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Correlations between the composition of modular fungal communities and litter decomposition-associated ecosystem functions

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ABSTRACT

Microbial co-occurrence network analyses provide important information on ecological interactions between different microbial Operational Taxonomic Units (OTUs), and often indicate that total microbial communities are sub-structured into modules. Here we investigate the fungal communities associated with beech leaf litter decomposition over 473 days using pyrotag sequencing of the fungal ITS rRNA genes. Our results demonstrate that the total fungal communities present during the two major decomposition stages are sub-structured into four modules each, giving eight modular fungal communities in total. These modular communities displayed different relationships with leaf litter physicochemical properties and ecological functions. During the early decomposition stage, modules 2 (dominated by *Gyoerffyella* sp.1 and *Mycosphaerella* sp.) and 4 (Xylariales OTU3, *Cylindrosympodium* sp.1 and Leotiomycetes OTU12) correlated significantly with both hydrolytic and oxidative enzyme activities ($P < 0.05$). During the later decomposition stage, module 7 (*Clitocybe phaeophthalma* and *Ceratobasidium* sp.) correlated significantly with the activities of laccase and general peroxidase ($P < 0.05$). Our results demonstrate that individual fungal subcommunities are largely responsible for providing specific ecosystem functions in litter decomposition, which improves our understanding of the factors that determine the distribution patterns of fungi and their potential roles in key ecosystem processes.

1. Introduction

Leaf litter decomposition is a complex ecological process that is regulated by three main drivers: climate, litter quality, and the decomposer communities (Coûteaux et al., 1995). Fungi are regarded as the primary decomposers of leaf litter because they secrete various digestive enzymes that breakdown polymeric organic plant compounds such as cellulose, hemicellulose and lignin (Keiblinger et al., 2012; Schneider et al., 2012; Voříšková and Baldrian, 2013; Purahong et al., 2014a). The distribution patterns of fungi provide important information about their potential roles in ecosystem functions and stability (Kubartová et al., 2012). Some studies reported that the overall composition of the fungal communities in leaf litter correlates with the physicochemical properties of the litter and with the microbially-mediated ecosystem functions (Burke et al., 2011; Peršoh, 2015; Purahong et al., 2015). However, it is not possible to generalize these observations as there is still no consistent support for this aspect and most of the studies may contain pseudocorrelations due to spatial distances, which is probably the most important driver of fungal community assembly (Talbot et al., 2014; Peršoh, 2015). Microbial communities are often built around complex networks of interactions such that the overall community is structured into smaller sub-communities or modules of co-occurring taxa. Studies on the relationships between such OTU co-occurrence modules and ecological variables can provide more significant insights into the ecosystem functions of fungi than could be obtained by considering relationships at the level of the whole fungal community (de Menezes et al., 2014). In addition, analyses of such modular- or sub-communities may enable better resolution of the factors that structure the microbial community in a given ecosystem while also facilitating the identification of different microbial functional groups and drivers of community composition (de Menezes et al., 2014).

Leaf litter decomposition is a key process of nutrient recycling

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that has two major phases (Berg, 2000). The first phase (the early decomposition stage) is regulated by the concentration of different nutrients and readily available carbon while the second phase (the later decomposition stage) is regulated by lignin decomposition. While several studies have explored the roles of fungal communities in litter decomposition, most of them have focused on the total fungal diversity (Steffen et al., 2007; Voříšková and Baldrian, 2013; Purahong et al., 2015). Here we present the first analysis of the co-occurrence networks within the litter-decomposing fungal community during the early and later stages of litter decomposition, and assess the composition of modular or sub-communities that are more specifically correlated with key enzymatic processes in litter decomposition.

A litterbag experiment was set up in a European beech (*Fagus sylvatica*) dominated forest. The experiment was maintained for 473 d to ensure that the collected litter reached the later stage of decomposition, which began after 284 d when significant lignin decomposition was observed (Purahong et al., 2014a). Indicators of the microbial communities' ecological functions were investigated by measuring eight enzyme activities at selected time points during the experiment (Purahong et al., 2014b). The fungal communities from the litter material were analyzed by pyrotag sequencing of the fungal ITS rDNA. Our aim was to: (i) characterize the fungal co-occurrence networks and modular communities existing during the early and later stages of decomposition; (ii) investigate the correlation between each fungal modular community and the litter's physicochemical parameters; and (iii) assess the link between the fungal modular communities and selected ecological functions including the activities of five hydrolytic enzymes important in the acquisition of carbon (β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), and xylosidase (EC 3.2.1.37)), nitrogen (N-acetylglucosaminidase (EC 3.1.6.1)), and phosphorus (acid phosphatase (EC 3.1.3.2)), as well as three oxidative enzymes important for lignin modification and degradation (laccase (EC 1.10.3.2), general peroxidase (EC 1.11.1.7), and manganese peroxidase (EC 1.11.1.13), MnP). We hypothesized that different fungal modular communities would correlate with different physicochemical properties and enzyme activities. We also expected that the correlations between the modular communities and the activities of specific enzymes would reflect the taxonomic and functional significance of the communities' member OTUs.

