Fungal Ecology xxx (2016) 1–8

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Are correlations between deadwood fungal community structure, wood physico-chemical properties and lignin-modifying enzymes stable across different geographical regions?

Witoon Purahong [a, 1], Tobias Arnstadt [b, 1], Tiemo Kahl [c], Jürgen Bauhus [c], Harald Kellner [b], Martin Hofrichter [b], Dirk Krüger [a], François Buscot [a, d], Björn Hoppe [a, *, 1]

[a] UFZ-Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany
[b] Dresden University of Technology (TU Dresden) – International Institute (IHI) Zittau, Markt 23, D-02763 Zittau, Germany
[c] University of Freiburg, Faculty of Environment and Natural Resources, Chair of Silviculture, Tennenbacherstr. 4, D-79085 Freiburg i. Brsg., Germany
[d] German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, D-04103 Leipzig, Germany

ARTICLE INFO

Article history:
Received 30 October 2015
Accepted 7 January 2016
Available online xxx

Corresponding editor: Kevin Hyde

Keywords:
Ecosystem processes
Enzyme activity
Wood decay
Wood physico-chemical properties
Fungal community structure

ABSTRACT

Wood-inhabiting fungi are major agents of wood decomposition. However, it is unclear which factors determine their distribution and enzyme production. Many studies that have addressed this issue suffer from a lack of geographic extent. Here, we investigate the fungal community structure of 117 *Fagus sylvatica* logs in relation to wood physico-chemical properties and secreted ligninolytic enzymes, across three distinct geographical regions of Germany. Our results revealed that fungal community structure was similar across different regions, but was nevertheless variable in all regions. The relationships between fungal community structure, wood physico-chemical properties and enzyme activities were not consistent across different regions. However, we identified that the wood physico-chemical properties (i.e. decay class, remaining mass, density, extractives, total lignin and pH) were the most important factors associated with the fungal community structure in all three regions. In contrast, the wood physico-chemical properties and the fungal community structure did not sufficiently explain variation in the detected enzymatic activities. Thus, we assume that interspecific interactions and recently described priority effects play more important roles in the production of lignin modifying enzymes.

1. Introduction

Decomposition of wood is essential in carbon dynamics and nutrient cycling in forest ecosystems (Cornelissen et al., 2012; Kahl et al., 2012; Rajala et al., 2012; van der Wal et al., 2013, 2014; Peršoh, 2015). It is a complex ecological process, which is regulated by different drivers: climate, substrate quality (tree species) and the abundance, composition and activity of the decomposer communities (Weedon et al., 2009; Herrmann and Bauhus, 2013; Liu et al., 2013). A recent work has demonstrated that climate alone failed to predict wood decomposition rates at regional scales, while local-scale factors were found to be much more important for explaining most of the variation (Bradford et al., 2014). Other studies indicate that substratum (e.g. litter) quality may be more important than climate in controlling decomposition rates across different biomes (Cornwell et al., 2008; Weedon et al., 2009; Bradford et al., 2014).

Due to its high amount of lignin, deadwood is difficult to decay (Floudas et al., 2012). Under natural conditions, only fungi substantially decompose deadwood. With their ability to use a battery of secreted oxidoreductases and hydrolases (wood decomposition enzymes), they are considered as the primary wood decomposers and among them are the only organisms which are able to decompose lignin (Cornelissen et al., 2012; Stokland et al., 2012; Purahong et al., 2014a, 2014b; Kubartová et al., 2015; Peršoh, 2015). Diverse bacteria also colonize deadwood and form at least commensal interactions with wood-inhabiting fungi, for example, by providing additional nitrogen (de Boer et al., 2005; Hoppe et al., 2014, 2015a). However, due to their limited ability to decompose polymeric lignocelluloses, bacteria are thought to play only a minor role in wood decomposition (Cornelissen et al., 2012). Distribution

* Corresponding author.
E-mail address: bjoern.hoppe@ufz.de (B. Hoppe).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.funeco.2016.01.002

patterns of wood-inhabiting fungi provide distinct information to predict their roles in ecosystem functions and stability (Kubartová et al., 2012). The fungal community structure can be influenced by various biotic and abiotic drivers. As one biotic driver, priority effects might play an important role for fungal community assembly (Boddy, 2000; Fukami et al., 2010; Dickie et al., 2012; Hiscox et al., 2015). The first arriving fungus significantly changes the assembly of the entire fungal community that follows, and thus affects wood decomposition rates. On the other hand, it is still unclear how abiotic factors regulate the wood-inhabiting fungal community structure. Based on a limited number of studies, wood physico-chemical factors such as decay stage, log diameter, volume, density, C:N ratio, lignin content, macronutrients and a few micronutrients significantly correlate with the structure of wood-inhabiting fungal communities (Rajala et al., 2010, 2011, 2012). As decay progresses, these wood physico-chemical parameters change, which in turn corresponds to changes in the wood-inhabiting fungal community structure (Rajala et al., 2012). However, most studies that have addressed this issue suffer from a lack of geographic extent. It has recently been shown that, for example, latitude and mean annual temperature of distinct regions correlate with wood decomposition types (Fukasawa, 2015), and might therefore also impact the decomposer community. Both, climatic and management associated factors have also been shown to impact diversity patterns of sporocarps across beech forests in Europe (Ódor et al., 2006). Furthermore, the relation of wood-inhabiting fungal community structure and corresponding ecological function such as lignin decomposition is still rarely studied.

