48.

Yurkov, A., Maximova, I., Chernov, I.Y., 2004. The comparative analysis of yeast communities in birch forests of the European part of Russia and Western Siberia.

Yurkov, A., Wehde, T., Kahl, T., Begerow, D., 2012a. Aboveground Deadwood Deposition Supports Development of Soil Yeasts. Diversity 4, 453.

Yurkov, A.M., Chernov, I., Tiunov, A.V., 2008. [Influence of *Lumbricus terrestris* earthworms on the structure of the yeast community of forest litter]. Mikrobiologiia 77, 121-125.

Yurkov, A.M., Kemler, M., Begerow, D., 2011. Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. PLoS One 6, e23671.

Yurkov, A.M., Kemler, M., Begerow, D., 2012b. Assessment of yeast diversity in soils under different management regimes. Fungal Ecology 5, 24-35.

Yurkov, A.M., Rohl, O., Pontes, A., Carvalho, C., Maldonado, C., Sampaio, J.P., 2016. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. FEMS Yeast Research 16, fov103.

Zhang, H., Skelton, A., Gardner, R.C., Goddard, M.R., 2010. *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* reside on oak trees in New Zealand: evidence for migration from Europe and interspecies hybrids. FEMS Yeast Research 10, 941-947.

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Research Article

RESEARCH ARTICLE

Drivers of yeast community composition in the litter and soil of a temperate forest

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One sentence summary: We used amplicon sequencing of environmental DNA to describe the composition of yeast communities in European temperate forest soil and to identify the potential biotic and abiotic drivers of community assembly.

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ABSTRACT

Fungi represent a group of soil microorganisms fulfilling important ecological functions. Although several studies have shown that yeasts represent a significant proportion of fungal communities, our current knowledge is based mainly on cultivation experiments. In this study, we used amplicon sequencing of environmental DNA to describe the composition of yeast communities in European temperate forest and to identify the potential biotic and abiotic drivers of community assembly. Based on the analysis of ITS2 PCR amplicons, yeasts represented a substantial proportion of fungal communities ranging from 0.4 to 14.3% of fungal sequences in soil and 0.2 to 9.9% in litter. The species richness at individual sites was 28 ± 9 in soil and 31 ± 11 in litter. The basidiomycetous yeasts dominated over ascomycetous ones. In litter, yeast communities differed significantly among beech-, oak- and spruce-dominated stands. Drivers of community assembly are probably more complex in soils and comprise the effects of environmental conditions and vegetation.

Keywords: yeast ecology; soil microbiology; metagenomics; yeasts in soil; microbial ecology; forest

INTRODUCTION

Fungi represent an important group of microorganisms in forest soil and litter. High-throughput sequencing methods have shown that a significant proportion of these communities is composed of yeasts (e.g. Buee *et al.* 2009; Voříšková and Baldrian 2013). Yeasts are a taxonomically heterogeneous group of fungi defined by their ability to propagate in a unicellular form and includes members of various orders within Ascomycota and Basidiomycota (e.g. Botha 2011; Kurtzman, Fell and Boekhout 2011). Despite their considerable abundance in forests, our knowledge of their ecological importance in this environment is limited.

Yeasts are found worldwide, and up to 130 yeast species have been reported specifically from soils (Yurkov, Kemler and Begerow 2012). Although their relevance for soil functioning is not yet fully understood (Botha 2011), it is known that soil yeasts are able to utilize a wide spectrum of carbon sources, including cellulose, hemicellulose and phenol, as well as products of the enzymatic hydrolysis of lignocellulosic plant materials (e.g. Sampaio 1999; Middelhoven, Scorzetti and Fell 2001; Margesin, Fonteyne and Redl 2005; DeRito and Madsen 2009; Štursová *et al.*

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2012). Yeasts have been also shown to influence soil aggregation by producing extracellular polysaccharides (Vishniac 1995) and to promote plant growth by their activity in the rhizosphere (e.g. Cloete *et al*. 2009; Botha 2011; Amprayn *et al*. 2012), and many interactions with soil animals including predation, mutualism and antagonistic interactions have been observed as well (e.g. Yurkov, Chernov and Tiunov 2008; Botha 2011).

Current knowledge of soil yeast communities is based mainly on cultivation experiments (e.g. Slavikova and Vadkertiova 2000; Yurkov *et al*. 2012, 2015; Yurkov, Kemler and Begerow 2012; Mestre, Fontenla and Rosa *et al*. 2014), which only allow us to describe cultivable taxa and, therefore, might give a biased picture of yeast community composition. Also, evidence for a strong association with soil-related substrates is lacking for many yeast species recovered from soils (Vishniac 2006). Unfortunately, despite the existence of high-throughput sequencing datasets, this method has not yet been used for specific analyses of yeasts in soils and only a few studies have mentioned the proportion of yeasts within fungal communities in temperate forest topsoils using environemntal sequencing. For example, Buee *et al*. (2009) sequenced six different soils in France and showed that *Saitozyma podzolica* and *Solicoccozyma terricola* were among the species with the highest sequence abundance at the studied sites. Also Yarwood, Bottomley and Myrold (2010) identified these two species as some of the most abundant taxa in forest soil soils from Oregon, USA. Sequences with their closest matches to yeasts represented between 0.2 and 16% and 3 and 27% of all fungal sequences in litter and soil, respectively, of forests developed under different trees in post-mining sites (Urbanová, Šnajdr and Baldrian 2015). Voříšková and Baldrian (2013) studied the development of a fungal community over 24 months of litter decomposition in a forest with dominant *Quercus petraea* and showed that the sequence abundance of yeasts increased with time. In their study, the yeast genus *Trichosporon* was one of the most abundant fungal genera. Also the yeasts formerly classified into the polyphyletic genus *Cryptococcus* appear to be both common and abundant in various temperate forest soils (e.g. Baldrian *et al*. 2012; Voříšková *et al*. 2014; Urbanová, Šnajdr and Baldrian 2015).

In contrast to mycelial fungi that are able to translocate resources via their mycelial cords over considerable distances (Cairney 2005), it is commonly assumed that the unicellular yeasts are less efficient in using bulky, recalcitrant substrates, much like bacteria. This can also affect their distribution in the environment, which is a result of two main processes: dispersal ability and the availability of a suitable niche. Microbial taxa differ widely with respect to both of these. Compared with bacteria, fungi appear to be much more stochastically distributed in the environment (Šťursová *et al*. 2016). These stochastic patterns may be partially a result of the fact that a considerable share of fungal taxa are specific to particular tree species (e.g. Peay, Kennedy and Bruns 2008; Tedersoo *et al*. 2008; Buee *et al*. 2009), which applies to both mycorrhizal symbionts and saprotrophs (Urbanová, Šnajdr and Baldrian 2015). Communities of both fungi and bacteria in forest litter and soil were found to be significantly associated with dominant tree species, but the effect of tree species on bacteria appeared to be much less pronounced and is likely mediated by substrate chemistry (Urbanová, Šnajdr and Baldrian 2015). Indeed, abiotic factors, especially the effect of pH, seem to be of high importance for the distribution of bacteria but not for fungi (Rousk *et al*. 2010).

The extent to which drivers of yeast community composition are similar to or different from those of the total fungal community remains unclear. The aim of this work was to describe yeast communities in soil and litter of a temperate forest, to identify dominant yeast species and to explore how yeast community composition changes across a range of abiotic and biotic factors including variable ground vegetation and composition of the tree layer (ranging from monocultures to mixed stands). Although high-throughput sequencing is commonly used to study composition of microbial communities, this study represents the first application of the high-throughput sequencing technique to assess specifically soil yeast community composition at the species level using up-to-date taxonomy, and address ecology and distribution of yeasts.

We hypothesized that composition of yeast communities will differ between soil and litter due to the large chemical and structural differences between these substrates. Based on the previous studies focused on yeast communities using isolation techniques (Birkhofer *et al*. 2012; Yurkov *et al*. 2016), yeasts are affected by dominant vegetation (tree species), especially in litter. In bulk soil, where yeast cells are not in direct contact with plant roots, we expect greater effect of soil chemistry than in litter.

MATERIALS AND METHODS

Study area, sampling sites and sample collection

Samples were collected in the Training Forest Enterprise Masaryk Forest Křtiny of Mendel University in Brno (Křtiny Forest). Křtiny Forest has a total area of 103 km^2 of mixed temperate forest (latitude 16°15′ E, longitude 49°15′ N) and is located north of Brno, Czech Republic.

The area has an altitude range of 210–575 m, a mean annual temperature of 7.5°C and a mean annual precipitation of 610 mm, and is characterized by a variety of natural geomorphological conditions. The forests are composed of approximately 54% broadleaf and 46% conifer trees, with the five most dominant species being *Fagus sylvatica*, *Quercus petraea* agg., *Picea abies*, *Pinus sylvestris* and *Carpinus betulus*.

At each site, vegetation was sampled in a 5 × 5 m plot with the soil cores placed inside the sampling area. Within each 25 m^2 plot, all vascular plant species were recorded, including those rooted outside the border but with branches extending over the sides of the plot. Cover-abundance of plant species was estimated on the nine-degree Braun-Blanquet scale (Westhoff and van der Maarel 1978). Vegetation cover was divided into two groups and these were correlated with yeast community composition: (i) tree layer (trees higher than shrub layer) and (ii) ground vegetation layer (herbs and seedlings or young trees and shrubs). Moisture was measured as a loss of mass after freeze-drying, organic matter content was measured after combustion at 650°C, and pH was measured in distilled water (1:10). Soil carbon (C) and nitrogen (N) content were measured by an external laboratory.

Two studies were carried out to assess yeast distribution at the study area. Both studies sampled the yeasts in litter and soil. The first study (Whole Area Experiment) was done to analyse the effects of abiotic properties and vegetation on yeast communities across the whole study area. Sixty-four forest covered sites were preselected covering an approximately 8 × 8 km grid with a distance of approximately 1 km between sampling sites. Areas with other vegetation types were excluded, as were clear-cut forests and stands with trees younger than 20 years, and respective sampling sites were relocated when necessary (Fig. 1). The 64 sampling sites across the study area exhibited high diversity in vegetation cover. Twenty sites were dominated by a single tree species: *Fagus sylvatica* (12×), *Picea abies* (5×), *Carpinus*

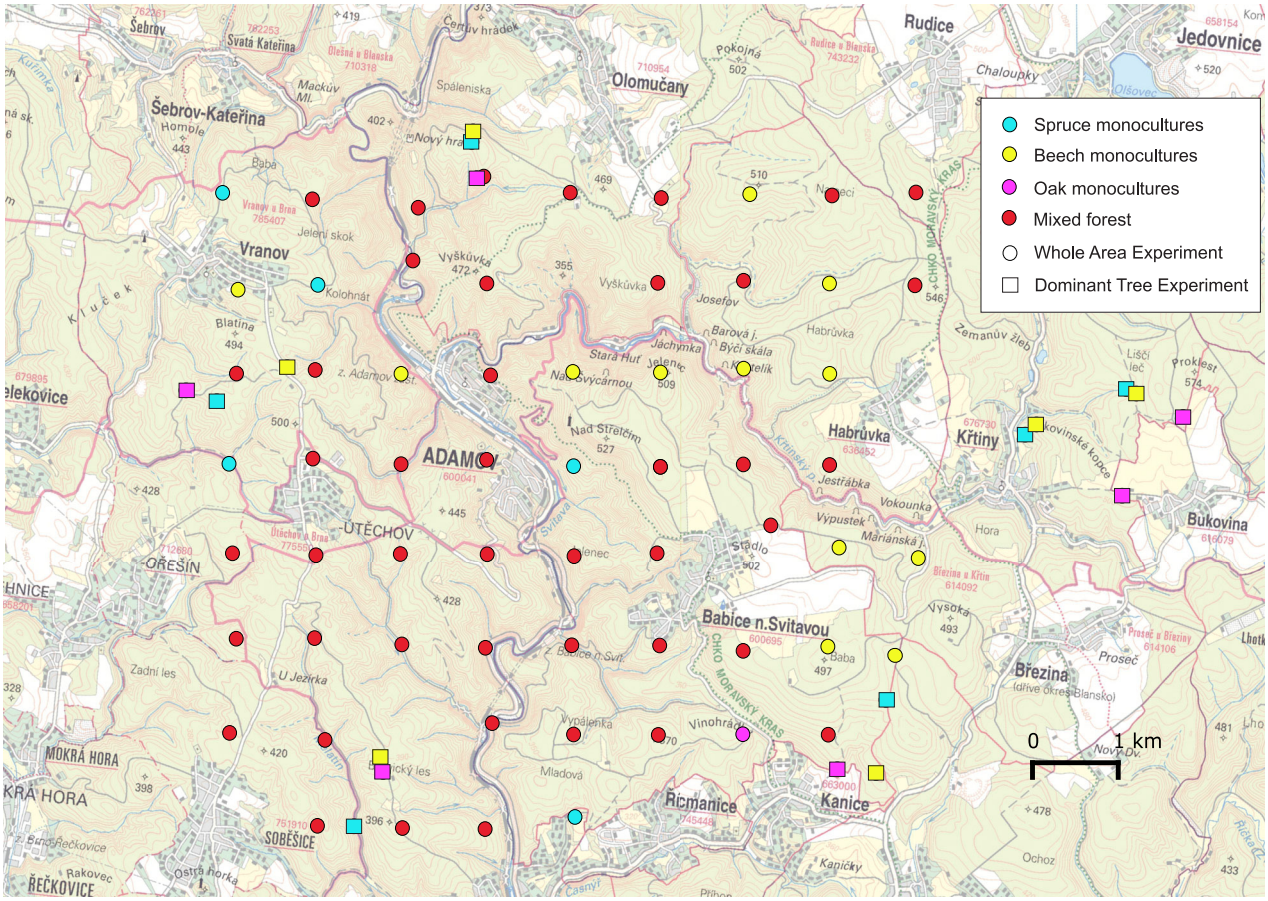

Figure 1. Localization of sampling sites in the Masaryk Forest Křtiny temperate forest, Czech Republic.

betulus, *Quercus petraea* and *Pinus sylvestris* (each 1×); another 20 were mixed forests of two species (most commonly *Fagus sylvatica* and either *Quercus petraea* or *Carpinus betulus*); 15 sites had three species; and nine sites had four or more species present. The sites also differed in ground vegetation composition (Supplementary Table S1). Sampling was performed in September 2013 late in the vegetation season.

The second experiment (Dominant Tree Experiment) was conducted to assess the effects of the dominant tree species on yeast communities in litter and soil. For this experiment, six blocks of single species stands of *Picea abies*, *Fagus sylvatica* and *Quercus petraea* were located to find stands as close to each other as possible, with the blocks being as distant across the study area as possible to cover the variation of other environmental factors. Tree stands older than 20 years with an area >1500 m^2 were selected for each of the three tree species (18 sites in total). For sampling, edges of the stands were avoided. Collection of soil and litter samples was performed in four seasons (December 2012, January 2013, June 2013 and October 2013) to capture the full seasonal variation in fungal communities that has been previously observed in both coniferous and broadleaved forests (e.g. Voříšková *et al.* 2014; Žifčáková *et al.* 2016).

In both experiments five soil cores of 4.5 cm diameter were collected at each site: a central core and four located at a distance of 2 m to the north, south, east and west from the central one. Cores were stored at 4°C and processed within 24 h after collection. Litter was collected as one sample, and the upper 10 cm of soil was used as the soil sample; material from all five cores within a site and sampling time were pooled to create composite samples of litter and soil. Litter was cut into *ca.* 0.25 cm^2 pieces, and soil was sieved through a 5-mm sieve. Soil and litter were freeze-dried and 2 g of fresh soil and litter material for DNA extractions were stored at –80°C.

DNA extraction and sequencing of PCR amplicons

Total genomic DNA was extracted in triplicate from 250 mg of fresh soil or litter material using a modified Miller method (Sagova-Mareckova *et al.* 2008). Triplicate DNA extracts were combined into one sample and PCR amplified in triplicate to reduce PCR bias. PCR amplification of the fungal ITS2 region from DNA was performed using barcoded gITS7 and ITS4 primers (Ihrmark *et al.* 2012) as described previously (Žifčáková *et al.* 2016). PCR reactions contained 2.5 μl of 10× buffer for DyNAzyme DNA Polymerase, 0.75 μl of BSA (20 mg ml^{-1}), 1 μl of each primer (0.01 mM), 0.5 μl of PCR Nucleotide Mix (10 mM each), 0.75 μl polymerase (2 U μl^{-1} DyNAzyme II DNA polymerase: Pfu DNA polymerase, 24:1) and 1 μl of template DNA. Cycling conditions were 94°C for 5 min, 35 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. Sequencing was performed on an Illumina MiSeq.

Processing of sequencing data

Sequence data were processed using the pipeline SEED 2.0 (Větrovský and Baldrian 2013). Briefly, pair-end reads were merged using fastq-join (Aronesty 2013) and the ITS2 region was extracted using ITS Extractor 1.0.8 (Nilsson *et al.* 2010) before

processing. Chimeric sequences were detected using UCHIME within Usearch 7.0.1090 (Edgar 2010) and deleted. For further analyses of both experiments only datasets containing 10 000 randomly chosen sequences from each sample were used. For the Dominant Tree Experiment, a fungal community dataset for each site was created by averaging the four seasonal samplings to cover the seasonal variability of the community composition. Sequence data were deposited in the MG-RAST public database (http://metagenomics.anl.gov/, data set number 4696490.3).

Retained sequences were clustered using UPARSE as implemented within Usearch (Edgar 2013) at a 97% similarity level. Consensus sequences were constructed for each cluster after the alignment of all sequences in the cluster, and the closest hits at the genus or species level were identified using UNITE (Koljalg *et al.* 2013) and the sequence database of ITS sequences of all yeast strains previously isolated from the studied area (Supplementary Document S1). There was a recent change in nomenclature of basidiomycetous yeast species. In this paper, we are using new species names. The conversion to the original names can be made by referring to Liu *et al.* (2015), Wang, Begerow and Groenewald (2015a) and Wang *et al.* (2015b).

Sequences of all clusters where the closest match was a yeast species according to Kurtzman, Fell and Boekhout (2011), or where the closest hit was a yeast isolate, were selected for further analyses (20 707 sequences after dereplication). Because basidiomycetous yeast species vary in their within-species variability of ITS2 (Liu *et al.* 2015), a combination of clustering and phylogenetic analyses was used to define operational taxonomic units (OTUs). First, all pre-selected unique ITS2 sequences, sequences of all best hits and of all yeast isolates were clustered into groups such that each sequence shared at least 90% similarity to at least one other sequence using the pipeline SCATA (https://scata.mykopat.slu.se/) suitable for the analysis of amplicon sequences that cannot be readily aligned across wide phylogenies, e.g. the ITS region. Only those clusters containing either the best hit sequence from UNITE or a sequence of a yeast isolate were considered as yeasts and processed further (11 508 sequences after dereplication, 155 clusters in total). Each cluster was used for the construction of phylogenetic trees using PhyML 3.0 (Guindon *et al.* 2010) as implemented in SEED (Větrovský and Baldrian 2013). Sequences were clustered using UPARSE both at 97% and 99% similarity and assigned to OTUs according to the following rules: (i) sequences clustering at 99% similarity were always assigned to the same OTU; (ii) sequences not clustering at 97% similarity were always classified to different OTUs; and (iii) sequences clustering at 97% and not clustering at 99% were clustered into different OTUs if the bootstrap support of internal branching was higher than 80%. In total, 56 353 yeasts sequences remained in the dataset. These sequences were clustered into 1921 OTUs (on average, 43 per sample) including 656 singletons. The abundance data reported in this paper are based on this dataset and sequence abundances should be taken as proxies of taxon abundance only with caution (Lindahl *et al.* 2013).

Statistical analyses

Past 2.17c (http://folk.uio.no/ohammer/past/) and RStudio 0.99.491 (https://www.rstudio.com/) were used for statistical analysis. Correlations between environmental variables were assessed using Pearson's correlation coefficients. A one-way analysis of similarities (ANOSIM) was used to compare relative abundance of yeasts sequences within fungal sequences in soil and litter and bellow different dominant tree types (Dominant Tree Experiment). Mantel tests with 99 999 permutations were used to test for linear correlation between measured biotic and abiotic variables and yeast sequence abundance (spatial effects were not considered). Because the majority of OTUs were represented by a very small number of reads, and because such read counts have been demonstrated to be not technically reproducible (Lundberg *et al.* 2012), only taxa with relative abundances >0.5% in >4 samples were considered in further analyses. The Bray–Curtis index was used as a measure of yeast community similarity between samples, the Jaccard index was used as a measure of tree and herb community similarity, and the Euclidean distances were used for all other variables. Graphs showing OTU preferences of the dominant tree species (Dominant Tree Experiment) were constructed in R using the package ggtern (Hamilton 2016). Only OTUs with abundance >0.5% on >3 sites were used in this analysis. Multilevel pattern analysis as implemented in the indicspecies package of R (De Caceres and Legendre 2009) was used to identify indicator species. Only species with $P < 0.001$ were selected. In all other cases, differences where $P < 0.05$ were regarded as statistically significant.

RESULTS

Whole Area Experiment

In total 32 221 sequences remained in the dataset after filtering and OTU construction. These sequences were clustered into 957 OTUs with best hits to 77 genera. The OTU richness per site was 20.4 ± 6.9 for soil samples and 25.6 ± 8.8 for litter samples.

Soils spanned a range of C and N contents from low to rich in organic matter, and pH varied from acidic to neutral. The same level of variation was also observed in litter (Table 1). Soil and litter pH showed negative correlations with C/N content, and positive correlations were observed between C and N, indicating samples with high organic matter content.

The proportion of yeast sequences within fungal sequences was significantly higher in the soil than in the litter as revealed by one-way ANOSIM ($P < 0.0001$). While soils harboured on average 4.6 ± 3.0% of yeast sequences (from 0.6 to 14.3%), only 1.6 ± 1.2% of yeast sequences were found in the litter samples (from 0.3 to 6.2%). No significant correlations between proportion of yeast sequences in soil and litter and chemistry or ground vegetation were observed, but the proportion of yeast sequences in soil was significantly correlated with tree layer vegetation (Mantel test, $P = 0.04$, $R = 0.1$).

Yeast communities differed significantly between litter and soil ($P < 0.0001$). Basidiomycetes strongly dominated the yeast communities in both litter and soil, representing between 32.1 and 100% of all sequences. Relative abundances of the observed yeast genera also varied considerably. Soil samples were dominated by members of the genera *Saitozyma* (40.9%), *Solicoccozyma* (33.1%) and *Apiotrichum* (11.6%). Relative abundances of other genera varied substantially among sampling sites, but never reached high proportions in more than a few samples (Supplementary Fig. S1). Even though yeast communities in litter contained more species with relatively higher abundance, *Saitozyma* (13.7%), *Solicoccozyma* (10.9%) and *Apiotrichum* (6.4%) were again the most abundant genera in most samples. Other genera with high sequence abundances included *Curvibasidium* (6.4%), *Vishniacozyma* (4.8%), *Fellozyma* (4.7%) and *Phaeotremella* (4.7%).

The composition of yeast communities in soil was most significantly affected by two factors: pH (Mantel test, $P = 0.0001$, $R = 0.3$) and tree composition ($P = 0.0002$, $R = 0.2$). The significant effect of ground vegetation ($P = 0.002$, $R =$

Table 1. Properties of soil and litter across the Whole Area Experiment sites in the study area.

	Soil			Litter		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
C	5.17 ± 1.99	2.43	12.31	18.39 ± 6.55	6.21	34.63
N	0.41 ± 0.16	0.19	1.23	1.23 ± 0.37	0.52	1.97
C/N	12.62 ± 2.62	8.26	25.08	14.95 ± 2.98	9.13	25.10
pH	5.20 ± 0.59	3.99	6.42	5.57 ± 0.35	4.80	6.48

0.2) was likely due to its strong dependence on the dominant tree species (ANOSIM, $P = 0.0001$). No significant effect of other chemical variables was observed. Yeast community composition in litter was most affected by the dominant tree (Mantel test, $P = 0.03$, $R = 0.1$) and ground vegetation ($P = 0.03$, $R = 0.1$) and by the combination of pH, C and N content ($P = 0.03$, $R = 0.1$); among individual variables, C content was the only significant factor ($P = 0.02$, $R = 0.1$).

Dominant Tree Experiment

In total 24 132 yeast sequences remained in the dataset after filtering and OTU building. These sequences were clustered into 1679 OTUs and assigned to 91 genera. The average OTU richness per site was 27.9 ± 10.6 (SD) for soil samples and 44.4 ± 9 (SD) for litter samples.

Relative abundance of yeast sequences was significantly affected by the dominant tree species. The relative abundance of yeast sequences in litter was significantly lower in beech sites compared with oak ($P = 0.00001$) and spruce ($P = 0.004$). Spruce soils exhibited higher relative abundance of yeast sequences than soils under beech ($P = 0.005$) and oak ($P = 0.0002$; Fig. 2). Yeast community compositions were similar to the Whole Area Experiment. Soils were dominated by the genera *Saitozyma* (represented by six OTUs with abundance higher than 0.5% on at least four sites; all the OTUs were assigned to one species, *So. podzolica*), *Solicoccozyma* (five OTUs, *So. fuscescens*, *Sa. terricola*) and *Apiotrichum* (two OTUs, *A. porosum*, *A. dulcitum*). Litter samples were more variable and were dominated by *Saitozyma* (four OTUs, *So. podzolica*), *Solicoccozyma* (four OTUs, *So. fuscescens* and *So. terricola*), *Apiotrichum* (two OTUs, *A. dulcitum*, *A.porosum*), *Fellozyma* (six OTUs, *F. inositophila*), *Leucosporidium* (three OTUs, *L. intermedium*) and *Curvibasidium* (three OTUs, *C. cygneicollum*, *C. pallidicorallinum*; Fig. 3).

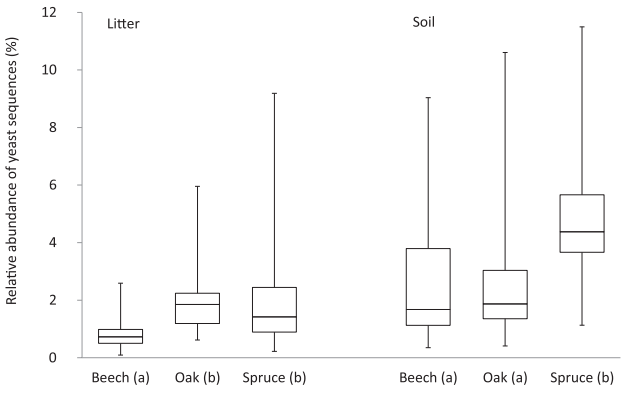

Figure 2. Relative abundance of yeasts in the temperate forest litter and soils in stands with different dominant trees. The results represent maxima, minima, upper and lower quartiles and medians from six sites per tree. Different letters indicate significant differences (ANOSIM, $P < 0.05$).

In soil, no significant difference in the community composition of yeasts was observed among different trees. Instead, soil pH ($P = 0.0003$, $R = 0.6$) and moisture ($P = 0.01$, $R = 0.6$) were found to have significant effects, as well as N content ($P = 0.002$, $R = 0.7$) and C content ($P = 0.001$, $R = 0.7$), but the last three of these variables were also correlated with the pH. In contrast, yeast communities in litter were significantly different between tree species ($P < 0.00001$, all pairwise differences were significant), while the effect of chemistry was lower (C/N content: $P = 0.008$, $R = 0.3$; pH: $P = 0.01$, $R = 0.2$).

Yeast communities in soils contained lower numbers of dominant OTUs than those in litter and revealed lower levels of tree specificity. OTUs assigned to the same genus in soil showed similar dominant tree preferences. *Saitozyma* and *Solicoccozyma* species equally inhabited sites dominated by different dominant tree species, even though *Saitozyma* showed a slight preference for spruce. *Apiotrichum* preferred sites dominated by deciduous trees, and *Leucosporidium* appeared to prefer sites with beech trees. In litter, *Phaeotremella* and *Curvibasidium* were most abundant under oak trees. Also in litter, *Fellozyma* species seemed not to prefer any dominant tree type, while *Saitozyma* preferred sites dominated by spruce and *Leucosporidium* was abundant underneath spruce and beech trees. *Piskurozyma* appeared to prefer litter of deciduous trees, especially of oaks (Fig. 4).

Indicator species analysis was used to assess the level of specificity of fungi for the three studied trees (only OTUs with $P < 0.001$ were selected). Eight OTUs were determined to be indicator species for oak litter: OTU15 (*Piskurozyma cylindrica*), OTU17 (*Fonsecazyma tronadorensis*), OTU24 (*Candida railenensis*), OTU35 (*Rhodosporidiobolus colostri*), OTU44 (closest hit to *Tremella* sp. – 88% similarity), OTU73 (*Fellozyma inositophila*), OTU65 (*Phaeotremella fagi*) and OTU124 (*Fellozyma inositophila*). OTU46 (*Piskurozyma capsuligena*) was identified as an indicator species for beech litter. Two OTUs, OTU20 (*Curvibasidium pallidicorallinum*) and OTU55 (*Piskurozyma cylindrica*), were shared by the two deciduous species *F. sylvatica* and *Quercus* spp. There were no indicator species identified for soil samples. Generally, there was little overlap between dominant and indicator taxa, and the abundance of most identified indicator taxa was low (Table 2).

DISCUSSION

Culture-based studies on yeast communities from forest sites have yielded mainly isolates belonging to the Basidiomycota (e.g. Maksimova and Chernov 2004; Yurkov, Kemler and Begerow 2012; Yurkov *et al.* 2012, 2015). This is in agreement with the present study, where Basidiomycota strongly dominated in both soil and litter. Yeast sequences showed higher relative abundance in soil than in litter, which has also been observed by Yurkov, Chernov and Tiunov (2008) and indicated by the analyses of amplicon sequencing data (Urbanová, Šnajdr and Baldrian 2015). The number of yeast OTUs obtained from one site ranged on average from 10 to 44. These numbers are comparable to the number of species obtained with cul-

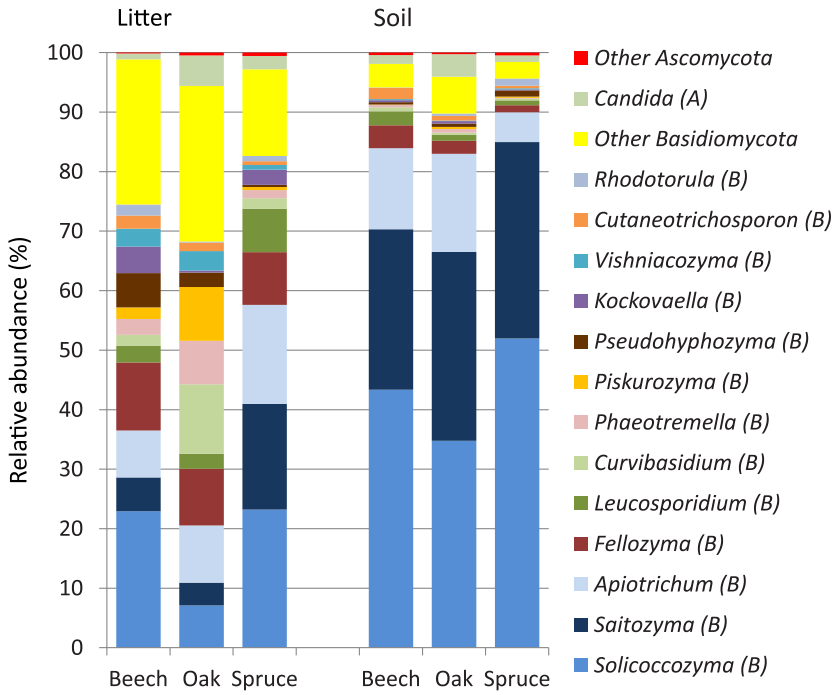

Figure 3. Communities of yeasts from the litter and soil of temperate beech, oak and spruce forests. OTU identifications are based on the taxonomy of the most closely related yeast taxon. The data represent mean values from six sites per tree. A, Ascomycota; B, Basidiomycota.

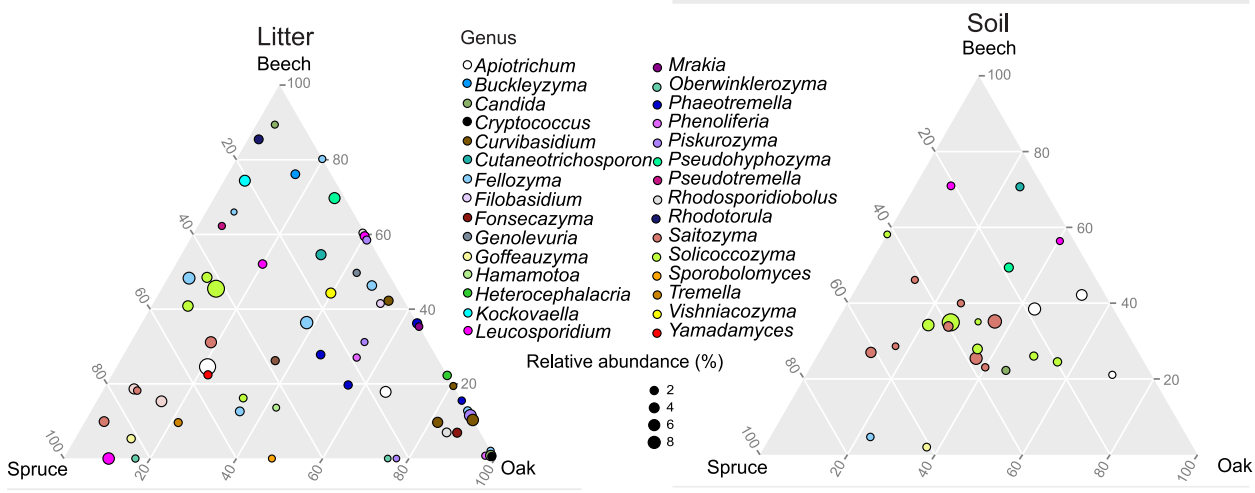

Figure 4. Association of yeast OTUs from the litter and soil of temperate forests with beech, oak and spruce. OTU identifications are based on the taxonomy of the most closely related yeast taxon. Each circle corresponds to one OTU, and only OTUs with abundance >0.5% on >3 sites are shown. Each vertex of the triangle represents one dominant tree type. OTUs that are placed in the middle of the triangle represent species that do not show preference for any tree type. The closer a circle is to the vertex, the stronger is their relationship. Sizes of the circles correspond to the relative abundance of sequences.

tivation approaches (e.g. Yurkov, Kemler and Begerow 2011; Yurkov *et al.* 2012; Yurkov, Kemler and Begerow 2012) and suggest that soil yeasts represent a group of well cultivable microorganisms.

The most abundant OTUs identified in this study were *Solicoccozyma terricola* (*Fillobasidiales*), *Saitozyma podzolica* (*Tremellales*), *Apiotrichum porosum* (*Trichosporonales*), *Apiotrichum dulcitum* (*Trichosporonales*), *Cutaneotrichosporon moniliiforme* (*Trichosporonales*) and *Fellozyma inositophila* (*Sporidiobolales*). *Solicoccozyma terricola* and *Saitozyma podzolica* are typical soil-borne yeasts that are commonly isolated from soils worldwide (e.g. Hong *et al.* 2006; Takashima *et al.* 2012; Yurkov *et al.* 2012; Yurkov, Kemler and Begerow 2012) and also detected in environmental sequencing studies (e.g. Lynch and Thorn 2006; Buee *et al.* 2009). *Apiotrichum porosum* has also been isolated from temperate forests and grasslands (Yurkov *et al.* 2012). This species has been shown to degrade phenolic compounds and hemicelluloses (Middelhoven, Scorzetti and Fell 2001) and is capable of producing antifungal cellobiose lipids, which suppress growth of other yeast species (Kulakovskaya *et al.* 2010). *Apiotrichum dulcitum* has a strong ability to decompose phenol (e.g. Margesin, Fonteyne and Redl 2005; DeRito and Madsen 2009) and benzene compounds (Middel-

Table 2. Identification of dominant yeast OTUs in soil and litter and their mean relative abundances. Forty OTUs with highest mean relative abundance were included. Data represents means and standard deviations. A, Ascomycota; B, Basidiomycota.

				Mean relative abundance (mean ± SD, %)							
				Whole Area Experiment		Dominant Tree Experiment					
OTU	Order	Best identified hit (accession number)	Closest type strain	Litter	Soil	Beech soil	Beech litter	Oak soil	Oak litter	Spruce soil	Spruce litter
OTU1	*Fillobasidiales* (B)	*Solicoccozyma terricola* (EU252550)	*Solicoccozyma terricola*/100%	7.17 ± 6.92	19.13 ± 10.13	11.25 ± 12.94	12.18 ± 8.8	3.35 ± 8.62	27.17 ± 1.28	24.38 ± 8.95	18.23 ± 5.51
OTU2	*Tremellales* (B)	*Saitozyma podzolica* (HQ220214)	*Saitozyma podzolica*/100%	4.46 ± 4.92	15.21 ± 12.80	3.68 ± 17.32	0.81 ± 0.64	0.79 ± 6.71	8.73 ± 0.44	10.68 ± 5.64	10.95 ± 6.45
OTU3	*Trichosporonales* (B)	*Apiotrichum porosum* (JX173716)	*Apiotrichum xylopini*/100%	6.48 ± 8.33	5.93 ± 7.88	12.6 ± 9.96	5.69 ± 3.14	4.86 ± 3.14	3.79 ± 3.70	8.8 ± 4.88	9.15 ± 11.33
OTU4	*Tremellales* (B)	*Saitozyma podzolica* (AB035576)	*Saitozyma podzolica*/99%	3.60 ± 6.57	14.23 ± 11.74	3.00 ± 5.94	0.75 ± 0.91	0.28 ± 8.35	7.98 ± 0.31	5.36 ± 5.91	7.72 ± 3.20
OTU5	*Trichosporonales* (B)	*Apiotrichum dulcitum* (KC009388)	*Apiotrichum dulcitum*/100%	3.50 ± 6.14	3.99 ± 6.27	0.91 ± 6.72	0.98 ± 1.43	3.61 ± 7.17	0.65 ± 4.95	5.8 ± 1.23	6.32 ± 1.49
OTU6	*Fillobasidiales* (B)	*Solicoccozyma terricola* (EU252550)	*Solicoccozyma terricola*/99%	1.20 ± 1.41	5.19 ± 4.82	2.36 ± 6.76	1.89 ± 0.98	0.39 ± 3.99	7.52 ± 0.33	5.79 ± 6.59	3.61 ± 1.92
OTU7	*Tremellales* (B)	*Saitozyma podzolica* (AB035576)	*Saitozyma podzolica*/99%	1.14 ± 2.62	3.61 ± 5.41	2.93 ± 2.93	0.34 ± 0.36	0.15 ± 1.15	6.28 ± 0.18	2.77 ± 6.61	1.22 ± 3.29
OTU8	*Tremellales* (B)	*Saitozyma podzolica* (DQ069015)	*Saitozyma podzolica*/91%	2.19 ± 3.51	2.31 ± 1.20	3.11 ± 1.91	1.92 ± 1.33	1.14 ± 1.51	2.75 ± 1.67	2.32 ± 2.46	1.79 ± 3.78
OTU9	*Fillobasidiales* (B)	*Solicoccozyma terricola* (EU252550)	*Solicoccozyma terricola*/100%	0.46 ± 0.80	1.65 ± 1.23	1.66 ± 1.56	1.89 ± 1.81	0.35 ± 2.69	2.78 ± 0.26	2.13 ± 0.94	2.73 ± 1.25
OTU10	*Trichosporonales* (B)	*Cutaneotrichosporon moniliiforme* (AB018029)	*Cutaneotrichosporon moniliiforme*/99%	1.83 ± 3.11	2.17 ± 4.30	0.52 ± 2.30	2.17 ± 2.54	1.28 ± 0.46	0.14 ± 2.39	1.84 ± 0.26	0.62 ± 0.80
OTU11	*Sporidiobolales* (B)	*Fellozyma inositophila* (AB038107)	*Fellozyma inositophila*/90%	0.33 ± 0.84	0.32 ± 0.82	3.72 ± 4.20	3.83 ± 7.11	0.38 ± 1.41	0.94 ± 0.55	2.51 ± 1.2	1.64 ± 4.43
OTU12	*Microbotriomycetes* (B)	*Curvibasidium cygneicollum* (FN298670)	*Curvibasidium cygneicollum*/100%	3.52 ± 5.23	0.53 ± 1.79	0.35 ± 0.06	0.39 ± 0.55	3.33 ± 0.21	0.01 ± 3.47	0.03 ± 0.02	0.11 ± 0.42
OTU13	*Tremellales* (B)	*Kockovaella chinensis* (AJ608651)	*Kockovaella chinensis*/98%	0.75 ± 1.20	0.00 ± 0.00	1.17 ± 0.32	4.18 ± 4.92	0.27 ± 0.92	0.01 ± 0.61	0.15 ± 0.03	0.41 ± 2.2
OTU14	*Sporidiobolales* (B)	*Fellozyma inositophila* (AB038107)	*Fellozyma inositophila*/97%	1.26 ± 2.50	0.06 ± 0.18	2.34 ± 1.36	3.34 ± 1.94	3.50 ± 0.37	0.02 ± 1.70	0.61 ± 0.05	0.24 ± 2.41
OTU15	*Fillobasidilaes* (B)	*Piskurozyma cylindrica* (FJ873596)	*Piskurozyma cylindrica*/89%	1.58 ± 3.30	0.04 ± 0.13	0.00 ± 0.00	0.90 ± 2.19	6.87 ± 0.33	0.03 ± 3.26	0.01 ± 0.05	0.25 ± 0.01
OTU16	*Leucosporidiales* (B)	*Leucosporidium intermedium* (AF444564)	*Leucosporidium intermedium*/93%	1.70 ± 2.41	0.34 ± 0.61	6.27 ± 0.50	0.00 ± 0.00	0.72 ± 0.58	0.13 ± 1.27	0.02 ± 0.14	0.24 ± 7.83
OTU17	*Tremellales* (B)	*Fonsecazyma tronadorensis* (GU997164)	*Cryptococcus cuniculi*/88%	0.26 ± 0.98	0.04 ± 0.20	0.12 ± 0.00	0.16 ± 0.31	1.98 ± 0.15	0.03 ± 1.21	0.07 ± 0.06	0.07 ± 0.25
OTU18	*Tremellales* (B)	*Vishniacozyma victoriae* (AB693909)	*Vishniacozyma victoriae*/100%	2.99 ± 5.71	0.17 ± 0.38	0.61 ± 0.26	1.70 ± 1.12	1.53 ± 0.17	0.15 ± 3.10	0.14 ± 0.19	0.09 ± 0.47
OTU19	*Microbotriomycetes* (B)	*Pseudohyphozyma pustula* (AF444531)	Pseudohyphozyma pustula/94%	1.78 ± 2.93	0.01 ± 0.04	0.14 ± 0.00	4.80 ± 2.98	1.63 ± 0.26	0.05 ± 1.17	0.00 ± 0.00	0.11 ± 0.34
OTU20	*Curvibasidium* (B)	*Curvibasidium pallidicorallinum* (JX188148)	*Leucosporidium fasciculatum*/95%	0.45 ± 1.31	0.01 ± 0.02	0.02 ± 0.00	0.65 ± 0.61	6.21 ± 0.28	0.03 ± 3.98	0.01 ± 0.05	0.14 ± 0.05
OTU21	*Tremellales* (B)	*Solicoccozyma fuscescens* (NR˙077081)	*Solicoccozyma fuscescens*/100%	0.58 ± 1.12	1.75 ± 2.15	0.47 ± 0.93	0.15 ± 0.24	0.31 ± 2.56	0.60 ± 0.39	0.75 ± 1.30	1.7 ± 0.74
OTU22	*Leucosporidiales* (B)	*Leucosporidium intermedium* (AF444564)	*Leucosporidium intermedium*/95%	1.50 ± 6.38	1.23 ± 2.53	0.47 ± 1.25	0.87 ± 0.61	0.33 ± 0.10	0.38 ± 0.47	1.28 ± 0.69	0.15 ± 0.8
OTU23	*Tremellales* (B)	*Tremella* sp. (FJ873437)	No significant similarity found	1.81 ± 6.74	0.47 ± 1.54	0.04 ± 0.19	2.82 ± 3.52	0.18 ± 0.13	0.03 ± 0.18	0.14 ± 0.08	0.08 ± 0.02
OTU24	*Saccharomycetales* (A)	*Candida railenensis* (JX993811)	*Candida railenensis*/99%	1.15 ± 8.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.44 ± 0.95	0.02 ± 4.8	0.08 ± 0.05	0.56 ± 0.09
OTU25	*Leucosporidiales* (B)	*Leucosporidium intermedium* (AF444564)	*Leucosporidium intermedium*/99%	0.95 ± 1.71	0.35 ± 0.90	0.20 ± 0.74	1.29 ± 1.91	0.86 ± 0.38	0.03 ± 0.88	0.46 ± 0.07	0.33 ± 0.05
OTU26	*Sporidiobolales* (B)	*Fellozyma inositophila* (AB038107)	*Fellozyma inositophila*/86%	1.10 ± 1.34	0.10 ± 0.25	0.16 ± 0.11	1.39 ± 1.37	1.45 ± 0.14	0.20 ± 0.95	0.05 ± 0.02	0.10 ± 0.21
OTU27	*Fillobasidiales* (B)	*Goffeauzyma gastrica* (AF145323)	*Goffeauzyma gastrica*/100%	0.80 ± 2.87	0.35 ± 0.60	1.20 ± 0.09	0.08 ± 0.19	0.19 ± 0.52	1.10 ± 0.23	0.04 ± 1.52	0.67 ± 2.00
OTU28	*Tremellomycetes* (B)	*Vishniacozyma tephrensis* (GU997157)	*Vishniacozyma tephrensis*/99%	1.53 ± 2.81	0.04 ± 0.27	0.09 ± 0.10	1.20 ± 0.58	1.30 ± 0.00	0.02 ± 1.92	0.04 ± 0.04	0.01 ± 0.13
OTU29	*Tremellales* (B)	*Solicoccozyma fuscescens* (NR_077081)	*Solicoccozyma fuscescens*/100%	0.13 ± 0.31	0.66 ± 0.87	0.56 ± 1.21	0.14 ± 0.34	0.06 ± 2.10	0.65 ± 0.10	0.69 ± 0.78	1.32 ± 0.89
OTU30	*Trichosporonales* (B)	*Apiotrichum laibachii* (JN831716)	*Apiotrichum laibachii*/100%	0.40 ± 1.51	0.75 ± 2.71	2.19 ± 0.15	0.16 ± 0.39	0.13 ± 0.17	0.41 ± 0.15	0.09 ± 0.94	0.09 ± 5.25
OTU31	*Curvibasidium* (B)	*Curvibasidium pallidicorallinum* (JX188148)	*Leucosporidium fasciculatum*/92%	1.74 ± 7.97	0.04 ± 0.21	0.49 ± 0.00	0.34 ± 0.67	0.46 ± 0.00	0.03 ± 1.70	0.04 ± 0.06	0.03 ± 0.86
OTU32	*Erythrobasidiales* (B)	*Buckleyzyma aurantiaca* (AB026015)	*Buckleyzyma aurantiaca*/98%	1.20 ± 1.86	0.16 ± 0.42	0.16 ± 0.00	1.44 ± 1.19	0.29 ± 0.04	0.03 ± 0.30	0.01 ± 0.08	0.02 ± 0.21
OTU33	*Tremellales* (B)	*Phaeotremella fagi* (DQ054534)	*Phaeotremella fagi*/95%	1.70 ± 1.71	0.02 ± 0.09	0.45 ± 0.00	0.47 ± 0.61	0.77 ± 0.26	0.02 ± 0.69	0.00 ± 0.04	0.15 ± 0.51
OTU34	*Microbotriomycetes* (B)	*Pseudohyphozyma buffonii* (AB038083)	*Pseudohyphozyma pustula*/91%	0.80 ± 1.94	0.07 ± 0.25	0.00 ± 0.00	0.98 ± 2.20	0.04 ± 0.17	0.04 ± 0.09	0.00 ± 0.00	0.07 ± 0.00
OTU35	*Sporidiobolales* (B)	*Rhodosporidiobolus colostri* (JN246563)	*Rhodosporidiobolus colostri*/100%	0.86 ± 1.80	0.13 ± 0.30	0.15 ± 0.00	0.14 ± 0.15	1.69 ± 0.03	0.04 ± 0.57	0.02 ± 0.07	0.01 ± 0.31
OTU36	*Cystobasidiales* (B)	*Rhodotorula laryngis* (AB693926)	*Cystobasidium pinicola*/100%	0.45 ± 2.11	0.03 ± 0.13	0.25 ± 0.00	1.80 ± 3.84	0.05 ± 0.02	0.50 ± 0.12	0.00 ± 0.00	0.01 ± 0.33
OTU37	*Tremellales* (B)	*Tremella diploschistina* (JN790587)	No significant similarity found	0.33 ± 0.78	0.07 ± 0.24	0.80 ± 0.07	0.11 ± 0.28	0.25 ± 1.27	0.02 ± 0.41	0.03 ± 0.04	0.52 ± 0.77
OTU38	*Sporidiobolales* (B)	*Yamadamyces rosulatus* (EU872492)	*Yamadamyces rosulatus*/100%	0.74 ± 1.33	0.12 ± 0.41	0.75 ± 0.00	0.30 ± 0.47	0.30 ± 0.39	0.04 ± 0.57	0.05 ± 0.07	0.16 ± 0.63
OTU39	*Trichosporonales* (B)	*Apiotrichum dulcitum* (KC009516)	*Apiotrichum dulcitum*/99%	0.36 ± 1.57	0.41 ± 1.55	0.09 ± 0.27	0.05 ± 0.13	0.67 ± 0.89	0.09 ± 1.45	0.20 ± 0.19	0.68 ± 0.23
OTU40	*Tremellales* (B)	*Phaeotremella skinneri* (AF444305)	*Phaeotremella skinneri*/92%	0.09 ± 0.95	0.71 ± 0.07	0.00 ± 0.07	0.93 ± 0.98	1.68 ± 0.05	0.03 ± 0.84	0.03 ± 0.07	0.02 ± 0.00

