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The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate

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ABSTRACT

We examine the relative importance of substrate quality and temperature in the establishment of aquatic hyphomycete assemblages and in their ability to decompose leaves. We used leaves of alder (*Alnus glutinosa*) and oak (*Quercus robur*) and we tested four temperatures (5°, 10°, 15° and 20 °C). Differences in decomposition rates and fungal assemblages were higher substrata than across temperatures. In both species, decomposition efficiency measured as the ratio of decay rate to fungal biomass, was greater at higher temperatures. Oak leaves were colonized by fewer aquatic hyphomycete species than was alder. Decomposition rates of oak increased with temperature but that of alder was not affected. We conclude that the substratum is a key driver of aquatic hyphomycete assemblages and can attenuate the effects of temperature differences on litter decomposition.

Introduction

Small woodland streams account for most of the total length of a river and are characteristically heterotrophic, given their dependence on terrestrial plant litter as a source of energy and carbon (Webster and Benfield, 1986; Abelho, 2001). Leaf litter decomposition in such streams is a key ecosystem-level process carried out by a varied assemblage of microbes (fungi and bacteria) and invertebrates (Gessner et al., 1999, 2010). The activity, diversity and productivity of aquatic hyphomycetes, a dominant group in early stages of leaf litter degradation, depends on the physico-chemical characteristics of the leaves and intraspecific variability in leaf traits, as well as being influenced by environmental factors (Gessner et al., 1999, 2007; Suberkropp, 2001; Baldy et al., 2002; Lecerf et al., 2005; Lecerf and Chauvet, 2008). It is generally accepted that microbial-mediated decomposition is retarded in low quality substrata (*i.e.* low nutrients and high lignin concentrations) while soft, highly nutritious leaves (*e.g.* N and P rich) tend to stimulate decomposition (Gessner and Chauvet, 1994; Canhoto and Graça, 1996; Ferreira et al., 2006).

Among the various environmental factors that have been identified as important regulators of stream ecosystem processes, temperature is of paramount relevance due to its role as a modulator of biological processes (*e.g.* Friberg et al., 2009; Woodward et al., 2010; Bergfur and Friberg, 2012). Evidence from terrestrial systems suggests that the ratio of labile to recalcitrant substances of a substratum may affect the

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temperature sensitivity of its decomposition (Conant et al., 2008, 2011), while a study in freshwater indicates that higher temperatures may overrule the importance of intrinsic differences in leaf characteristics on microbial-mediated decomposition (Fernandes et al., 2012). Experiments with European alder leaves (high-nutrient content) revealed that raising the temperature lead to an increase in microbial-mediated decomposition (*e.g.* Fernandes et al., 2009, 2012; Ferreira and Chauvet, 2011a,b; Geraldes et al., 2012), in spite of a general reduction of fungal diversity and the consequent changes in the structure of fungal communities (Bärlocher et al., 2008; Fernandes et al., 2009, 2012; Ferreira and Chauvet, 2011a). It seems plausible that temperature changes may result in species replacement (probably related to fungal species-specific thermal physiological limits) with the overall ecological functions remaining intact. Furthermore, the effects of temperature on fungal performance are not straightforward: oscillations could causes differ compared with continuous gradients, since exposure to extreme values may inhibit fungal activity (Rajashekhar and Kaveriappa, 2000; Bergfur and Friberg, 2012; Bärlocher et al., 2013), promoting significant changes in litter decomposition with cascading effects throughout stream food webs.

We used a microcosm experiment to assess leaf mass loss and associated aquatic hyphomycete assemblages of alder (*Alnus glutinosa*; high quality) and oak (*Quercus robur*; low quality) leaves across a temperature range from 5 to 20 °C. Both species have been commonly used as test-species in decomposition experiments (*e.g.* Gessner and Chauvet, 1994; Ferreira et al., 2006; Gulis et al., 2006; Lecerf et al., 2007). We hypothesize that deviation from the prevailing stream temperature from which the aquatic hyphomycete assemblages were obtained will affect fungal diversity, which in turn will affect both the activity and decomposition of the 2 leaf species accordingly. Differences across temperatures are expected to be more striking on the more recalcitrant leaf.