2. Materials and methods

2.1. Study site

This study was carried out at the Hainich-Dün Biodiversity Exploratory (about 1300 km^2; 51°16′N 10°47′E) in Central Germany (Fischer et al., 2010). The main soil type is Stagnosol on a limestone bedrock (Fischer et al., 2010). The soil pH is weakly acidic (5.1 ± 1.1; mean ± SD) with a litter layer of 2 cm–5 cm. The annual mean temperature and precipitation ranges from 6.5 °C – 8 °C and 500 mm–800 mm respectively. All information pertaining to the experimental area has been described in detail in Fischer et al. (2010). We assigned three replicate study plots (2 m × 8 m) located on flat land within the experimental site (HEW12) characterized as an unmanaged deciduous forest reserve of 10,000 m^2 dominated by European beech with an uneven-age distribution (tree age up to >100 years) (Purahong et al., 2015).

2.2. Litterbag design and sampling

Freshly fallen leaves of European beech were collected from the study site in October 2009. The leaves were air dried to constant weight at room temperature. Ten grams of air-dried leaves were placed into nylon litterbags (25 cm × 25 cm, mesh size 2 mm). At the end of the litter fall period (13 November 2009) 15 litterbags were placed horizontally in the upper litter horizon of each replicate study plot. Nine additional litterbags were retained to determine the initial dry mass (oven-dried at 105 °C ≥ 24 h until constant weight), nutrient element concentrations, and lignin content of the litter. The chemical composition of the litter material is given in Supplementary Table S1. Three randomly chosen litterbags per plot (nine litterbags in total) were retrieved on five sampling dates: in 2010 on February 10th (89 d), May 12th (180 d), August 24th (284 d), November 10th (362 d), and in 2011 on March 1st (473 d). Each bag was placed into a separate clean plastic bag to reduce the loss of small fragments, and transported in an ice-box (0 °C) to the laboratory within 4 h, and processed immediately. First, the three replicate litterbags retrieved from the same plot were pooled and their wet weight was determined. Thus, three composite samples were obtained for each sampling time. Each composite sample was then homogenized, subsampled into two parts (original homogenized and freeze-dried samples), and stored at −20 °C for further analysis.

2.3. Physicochemical analysis of leaf litter

The water content and pH (in 0.01 M $CaCl_2$) of leaf litter samples were determined. Total C and N were measured by dry combustion at 1000 °C with an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) according to DIN/ISO 10694. Nutrient ions (Mg, K, P, Ca, Fe, Cu, V, Mn, Co) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS) according to the manufacturers' specifications. Total lignin was calculated by summing Klason lignin (acid insoluble lignin) and acid soluble lignin (Raiskila et al., 2007). Klason lignin content was determined gravimetrically as the dry mass of solids after sequential hydrolysis with sulfuric acid (72% w/w); in a second step, acid soluble lignin was measured UV-photometrically in 4% H_2SO_4 (Effland, 1977; Liers et al., 2011). All physicochemical analyses were conducted in triplicate on the same subsample.

2.4. Enzyme assays

A total of eight potential enzyme activities were measured from original homogenized leaf litter samples (five sampling dates: 89–473 d). These include five hydrolytic enzymes important for the acquisition of polymeric carbon (β-glucosidase, cellobiohydrolase, and xylosidase), nitrogen (N-acetylglucosaminidase) and phosphorus (acid phosphatase), and three oxidative enzymes related to the chemical modification of lignin (laccase, general peroxidase, and MnP). Hydrolytic enzyme analyses were done using 4-methylumbelliferone (MUB) substrate as described elsewhere (Sinsabaugh et al., 2003). The protocol was slightly modified by pretesting (German et al., 2011). Oxidative enzyme analyses were done using ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) substrate (Purahong et al., 2014a, 2016).

2.5. Microbial DNA extraction and sequence library preparation

DNA was extracted from 100 mg of each homogenized and freeze-dried leaf litter sample using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The presence and quantity of genomic DNA was checked using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Dreieich, Germany). DNA extracts were then stored at −20 °C for further analysis.

Fungal amplicon libraries were obtained for pyrosequencing

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