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Ecology and diversity of leaf litter fungi during early-stage decomposition in a seasonally dry tropical forest

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ARTICLE INFO

Article history:
Received 10 January 2015
Revision received 27 April 2015
Accepted 30 April 2015
Available online 8 July 2015
Corresponding editor:
Gareth W. Griffith

Keywords:
Dry tropical forest
Forest fire
Fungal diversity
Plant biomass
Saprotrophic fungi

ABSTRACT

Leaf litter samples of 12 dicotyledonous tree species (belonging to eight families) growing in a dry tropical forest and in early stages of decomposition were studied for the presence of litter fungi. Equal-sized segments of the leaves incubated in moist chambers were observed every day for 30 d for the presence of fungi. Invariably, the fungal assemblage on the litter of each tree species was dominated by a given fungal species. The diversity of fungi present in the litter varied with the tree species although many species of fungi occurred in the litter of all 12 species. A *Pestalotiopsis* species dominated the litter fungal assemblage of five trees and was common in the litter of all tree species. The present study and earlier studies from our lab indicate that fungi have evolved traits such as thermotolerant spores, ability to utilize toxic furaldehydes, ability to produce cell wall destructuring enzymes and an endophyte-litter fungus life style to survive and establish themselves in fire-prone forests such as the one studied here. This study shows that in the dry tropical forest, the leaf litter fungal assemblage is governed more by the environment than by the plant species.

Introduction

Microbial communities play a major role in the decomposition of plant litter in terrestrial ecosystems. Fungi contribute substantially to this complex phenomenon of nutrient recycling in forest ecosystems as they elaborate an array of extracellular enzymes that deconstruct the different types of organic compounds in the litter (Baldrian and Lindahal 2011; Ţifčáková *et al.* 2011) including lignocellulose which other organisms are unable to decompose (de Boer *et al.* 2005). Studies on litter fungi of tropical ecosystems are limited compared to those in temperate ecosystems (Sayer 2006; McGuire *et al.* 2012; Xu *et al.* 2013). Many studies on litter fungi in the tropics pertain to fungi occurring in the litter of individual plant species such as *Maglielia garrettii* (Promputtha *et al.* 2002), *Magnolia liliifera* (Kodsueb *et al.* 2008), *Pandanus* sp. (Thongkantha *et al.* 2008), *Ficus* sp. (Wang *et al.* 2008), *Hevea brasiliensis* (Seephueak *et al.* 2010), *Anacardium occidentale* and *Pavetta indica* (Shanthi and Vittal 2010a, 2010b). Most of the studies from India on litter fungi are concerned with

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http://dx.doi.org/10.1016/j.funeco.2015.05.004

identification of new fungal taxa or new reports for a region (Subramanian and Ramakrishnan 1953; Subramanian and Natarajan 1975; Subramanian and Sudha 1978; Subramanian and Bhat 1987; Subramanian 1992; D'Souza and Bhat 2002, 2013); very few investigations address the ecology of litter fungi (Sinsabaugh *et al.* 2002; Ananda and Sridhar 2004; Suryanarayanan *et al.* 2009). We designed a study to assess the diversity and distribution of litter fungi in a dry tropical forest by comparing the fungi occurring in the leaf litter of 12 different dicotyledonous tree species.

As the litter decomposes due to microbial activity, its chemical makeup is altered which in turn selects the species of microbes (including fungi) that are adapted to occupy this progressively decaying and dynamic substratum. This continues until decomposition is complete, and entails a succession of fungal species on litter which are arbitrarily classified as early, intermediate and late colonizers (Frankland 1998; Dickie *et al.* 2012). Hence, long-term monitoring is essential to determine changes in the fungal community during litter decomposition (Treseder *et al.* 2013). One-time samplings of litter for their fungal community have also been undertaken to answer various questions regarding their ecology (McGuire *et al.* 2012; D'Souza and Bhat 2013; Osono *et al.* 2013). In the present study we chose a one-time sampling method. Although this would not reveal the extent of contribution of different fungi to the complete decomposition of the litter, it helped to minimize the influence of environment on litter fungal community and facilitated comparison across tree species.