hoven, Koorevaar and Schuur 1992), and has been frequently isolated from temperate forest soils (Yurkov *et al.* 2012). *Apiotrichum porosum*, *Solicoccozyma terricola* and *Saitozyma podzolica* were among the most abundant Basidiomycetes in the soil and litter from the spruce forests and were able to incorporate carbon from cellulose (Šťursová *et al.* 2012), which indicates their involvement in the decomposition of dead plant biomass.

The effect of the dominant tree on the composition of yeast communities in temperate forest has been also addressed. It is known that soil yeast communities may be highly variable over small spatial scales. Due to their small size, yeasts are expected to occupy microniches and be highly affected by patchiness of soil properties such as acidity, water activity and availability of nitrogen and carbon sources rather than by direct influence of plant roots. On the other hand, it has been shown that tree species in a forest determine the yeast community composition, at least of some taxa. Numerous studies investigating the diversity of yeasts report differences of yeast communities among forests with different tree species (e.g. Wuczkowski and Prillinger 2004; Yurkov, Maksimova and Chernov 2004; Yurkov *et al.* 2012; Mestre, Fontenla and Rosa *et al.* 2014). For example, Maksimova and Chernov (2004) studied yeast communities of boreal forests and found that yeast communities observed in spruce forests differed from those in birch and alder forests. Moreover, yeast communities in birch forests with similar climatic conditions, soil type and vegetation of two geographically separated regions of Russia were notably similar (Yurkov, Maksimova and Chernov 2004). Several studies have reported that yeast communities exhibit low diversity within sites (i.e., low alpha diversity), but high variability at larger geographical scales (i.e. high beta diversity) (e.g. Slavikova and Vadkertiova 2000; Vishniac 2006; Yurkov *et al.* 2012).

In the present study, composition of yeast communities in soils was affected by pH, which has been shown to be the main factor affecting community composition of bacteria (Rousk *et al.* 2010). Soil communities from different stands were also affected by moisture, which agrees with previously reported results (e.g. Vishniac 2006; Yurkov *et al.* 2016). The effects of abiotic conditions on yeast community composition have been also addressed in cultivation-based studies. An important factor affecting yeast communities is moisture. The structure of yeast communities along vast latitudinal gradients showed that species composition changed significantly with rainfall intensity (Vishniac 2006). Additionally, Yurkov *et al.* (2016) studied yeasts in Mediterranean forests and reported that communities significantly differed between three forests, constrained by precipitation level. Birkhofer *et al.* (2012) showed that neither soil yeast community composition nor abundance across different land use types in Germany was significantly related to soil properties, including pH, N content and C/N ratio. However, yeasts were, in contrast to other fungi, highly abundant in forest soils of the Schorfheide region with low pH (3.2) and average annual precipitation (520–600 mm). Also Yarwood, Bottomley and Myrold (2010) showed that basidiomycetous yeasts were abundant in nutrient rich and well drained soils with low pH.

Litter communities of yeasts were mainly affected by the dominant tree type. It is known that a high proportion of litter-associated yeast communities are composed of taxa that are not restricted to this habitat and may passively enter the soil (e.g. Vishniac 2006; Yurkov *et al.* 2012). Litter input may contribute substantially to the composition of yeast communities because phylloplanes contain abundant tree species-specific yeast populations reaching up to millions of cells per gram (Yurkov, Chernov and Tiunov 2008). It is also likely that yeasts are selected by the composition of secondary metabolites in litter rather than the content of nutrients, indicating that these secondary metabolites are the source of their nutrition. Indeed, litters with similar nutrient content but of different origin were found to host different microbial communities (e.g. Urbanová *et al.* 2014; Urbanová, Šnajdr and Baldrian 2015). Also Yurkov *et al.* (2012) showed that soil collected underneath litter hosted more variable communities than soils underneath logs where selective and stable conditions promote growth of few highly specialized soil-borne yeasts. While the effect of tree layer vegetation was also significant in the Whole Area Experiment soil samples, yeast communities under beech, oak and spruce stands were similar (Fig. 4) and no indicator species specific to any of the trees were recorded. These results suggest that relationships between dominant trees and yeast communities in soil may be more complex, and perhaps result from the strong effect of some tree species while others may share similar taxa. This would not be surprising, as stands of different trees exhibit different levels of specificity of their soil fungal communities (Urbanová, Šnajdr and Baldrian 2015).

In this study, we showed that yeasts represent a considerable proportion of the fungal communities in soil. Comparisons of our results with data obtained in cultivation studies suggests that yeasts from temperate forest soil represent a well cultivable group of organisms. Communities in litter are highly affected by composition of tree species. Drivers in soil are likely more complex and combine the effects of abiotic properties of soil and vegetation, and therefore, further research is required. Exploration of yeast nutritional traits may provide the most important clue for answering questions regarding the drivers of their occurrence and distribution in the environment.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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REFERENCES

Amprayn K, Rose MT, Kecskés M *et al.* Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. *Appl Soil Ecol* 2012;**61**:295–9.

Aronesty E. Comparison of sequencing utility programs. *Open Bioinforma J* 2013 **7**:1–8.

Baldrian P, Kolařík M, Šťursová M *et al.* Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J* 2012;**6**:248–58.

Birkhofer K, Schoning I, Alt F *et al.* General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. *PLoS One* 2012;**7**:e43292.

Botha A. The importance and ecology of yeasts in soil. *Soil Biol Biochem* 2011;**43**:1–8.

Buee M, Reich M, Murat C *et al.* 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytol* 2009;**184**:449–56.

Cairney JWG. Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. *Mycol Res* 2005;**109**:7–20.

Cloete KJ, Valentine AJ, Stander MA *et al.* Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans. *Microbial Ecol* 2009;**57**:624–32.

De Caceres M, Legendre P. Associations between species and groups of sites: indices and statistical inference. *Ecology* 2009;**90**:3566–74.

DeRito CM, Madsen EL. Stable isotope probing reveals *Trichosporon* yeast to be active in situ in soil phenol metabolism. *ISME J* 2009;**3**:477–85.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013;**10**:996–8.

Guindon S, Dufayard JF, Lefort V *et al.* New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 2010;**59**:307–21.

Hamilton N. Ggtern: An Extension to 'ggplot2', for the Creation of Ternary Diagrams. R package version 2.1.1. 2016. http://CRAN.R-project.org/package=ggtern. (16 February 2016, date last accessed).

Hong SG, Lee KH, Kwak J *et al.* Diversity of yeasts associated with Panax ginseng. *J Microbiol* 2006;**44**:674–9.

Ihrmark K, Bodeker IT, Cruz-Martinez K *et al.* New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* 2012;**82**:666–77.

Koljalg U, Nilsson RH, Abarenkov K *et al.* Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* 2013;**22**:5271–7.

Kulakovskaya TV, Golubev WI, Tomashevskaya MA *et al.* Production of antifungal cellobiose lipids by Trichosporon porosum. *Mycopathologia* 2010;**169**:117–23.

Kurtzman C, Fell JW, Boekhout T. *The Yeasts: A Taxonomic Study*, 5th edn. Amsterdam: Elsevier Science, 2011.

Liu XZ, Wang QM, Göker *et al.* Towards an integrated phylogenetic classification of the Tremellomycetes. *Stud Mycol* 2015;**81**:85–147.

Lindahl BD, Nilsson RH, Tedersoo L *et al.* Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytol* 2013;**199**:288–99.

Lundberg DS, Lebeis SL, Paredes SH *et al.* Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 2012;**488**:86–90.

Lynch MD, Thorn RG. Diversity of basidiomycetes in Michigan agricultural soils. *Appl Environ Microbiol* 2006;**72**:7050–6.

Maksimova I, Chernov IY. Community structure of yeast fungi in forest biogeocenoses. *Microbiology* 2004;**73**:474–81.

Margesin R, Fonteyne PA, Redl B. Low-temperature biodegradation of high amounts of phenol by Rhodococcus spp. and basidiomycetous yeasts. *Res Microbiol* 2005;**156**:68–75.

Mestre MC, Fontenla S, Rosa CA. Ecology of cultivable yeasts in pristine forests in northern Patagonia (Argentina) influenced by different environmental factors. *Can J Microbiol* 2014;**60**:371–82.

Middelhoven WJ, Koorevaar M, Schuur GW. Degradation of benzene compounds by yeasts in acidic soils. *Plant Soil* 1992;**145**:37–43.

Middelhoven WJ, Scorzetti G, Fell JW. *Trichosporon porosum* comb. nov., an anamorphic basidiomycetous yeast inhabiting soil, related to the *loubieri/laibachii* group of species that assimilate hemicelluloses and phenolic compounds. *FEMS Yeast Res* 2001;**1**:15–22.

Nilsson RH, Veldre V, Hartmann M *et al.* An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. *Fungal Ecol* 2010;**3**:284–7.

Peay KG, Kennedy PG, Bruns TD. Fungal community ecology: A hybrid beast with a molecular master. *Bioscience* 2008;**58**:799–810.

Rousk J, Baath E, Brookes PC *et al.* Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 2010;**4**:1340–51.

Sagova-Mareckova M, Cermak L, Novotna J *et al.* Innovative methods for soil DNA purification tested in soils with widely differing characteristics. *Applied Environ Microb* 2008;**74**:2902–7.

Sampaio JP. Utilization of low molecular weight aromatic compounds by heterobasidiomycetous yeasts: taxonomic implications. *Can J Microbiol* 1999;**45**:491–512.

Slavikova E, Vadkertiova R. The occurrence of yeasts in the forest soils. *J Basic Microbiol* 2000;**40**:207–12.

Štursová M, Bárta J, Šantrůčková H *et al.* Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. *FEMS Microbiol Ecol* 2016, DOI: 10.1093/femsec/fiw185.

Štursová M, Žifčáková L, Leigh MB *et al.* Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiol Ecol* 2012;**80**:735–46.

Takashima M, Sugita T, Van BH *et al.* Taxonomic richness of yeasts in Japan within subtropical and cool temperate areas. *PLoS One* 2012;**7**:e50784.

Tedersoo L, Jairus T, Horton BM *et al.* Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytol* 2008;**180**:479–90.

Urbanová M, Šnajdr J, Baldrian P. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biol Biochem* 2015;**84**:53–64.

Urbanová M, Šnajdr J, Brabcová V *et al.* Litter decomposition along a primary post-mining chronosequence. *Biol Fertil Soils* 2014;**50**:827–37.

Větrovský T, Baldrian P. Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. *Biol Fertil Soils* 2013;**49**:1027–37.

Vishniac HS. Simulated in situ competitive ability and survival of a representative soil yeast, *Cryptococcus albidus*. *Microbial Ecol* 1995;**30**:309–20.

Vishniac HS. A multivariate analysis of soil yeasts isolated from a latitudinal gradient. *Microbial Ecol* 2006;**52**:90–103.

Voříšková J, Baldrian P. Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME J* 2013 **7**:477–86.

Voříšková J, Brabcová V, Cajthaml T *et al.* Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytol* 2014;**201**:269–78.

Wang Q-M, Begerow D, Groenewald M. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the *Ustilaginomycotina*. *Stud Mycol* 2015a **81**:55–83.

Wang Q-M, Yurkov AM, Göker M *et al.* Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. *Stud Mycol* 2015b;**81**:149–89.

Westhoff V, van der Maarel E. The Braun-Blanquet approach. In: Whittaker RH (ed.). *Classification of Plant Communities*. The Hague: Dr W. Junk Publishers, 1978, 287–399

Wuczkowski M, Prillinger H. Molecular identification of yeasts from soils of the alluvial forest national park along the river Danube downstream of Vienna, Austria (Nationalpark Donauauen"). *Microbiol Res* 2004;**159**:263–75.

Yarwood S, Bottomley P, Myrold D. Soil microbial communities associated with douglas-fir and red alder stands at high- and low-productivity forest sites in Oregon, USA. *Microbial Ecol* 2010;**60**:606–17.

Yurkov AM, Chernov I, Tiunov AV. Influence of Lumbricus terrestris earthworms on the structure of the yeast community of forest litter. *Mikrobiologia* 2008;**77**:121–5.

Yurkov AM, Inacio J, Chernov *et al*. Yeast biogeography and the effects of species recognition approaches: the case study of widespread basidiomycetous species from birch forests in Russia. *Curr Microbiol* 2015;**70**:587–601.

Yurkov AM, Kemler M, Begerow D. Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. *PLoS One* 2011;**6**:e23671.

Yurkov AM, Kemler M, Begerow D. Assessment of yeast diversity in soils under different management regimes. *Fungal Ecol* 2012;**5**:24–35.

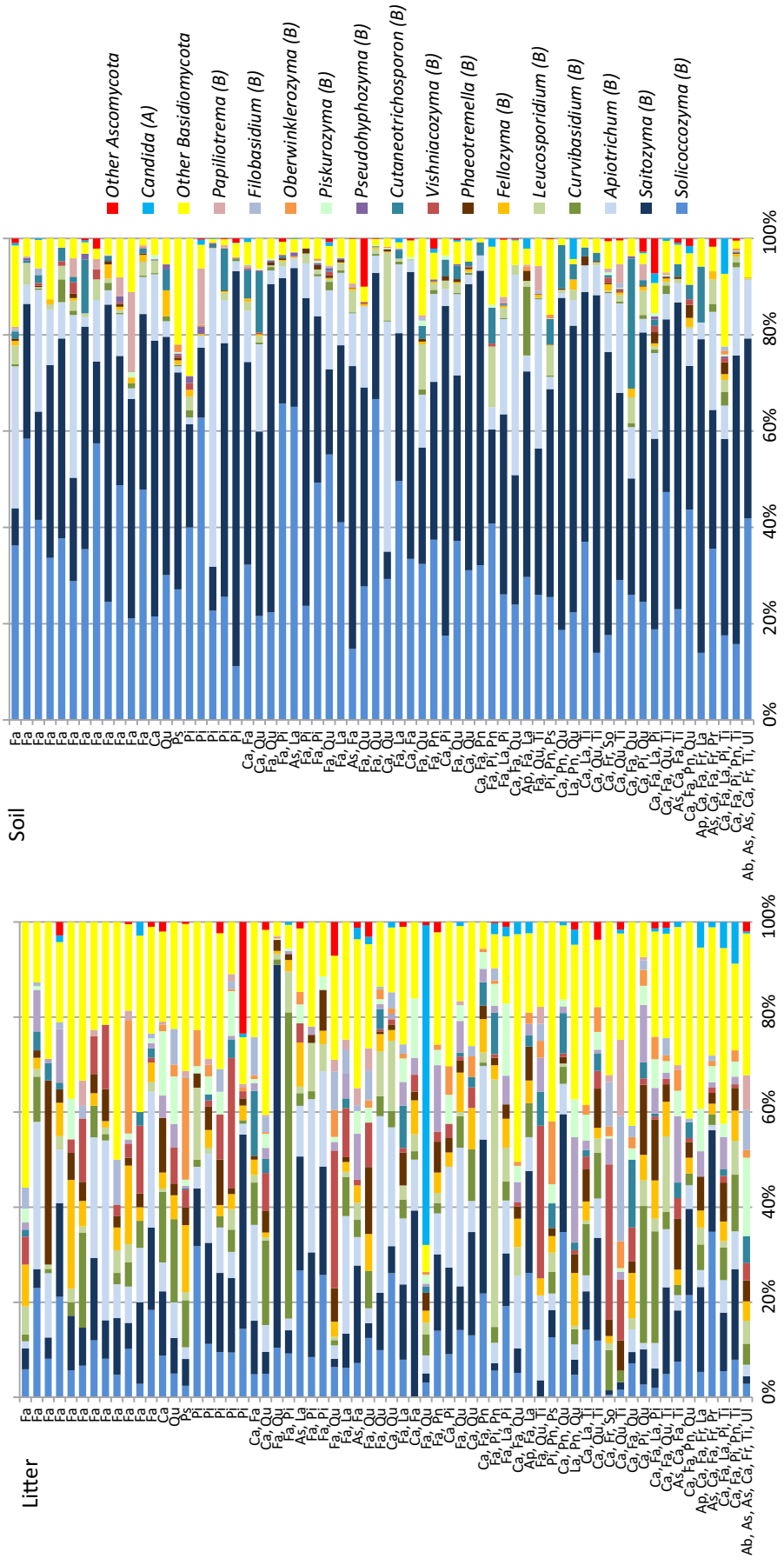
Yurkov AM, Maksimova IA, Chernov I. The comparative analysis of yeast communities structure in birch forests of European of European Russia and Western Siberia (English abstract). *Mikol Fitopatol* 2004;**38**:71–79.

Yurkov AM, Rohl O, Pontes A *et al*. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. *FEMS Yeast Res* 2016; **16**:fov103.

Yurkov AM, Wehde T, Kahl T *et al*. Aboveground deadwood deposition supports develompment of soil yeasts. *Diversity* 2012;**4**:453–74.

Žifčáková L, Větrovský T, Howe A *et al*. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environ Microbiol* 2016;**18**: 288–301.

Supplementary Figure 1: Taxonomic assignment of yeast sequences from litter and soil in the temperate forests across the study area. Abbreviations are as follows: Ab = *Abies alba*, Ap = *Acer platanoides*, As = *Acer pseudoplatanus*, Ca = *Carpinus betulus*, Fa = *Fagus sylvatica*, Fr = *Fraxinus excelsior*, La = *Larix decidua*, Qu = *Quercus robur*, Pi = *Picea abies*, Pr = *Prunus avium*, Pn = *Pinus nigra*, Ps = *Pinus silvestris*, So = *Sorbus torminalis*, Ti = *Tilia cordata*, Ul = *Ulmus glabra*.



II

Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest.

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Research Highlights

- Fungal community composition differed in litter and soils and among stand types
- Litter communities were strongly coupled to dominant species regardless of location
- Soil communities were coupled to dominant tree species and local abiotic variables
- Stand types were well-characterized by a set of low-abundant but highly specific taxa
- Community variation in time was similar between coniferous and deciduous stands

Abstract

Despite the progress in the past few years, the drivers of the composition of fungal communities in temperate forest soils are not fully identified. Here we have explored the factors driving the variation in natural-occurring fungal communities in litter and soils by sampling sites dominated by either spruce, beech or oak across a temperate forest. Randomized complete-block design with six replicates blocks spanning a 100km^2 forested area was used and sampling was performe four times over a one-year period to account for temporal variability. Fungal communities were characterized using amplicon sequencing. We found that fungal community composition differed between litter and soil and among stand types but community structure (richness, functional-guilds) was similar. Litter community composition was strongly coupled to dominant tree species. In soil communities, both dominant tree and abiotic variables were important with each variable explaining a unique parts of the variation in the community composition. Analyses of the community by functional-group subsets of showed some variation to these overall community patterns. Both the litter and soil communities of given stand type were well-characterized by a set of low-abundance indicator species with consistent presence, regardless of location, suggesting stand type is an important local filter. The marked difference in annual growth patterns between coniferous and deciduous stands were not found to correlate with changes in fungal community composition, however during our sampling period, a common time-dependent trend was found across all soil communities and among all soil functional-group subsets regardless of the dominant tree type.

1. Introduction

Fungi comprise a highly diverse group of organisms that play pivotal roles in ecosystem functioning (Hawksworth, 2001). They contribute to soil ecosystem processes through several pathways: i) by facilitation of nutrient cycling through their ability to decompose recalcitrant plant biomass; ii) by translocation of nutrients and moisture via their extensive hyphal networks; iii) by assisting plants in nutrient uptake and providing protection from disease and drought through their associations to plant roots; iv) by formation of soil aggregates and thus soil structure via their hyphal growth form; and v) in some cases, by mediation of pathogenic activity on plants and higher organisms.

Despite their known roles in ecosystems across the globe, the patterns of variation in soil fungal distribution and abundance and possible drivers of these patterns are insufficiently known (Martiny et al., 2006; Nemergut et al., 2013; Taylor et al., 2014). What we do know, however, is that fungal communities have quite a variable taxonomic composition across small as well as large distances. This high level of spatial variation has often been attributed to the patchy growth form of fungal individuals (e.g., mycorrhizal mats), the natural heterogeneity of resources in the soil matrix (Buée et al., 2009; Robertson et al., 1993), and the fine-scale spatial variation of abiotic variables (Robertson et al., 1993), all of which may lead to fine-scale species colonization patterns. Furthermore, fungal communities are known to be stratified vertically; distinct communities have been identified from the litter, organic soil and mineral soil horizons (Baldrian et al., 2012; Lindahl et al., 2007; McGuire et al., 2012; O'Brien et al., 2005) with fungal communities in litter tightly linked to the litter type (Aneja, 2005; Kubartová et al., 2009; Urbanová et al., 2015). This is thought to be due to the physical and chemical structures of the specific litter and the latent saprobes present in the leaf before leaf drop. More recently, seasonal

fluctuations, which has been largely neglected in fungal community ecology studies, has been demonstrated in these communities. Fluctuation over time has been ascribed to changes in litter quality and root exudates (Jumpponen et al., 2010; Voříšková et al., 2014). However, important questions about how these biotic and abiotic variables shape fungal communities in various ecosystems over space and time, remain unanswered.

Insights into soil fungal community structure and dynamics have been hampered by the high cost of fungal community studies which has restricted sample sizes as well as the temporal and the spatial extent of replication. However, cost of sample processing and sequencing are declining (Hibbett and Ohman, 2009). Thus, over the last 10 years we have seen a strong increase in studies that aim to identify the important drivers shaping fungal communities in natural systems over much broader spatial, and, in some cases, temporal, scales. The spatial extent of these studies can be divided into three main groups: i) global: assessing community variation across many distinct habitats (e.g. Tedersoo *et al.*, 2012); ii) landscape: assessing community variation in adjoining habitats (e.g. Peay *et al.*, 2013; Sterkenburg *et al.*, 2015); and iii) local: assessing community variation in a specific habitat (e.g. Clemmensen *et al.*, 2013; Adams *et al.*, 2013; Voříšková *et al.*, 2014; Šťursová *et al.*, 2016). While some of these studies have also included a temporal component (e.g. Voříšková *et al.*, 2014; Peay & Bruns, 2014) most are based on one-time sampling (e.g. Taylor *et al.*, 2014; Sterkenburg *et al.*, 2015). These recent studies demonstrate a progression towards increasing depth of spatial, temporal and taxonomic sampling, which has brought some clarity to drivers of biodiversity and community. Examples of abiotic drivers that are now known are calcium availability, precipitation, and soil fertility, while biotic drivers include litter type and composition of local vegetation. In addition to these extrinsic drivers, intrinsic population processes, such as spore dispersal, have also been shown to affect

fungal composition (Peay et al., 2016). The relative importance of these drivers are found to vary across studies, highlighting the complexity of fungal community dynamics over space and time.

Gaps remain in our knowledge of fungal community variation over landscape scales, specifically with respect to how fungal communities respond to variation in soil properties, and plant community composition and whether this response varies seasonally.

To understand the influence of these factors at a landscape scale, we sampled a series of spruce, beech and oak stands across a 100 km^2 forest landscape at four sampling times throughout a one-year period. We used a randomized complete block design along with amplicon sequencing to evaluate the following hypotheses:

1) On a landscape scale, fungal communities are primarily structured by the identity of the dominant vegetation, as motivated by the close association of the fungal community with vascular plant composition.
2) Litter communities are more tightly coupled to above-ground vegetation type (and, hence, litter type) than soil communities.
3) Temporal effects are more pronounced in deciduous than in coniferous stands due to the seasonal effects of leaf senescence and the expected greater fluctuation in both photosynthetic and root activity.
4) Spatial patterns in fungal community composition are detectable even after the effects of vegetation and local habitat conditions have been partialled out.

2. Materials and Methods

2.1 Study area

This study was conducted in the Masaryk Forest Křtiny, (latitude 49°15 N, longitude 16°15' E) near Brno in the Czech Republic which contains 103 km^2 of mixed temperate forest. The area is situated in the South Moravian Bioclimatic Region, with mean annual temperatures of 8.0–9.5 °C and mean annual precipitation of 400–500mm. The area is covered by approximately 46% conifer and 54% broadleaf tree species and includes parent rock types of granodiorites, culmian greywacke and limestone (Svatek, 2004). A comprehensive set of spatially explicit forest descriptive data is maintained by the Training Forest Enterprise (TFE-Křtiny), an organizational unit of Mendel University in Brno.

2.2 Study sites and sampling

Study sites were chosen based on dominance of three tree taxa (hereatfter referred to as 'species'): spruce (*Picea abies),* beech (*Fagus sylvatica),* and oak (*Quercus* spp). A tree species was considered dominant when it comprised > 80 % cover at a site. All polygons > 1000 m^2 with > 80 % dominance of one of these tree species, with tree age > 20 years according to the TFE-Křtiny forestry database were potential sampling sites.

We sampled according to a randomized complete block design by first dividing the forest map into 6 equal-sized blocks. In each block we selected the set of three polygons, dominated by different tree species, that were situated as close as possible to within a 1 km radius (Fig. 1; Table. S1). The average distance between sites (in different blocks) dominated by the same tree species was 6.9 km (range: 1.1–11.7 km) and sites within blocks were, on average, 700 m apart (range: 16–1860 m) (Fig. 1).

Sites were sampled four times throughout 2013 (January, April, June, October). At each site we collected five 4.5-cm diameter soil cores by establishing one central core location and then collecting four additional cores 2 m from this central core at the four cardinal points. For the first sampling, the center core was located at the center of the mapped polygon. In each consecutive sampling period, we shifted the central core location 20cm in one of the cardinal directions, to avoid resampling directly on top of the soil disturbed by the prior sampling. Each sampling period was completed in one day and cores were stored at 4 °C before processing in the laboratory within 24 h.

Samples were separated into litter (including the fermentation layer) and soil (the upper 10 cm of the soil core). Composite samples from each of the 18 sites were prepared by combining the material from all cores and homogenizing it either by cutting the litter into ca. 0.25-cm^2 pieces or passing the soil through a 5-mm sieve. Samples for DNA extraction were frozen and stored at –80 °C before processing. The remaining material was lyophilized and stored at –40 °C for further chemical analyses.

2.3 Site characterization: recording of explanatory variables

Dry mass content was measured as a loss of mass during lyophilization. Organic matter content was measured after combustion at 650 °C, and pH was measured in distilled water (1:10 soil to water). Total N and organic C were measured in an external laboratory from samples collected in June. Total ergosterol was extracted from all samples using 10% KOH in methanol. Extracts were analyzed by HPLC (Šnajdr et al., 2008) and used as a proxy of fungal biomass (Bååth, 2001; Högberg, 2006). Cover-abundance of all vascular plant species that covered each

$25m^2$-plot was estimated in the summer of 2013 using the nine-degree Braun-Blanquet scale (Westhoff and Van Der Maarel, 1978).

2.4 DNA extraction and sequencing of PCR amplicons

Total genomic DNA was extracted in triplicate from 250 mg of fresh soil or litter material using a modified Miller method (Sagova-Mareckova et al., 2008). Triplicate DNA extracts were combined into one sample and amplified, again in triplicate, to reduce single-sample amplification bias. The fungal ITS2 region was amplified according to the method described by Ihrmark *et al.* (2012) using barcoded gITS7 and ITS4 primers as described previously (Ihrmark et al., 2012; Zifcakova et al., 2016) with the exception that the number of cycles were lowered from 35 to 25, to reduce amplification bias. Sequencing was performed on an Illumina MiSeq.

2.5 Processing of sequence data

Pair-end reads were merged using fastq-join (Aronesty, 2013) and the ITS2 region was extracted using ITS Extractor 1.0.8 (Nilsson et al., 2010) Sequences were clustered into Operational Taxonomic Units (OTUs) according to the UPARSE pipeline (http://drive5.com/uparse/) implemented in USEARCH7 (Edgar, 2013) with one addition: after de-replication and removal of singletons, sequences were tested for fungal origin using the ITSx program (Bengtsson-Palme et al., 2013) as is recommend for this region (Lindahl et al., 2013). Chimera checking was implemented during ITSx filtering (Nilsson et al., 2014) and in the UPARSE pipeline. OTU taxonomy was assigned using the UNITE database (Abarenkov et al., 2010) except for yeasts which follow the more recently proposed taxonomy (Liu et al., 2015;

Wang et al., 2015a, 2015b). OTU assignment to putative ecological functional groups (e.g., arbuscular mycorrhiza (AM), ectomycorrhizal (EcM), saprotroph, yeast) was based on the genus-level classification of Tedersoo *et al.*(2014). Sequence data were deposited in the MG-RAST public database (http://metagenomics.anl.gov/, data set number 4696490.3).

After removing singletons and OTUs with low prevalence (occurrence less than 3% of samples), variation in sequencing depth among samples (common in HTS output) was managed using the variance stabilized transformation (VST) available in the DESeq2 package for R (Love et al., 2014) according to the Phyloseq package for R vignette on differential abundance testing (McMurdie and Holmes, 2013). This transformation is recommended over rarefying and proportional abundance methods for equalizing variation that occurs in amplicon library size as it better maintains resolution of the community composition (McMurdie and Holmes, 2014).

2.6 Data analysis

All statistical analyses were carried out in R (R Development Core Team, 2011). OTU and vascular plant species richness were used as measures of alpha diversity of the fungal and plant communities, respectively. Jaccard's distance (Ramette, 2007), calculated by use of species presence-absence data, was used as a measure of the fraction of shared species among sites. Species compositional patterns across sites and sampling times were extracted separately for the soil and litter fungal communities, and above-ground vegetation using parallel global non-metric multidimensional scaling (gNMDS) and detrended correspondence analysis (DCA) ordination methods (Økland, 1990; Van Son and Halvorsen, 2014). Cover-abundance data were used as input for the analyses of vegetation communities and the variance-stabilized transformed data were used as input for the analysis of fungal community data.

Specifications of gNMDS ordination was similar to Rämä *et al.* (2016), except maximum iterations were set to 200. Solutions were constrained to two dimensions and parallel ordinations were tested for similarity via Procrustes rotations and Monte Carlo test of significance (protest() command vegan package for R (Oksanen et al., 2015; Peres-Neto and Jackson, 2001) as well as pairwise Kendall's rank correlation coefficients (τ) between axes to identify possible artifacts in ordination patterns and thus avoid interpreting spurious patterns (van Son & Halvorsen, 2014) (see Tables S4 and S6). The envfit() function in the vegan package was used to fit all measured environmental variables onto ordinations and Kendall's rank correlation coefficients τ were calculated for each axis between site centroids and averages for environmental variables that were measured at each sampling time. The hypothesis that the soil fungal community is influenced by the understory vegetation was tested by using site scores from axes 1 and 2 of the vascular plant ordination as explanatory variables in the interpretation of the fungal community ordination.

Further ordinations were carried out on the prevalent functional guild subsets of the community to explore whether the unique biology of each functional guild might change the relative importance of the explanatory variables. We used gNMDS with the same options and settings as was used to ordinate the full community. We calculated the proportion of functional guilds in each stand type by dividing the raw sequence counts of a given functional guild in a given stand type by the total number of sequences recovered in that stand type, and tested for difference among stand types and over time using repeated measures ANOVA.

To identify fungal OTUs showing preferences for specific tree species we used Indicator Species Analysis (De Cáceres & Legendre 2009) as implemented in the indicspecies package for R (De Cáceres, 2013).We used the multipatt() command with the default Indicator Value described by Dufrêne & Legendre (1997).

Variation partitioning analysis (VPA) based on canonical correspondence analysis (ter Braak, 1986) was used to quantify the relative, shared, and unique contribution of predictor variables to explain fungal community compositional variation in litter and soil (Økland, 2003). Predictor variables included i) dominant tree species, ii) sampling time and iii) an abiotic group-variable. For the abiotic group-variable forward selection of the individually measured abiotic variables (eg. moisture content, pH) was used to ensure that each new variable added to the group-variable contributed additional significant explanatory power.

The explanatory power of a set of predictor variables in VPA is reported as the fraction of the variation explained by all sets of explanatory variables included in the analyses (FTVE) rather than as the fraction of the total inertia (Økland, 2003). The reason for this is that polynomial distortions contribute 40–65% of the total inertia in a given data set (Økland, 2003) and, accordingly, that ecologically meaningful comparisons can only be made relative to explained variation. Significance of the FTVEs of each group were tested by use of the Monte Carlo permutation test (permutest.cca () command) in package 'vegan' (see Legendre, 2007).

We examined temporal patterns in the fungal community under the different dominant tree species using Principal Response Curves (PRC) as described van den Brink *et al.* (2009), by which a zero-reference point is used to create a baseline for treatment comparison. PRC is a variation on constrained ordination where time is displayed as a single vector allowing site scores from the constrained ordination, representing given time points, to be compared. To further explore temporal patterns in both litter and soils we carried out species turnover analysis using the turnover() command in the R package codyn (Hallett et al., 2016), by which both the appearance and disappearance of taxa between consecutive temporal observations at each site are calculated. We used repeated measures two-way ANOVA to test if turnover patterns differed over time among sites and dominant tree types.

To test for spatial trends in community composition across our study area we ran trend surface analysis separately on litter and soil. We further explored finer-scale spatial patterns in these communities using Principal Coordinates of Neighbour Matrices (PCNM) (Borcard et al., 2011).

3. Results

3.1 Site properties

Among the three stand types, a significant difference in pH was found between spruce- and beech-dominated sites, both in litter and soil. Moisture, C/N ratio, lignin and soil organic matter content did not significantly differ among stand types (Table 1).

Understory plant species richness varied from 4 to 28 species among sites (mean=16.3, sd=7.2; Table 2) with a total richness of 102 species across all sites. Evenness and richness were higher in oak-dominated sites but species richness varied considerably among plots of the same tree type. For example, sites Beech 1 and 6 and Spruce 1 contained < 10 taxa) while Spruce 3, and Beech 2 and 3 were relatively species-rich (Fig. S1).

Axes of parallel ordinations (by gNMDS and DCA) of sites by understory vegetation were significantly correlated (Procrustes correlation = 0.53, Table S2). Litter CN ratio was correlated with axis 1 and moisture as well as the CN ratio in litter were correlated with axis 2 (Fig. 2; Table S2). *Galium aparine* and *Poa nemoralis* were identified as indicator species (alpha=0.01), both in oak-dominated sites. The understory community in oak-dominated sites was significantly different from beech and spruce (Table S2).

3.2 Characteristics of fungal communities in litter and soil

Fungal biomass was substantially higher in litter than in soil (40-fold increase in spruce stands; 20-fold increase in broadleaf) and was significantly higher in beech stands compared to spruce stands (Table 1).

Sequence clustering resulted in a total of 7309 OTUs (plus 279 additional singletons, not included in the analyses) of which 65% were recovered from both soil and litter. After filtering rare taxa, 5070 OTUs (litter: 3616; soil: 3301) were retained. These accounted for approximately 95% of all reads.

Fungal OTU richness in soils (the number of OTUs per site) ranges from 559 to 1363 and Pielous's evenness index ranges from 0.622 to 0.786. Neither index differed significantly among stand types (Table 2). Fungal OTU richness in litter is similar to soil and ranges from 757 to 1316 OTUs. Pielous's evenness index is ca. 0.99. As with soil, neither richness nor evenness differs significantly among stand types (Table 2) but litter communities have significantly higher evenness than soil communities and both broadleaf stands have higher mean OTU richness in litter than in soil communities.

The OTUs in each of the horizons were best identified to 772 genera in litter and 750 genera in soil. Sixteen genera in litter and 19 genera in soils had mean relative abundances of ≥ 1% (Table 3). Many genera that were abundant in both litter and soil were saprotrophs. In total, the 26 genera listed in Table 3 accounted for 37% of fungal sequences in litter and 44% in soil. In litter, most sequences were associated with saprotrophic genera (67%), while 3% and 6% of sequences were associated with yeast and ectomycorrhizal genera, respectively. In soil, most sequences were also associated with saprotrophic genera (53%), with 36% and ca. 5% of sequences associated with ectomycorrhizal and yeast genera, respectively.

Among dominant tree species (stand types), beech soils differed from spruce soils by their higher proportions of ectomycorrhizal and lower proportions of saprotrophs sequences (Fig.

S2(a)). In litter, spruce and oak litter differed from beech litter with a significantly higher proportions of saprotrophs and yeasts sequences, and lower proportions of arbuscular mycorrhizal fungi sequences (Fig. S2(b)).