Methodology

Microcosms and experimental setup

Alder (*A. glutinosa*) and oak (*Q. robur*) leaves were collected from the forest floor immediately after fall and air-dried in the dark at room temperature. To stimulate the loss of secondary compounds that usually retard fungal colonization in streams, leaves were leached for 48 hr in aerated distilled water in the lab and oven-dried (60 °C; 48 hr). Portions of leaves were ground up and analyzed for phosphorus, nitrogen, carbon, total polyphenolics (% leaf dry mass; as described in Graça et al., 2005) and lignin (% leaf dry mass; as described in Goering and Van Soest, 1970). Additionally, initial toughness was measured as penetrance (Graça et al., 2005), *i.e.* the required mass (g of water in a container) to push a 1 mm diameter metal shaft through the leaf disc.

The remaining leaves were used to obtain pairs of 12 mm diameter leaf discs, which were punched out symmetrically in relation to the main leaf vein with a cork borer. Groups of 20 discs from each species were oven-dried (105 °C; 48 hr), pre-weighed (±0.1 mg), rehydrated by spraying with distilled water and enclosed in 6 × 6 cm fine mesh bags (0.5 mm). In each bag, two groups of five discs (each one with known symmetrical pairs) were separately marked with nylon lines to allow identification in subsequent experiments (see below).

A total of 192 bags, each with 20 leaf discs (96 with alder and 96 with oak) were tied to nylon ropes and immersed in the Candal stream (a 2nd order stream located in Serra da Lousã at 617 m asl; Central Portugal; W 40° 4′ 44″ N and 8° 12′ 10″ W) to allow microbial colonization for 7 d. During the incubation, the water was 90 % saturated with oxygen, the temperature was 9 °C (Jenway 9200 oxygen meter; Jenway, UK), the pH was 6.6 (Jenway 3150; Jenway, UK) and the conductivity reached 25.7 μS cm^{-1} (WTW LF 330; WTW, Germany) (mean of two measurements on days 1 and 4). Stream water samples were transported to the laboratory in a cooler, filtered (glass fiber filter, 0.7 μm pore size, Millipore APFF) and frozen at −18 °C for determination of cations and anions by ion chromatography (Dionex DX-120, Sunnyvale, California, USA) and soluble reactive phosphorus (SRP) concentration (Allan and Castillo, 2007). Stream values for nitrate, ammonia, sulfate, nitrite and phosphate SRP were: 0.13 mg l^{-1}, 0.96 mg l^{-1}, 1.48 mg l^{-1}, <100 μg l^{-1} and 45.33 μg l^{-1}, respectively. Additional stream water samples were collected, filtered as described above and autoclaved (120 °C, 1 hr) for microcosm assays (see below). After stream incubation, all the bags were brought to the laboratory and the leaf discs rinsed with deionized water. From the 20 discs in each bag, one of the marked sets of five discs was used as a control to account for mass losses in the stream and to determine initial mass for subsequent laboratory experiments (see below); the corresponding symmetrical set was further immersed in the microcosm along with the other 10 discs from each bag. These 15 leaf discs from each replicate were placed in sterilized 100 ml Erlenmeyer flasks with 40 ml of sterile stream water. Batches of 48 microcosms (24 for each leaf species) were exposed to temperatures of 5, 10, 15 or 20 °C, which encompass the annual temperature range of local streams.

The microcosms were incubated on shakers (120 rpm) under a 12 hr light: 12 hr dark photoperiod for 56 d. Stream water was replaced every 2 d and spore suspensions were saved and combined to determine the total spore production and fungal diversity from each microcosm. After 7, 21, 35 and 56 d, six replicates of each treatment were sacrificed to estimate leaf mass loss, microbial respiration and fungal biomass.

Mass loss

Leaf discs (control and exposed in microcosms) were oven-dried (48 hr at 105 °C), weighed (±0.1 mg), ashed (5 hr at 550 °C) and reweighed to obtain ash free dry mass remaining (AFDMr). Mass loss was estimated as the % difference between the dry mass of the discs not exposed and the discs exposed in the microcosms for 56 d.

Microbial respiration

One set of five leaf discs from each microcosm was used to determine leaf associated microbial respiration. This was done