Science of the Total Environment 565 (2016) 489–495

Contents lists available at ScienceDirect

Science of the Total Environment

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

Ethanol and phenanthrene increase the biomass of fungal assemblages and decrease plant litter decomposition in streams

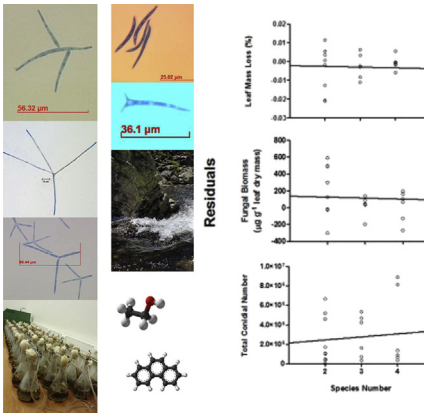
Diana Barros [a,b], Patrícia Oliveira [a], Cláudia Pascoal [a,b], Fernanda Cássio [a,b,*]

[a] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

HIGHLIGHTS

- Ethanol and phenathrene increased fungal biomass
- Ethanol and phenathrene inhibited leaf decomposition and fungal reproduction
- Fungal activity tended to be higher in polycultures than in monocultures
- Fungi in polycultures did not exceeded the activity of the most productive species

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 26 April 2016
Accepted 28 April 2016
Available online xxxx

Editor: Jay Gan

Keywords:
Fungal consortia
Phenanthrene
Ethanol
Ecosystem function
Streams

ABSTRACT

Fungi, particularly aquatic hyphomycetes, have been recognized as playing a dominant role in microbial decomposition of plant litter in streams. In this study, we used a microcosm experiment with different levels of fungal diversity (species number and identity) using monocultures and combinations with up to five aquatic hyphomycete species (*Articulospora tetracladia*, *Tricladium splendens*, *Heliscus submersus*, *Tetrachaetum elegans* and *Flagellospora curta*) to assess the effects of ethanol and phenanthrene on three functional measures: plant litter decomposition, fungal biomass accrual and reproduction. Alder leaves were conditioned by fungi for 7 days and then were exposed to phenanthrene (1 mg L^{-1}) dissolved in ethanol (0.1% final concentration) or ethanol (at the concentration used to solubilise phenanthrene) for further 24 days. Exposure to ethanol alone or in combination with phenanthrene decreased leaf decomposition and fungal reproduction, but increased fungal biomass produced. All aspects of fungal activity varied with species number. Fungal activity in polycultures was generally higher than that expected from the sum of the weighted performances of participating species in monoculture, suggesting complementarity between species. However, the activity of fungi in polycultures did not exceed the activity of the most productive species either in the absence or presence of ethanol alone or with phenanthrene.

* Corresponding author.
E-mail address: fcassio@bio.uminho.pt (F. Cássio).

http://dx.doi.org/10.1016/j.scitotenv.2016.04.190
0048-9697/

1. Introduction

In low-order forest streams with reduced primary production, allochthonous organic matter, particularly leaf litter, is the main source of carbon and energy for the stream biota (Tank et al., 2010). Decomposition of leaf litter is a complex process that involves physical and chemical transformations driven by bacteria, fungi and invertebrates (Gessner et al., 1999; Graça, 2001; Pascoal et al., 2005a).

Aquatic hyphomycetes are among the most active group of microorganisms in the mineralization of leaf litter due to their ability to produce extracellular enzymes able to degrade the most recalcitrant polymers of plant cell walls (Romaní et al., 2006). They also play a crucial role in trophic food webs because, together with bacteria, they convert leaf carbon into microbial biomass, an important component of the diet of invertebrate detritivores (Gessner et al., 1999; Graça, 2001). When microbial community colonizing leaf litter is altered, litter decomposition and consumption by detritivores is most certainly affected with impacts on food-web structure and function (Batista et al., 2012).

The knowledge on the relationships between biodiversity of aquatic hyphomycetes and their ecological functions are of particular concern in polluted environments, where a decrease in biodiversity is often found (Pascoal and Cássio, 2004; Pascoal et al., 2010; Sridhar et al., 2009). Species loss does not necessarily result in alterations of ecosystem processes because there is considerable redundancy among aquatic fungal species (Pascoal et al., 2005b). Moreover, when subjected to environmental stressors, biodiversity is expected to decline faster than ecosystem processes, which remain relatively stable at least until moderate levels of stress (Niyogi et al., 2002; Duarte et al., 2008).

Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrophobic organic compounds, with two or more combined benzene rings, which are spread across ecosystems where they can persist for many years (Peng et al., 2008). There are more than 100 PAH compounds that can be originated either from natural sources, such as forest fires and volcanic eruptions, or from anthropogenic sources, as burning of fossil fuels, wood and garbage and petroleum spills (Peng et al., 2008). They are considered pollutants of great concern due to their potential toxicity, mutagenicity and carcinogenicity (Holoubek, 2000; Peng et al., 2008).