Indicator species analysis identified 38 OTUs in soil ($p<0.01$) (11 with spruce, 10 with oak 3 with beech and 14 with more than one type; Table S3) and 65 OTUs in litter ($p<0.001$) (25 with beech, 26 with oak, 10 with spruce and 4 with pairs thereof; Table S3). All tree-specific OTUs occurred at low relative abundances (average relative abundance = 0.0005) and most were associated with saprotrophic genera (Table S3).

3.3 Factors associated with fungal community composition in soil

The variable explaining most of the variation in the community composition is pH (axis 1: $\tau = 0.673$, $p<0.001$). The random effect of block is also important in explaining site positions along axis 1 (ANOVA: $F_{5,12} = 3.38$, $p=0.39$). Dominant tree species (ANOVA: $F_{2,15} = 14.16$, $p< 0.001$) and understory vegetation site scores-2 ($\tau = 0.399$, $p=0.02$) explain site positions along axis 2.

In VPA, as revealed by forward selection of variables, tree species, abiotic variables and sampling time contribute independently to explaining variation in the soil community (Table S5(a,b)). Together, these variables account for 25% of the total variation in community composition. Stand type makes the most significant contribution to explaining fungal community variation (38.7% of total variation explained (TVE); Table S5(c,d)). Abiotic variables are also significant in explaining community variation (43.5% of TVE) and share part of the explained variation with stand type (6.5% of the abiotic fraction of TVE). Sampling time explains 23.7% of TVE and shares no explained variation with the other variables (Table S5; Fig. 7).

The ordinations of soil samples by functional guild OTU subsets finds only EcM communities clearly differentiated according to stand type (axis 2: $F_{(2,15)}$=15.04 , p= 0.0003) with spruce EcM communities separating from those of beech and oak (Fig. 4). The influence of dominant tree is apparent for soil yeasts and saprotrophs, although communities under the three dominant tree types are not significantly different (axis 2: yeast $F_{(2,15)}$=3.19, p= 0.07; sap $F_{(2,15)}$=2.73, p= 0.1). All three of these functional guilds (yeasts, sap and EcM) show association to the understory vegetation site scores-2 on axis 1 (yeast τ =0.438 p=0.01, sap τ =0.386, p=0.03, EcM τ =0.412, p=0.02) with saprotrophs and EcM communities also showing association with understory vegetation site scores-1 on axis 2 (sap τ =0.477 p=0.005, EcM τ =0.429, p=0.01). pH is associated with placement of sites for saprotrophs and yeasts (Table. S9). We find no measured variables to be significant for the placement of soil AM communities.

3.4 Factors associated with fungal community composition in litter

The variable explaining most of the variation in community composition in litter is stand type (axis 1: $F_{(2,15)}$=123.9 ,p<0.001; axis 2: $F_{(2,15)}$=49.05 , p<0.001). Both understory vegetation scores-1 as well as percent of graminoid cover are correlated with the positions of sites on axis 1 (vegetation scores-1: τ =0.569, p=0.001; graminoid cover: τ =0.439, p=0.01). Percent graminoid cover as well as non-woody vegetation richness are correlated with site positions along axis 2 (graminoid cover: τ =-0.385, p=0.03; non-woody veg.: τ =-0.41, p=0.02).

In VPA, as revealed by forward selection of variables, stand type and abiotic variables contribute independently to explaining variation in the litter community. Together, these variables account for 30% of the total variation in litter community composition. Stand type

makes the most significant contribution to explaining fungal community variation (Table S7, Fig 7). Time of sampling does not explain significant variation in our litter community composition.

The ordination of litter samples by functional-group OTU subsets show a similar pattern to that of the full litter community (Fig. 6). Significant segregation by stand types along axes 1 and 2 are observed for yeasts, saprotrophs and EcM communities, while relationships are less strong for AM communities (Table. S9). Results from post-hoc tests indicate that saprotroph and yeast communities are significantly different in each stand type. Saprotrophs and yeast communities also correlate with understory vegetation scores-1 on axis 1 (Sap: $\tau = 0.595$, $p<0.001$; Yeasts: $\tau =0.634$, $p<0.001$). Placement of AM communities on axis 1 is correlated with moisture and understory vegetation scores-2, and on axis 2 with several understory vegetation-related variables including understory vegetation scores-1, understory richness and percent graminoid, as well as stand type. Placement of saprotroph communities on axis 2 is significantly correlated with the understory vegetation-related variables of percent graminoid cover and non-woody richness (Table. S9). Litter EcM communities significantly correlated with pH (axis 2: $\tau =-0.412$, $p=0.02$).

3.5 Temporal patterns in fungal community composition – soil and litter

No clear shifts in site positions in ordination space over sampling times are observed for litter or soil communities (Figs 3 and 5) and PRC analysis reveals no difference in the extent of temporal change of communities among stand types or between coniferous and deciduous stands. We do, however, find a temporal trend in soil communities reflected in compositional change from sampling times between January and April and between July and September. This

corroborates the VPA results where time of sampling is significant for soil communities (Fig. S7).

Species turnover analysis reveals differences in average OTU turnover (i.e gain and loss of species) among dominant tree types for EcM communities in soils and litter, and for yeasts communities in litter. EcM communities in soils show lower average loss of species in beech soils and significant lower addition of species in spruce litter. In litter we also find a significantly higher addition of species in yeast communities among beech litter as compared to the other stand types (Fig. S5).

Differences in turnover patterns between time points are found for all functional guilds in soil, where we see a significantly greater loss of species between January and April sampling periods and significantly greater gain of species between July and September sampling periods. This pattern is similar among all stand types (Table S8, Fig. S3) and is corroborated by the outcomes of VPA and PRC analyses. In litter, differences between time point turnover is only found in the yeast community, where we find a greater gain of yeast OTUs between July and September sampling periods (Table S8).

3.6 Spatial dependencies in fungal community composition– soil and litter

Trend surface analysis revealed one significant spatial gradient in soil community composition which runs in a north-south direction across sites and is related to pH. No further significant finer-scale spatial patterns were detected in the soil community by PCNM analysis. No significant spatial pattern was found in the litter community.

4. Discussion

4.1 Characteristic of fungal communities in litter and soil

Of the 5070 OTUs recovered in this study 26% were restricted to litter and 19% to soils providing evidence for distinct communities. This vertical stratification of fungal communities is consistent with previously reported compositional patterns (O'Brien et al., 2005; Prescott and Grayston, 2013). Although these communities are statistically distinct, over half (~53%) of the OTUs occur in both litter and soil. This finding likely reflects the generalist ecology of many taxa, such as saprotrophs, which dominate both litter and soil communities. Four of the six most abundant genera, all saprotrophic, were dominant in both litter and soil.

The clear separation of stand types based on abundance data supports our first hypothesis (H1) that, stand type is an important driver of fungal community composition. At the OTU level, two interesting OTU-level preference patterns are apparent from our indicator analysis: i) spruce and oak soils share more indicator species as compared to beech soils and, ii) beech and oak litter share more indicator species as compared to spruce litter. These patterns may suggest resource similarities between spruce and oak soils and beech and oak litter which, due to their similar nature, can be exploited by the same species. Prescott & Grayston (2013) note that the greater base cation content in beech soils may have an important effect on the microbial community which may explain the separation of beech soils. In litter communities, we postulate that physical structure and chemistry of oak and beech which have been shown to be similar (Carnol and Bazgir, 2013), may be exploited equally well by certain species. Ecological meaningful interpretation of these preferences to specific stands is limited due to the lack of life history information and taxonomic identity at the OTU-level, although these enigmatic fungi are consistently found in molecular studies (Peay, 2014; Prosser, 2012).

4.2 Factors associated with fungal community composition in litter and soil

Both litter and soil communities are influenced by the dominant tree species. Litter communities also correlate with patterns in the understory vegetation while soil communities correlate with pH. Significant differences in fungal communities are found among all three stand types in both litter and soil, but VPA analyses finds a stronger stand type effect in litter. These results support our second hypothesis (H2) and are consistent with previous findings (Peršoh, 2013; Urbanová et al., 2015).

Abiotic variables explain additional variation in litter communities, but much of this explained variation is shared with stand type. This is likely due to the fact that abiotic variables in the litter layer are highly influenced by the dominant tree species as the dominant tree typically contributes ca. 85% to the litterfall (Carnol and Bazgir, 2013). The variation explained in soil fungal communities by the abiotic variables, unlike that of the litter community, is not shared with the dominant tree effects. Urbanová *et al.* (2015) also found that dominant tree species explained less variation for communities in soils compared to those in litter.

We find temporal community compositional shifts in soils are mainly driven by a loss of species from winter to spring and a gain in species from summer to fall. The pattern detected equates to a decrease in soil community richness during the active-growth period of aboveground vegetation, typically thought to be associated with increased microbial activity in soils due to the higher production of root exudates (Abramoff and Finzi, 2016). Högberg *et al.* (2010) found a large contribution of root-exudates to the soil carbon pool along with assimilation by soil fungi. This flush of resources may lead us to expect greater fungal diversity during the months of high photosynthetic activity. However, Hobbie & Hobbie (2013) have questioned the accuracy of past

root-exudate measurements and when Strickland *et al.* (2015) used Hobbie & Hobbie (2013) concentrations in their controlled microcosm experiment, they found little effect of root exudates on community structure. We conclude that drivers of temporal change in fungal communities remain unclear and the reported mixed response to seasonal changes in vegetation inputs suggest that other unmeasured variables are more important. In the present study, we find similar temporal changes under all stand types in the soil community, and the absences of clear temporal changes in the litter community, which leads us to reject our third hypothesis (H3) that fungal communities in deciduous stands have different temporal patterns from those in coniferous stands.

Contrasting to our fourth hypothesis (H4) we did not detect spatial patterns in community composition. This may be due to the spatial resolution of our sampling design. Lilleskov *et al.* (2004) found that an inter-sample distance of 3m or less was required to detect spatial patterns in EcM taxa. Thus, it is likely that spatial dependencies exist at scales much finer than our inter-site distances.

4.3 Fungal functional guild composition in forest litter and soil

Saprotrophs account for over half of the sequences in both soil and litter communities, whereas other functional guilds showed more variability. The greater proportion and richness of EcM taxa in soils versus litter (soil: 495 OTUs; litter: 351 OTUs) is congruent with our understanding of EcM taxa as root-associated symbionts. While the occurrence of EcM fungi in litter is lower than in soil, it is not negligible and similar patterns have been reported previously (Sterkenburg et al., 2015; Tedersoo et al., 2003; Voříšková et al., 2014; Voříšková and Baldrian, 2013). These findings are consistent with the theory that EcM taxa are not only capable using nutrient from organic resources in litter (Lindahl and Tunlid, 2015), but access this source more than originally

thought (Courty et al., 2010; Peršoh, 2015). The higher richness of yeast taxa in litter (soil: 194 OTUs; litter: 320 OTUs) is also congruent with our understanding of this group. Yeasts, defined primarily by their single-cell morphology, are better adapted to habitats where moisture is high and nutrient-rich inputs are common such as fallen fruit and leaf-drop on the forest floor (Botha, 2011).

4.4 Factors associated with functional guild composition in soil

In soils, we find EcM communities to be the only guild for which stand type is a significant driver of composition. This has been previously reported (Schirkonyer et al., 2013; Tedersoo et al., 2014, 2012) and is perhaps expected given the symbiotic, sometimes species-specific, relationship between EcM fungi and their host plants. EcM communities also correlate to fungal ergosterol-measured biomass which was also reported by Sterkenburg *et al.* (2015) who found EcM community composition to be associated with their index of soil fertility, to which ergosterol content was positively and significantly correlated.

EcM fungi, saprotrophs and, yeasts in soils all correlate with the site scores obtained from our vegetation axis 2. While visually this variable seems to be related to spruce-dominated sites, none of our measured variables (including stand type) were correlated with this vegetation axis. From this we can only conclude that an aspect of understory vegetation is more important in shaping soil communities than litter.

Variation in the community composition of soil saprotrophs is correlated with pH and understory vegetation. pH likely affects the ability of saprotrophs to access resources in soil due to interactions between pH and base cation availability. A strong influence of calcium availability

on fungal communities has been demonstrated previously (Prescott and Grayston, 2013; Tedersoo et al., 2014).

All functional guild subsets in soil show loss of species from winter to spring and a gain in species from summer to fall. This suggests that this previously noted pattern in the general fungal community, affects all functional guilds similarly.

4.5 Factors associated with functional guild composition in litter

In litter, both saprotroph and yeast communities are distinct among all three stand types. Specificity of the litter decomposer community to a particular dominant tree species has been previously reported and attributed to the pre-existing endophytic community present in the leaf before leaf drop (Peršoh, 2013) as well as differences in nutrient availability or specific litter chemistry (Bray et al., 2012). The primary axes of the ordinations for these guilds suggest that the major variation in the saprotroph community is associated with the differences between broad-leaf and coniferous litter while the major variation in the yeasts community is associated with the differences among each litter type. This may be evidence of the generalist nature of saprotroph ecology where species are known to be adept at accessing resources under a broad range of conditions by releasing extracellular oxidative and hydrolytic enzymes. These enzymes act on the general structure of a compound (for example, cellulose) rather than requiring species-specific conditions for nutrition acquisition. The specificity of yeast communities to stand types indicates this group may have unique ecologies in forest ecosystems which may be related to their unicellular growth form and this has garnered particular research interest (Botha, 2011; Mašínová et al., 2017; Yurkov et al., 2012).

EcM community composition correlates with litter chemistry (pH and C:N ratio) and stand type. The significant differences in beech litter pH in our study makes it impossible to separate the influence of stand type from litter chemistry but previous studies have noted the importance of both these variables. For example, Tedersoo *et al.* (2014) found EcM host species richness and pH to be the strongest predictors of EcM communities globally, and Sterkenburg *et al.* (2015) reported effect of litter C:N ratio on the EcM community along a fertility gradient in boreal forest and, similar to our study, found much of this variation to be explained by the dominant tree species.

We find that variation in the AM community correlates with understory vegetation and that oak-dominated sites are distinct from beech and spruce. AM fungi mainly establish associations with herbaceous plants (Buée et al., 2009), thus the unique oak AM communities may be an indirect result of the significantly different understory vegetation in oak-dominated sites which had higher cover of graminoids. Such relationship between AM communities and plant communities have been shown previously (Öpik et al., 2009).

We observe temporal changes in yeast turnover with a significant gain of species between the July and September sampling dates. The fact that temporal shifts in turnover were only observed in litter may be due to the yeasts' ability to respond quickly to suitable conditions with rapid growth (Lachance, 2011). Our study did not find other temporal patterns that have been previously reported for deciduous litter. For example, Voříšková *et al*. (2014) noted that the proportion of EcM sequences was significantly higher in summer and abundance of *Mycena spp*. was significantly higher in spring under oak trees. We detect no significant difference in proportion of EcM sequences over our sampling times and observe relatively little change in *Mycena spp* abundance in oak litter (see Fig. S4). Repeated sampling and replicate sites were incorporated in both of these studies but idiosyncratic differences in study location and

environmental conditions at sampling times may be responsible for the apparent conflicting patterns. The findings may also indicate that we did not measure variables at a temporal and/or spatial scales to which fungi may be responding.

5. Conclusions

We find that non-contiguous forest stands dominated by mature beech, oak or spruce harbor distinct fungal communities that are relatively stable over a one-year period. Both litter and soil communities in each stand type are characterized by a subset of indicator species with high fidelity but relatively low abundance. In litter, the main factor driving fungal community composition is the dominant tree species. In soils both the dominant tree species and local abiotic variables are important drivers. Functional-guild subsets of the community show some novel variation, different from that of the general fungal community. We detect no differences in temporal patterns between deciduous and coniferous stands but do detect a temporal change common to all soil communities.

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References

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Hoiland,

K., Kjoller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vralstad, T., Liimatainen, K., Peintner, U., Koljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. NEW PHYTOLOGIST 186, 281–285. doi:10.1111/j.1469-8137.2009.03160.x

Abramoff, R.Z., Finzi, A.C., 2016. Seasonality and partitioning of Root allocation to rhizosphere soils in a midlatitude forest. Ecosphere 7. doi:10.1002/ecs2.1547

Adams, R.I., Miletto, M., Taylor, J.W., Bruns, T.D., 2013. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. The ISME Journal 7, 1262–73. doi:10.1038/ismej.2013.28

Aneja, M.K., 2005. Degradation of beech and spruce litter - Influence of soil site and litter quality on microbial communities.

Aronesty, E., 2013. Comparison of Sequencing Utility Programs. The Open Bioinformatics Journal 7, 1–8. doi:10.2174/1875036201307010001

Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology & Biochemistry 33, 2011–2018. doi:10.1016/s0038-0717(01)00137-7

Baldrian, P., Kolařík, M., Stursová, M., Kopecký, J., Valášková, V., Větrovský, T., Zifčáková, L., Snajdr, J., Rídl, J., Vlček, C., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–58. doi:10.1038/ismej.2011.95

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sanchez-Garcia, M., Ebersberger, I., de Sousa, F., Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. METHODS IN ECOLOGY AND EVOLUTION 4, 914–919. doi:10.1111/2041-210X.12073

Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R. Springer New York, New York. doi:10.1007/978-0-387-78171-6

Botha, A., 2011. The importance and ecology of yeasts in soil. Soil Biology and Biochemistry 43, 1–8. doi:10.1016/j.soilbio.2010.10.001

Bray, S.R., Kitajima, K., Mack, M.C., 2012. Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. Soil Biology and Biochemistry 49, 30–37. doi:10.1016/j.soilbio.2012.02.009

Buée, M., Boer, W., Martin, F., Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi:10.1007/s11104-009-9991-3

Carnol, M., Bazgir, M., 2013. Nutrient return to the forest floor through litter and throughfall under 7 forest species after conversion from Norway spruce. Forest Ecology and Management 309, 66–75. doi:10.1016/j.foreco.2013.04.008

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D. a, Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science (New York, N.Y.) 339, 1615–8. doi:10.1126/science.1231923

Courty, P.E., Buee, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology & Biochemistry 42, 679–698. doi:Doi 10.1016/J.Soilbio.2009.12.006

De Cáceres, M., 2013. How to use the indicspecies package (ver. 1.7.1). R Project 29.

De Cáceres, M., Legendre, P., 2009. Associations between species and groups of sites : indices and statistical inference Author (s): Miquel De Caceres and Pierre Legendre Associations species and groups of sites : indices and statistical inference. Ecology 90, 3566–3574.

Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67, 345–366.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Meth 10, 996–998.

Hallett, L.M., Jones, S.K., MacDonald, A.A.M., Jones, M.B., Flynn, D.F.B., Ripplinger, J., Slaughter, P., Gries, C., Collins, S.L., 2016. CODYN: AnR package of community dynamics metrics. METHODS IN ECOLOGY AND EVOLUTION 7, 1146–1151. doi:10.1111/2041-210X.12569

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate

revisited. Mycological Research 105, 1422–1432. doi:10.1017/S0953756201004725

Hibbett, D., Ohman, P.M.K., 2009. Fungal ecology catches fir. New Phytologist 184, 279–282. doi:10.1111/j.1469-8137.2009.03042.x

Hobbie, J.E., Hobbie, E. a, 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Frontiers in Microbiology 4, 324. doi:10.3389/fmicb.2013.00324

Högberg, M.N., 2006. Discrepancies between ergosterol and the phospholipid fatty acid 18:2ω6,9 as biomarkers for fungi in boreal forest soils. Soil Biology and Biochemistry 38, 3431–3435. doi:10.1016/j.soilbio.2006.06.002

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., N??sholm, T., H??gberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493. doi:10.1111/j.1469-8137.2010.03274.x

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82, 666–77. doi:10.1111/j.1574-6941.2012.01437.x

Jumpponen, A., Jones, K.L., David Mattox, J., Yaege, C., 2010. Massively parallel 454-sequencing of fungal communities in Quercus spp. ectomycorrhizas indicates seasonal dynamics in urban and rural sites. Molecular Ecology 19 Suppl 1, 41–53. doi:10.1111/j.1365-294X.2009.04483.x

Kubartová, A., Ranger, J., Berthelin, J., Beguiristain, T., 2009. Diversity and decomposing ability of saprophytic fungi from temperate forest litter. Microbial Ecology 58, 98–107. doi:10.1007/s00248-008-9458-8

Lachance, M.-A., 2011. Yeast, in: eLS. John Wiley & Sons, Ltd., Chichester. doi:10.1002/9780470015902.a0000380.pub2

Legendre, P., 2007. Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. Journal of Plant Ecology 1, 3–8. doi:10.1093/jpe/rtm001

Lilleskov, E.A., Bruns, T.D., Horton, T.R., Taylor, D.L., Grogan, P., 2004. Detection of forest

stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiology Ecology 49, 319–332. doi:10.1016/j.femsec.2004.04.004

Lindahl, B., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjoller, R., Koljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers-a user ’s guide. New Phytologist 199, 288–299.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. The New Phytologist 173, 611–20. doi:10.1111/j.1469-8137.2006.01936.x

Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. New Phytologist 205, 1443–1447. doi:10.1111/nph.13201

Liu, X.Z., Wang, Q.M., Göker, M., Groenewald, M., Kachalkin, A. V., Lumbsch, H.T., Millanes, A.M., Wedin, M., Yurkov, A.M., Boekhout, T., Bai, F.Y., 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. Studies in Mycology 81, 85–147. doi:10.1016/j.simyco.2015.12.001

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 550–558.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J. a, Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreås, L., Reysenbach, A.-L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nature Reviews. Microbiology 4, 102–112. doi:10.1038/nrmicro1341

Mašínová, T., Bahnmann, B.D., Větrovský, T., Tomšovský, M., Merunková, K., Baldrian, P., 2017. Drivers of yeast community composition in the litter and soil of a temperate forest. FEMS Microbiology Ecology 93, fiw223-fiw223.

McGuire, K.L., Fierer, N., Bateman, C., Treseder, K.K., Turner, B.L., 2012. Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. Microbial Ecology 63, 804–12. doi:10.1007/s00248-011-9973-x

McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Computational Biology 10, e1003531. doi:10.1371/journal.pcbi.1003531

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., Ferrenberg, S., 2013. Patterns and Processes of Microbial Community Assembly. Microbiology and Molecular Biology Reviews 77, 342–356. doi:10.1128/MMBR.00051-12

Nilsson, R.H., Hyde, K.D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A.B., Alias, S. a., Alves, A., Anderson, C.L., Antonelli, A., Arnold, a. E., Bahnmann, B., Bahram, M., Bengtsson-Palme, J., Berlin, A., Branco, S., Chomnunti, P., Dissanayake, A., Drenkhan, R., Friberg, H., Frøslev, T.G., Halwachs, B., Hartmann, M., Henricot, B., Jayawardena, R., Jumpponen, A., Kauserud, H., Koskela, S., Kulik, T., Liimatainen, K., Lindahl, B.D., Lindner, D., Liu, J.-K., Maharachchikumbura, S., Manamgoda, D., Martinsson, S., Neves, M.A., Niskanen, T., Nylinder, S., Pereira, O.L., Pinho, D.B., Porter, T.M., Queloz, V., Riit, T., Sánchez-García, M., de Sousa, F., Stefańczyk, E., Tadych, M., Takamatsu, S., Tian, Q., Udayanga, D., Unterseher, M., Wang, Z., Wikee, S., Yan, J., Larsson, E., Larsson, K.-H., Kõljalg, U., Abarenkov, K., 2014. Improving ITS sequence data for identification of plant pathogenic fungi. Fungal Diversity. doi:10.1007/s13225-014-0291-8

Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K., 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecology 3, 284–287. doi:10.1016/j.funeco.2010.05.002

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J., Vilgalys, R., 2005. Fungal Community Analysis by Large-Scale Sequencing of Environmental Samples. Applied and Environmental Microbiology 71, 5544–5550. doi:10.1128/AEM.71.9.5544

Økland, R.H., 2003. Partitioning the variation in a plot-by-species data matrix that is related to n sets of explanatory variables. Journal of Vegetation Science 14, 693–700. doi:10.1658/1100-9233(2003)014[0693:PTVIAP]2.0.CO;2

Økland, R.H., 1990. Vegetation ecology: theory, methods and applications with reference to Fennoscandia. Sommerfeltia Supplement 1:233.

Oksanen, A.J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., Hara, R.B.O., Simpson,

G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. vegan: Community Ecology Package.

Öpik, M., Metsis, M., Daniell, T.J., Zobel, M., Moora, M., 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytologist 184, 424–437. doi:10.1111/j.1469-8137.2009.02920.x

Peay, K.G., 2014. Back to the future: natural history and the way forward in modern fungal ecology. Fungal Ecology 1–6. doi:10.1016/j.funeco.2014.06.001

Peay, K.G., Baraloto, C., Fine, P.V. a, 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. The ISME Journal 7, 1852–61. doi:10.1038/ismej.2013.66

Peay, K.G., Bruns, T.D., 2014. Spore dispersal of basidiomycete fungi at the landscape scale is driven by stochastic and deterministic processes and generates variability in plant-fungal interactions. The New Phytologist. doi:10.1111/nph.12906

Peay, K.G., Kennedy, P.G., Talbot, J.M., 2016. Dimensions of biodiversity in the Earth mycobiome. Nature Reviews Microbiology 14, 434–447. doi:10.1038/nrmicro.2016.59

Peres-Neto, P.R., Jackson, D. a., 2001. How well do multivariate data sets match? The advantages of a procrustean superimposition approach over the Mantel test. Oecologia 129, 169–178. doi:10.1007/s004420100720

Peršoh, D., 2015. Plant-associated fungal communities in the light of meta'omics. Fungal Diversity. doi:10.1007/s13225-015-0334-9

Peršoh, D., 2013. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. Fungal Diversity 60, 55–69. doi:10.1007/s13225-013-0225-x

Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. Forest Ecology and Management 309, 19–27. doi:10.1016/j.foreco.2013.02.034

Prosser, J.I., 2012. Ecosystem processes and interactions in a morass of diversity. FEMS Microbiology Ecology 81, 507–19. doi:10.1111/j.1574-6941.2012.01435.x

R Development Core Team, R., 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, R Foundation for Statistical Computing. doi:10.1007/978-3-540-74686-7

Rämä, T., Davey, M.L., Nordén, J., Halvorsen, R., Blaalid, R., Mathiassen, G.H., Alsos, I.G., Kauserud, H., 2016. Fungi Sailing the Arctic Ocean: Speciose Communities in North Atlantic Driftwood as Revealed by High-Throughput Amplicon Sequencing. Microbial Ecology 72, 295–304. doi:10.1007/s00248-016-0778-9

Ramette, A., 2007. Multivariate analyses in microbial ecology. Fems Micriobiology Ecology 62, 142–160. doi:10.1111/j.1574-6941.2007.00375.x

Robertson, G.P., Crum, J.R., Ellis, B.G., 1993. The spatial variability of soil resources following long-term disturbance. Oecologia 96, 451–456. doi:10.1007/BF00320501

Sagova-Mareckova, M., Cermak, L., Novotna, J., Plhackova, K., Forstova, J., Kopecky, J., 2008. Innovative methods for soil DNA purification tested in soils with widely differing characteristics. Applied and Environmental Microbiology 74, 2902–2907. doi:10.1128/AEM.02161-07

Schirkonyer, U., Bauer, C., Rothe, G.M., 2013. Ectomycorrhizal diversity at five different tree species in forests of the Taunus Mountains in Central Germany. Open Journal of Ecology 3, 66–81. doi:10.4236/oje.2013.31009

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry 40, 2068–2075. doi:doi:10.1016/j.soilbio.2008.01.015

Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytologist n/a-n/a. doi:10.1111/nph.13426

Strickland, M.S., McCulley, R.L., Nelson, J.A., Bradford, M.A., 2015. Compositional differences in simulated root exudates elicit a limited functional and compositional response in soil microbial communities. Frontiers in Microbiology 6, 1–9. doi:10.3389/fmicb.2015.00817

Štursová, M., Bárta, J., Šantrůčková, H., Baldrian, P., 2016. Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. FEMS MICROBIOLOGY ECOLOGY 92. doi:10.1093/femsec/fiw185

Svatek, M., 2004. Landscape-ecological approach to the contemporary state and management evaluation of protected areas. EKOLOGIA-BRATISLAVA 23, 340–350.

Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C., Ruess, R.W., 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs 84, 3–20.

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios, a. M., Thu, P.Q., Suija, a., Smith, M.E., Sharp, C., Saluveer, E., Saitta, a., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Poldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Partel, K., Otsing, E., Nouhra, E., Njouonkou, a. L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L. -d., Greslebin, a., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, a., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346, 1256688–1256688. doi:10.1126/science.1256688

Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A.G., Henkel, T.W., Kjøller, R., Morris, M.H., Nara, K., Nouhra, E., Peay, K.G., Põlme, S., Ryberg, M., Smith, M.E., Kõljalg, U., 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21, 4160–70. doi:10.1111/j.1365-294X.2012.05602.x

Tedersoo, L., Koljalg, U., Hallenberg, N., Larsson, K.H., 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. NEW PHYTOLOGIST 159, 153–165. doi:10.1046/j.0028-646x.2003.00792.x

ter Braak, C., 1986. Canonical Correspondence Analysis : A New Eigenvector Technique for Multivariate Direct Gradient Analysis Stable URL : http://www.jstor.org/stable/1938672 REFERENCES Linked references are available on JSTOR for this article : You may need to log in to JST. Ecology 67, 1167–1179.

Urbanová, M., Šnajdr, J., Baldrian, P., 2015. Soil Biology & Biochemistry Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees 84, 53–64. doi:10.1016/j.soilbio.2015.02.011

van den Brink, P.J., den Besten, P.J., bij de Vaate, A., ter Braak, C.J.F., 2009. Principal response curves technique for the analysis of multivariate biomonitoring time series. Environmental Monitoring and Assessment 152, 271–281. doi:10.1007/s10661-008-0314-6

Van Son, T.C., Halvorsen, R., 2014. Multiple parallel ordinations: the importance of choice of

ordination method and weighting of species abundance data, Sommerfeltia. doi:10.2478/som-2014-0001

Voříšková, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME JOURNAL 7, 477–486. doi:10.1038/ismej.2012.116

Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. The New Phytologist 201, 269–78. doi:10.1111/nph.12481

Wang, Q.M., Begerow, D., Groenewald, M., Liu, X.Z., Theelen, B., Bai, F.Y., Boekhout, T., 2015a. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Studies in Mycology 81, 55–83. doi:10.1016/j.simyco.2015.10.004

Wang, Q.M., Yurkov, A.M., Göker, M., Lumbsch, H.T., Leavitt, S.D., Groenewald, M., Theelen, B., Liu, X.Z., Boekhout, T., Bai, F.Y., 2015b. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. Studies in Mycology 81, 149–189. doi:10.1016/j.simyco.2015.12.002

Westhoff, V., Van Der Maarel, E., 1978. The Braun-Blanquet Approach, in: Whittaker, R.H. (Ed.), Classification of Plant Communities. Springer Netherlands, Dordrecht, pp. 287–399. doi:10.1007/978-94-009-9183-5_9

Yurkov, A., Wehde, T., Kahl, T., Begerow, D., 2012. Aboveground deadwood deposition supports development of soil yeasts. Diversity 4, 453–474. doi:10.3390/d4040453

Zifčákova, L., Vetrovsky, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environmental Microbiology 18, 288–301. doi:10.1111/1462-2920.13026

Table 1. Characteristics of litter soil and vegetation across study sites in a temperate forest. The values are means and standard deviations. Different superscript letters indicate significant differences among stand types (ANOVA followed by Tukey HSD *post-hoc* test).

	Environmental variable name	Beech	Spruce	Oak
litter	pH	5.6 (0.4)^{a}	5.1 (0.5)^{b}	5.3 (0.4)^{a,b}
	C/N (% dry mass)	17.8 (3.4)	15.6 (3.5)	20.2 (5.7)
	Moisture content (%)	53.3 (11.3)	49.5 (12.2)	48.2 (11.7)
	Lignin	63.6 (10.0)	64.0 (8.5)	63.5 (7.1)
	Ergosterol (μg g^{-1})	1118 (371)^{a}	808 (255)^{b}	1074 (283)^{a,b}
soil	pH	5.1 (0.6)^{a}	4.6 (0.5)^{b}	5.1 (0.5)^{a}
	Organic matter content (%)	13.0 (9.0)	9.4 (1.6)	10.3 (2.5)
	C/N (% dry mass)	13.2 (1.6)	14.0 (1.8)	13.0 (0.6)
	Moisture content (%)	26.2 (8.3)	22.2 (5.3)	22.2 (6.3)
	Ergosterol (μg g^{-1})	62.5 (66.6)^{a}	20.1 (13.3)^{b}	47.8 (24.8)^{a,b}
vegetation	Total shrub cover (%)	9 (7)	6(2)	8 (5)
	Total vegetation cover(%)	31 (25)	25 (14)	62 (35)
	Total graminoid cover(%)	5 (4)	4 (5)	25 (24)

Table 2. Richness and evenness of understory vegetation and fungi in litter and soil across study sites in a temperate forest.

		Understory vegetation		Litter fungi		Soil fungi	
Block	Stand	No. of spp.	Pielous even	No. of OTUs	Pielous even	No. of OTUs	Pielous even
1	Spruce	4	0.9591	757	0.9930	716	0.6252
1	Beech	17	0.8263	1316	0.9908	819	0.7857
1	Oak	18	0.9804	1122	0.9906	852	0.6625
2	Spruce	28	0.6670	1068	0.9909	1112	0.7635
2	Beech	25	0.9841	1174	0.9908	947	0.7805
2	Oak	22	0.9489	826	0.9922	661	0.7034
3	Spruce	15	0.9852	1001	0.9901	1363	0.7290
3	Beech	10	0.9788	993	0.9913	1262	0.7345
3	Oak	12	0.9683	910	0.9921	927	0.7259
4	Spruce	13	0.9837	1299	0.9914	1285	0.7691
4	Beech	11	0.9056	947	0.9927	1008	0.6699
4	Oak	21	0.8722	922	0.9908	694	0.6224
5	Spruce	11	0.9767	786	0.9924	1051	0.7166
5	Beech	9	0.9709	782	0.9926	704	0.6834
5	Oak	12	0.9616	930	0.9919	559	0.6940
6	Spruce	26	0.8741	881	0.9915	1031	0.7523
6	Beech	11	0.7304	1130	0.9905	847	0.6467
6	Oak	28	0.9337	921	0.9921	1016	0.7014

Table 3. Comparison of the most abundant genera (≥ 1% proportion of community) in litter and soil across the study area. Numbers represent proportions. Shaded cells indicate genera with ≥ 1% proportion in either litter or soil based on variance-stabilized transformed data (VST) of original sequence count data (McMurdie and Holmes, 2014). Unclassified OTUs accounted for a 0.002 proportion of the soil community and a 0.003 proportion of the litter community.

Best genus hit	soil	litter	Ecology	No. of OTUs with best hit
Penicillium	5.1	3.4	Saprotroph	111
Oidiodendron	5.1	2.0	Saprotroph	98
Mortierella	4.9	2.5	Saprotroph	124
Cladophialophora	2.9	3.0	Saprotroph	131
Umbelopsis	2.6	1.5	Saprotroph	38
Russula	2.3	0.6	EcM	102
Mycena	2.2	3.6	Saprotroph	120
Phialophora	1.8	1.8	Saprotroph	94
Cenococcum	1.6	0.6	EcM	22
Trichoderma	1.5	1.1	Saprotroph/Mycoparasite	55
Pseudogymnoascus	1.5	1.0	Saprotroph	19
Cadophora	1.4	1.0	Saprotroph	44
Tomentella	1.4	0.9	EcM	140
Cortinarius	1.1	0.4	EcM	117
Meliniomyces	1.1	0.9	Saprotroph	44
Trechispora	1.0	0.9	Saprotroph	53
Rhinocladiella	1.0	0.4	Saprotroph	25
Piloderma	1.0	0.4	EcM	40
Scleropezicula	0.6	1.7	Saprotroph	37
Exophiala	0.8	1.4	Saprotroph	77

Naevala	0.4	1.4	Saprotroph	16
Gorgomyces	0.5	1.3	Uncertain	23
Herpotrichia	0.5	1.3	Plant pathogen	29
Chalara	0.5	1.1	Saprotroph	53
Mycoarthris	0.4	1.0	Aquatic	23
Total	44.4	37.7		

Figure 1: Location of sampling blocks in Masaryk Forest, CR. Black ovals indicate replicate sampling blocks (n=6) comprising each of three stand types (total sites = 18)

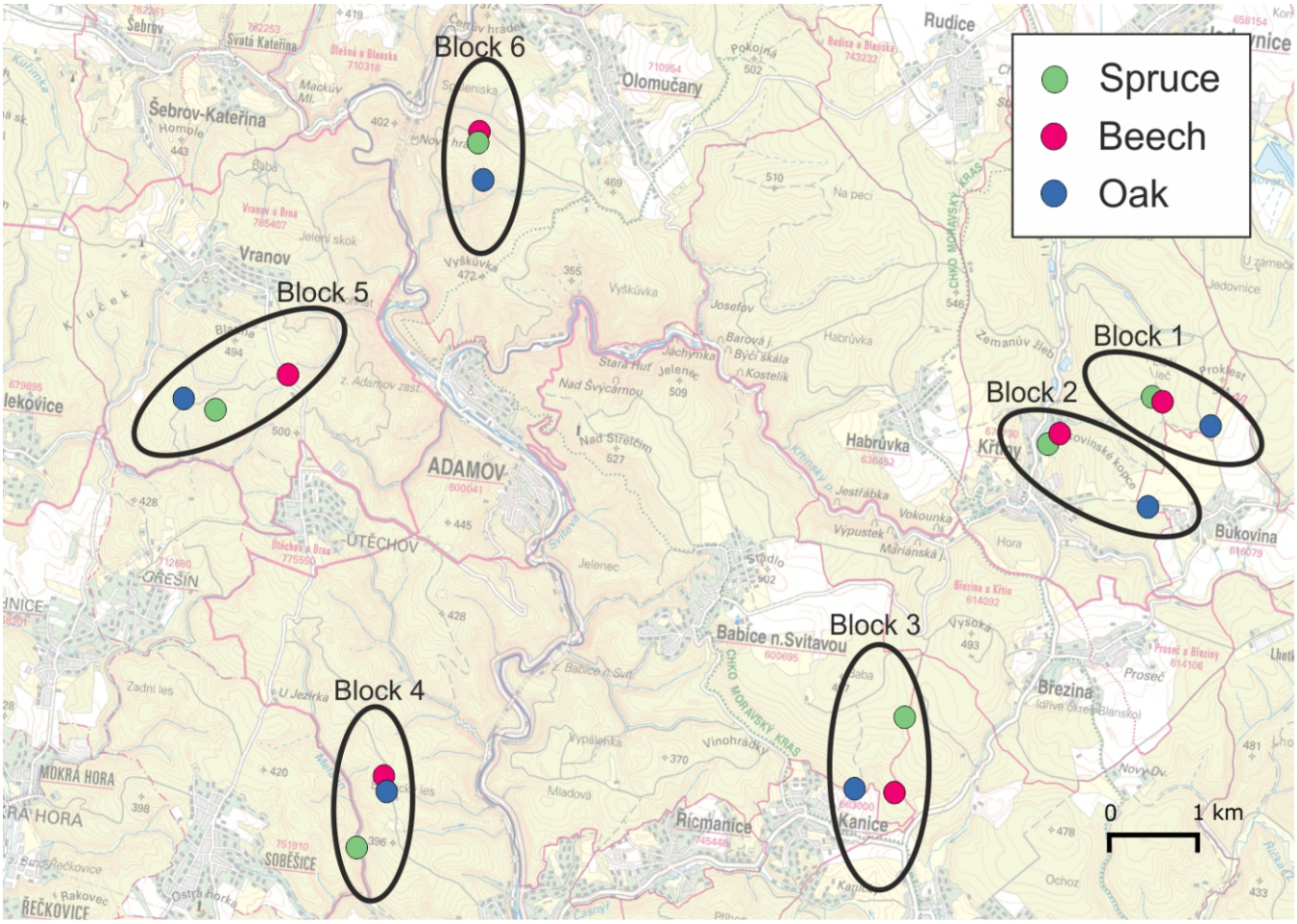


Figure 2: gNMDS ordination of understory vegetation composition (n=18, stress= 0.1800, k=2). All sites of a given dominant tree are connected by dotted lines; centroids represent stand type averages of gNMDS scores. Environmental variables with significant correlations are shown as vectors in black. Understory vegetation species with significant indicator values are shown in as vectors in grey ($p<0.01$). Axes are scaled in half-change units.

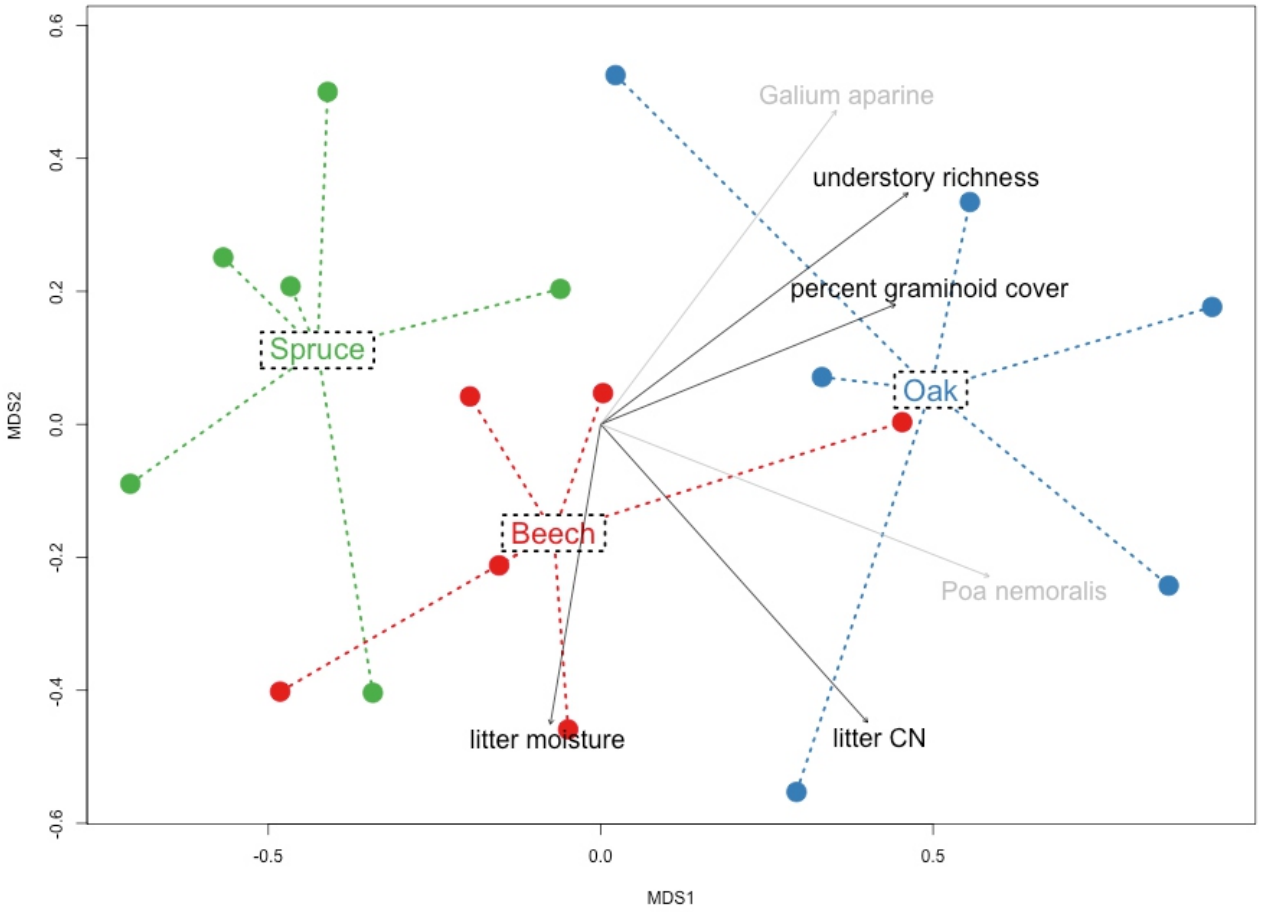


Figure 3: Non-metric multidimensional scaling ordination of fungal communities in soil under natural stands of beech, oak and spruce (stress=0.2437, k=2), n=18, sampling time=4, samples= 72, total spp.=3301). Repeated samples are connected to the site centroids by dotted lines; centroids represents the site score averages. Spruce=green, Beech=red, Oak=blue. Environmental variables with significant correlations are shown as vectors in black.