In this study, we investigated the correlation between fungal community structure, wood physico-chemical properties and ligninolytic enzyme activities in natural deadwood of *Fagus sylvatica* in temperate forests across different geographical regions of Germany (north to south gradient of approximately 600 km). Diverse wood physico-chemical properties, which were already reported to correlate with wood-inhabiting fungal community structure in previous publications are included in this present study (Rajala et al., 2010, 2011, 2012). Activities of three lignin-modifying enzymes: laccase (EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13) and manganese independent peroxidases (MiP, EC 1.11.1.7/14/16 representing distinct protein families e.g. Kellner et al., 2014) were used as a proxy for fungal-mediated ecosystem functions. We hypothesize that different geographical regions of Germany harbour distinct fungal communities, which in turn results in consistent ecosystem functionality and thus consistent correlations with environmental factors.

2. Materials and methods

2.1. Sampling design

The study was conducted within the experimental research platform of the 'biodiversity-exploratories' in Germany (Fischer et al. 2010). Thirty forest plots (100 × 100 m) at three locations in different geographical regions were selected: the UNESCO Biosphere Area Schwäbische Alb (ALB) in south-western Germany (460–860 m a.s.l.), the National Park Hainich and its surrounding areas (Hainich-Dün) (HAI) in central Germany (285–550 m a.s.l.), and the UNESCO Biosphere Reserve Schorfheide-Chorin (SCH) in north-eastern Germany (3–140 m a.s.l.). The annual mean temperatures are in the range of 8–8.5 °C (SCH), 6.5–8 °C (HAI) and 6–7 °C (ALB) and the annual mean precipitation varies between 500 and 600 mm (SCH), 500 and 800 mm (HAI) and 700 and 1000 mm (ALB). The distances between different forest plots ranged from 0.315 km (within one experimental site) to 626.9 km (longest distance between plots in north-eastern and south-western Germany) (Fig. 1).

In May 2009, 117 naturally occurring downed deadwood logs of *F. sylvatica* were randomly selected in 30 forest plots in all three exploratories (Table S1). Three to seven wood samples were taken from each log (to better represent the entire log dimension) using a cordless Makita BDF451 drill (Makita, Anja, Japan) equipped with a 2 × 42 cm wood auger as described in Hoppe et al. (2014, 2015) and Purahong et al. (2014a, b). Data pertaining to the experimental forest plots and wood sampling are summarized in the supplementary data section and are explained in detail in Hoppe et al., 2014, 2015a and Purahong et al., 2014a, 2014b.

2.2. DNA isolation, PCR and ARISA (automated ribosomal intergenic spacer analysis) fingerprints

The total community DNA from 1 g wood powder of each sample was isolated using a modified CTAB-protocol (Doyle and Doyle, 1987). 900 μl of CTAB was added to the sample and the nucleic acid was separated from proteins and cell debris by adding 500 μl of 24:1 chloroform: isoamyl alcohol (Carl Roth, Karlsruhe, Germany) followed by another chloroform step (Carl Roth, Karlsruhe, Germany). DNA was precipitated by washing twice with ethanol (Merck, Darmstadt, Germany). Dried pellets were eluted in 100 μl molecular water (AppliChem, Darmstadt, Germany).

F-ARISA PCR (Ranjard et al., 2001) of each DNA extract was done in two replicates using a carboxyfluorescein FAM-labelled primer ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′, Gardes and Bruns, 1993) and unlabelled ITS4 (5′-TCCTCCGCTTATTGATATGC-3′, White et al., 1990) in 30 μl reaction mixtures containing 6 μl FIREPol 5x Master Mix (Solis BioDyne, Tartu, Estonia), 15 μM of each primer and 1 μl template DNA. PCR was performed with an initial denaturation step at 95 °C for 5 min followed by 35 cycles at 95 °C for 60 s, 55 °C for 60 s and 72 °C for 75 s. Elongation was completed with a final step of 72 °C for 7 min.

PCR products were purified using the E.Z.N.A.® Cycle-Pure Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). 10 ng of each purified PCR product was dissolved in 14 μl of deionized Hi-Di formamide (Applied Biosystems, Foster City, CA) with 0.1 μl of internal size standard Map Marker 1000 ROX (BioVentures, Inc., Murfreesboro, TN, USA). After denaturation for 5 min at 95 °C samples were chilled on ice for at least 10 min. Length heterogeneity of fungal ITS fragments was detected by capillary electrophoresis (ABI 3730xl, Applied Biosystems). Electrophoretic conditions were as follows: 7 s injection at 1.6 kV and separation at 15 kV for 3800 s. Row profiles were analysed using Gene Mapper software 4.0 (Applied Biosystems). All peaks above a threshold of 100 fluorescence units, which were presented in both technical replicates, were considered for further analyses (Purahong et al., 2015a, Moll et al., 2015). OTU binning was performed with the interactive and automatic R binning script (Ramette, 2009) using R (The R Foundation for Statistical Computing [http://cran.r-project.org/]). According to the script and its correlation values a window size of two was chosen.

2.3. Wood physico-chemical properties and enzyme assays

The concentration of C and N in wood samples was determined by total combustion using a Truspec elemental analyzer (Leco, St. Joseph, MI, USA). Klason lignin content was determined gravimetrically as the dry mass of solids remaining after sequential hydrolysis with 72% (v/v) sulphuric acid at 30 °C for 1 h followed with 2.4% H_2SO_4 (v/v) at 120 °C for 1 h (Effland, 1977; Liers et al., 2011). In a second step, acid soluble lignin was measured by UV-photometry according to Dence (1992) in the obtained hydrolysate. Total lignin was obtained by summing acid insoluble Klason lignin and acid

Please cite this article in press as: Purahong, W., et al., Are correlations between deadwood fungal community structure, wood physico-chemical properties and lignin-modifying enzymes stable across different geographical regions?, Fungal Ecology (2016), http://dx.doi.org/10.1016/j.funeco.2016.01.002