Several studies have attributed the ecological recovery of PAH-contaminated sites to both bacteria and fungi due to their ability to metabolize toxic organic compounds, generating less toxic and complex metabolites (Leys et al., 2005; Haritash and Kaushik, 2009). Some species of bacteria and fungi can even utilize PAHs as sole carbon source (Kazunga and Aitken, 2000; Johnsen et al., 2002). For instance, the bacterium *Pseudomonas aeruginosa* and the yeast *Rhodotorula glutinis* were able to metabolize phenanthrene, and an almost complete degradation was observed during one month of incubation in liquid medium with up to 200 mg L^{-1} of phenanthrene (Romero et al., 1998). Moreover, there is evidence that aquatic hyphomycetes have the potential to tolerate or even degrade organic xenobiotics (Junghanns et al., 2005; D'Annibale et al., 2006).

Owing to the low aqueous solubility of PAHs and their absorption to solid particles, they tend to become potentially unavailable for microbial degradation. However, some microorganisms have developed different strategies to increase its bioavailability by, for instance, producing biosurfactants (Holoubek, 2000; Cuny et al., 2004; Huesemann et al., 2004; Pozdnyakova et al., 2006). Moreover, PAHs can become bioavailable in the presence of organic solvents, such as ethanol.

Ethanol can be used as a source of carbon and energy by microorganisms, such as *Saccharomyces cerevisiae*, and even humans are able to metabolize it for energy release (Boulton et al., 1998). On the other hand, ethanol reaches all body tissues and compromises most vital functions (Lieber, 1997), its negative effects on cells are widely known and lead, for example, to cell viability reduction (Canetta et al., 2006). Moreover, ethanol inhibits the activity of glycolytic enzymes (Duruibe and Tejwani, 1981), stimulates the ATPase activity (Rosa and Sá-Correia, 1991) and causes damage in mitochondrial DNA (Ibeas and Jimenez, 1997).

The largest single use of ethanol is as a motor fuel and fuel additive, at levels from ca. 10% up to 85% (Galbe and Zacchi, 2007). In 2010, the world's top ethanol fuel producers were the United States and Brazil, accounting together for 88% of world production, and strong incentives are giving rise to fledgling ethanol industries in other countries in Europe, Asia and America (Lichts, 2010). Although beneficial in reducing atmospheric pollution, the use of ethanol as fuel has increased groundwater contamination, due to the co-solvency of petroleum hydrocarbons and by the provision of a preferential substrate for microbial utilization (Adam et al., 2002; Bhanu and Philip, 2011). Although PAHs are ubiquitous, little information is available on the responses of aquatic hyphomycetes to PAHs (but see Moreirinha et al., 2011). Previous studies assessed the effects of stressors, including eutrophication (Pascoal et al., 2003; Pascoal and Cássio, 2004), metals (Azevedo et al., 2007; Azevedo et al., 2009), organic xenobiotics (Junghanns et al., 2005; Krauss et al., 2005) on the structure of aquatic hyphomycete communities and the ecological processes they drive; however, only one study assessed the effects of phenanthrene on aquatic hyphomycetes (Moreirinha et al., 2011) and no data is available on the effects of ethanol.

We used a microcosm experiment with monocultures and combinations with up to five aquatic hyphomycete species to examine the effects of phenanthrene and ethanol alone or in mixtures under different levels of fungal diversity (species number and identity) on three aspects of ecosystem functioning: litter decomposition, fungal biomass accrual and reproduction. We hypothesized that phenanthrene and/or ethanol could be used as a source of carbon and energy for aquatic fungi and, if so, plant litter decomposition in streams would decrease while fungal biomass production would increase. However, if ethanol and/or phenanthrene had toxic effects on fungi, fungal activity and leaf litter decomposition would decrease.

2. Materials and methods

2.1. Microcosms

Leaves of *Alnus glutinosa* (L.) Gaertn., collected in October 2006, were air dried and kept at room temperature. The leaves were leached in deionised water for 2 days and cut into 22 mm diameter disks. Sets of 20 disks were placed in 250 mL Erlenmeyer flasks and autoclaved for 20 min. To each Erlenmeyer flask, 80 mL of a mineral solution (0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 2H_2O$, 0.01 g KNO_3, 0.01 g K_2HPO_4, and 0.5 g 2-[*N*-morpholino] ethanesulfonic acid per 1 L, pH 6.0) was added aseptically.

The aquatic hyphomycetes *Articulospora tetracladia* Ingold (UMB-61.01), *Flagellospora curta* J. Webster (UMB-39.01), *Tricladium splendens* Ingold (UMB-103.01), *Tetrachetum elegans* Ingold (UMB-138-01) and *Heliscus submersus* H. J. Huds. (UMB-01.99) were isolated from single spores collected in the Este River (NW Portugal) and grown on 1% malt extract agar.

Microcosms were inoculated with agar plugs collected from the edge of 20-days-old colonies of the five fungi as follows: monocultures of the five species (At, *A. tetracladia*; Fc, *F. curta*; Ts, *T. splendens*; Te, *T. elegans*; and Hs, *H. submersus*), three combinations of two species (At + Hs, Ts + Fc and Te + Hs), two combinations of three species (At + Fc + Ts and Hs + Ts + Te), two combinations of four species (Hs + At + Ts + Te and Fc + At + Ts + Te) and all species combined. The species in polycultures were chosen at random. Single species microcosms were inoculated with 7-mm-diameter plugs. For polyculture microcosms, the plugs were divided equally among species maintaining the total inoculum size constant. For each treatment, three replicate microcosms were used. Alder leaf disks were conditioned by fungi for 7 days and then were exposed to ethanol (0.1% v/v; Panraec) alone or in mixture with phenanthrene (1 mg L^{-1}; Sigma) for further 24 days.