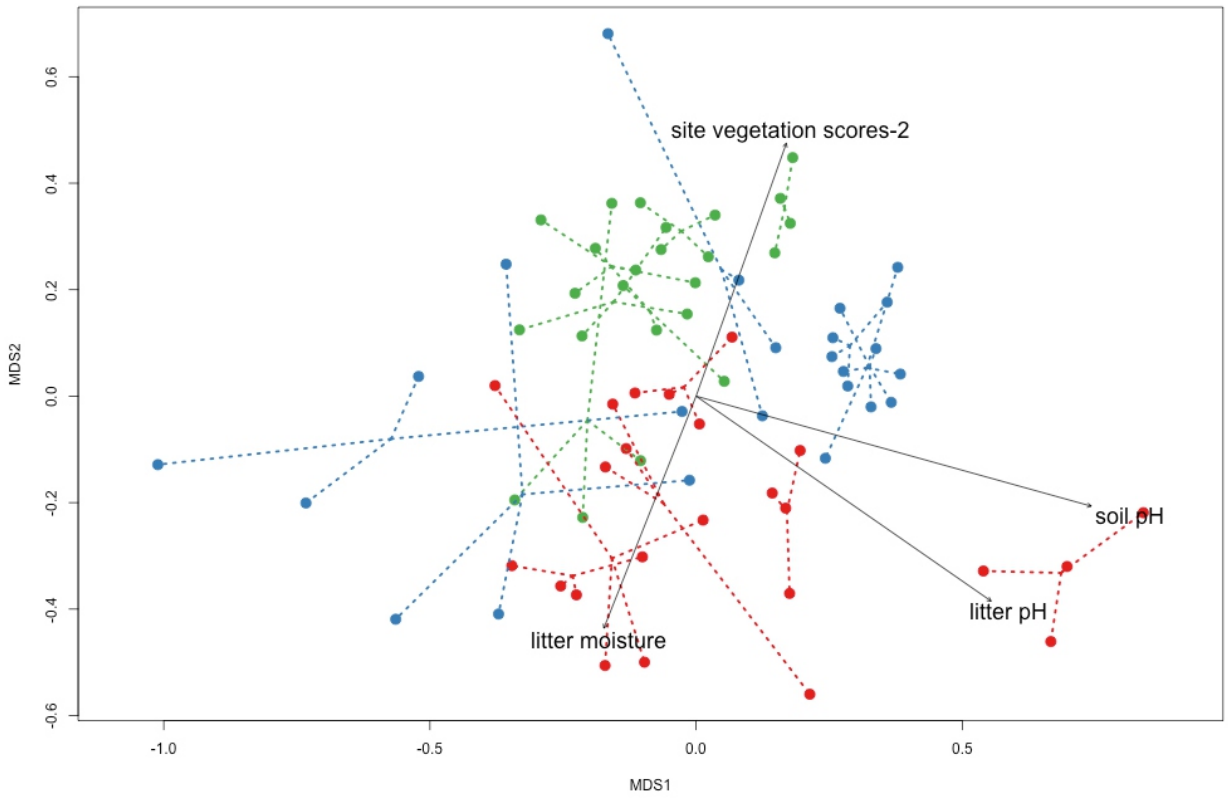


Figure 4: Non-metric multidimensional scaling ordination of fungal communities by functional group in soils under natural stands of beech, oak and spruce; n=18, sampling time=4, k=2. Spruce=green, Beech=red, Oak=blue. Repeated samples are connected to the site centroids by dotted lines; centroids represent the site score averages. Environmental variables with significant correlations are shown as vectors in black. Saprotrophs: samples=72, total spp=1917 stress= 0.2422; Yeasts: samples =72 total spp=194 stress=0.2460; EcM: samples =72, total spp =495, stress=0.2471; AMF samples =57, total spp=23, stress=0.0914.

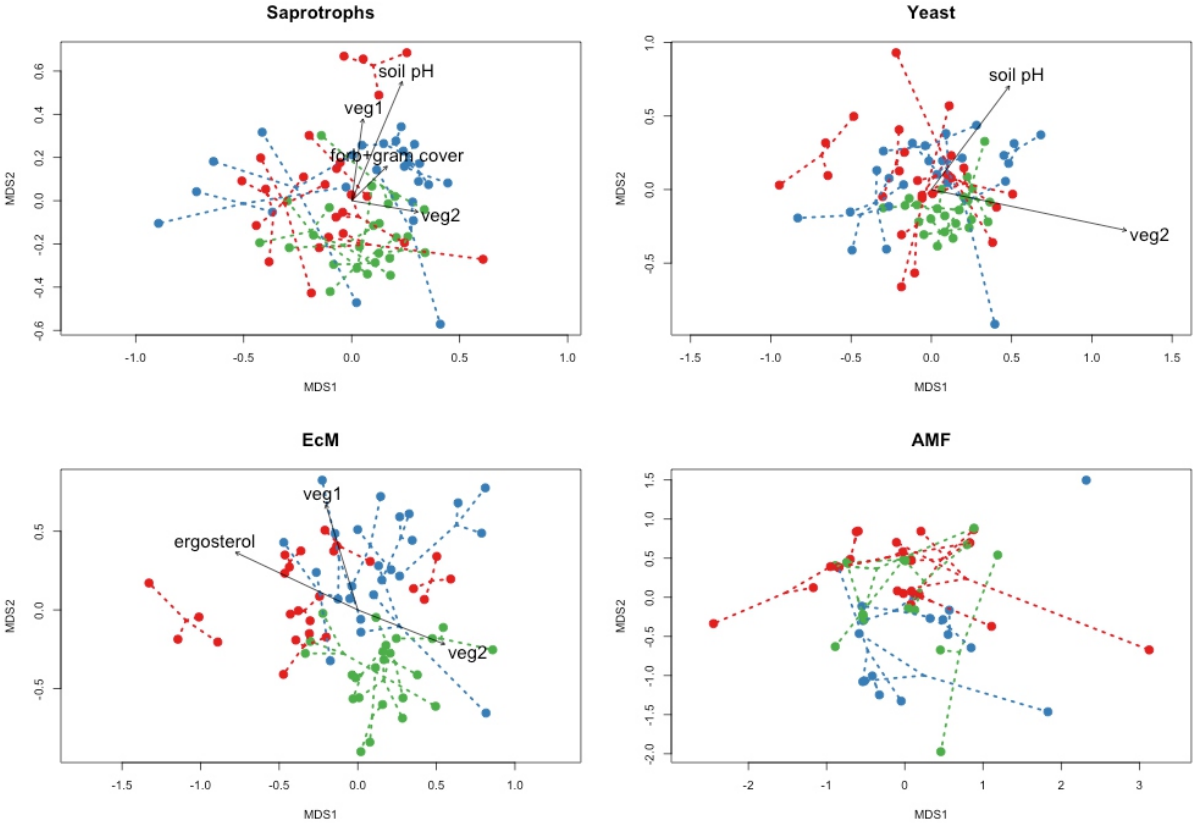


Figure 5: Non-metric multidimensional scaling ordination of fungal communities in litter under natural stands of beech, oak and spruce (stress=0.1424, k=2), n=18, sampling time=4, samples= 72, total spp.=3616). Spruce=green, Beech=red, Oak=blue. Repeated samples are connected to the site centroids by dotted lines; centroids represent the site score averages. Environmental variables with significant correlations are shown as vectors in black.

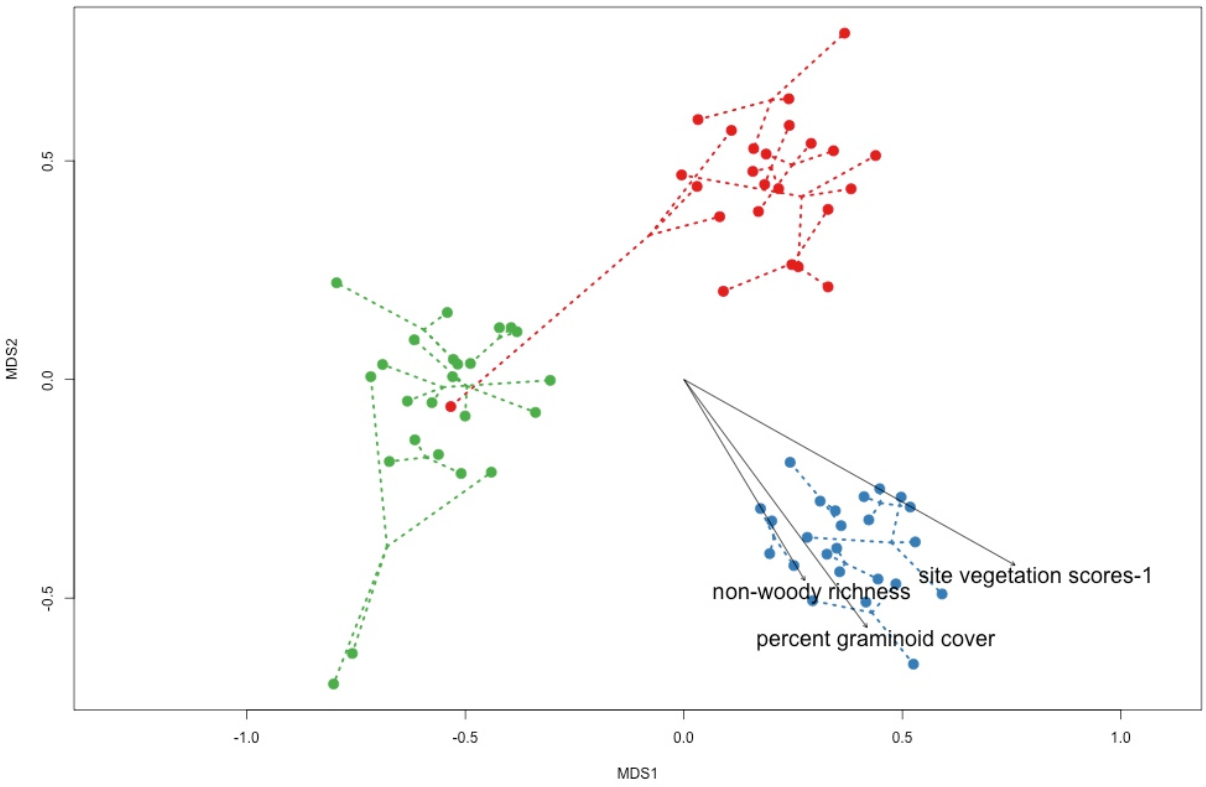


Figure 6: Non-metric multidimensional scaling ordination of fungal communities by functional group in litter under natural stands of beech, oak and spruce; n=18, sampling time=4, k=2. Spruce=green, Beech=red, Oak=blue. Repeated samples are connected to the site centroids by dotted lines; centroids represent the site score averages. Environmental variables with significant correlations are shown as vectors in black. Saprotrophs: samples=72, total spp=2079 stress= 0.1535; Yeasts: samples =72 total spp=320 stress=0.2048; EcM: samples =72, total spp =351, stress=0.2815; AMF samples =66, total spp=18, stress=0.1565.

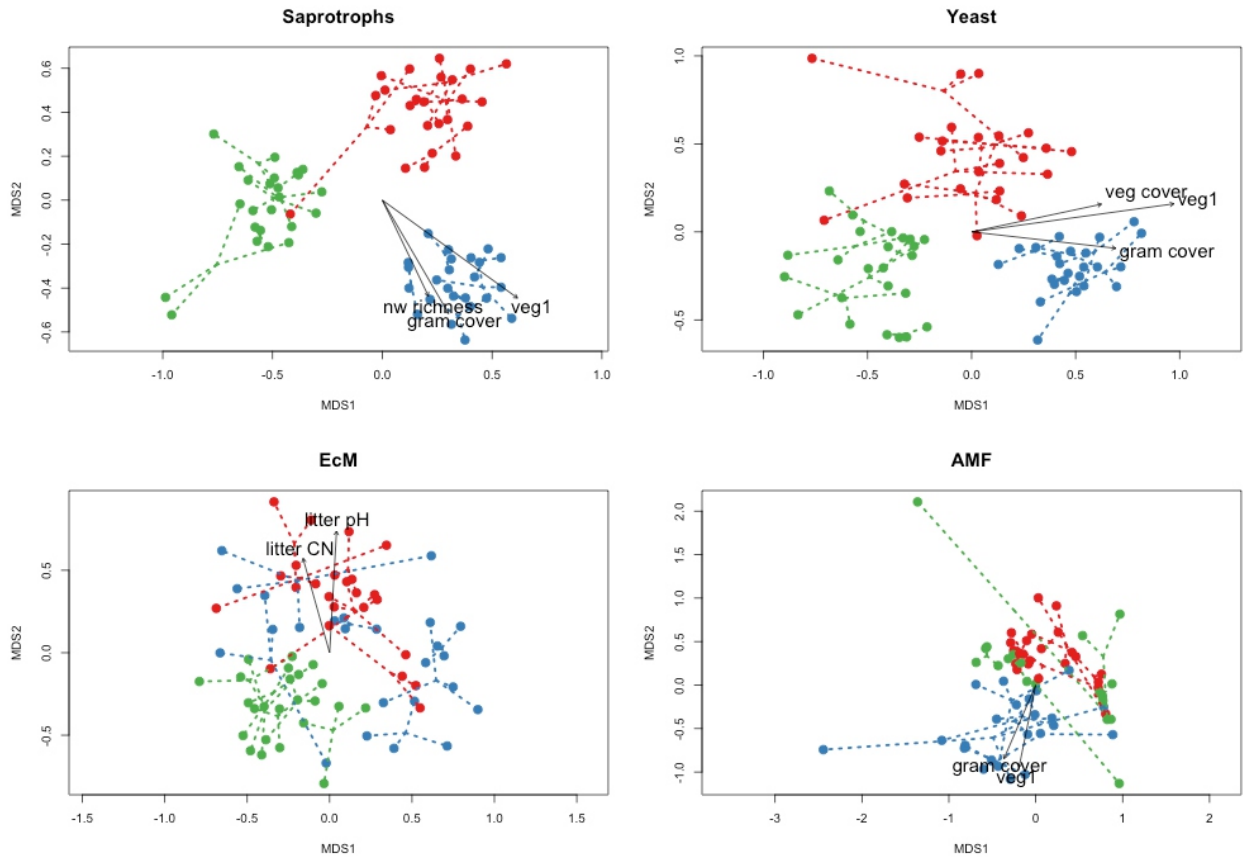
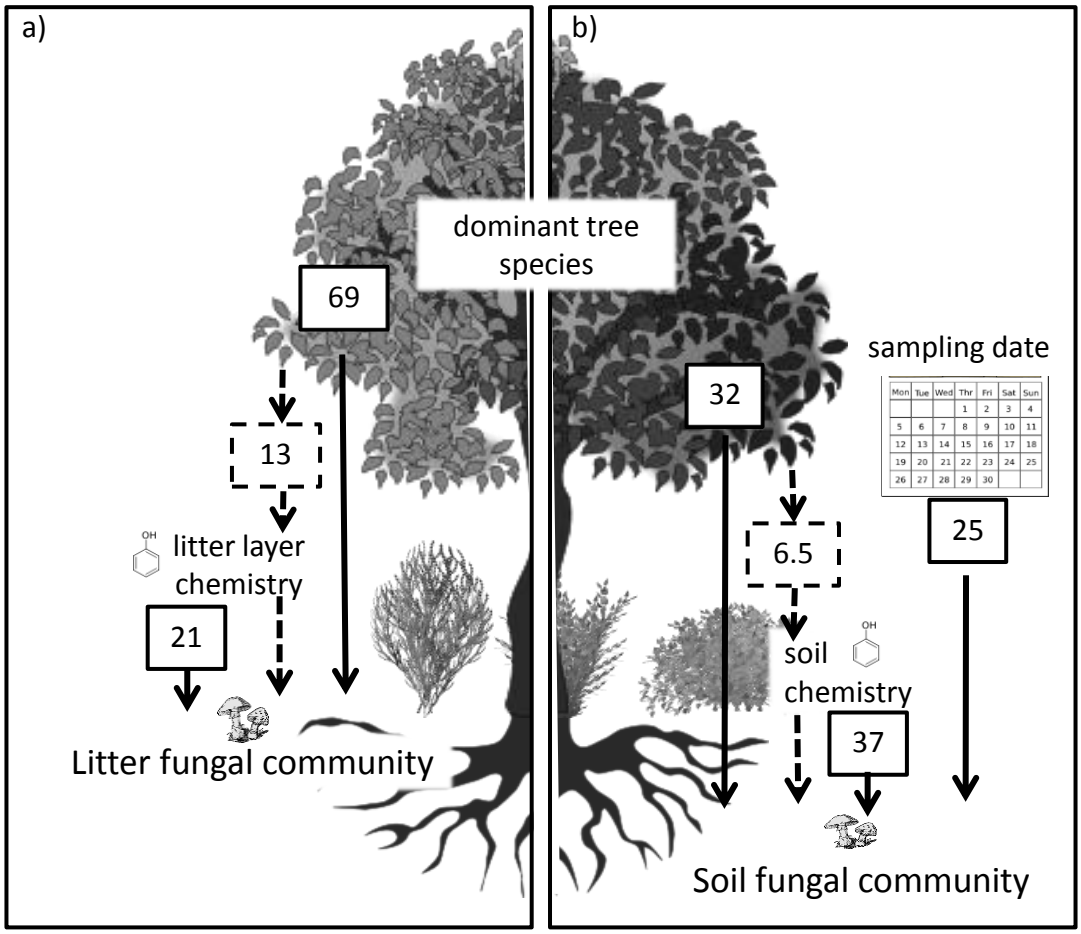


Figure 7: Relative contributions of explanatory factors associated with fungal community structure in litter (a) and soil (b). Numbers within solid-line circles represent explanatory power that is uniquely associated with a single variable. Numbers within dashed-lined boxes represent explanatory power that is shared between two variables. Numbers represent the fraction of the total variation explained (FTVE) by the variable (or combination thereof) from the total variation explained by all variables in the analysis (Table S5 and S7).



Supporting Information

Article title: Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest

Authors: Bahnmann, Barbara[1] Mašínová, Tereza[1] Halvorsen, Rune[2] Davey, Marie[3] Sedlák, Petr[4] Tomšovský, Michal[4] Baldrian, Petr[1]

The following Supporting Information is available for this article:

Fig. S1 Examples of the range of the above ground vegetation conditions within stand types where sampling occurred.

Fig. S2 Proportion of fungi in soil by functional taxonomic guild under dominant tree types across sampling periods.

Fig. S3 Trend of significant time interaction on turnover patterns of OTUs in soils.

Fig. S4 Variation by stand type and sampling time of the six most abundant taxa in soil and litter**.**

Fig. S5 Turnover rates for functional guild subsets with significant differences among dominant tree species.

Table S1 Sampling locations in Masaryk forest, Czech Republic

Table S2 Understory vegetation ordination results

Table S3 List of Indicator Species by dominant tree type

Table S4 Soil community ordination results

Table S5 Results of Variation partitioning analysis of the Soil community

Table S6 Litter community ordination results

Table S7 Results of Variation partitioning analysis of the Litter community

Table S8 ANOVA results of the interaction time and stand on turnover patterns of OTUs in litter and soil communities

Table S9 Kendall's Tau rank correlation and ANOVA results of measured variables to axes 1 and 2 of ordination of samples by functional guild subsets in Litter and Soil communities

Fig. S1 Examples of the range of the above ground vegetation conditions within stand types where sampling occurred. Beech stands ranged from low to high understory cover (B1, B2, B3); oak stands ranged from moderate to high understory vegetation cover (O1, O2, O3); spruce stands ranged from low to high understory vegetation cover (S1, S2, S3).

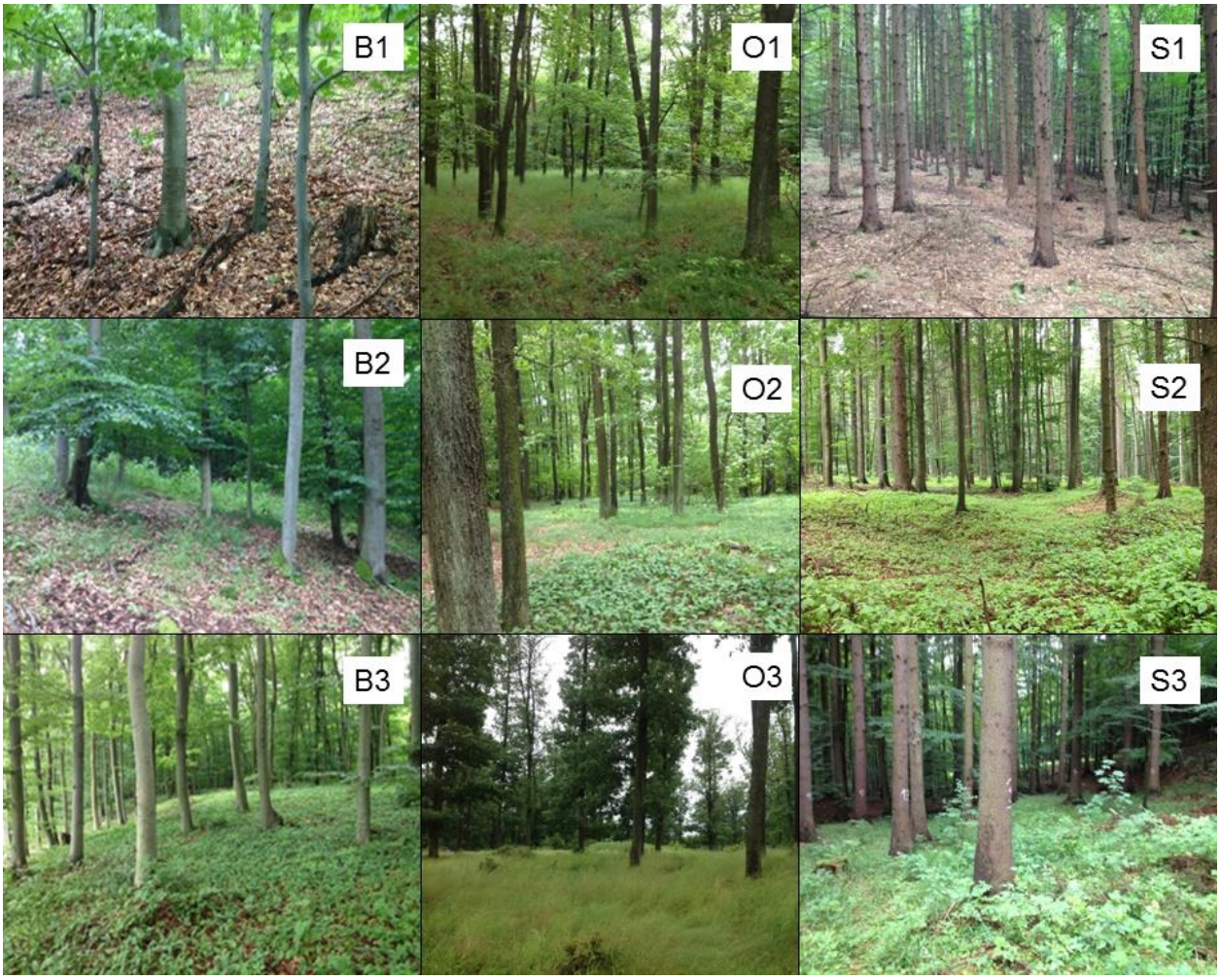
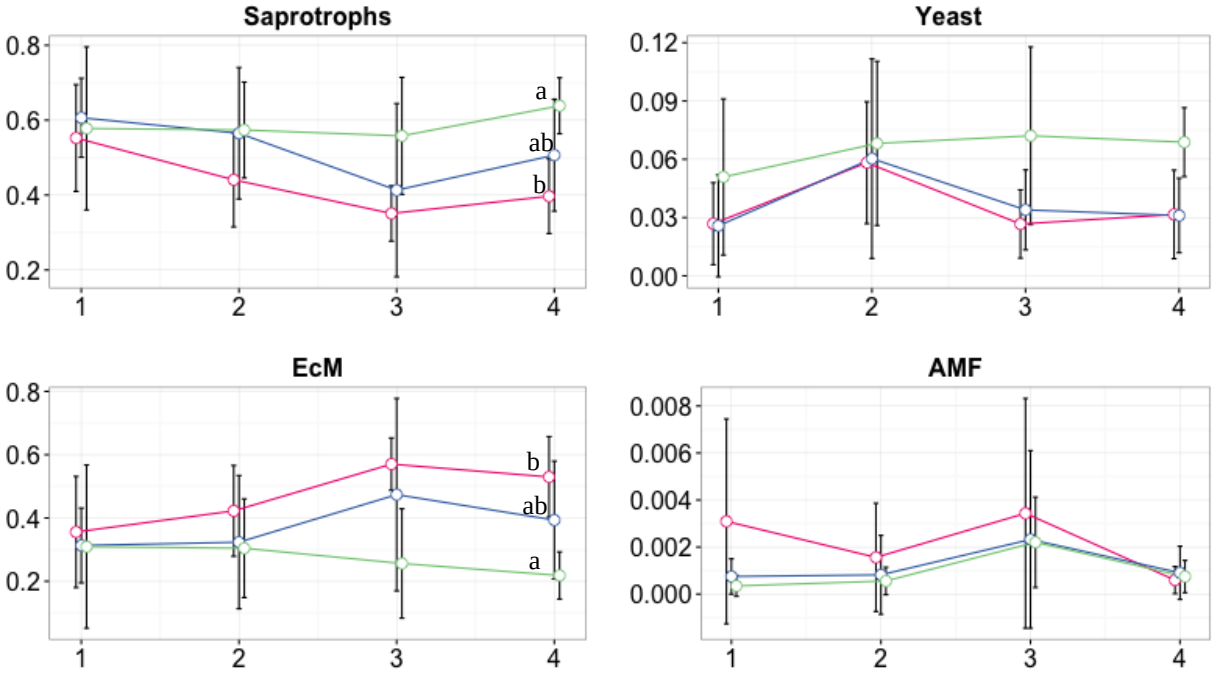


Fig. S2 Proportion of fungi in soil by functional taxonomic guild under dominant tree types across sampling periods; a) soils and b) litter. Error bars represent 95% confidence intervals of biological stand replicates (n=6). Overall significant differences in proportions under a given dominant tree types are noted with superscripts letters on graph. No significant differences were found between consecutive sampling times. spruce= green, beech = pink, oak=blue.

a)



b)

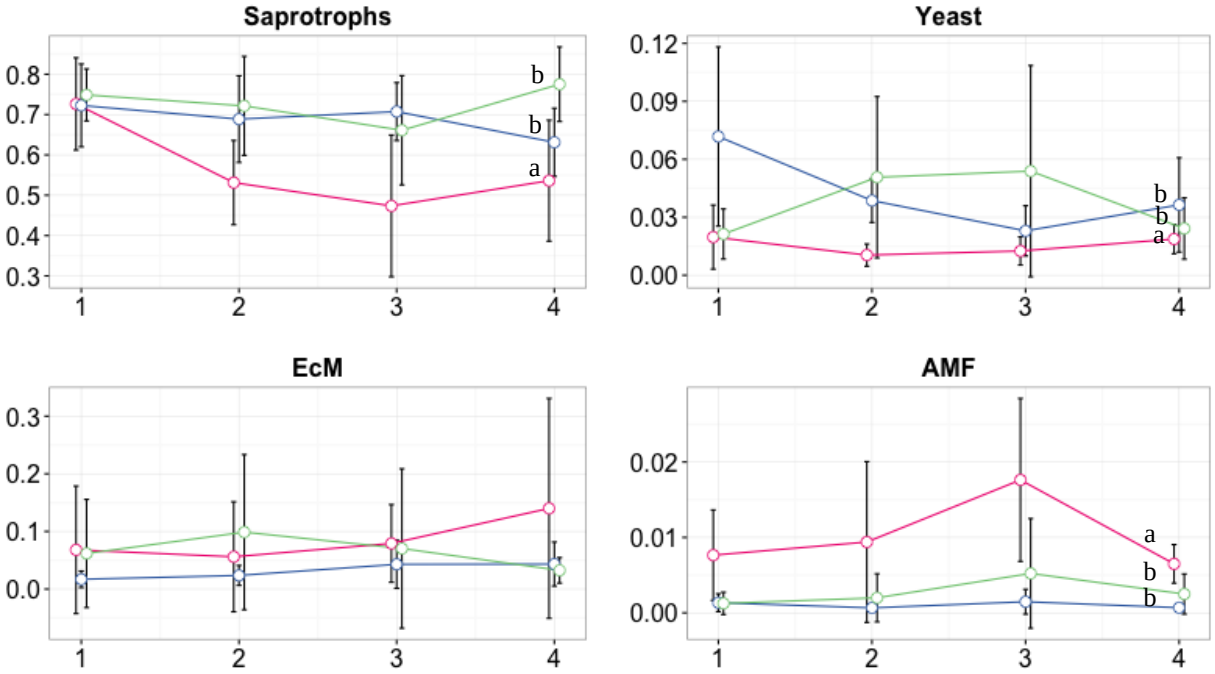


Fig. S3 Interaction of time on turnover rates of OTUs in soils. Where 'turnover +' = mean proportion of new OTUs and 'turnover - '= mean proportion of lost OTUs between consecutive sampling times. Analyses was carried out in the R package 'codyn' with the command turnover(). Error bars represent 95% confidence intervals. Functional guild subsets in the soil community showed similar pattern. Repeated measures ANOVA results are reported in Table S8.

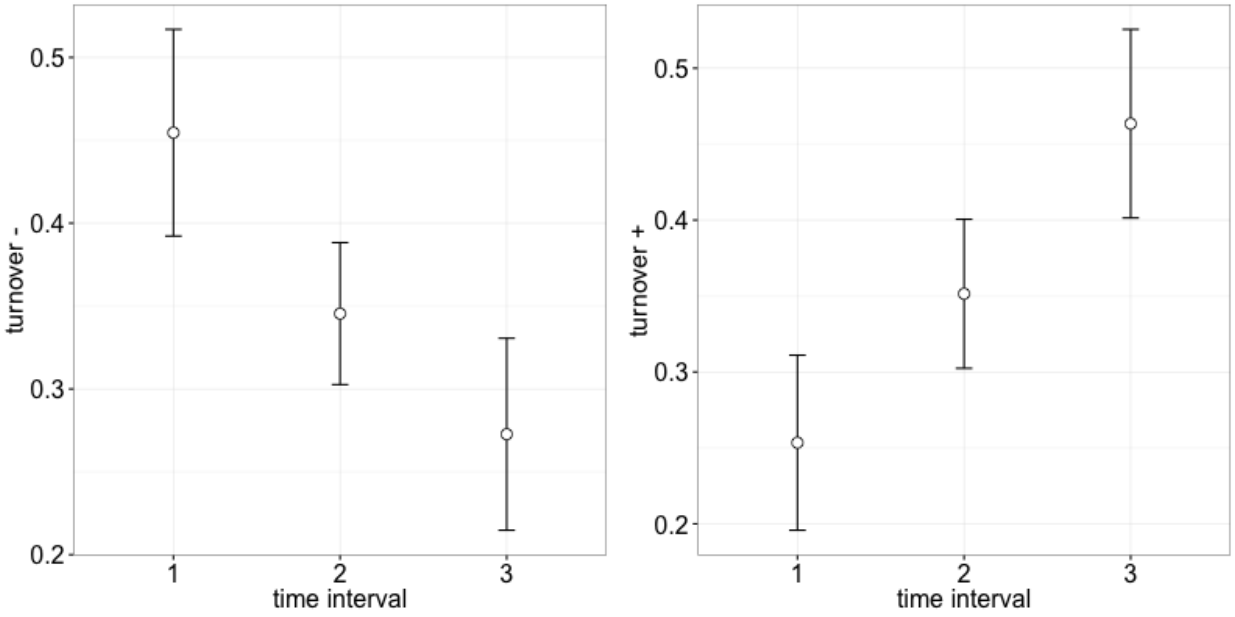
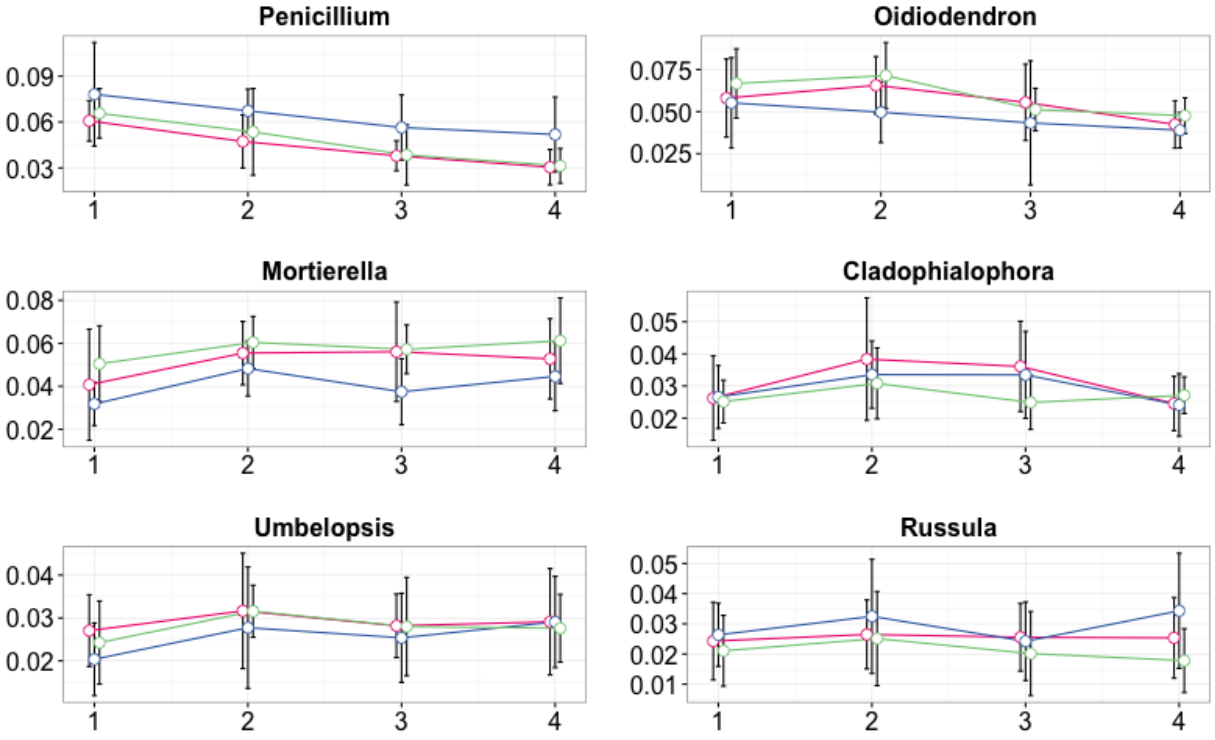
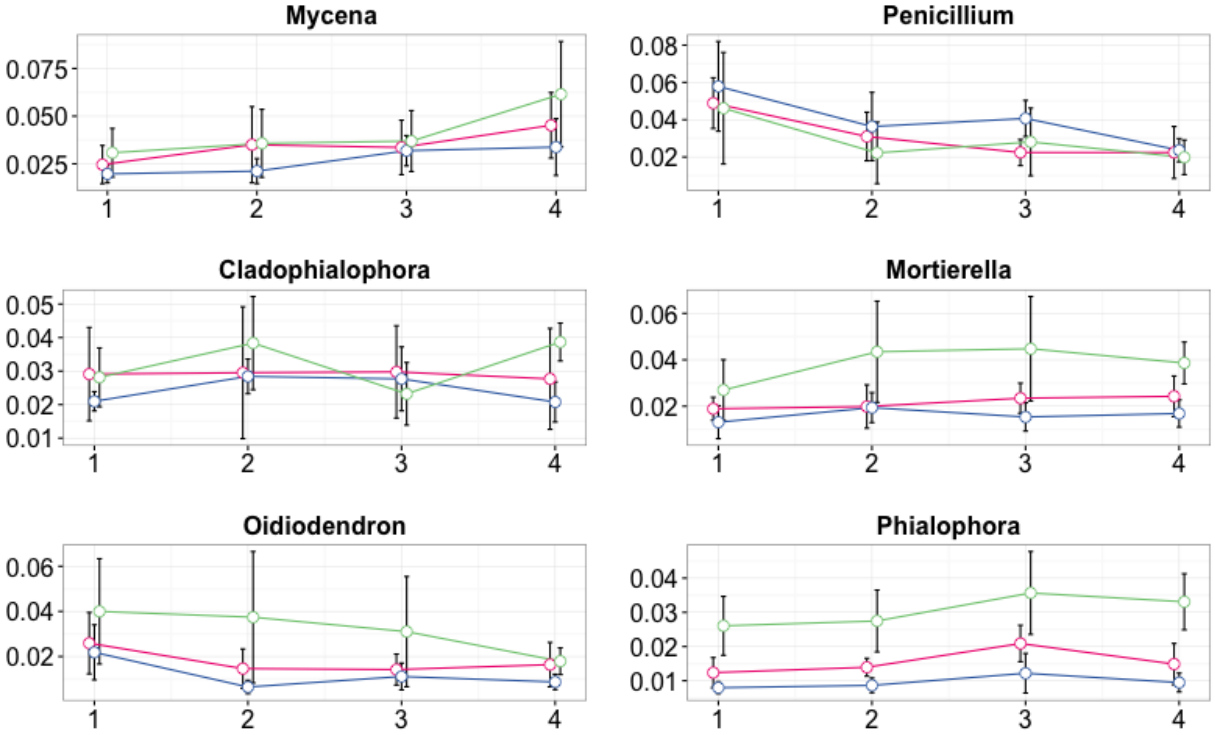


Fig. S4. Variation by stand type and sampling time of the six most abundant taxa in a) Soils and b) Litter**.** Graphs ordered according to relative abundance in community. Error bars are standard error of 6 replicate sites.

a)



b)



S5. Turnover among functional guilds with significant differences among dominant tree species. Bar represent 95% confidence intervals; letters represent significant differences.

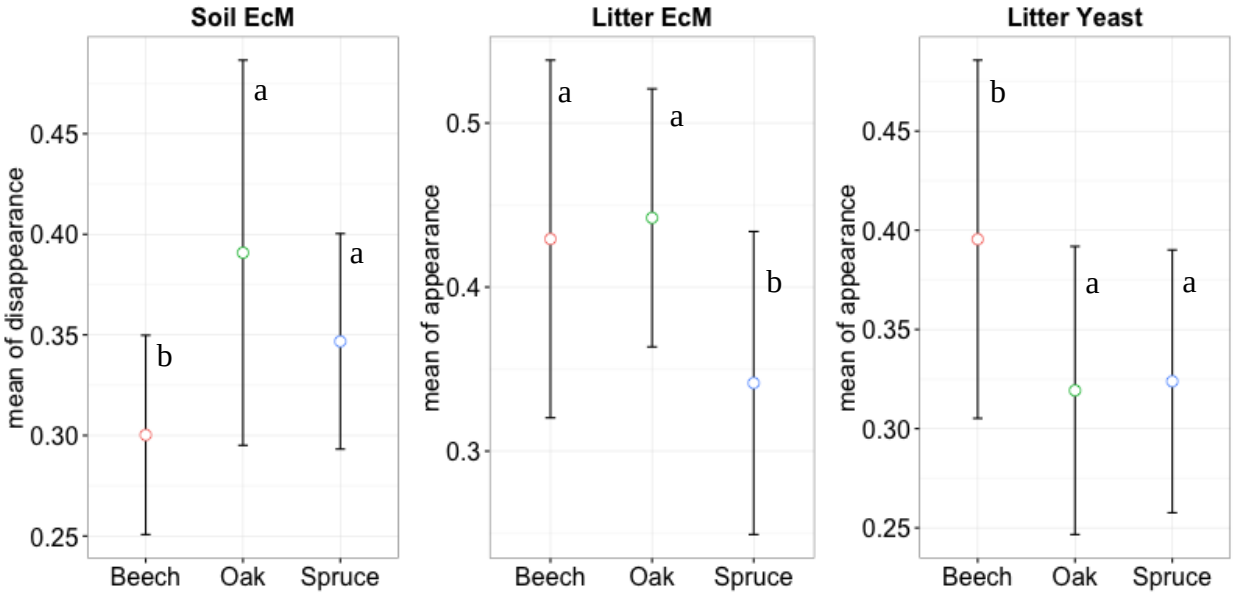


Table S1 Sampling locations in Masaryk forest, Czech Republic

			UTM coordinates		
Site	**Block**	**Stand type**	**Zone**	**X**	**Y**
1		Spruce	33N	628014	5463165
2	1	Beech	33N	628138	5463129
3		Oak	33N	628712	5462941
4		Spruce	33N	626909	5462511
5	2	Beech	33N	627027	5462640
6		Oak	33N	628119	5461987
7		Spruce	33N	625674	5459465
8	3	Beech	33N	625667	5458575
9		Oak	33N	625214	5458557
10		Spruce	33N	619704	5457206
11	4	Beech	33N	619910	5458007
12		Oak	33N	619963	5457847
13		Spruce	33N	617512	5461665
14	5	Beech	33N	618282	5462130
15		Oak	33N	617140	5461738
16		Spruce	33N	620095	5464892
17	6	Beech	33N	620109	5464902
18		Oak	33N	620202	5464503

Table S2. Understory vegetation ordination results: a)Procrustes test results for comparison of gNMDS and DCA ordinations, b) Kendall's Tau rank correlation coefficient between axes, c) Kendall's Tau rank correlation coefficient between explanatory variables and site values on gNMDS axes, d) Analysis of variance for stand type and site values on gNMDS axes, e)Tukey post hoc test for significant factors.

a)

Procrustes Sum of Squares ($m_{1,2}^2$):	0.7163
Correlation in a symmetric Procrustes rotation:	0.5326
Significance	0.003

b)

	DCA1	DCA2	DCA3	DCA4
gnmds1	0.373	-0.333	0.242	-0.033
gnmds2	0.373	-0.255	-0.124	0.124

c)

	Axis 1			Axis 2		
Variable	p.val	tau.val	p	p.val	tau.val	p
UnderstoryRichness	0.704	0.066		-0.066	5.000	
NonwoodyRichness	0.517	0.114		0.517	-0.114	
WoodyRichness	0.532	0.114		0.435	0.143	
Total_veg_cover	1.000	0.007		0.654	-0.085	
Total_woody_cover	0.493	0.120		0.939	-0.013	
Total_forbGraminoid_cover	0.820	0.040		0.649	-0.079	
Total_forb_cover	0.791	0.046		0.676	-0.072	
Total_graminoid_cover	0.970	0.007		0.909	0.020	
moistAVG	0.709	-0.072		0.014	-0.425	***
pH_AVG	0.131	0.268		0.941	0.020	
CNsoil	0.909	0.020		0.184	-0.230	
CNlitter	0.015	0.432	***	0.034	-0.377	***

d)

	Df	SumSq	MeanSq	F value	Pr(>F)	Axis
Stand type	2	2.598	1.299	14.790	**0.0003**	1[1]
Residuals	15	1.318	0.088			1

[1]Stand type is not significant for axis 2 (data not shown)

e)

	diff	lwr	upr	p adj
Oak-Beech	0.57	0.12	1.01	**0.012**
Spruce-Beech	-0.36	-0.80	0.09	0.128
Spruce-Oak	-0.92	-1.37	-0.48	**0.0002**

Table S3. List of Indicator Species by dominant tree type where part A indicates group fidelity and part B indicates coverage of sites within specified group. A=1 indicates the otu occurs only in sites belonging to specified group. B=1 indicates the otu occurs in all sites of specified group.

OTU	A	B	P	Stand	Hor	Taxonomy	Best hit	Sim	Cov	Division	Eco	Prop
otu_6430	0.75	1.00	0.001	Beech	S	Rhizoctonia butinii	KF386034	95.2	100	Basidiomycota	PP	0.0005
otu_1674	0.75	0.83	0.01	Beech	S	Umbelopsis vinacea	JN206376	98.2	100	Mucoromycotina	SAP	0.0004
otu_971	0.73	0.83	0.009	Beech	S	Varicosporium delicatum	DQ202516	91	87.1	Ascomycota	SAP	0.0003
otu_2149	1.00	0.83	0.003	Oak	S	Dactylella rhopalota	DQ494370	95.4	100	Ascomycota	SAP	0.0003
otu_667	0.77	1.00	0.001	Oak	S	Trichoderma rossicum	HQ342416	98.8	100	Ascomycota	SAP	0.0010
otu_496	0.76	1.00	0.004	Oak	S	Hymenoscyphus epiphyllus	DQ431180	99.3	100	Ascomycota	SAP	0.0005
otu_181	0.76	1.00	0.001	Oak	S	Talaromyces purpurogenus	AB872822	99.3	96.2	Ascomycota	SAP	0.0008
otu_3090	0.88	0.83	0.005	Oak	S	Talaromyces purpurogenus	AB872822	98.7	100	Ascomycota	SAP	0.0004
otu_791	0.87	0.83	0.009	Oak	S	Chaunopycnis alba	JN628073	100	100	Ascomycota	MycoP	0.0003
otu_562	0.81	0.83	0.004	Oak	S	Russula sp. ue53	AF418629	99.6	100	Basidiomycota	EcM	0.0005
otu_452	0.76	0.83	0.009	Oak	S	Coleophoma empetri	FJ480121	86.9	100	Ascomycota	SAP	0.0004
otu_1851	0.75	0.83	0.008	Oak	S	Gorgomyces honrubiae	KC834057	89.4	95.9	Ascomycota		0.0005
otu_3922	0.74	0.83	0.009	Oak	S	Rhinocladiella sp. YH-2009a	FJ948175	82.2	86.9	Ascomycota	SAP	0.0003
otu_6707	1.00	1.00	0.001	Spruce	S	Phialocephala virens	NR_103564	92.4	100	Ascomycota	SAP	0.0005
otu_2062	0.88	0.83	0.006	Spruce	S	Hirsutella sp. ICMP14250	EF029185	93.5	92.2	Ascomycota	AP	0.0003
otu_1818	0.88	0.83	0.001	Spruce	S	Lecanicillium cf. psalliotae	AB517935	100	100	Ascomycota	AP	0.0004
otu_647	0.87	0.83	0.009	Spruce	S	Mortierella parvispora	EU484279	98.3	100	Mortierellomycotina	SAP	0.0005
otu_800	0.85	0.83	0.002	Spruce	S	Phialocephala virens	NR_103564	91.7	100	Ascomycota	SAP	0.0003
otu_981	0.84	0.83	0.006	Spruce	S	Leptodontidium boreale	AY129284	93	96.6	Ascomycota	SAP	0.0005
otu_1579	0.80	0.83	0.006	Spruce	S	Phialophora sp. GS6N4b	AY465455	96.6	100	Ascomycota	SAP	0.0004
otu_4956	0.78	0.83	0.006	Spruce	S	Thelephora terrestris	AY750163	100	100	Basidiomycota	EcM	0.0003
otu_1777	0.76	0.83	0.006	Spruce	S	Oidiodendron cerealis	HQ115707	93.5	100	Ascomycota	SAP	0.0004
otu_4512	0.75	0.83	0.005	Spruce	S	Meliniomyces sp. B101	FN669230	92	98.6	Ascomycota	SAP	0.0003
otu_389	0.75	0.83	0.008	Spruce	S	Cystodendron sp. EXP0561F	DQ914672	94	100	Ascomycota	SAP	0.0002
otu_352	0.91	1.00	0.001	Be + Ok	S	Meliniomyces sp. 2 RT-2012	JQ711936	95	97.2	Ascomycota	SAP	0.0010
otu_3206	0.88	1.00	0.003	Be + Ok	S	Apodus deciduus	AY681199	100	100	Ascomycota	SAP	0.0009
otu_615	0.88	0.92	0.003	Be + Ok	S	Mortierella echinula	JX975948	95	87.6	Mortierellomycotina	SAP	0.0010
otu_1481	0.87	0.92	0.004	Be + Ok	S	Leptodontidium orchidicola	AY606312	100	100	Ascomycota	SAP	0.0008
otu_1915	0.87	1.00	0.006	Be + Sp	S	Polyporus brumalis	EU162059	99.5	100	Basidiomycota	SAP	0.0005
otu_33	0.85	1.00	0.006	Be + Sp	S	Naevala minutissima	AY853228	97.2	97.3	Ascomycota	SAP	0.0012
otu_304	0.93	1.00	0.002	Ok + Sp	S	Gorgomyces honrubiae	KC834057	89.4	95.9	Ascomycota		0.0006
otu_2480	0.89	1.00	0.002	Ok + Sp	S	Penicillium verrucosum	AB479317	100	100	Ascomycota	SAP	0.0009
otu_324	0.88	1.00	0.004	Ok + Sp	S	Phialophora sp. GS6N4b	AY465455	94.4	100	Ascomycota	SAP	0.0012
otu_7994	0.96	0.92	0.002	Ok + Sp	S	Penicillium quercetorum	KC009095	100	100	Ascomycota	SAP	0.0013
otu_468	0.91	0.92	0.007	Ok + Sp	S	Exophiala sp. EXP0542F	DQ914677	99.4	100	Ascomycota	SAP	0.0006
otu_130	1.00	0.83	0.007	Ok + Sp	S	Wilcoxina sp. aurim735	DQ069051	96.9	98.1	Ascomycota	EcM	0.0012
otu_1338	1.00	0.83	0.005	Ok + Sp	S	Phialophora sp. p3901	AF083199	92.7	95.1	Ascomycota	SAP	0.0005
otu_2223	0.93	0.83	0.008	Ok + Sp	S	Chalara vaccinii	KC881073	89.6	68.9	Ascomycota	SAP	0.0004
otu_641	1.00	1.00	0.001	Beech	L	Herpotrichia parasitica	AF525676	87	64.5	Ascomycota	SAP	0.0003
otu_1326	1.00	1.00	0.001	Beech	L	Tubaria hiemalis var. major	DQ987910	100	100	Basidiomycota	SAP	0.0003
otu_7921	1.00	1.00	0.001	Beech	L	Coccomyces sp. V12W11	AB366644	99.3	100	Ascomycota	PP	0.0004
otu_8582	1.00	1.00	0.001	Beech	L	Calycellina punctata	U57494	93.9	100	Ascomycota		0.0003

OTU	A	B	p	Stand	Hor	Taxonomy	Best hit	Sim	Cov	Division	Eco	Prop
otu_9003	1.00	1.00	0.001	Beech	L	Syzygospora effibulata	JN053499	97.4	100	Basidiomycota	Y	0.0003
otu_6430	0.94	1.00	0.001	Beech	L	Rhizoctonia butinii	KF386034	95.2	100	Basidiomycota	PP	0.0011
otu_9340	0.89	1.00	0.001	Beech	L	Calycellina punctata	U57494	93.8	98	Ascomycota		0.0003
otu_3225	0.89	1.00	0.001	Beech	L	Rhodotorula sp. CBS 6372	AF444621	91.7	88.5	Basidiomycota	Y	0.0003
otu_971	0.89	1.00	0.001	Beech	L	Varicosporium delicatum	DQ202516	91	87.1	Ascomycota	SAP	0.0007
otu_662	0.89	1.00	0.001	Beech	L	Neonectria sp. E9615A	JN564481	86.9	96.4	Ascomycota	PP	0.0006
otu_172	0.86	1.00	0.001	Beech	L	Hymenoscyphus immutabilis	AY348584	100	100	Ascomycota	SAP	0.0008
otu_197	0.85	1.00	0.001	Beech	L	Lactarius blennius	EF493301	100	100	Basidiomycota	EcM	0.0008
otu_921	0.85	1.00	0.001	Beech	L	Veronaea compacta	EU041819	92	100	Ascomycota	SAP	0.0006
otu_7400	0.84	1.00	0.001	Beech	L	Alatospora acuminata	AY204588	91.8	100	Ascomycota	SAP	0.0006
otu_9906	0.83	1.00	0.001	Beech	L	Mycoarthris corallina	AF128440	99.3	93.1	Ascomycota		0.0008
otu_627	0.83	1.00	0.001	Beech	L	Stomiopeltis betulae	GU214701	84.3	92.4	Ascomycota	SAP	0.0006
otu_291	0.82	1.00	0.001	Beech	L	Rhizoctonia butinii	KF386034	94.7	100	Basidiomycota	PP	0.0010
otu_1850	0.81	1.00	0.001	Beech	L	Tubaria sp. PBM3355	HQ839739	92.5	75	Basidiomycota	SAP	0.0004
otu_847	0.80	1.00	0.001	Beech	L	Dactylaria appendiculata	AY265339	91.5	100	Ascomycota		0.0007
otu_1378	0.80	1.00	0.001	Beech	L	Phialophora sp. olrim289	AY781234	100	100	Ascomycota	SAP	0.0005
otu_1184	0.80	1.00	0.001	Beech	L	Myrothecium verrucaria	AB693919	93.1	92.3	Ascomycota	SAP	0.0003
otu_1241	0.78	1.00	0.001	Beech	L	Sarea resinae	AY781237	100	20	Ascomycota	Lich	0.0008
otu_630	0.78	1.00	0.001	Beech	L	Lachnum asiaticum	AB481251	94.3	97.9	Ascomycota	SAP	0.0006
otu_909	0.91	0.83	0.001	Beech	L	Rhodotorula sp. SJ13L05	FJ153202	93.8	36.1	Basidiomycota	Y	0.0005
otu_8349	0.76	1.00	0.001	Beech	L	Helicodendron luteoalbum	EF029237	97.2	100	Ascomycota	SAP	0.0005
otu_922	1.00	1.00	0.001	Oak	L	Arachnopeziza variepilosa	EU940163	89	57.7	Ascomycota	SAP	0.0008
otu_8963	1.00	1.00	0.001	Oak	L	Cryptococcus sp. TSN-649	HG324303	97.3	100	Basidiomycota	Y	0.0003
otu_639	0.93	1.00	0.001	Oak	L	Athelia arachnoidea	U85791	98.5	100	Basidiomycota	SAP	0.0006
otu_844	0.93	1.00	0.001	Oak	L	Cryptococcus sp. CBS 9089	AF444487	92	84.5	Basidiomycota	Y	0.0009
otu_3090	0.90	1.00	0.001	Oak	L	Talaromyces purpurogenus	AB872822	98.7	100	Ascomycota	SAP	0.0005
otu_3007	0.90	1.00	0.001	Oak	L	Rhodotorula sp. TP-Snow-Y129	JQ768940	95.1	36	Basidiomycota	Y	0.0004
otu_2739	0.89	1.00	0.001	Oak	L	Cryptococcus sp. TSN-649	HG324303	96	97.8	Basidiomycota	Y	0.0004
otu_326	0.89	1.00	0.001	Oak	L	Fibulorhizoctonia sp. TMB	DQ493753	96.5	100	Basidiomycota	SAP	0.0009
otu_410	0.86	1.00	0.001	Oak	L	Gorgomyces honrubiae	KC834057	93.9	100	Ascomycota		0.0011
otu_258	0.86	1.00	0.001	Oak	L	Chalara microspora	FR667227	95.9	100	Ascomycota	SAP	0.0005
otu_2224	1.00	0.83	0.001	Oak	L	Pyrenochaetopsis microspora	HM751085	95.6	100	Ascomycota	SAP	0.0002
otu_2642	1.00	0.83	0.001	Oak	L	Mycena polyadelpha	JF908456	99.5	100	Basidiomycota	SAP	0.0003
otu_2885	1.00	0.83	0.001	Oak	L	Poculum sydowianum	KC533544	100	100	Ascomycota	SAP	0.0003
otu_2413	0.82	1.00	0.001	Oak	L	Sporobolomyces inositophilus	AB038107	88.5	95.9	Basidiomycota	Y	0.0006
otu_1887	0.81	1.00	0.001	Oak	L	Rhodotorula buffonii	AB038083	85.3	100	Basidiomycota	Y	0.0005
otu_264	0.81	1.00	0.001	Oak	L	Sympodiella acicola	EU449953	93.7	91.4	Ascomycota	SAP	0.0010
otu_387	0.80	1.00	0.001	Oak	L	Sympodiella acicola	EU449953	89.1	92.8	Ascomycota	SAP	0.0005
otu_452	0.79	1.00	0.001	Oak	L	Coleophoma empetri	FJ480121	86.9	100	Ascomycota	SAP	0.0009
otu_2258	0.79	1.00	0.001	Oak	L	Tremella phaeophysciae	JN053479	86	79.6	Basidiomycota	Y	0.0003
otu_484	0.79	1.00	0.001	Oak	L	Coleophoma empetri	FJ480129	95.1	97.9	Ascomycota	SAP	0.0010
otu_759	0.78	1.00	0.001	Oak	L	Alatospora acuminata	AY204588	92.5	100	Ascomycota	SAP	0.0009
otu_1851	0.78	1.00	0.001	Oak	L	Gorgomyces honrubiae	KC834057	89.4	95.9	Ascomycota		0.0010
otu_1477	0.78	1.00	0.001	Oak	L	Cryptococcus huempii	NR_073214	93.1	84.9	Basidiomycota	Y	0.0007
otu_1183	0.77	1.00	0.001	Oak	L	Cryptococcus sp. CBS 9089	AF444487	90.9	100	Basidiomycota	Y	0.0009
otu_1037	0.77	1.00	0.001	Oak	L	Flagellospora leucorhynchos	KC834049	92.3	96.6	Ascomycota		0.0010

OTU	A	B	p	Stand	Hor	Taxonomy	Best hit	Sim	Cov	Division	Eco	Prop
otu_8695	0.77	1.00	0.001	Oak	L	Cylindrium elongatum	AY853244	98.6	100	Ascomycota	PP	0.0009
otu_1579	1.00	1.00	0.001	Spruce	L	Phialophora sp. GS6N4b	AY465455	96.6	100	Ascomycota	SAP	0.0004
otu_2950	1.00	1.00	0.001	Spruce	L	Cladophialophora sp. TRN488	AY843173	86.8	100	Ascomycota	SAP	0.0002
otu_5131	1.00	1.00	0.001	Spruce	L	Phialophora sp. GS6N4b	AY465455	95.9	100	Ascomycota	SAP	0.0003
otu_8742	1.00	1.00	0.001	Spruce	L	Xenochalara juniperi	JN604462	100	100	Ascomycota	SAP	0.0005
otu_8050	0.90	1.00	0.001	Spruce	L	Slimacomyces isiola	AB597216	92.5	100	Ascomycota		0.0005
otu_912	0.82	1.00	0.001	Spruce	L	Devriesia sp. NG_p52	HQ115717	87.9	100	Ascomycota	PP	0.0006
otu_1105	0.81	1.00	0.001	Spruce	L	Phialocephala europaea	AY347402	100	100	Ascomycota	SAP	0.0005
otu_594	0.80	1.00	0.001	Spruce	L	Ochroconis humicola	AY265334	91.7	98.7	Ascomycota	SAP	0.0007
otu_820	0.80	1.00	0.001	Spruce	L	Chalara hyalocuspica	FR667220	100	100	Ascomycota	SAP	0.0008
otu_9849	0.76	1.00	0.001	Spruce	L	Helicodendron websteri	EF029229	99.3	100	Ascomycota	SAP	0.0005
otu_2809	0.94	1.00	0.001	Be + Ok	L	Aureobasidium pullulans	AB693813	100	100	Ascomycota	SAP	0.0005
otu_352	0.88	1.00	0.001	Be + Ok	L	Meliniomyces sp. 2 RT-2012	JQ711936	95	97.2	Ascomycota	SAP	0.0018
otu_2610	0.93	0.92	0.001	Be + Ok	L	Cryptococcus sp. TSN-649	HG324303	91.4	100	Basidiomycota	Y	0.0007
otu_186	1.00	1.00	0.001	Ok + Sp	L	Phialocephala dimorphospora	AY606306	90.6	97.2	Ascomycota	SAP	0.0010

Table S4. Soil community ordination results: a) Procrustes test results for comparison of gNMDS and DCA ordinations, b) Kendall's Tau rank correlation coefficient between axes, c) Kendall's Tau rank correlation coefficient and ANOVA between explanatory variables and centroid values for site (from four sampling times) on gNMDS axes.

a)

Procrustes Sum of Squares ($m_{1,2}^2$):	0.6159
Correlation in a symmetric Procrustes rotation:	0.6197
Significance:	0.001

b)

	DCA1	DCA2	DCA3	DCA4
gnmds1	0.376	0.357	-0.257	-0.036
gnmds2	-0.410	-0.119	-0.468	-0.230

c)

		Axis1		Axis 2	
Variable		tau.val	p	tau.val	p
pH		0.673	***	-0.098	
OM		-0.059		-0.124	
CN ratio		-0.033		0.086	
C		0.059		-0.072	
N		0.072		0.007	
Total understory richness		0.186		0.080	
Non-woody understory richness		0.168		0.208	
Woody understory richness		-0.186		-0.257	
Total vegetation cover		0.190		-0.007	
Total woody veg. cover		-0.013		-0.201	
Total forb + graminoid cover		0.158		0.013	
Total forb cover		0.243		0.125	
Total graminoid cover		-0.047		-0.020	
Site vegetation scores axis 1		0.163		-0.163	
Site vegetation scores axis 2		0.229		0.399	**
ANOVA	Df	Sumsq	MnSq	F val	p
Stand (ax1)	2	0.062	0.031	0.337	
Stand (ax2)	2	0.56	0.28	14.16	***
Block (ax1)	5	0.841	0.168	3.382	*
Block (ax2)	5	0.156	0.031	0.537	

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Table S5. a) Results from variation partitioning analysis on fungal community in soils. a) Forward selection of variables to be included in the environmental group variable; b) Significance testing of selected variables where 1=stand type, 2=environmental variables, 3= sampling time; c) Significance testing of all group combinations via canonical correspondence analysis; d) Unique and shared portions of variation explained (VE) by selected variables as the fraction of the total VE (FVE). All variance values are shown with multiplier of 1000 (i.e. 2.252 = 2525).

a)

Round	No. of comparisons	Variable	VE	Pseudo F	df	sig	padj
1	3	pH	130	3.6934	1.68	0.001	0.017
1	3	CN	89	2.4719	1.68	0.001	0.017
1	3	Moisture	62	1.7266	1.68	0.001	0.017
2	5	CN	83	2.4119	1.67	0.001	0.010
2	5	Moisture	58	1.6638	1.67	0.002	0.010
3	6	Moisture	57	1.68279	1.66	0.001	0.008

b)

Variable	Total Variation (Total Inertia)	VE	Pseudo F	df	sig (999 perm)
Stand	2525	241	3.5312	2.67	0.001
Env	2525	271	2.6426	3.66	0.001
Time	2525	153	1.4228	3.66	0.001
All	2525	623	2.9675	5.64	0.001

c)

Condition	Constraining	Variation (condition)	VE (constraining)	pseudo F	sig
0	1		241	3.5312	0.001
0	2		271	2.6426	0.001
0	3		153	1.4228	0.001
0	1,2,3		622	2.9675	0.001
2.3	1	424	198	3.1831	0.001
1.3	2	392	230	2.4621	0.001
1.2	3	475	147	1.5751	0.001
3	1.2	153	469	3.0089	0.001
2	1.3	271	352	2.2561	0.001
1	2.3	241	382	2.0404	0.001

d)

Section	FVE
All groups	100.0
Stand	38.7
Env	43.5
Time	24.6
Stand unique	31.9
Env unique	37.0
Time unique	23.7
Stand + Env shared	6.5
Stand + Time shared	0.0
Env + Time shared	0.0
Shared by all	0.0

Table S6. Litter community ordination results: a) Procrustes test results for comparison of gNMDS and DCA ordinations, b) Kendall's Tau rank correlation coefficient between axes, c) Kendall's Tau rank correlation coefficient and ANOVA between explanatory variables and centroid values for site (from four sampling times) on gNMDS axes.

a)

Procrustes Sum of Squares ($m_{1,2}^2$):	0.4799
Correlation in a symmetric Procrustes rotation:	0.7112
Significance:	0.001

b)

	DCA1	DCA2	DCA3	DCA4
gnmds1	-0.800	-0.028	0.114	0.054
gnmds2	0.201	0.243	-0.244	0.090

c)

	Axis1		Axis 2	
Variable	tau.val	p	tau.val	p
pH	0.229		0.163	
Moisture	-0.242		0.294	
lignin	0.19		0.046	
CN ratio	0.281		-0.103	
C	0.020		0.033	
N	0.007		-0.007	
Lignin:N	0.072		-0.046	
Total understory richness	0.239		-0.266	
Non-woody understory richness	0.168		-0.41	*
Woody understory richness	0.029		0.186	
Total vegetation cover	0.281		-0.255	
Total woody veg. cover	0.147		0.04	
Total forb + graminoid cover	0.304		-0.304	
Total forb cover	0.112		-0.204	
Total graminoid cover	0.439	*	-0.385	*
Site vegetation scores axis 1	0.569	***	-0.281	
Site vegetation scores axis 2	-0.072		-0.268	

ANOVA	Df	Sumsq	MnSq	F val	p
Stand (ax1)	2	2.891	1.446	123.9	***
Stand (ax2)	2	2.018	1.009	49.05	***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Table S7. Results from variation partitioning analysis on fungal community in litter. a) Forward selection of variables to be included in the environmental group variable; b) Significance testing of selected variables, all values shown with multiplier of 1000 (i.e. 2.252 = 2525); c) Significance testing of all group combinations via canonical correspondence analysis; d) Unique and shared portions of variation explained by selected variables as the fraction of the total VE (FVE).

a)

Round	No. of comparisons		VE	Pseudo F	df	sig	padj
1	3	pH	143	3.7276	1.69	0.001	0.017
1	3	CN	92	2.3594	1.69	0.003	0.017
1	*3*	*Moisture*	*56*	*1.4246*	*1.69*	***0.07***	*0.017*
2	5	CN	94	2.5045	1.68	0.001	0.010

b)

Variable	Total Variation (Total Inertia)	VE	Pseudo F	df	sig (999 perm)
Stand	2793	608	9.4692	2.68	0.001
Env	2793	237	3.1568	2.68	0.001
All	2793	767	2.9675	5.64	0.001

c)

Condition	Constraining	Variation (condition)	VE (constraining)	pseudo F	sig
0	1		608	9.4692	0.001
0	2		237	3.1568	0.001
0	1.2		767	6.2468	0.001
2	1	237	529	8.6286	0.001
1	2	608	158	2.5834	0.001

d)

Section	FVE
All groups	100.0
Stand	79.3
Env	30.9
Stand unique	69.1
Env unique	20.7
Stand + Env shared	12.6

Table S8. Significance of time and time + stand interaction on turnover patterns of OTUs. Where 'turnover +' = proportion of new OTUs and 'turnover - '= proportion of lost OTUs between consecutive sampling times at a given site. Analyses was carried out in the R package 'codyn' with the command turnover(): a) total soil and total litter community, b) soil community by functional guild, c) litter community by functional guild. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.'. Significant time pattern in soil community is presented in graph form in Fig S3.

a)

Turnover +		Df	Sum Sq	Mean Sq	F value	p
Soil	time	2	0.3977	0.19885	11.126	***
	stand + time	4	0.0281	0.00703	0.393	
Litter	time	2	0.1067	0.05334	2.462	
	stand + time	4	0.0347	0.00867	0.4	
Turnover -		Df	Sum Sq	Mean Sq	F value	p
Soil	time	2	0.3017	0.15086	10.099	***
	stand + time	4	0.0153	0.00383	0.256	
Litter	time	2	0.0898	0.04492	2.402	
	stand + time	4	0.0468	0.01171	0.626	

b)

Turnover +		Df	Sum Sq	Mean Sq	F value	p
SAP	time	2	0.4072	0.20359	10.4	***
	stand + time	4	0.0242	0.00605	0.309	
EcM	time	2	0.3954	0.19771	10.487	***
	stand + time	4	0.0482	0.01205	0.639	
Yeast	time	2	0.3336	0.16682	7.14	***
	stand + time	4	0.0195	0.00488	0.209	
Turnover -		Df	Sum Sq	Mean Sq	F value	p
SAP	time	2	0.2993	0.14965	9.208	***
	stand + time	4	0.0155	0.00389	0.239	
EcM	time	2	0.408	0.20398	12.504	***
	stand + time	4	0.0344	0.00859	0.527	
Yeast	time	2	0.1061	0.05304	2.883	.
	stand + time	4	0.009	0.00226	0.123	

c)

Turnover +		Df	Sum Sq	Mean Sq	F value	p
SAP	time	2	0.1074	0.05371	2.4	
	stand + time	4	0.0373	0.00932	0.417	
EcM	time	2	0.0746	0.03728	0.825	
	stand + time	4	0.2776	0.06939	1.535	
Yeast	time	2	0.1932	0.09662	3.334	*
	stand + time	4	0.0633	0.01583	0.546	
Turnover -		Df	Sum Sq	Mean Sq	F value	p
SAP	time	2	0.0748	0.03739	1.957	
	stand + time	4	0.0516	0.01289	0.674	
EcM	time	2	0.121	0.06051	1.354	
	stand + time	4	0.3206	0.08016	1.794	
Yeast	time	2	0.1802	0.09008	3.041	
	stand + time	4	0.1167	0.02918	0.985	

Table S9. Kendall's Tau rank correlation and ANOVA results of measured variables to axes 1 and 2 of ordination of samples by functional guild subsets. Tests used site centroids and average values for variables with multiple measurements. a) soil community b) litter community. Significance codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.'. Block was not significant for any litter community subset (data not shown).

a)

Soil		**Saprotrophs**				**EcM**				**Yeasts**				**AM**			
		axis 1		axis 2		axis 1		axis 2		axis 1		axis 2		axis 1		axis 2	
Variable		tau	p	tau	p	tau	p	tau	p	tau	p	tau	p	tau	p	tau	p
pH		0.359	*	0.595	***	-0.294		0.203		0.176		-0.399	*	-0.02		-0.294	
Moisture		0.137		0.059		-0.255		-0.098		0.059		-0.02		-0.085		0.163	
OM		0.098		-0.085		-0.111		-0.059		-0.033		0.281		-0.02		0.02	
CN ratio		-0.086		-0.007		-0.112		-0.191		-0.112		0.336		-0.151		-0.046	
C		0.217		0.007		0.112		0.072		0.204		0.007		0.243		-0.059	
N		0.255		-0.033		0.124		0.02		0.203		0.719		0.242		-0.059	
Total understory richness		0.173		0.292		0.12		0.106		-0.013		0.08		-0.053		-0.292	
Non-woody understory richness		0.275		0.208		0.141		0.047		0.06		0.034		-0.02		-0.302	
Woody understory richness		-0.3		0.1		0		0.086		-0.329		0.214		0.029		0.1	
Total vegetation cover		0.19		0.346	*	0.085		0.137		-0.046		0.033		-0.007		-0.15	
Total woody veg. cover		-0.107		0.268		0.04		0.12		-0.201		0.027		0.134		-0.013	
Total forb + graminoid cover		0.224		0.356	*	0.092		0.172		-0.026		0		0.013		-0.158	
Total forb cover		0.243		0.204		0.164		0.046		0.007		0.151		0.007		-0.059	
Total graminoid cover		0.007		0.169		0.034		0.264		-0.169		-0.155		0.007		-0.128	
Understory vegetation scores-1		0.163		0.477	***	-0.046		0.425	***	-0.098		-0.229		0.046		-0.255	
Understory vegetation scores-2		0.386	**	0.020		0.412	**	-0.033		0.438	**	0.007		0.163		-0.085	
ANOVA	Df	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p
Stand (ax1)	2	0.07	0.03	0.58		0.85	0.43	3.2		0.13	0.06	0.75		0.98	0.49	0.72	
Stand (ax2)	2	0.22	0.11	2.73	.	1.46	0.73	15.04	***	0.17	0.08	3.19	.	1.23	0.61	1.56	
Block (ax1)	5	0.5	0.1	2.62	.	1.21	0.24	1.77		0.8	0.16	3.23	*	3.49	0.7	1.09	
Block (ax2)	5	0.27	0.05	1.15		0.14	0.03	0.17		0.06	0.01	0.31		1.57	0.31	0.68	

b)

Litter		Saprotrophs				EcM				Yeasts				AM			
		axis 1		axis 2		axis 1		axis 2		axis 1		axis 2		axis 1		axis 2	
Variable		tau	p	tau	p	tau	p	tau	p	tau	p	tau	p	tau	p	tau	p
pH		0.33		0.11		0.05		0.41	*	0.24		-0.11		-0.06		-0.03	
Moisture		-0.19		0.27		0.02		0.15		-0.25		-0.29		0.542	***	0.15	
lignin		0.19		0.05		0.14		-0.2		0.12		0.06		-0.1		-0.05	
CN ratio		0.27		-0.14		-0.09		0.34		0.28		-0.17		0.17		-0.31	
C		0.05		0.01		0.07		0.12		0.01		-0.24		0.25		-0.14	
N		0.03		0.02		0.22		-0.15		-0.01		-0.1		0.11		-0.07	
Total understory richness		0.28		-0.29		-0.05		0.11		0.31		0.01		-0.11		-0.492	**
Non-woody understory richness		0.17		-0.44	**	-0.15		-0.03		0.23		0.17		-0.22		-0.48	
Woody understory richness		0.06		0.19		0.06		0.16		0.03		-0.3		0.3		-0.19	
Total vegetation cover		0.28		-0.28		0.07		0.18		0.37	*	-0.08		-0.08		-0.529	**
Total woody veg. cover		0.17		0.01		0.01		0.23		0.2		-0.21		0.11		-0.24	
Total forb + graminoid cover		0.3		-0.33		0.03		0.16		0.4	*	-0.05		-0.11		-0.541	**
Total forb cover		0.14		-0.23		-0.07		0.03		0.18		-0.06		-0.09		-0.38	*
Total graminoid cover		0.32		-0.44	**	0.21		0.11		0.534	**	-0.05		-0.09		-0.629	***
Understory vegetation scores-1		0.595	***	-0.307		0.281		0.229		0.634	***	-0.085		-0.190		-0.608	***
Understory vegetation scores-2		-0.046		-0.242		-0.072		-0.307		-0.033		0.268		-0.386	**	-0.072	
ANOVA	Df	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p	Sumsq	MnSq	F val	p
Stand (ax1)	2	2.67	1.33	81.21	***	0.81	0.41	4.45	*	2.76	1.38	115.44	***	0.77	0.39	2.33	
Stand (ax2)	2	1.83	0.92	51.01	***	1.24	0.62	10.06	**	1.63	0.82	22.02	***	2.3	1.15	11.56	***

III

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NOTE

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Libkindia masarykiana gen. et sp. nov., *Yurkovia mendeliana* gen. et sp. nov. and *Leucosporidium krtinense* f.a. sp. nov., isolated from temperate forest soils

Tereza Mašínová,[1,*] Ana Pontes,[2] Cláudia Carvalho,[2] José Paulo Sampaio[2] and Petr Baldrian[1]

Abstract

One hundred and ninety-eight isolates of soil yeasts were isolated from mixed temperate forests in the Czech Republic, and their abundance and distribution in the litter and soil were evaluated using amplicon sequencing of soil fungal communities. Abundant taxa with no close identified hits were selected for further characterization as potential novel species of yeasts. Phylogenetic analyses using sequences of the D1/D2 domain, the ITS region and *RPB1* and *TEF1* genes support the recognition of the following three novel species belonging to the subphylum Pucciniomycotina, class Microbotryomycetes: *Leucosporidium krtinense* f.a. sp. nov. (type strain PYCC 6879^T=KT96^T=CBS 14304^T=DSM 101892^T), *Yurkovia mendeliana* sp. nov. (type strain PYCC 6884^T=KT152^T=CBS 14273^T=DSM 101889^T) and *Libkindia masarykiana* sp. nov. (type strain PYCC 6886^T=KT310^T=CBS 14275^T=DSM 101891^T). Since the latter two novel taxa cannot be assigned to existing genera, two new genera, *Libkindia* gen. nov. and *Yurkovia* gen. nov., are also described.

Yeasts represent a significant part of soil fungal communities [1], and although approximately 130 species have been reported to be associated with soil worldwide (reviewed by Yurkov *et al.* [2]), their diversity in soil ecosystems is poorly known [1]. Moreover, although approximately 1500 yeast species have been described up to 2011 [3], estimates of the total number of species suggest a number 10 times higher [4]. This uncharted diversity calls for the introduction of novel techniques to foster the global inventory of yeast diversity. Among these techniques, direct detection methods that avoid the cultivation approach are at the forefront [5]. For example, the direct amplification and sequencing of DNA from environmental samples constitute a suitable tool to study the composition of microbial communities and ecological associations of species [5, 6]. This approach provides valuable insights into the community composition and clues to identify the drivers of species distribution. A negative aspect of such methodologies is the absence of cultures for phenotypic analysis and, more specifically, for the investigation of interesting biotechnological properties. One way of circumventing this problem is to combine, in a single study, a cultivation-independent approach that typically has high throughput and a more conventional cultivation-dependent method that yields cultures for further study.

We conducted such a study combining the two approaches using soil samples from forests located north of Brno, in the Czech Republic [7]. Here we analyse, with the combined approach, the occurrence of three novel species, two of them described as new genera. The culture-independent approach revealed that the new yeast species appear to be relatively frequent in the studied sites, therefore, illustrating their importance in temperate forests. Representatives of the novel taxa were present among the cultures obtained in this study but, in contrast with metagenomics, were rarely isolated. They are described as *Leucosporidium krtinense* f.a. sp. nov., *Libkindia masarykiana* gen. et sp. nov. and *Yurkovia mendeliana* gen. et sp. nov.

Soil and litter samples were obtained from the Training Forest Enterprise Masaryk Forest Křtiny. The Masaryk Forest Křtiny has a total area of 103 km^2 (16° 15′ E 49° 15′ N) and

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Keywords: new yeast species; cultivation and metagenomics methods; yeast ecology; soil microbiology.
Abbreviations: ITS, internal transcribed spacer; OTU, operational taxonomic unit.
The GenBank/EMBL/DDBJ accession numbers for the LSU sequences of *Yurkovia mendeliana*, *Leucosporidium krtinense* f.a. and *Libkindia masarykiana* are KU187888, KU187886 and KU187889, respectively; ITS sequences of *Yurkovia mendeliana*, *Leucosporidium krtinense* f.a. and *Libkindia masarykiana* are KU187884, KU187882 and KU187885, respectively; *TEF1* sequences of *Yurkovia mendeliana* and *Libkindia masarykiana* are KX620743 and KX620745, respectively; *RPB1* sequences of *Yurkovia mendeliana* and *Libkindia masarykiana* are KX620742 and KX620744, respectively.
One supplementary figure and two supplementary tables are available with the online Supplementary Material.

is located north of Brno, Czech Republic. Climatically, the area is part of the South Moravian Region with a mean annual temperature of 8–9.5 °C (mean annual temperature of the coldest and warmest months of 2014 was 0.5 and 20.5 °C, respectively) and a mean annual precipitation of 400–500 mm. The area is covered by approximately 46 % conifer and 54 % broadleaf tree species and includes the parent rocks of granodiorites, culmian greywacke and limestone types.

In this area, 80 study sites [11 spruce stands, 18 beech stands, 7 oak stands and 44 sites with mixed vegetation; Fig. S1 (available in the online Supplementary Material)] were sampled in September and October 2013 and used for the extraction of environmental DNA. Yeast cultures were isolated from 18 selected sites (six stands each of beech, oak and spruce) in October 2013 and April 2014. At each site, five soil cores of 4.5 cm in diameter were collected up to a depth of 10 cm, including one from the centre and four located at a distance of 2 m to the north, south, east and west from the central core. The cores were stored at 4 °C and processed within 24 h after collection. The material was divided into soil and litter fractions, which were processed separately. The litter was cut into ca. 0.25 cm^2 pieces. The soil was sieved through a 5 mm sieve to remove stones, roots and other big particles and to homogenize the sieved soil material. The samples used for yeast isolation were stored at 4 °C and were processed within 48 h. The samples for DNA extractions were stored at −80 °C.

For the analysis of the fungal community by high-throughput sequencing, the total genomic DNA was extracted in triplicate from 250 mg soil using a modified Miller method [8] and a previously described protocol [9]. The PCR amplification of the fungal internal transcribed spacer 2 (ITS2) region from DNA was performed using barcode primers gITS7 and ITS4 [10] in three reactions per sample.

The sequencing of the fungal amplicons was performed on an Illumina MiSeq. The sequencing data were processed using the SEED 1.2.1 pipeline [11]. Briefly, pair-end reads were merged using fastq-join [12], and the ITS2 region was extracted using the ITS Extractor 1.0.8 [13] before processing. Chimeric sequences were detected using Usearch 7.0.1090 [14] and then deleted. Afterwards, the environmental sequences and sequences of obtained isolates were clustered using UPARSE implemented within Usearch [14] at a 97 % similarity level [15]. Consensus sequences were constructed for each cluster, and the closest hits at the genus or species level were identified using UNITE [16].

Isolates were clustered into operational taxonomic units (OTUs) based on 97 % sequence similarity. Those isolates that belonged to OTUs with a relative abundance >0.5 % in at least one environmental sample and with no close identified hits (<90 % coverage, <97 % similarity) were selected for further characterization as potential novel species of yeasts.

For yeast isolation, 1 g soil was suspended in 5 ml demineralized water, serially diluted and plated on yeast glucose (YG) agar [17] supplemented with chloramphenicol (0.2 g l^{-1}). Plates were inoculated in triplicate and incubated at 4 °C for 14 days. The growing yeast colonies were transferred to fresh YG agar plates for culture purification. For the preliminary molecular identification of the strains, total DNA was extracted with the ArchivePure DNA Yeast/Gram-positive Bacteria kit (5 PRIME). The ITS1F and NL4 primers [18, 19] were used to amplify a region of the nuclear ribosomal DNA (rDNA) which spans the ITS1, the 5.8S RNA gene, the ITS2 and the D1/D2 region of the large subunit of the 28S rDNA. Each 25 µl reaction contained 2.5 µl 10× polymerase buffer, 1 µl of each primer (0.01 mM), 0.5 µl PCR Nucleotide Mix (10 mM), 0.75 µl polymerase (2 U μl^{-1} DyNAzyme polymerase) and 1 µl genomic DNA with concentration between 10 and 100 ng μl^{-1}. The cycling conditions were 94 °C for 4 min and 35 cycles of 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min, followed by an extension step at 72 °C for 10 min. PCR amplicons were sequenced at an external facility (GATC Biotech), and the sequences were manually edited.

Assimilation tests of carbon and nitrogen sources and additional standard tests used in phenotypic characterization were performed in liquid media following the procedures described by Kurtzman *et al.* [3]. For microscopy, cultures were grown at 17–22 °C on YM agar and on corn meal agar (CMA; Difco) and studied with phase-contrast optics.

For the phylogenetic analyses, total DNA of isolates that potentially represented novel species was extracted using the protocol described by Sampaio *et al.* [20] and purified by illustra GFX PCR DNA and a Gel Band Purification kit (GE Healthcare). The DNA was amplified with the LR6 and ITS5 primer pairs. The sequencing of the D1/D2 domain of the 26S rDNA was performed with the NL4 primer. The ITS region of the rDNA was sequenced with the ITS4 primer. For the amplification and sequencing of the genes *RPB1* and *TEF1*, the primers used were RPB1-Af (5′-GARTG YCCDGGDCAYTTYGG-3′) and RPB1-Cr (5′-CCNGCDA TNTCRTTRTCCATRTA-3′) and Al_34_EF1_300_F1 (5′-TTCATCAAGAACATGAT-3′) and AL34_EF1_1050_R_-Tail (5′- GCTATCATCACAATGGACGTTCTTGGAG-3′), respectively. Phylogenetic analyses were based on the D1/D2 domains of the 26S rDNA (LSU) and the ITS region, which included the 5.8S rRNA gene, and also on a concatenated alignment of D1/D2, ITS, *RPB1* (subunit 1 of RNA polymerase II) and *TEF1* (translation elongation factor 1-α). The maximum-likelihood method as implemented in RAxML with the GTRGAMMA model of sequence evolution was used in all cases.

In total, 198 yeast strains were isolated and tentatively identified using BLASTn against the UNITE and GenBank (www.ncbi.nlm.nih.gov) databases. To ensure a clear identification, only the hits with >90 % coverage and >97 % similarity were retained. This criterion was fulfilled for 128 isolates that belonged to 24 genera, whose designations follow recent

taxonomic changes [21, 22]: *Candida*, *Cryptococcus*, *Cutaneotrichosporon*, *Cystofilobasidium*, *Dioszegia*, *Fellozyma*, *Filobasidium*, *Heterocephalacria*, *Holtermanniella*, *Itersonilia*, *Kwoniella*, *Lachancea*, *Leucosporidium*, *Naganishia*, *Oberwinklerozyma*, *Pichia*, *Piskurozyma*, *Rhodosporidiobolus*, *Saitozyma*, *Sporobolomyces*, *Solicoccozyma*, *Trichosporon*, *Vishniacozyma* and *Yamadamyces* (Table S1). Based on analyses of rDNA sequences, three strains were preliminarily classified as novel basidiomycetous yeast species belonging to the subphylum Pucciniomycotina and class Microbotryomycetes. These three strains were isolated from litter collected either in October 2013 or April 2014 and were distinctly different from their closest relatives (Fig. 1). Strain PYCC 6879^T was most closely related to *Leucosporidium intermedium* CBS 7226^T, but the two strains differed by 4 nucleotide substitutions (0.8 %) in the D1/D2 domain of the 26S rDNA and by 23 nucleotide substitutions in the complete ITS region (3.9 %). The differences between PYCC 6884^T and its closest relatives, *Bannozyma arctica*, *Bannozyma yamatoana*, *Chrysozyma griseoflava*, *Fellozyma inositophila*, *Hamamotoa singularis* and *Hamamotoa lignophila*, ranged between 3 and 5 % in the D1/D2 domain and 8 and 12 % in the complete ITS region. The strain PYCC 6886^T occupied a somewhat isolated position and was loosely related to *Phenoliferia* and to *Yamadamyces.*

Whereas the generic placement of PYCC 6879^T within *Leucosporidium* offered no doubts, to clarify the generic assignments of the remaining new yeast species, we reconstructed more restricted phylogenetic trees that included concatenated sequences of *RPB1*, *TEF1*, D1/D2 and ITS and that are also depicted in Fig. 1. In this analysis, PYCC 6886^T appears not to belong to *Phenoliferia* or to *Yamadamyces* (Fig. 1b). This strain differed from the four species of *Phenoliferia* by 8–11 substitutions (1.6–2.3 %) in the D1/D2 domain and by 31–34 substitutions in the complete ITS region (5.8–6.5 %). PYCC 6886^T differed from *Yamadamyces rosulatus* by 16 substitutions (3 %) in the D1/D2 domain and 12 substitutions (3 %) in the ITS region. Therefore, given the isolated position of PYCC 6886^T, we opt to describe it in a new genus as *Libkindia masarykiana* gen. et sp. nov. As for PYCC 6884^T, the multigene analysis shown in Fig. 1(c) places this strain in an isolated position, having as near relatives the genera *Bannozyma*, *Chrysozyma*, *Fellozyma* and *Hamamotoa*. Since in our analyses, none of these genera appear as an obvious candidate to accommodate the novel species, we opt to describe it in a new genus as *Yurkovia mendeliana* gen. et sp. nov. The sequence data for the new taxa were deposited in GenBank (Table S2). The detection of *Fellozyma inositophila*, represented by strain PYCC 6882, is of notice. This species was originally found in a bamboo leaf, about 30 years ago in Japan, and was until now known from a single strain [23].

Based on the analysis of environmental DNA, the novel species constitute common and abundant taxa in the studied environment (Table S2). If we consider the fact that the mean relative abundance of the three most abundant genera, i.e *Saitozyma*, *Solicoccozyma* and *Apiotrichum*, was 85.6 % in the soil samples and 36.7 % in the litter samples, the novel species represented an important share of the rest of the yeast community, which indicates their high environmental relevance in the temperate mixed forests ecosystems. Therefore and in spite of basing their respective formal descriptions on single strains, the metagenomics data indicated that these are indeed relatively frequent species in the type of soils studied. Interestingly, among the total number of yeast strains isolated (198), the novel species were poorly represented. In two cases, only a single strain was found, and for *Leucosporidium krtinense*, six additional strains were isolated and molecularly identified, having identical ITS sequences, but lost viability subsequently. This discrepancy between culture-dependent and culture-independent methods might be caused by a lower fitness of the three novel taxa in conventional culture media, a topic worthy of further research. The strains representing the novel species were characterized with the standard tests used in yeast taxonomy.

DESCRIPTION OF *LEUCOSPORIDIUM KRTINENSE* F.A., SP. NOV. MAŠÍNOVÁ, PONTES, CARVALHO, SAMPAIO AND BALDRIAN

Leucosporidium krtinense (kr.tin.en′se. N.L. neut. adj. *krtinense* referring to the Training Forest Enterprise Masaryk Forest Křtiny where the novel species was found).

After 3 days at 25 °C in YG agar, the cells are subglobose to ovoid, 2.5–4.0×3.5–9 µm, occurring singly or in pairs, and budding is predominantly polar (Fig. 2a). After 1 month at 25 °C, the streak culture is brownish cream; the surface is rough with a membranous structure, and the margin is entire. Pseudohyphae are observed after 1 month in CMA at room temperature (Fig. 2b). Fermentation is absent. The following compounds are assimilated: D-glucose, D-galactose, L-sorbose (delayed), D-ribose (delayed), D-xylose, L-arabinose, sucrose, maltose, trehalose, melezitose, inulin, glycerol, ribitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, DL-lactate (delayed), succinate, citrate, ethanol, L-malic acid, protocatechuic acid, nitrate, ethylamine, L-lysine and cadaverine. The following compounds are not assimilated: D-glucosamine, D-arabinose, L-rhamnose, methyl α-D-glucoside, cellobiose, starch, salicin, melibiose, lactose, raffinose, erythritol, xylitol, galactitol, *myo*-inositol, methanol, L-tartaric acid, nitrite, creatine and creatinine. Growth in vitamin-free medium is negative. Growth in the presence of 0.1 and 0.01 % cycloheximide is positive. Hydrolysis of urea is positive. Growth in the presence of 10 % NaCl is weak. Growth at 30 °C is positive but is negative at 35 °C. The diazonium blue B reaction is positive. In the genus *Leucosporidium*, the sexual stage normally develops after mating of sexually compatible strains, although in some cases, self-fertile strains have been reported. Since in the present case a sexual stage

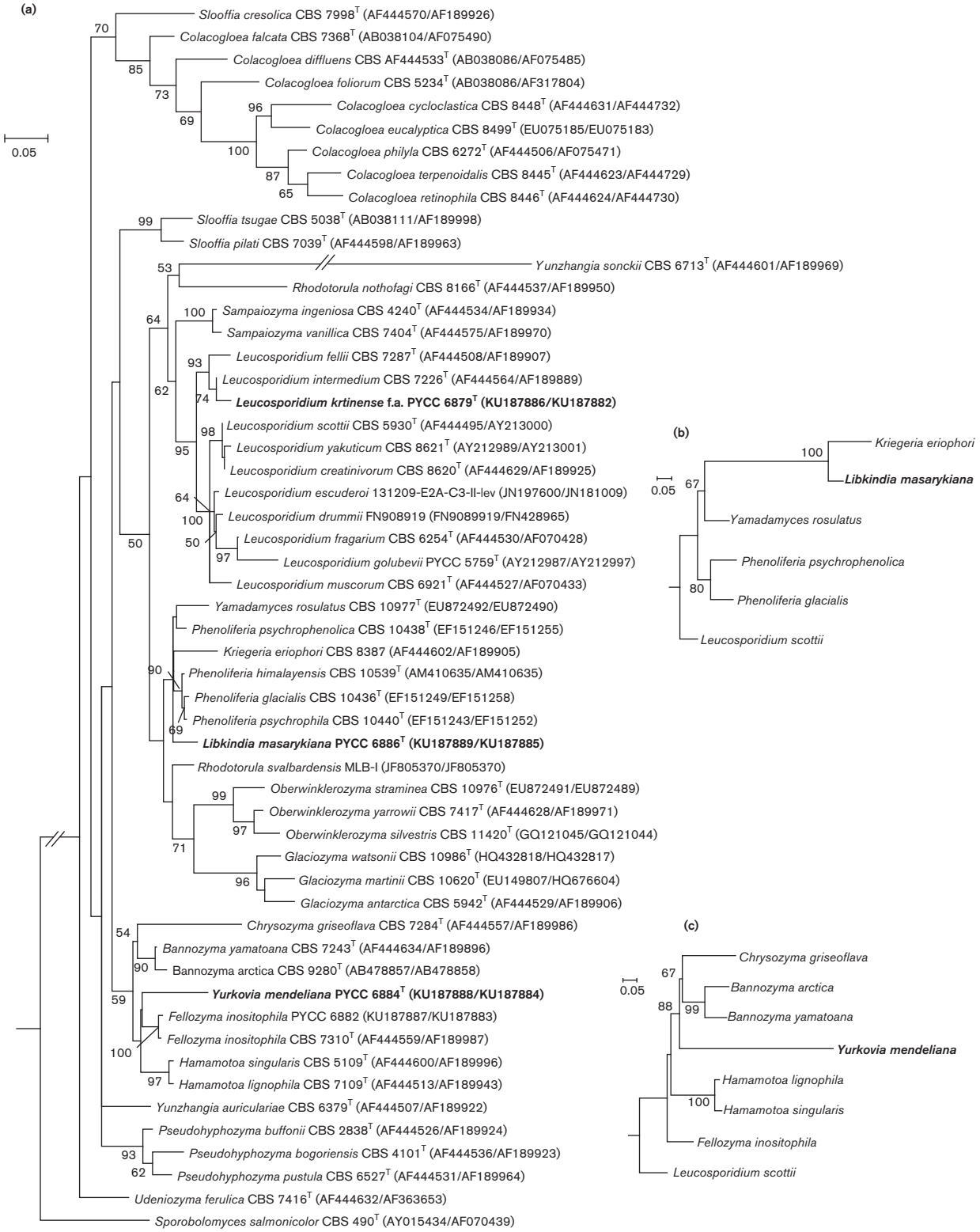

Fig. 1. Phylogenetic trees based on (a) an alignment of the D1/D2 domains of the 26S rDNA and the complete ITS region and (b, c) a concatenated alignment of those sequences and also *RPB1* (subunit 1 of RNA polymerase II) and *TEF1* (translation elongation factor 1-α), showing the placement of *Leucosporidium krtinense* f.a. sp. nov., *Libkindia masarykiana* gen. et sp. nov. and *Yurkovia mendeliana* gen. et sp. nov. (a) General tree rooted with *Sporobolomyces salmonicolor* showing the phylogenetic placement of the new taxa within a comprehensive group of closely related species. (b) Detailed phylogeny rooted with *Leucosporidium scottii* showing the phylogenetic position of *Libkindia masarykiana* gen. et sp. nov. (c) Detailed phylogeny rooted with *Leucosporidium scottii* showing the phylogenetic

position of *Yurkovia mendeliana* gen. et sp. nov. All phylogenies were reconstructed using the maximum-likelihood method as implemented in RAxML with the GTRGAMMA model of sequence evolution. Bootstrap values (100 replicates) are shown as percentages near tree branches. The scalebar represents number of expected substitutions accumulated per site.

was not observed, the novel species is described as *forma asexualis* (f.a.).

The type strain PYCC 6879^T (=KT96^T=CBS 14304^T=DSM 101892^T) was isolated from beech litter in October 2013 in the Křtiny forest area (49° 18′ 11.8″ N 16° 44′ 50.6″ E), Czech Republic. The type strain was deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands. The MycoBank accession number is MB815370.

DESCRIPTION OF *LIBKINDIA* GEN. NOV. MAŠÍNOVÁ, PONTES, SAMPAIO AND BALDRIAN

Libkindia (Lib.kin′di.a. N.L. fem. n. *Libkindia*named in honour of D. Libkind for his contributions to yeast taxonomy).

The genus is circumscribed by the phylogenetic analysis shown in Fig. 1, having therefore as closest relatives the genera *Kriegeria*, *Phenoliferia* and *Yamadamyces*. Cultures are brownish cream coloured. Sexual reproduction is not known. Cells divide by budding. Hyphae, pseudohyphae and ballistoconidia are not formed.

The MycoBank accession number is MB817423.

Type species: *Libkindia masarykiana* Mašínová, Pontes, Carvalho, Sampaio and Baldrian.

Species accepted: *Libkindia masarykiana* Mašínová, Pontes, Carvalho, Sampaio and Baldrian MB815373.

DESCRIPTION OF *LIBKINDIA MASARYKIANA* SP. NOV. MAŠÍNOVÁ, PONTES, CARVALHO, SAMPAIO AND BALDRIAN

Libkindia masarykiana (ma.sa.ryk.i.a′na. N.L. fem. adj. *masarykiana* referring to the Training Forest Enterprise Masaryk Forest Křtiny where the novel species was found).

After 3 days at 25 °C in YG agar, the cells are elongated to cylindrical, 2–3×8.5–12 µm, occurring singly or in pairs, and budding is predominantly polar (Fig. 2c). After 1 month at 25 °C, the streak culture is brownish cream; the surface is rough, and the margin is entire or rarely wrinkled. Hyphae, pseudohyphae and ballistoconidia are not formed. Fermentation is absent. The following compounds are assimilated: D-glucose, D-galactose, L-sorbose (delayed), D-xylose, L-arabinose, sucrose, maltose, melezitose, trehalose (delayed), methyl α-D-glucoside (delayed), melezitose, glycerol, ribitol, xylitol (delayed), D-glucitol, D-mannitol, D-glucono-1,5-lactone (delayed), D-gluconate, succinate, citrate (weak), ethanol, L-malic acid, protocaechuic acid, ethylamine and cadaverine. The following compounds are not assimilated: D-glucosamine, D-ribose, D-arabinose, L-rhamnose, cellobiose, salicin, melibiose, lactose, raffinose, starch, erythritol, galactitol, *myo*-inositol, D-glucuronate, DL-lactate, methanol, L-tartaric acid, nitrate, nitrite, L-lysine, creatine and creatinine. Growth in vitamin-free medium is positive and in the presence of 10 % NaCl is weak. Growth in the presence of 0.01 % cycloheximide is delayed and weak and in the presence of 0.1 % cycloheximide is negative. Growth at 25 °C is positive and at 30 °C is negative. The diazonium blue B reaction is positive.

The type strain, PYCC 6886^T (=KT310^T=CBS 14275^T=DSM 101891^T) was isolated from oak litter in April 2014 in the Křtiny forest area (49° 16′ 01.0″ N 16° 43′ 16.2″ E), Czech Republic. The type strain was deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands. The MycoBank accession number is MB815373.

DESCRIPTION OF *YURKOVIA* GEN. NOV. MAŠÍNOVÁ, PONTES, SAMPAIO AND BALDRIAN

Yurkovia (Yur.ko′vi.a. N.L. fem. n. *Yurkovia* named in honour of A. Yurkov for his contributions to yeast taxonomy).

The genus is circumscribed by the phylogenetic analysis shown in Fig. 1, having therefore as closest relatives the genera *Bannozyma*, *Chrysozyma*, *Fellozyma* and *Hamamotoa*. Cultures are brownish-cream coloured. Sexual reproduction is

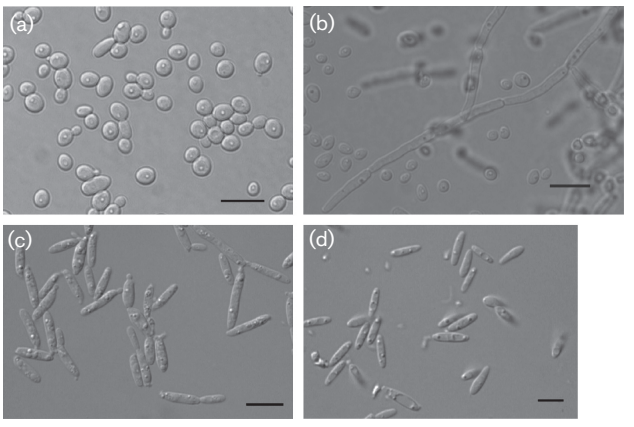

Fig. 2. Micrographs of *Leucosporidium krtinense* f.a. PYCC 6879^T, *Libkindia masarykiana* PYCC 6886^T and *Yurkovia mendeliana* PYCC 6884^T. (a) Yeast cells of *Leucosporidium krtinense* on YG agar. (b) Pseudomycelium of *Leucosporidium krtinense* on CMA. (c) Yeast cells of *Yurkovia mendeliana* on YG agar. (d) Yeast cells of *Libkindia masarykiana* on YG agar. Scale bar, 10 µm.

not known. Cells divide by budding. Hyphae, pseudohyphae and ballistoconidia are not formed.

The MycoBank accession number is MB816538.

Type species: *Yurkovia mendeliana* Mašínová, Pontes, Carvalho, Sampaio and Baldrian.

Species accepted: *Yurkovia mendeliana* Mašínová, Pontes, Carvalho, Sampaio and Baldrian MB815372.

DESCRIPTION OF *YURKOVIA MENDELIANA* SP. NOV. MAŠÍNOVÁ, PONTES, CARVALHO, SAMPAIO AND BALDRIAN

Yurkovia mendeliana (men.del.i.a′na. N.L. fem. adj. *mendeliana* in honour of Johann Gregor Mendel, the founder of genetics, whose name is carried by the university that owns the forest where the type strain was isolated).

After 3 days at 25 °C in YG agar, the cells are elongate to ellipsoidal, 2–4×8–11 µm, occurring singly or in pairs, and budding is predominantly polar (Fig. 2d). After 1 month at 25 °C, the streak culture is brownish-cream coloured; the surface is smooth, and the margin is entirely or rarely wrinkled. Hyphae, pseudohyphae and ballistoconidia are not formed. Fermentation is absent. The following compounds are assimilated: D-glucose, D-galactose, L-sorbose (delayed), D-xylose, L-arabinose, sucrose, maltose, trehalose, methyl α-D-glucoside (delayed), raffinose (weak), melezitose, inulin, starch (delayed), glycerol, ribitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, D-gluconate (delayed), succinate, citrate (weak), ethanol, L-malic acid, protocatechuic acid, 0.01 % cycloheximide, D-gucosamine (weak), ethylamine, L-lysine and cadaverine. The following compounds are not assimilated: D-ribose, D-arabinose, L-rhamnose, cellobiose, salicin, melibiose, lactose, erythritol, xylitol, galactitol, *myo*-inositol, D-glucuronate, DL-lactate, methanol, L-tartaric acid, nitrate, nitrite, creatine and creatinine. Growth in vitamin-free medium is positive and in the presence of 10 % NaCl is weak. Growth in the presence of 0.01 and 0.1 % cycloheximide is delayed and positive, respectively. Growth at 25, 30 and 35 °C is positive. The diazonium blue B reaction is positive.

The type strain, PYCC 6884^T (=KT152^T=CBS 14273^T=DSM 101889^T) was isolated from beech litter in October 2013 in the Křtiny forest area (49° 19′ 29.8″ N 16° 39′ 09.9″ E), Czech Republic. The type strain was deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands. The MycoBank accession number is MB815372.

Leucosporidium krtinense f.a. differed from *Leucosporidium intermedium* in D-xylose, L-arabinose and ribitol assimilation and in the ability to use nitrate as the sole nitrogen source. In the temperate forests studied, *Leucosporidium krtinense* f.a. occurred at sites dominated by all the dominant tree types, even though it was infrequent under oak. No clear preference for soil or litter was observed (Table S2). This species is abundant mainly in litter, where it may dominate the yeast community (Table S2). In the studied temperate forests, *Libkindia masarykiana* occurred at sites dominated by all dominant tree types. This species is abundant mainly in litter (Table S2). An identical ITS sequence (GenBank accession no. JN889786) was detected in a study [24] comparing fungal communities between AK (USA) and Guyana. A D1/D2 sequence that differed by 2 nucleotides (GenBank accession no. KC588725) was detected in *Acer saccharum*-dominated sites in MI (USA), and a sequence that differed by 5 nucleotides (GenBank accession no. KF567265) was found in a pine forest in NC, USA. In spite of the association of *Libkindia* with *Kriegeria* in the concatenated phylogeny of Fig. 1(b), we believe that the novel taxon should not be described in *Kriegeria* because this genus includes a unique plant pathogen with a distinctive life cycle and very peculiar yeast cells [25]. *Yurkovia mendeliana* occurred mainly in litter and revealed no clear preference for dominant tree species (Table S2). It is likely that other species will be described in *Yurkovia* in the near future. For example, a sequence of the D1/D2 domain with 6 nucleotide substitutions (GenBank accession no. JX242144) was detected in Hawaiian plants, and a soil yeast (AY 214) found in Germany had 2 and 36 substitutions in the D1/D2 (GenBank accession no. FN428969) and ITS (GenBank accession no. KY083054) regions, respectively. Another soil isolate, DBVPG 10602, whose D1/D2 and ITS sequences were released during the revision of this manuscript (GenBank accession nos KU745307 and KU745372, respectively), is probably an additional representative of *Yurkovia mendeliana*. The two strains differ just by one substitution in the D1/D2 region (but additional sequencing is needed because sequence coverage was only 87 %) and three substitutions in the ITS region.

Our results demonstrate that a combination of metagenomic approaches with classical yeast isolation and cultivation techniques is an effective methodology for assessing yeast diversity in the soil ecosystem and for uncovering novel yeast taxa that are, apparently, less prone to yield cultures during the isolation process.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Botha A.** The importance and ecology of yeasts in soil. *Soil Biol Biochem* 2011;43:1–8.
2. **Yurkov AM, Kemler M, Begerow D.** Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. *PLoS One* 2011;6:e23671.
3. **Kurtzman C, Fell JW, Boekhout T.** *The Yeasts: A Taxonomic Study*, 5th ed. Amsterdam: Elsevier Science; 2011.
4. **Lachance MA.** Yeast biodiversity: how many and how much? In: Péter G and Rosa C (editors). *Biodiversity and Ecophysiology of Yeasts*. Springer Berlin Heidelberg; 2006. pp. 1–9.
5. **Lindahl BD, Nilsson RH, Tedersoo L, Abarenkov K, Carlsen T *et al.*** Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytol* 2013;199:288–299.
6. **Peay KG.** Back to the future: natural history and the way forward in modern fungal ecology. *Fungal Ecol* 2014;12:4–9.
7. **Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K *et al.*** Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiol Ecol* 2016;93:fiw223.
8. **Sagova-Mareckova M, Cermak L, Novotna J, Plhackova K, Forstova J *et al.*** Innovative methods for soil DNA purification tested in soils with widely differing characteristics. *Appl Environ Microbiol* 2008;74:2902–2907.
9. **Žifčáková L, Větrovský T, Howe A, Baldrian P.** Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environ Microbiol* 2016;18:288–301.
10. **Ihrmark K, Bödeker IT, Cruz-Martinez K, Friberg H, Kubartova A *et al.*** New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* 2012;82:666–677.
11. **Větrovský T, Baldrian P.** Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. *Biol Fertil Soils* 2013;49:1027–1037.
12. **Aronesty E.** Comparison of sequencing utility programs. *Open Bioinforma J* 2013;7:1–8.
13. **Nilsson RH, Veldre V, Hartmann M, Unterseher M, Amend A *et al.*** An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. *Fungal Ecol* 2010;3:284–287.
14. **Edgar RC.** Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;26:2460–2461.
15. **O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R.** Fungal community analysis by large-scale sequencing of environmental samples. *Appl Environ Microbiol* 2005;71:5544–5550.
16. **Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF *et al.*** Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* 2013;22:5271–5277.
17. **Cooney DG, Emerson R.** *Thermophilic Fungi: An Account of their Biology, Activities, and Classification*. San Francisco, London: W. H. Freeman & Co; 1964.
18. **White T, Bruns T, Lee S, Taylor J.** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Shinsky J and White T (editors). *PCR Protocols: A Guide to Methods and Applications*. London: Academic Press; 1990.
19. **Gardes M, Bruns TD.** ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Mol Ecol* 1993;2:113–118.
20. **Sampaio JP, Gadanho M, Santos S, Duarte FL, Pais C *et al.*** Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *Rhodosporidium kratochvilovae* and related anamorphic species. *Int J Syst Evol Microbiol* 2001;51:687–697.
21. **Liu XZ, Wang QM, Göker M, Groenewald M, Kachalkin AV *et al.*** Towards an integrated phylogenetic classification of the *Tremellomycetes*. *Stud Mycol* 2015;81:85–147.
22. **Wang QM, Yurkov AM, Göker M, Lumbsch HT, Leavitt SD *et al.*** Phylogenetic classification of yeasts and related taxa within *Pucciniomycotina*. *Stud Mycol* 2015;81:149–189.
23. **Nakase T, Suzuki M.** *Sporobolomyces inositophilus*, a new species of ballistosporous yeast isolated from a dead leaf of *Sasa* sp. in Japan. *Antonie van Leeuwenhoek* 1987;53:245–251.
24. **Mcguire KL, Allison SD, Fierer N, Treseder KK.** Ectomycorrhizal-dominated boreal and tropical forests have distinct fungal communities, but analogous spatial patterns across soil horizons. *PLoS One* 2013;8:e68278.
25. **Sampaio JP, Oberwinkler F.** *Kriegeria* Bresadola (1891). In: Kurtzman CP (editor). *The Yeasts, A Taxonomic Study*, 5th ed. Amsterdam: Elsevier; 2011. pp. 1477–1479.

IJSEM Supplementary materials for

Libkindia masarykiana gen. nov. et sp. nov., *Yurkovia mendeliana* gen. nov. et sp. nov., and *Leucosporidium krtinense* f.a. sp. nov., isolated from temperate forest soils

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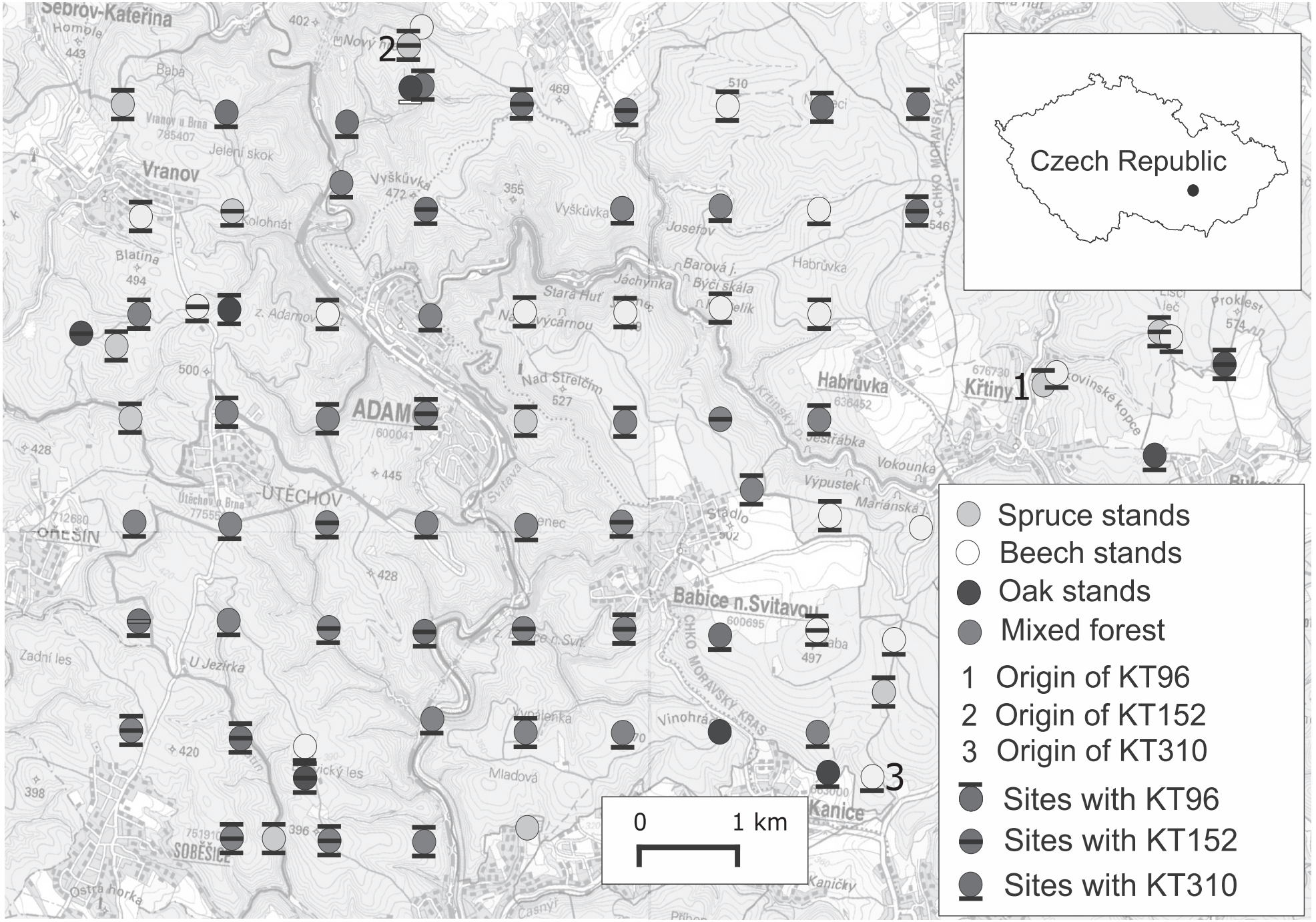
Supplementery Figure 1: Localization of the sampled sites in the temperate Masaryk Forest Křtiny area, Czech Republic, and origin of the studied strains.



Supplementary table 1: List of isolated yeast strains from litter and soil in the studied temperate forest and strains co-isolated with novel species.

Strain ID	Identification results	Closests match	Closest type	Co-isolated
KT224	*Apiotrichosporon porosum*	KU728171	100%, NR_073209	
KT225	*Apiotrichosporon porosum*	KU728171	100%, NR_073209	
KT240	*Apiotrichosporon porosum*	KU728171	100%, NR_073209	
KT202	*Asterotremella* sp.	HF558654	96%, FJ153164	
KT203	*Asterotremella* sp.	HF558654	96%, FJ153164	
KT204	*Asterotremella* sp.	HF558654	96%, FJ153164	
KT205	*Asterotremella* sp.	HF558654	96%, FJ153164	
KT227	*Asterotremella* sp.	HF558654	96%, FJ153164	
KT256	*Aureobasidium pullulans*	KU301224	99%, AJ244232	
KT259	*Aureobasidium pullulans*	KT722607	100%, AJ244232	
KT276	*Aureobasidium pullulans*	KT722607	100%, AJ244232	
KT288	*Aureobasidium pullulans*	KT722607	100%, AJ244232	
KT289	*Aureobasidium pullulans*	KT693743	100%, AJ244232	
KT328	*Aureobasidium* sp.	LN906592	97%, AJ244232	
KT309	*Aureobasidium pullulans*	KT722605	100%, AJ244232	*L. masarykiana*
KT291	*Aureobasidium pullulans*	KT693743	100%, FJ150906	
KT319	*Aureobasidium* sp.	KT693736	98%, FJ150895	
KT315	*Aureobasidium subglaciale*	JX188099	99%, FJ150895	
KT148	*Bannozyma* sp.	AF444634	93%, AF444634	
KT169	*Bannozyma* sp.	AF444634	93%, AF444634	
KT209	*Bannozyma* sp.	AF444634	93%, AF444634	
KT595	*Candida railenensis*	KC349937	99%, FM178302	
KT593	*Candida railenensis*	KC349937	99%, FM178302	
KT592	*Candida railenensis*	KC349937	99%, FM178302	
KT591	*Candida railenensis*	KC349937	99%, FM178302	
KT589	*Candida railenensis*	KC349937	99%, FM178302	
KT518	*Candida railenensis*	KC349937	99%, FM178302	
KT511	*Candida railenensis*	KC349937	99%, FM178302	
KT509	*Candida railenensis*	KC349937	99%, FM178302	
KT501	*Candida railenensis*	KC349937	99%, FM178302	
KT519	*Candida railenensis*	KC349937	99%, FM178302	
KT142	*Colacogloea* sp.	NR_073297	91%, NR_073297	
KT160	*Colacogloea* sp.	NR_073297	91%, NR_073297	
KT301	*Cryptococcus* sp.	KM079158	92%, KM079158	*L. masarykiana*
KT144	*Curvibasidium cygneicollum*	KM384465	99%, NR_111077	
KT176	*Curvibasidium cygneicollum*	KM384465	100%, NR_111077	
KT180	*Curvibasidium cygneicollum*	KM384465	100%, NR_111077	
KT182	*Curvibasidium cygneicollum*	KM384465	100%, NR_111077	
KT249	*Curvibasidium cygneicollum*	KM384465	99%, NR_111077	
KT280	*Curvibasidium cygneicollum*	KM384465	100%, NR_111077	
KT296	*Curvibasidium cygneicollum*	KM384465	100%, NR_111077	
KT303	*Curvibasidium cygneicollum*	AF444572	99%, NR_111077	*L. masarykiana*
KT268	*Curvibasidium* sp.	KM384465	96%, NR_111077	
KT290	*Cutaneotrichosporon moniliiforme*	NR_073240	99%, NR_073240	
KT243	*Cutaneotrichosporon moniliiforme*	AB180198	99%, NR_073240	
KT93	*Cystofilobasidium capitatum*	AY052491	100%, FJ545253	
KT115	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	
KT150	*Cystofilobasidium capitatum*	FJ545253	99%, FJ545253	*Y. mendeliana*
KT151	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	*Y. mendeliana*
KT244	*Cystofilobasidium capitatum*	FJ545253	99%, FJ545253	
KT264	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	
KT267	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT271	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT272A	*Cystofilobasidium capitatum*	AY052491	100%, FJ545253	

KT272B	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT275	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT282	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT284	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT298	*Cystofilobasidium capitatum*	KM384144	99%, FJ545253	*L. masarykiana*
KT326	*Cystofilobasidium capitatum*	HG937044	100%, FJ545253	
KT325	*Cystofilobasidium capitatum*	HG937044	99%, NR_111042	
KT323	*Cystofilobasidium capitatum*	NR_111042	99%, NR_111042	
KT322	*Cystofilobasidium capitatum*	AY052491	99%, NR_111042	
KT320	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	
KT270	*Cystofilobasidium capitatum*	KM384144	100%, FJ545253	
KT279	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	
KT285	*Cystofilobasidium capitatum*	FJ545253	100%, FJ545253	
KT155	*Dioszegia crocea*	LT548261	99%, AF444406	
KT158	*Dioszegia crocea*	LT548261	99%, AF444406	
KT210A	*Dioszegia crocea*	LT548261	99%, AF444406	
KT212A	*Dioszegia crocea*	LT548261	99%, AF444406	
KT165	*Fellozyma* sp.	NR_073305	92%, NR_073305	
KT183	*Fellozyma* sp.	NR_073305	98%, NR_073305	
KT211	*Fellozyma* sp.	NR_073305	92%, NR_073305	
KT145	*Fellozyma* sp.	NR_073305	93%, NR_073305	
KT255	*Fellozyma* sp.	LT548259	92%, NR_073305	
KT258	*Fellozyma* sp.	LT548259	91%, NR_073305	
KT274	*Fellozyma* sp.	NR_073305	88%, NR_073305	
KT286	*Fellozyma* sp.	NR_073305	92%, NR_073305	
KT294	*Fellozyma* sp.	NR_073305	92%, NR_073305	
KT318	*Fellozyma* sp.	LT548259	92%, NR_073305	
KT311	*Fellozyma* sp.	NR_073305	93%, NR_073305	*L. masarykiana*
KT305	*Fellozyma* sp.	LT548259	92%, NR_073305	*L. masarykiana*
KT300	*Fellozyma* sp.	LT548259	91%, NR_073305	*L. krtinyense*
KT257	*Filobasidium* sp.	KF981864	93%, NR_111207	
KT260	*Filobasidium* sp.	KF981864	94%, NR_111207	
KT292	*Filobasidium* sp.	KF981864	93%, NR_111207	
KT278	*Filobasidium* sp.	KF981864	95%, NR_111207	
KT181	*Heterocephalacria* sp.	NR_073225	97%, NR_073225	
KT29	*Heterocephalacria* sp.	NR_073225	96%, NR_073225	
KT97	*Heterocephalacria* sp.	NR_073225	92%, NR_073225	*L. krtinyense*
KT242	*Holtermanniella nyarrowii*	FN430734	99%, AY006481	
KT246	*Holtermanniella nyarrowii*	AY749434	99%, AY006481	
KT239	*Holtermanniella nyarrowii*	AY749434	99%, AY006481	
KT177	*Holtermanniella takashimae*	AY749434	99%, FM246501	
KT154	*Itersonilia pannonica*	NR_077110	100%, NR_077110	
KT99	*Kluyveromyces dobzhanski*	KC810947	99%, AY046215	*L. krtinyense*
KT184	*Kluyveromyces dobzhanskii*	KC810947	99%, AY046215	
KT277	*Kluyveromyces dobzhanskii*	KC810947	99%, AY046215	
KT418	*Kregervanrija* sp.	KF057703	95%, AY923249	
KT281	*Kwoniella* sp.	KP638739	91%, GU585748	
KT266	*Kwoniella pini*	NR_111269	100%, NR_111269	
KT437	*Lachancea quebecensis*	KX015903	99%, KP793243	
KT436	*Lachancea quebecensis*	KX015903	99%, KP793243	
KT419	*Lachancea quebecensis*	KX015903	99%, KP793243	
KT295	*Leucosporidium drummii*	JQ272411	100%, FN908919	
KT226	*Leucosporidium drummii*	NR_137036	100%, FN908919	
KT86	*Leucosporidium intermedium*	FR719968	99%, NR_073309	
KT85	*Leucosporidium krtinyense* sp. nov.			
KT90	*Leucosporidium krtinyense* sp. nov.			
KT91	*Leucosporidium krtinyense* sp. nov.			
KT92	*Leucosporidium krtinyense* sp. nov.			
KT111	*Leucosporidium* sp.	FR719968	97%, NR_073309	

KT95	*Leucosporidium krtinyense* sp. nov.			*L. krtinyense*
KT102	*Leucosporidium krtinyense* sp. nov.			*L. krtinyense*
KT143	*Mrakia curviuscula*	KF036599	100%, KF036599	
KT141	*Mrakia* sp.	KF036599	98%, KF036599	
KT140	*Mrakia* sp.	KF036599	98%, KF036599	
KT136	*Oberwinklerozyma silvestris*	GQ121045	100%, GQ121045	
KT6	*Oberwinklerozyma* sp.	FJ153202	95%, EU872491	
KTG08_1	*Oberwinklerozyma* sp.	NR_073328	90%, NR_073328	
KT248	*Oberwinklerozyma yarrowii*	NR_073328	99%, NR_073328	
KT313	*Oberwinklerozyma yarrowii*	NR_073328	99%, NR_073328	*L. masarykiana*
KT138	*Oberwinklerozyma* sp.	NR_073328	94%, NR_073328	
KT179	*Phaeotremella* sp.	NR_119558	95%, NR_119558	
KT116	*Phaeotremella* sp.	NR_119558	96%, NR_119558	
KT125	*Phaeotremella* sp.	FJ873574	93%, EU252549	
KT127	*Phaeotremella* sp.	FJ873574	93%, NR_073211	
KT128	*Phaeotremella* sp.	EU252549	92%, EU252549	
KT129	*Phaeotremella* sp.	HQ890367	94%, NR_073211	
KT131	*Phaeotremella* sp.	HQ890367	94%, NR_073211	
KT173	*Phaeotremella* sp.	FJ873574	93%, NR_073211	
KT126	*Phaeotremella* sp.	HQ890367	93%, NR_073211	
KT139	*Phenoliferia* sp.	EF151245	97%, EF151249	
KT428	*Pichia* sp.	DQ104712	92%, DQ104714	
KT427	*Pichia* sp.	EF061131	88%, NR_111195	
KT162	*Piskurozyma* sp.	HG324303	84%, NR_073229	
KT168	*Piskurozyma* sp.	HG324303	85%, NR_073229	
KT178	*Piskurozyma* sp.	HG324303	85%, NR_073229	
KT41	*Piskurozyma* sp.	HQ623605	92%, NR_073224	
KT71	*Piskurozyma* sp.	HQ623605	92%, NR_073224	
KT123	*Piskurozyma* sp.	HQ623605	92%, NR_073224	
KT171	*Piskurozyma* sp.	HQ623605	91%, NR_073224	
KT164	*Piskurozyma* sp.	HQ623605	91%, NR_073224	
KT170	*Piskurozyma* sp.	AF444487	94%, NR_073224	
KT172	*Piskurozyma* sp.	AF444487	95%, NR_073224	
KT174	*Piskurozyma* sp.	AF444487	95%, NR_073224	
KT287	*Piskurozyma* sp.	AF444487	94%, NR_073224	
KT297	*Piskurozyma* sp.	EU433985	94%, NR_073224	
KT312	*Piskurozyma* sp.	AF444487	94%, NR_073224	*L. masarykiana*
KT308	*Piskurozyma* sp.	AF444487	95%, NR_073224	*L. masarykiana*
KT307	*Piskurozyma* sp.	AF444487	95%, NR_073224	*L. masarykiana*
KT149	*Piskurozyma* sp.	NR_073224	98%, NR_073224	*Y. mendeliana*
KT146	*Piskurozyma* sp.	FN908210	91%, NR_073224	
KT45	*Pseudohyphozyma* sp.	AB038083	91%, NR_073288	
KT46	*Pseudohyphozyma* sp.	AB038083	91%, NR_073288	
KT324	*Pseudohyphozyma* sp.	AY052491	91%, NR_073288	
KT83	*Pseudohyphozyma* sp.	AB038083	92%, NR_073288	
KT241	*Rhodosporidiobolus colostri*	KX079879	100%, JN246563	
KT269	*Rhodosporidiobolus colostri*	JX188223	100%, JN246563	
KT283	*Rhodosporidiobolus colostri*	KX079888	100%, N246563	
KT321	*Rhodosporidiobolus colostri*	JN636813	100%, JN246563	
KT304	*Rhodosporidiobolus colostri*	JX188223	100%, JN246563	*L. masarykiana*
KT299	*Rhodosporidiobolus colostri*	JX188223	100%, JN246563	*L. masarykiana*
KT265	*Rhodosporidiobolus colostri*	KP714626	99%, JN246563	
KT273	*Rhodosporidiobolus colostri*	KP714626	99%, JN246563	
KT293	*Rhodosporidiobolus colostri*	KX079888	100%, JN246563	
KT27	*Rhodotorula bacarum*	DQ317624	99%, DQ317629	
KT245	*Rhodotorula fujisanensis*	KC865286	100%, AB038090	
KT208	*Saitozyma podzolica*	KC007289	99%, NR_073213	
KT147	*Saitozyma* sp.	KC171330	87%, NR_073213	
KT216	*Saitozyma podzolica*	KM113758	99%, NR_073213	

KT217	*Saitozyma podzolica*	HF558652	99%, NR_073213	
KT218	*Saitozyma podzolica*	HF558652	99%, NR_073213	
KT219	*Saitozyma podzolica*	HF558652	99%, NR_073213	
KT201	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT202	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT203	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT206	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT114	*Solicoccozyma terricola*	JN942242	99%, NR_073221	
KT228	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT203A	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT223	*Solicoccozyma terricola*	HF558655	100%, NR_073221	
KT113	*Sporobolomyces roseus*	KU504594	100%, AY015438	
KT156	*Sporobolomyces roseus*	AM160644	99%, AY015438	
KT124	*Vishniacozyma victoriae*	KU168773	99%, NR_073260	
KT157	*Vishniacozyma victoriae*	KU168773	100%, NR_073260	
KT261	*Vishniacozyma victoriae*	KU182507	100%, NR_073260	
KT262	*Vishniacozyma victoriae*	JX188144	100%, NR_073260	
KT65	*Yamadamyces rosulatus*	FN400944	100%, EU872492	
KT175	*Yamadamyces* sp.	LC126440	93%, EU872492	
KT132	*Yamadamyces* sp.	JQ857037	97%, EU872492	
KT79	*Yamadamyces rosulatus*	FN400944	99%, EU872492	
KT167	*Yamadamyces rosulatus*	FN400944	100%, EU872492	
KT94	*Yamadazyma mexicana*	EU343839	99%, EF568069	*L. krtinyense*

Suplementary table 2: Frequency of occurrence and sequence abundance of *Yurkovia mendeliana, Leucosporidium krtinense* f.a. and *Libkindia masarykiana* in the litter and soil of the studied temperate forest sites according to sequencing results of environmental DNA. The frequency of occurrence indicate the percentage of the sites in which the taxon was observed. The abundance values (mean and SD) represent the share of the total yeast community and are expressed as percentages. Numbers in brackets indicate the number of sites where the sequences of a given species were identified

		Yurkovia mendeliana	*Leucosporidium krtinense* f.a	*Libkindia masarykiana*
	PYCC	6884	6879	6886
	CBS	14273	14304	14275
	DSM	DSM101889	DSM101892	DSM101891
	Source	Beech litter	Beech litter	Oak litter
GenBank accession numbers	ITS	KU187884	KU187882	KU187885
	D1/D2	KU187888	KU187886	KU187889
	TEF1	KX620743		KX620745
	RPB1	KX620742		KX620744
Litter	Mean relative abundance (%) ± SD	2.0 ± 1.6 (28)	6.2 ± 12.2 (27)	2.2 ± 1.5 (68)
	Maximal relative abundance (%)	6.7	63.4	5.7
	Sites where relative abundance > 10%	-	3	-
	Frequency of occurrence on spruce sites (n = 11) (%)	27.3	36.4	18.2
	Frequency of occurrence on beech sites (n = 18) (%)	5.6	27.8	27.8
	Frequency of occurrence on oak sites (n = 7) (%)	14.3	14.3	42.9
	Frequency of occurrence on mixed sites (n = 44) (%)	27.3	34.1	45.5
Soil	Mean Relative abundance (%) ± SD	0.3 ± 0.1 (7)	0.8 ± 0.7 (30)	0.6 ± 0.3 (56)
	Maximal relative abundance (%)	0.4	2.8	1.1
	Sites where relative abundance > 2%	-	3	-
	Frequency of occurrence on spruce sites (n = 11) (%)	9.1	36.4	18.2
	Frequency of occurrence on beech sites (n = 18) (%)	0.0	50	0.0
	Frequency of occurrence on oak sites (n = 7) (%)	0.0	14.3	0.0
	Frequency of occurrence on mixed sites (n = 44) (%)	13.6	34.1	6.8

IV

Forest soil yeasts: Decomposition potential and the utilization of carbon sources

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Research Highlights

- Yeasts have specific nutritional strategy dissimilar from other soil fungi
- The ability of yeasts to decompose biopolymers appears to be limited
- Enzyme activity of yeasts is largely associated with yeast cell surfaces
- Yeasts can utilize wide spectrum of simple carbon sources
- Yeasts may act as mutualists utilizing products of decomposition provided by others

Abstract

Fungi that inhabit upper forest soil horizons are important decomposers of dead plant organic matter. Fungi living in soils can be divided into two functional groups: filamentous, multicellular fungi and predominantly unicellular yeasts. Due to an inability to efficiently translocate nutrients, the nutritional mode and realized niche of yeasts in the soil is expected to differ from that of filamentous fungi. Soil yeasts comprise a systematically artificial group of fungi, some of which are able to switch between filamentous and unicellular growth. In this study, we explored the decomposition potential and carbon utilization profiles of twenty-five dominant yeasts from the topsoil of a temperate forest. The results indicated that despite taxonomic heterogeneity, yeasts represent a fungal group with a specific nutritional strategy that is dissimilar from other tested soil fungi. Yeast isolates frequently produced enzymes involved in the degradation of hemicellulose: β-xylosidase, α-galactosidase, β-galactosidase, β-mannosidase, β-glucuronidase, and arabinosidase activity was observed in 44-92% of strains. The ability to utilize cellulose was relatively common, with 84% of strains producing exocellulase, and all of the tested yeast strains exhibited high β-glucosidase activity. The activity of laccase, an enzyme that potentially contributes to the transformation of lignin and other phenolics, was rarely observed, and only in association with yeast cell walls. Chitinase activity was present in 72% of yeast strains, although it was typically low. While the efficient decomposition of hemicellulose, cellulose or chitin appeared to be restricted to only a few taxa, the results of carbon source utilization assays indicated that most yeasts could efficiently act as mutualists, utilizing the decomposition products generated by other microbes. Importantly, a large fraction of total enzyme activity was associated with yeast cell surfaces. This adaptation likely ensures that the

decomposition products are produced at the cell surface of the unicellular microorganisms and are readily available to the organisms producing the enzymes.

1. Introduction

The soil microbial community is a complex assemblage of prokaryotic and eukaryotic organisms, including archaea, bacteria, algae, protists and fungi. Each of these groups is more prominent in habitats that specifically favour their survival. For example, mineral soil layers are more suitable for unicellular organisms (bacteria, archaea and some yeasts) than for filamentous fungi, which primarily inhabit litter and coarse woody debris (Baldrian, 2017; Yurkov, 2017). A diversity of traits, both morphological and physiological, results in a more efficient distribution of microorganisms between microhabitats in the soil and adjacent substrates. Fungi living in soils can be divided into two functional groups, filamentous, multicellular fungi and predominantly unicellular yeasts. The hyphae of filamentous fungi allow them to bridge sites with unfavourable conditions or limited nutrients to access, translocate and utilize heterogeneously distributed resources. Filamentous growth enables fast, horizontal dispersal and is important for colonization of certain niches, e.g., decomposing bulky substrates (Boer et al., 2005). Functionally, yeasts can be considered to be especially adapted to thrive in liquid or semi liquid mediums with high concentrations of easy-to-use nutrients (Lachance & Starmer, 1998). However, some yeast taxa are capable of forming a filamentous stage and are thus dimorphic during different stages of their life cycle. Whether or not the transition between filamentous and unicellular yeast growth has direct implications on the physiological adaptations and functions of soil fungi is unclear.

Substrate decomposition and carbon utilization by various groups of filamentous fungi that are considered to be primary degraders of organic matter in temperate forest soil and litter have been frequently investigated (e.g., Martinez et al., 2005; Baldrian et al., 2011; Eichlerová et al., 2015). However, despite the evidence that yeasts represent an integral part of soil fungal communities (e.g., Yurkov et al., 2012b; Yurkov et al., 2016b; Mašínová et al., 2017a), the role of unicellular fungi in the decomposition and dissemination of soil carbon in soils has received less attention. The majority of soil yeasts have been regarded as saprotrophs that contribute to C mineralization processes in the environment by utilizing various carbon compounds. In his review on soil yeasts, Botha (2006) noted that most are able to utilize the products of the enzymatic hydrolysis of lignocellulosic plant materials, such as L-arabinose, D-xylose, cellobiose and intermediates of lignin degradation, i.e., ferulic acid, 4-hydroxybenzoic acid and vanillic acid (e.g., Henderson, 1961; Sampaio, 1999; Slavikova and Vadkertiova, 2000; Mestre et al., 2011). On average, basidiomycetous yeasts have been reported to utilize a wider spectrum of carbon sources, including low-weight aromatic compounds, than ascomycetous yeasts, which have a copiotrophic lifestyle (Fonseca, 1992; Sampaio, 1999; Middelhoven, 2006; Botha 2006).

Although yeasts have repeatedly been isolated from decaying plant material, these studies were limited to the description of novel species (e.g., Kurtzman, 2001; Middelhoven, 2006; Middelhoven and Kurtzman, 2007; Peter et al., 2003). Our knowledge of the functional relationships of yeasts with respect to wood degradation is limited to a few studies that demonstrated either utilization of plant-derived compounds (e.g., Sampaio 1999; Middelhoven, 2006) or community alteration in response to coarse woody debris (e.g., Yurkov et al., 2012). A growing number of studies have utilized culture-independent techniques to assess fungal

communities in soils or in the context of wood decomposition (e.g., Buee et al., 2009; Voříšková and Baldrian, 2013; Mašínová et al., 2017a). Basidiomycetous yeasts have been reported to be among the most numerous fungal operational taxonomic units (OTU, as a proxy for species) in culture-independent surveys (e.g., Buee et al., 2009; Voříšková and Baldrian, 2013; Mašínová et al., 2017a). Yeasts assigned to the polyphyletic genera *Cryptococcus* and *Trichosporon* were demonstrated to obtain carbon from cellulose (Štursová et al., 2012), and the relative abundance of *Trichosporon* was also observed to increase during the decomposition of oak litter following the depletion of sugars (Voříšková and Baldrian, 2013). Notwithstanding the above evidence, the role of yeasts in the decomposition of recalcitrant biopolymers is often neglected in the literature. Yeasts are evened with saccharolytic organisms resident to soils or associated only with nutrient-rich habitats.

The aim of this study was to characterize the decomposition potential and the spectrum of carbon source utilization (metabolic fingerprints) by yeasts isolated from the topsoil of a temperate forest. Strains representing the 25 most prominent yeast species were selected based on their abundance in the analysis of environmental DNA (Mašínová et al., 2017a). We review common views on the role of yeasts in soil and challenge them using our experimental data to demonstrate that (1) soil yeasts are able to utilize wide spectra of carbon sources, including mono- and oligosaccharides and some low-weight aromatic compounds, while their ability to decompose recalcitrant polymers is frequently regarded as low; and (2) soil yeasts produce only a limited spectrum of extracellular enzymes, and the activity of these enzymes is lower than those in filamentous fungi. Additionally, we hypothesize that the adaptation to unicellular growth also affects the enzymatic capabilities of yeast. Because yeasts have limited horizontal dispersal

abilities, they assimilate nutrients by coming into direct contact with a substrate and do not translocate nutrients as filamentous fungi do. We hypothesize that a substantial portion of enzymatic activity may be associated with yeast cell walls to secure products of enzymatic degradation for the enzyme-producing cell. This strategy is different from those of filamentous decomposers, such as rot fungi, which secrete most of the hydrolytic enzymes into the environment.

2. Materials and Methods

2.1 Study site, sample collection and soil analysis

Samples were collected in the area of the Training Forest Enterprise Masaryk Forest Křtiny (Křtiny Forest). Křtiny Forest, located north of Brno, Czech Republic (16°15' E, 49°15 N), has a total area of 103 km^2 and is covered by a mixed temperate forest, with beech, oak and spruce as the most common tree species. Sampling for fungal DNA community analyses was performed at 80 sites during 2013 in a preceding study (Mašínová et al., 2017a). Soil and litter samples were collected in monoculture stands of *Fagus sylvatica*, *Quercus petraea* agg., and *Picea abies* as well as in mixed tree stands. At 18 of these sites (dominated by monocultures of *Fagus sylvatica*, *Quercus petraea* agg., or *Picea abies* [6 each]), samples were collected four times during a year. Litter samples were cut into approximately 0.25-cm^2 pieces, and soil was sieved through a 5-mm sieve and homogenized. Soil was freeze-dried and stored at -80°C until

DNA extraction. Physicochemical parameters properties of the soils and litter were measured and published previously (Mašínová et al., 2017a).

The collection of soil and litter used to isolate yeast strains was performed at the 18 monoculture sites multiple times between October 2013 and April 2014. Fresh samples were transferred to the laboratory and maintained at 4°C. Litter and soil samples were processed as described above. The isolation of yeasts was carried out within 48 h of sample collection.

2.2 Extraction and analysis of environmental DNA

Details of the DNA isolation from soil and litter samples, ITS2 amplification, sequencing and data analyses were described by Mašínová et al. (2017a). Briefly, total DNA was extracted in triplicate from 250 mg of soil using a modified Miller method (Ságová-Marečková et al., 2008) and a previously described protocol (Žifčáková et al., 2016). The PCR amplification of the fungal ITS2 region from DNA was performed using gITS7 and ITS4 barcoded primers (Ihrmark et al., 2012), with three PCR reactions per sample. The sequencing of the fungal amplicons was performed on an Illumina MiSeq. The sequencing data were processed using the SEED 1.2.1 pipeline (Větrovský and Baldrian, 2013). Pair-end reads were merged using fastq-join (Aronesty, 2013), and the ITS2 region was extracted using the ITS Extractor 1.0.8 (Nilsson et al., 2010) before processing. Chimeric sequences were detected using UCHIME within Usearch 7.0.1090 (Edgar, 2013) and excluded from the subsequent analyses. For further analyses, datasets containing 10,000 randomly chosen sequences from each sample were used. For the 18 repeatedly sampled sites, a fungal community dataset for each site was created by averaging the

four seasonal samplings to cover the seasonal variability of the community composition. OTUs were constructed at a 97% similarity level and yeast OTUs were further identified to the species level with a phylogenetic analysis (Mašínová et al., 2017a) using the UNITE version 7.1. dataset (Koljalg et al., 2013).

2.3 Isolation of yeast strains

One gram of soil or litter was suspended in 5 mL of sterile, demineralized water, serially diluted and plated on yeast glucose agar (YG; Cooney and Emerson, 1964) supplemented with chloramphenicol (0.2 g L^{-1}). Plates were inoculated in triplicate and incubated at 4°C for 14 days to prevent the rapid development of moulds. Colonies were differentiated into macro-morphological types using dissection microscopy and counted, and 1-2 representatives of each colony type per plate were transferred into pure culture. Yeast strains have been preserved in the fungal collection of the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

2.4 Yeast strain identification and selection of strains for physiological characterization

For the molecular identification of isolates, total genomic DNA was extracted using an ArchivePure DNA Yeast/Gram positive Bacteria Kit (5 PRIME, Inc. Gaithersburg, USA). The ITS1F and NL4 primers (White et al., 1990; Gardes and Bruns, 1993) were used to amplify a region of the nuclear ribosomal DNA (rDNA), which covers the ITS region (i.e., internal

transcribed spacers 1 and 2 (ITS1, ITS2), and the 5.8S rRNA gene) and the D1/D2 domains of the large subunit (26S/28S or LSU). Further details of the PCR conditions and sequencing are described in Mašínová et al. (2017b). For species identification, the obtained nucleotide sequences (both ITS and LSU) were compared with sequences deposited in the NCBI GenBank (http://www.ncbi.nlm.nih.gov), UNITE (https://unite.ut.ee/index.php) and MycoBank (www.mycobank.org) databases. ITS2 sequences obtained by amplicon sequencing of soil and litter samples were matched to ITS2 sequences of the identified isolates (best hits with a similarity of > 97% and a coverage of > 90%). Isolates representing the most frequent OTUs in the environmental DNA libraries were selected for further characterization. The number of OTU reads matching an isolate was taken as a measure of its abundance.

2.5 Growth and enzyme production of the yeast isolates

Yeast strains were cultivated in 100-mL flasks with 10 mL of liquid malt extract media. Triplicate flasks were inoculated with 200 µL of cell suspension that was adjusted to an optical density at a wavelength of 600 nm (OD_{600}) of 0.1 and were cultivated for 14 days at 15°C in the dark. The OD_{600} was measured after 3, 5, 8, 10, 12, and 15 days of incubation as a proxy for yeast abundance. After 15 days, cultures were harvested and used for enzyme activity measurements. For each yeast culture, a 10 mL aliquot was centrifuged for 2 minutes at 11,357 g, after which the yeast cells were separated from the culture supernatant, washed twice in 50 mM acetate buffer (pH = 5) and resuspended in 20 mL of the same buffer. In addition, 10 mL of acetate buffer was used to adjust the pH of the culture supernatant. Both the resuspended cell pellet and the culture

liquid were used for measurements of enzyme activities to represent the cell wall-bound and extracellular fractions of enzymatic activity, respectively. The activities of α-galactosidase (EC 3.2.1.22), β-galactosidase (EC 3.2.1.23), α-glucosidase (EC 3.1.2.20), β-glucosidase (EC 3.2.1.21), β-glucuronidase (EC 3.2.1.31), β-mannosidase (EC 3.2.1.25), β-xylosidase (EC 3.2.1.37), acidic phosphomonoesterase (phosphatase, EC 3.1.3.2), arabinosidase (EC 3.2.1.55), cellobiohydrolase (exocellulase, EC 3.2.1.91), chitinase (EC 3.2.1.14), and lipase (EC 3.1.1.3) were assayed as previously described (Baldrian, 2009) using methylumbelliferyl-based fluorescent substrates. The laccase activity in extracts was measured by monitoring the oxidation of ABTS in 50 mM of acetate buffer (pH = 5) at 420 nm (Bourbonnais and Paice, 1990). Total enzyme activities were calculated as sums of the activity in the extracellular supernatant and on yeast cells. Enzymes activities below the detection limit (<2 nM min^{-1} ml^{-1}) were considered as a lack of activity.

API ZYM™ (Biomerieux, France), a laboratory kit for the semi-quantitative analysis of the production of the selected hydrolytic enzymes by microorganisms, was used to compare enzyme production of yeasts with those of other fungi. The tests were performed according to Eichlerová et al. (2015). Yeast strains were cultured on MEA (20 g L^{-1} malt extract, 20 g L^{-1} agar) at 25°C for 14 days and cells from 1 cm^2 of the surface of a culture were harvested and suspended in 2 mL of distilled H_2O. A 65 µL aliquot of the resulting suspension was inoculated into each well of an API-ZYM strip. Enzyme activities were evaluated after 4 hours of incubation at 37°C and were recorded on a scale of 0 (no activity) to 5 (≥40 nmol of product released).

Phenotype microarray testing of carbon sources was examined using the BIOLOG MicroStation and YT MicroPlates following the manufacturer's instructions (Biolog Inc.,

Hayward, CA, USA). Yeasts were incubated on potato-dextrose agar (PDA, Difco) at room temperature. Yeast biomass was harvested from PDA, suspended in sterile water and the turbidity was adjusted to the transmittance value provided by the manufacturer. YT MicroPlates were incubated at 20°C and measured after 1, 2 and 3 weeks, with the optical density recorded at 590 and 750 nm. Some yeast species are inhibited by the tetrazolium violet redox used in Biolog MicroPlates, so the YT MicroPlate is configured with both metabolism and turbidity tests. The ability to utilize particular substrates by individual strains was recorded as positive when the activity was rated as "borderline" or "positive" by the scanning device.

2.6 Statistics

The software package Past 3.15 (http://folk.uio.no/ohammer/past/) was used for statistical analyses. Three dimensional non-metric multidimensional scaling (NMDS) with Euclidean distances was used to analyse the variability in enzyme production among yeasts and other previously characterized ecophysiological groups of fungi (Eichlerová et al., 2015). One-way PERMANOVA was used to test for the differences in enzyme production among yeasts, saprotrophic soil micromycetes and litter-decomposing basidiomycetes. Differences in the production of individual enzymes were tested using the Mann-Whitney U test, which assumes that the measurements are on a rank-order scale but does not assume normality of data. In all cases, differences at $P < 0.05$ were regarded as statistically significant.

3. Results

Cultivable yeast communities

A total of 205 yeasts strains were isolated from YEG plates inoculated with soil and litter samples (for additional details, see Mašínová et al., 2017b). Among them, ITS2 sequences of 45 cultures matched the molecular OTUs with a similarity > 97% and coverage > 90%. Out of 45 cultures that matched to the molecular OTUs, 25 strains represented OTUs with the highest read numbers and were selected for further characterization.

The 25 yeast strains belonged to three lineages of fungi, Saccharomycotina, class Saccharomycetes (four species), Agaricomycotina, class Tremellomycetes (16 species) and Pucciniomycotyna, class Microbotryomycetes (five species) (Fig. S1; Tab. 1). According to the genetic distances and the physiological profiles, eleven yeast taxa represented potential new species, out of which two novel species have been recently described as *Libkindia masarykiana* and *Yurkovia mendeliana* (Mašínová et al., 2017b).

Yeast strains isolated in this study represented OTUs that accounted for 42.3 and 29.4% of the total read counts corresponding to yeasts in soil and litter, respectively. The three most abundant species were *Solicoccozyma terricola* (strain KT203A), *Apiotrichum porosum* (strain KT240), and *Cutaneotrichoporon moniliforme* (strain KT290), while other yeasts were observed with lower read numbers in the analysed environmental DNA libraries (Tab. 1).

The doubling times of isolates ranged from 12.5 h for *Oberwinklerozyma yarrowii* KT313 to 33.6 h for *Heterocephalacria* sp. KT97. Doubling rates of 13 yeasts were lower than 20 hours

(Tab. 1). *Tremella* sp. KT147 and *Piskurozyma* sp. KT162 formed pellets under the selected conditions such that reliable OD measurements could not be obtained during the experiment.

3.1 Production of enzymes by soil yeasts

Among the enzyme activities assayed semi-quantitatively using API ZYM^{TM}, most yeast isolates showed high activity of leucine aminopeptidase, acid phosphatase, α-glucosidase and β-glucosidase, while the activities of α-fucosidase, α-manosidase, β-galactosidase and β-glucuronidase were either absent or low. None of the studied strains produced all 15 assayed enzymes. A total of 14 enzymes were produced by *Apiotrichum porosum* (strain KT240, negative for α-fucosidase), and 13 enzymes were produced by *Phaeotremella* sp. KT179, *Cutaneotrichosporon moniliforme* (strain KT290), *Solicoccozyma terricola* (strain KT203A), *Piskurozyma* sp. KT312 and *Piskurozyma taiwanensis* (strain KT170). Strains *Leucosporidium krtinense* (strain KT96) and *Bannozyma* sp. KT138 produced only 6 and 8 enzymes, respectively (Fig. S2).

The NMDS analysis showed that the composition and activity of enzymes produced by soil yeasts were different from other ecophysiological groups of filamentous fungi, i.e., litter decomposing Basidiomycota, white-rot fungi, and brown-rot fungi (Fig. 1). Wood-associated ascomycetes and saprotrophic micromycetes showed enzymatic capabilities that overlapped with those of yeasts. However, a quantitative approach showed that the enzymatic abilities of yeast were significantly different from all other functional groups of fungi (PERMANOVA, $p<0.0001$). Overall, enzyme activities measured in yeast cultures were lower than in other groups of fungi.

Significant differences between the three phylogenetic groups of yeasts (i.e., classes Tremellomycetes, Mycrobotryomycetes and Saccharomycetes) were not observed (Fig. 1).

Compared to fungi originating from the same environment (saprotrophic micromycetes and litter-decomposing basidiomycetes), yeast exhibited lower activities of N-acetylglucosaminidase, alkaline phosphatase and β-galactosidase but higher activities of leucine and valine aminopeptidase and α-glucosidase. The activities of esterase, lipase, β-glucuronidase, α-mannosidase, acid phosphatase, α-galactosidase and β-glucosidase in yeasts were lower than in the litter-decomposing fungi. α-Fucosidase activity was very low in all studied groups of fungi (Fig. 2).

The production of 13 extracellular and cell-wall associated enzymes was measured in liquid cultures. Only *Leucosporidium krtinense* (strain KT96) and *Heterocephalacria* sp. KT97 produced all enzymes. A total of 18 species produced 8 to 12 enzymes, and 5 species produced between 4 and 7 enzymes. β-Glucosidase, acid phosphatase and lipase were produced by all strains and exhibited the highest activities. The least produced enzyme was laccase, which was only produced by five strains. Its activity was typically low, as was the case for hemicellulases β-galactosidase, β-glucuronidase and arabinosidase. The analyses showed that a substantial fraction of enzymes was bound to the cell wall.

Over 70% of the activity of acid phosphatase, β-glucosidase and laccase was associated with the cell wall fraction, whereas less than 30% of α-galactosidase, β-mannosidase, and β-glucuronidase activity was in the bound fraction. Interestingly, laccase was only detected as a cell wall-associated enzyme (Fig. 3, Tab. S1).

3.2 Microarray phenotyping of yeasts

Metabolic fingerprints obtained with Biolog YT MicroPlates were distinct between the tested yeasts (Fig. 4, Tab. S2). The least oxidized substrates were carboxylic acids, which were only utilized by 4-5 yeasts. Among these compounds, succinic and D-gluconic acids were oxidized more frequently. The oxidation of glycerol was observed in 8 of the 25 assayed yeasts. Although a colour reaction was visible in the aforementioned experiments, the level of oxidation was very low. Substrates that were oxidized by more than 20 strains were α-glucose-containing carbohydrates, such as the disaccharides sucrose and trehalose, the trisaccharide D-melezitose and the polysaccharides dextrin and inulin. Six yeasts, *Sporobolomyces roseus* (strain KT156), *Holtermaniella wattica* (strain KT242), *Piskurozyma taiwanensis* (strain KT170), *Piskurozyma* sp. KT162, *Cutaneotrichosporon moniliforme* (strain KT290) and *Tremella* sp. KT147, were able to oxidize 30 or more substrates. In contrast, *Phaeotremella* sp. KT179 and *Fonsecazyma* sp. KT301 were able to oxidize a very limited number of substrates, 2 and 7, respectively. None of the tested yeasts assimilated all of the tested carbon substrates. The monosaccharides α-D-glucose and D-xylose, the disaccharides sucrose, maltose and D-trehalose, the trisaccharides D-melezitose and maltotriose, and the polysaccharides dextrin and pentose alcohol ribitol (adonitol) were assimilated by most strains (21 out of 25). The polyols glycerol and i-erythritol were utilized by only 10 and 9 yeasts, respectively. Yeasts that were rapidly able to convert substrates into biomass (strong coloration and high turbidity values) included *Piskurozyma* sp. KT162, *Kwoniella pini* (strain KT266), *Holtermaniella wattica* (strain KT242) and *Piskurozyma taiwanensis* (strain KT170). The least number of substrates was assimilated by *Candida*

railenensis (strain KT592), *Fonsecazyma* sp. KT301, and *Tremella* sp. KT147. Interestingly, *Phaeotremella* sp. KT179, which was able to oxidize only 2 substrates, was able to assimilate 29 substrates. Some yeast species are inhibited by the tetrazolium violet redox reagent used in Biolog MicroPlates, thus, discrepancies between the oxidation and turbidity tests in YT MicroPlates are expected.

4. Discussion

Almost a half of the total terrestrial carbon (C) is concentrated in forests, with the majority shared between soils and live biomass (44% and 42%, respectively) followed by deadwood (8%) and litter (5%) (Pan et al., 2011). Geographically, approximately half of the C pool (55%) is stored in tropical forests, whereas boreal and temperate forests contribute 32 and 14%, respectively. While tropical forests primarily store C in live biomass (56%), boreal and temperate forests show the opposite trend, with major C stores located in soils and dead plant material (Pan et al., 2011). It has been estimated that up to ninety percent of the annual plant biomass is not consumed by herbivores and enters the dead organic matter pool (Gessner et al., 2010). This organic carbon is trapped in complex substances, including lignin, cellulose and humic acids, which are not readily available for soil inhabitants, such as invertebrates and protists.

Fungi that inhabit forest topsoil are the most important decomposers of dead plant material, converting recalcitrant lignocellulosic compounds into smaller molecules that are available for other organisms. Yeast fungi occur in various soils worldwide and are more

prominent in the temperate zone and in tundra (Vishniac, 2006; Botha, 2011; Yurkov et al., 2012a). Even though yeasts represent a substantial part of these communities (Bueé et al., 2009; Yarwood et al., 2010; Voříšková and Baldrian, 2013; Mašínová et al., 2017a), their involvement in decomposition processes remains largely unknown. Our current understanding suggests that over 100 yeast species use forest soils as their primary habitat (Yurkov, 2017). However, soils also serve as a refugium for allochthonous species entering this habitat with fruits and plant residues (Lachance and Starmer, 1998; Botha, 2011; Yurkov et al., 2008). Considerable effort has been made in the past to distinguish between autochthonous and allochthonous soil yeasts based on their isolation frequencies, abundance and physiological traits (reviewed in Phaff and Starmer, 1987; Babjeva and Chernov, 1995). However, this task is difficult, or even impossible, in culture-independent surveys. Due to several experimental biases, sequence read counts may not well reflect total numbers (abundance) of distantly related species, but they are often regarded as a good proxy for species occurrence. For example, low read numbers observed for some OTUs in our study suggests an allochthonous origin of these yeasts. However, these OTUs represented well-known soil-borne yeasts (e.g., *Apiotrichum porosum*, *Cutaneotrichosporon moniliforme*, *Holtermanniella wattica* and *Solicoccozyma terricola*) that were isolated with manageable effort in the present study and in numerous surveys in the past (Slavikova and Vadkertiova, 2000; Vishniac, 2006; Mestre et al., 2011; Takashima et al., 2012; Yurkov et al., 2012b; Yurkov et al., 2016a; Yurkov et al., 2016b; Mašínová et al., 2017a; Mašínová et al., 2017b). Other species, such as *Leucosporidium krtinense*, *Phaeotremella* spp. and *Piskurozyma* spp., were detected with a large number of reads (Mašínová et al., 2017a; Tab. 1) and their association with forest soils appears to be important.

Our previous high-throughput sequencing study demonstrated that the soil yeast community in the studied temperate forest was highly uneven, represented by a few highly abundant strains and many rare taxa, while the yeast community in litter was more uniform (Mašínová et al., 2017a). Yeast strains isolated in this study together accounted for 42.3 and 29.4% of total read counts corresponding to yeasts in soil and litter, respectively. Most of the yeast species belonged to the phylum Basidiomycota, in agreement with previous reports concerning forest soils (e.g., Maksimova and Chernov, 2004; Yurkov et al., 2012; Yurkov et al., 2012b; Yurkov 2017). Basidiomycetous yeasts accounted for more than 90% of sequence reads in the previous study by Mašínová et al. (2017a) and were among dominating OTUs in other culture-independent surveys (e.g., Bueé et al., 2009; Yarwood et al., 2010). This is the first study to compare directly yeast biodiversity assessments in soils using conventional cultivation and environmental DNA sequencing techniques. The present study showed good overlap between cultivated and detected species, confirming that soil yeasts can be fairly well cultured (Yurkov et al., 2012; Mašínová et al., 2017a).

Several adaptations may facilitate the ability of yeast capability for surviving in soil substrates. For example, species frequently observed in soil have been shown to be able to grow in media with low concentrations of nutrients (Vishniac 1982; Kimura et al. 1998; Botha, 2006). Oligotrophy is believed to provide an advantage during competition with other soil microbes. Sugar turnover rates in soils are very high, therefore the uptake of monosaccharides by microorganisms occurs in seconds to minutes (Gunina and Kuzyakov 2015). However, our results show that the *in vitro* growth rates do not correlate with the detected abundance of yeasts, and the most abundant yeast (*Solicoccozyma terricola*) displayed one of the slowest growth rates

in the study. In addition, compared to soil bacteria and microfungi, the growth rates recorded for the studied soil yeasts were within a somewhat narrow range (Baldrian et al., 2011; Lladó et al., 2016).

It has been repeatedly demonstrated that saprotrophic basidiomycetous fungi are able to break down plant litter and wood more rapidly and efficiently than Ascomycetes (e.g., Osono and Takeda, 2002, 2006). The soil yeasts assayed in this study showed a similar trend, with higher and broader enzymatic activity observed in basidiomycetous yeasts. Although some exceptions exist, most ascomycetous yeasts tested to date have efficiently utilized simple sugars, while their ability to degrade complex polysaccharides was reported as absent or low. Therefore, they are often considered to be primary inhabitants of fresh litter or rhizospheres (e.g., Middelhoven, 2006; Mestre et al., 2011). Nevertheless, a few ascomycetous yeasts have been isolated from forest soils, including *Candida railenensis* and *Lachansea thermotolerans*. Here, the activity of four ascomycetous yeasts did not differ substantially from basidiomycetous yeasts, some of which also displayed a limited physiological capability.

In this study, yeasts frequently produced enzymes involved in the degradation of hemicellulose, with β-Xylosidase, α-galactosidase, β-galactosidase, β-mannosidase, β-glucuronidase, and arabinosidase produced by 44-92% of strains. This observation is in line with earlier reports of the ability of some yeasts isolated from forests to assimilate different types of hemicelluloses, such as xylan or galactomannan (Jiménez et al., 1991; Shubakov, 2000; Middelhoven, 2006). Although the activity of hemicellulases in the analysed strains was low in most cases, yeasts showed a strong ability to utilize hemicellulose-derived monosaccharides, such as arabinose and xylose (see also Kurtzman et al., 2011; Mestre et al., 2011). The ability to

utilize cellulose was rather common among the studied species, with 84% of strains producing exocellulases, although their activity was often low. Interestingly, a yet to be described Tremellomycete (*Tremella* sp. KT147) exhibited the considerable activity of this enzyme. Cellulolytic activity has only rarely been reported in yeasts isolated from rotten wood (Jiménez et al., 1991; Middelhoven, 2006), but Štursová et al. (2012) showed that tremelloid yeasts assigned to the previously polyphyletic genera *Cryptococcus* and *Trichosporon* acquired C from ^{13}C-cellulose added to soil. In our study, all yeasts displayed a high activity of β-glucosidase. This enzyme can hydrolyse cellobiose (a product of cellulose hydrolysis) into two glucose molecules. The ability to utilize this disaccharide seems to be relatively common among yeasts (reviewed by Botha, 2006; Kurtzman et al., 2011), and most of the strains tested in our experiments oxidized and assimilated this compound. These observations may indicate that even though cellulose decomposition by yeasts is probably rare, they can efficiently use products of cellulose degradation and act as potential commensals of cellulolytic filamentous fungi.

The activity of laccase, an enzyme that potentially contributes to the transformation of lignin and other aromatic compounds, is rather widespread among filamentous fungi (Eichlerová et al., 2015). A limited number of yeasts have been tested for this enzyme, although laccase plays an important role in the virulence of the human pathogen *Cryptococcus neoformans* (Zhu and Williamson, 2004). Studies performed to date suggest that laccase activity is present in other Tremellomycetes, including the soil-related yeasts *Naganishia albida* (cited as *Cryptococcus albidus*), *Papiliotrema laurentii* (cited as *Cryptococcus laurentii*), *Saitozyma podzolica* (cited as *Cryptococcus podzolicus*), and *Apiotrichum multisporum* (cited as *Trichosporon akiyoshidainum*) (Petter et al., 2001; Ikeda et al., 2002; Bovers et al., 2008; Pajot et al., 2011). In our study,

laccase activity was detected in a few species, always in association with yeast cell walls. Interestingly, the laccase of *Cryptococcus neoformans* is localized in the cell wall (Zhu and Williamson, 2004). Although lignin decomposition is not a frequently reported trait in yeasts, some indications of the utilization of this compound exist (Jiménez et al., 1991). Several yeasts have previously been reported to utilize phenolic compounds that arise during lignin degradation (e.g., Henderson, 1961; Sampaio 1999; Middelhoven, 2004; Middelhoven, 2006). Whether laccases or other hydrolytic enzymes are involved in the degradation of aromatic compounds by yeasts requires additional studies. We also believe that whole-genome analyses will shed the light on the diversity of enzymes in soil yeasts and increase the number of yeasts known to bear laccases.

Even though the ability to produce chitinase (N-acetylglucosaminidase) was present in 72% of tested yeast strains, the activity of this enzyme was typically low, with just a few exceptions. Similarly, yeasts from rotten wood, which is rich in fungal biomass, were unable to grow on colloidal chitin in a previous study (Middelhoven 2006). It has been previously reported that the production of chitinolytic enzymes is widespread among both ascomycetous and basidiomycetous filamentous fungi isolated from the forest environment (Lindahl and Finlay, 2006; Baldrian et al., 2011). Therefore, the soil yeasts assayed in this study seem to be an exception in this respect. Chitinases were reported to be essential for the sexual development of *Cryptococcus neoformans* (Baker et al., 2009). A possible role of chitinases in defence against other fungi has been demonstrated with the yeasts *Papiliotrema laurentii* and *Candida oleophila* (Bar-Shimon et al., 2004; Yu et al., 2008). Several soil-related yeasts, including *Naganishia albida* (cited as *Cryptococcus albidus*), *Papiliotrema laurentii* (cited as *Cryptococcus laurentii*)

and *Vanrija humicola* (cited as *Cryptococcus humicolus*) have been reported to express chitinases (Buzzini and Martini, 2002). Our results show that many yeasts were able to use the final product of chitin degradation (N-acetylglucosamine), suggesting that the importance of chitinases for soil yeasts, either as enzyme producers or as commensals, cannot be ruled out. It is important to note that multiple yeasts, identified as members of the polyphyletic genera *Candida*, *Cryptococcus*, *Trichosporon* and *Asterotremella* (currently *Vanrija*; Trichosporonales) were found to be enriched on fungal mycelia that decomposed in the soil (Brabcová et al., 2016). The utilization of chitin offers access to carbon and nitrogen, making it highly attractive in the nutrient-limited forest environment (Date, 1973; Reich et al., 2006). Compared to saprotrophic soil micromycetes and litter decomposing Basidiomycota, yeasts exhibited higher activities of leucine and valine aminopeptidases, thus, it seems that proteins are important C and N sources for them.

The results of our study suggest that yeasts represent a fungal group with a unique nutritional strategy that is dissimilar from other fungi. While the efficient decomposition of cellulose, chitin or hemicelluloses is probably restricted to only to a few yeast taxa, most yeasts can efficiently act as mutualists that utilize the products of decomposition provided by other microbes. This view is supported by the observations of yeasts associated with decomposing litter and fungal mycelia (Voříšková and Baldrian, 2013; Brabcová *et al.*, 2016) as well as the accumulation of C from cellulose (Štursová *et al.*, 2012). Considering the inability of yeasts to translocate nutrients in space, a combination of the localization of enzymes in the cell wall, the acquisition nutrients from microbial recycling and oligotrophy results in an efficient living strategy. Both culture-dependent and culture-independent studies suggest that soil yeasts are more prominent in temperate and boreal soils, where decomposition rates are slow. Although the

C sources most frequently utilized by yeasts in our experiment were mono- and oligosaccharides, many soil yeasts are polytrophic. There is a growing body of evidence that basidiomycetous yeasts possess diverse enzymes that can be activated during certain stages of their life cycle. It is important to recall that many basidiomycetous yeasts (including relatives of some soil-related lineages) are dimorphic mycoparasites that switch between filamentous and unicellular stages (Begerow et al., 2017; Oberwinkler 2017). Accordingly, their interactions with wood decomposers might change from commensalism to parasitism, which can explain the polytrophy and diverse enzymatic capabilities observed in our study. It further suggests that the role of yeasts in soils is more than copiotrophs relying on nutrients from filamentous fungi and soil bacteria. The capabilities and functions of soil yeasts are far more diverse than those routinely measured in the lab. Thus, future studies that address decomposition in forest soils using meso- and microcosms, metatranscriptomics and stable isotope probing should include fungi commonly considered as yeasts.

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References

Aronesty, E., 2013. Comparison of Sequencing Utility Programs. The Open Bioinformatics Journal 7, 1-8.

Baker, L.G., Specht, C.A., Lodge, J.K., 2009. Chitinases are essential for sexual development but not vegetative growth in *Cryptococcus neoformans*. Eukaryotic cell 8, 1692-1705.

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109-130.

Baldrian, P., Voříšková, J., Dobiášová, P., Merhautová, V., Lisá, L., Valášková, V., 2011. Production of extracellular enzymes and degradation of biopolymers by saprotrophic microfungi from the upper layers of forest soil. Plant and Soil 338, 111-125.

Bar-Shimon, M., Yehuda, H., Cohen, L., Weiss, B., Kobeshnikov, A., Daus, A., Goldway ,M., Wisniewski, M., Droby, S., 2004. Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent *Candida oleophila*. Current genetics, 45, 140-148.

Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbioly Reviews 29, 795-811.

Botha, A., 2006. Yeasts in Soil, In: Péter, G., Rosa, C. (Eds.), Biodiversity and Ecophysiology of Yeasts. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 221-240.

Botha, A., 2011. The importance and ecology of yeasts in soil. Soil Biology and Biochemistry 43, 1-8.

Bourbonnais, R., Paice, M.G., 1990. Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. FEBS Letters 267, 99-102.

Bovers, M., Hagen, F., Kuramae, E.E., Boekhout, T., 2008. Six monophyletic lineages identified

within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. Fungal Genetics and Biology 45, 400-421.

Brabcová, V., Nováková, M., Davidová, A., Baldrian, P., 2016. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytologist 210, 1369-1381.

Bueé, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184, 449-456.

Buzzini, P., Martini, A., 2002. Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. Journal of Applied Microbiology, 93, 1020-1025.

Cooney, D.G., Emerson, R., 1964. Thermophilic fungi: an account of their biology, activities, and classification. W. H. Freeman.

Date, R.A., 1973. Nitrogen, a major limitation in the productivity of natural communities, crops and pastures in the pacific area. Soil Biology and Biochemistry 5, 5-18.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996-998.

Eichlerová, I., Homolka, L., Žifčáková, L., Lisá, L., Dobiášová, P., Baldrian, P., 2015. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. Fungal Ecology 13, 10-22.

Fonseca, A., 1992. Utilization of tartaric acid and related compounds by yeasts: taxonomic implications. Canadian Journal of Microbiology 38, 1242-1251.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113-118.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology & Evolution 25, 372-380.

Henderson, M.E., 1961. The metabolism of aromatic compounds related to lignin by some hyphomycetes and yeast-like fungi of soil. Journal of General Microbiology 26, 155-165.

Ihrmark, K., Bodeker, I.T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandstrom-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82, 666-677.

Ikeda, R., Sugita, T., Jacobson, E. S., Shinoda, T., 2002. Laccase and melanization in clinically important *Cryptococcus* species other than *Cryptococcus neoformans*. Journal of clinical microbiology 40, 1214-1218.

Jiménez, M., González, A.E., Martínez, M.J., Martínez, A.T., Dale, B.E., 1991. Screening of yeasts isolated from decayed wood for lignocellulose-degrading enzyme activities. Mycological Research 95, 1299-1302.

Kimura, Y., Nakano, Y., Fujita, K., Miyabe, S., Imasaka, S., Ishikawa, Y., Sato, M.,1998. Isolation and characteristics of yeast able to grow at low concentrations of nutrients. Yeasts 14, 233-238.

Kurtzman, C., Fell, J.W., Boekhout, T., 2011. The Yeasts: A Taxonomic Study. Elsevier Science.

Lindahl, B.D., Finlay, R.D., 2006. Activities of chitinolytic enzymes during primary and secondary colonization of wood by basidiomycetous fungi. New Phytologist 169, 389-397.

Lladó, S., Žifčáková, L., Větrovský, T., Eichlerová, I., Baldrian, P., 2016. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. Biology and Fertility of Soils 52, 251-260.

Maksimova, I.A., Chernov, I.Y., 2004. Community Structure of Yeast Fungi in Forest Biogeocenoses. Microbiology 73, 474-481.

Martinez, A.T., Speranza, M., Ruiz-Duenas, F.J., Ferreira, P., Camarero, S., Guillen, F., Martinez, M.J., Gutierrez, A., del Rio, J.C., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. International Microbiology 8, 195-204.

Mašínová, T., Bahnmann, B.D., Větrovský, T., Tomšovský, M., Merunková, K., Baldrian, P., 2017a. Drivers of yeast community composition in the litter and soil of a temperate forest. FEMS Microbiology Ecology 93.

Mašínová, T., Pontes, A., Carvalho, C., Sampaio, J.P., Baldrian, P., 2017b. *Libkindia masarykiana* gen. et sp. nov., *Yurkovia mendeliana* gen. et sp. nov. and *Leucosporidium krtinense* f.a. sp. nov., isolated from temperate forest soils. International Journal of Systematic and Evolutionary Microbiology 67, 902-908.

Mestre, M.C., Fontenla, S., Rosa, C.A., 2014. Ecology of cultivable yeasts in pristine forests in northern Patagonia (Argentina) influenced by different environmental factors. Canadian Journal of Microbiology 60, 371-382.