Materials and methods

Sample collection

Leaf litter samples of 12 tree species (belonging to eight families) from private lands located adjacent to the dry deciduous forest (DD) of the Mudumalai Wildlife Sanctuary (Lat. 11°32′ and 11°43′ N, Long. 76° 22′ and 76° 45′ E), which receives 1 200 mm of rainfall per annum were studied (Table 1). These were the most common tree species growing in this forest (Suryanarayanan *et al.* 2011a). DD constitutes the largest expanse of Mudumalai Wildlife Sanctuary and experiences a continuous dry period from Nov. to Apr. Leaves are completely shed in Jan. and Feb. and new leaves are flushed by the end of Apr (Murali and Sukumar 1993). For each tree species, 20 mature, hard, brown leaves-representing neither freshly fallen nor in a state of advanced decay-from the floor of the forest (O horizon) were collected during Mar.—Apr. and processed as follows.

Table 1 – Leaf litter of tree species studied for the presence of litter fungi

Tree species	Family	Code
Anogeissus latifolia	Combretaceae	AL
Cassia fistula	Caesalpiniaceae	CF
Cordia wallichii	Boraginaceae	CW
Lagerstroemia microcarpa	Lythraceae	LM
Lagerstroemia parviflora	Lythraceae	LP
Ougeinia oojeinensis	Papilionaceae	OO
Premna tomentosa	Verbenaceae	PT
Shorea roxburghii	Dipterocarpaceae	SR
Syzygium cumini	Myrtaceae	SC
Tectona grandis	Verbenaceae	TG
Terminalia bellerica	Combretaceae	TA
Vitexa ltissima	Verbenaceae	VA

Isolation and identification of litter fungi

The moist chamber technique (Cannon and Sutton 2004) was used for isolating fungi from the leaf litter. Twenty fallen leaves were collected from the litter layer for each tree species (Table 1) and from each leaf five segments (approx. 0.5 cm^2) were cut from the lamina region. The one hundred tissue segments thus obtained for each tree species were rinsed in sterile water. From these 100 segments, 90 were randomly selected and incubated in Petri dishes (9 cm dia) containing three layers of filter papers moistened with sterile water. Each Petri dish had nine tissue segments and the Petri dishes were sealed using Parafilm™ and incubated in a light chamber with a 12 hr light: 12 hr dark cycle at 26 ± 1 °C (Suryanarayanan 1992) for 30 d. The light chamber had a bank of three four foot Philips day light fluorescent lamps. The tissue segments received about 2 200 lux of light through the Petri dish lid. Three litter segments were observed under a microscope daily for the presence of fungal spores from 3 d of incubation onwards up to 30 d. The leaf litter segment was comminuted using sterile water and a scalpel, placed on a glass slide, stained with lactophenol and observed under a bright field microscope (×400, Nikon, Labophot 2) for the presence of fungal spores.

Fungi were identified based on their spore morphology and spore development. Every time a particular fungus was observed from a leaf segment, it was recorded as one isolate. The isolated fungi were identified using standard taxonomic keys (Ellis 1976; Subramanian 1971; Sutton 1980; Onions *et al.* 1981; Ellis and Ellis 1988; Nag Raj 1993; Hyde *et al.* 2000). Fungi that could not be identified were given codes (DLF 001, 002, 003, etc.) based on the size, shape, septation, ornamentation and pigmentation of the spores. Spores exhibiting similar morphology were grouped under one morphospecies (Arnold *et al.* 2000).

Detection of extracellular enzyme production by litter fungi

The method of Rohrmann and Molitoris (1992) and Kumaresan *et al.* (2002) were used for qualitative screening of the fungi for the production of amylase, cellulase, laccase, lipase, pectinase, pectate transeliminase, and protease enzymes. The methods involved growing the fungus in an agar medium amended with a suitable substrate and visually detecting the loss of substrate or the formation of the product due to enzyme action.

Statistical methods used

Percentage of Abundance (PA) (Van Ryckegem and Verbeken 2005) was given by: