



# The replacement of native plants by exotic species may affect the colonization and reproduction of aquatic hyphomycetes



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## ABSTRACT

The tropical riparian zone has a high diversity of plant species that produce a wide variety of chemical compounds, which may be released into streams. However, in recent decades there has been an extensive replacement of tropical native vegetation by *Eucalyptus* monocultures. Our objective was to compare fungal colonization of *Eucalyptus camaldulensis* leaves with fungal colonization of native plant species from riparian zones in Brazilian Cerrado (savannah) streams. The fungal colonization and enzymatic activity significantly influenced leaf litter decomposition. Fungal sporulation rates from leaf litter varied significantly with leaf species, with *E. camaldulensis* showing the highest sporulation rate (1226 conidia  $\text{mg}^{-1}$  AFDM  $\text{day}^{-1}$ ) and leaf mass loss ( $23.2 \pm 0.9\%$ ). This species has the lowest lignin content and highest N concentration among the studied species. Among the studied native species, we observed the highest sporulation rate for *Protium spruceanum* (271 conidia  $\text{mg}^{-1}$  AFDM  $\text{day}^{-1}$ ), *Maprounea guianensis* (268 conidia  $\text{mg}^{-1}$  AFDM  $\text{day}^{-1}$ ) and *Copaifera langsdorffii* (196 conidia  $\text{mg}^{-1}$  AFDM  $\text{day}^{-1}$ ). Overall, native plant species of the Brazilian Cerrado exhibited recalcitrant characteristics and a higher lignin:N ratio. Therefore, variations in the physical and chemical characteristics of the leaf litter could explain the higher decay rate and reproductive activity observed for *E. camaldulensis*. However, the detritus of this species were colonized almost exclusively by *Anguillospora filiformis* ( $99.6 \pm 0.4\%$ ) and exhibited a reduction in aquatic hyphomycetes species diversity. Our results suggest that the disturbance in the composition of riparian vegetation and consequently, in the diversity of leaf litter input into streams, could change the patterns and rates of leaf litter utilization by microbial decomposers. These changes may have important consequences in the processing of organic matter and, consequently, in the functioning of freshwater ecosystems.

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## 1. Introduction

Headwater streams are typically heterotrophic systems due to their limited primary productivity; thus the organic matter from the riparian zone is the energy source and nutritional base of stream food webs (Vannote et al., 1980; Abelho, 2001). Leaf breakdown induced by chemical and physical processes (e.g., leaching and physical abrasion) and by biological factors (i.e., microorganisms and invertebrate detritivores) are key to the functioning of these stream ecosystems (Gessner et al., 1999; Graça et al., 2002). Microbial conditioning modifies the leaf matrix directly by accelerating decomposition through leaf degradation and metabolization and indirectly by increasing the palatability and nutritional value of the

litter to invertebrate detritivores (Gessner et al., 1999; Krauss et al., 2011).

Fungi and bacteria are the primary microorganisms involved in the decomposition of leaf litter in streams (Hieber and Gessner, 2002; Gonçalves et al., 2006). Despite the importance of bacteria, aquatic hyphomycetes are the main microbial drivers of this process (Kearns and Bärlocher, 2008; Krauss et al., 2011). This group of fungi belongs to a phylogenetically heterogeneous group that develops in decaying leaves and produces a high number of asexual spores that are released into the water column (Bärlocher, 2009; Findlay, 2010). Rapid colonization of a new substrate is, therefore, fundamental to the survival of these microorganisms, which invest an average of 50% of their biomass production into spore formation (Sridhar and Bärlocher, 2000; Kearns and Bärlocher, 2008).

The unidirectional flow in lotic environments is a common challenge for many organisms, such as aquatic hyphomycetes, that might be carried downstream (Bärlocher, 2009). Moreover, con-

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dia are relatively fragile, and fungal germline viability is limited (Sridhar and Bärlocher, 1994); thus, the survival of hyphomycetes depends on the efficient attachment of conidia to a suitable substrate (Dang et al., 2007). An important hyphomycete adaptation to turbulent lotic environments is the production of relatively large conidia of various shapes, including tetra- and sigmoid (Kearns and Bärlocher, 2008; Sudheep and Sridhar, 2013). Moreover, hyphomycete conidia produce mucilage, which is a mixture of several polysaccharides that facilitate adhesion to the substrate (Kearns and Bärlocher, 2008). These adaptations provide a greater capacity for colonization of different types of leaf substrate (Dang et al., 2007).

Other factors, such as the chemical properties and physical structure of the leaf surface, also influence fungal colonization (Dang et al., 2007; Ferreira et al., 2012). Overall, leaf litter with a lower lignin:N ratio is considered to be of high chemical quality, while higher lignin:N ratios indicate low quality (Lecerf and Chauvet, 2008; Talbot and Treseder, 2012). Thus, leaves of lower chemical quality may promote selective colonization, thereby inhibiting leaf litter decomposition by some fungal species (Ardón and Pringle, 2008; Lecerf and Chauvet, 2008). Moreover, the leaves of some species have very thick cuticles, which also create a barrier to fungal colonization (Canhoto and Graça, 1999). Conversely, the presence of various epidermal appendages (e.g., hair, scales and buds) increase leaf surface roughness and, consequently, provide opportunities for conidial adhesion to the substrate (Dang et al., 2007; Kearns and Bärlocher, 2008).

Understanding the relationship between the quality of leaves entering a stream and their decomposition is important because the encroachment of human settlements into riparian areas has led to major changes in plant composition (García et al., 2012). Change in riparian plants leads to modifications of the quality of organic matter input into freshwater ecosystems (Tank et al., 2010) because various types of litter differ in their chemical composition. Decomposers prefer high quality substrates, and therefore, shifts in the composition and diversity of riparian vegetation may affect the colonization and decomposition of leaf litter by aquatic hyphomycetes and the consumption by invertebrate detritivores (Ferreira et al., 2012, 2015).

Some studies have reported a significant reduction in leaf litter processing capacity in streams where the native vegetation has been replaced by monocultures of exotic species (Martínez et al., 2013; Menéndez et al., 2013). However, studies examining the effects of exotic species on leaf breakdown or decomposer communities have typically compared the breakdown of the leaf litter of exotic plant species, especially of the genus *Eucalyptus*, with a reduced number of native species (around five native species; Larrañaga et al., 2009; Pérez et al., 2014; Ferreira et al., 2015). In the tropical riparian zone, we found a higher diversity of plant species that produce a wide variety of chemical compounds, which consequently release different compounds into streams. Thus, the objective of this investigation was to compare fungal colonization of *Eucalyptus camaldulensis* leaves with fungal colonization of native plant species from riparian zones in Brazilian Cerrado (savannah) streams. Our hypothesis was that the increased input of exotic leaves into streams will decrease fungal diversity and activity.

## 2. Materials and methods

### 2.1. Study site

The Botanical Garden of Brasília (Jardim Botânico de Brasília, JBB) (15°50′–15°55′S, 47°49′–47°55′W) is an important preservation area within the Federal District (Distrito Federal, DF) of

Brazil. The JBB is located 1056 m above sea level and is part of the Environmental Protection Area Gama Cabeça de veado (SEMATEC/CODEPLAN, 1994). The climate is classified as tropical Aw with a dry season (April to September) and a rainy season (October to March) (Eiten, 1972). The Ecological Station of the Botanical Garden of Brasília (EEJBB) has an area of approximately 4500 ha and is composed of substantial areas representing many of the Cerrado physiognomies (Fonseca and Silva-Júnior, 2004). The headwaters and upper and middle courses of the Cabeça de veado stream are located within the Ecological Station, and this stream has a width ranging from 5 to 50 m and tree cover of 80–100% for its entire length (approximately 7 km) (Plano de Manejo da EEJBB, 2010). A leaf litter incubation experiment was carried out in a 2nd order stretch of this stream.

### 2.2. Experimental plant species

A total of 14 riparian plant species from the Cerrado biome were selected: *Aspidosperma discolor* A. DC.; *Calophyllum brasiliense* Cambess.; *Copaifera langsdorffii* Desf.; *Emmotum nitens* (Benth.) Miers; *Hyeronima alchorneoides* Freire Allemão; *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang; *Inga laurina* (Sw.) Willd.; *Maprounea guianensis* Aubl.; *Paullinia carpopodea* Cambess.; *Protium heptaphyllum* (Aubl.) March.; *Protium spruceanum* (Benth.) Engl.; *Richeria grandis* Vahl; *Tapirira guianensis* Aubl.; and *Vochysia pyramidalis* Mart. These species were selected because they are common riparian vegetation of Cerrado streams (Silva-Júnior et al., 2001). In addition, we used an exotic species (*Eucalyptus camaldulensis* Dehnh.) in the experiment due to the extensive replacement of native vegetation by *Eucalyptus* monocultures worldwide (Graça et al., 2002). The replacement of native vegetation by *Eucalyptus* monocultures is very common in tropical environments, such as the Brazilian Cerrado, leading to important changes in the ecological processes of aquatic ecosystems (Gonçalves et al., 2012).

The leaves were sampled monthly, from April 2011 to March 2012, using nets (1 m<sup>2</sup>, 10 mm mesh) fixed approximately 1.5 m from the ground in riparian zones within the Gama Cabeça de veado. Collected leaves were transported in plastic bags to the laboratory, where they were sorted, identified, and air-dried. Leaves were then totality homogenized and wrapped in thermal boxes prior to being placed into litter bags.

### 2.3. Leaf chemical composition (microcosm experiment)

The initial chemical composition of each plant species was assessed. After 10 days of incubation in a microcosm with flowing continuum, the chemical composition and mass loss of the leaf detritus were analysed. The water used in the microcosms was collected in the stream Cabeça de veado and maintained in 60 L tanks (35 cm × 50 cm × 35 cm) at 18.4 ± 3.6 °C. We used aquarium pumps to oxygenate and agitate the water; this process creates turbulence and keeps the detritus in permanent agitation. Three litter bags (20 × 20 cm, 10 mm mesh width) containing 2.00 ± 0.01 g of each leaf species were incubated separately in three aquariums. For each species, three additional litter bags were prepared but not incubated to determine the initial ash free dry mass (AFDM) and to account for any mass losses due to manipulation. After 10 days, a litter bag was removed from each aquarium. Five leaves were taken at random from each litter bag. A leaf disk (diameter 12 mm) was taken from each leaf, resulting in a set of five leaf disks per species for use in determining AFDM. The disks were dried in a stove at 60 °C for 72 h to determine the remaining dry mass (DM). The disks were then incinerated in a muffle furnace at 550 °C for 4 h to determine the ash mass (AM). The calculation of the remaining AFDM was estimated as the difference between the DM and AM expressed as a percentage. The detritus was then ground for the chemical

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