Mestre, M.C., Rosa, C.A., Safar, S.V., Libkind, D., Fontenla, S.B., 2011. Yeast communities associated with the bulk-soil, rhizosphere and ectomycorrhizosphere of a *Nothofagus pumilio* forest in northwestern Patagonia, Argentina. FEMS Microbiology Ecology 78, 531-541.

Middelhoven, W.J., 2004. The yeast flora of some decaying mushrooms on trunks of living trees. Folia Microbiologica (Praha) 49, 569-573.

Middelhoven, W.J., 2006. Polysaccharides and phenolic compounds as substrate for yeasts isolated from rotten wood and description of *Cryptococcus fagi* sp.nov. Antonie van Leeuwenhoek 90, 57-67.

Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K., 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecology 3, 284-287.

Oberwinkler, F., 2017. Yeasts in Pucciniomycotina. Mycological Progress, 1-26.

Osono, T., Takeda, H., 2002. Comparison of litter decomposing ability among diverse fungi in a cool temperate deciduous forest in Japan. Mycologia 94, 421-427.

Osono, T., Takeda, H., 2006. Fungal decomposition of Abies needle and Betula leaf litter. Mycologia 98, 172-179.

Pajot, H.F., Fariña, J.I., de Figueroa, L.I.C., 2011. Evidence on manganese peroxidase and tyrosinase expression during decolorization of textile industry dyes by *Trichosporon akiyoshidainum*. International Biodeterioration & Biodegradation 65, 1199-1207.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire,

A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D.,, 2011. A large and persistent carbon sink in the world's forests. Science 333. 988-993.

Petter, R., Kang, B.S., Boekhout, T., Davis, B. J., Kwon-Chung, K. J., 2001. A survey of heterobasidiomycetous yeasts for the presence of the genes homologous to virulence factors of *Filobasidiella neoformans*, CNLAC1 and CAP59b. Microbiology 147, 2029-2036.

Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M.H., Naeem, S., Trost, J., 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440, 922-925.

Ságová-Marečková, M., Čermák, L., Novotná, J., Plháčková, K., Forstová, J., Kopecký, J., 2008. Innovative methods for soil DNA purification tested in soils with widely differing characteristics. Applied Environmental Microbiology 74, 2902-2907.

Sampaio, J.P., 1999. Utilization of low molecular weight aromatic compounds by heterobasidiomycetous yeasts: taxonomic implications. Can J Microbiol 45, 491-512.

Shubakov, A.A., 2000. The β-xylosidase production by yeast *Cryptococcus podzolicus*. Russian Journal of Bioorganic Chemistry 26, 550-552.

Slavikova, E., Vadkertiova, R., 2000. The occurrence of yeasts in the forest soils. Journal of Basic Microbiology 40, 207-212.

Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80, 735-746.

Takashima, M., Sugita, T., Van, B.H., Nakamura, M., Endoh, R., Ohkuma, M., 2012. Taxonomic richness of yeasts in Japan within subtropical and cool temperate areas. PLoS One 7, e50784.

Větrovský, T., Baldrian, P., 2013. Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. Biology and Fertility of Soils 49, 1027-1037.

Vishniac, H.S., 1982, An enation system for the isolation of Antarctic yeasts inhibited by conventional media. Canadian Journal of Microbiology 29, 90-95.

Vishniac, H.S., 2006. A multivariate analysis of soil yeasts isolated from a latitudinal gradient. Microbial Ecology 52, 90-103.

Voříšková, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME Journal 7, 477-486.

White, T., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, In: Innis, M., Gelfand, D., Shinsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, pp. 315-322.

Yu, T., Wang, L., Yin, Y., Wang, Y., Zheng, X., 2008. Effect of chitin on the antagonistic activity of *Cryptococcus laurent*ii against *Penicillium expansum* in pear fruit. International Journal of food Microbiology 122, 44-48.

Yurkov, A.M., Chernov, I.Y. Tiunov, A.V.,2008. Influence of *Lumbricus terrestris* earthworms on the structure of the yeast community of forest litter. Microbiology 77, 107-111.

Yurkov, A., Kemler, M., Begerow, D., 2012a. Assessment of yeast diversity in soils under different management regimes. Fungal Ecology 5, 24-35.

Yurkov, A.M., Rohl, O., Pontes, A., Carvalho, C., Maldonado, C., Sampaio, J.P., 2016a. Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. FEMS Yeast Research 16, fov103.

Yurkov, A.M., Wehde, T., Federici, J., Schäfer, A.M., Ebinghaus, M., Lotze-Engelhard, S., Mittelbach, M., Prior, R., Richter, C., Röhl, O., Begerow, D., 2016b. Yeast diversity and species recovery rates from beech forest soils. Mycological Progress 15, 845-859.

Zhu, X., Williamson, P.R., 2004. Role of laccase in the biology and virulence of *Cryptococcus neoformans*. FEMS yeast research 5, 1-10.

Žifčáková, L., Větrovský, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environmental Microbiology 18, 288-301.

Table1: The taxonomic identity of yeast isolates from temperate forest topsoil that were characterized in this study, D1/and ITS Genbank Acession numbers, the mean relative abundances and standard deviations of OTUs with similarity > 97% and coverage > 90% to the ITS2 sequence of the best hit and number of sites where these OTUs occured in the studied environment.

						Relative OTU abundance (%)		OTU occurrence	
Isolate	**Identification**	**Taxonomy**	**DSMZ Accession**	**GenBank Accession**	**Doubling time (h)**	**Soil**	**Litter**	**Soil**	**Litter**
KT592	*Candida railenensis*	Saccharomycetes (A)	DSM 103189	KY607883	15.4 ± 0.8	0.14±0.76	1.34±8.17	10	15
KT532	*Kluyveromyces dobzhanskii*	Saccharomycetes (A)	DSM 101892	KU187882, KU187886	13.5 ± 0.5	0.00±0.02	0.03±0.19	1	4
KT419	*Lachancea thermotolerans*	Saccharomycetes (A)	DSM 103190	KY558337	15.3 ± 0.2	0.00±0.02	0.01±0.04	2	3
KT94	*Yamadazyma mexicana*	Saccharomycetes (A)	DSM 103192	KY558339	15.6 ± 0.7	0.01±0.05	0.00±0.00	1	0
KT138	*Bannozyma* sp.	Microbotryomycetes (B)	DSM 103195	KY558342	24.8 ± 3.3	0.00±0.02	0.02±0.09	2	3
KT96	*Leucosporidium krtinense*	Microbotryomycetes (B)	DSM 103197	KY558344	16.1 ± 1.1	0.30±0.57	1.38±3.32	32	29
KT313	*Oberwinklerozyma yarrowii*	Microbotryomycetes (B)	DSM 103198	KY558345	12.5 ± 0.2	0.03±0.13	0.31±0.85	5	16
KT156	*Sporobolomyces roseus*	Microbotryomycetes (B)	DSM 103199	KY558346	23.7 ± 1.5	0.06±0.19	0.17±0.51	12	12
KT152	*Yurkovia mendeliana*	Microbotryomycetes (B)	DSM 101889	KU187884, KU187888	18.3 ± 0.8	0.03±0.15	0.51±2.24	8	29
KT240	*Apiotrichum porosum*	Tremellomycetes (B)	DSM 103200	KY558347	13.7 ± 0.5	6.19±7.90	6.92±8.95	77	70
KT290	*Cutaneotrichosporon moniliiforme*	Tremellomycetes (B)	DSM 103201	KY558348	17.6 ± 3.7	1.98±4.15	1.84±3.41	50	44
KT301	*Fonsecazyma* sp.	Tremellomycetes (B)	DSM 103203	KY558350	23.7 ± 1.2	0.04±0.20	0.50±1.87	8	20
KT97	*Heterocephalacria* sp.	Tremellomycetes (B)	DSM 103204	KY558351	33.6 ± 0.7	0.00±0.00	0.44±2.63	0	12
KT242	*Holtermanniella wattica*	Tremellomycetes (B)	DSM 103205	KY558367	20.7 ± 0.4	0.00±0.02	0.11±0.62	2	6
KT266	*Kwoniella pini*	Tremellomycetes (B)	DSM 103207	KY558352	17.1 ± 2.0	0.00±0.00	0.19±1.18	0	7
KT281	*Kwoniella* sp.	Tremellomycetes (B)	DSM 104104	KY558353	19.0 ± 0.2	0.01±0.05	0.23±1.28	3	14
KT116	*Phaeotremella* sp.	Tremellomycetes (B)	DSM 103208	KY558354	19.4 ± 1.6	0.06±0.32	0.55±1.36	7	29
KT179	*Phaeotremella* sp.	Tremellomycetes (B)	DSM 103209	KY558355	29.6 ± 0.8	0.01±0.05	0.09±0.44	2	9
KT149	*Piskurozyma cylindrica*	Tremellomycetes (B)	DSM 103211	KY558357	21.9 ± 3.1	0.01±0.09	0.25±1.25	4	19
KT146	*Piskurozyma* sp.	Tremellomycetes (B)	DSM 103212	KY558358	19.8 ± 1.1	0.05±0.15	1.63±3.10	11	29
KT162	*Piskurozyma* sp.	Tremellomycetes (B)	DSM 103214	KY558360	-	0.04±0.23	0.23±0.89	4	6
KT312	*Piskurozyma* sp.	Tremellomycetes (B)	DSM 103215	KY558361	24.1 ± 0.2	0.02±0.10	0.33±0.80	5	28
KT170	*Piskurozyma taiwanensis*	Tremellomycetes (B)	DSM 103216	KY558362	20.3 ± 1.3	0.02±0.14	0.19±0.82	18	25
KT203A	*Solicoccozyma terricola*	Tremellomycetes (B)	DSM 104106	KY558363	23.7 ± 2.0	33.11±40.11	11.77±21.32	80	78
KT147	*Tremella* sp.	Tremellomycetes (B)	DSM 103217	KY558366	-	0.20±0.48	0.43±0.95	22	20

Figure 1: Three dimensional non-metric multidimensional scaling (NMDS) of enzyme production by fungi belonging to six ecophysiological groups. Each point represents one fungal species. Brown squares represent individual enzymes. Data for non-yeast fungi are from Eichlerová et al. (2015)

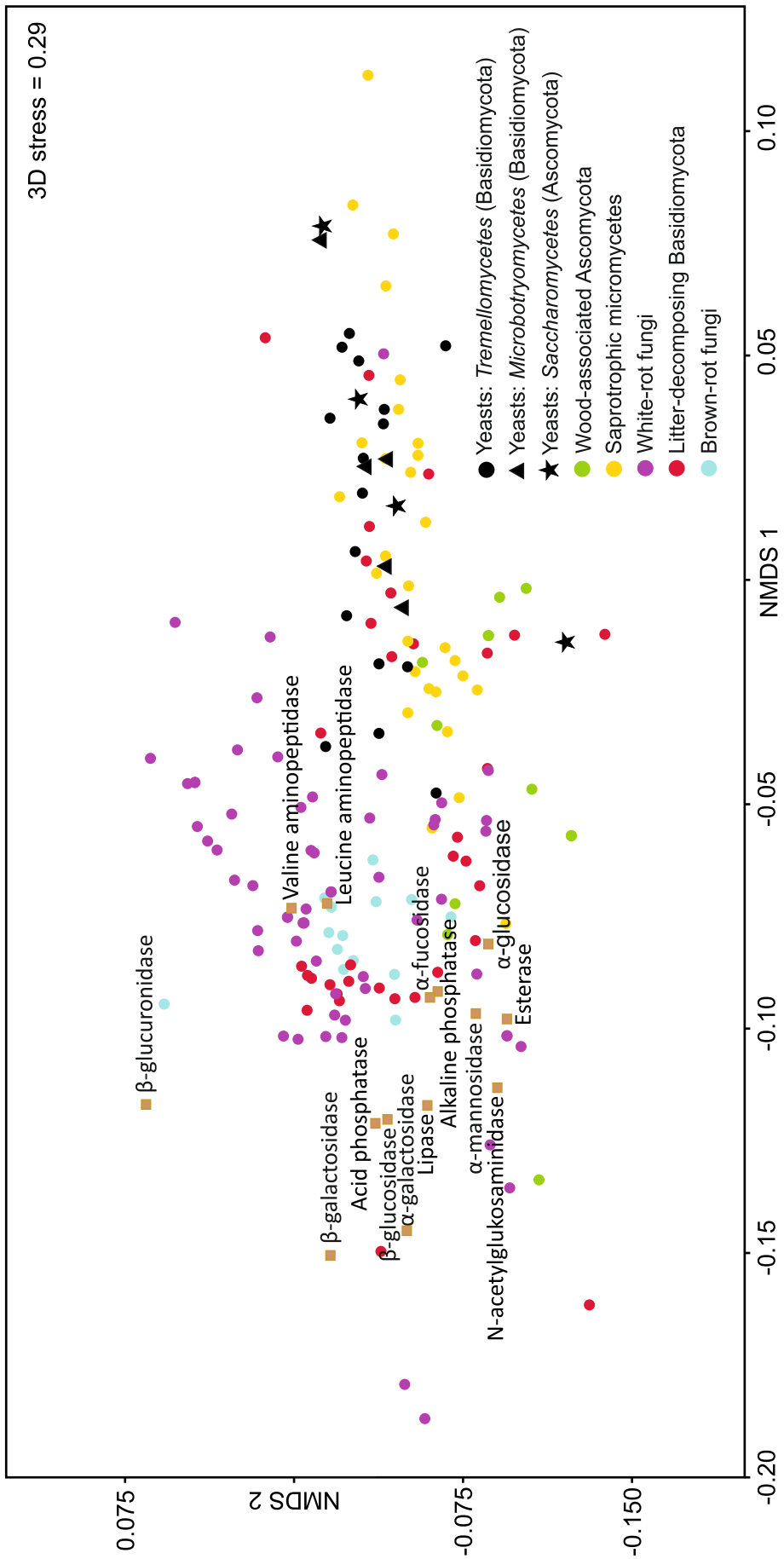


Figure 2: Production of enzymes by yeasts (Y), saprotrophic micromycetes (SA), and litter-decomposing Basidiomycota (LDF). The data represent medians and quartiles. Different letters indicate significant differences in the enzyme activity among groups ($P < 0.05$).

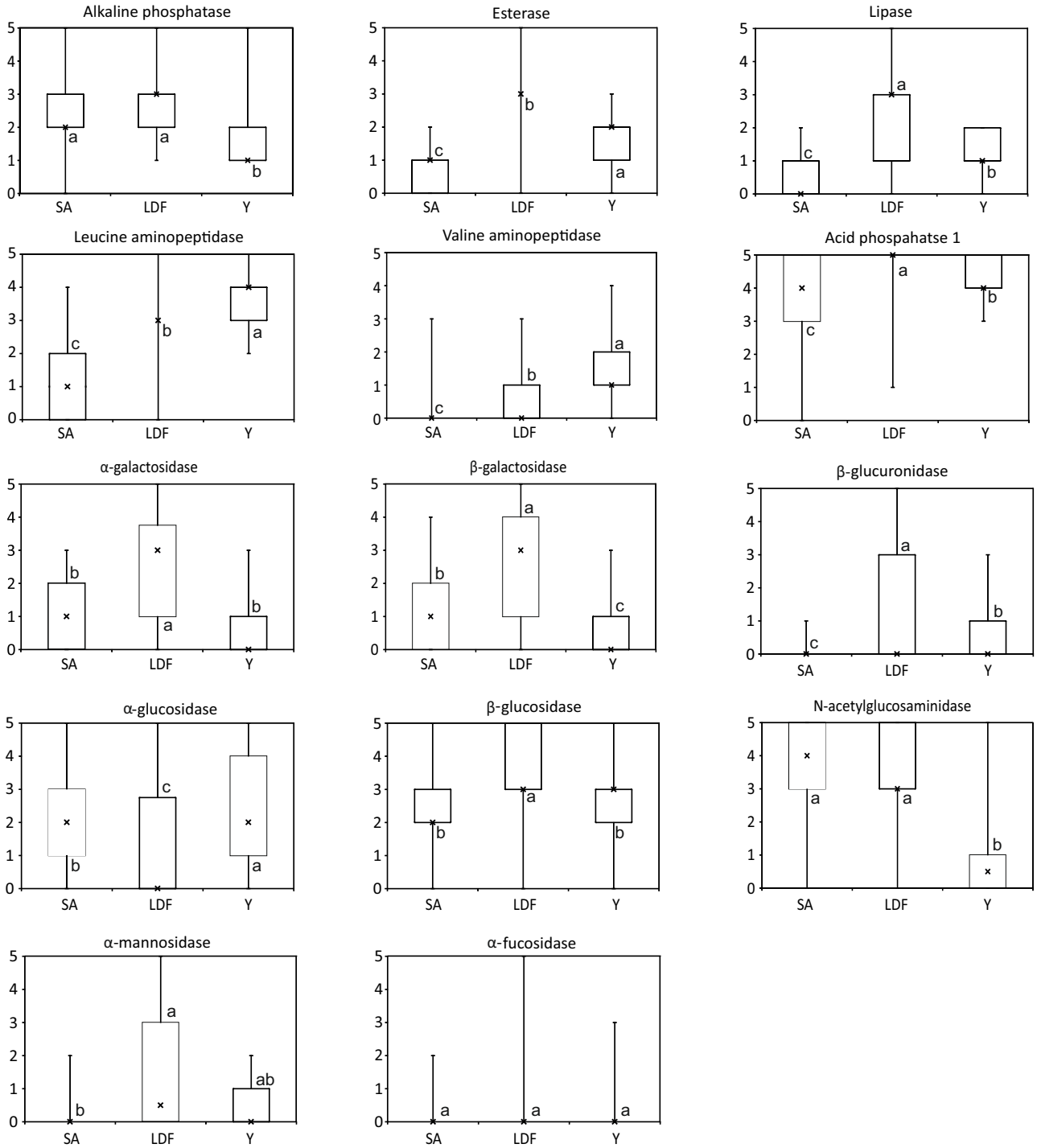


Figure 3: Enzyme activities in cultures of yeasts isolates from temperate forest topsoil. The data represent means of three replicate cultures and are expressed in nM min^{-1} ml^{-1}. The Neighbor-Joining tree was gained from the analysis of 28S rRNA gene.

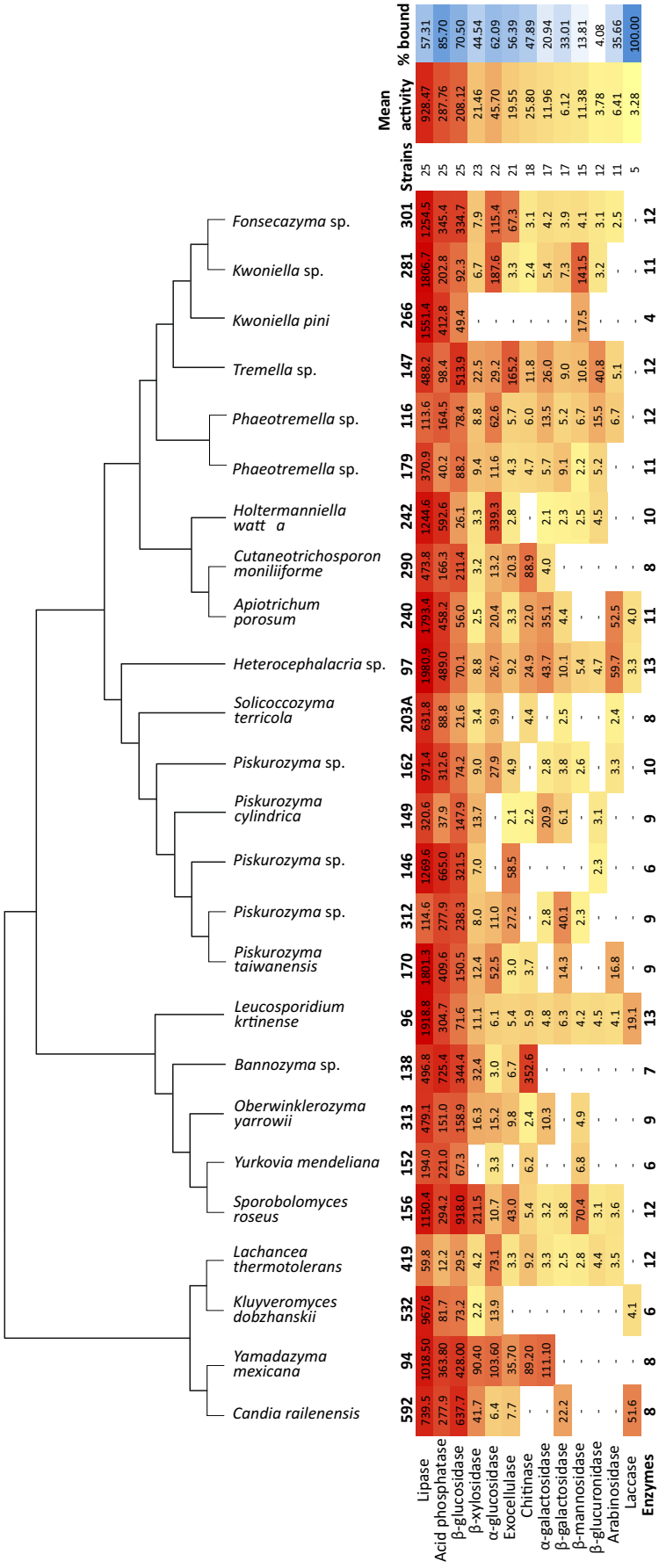
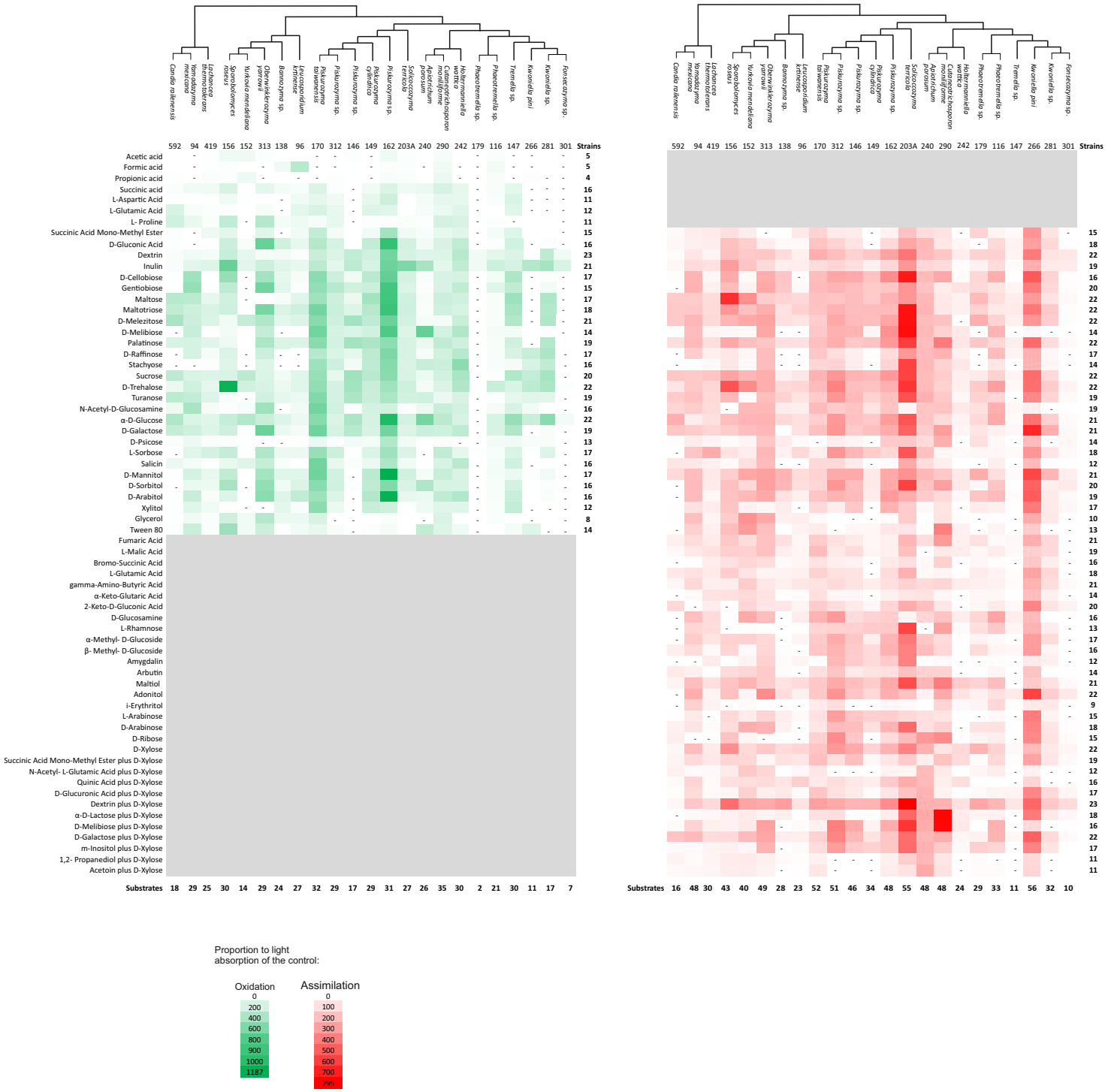
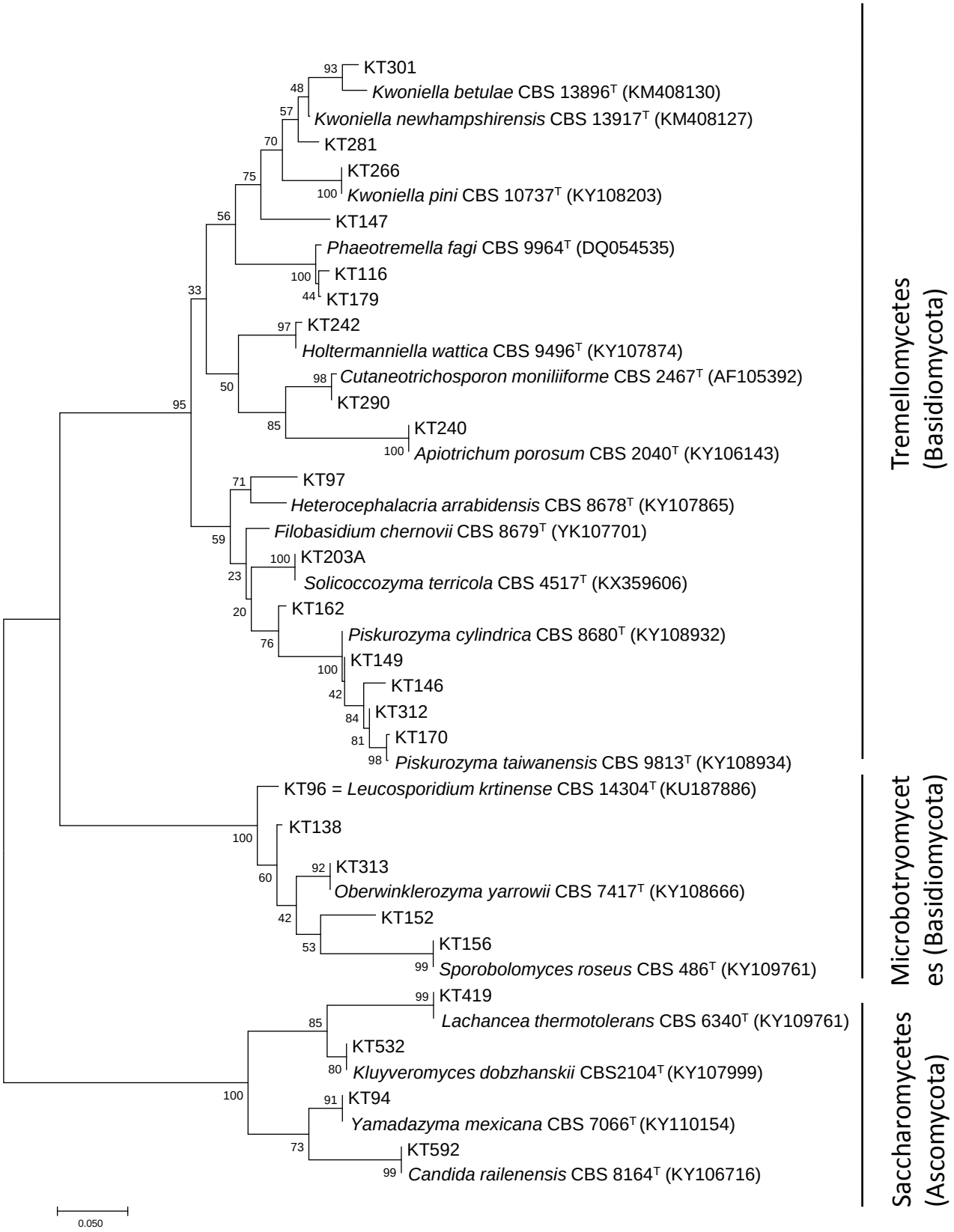


Figure 4: Ability of yeast isolates from temperate forest topsoil to oxidize and assimilate different carbon sources assayed using Bi olog YT Microplates. "-" indicates values that were lower than 0 or were not measured. Grey fields indicate substrates that were not tested. The Neighbor-Joining tree was gained from the analysis of 28S rRNA gene.



Supplementary Figure 1: Phylogenetic tree of the partial 28S rRNA gene sequences showing the placement of yeast isolates from the Křtiny forest litter and soil. The Neighbor-Joining tree was calculated from an alignment of 405 nucleotide positions using MEGA 7. Bootstrap values (1000 replicates) are shown next to the branches. The evolutionary distances were inferred using the Jukes-Cantor method. GenBank accession numbers are displayed in parentheses. The bar indicates 0.05 substitutions per nucleotide position.



Supplementary Figure 2: Production of extracellular enzymes by yeast strains from temperate forest topsoil assayed using API ZYM ™.

	Fonsecazyma sp.	*Kwoniella* sp.	*Kwoniella pini*	*Tremella* sp.	*Phaeotremella* sp.	*Phaeotremella* sp.	*Cutaneotrichosporon moniliiforme*	*Apiotrichum porosum*	*Heterocephalacria* sp.	*Solicoccozyma terricola*	*Piskurozyma* sp.	*Piskurozyma cylindrica*	*Piskurozyma* sp.	*Piskurozyma* sp.	*Piskurozyma taiwanensis*	*Holtermanniella wattica*	*Leucosporidium krtinense*	*Oberwinklerozyma yarrowii*	*Bannozyma* sp.	*Yurkovia mendeliana*	*Sporobolomyces roseus*	*Lachancea thermotolerans*	*Kluyveromyces dobzhanskii*	*Yamadazyma mexicana*	*Candida railenensis*	
	301	281	266	147	116	179	290	240	97	203A	162	149	146	312	170	242	96	313	138	152	156	419	532	94	592	**Mean**
Alkaline phosphatase	1	1	1	1	1	1	2	1	1	3	1	5	2	3	2	1	2	1	1	2	2	1	2	1	3	**1.68**
Esterase	3	2	2	1	1	2	2	2	1	3	1	2	1	2	2	2	0	1	1	2	2	1	2	2	2	**1.68**
Lipase	2	1	1	1	1	1	2	2	1	2	1	1	1	1	2	1	0	1	1	2	2	1	2	2	1	**1.32**
Leucine aminopeptidase	2	4	3	3	4	3	3	4	2	4	5	4	4	4	3	4	4	4	2	3	2	4	3	5	3	**3.44**
Valine aminopeptidase	1	1	1	1	1	1	2	1	1	3	1	2	2	1	4	3	1	1	0	2	3	3	2	1	2	**1.64**
Acid phosphatase	4	5	4	3	4	4	4	4	5	4	4	4	5	5	4	5	4	5	5	4	4	3	4	5	4	**4.24**
α-galactosidase	0	0	0	1	1	1	3	1	0	3	0	3	0	1	2	0	0	2	0	0	0	0	0	1	0	**0.76**
β-galactosidase	0	0	0	0	0	1	1	1	0	0	0	3	0	1	2	0	0	0	0	0	1	0	0	0	0	**0.40**
β-glucuronidase	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	**0.28**
α-glucosidase	5	2	1	1	2	2	4	2	0	5	2	1	1	1	3	4	1	1	0	2	2	4	4	5	3	**2.32**
β-glucosidase	4	1	3	2	1	2	3	3	1	4	2	4	2	4	3	3	0	1	2	3	4	1	3	5	3	**2.56**
N-acetylglucosaminidase	0	0	1	0	0	0	1	3	0	3	0	0	1	0	1	2	0	1	2	3	1	0	0	5	1	**1.00**
α-mannosidase	1	0	0	0	0	1	1	1	0	1	0	0	0	0	2	1	0	0	0	1	1	0	0	1	1	**0.48**
α-fucosidase	0	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	**0.16**
Enzymes	**9**	**8**	**10**	**9**	**10**	**12**	**12**	**13**	**7**	**12**	**8**	**11**	**9**	**12**	**12**	**11**	**5**	**10**	**7**	**10**	**11**	**8**	**8**	**11**	**10**	

Supplementary Table 1: Extracellular, cell wall-associated and total activity of extracellular enzymes in cultures of yeast strains from temperate forest topsoil. The data represent means of three replicate cultures and are expressed in nM $min^{-1}ml^{-1}$.

Strain	94			96			97			114			116		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	1018.5	19.4	80.6	1918.8	48.5	51.5	1980.9	49.4	50.6	65.8	63.4	36.6	113.6	39.8	60.2
Acid phosphatase	363.8	73.8	26.2	304.7	92.8	7.2	489.0	92.9	7.1	238.6	94.7	5.3	164.5	93.7	6.3
β-glucosidase	428.0	90.9	9.1	71.6	53.1	46.9	70.1	91.9	8.1	40.3	62.3	37.7	78.4	66.5	33.5
β-xylosidase	90.4	95.8	4.2	11.1	23.4	76.6	8.8	26.1	73.9	6.2	-	100.0	8.8	-	100.0
α-glucosidase	103.6	93.8	6.2	6.1	-	100.0	26.7	84.6	15.4	125.6	95.8	4.2	62.6	-	100.0
Cellobiohydrolase	35.7	100.0	-	5.4	-	100.0	9.2	37.0	63.0	3.6	-	100.0	5.7	-	100.0
Chitinase	89.2	85.8	14.2	5.9	-	100.0	24.9	59.4	40.6	11.0	42.7	57.3	6.0	-	100.0
α-galactosidase	111.1	96.7	3.3	4.8	-	100.0	43.7	85.4	14.6	3.5	-	100.0	13.5	-	100.0
β-galactosidase	-	-	-	6.3	-	100.0	10.1	45.5	54.5	4.0	-	100.0	5.2	-	100.0
β-mannosidase	-	-	-	4.2	-	100.0	5.4	-	100.0	3.0	-	100.0	6.7	-	100.0
β-glucuronidase	-	-	-	4.5	-	100.0	4.7	-	100.0	2.9	-	100.0	15.5	-	100.0
Arabinosidase	-	-	-	4.1	-	100.0	59.7	92.3	7.7	8.2	56.1	43.9	6.7	-	100.0
Laccase	-	-	-	19.1	100.0	-	3.3	100.0	-	-	-	-	-	-	-

Strain	146			147			149			152			156		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	1269.6	53.0	47.0	488.2	53.8	46.2	320.6	65.9	34.1	194.0	53.0	47.0	1150.4	62.5	37.5
Acid phosphatase	665.0	71.9	28.1	98.4	88.0	12.0	37.9	85.5	14.5	221.0	85.9	14.1	294.2	96.6	3.4
β-glucosidase	321.5	82.0	18.0	513.9	62.3	37.7	147.9	45.6	54.4	67.3	79.3	20.7	918.0	47.8	52.2
β-xylosidase	7.0	58.6	41.4	22.5	21.8	78.2	13.7	25.5	74.5	-	-	-	211.5	55.6	44.4
α-glucosidase	-	-	-	29.2	16.1	83.9	-	-	-	3.3	100.0	-	10.7	22.4	77.6
Cellobiohydrolase	58.5	91.1	8.9	165.2	69.6	30.4	2.1	100.0	-	-	-	-	43.0	38.1	61.9
Chitinase	-	-	-	11.8	72.9	27.1	2.2	100.0	-	6.2	100.0	-	5.4	-	100.0
α-galactosidase	-	-	-	26.0	-	100.0	20.9	-	100.0	-	-	-	3.2	-	100.0
β-galactosidase	-	-	-	9.0	-	100.0	6.1	100.0	-	-	-	-	3.8	-	100.0
β-mannosidase	-	-	-	10.6	-	100.0	-	-	-	6.8	100.0	-	70.4	81.4	18.6
β-glucuronidase	2.3	-	100.0	40.8	-	100.0	3.1	-	100.0	-	-	-	3.1	-	100.0
Arabinosidase	-	-	-	5.1	-	100.0	-	-	-	-	-	-	3.6	-	100.0
Laccase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain	168			170			179			203			240		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	1272.2	40.9	59.1	1801.3	56.0	44.0	370.9	56.9	43.1	631.8	87.1	12.9	1793.4	50.0	50.0
Acid phosphatase	568.2	92.5	7.5	409.6	89.2	10.8	40.2	80.6	19.4	88.8	94.6	5.4	458.2	92.7	7.3
β-glucosidase	139.1	77.8	22.2	150.5	78.9	21.1	88.2	76.4	23.6	21.6	68.5	31.5	56.0	100.0	-
β-xylosidase	36.8	54.6	45.4	12.4	68.5	31.5	9.4	37.2	62.8	3.4	-	100.0	2.5	100.0	-
α-glucosidase	12.8	22.7	77.3	52.5	100.0	-	11.6	-	100.0	9.9	100.0	-	20.4	100.0	-
Cellobiohydrolase	15.6	46.2	53.8	3.0	100.0	-	4.3	48.8	51.2	-	-	-	3.3	100.0	-
Chitinase	13.6	38.2	61.8	3.7	100.0	-	4.7	46.8	53.2	4.4	100.0	-	22.0	67.3	32.7
α-galactosidase	6.2	-	100.0	-	-	-	5.7	-	100.0	-	-	-	35.1	100.0	-
β-galactosidase	9.8	-	100.0	14.3	100.0	-	9.1	67.0	33.0	2.5	-	100.0	4.4	100.0	-
β-mannosidase	6.3	-	100.0	-	-	-	2.2	-	100.0	-	-	-	-	-	-
β-glucuronidase	8.9	-	100.0	-	-	-	5.2	-	100.0	-	-	-	-	-	-
Arabinosidase	5.2	-	100.0	16.8	100.0	-	-	-	-	2.4	100.0	-	52.5	100.0	-
Laccase	-	-	-	-	-	-	-	-	-	-	-	-	4.0	100.0	-

Strain	266			281			290			301			311		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	1551.4	45.2	54.8	1806.7	53.2	46.8	473.8	73.4	26.6	1254.5	53.7	46.3	393.5	76.4	23.6
Acid phosphatase	412.8	94.6	5.4	202.8	95.6	4.4	166.3	87.7	12.3	345.4	73.5	26.5	50.4	84.5	15.5
β-glucosidase	49.4	90.7	9.3	92.3	52.1	47.9	211.4	50.9	49.1	334.7	87.9	12.1	99.3	64.5	35.5
β-xylosidase	-	-	-	6.7	-	100.0	3.2	100.0	-	7.9	34.2	65.8	9.9	22.2	77.8
α-glucosidase	-	-	-	187.6	90.6	9.4	13.2	28.8	71.2	115.4	95.8	4.2	8.9	25.8	74.2
Cellobiohydrolase	-	-	-	3.3	-	100.0	20.3	62.6	37.4	67.3	90.6	9.4	8.7	28.7	71.3
Chitinase	-	-	-	2.4	-	100.0	88.9	69.4	30.6	3.1	-	100.0	10.6	36.8	63.2
α-galactosidase	-	-	-	5.4	-	100.0	4.0	-	100.0	4.2	-	100.0	16.0	-	100.0
β-galactosidase	-	-	-	7.3	-	100.0	-	-	-	3.9	-	100.0	8.1	-	100.0
β-mannosidase	17.5	25.7	74.3	141.5	-	100.0	-	-	-	4.1	-	100.0	5.5	-	100.0
β-glucuronidase	-	-	-	3.2	-	100.0	-	-	-	3.1	-	100.0	6.9	-	100.0
Arabinosidase	-	-	-	-	-	-	-	-	-	2.5	-	100.0	4.7	-	100.0
Laccase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Strain	313			419			532			592		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	479.1	27.5	72.5	59.8	37.5	62.5	967.6	94.8	5.2	739.5	83.6	16.4
Acid phosphatase	151.0	91.0	9.0	12.2	-	100.0	81.7	95.8	4.2	277.9	83.7	16.3
β-glucosidase	158.9	45.1	54.9	29.5	37.3	62.7	73.2	74.7	25.3	637.7	76.1	23.9
β-xylosidase	16.3	-	100.0	4.2	-	100.0	2.2	100.0	-	41.7	59.5	40.5
α-glucosidase	15.2	-	100.0	73.1	-	100.0	13.9	100.0	-	6.4	50.0	50.0
Cellobiohydrolase	9.8	48.0	52.0	3.3	-	100.0	-	-	-	7.7	63.6	36.4
Chitinase	2.4	-	100.0	9.2	59.8	40.2	-	-	-	-	-	-
α-galactosidase	10.3	73.8	26.2	3.3	-	100.0	-	-	-	-	-	-
β-galactosidase	-	-	-	2.5	-	100.0	-	-	-	22.2	69.8	30.2
β-mannosidase	4.9	-	100.0	2.8	-	100.0	-	-	-	-	-	-
β-glucuronidase	-	-	-	4.4	-	100.0	-	-	-	-	-	-
Arabinosidase	-	-	-	3.5	-	100.0	-	-	-	-	-	-
Laccase	-	-	-	-	-	-	4.1	100.0	-	51.6	100.0	-

Strain	138			162			242			312		
	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular	Total activity	% Bound	% Extracellular
Lipase	496.8	100.0	-	971.4	73.1	26.9	1244.6	37.4	62.6	114.6	58.1	41.9
Acid phosphatase	725.4	99.6	0.4	312.6	95.1	4.9	592.6	96.8	3.2	277.9	91.0	9.0
β-glucosidase	344.4	37.7	62.3	74.2	85.7	14.3	26.1	89.7	10.3	238.3	91.4	8.6
β-xylosidase	32.4	100.0	-	9.0	54.4	45.6	3.3	-	100.0	8.0	63.8	36.3
α-glucosidase	3.0	100.0	-	27.9	90.0	10.0	339.3	93.8	6.2	11.0	100.0	-
Cellobiohydrolase	6.7	100.0	-	4.9	42.9	57.1	2.8	-	100.0	27.2	91.9	8.1
Chitinase	352.6	0.7	99.3	-	-	-	-	-	-	-	-	-
α-galactosidase	-	-	-	2.8	-	100.0	2.1	-	100.0	2.8	-	100.0
β-galactosidase	-	-	-	3.8	-	100.0	2.3	-	100.0	40.1	78.8	21.2
β-mannosidase	-	-	-	2.6	-	100.0	2.5	-	100.0	2.3	-	100.0
β-glucuronidase	-	-	-	-	-	-	4.5	48.9	51.1	-	-	-
Arabinosidase	-	-	-	3.3	-	100.0	-	-	-	-	-	-
Laccase	-	-	-	-	-	-	-	-	-	-	-	-