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Effects of whole-stream nitrogen enrichment and litter species mixing on litter decomposition and associated fungi

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ABSTRACT

Nutrient enrichment and changes in riparian tree species composition affect many streams worldwide but their combined effects on decomposers and litter decomposition have been rarely assessed. In this study we assessed the effects of experimental nitrogen (N) enrichment of a small forest stream on the decomposition of three leaf litter species differing in initial chemical composition [alder (*Alnus glutinosa*), chestnut (*Castanea sativa*) and poplar (*Populus nigra*)], incubated individually and in 2-species mixtures during late spring-early summer. To better understand the effects of litter mixing on litter decomposition, component litter species were processed individually for remaining mass and fungal reproductive activity. Litter decomposition rates were high. Nitrogen enrichment significantly stimulated litter decomposition only for alder incubated individually. Differences among litter treatments were found only at the N enriched site where the nutrient rich alder litter decomposed faster than all other litter treatments; only at this site was there a significant relationship between litter decomposition and initial litter N concentration. Decomposition rates of all litter mixtures were lower than those expected from the decomposition rates of the component litter species incubated individually, at the N enriched and reference sites, suggesting antagonistic effects of litter mixing. Conidial production by aquatic hyphomycetes for each sampling date was not affected by nutrient enrichment, litter species or mixing. Aquatic hyphomycetes species richness for each sampling date was higher at the N enriched site than at the reference site and higher for alder litter than for chestnut and poplar, but no effect of mixing was found. Aquatic hyphomycetes communities were structured by litter identity and to a lesser extent by N enrichment, with no effect of mixing. This study suggests that nutrient enrichment and litter quality may not have such strong effects on decomposers and litter decomposition in warmer seasons contrary to what has been reported for autumn-winter. Changes in the composition of the riparian vegetation may have unpredictable effects on litter decomposition independently of streams trophic state.

1. Introduction

Small forest streams make up most of the water courses in temperate catchments (Allan and Castillo, 2007). In these streams, the aquatic food web is greatly fuelled by the autumnal leaf litter input derived from the riparian vegetation (Wallace et al., 1997). Once in water, this organic matter is rapidly colonized by microbial decomposers, especially aquatic hyphomycetes, who mineralize it and convert it into biomass (mycelium and conidia) leading to substantial litter mass loss (Gulis and Suberkropp, 2003). By the activities of their external enzymes and build up of mycelium, aquatic hyphomycetes also increase litter palatability to invertebrate detritivores, establishing the link between organic matter and secondary production (Canhoto and Graça, 2008).

The community structure and performance of aquatic hyphomycetes and litter decomposition are affected by environmental changes (Ferreira et al., 2014; Canhoto et al., 2016; Ferreira and Voronina, 2016). In particular, moderate increases in dissolved nutrients concentration generally modify aquatic hyphomycetes community structure (Gulis and Suberkropp, 2003; Castela et al., 2008; Lima-Fernandes et al., 2015) and stimulate fungal activities (with reproductive activity being most sensitive) and litter decomposition (Suberkropp and Chauvet, 1995; Gulis and Suberkropp, 2003; Gulis et al., 2006; Woodward et al., 2012; Ferreira et al., 2015). Increased nutrients concentration presently affects many streams (Woodward et al., 2012) and generally results

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from agriculture, urbanization and atmospheric nitrogen (N) deposition (Rockström et al., 2009). Given the predicted increase in human population over this century, and consequent increases in the needs for food, water, transport and housing, nutrient loads to streams are expected to increase (MEA, 2005). Thus, many more streams will likely be affected by nutrient enrichment in the future, with consequences for aquatic communities and litter decomposition.

The performance of aquatic hyphomycetes and litter decomposition also depend on litter characteristics, with soft, high quality (low carbon: nutrients ratio, low lignin concentration) litter being colonized and decomposed faster than hard, low quality litter (Gessner and Chauvet, 1994; Lecerf and Chauvet, 2008; Schindler and Gessner, 2009; Frainer et al., 2015). Aquatic hyphomycete community structure often also differs between litter species (Canhoto and Graça, 1996; Gulis, 2001; Ferreira et al., 2006). Thus, an interaction between litter species and dissolved nutrients concentration on microbial activity and litter decomposition is expected. Indeed, the effects of nutrient enrichment on litter decomposition are usually stronger for low quality litter where microbial activity is likely nutrient limited (Gulis and Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006). However, most studies have addressed the effects of nutrient enrichment on the decomposition of litter species incubated individually (but see Rosemond et al., 2010; Lima-Fernandes et al., 2015), despite the fact that litter usually make multi-species mixtures in streambeds (Swan and Palmer, 2004; Molinero and Pozo, 2006).

Forestry practices, pathogens, species invasions and climate changes may promote modifications in the composition of riparian forests with species replacement or loss, and the consequent alteration of the identity and/or number of litter species in streambeds (Graça et al., 2002; Kominoski et al., 2013). Many studies have shown that the interactions between component litter species in litter mixtures commonly lead to non-additive effects of mixing on litter decomposition and that this often depends on the identity of the component litter species (reviewed by Lecerf et al., 2011). Non-additive effects of litter mixing on litter decomposition may be due to selective feeding by detritivores on the more palatable litter, nutrient transfer between litter species by fungal mycelium that may reduce nutrient limitation of microbial activities in the nutrient poor litter or leaching of secondary compounds (e.g. polyphenols) that can inhibit fungal exoenzymes in the neighbor litter (Gessner et al., 2010).

Since nutrient enrichment of streams and changes in riparian vegetation composition are likely to occur simultaneously in the future (MEA, 2005), it is important to assess the effects of nutrient enrichment and leaf species mixing on litter decomposition and associated fungi if we want to better predict the response of stream ecosystems to environmental change. The two studies that have so far assessed the interaction between stream nutrient enrichment and litter species mixing on litter decomposition and associated biota have found non-additive effects of mixing under low nutrient conditions but not under nutrient enrichment in autumn-winter (Rosemond et al., 2010; Lima-Fernandes et al., 2015).

In this study we assessed the effects of experimental N enrichment of a small forest stream and litter species mixing on the decomposition of 2-species mixtures and of component litter species incubated individually, and reproductive activity of fungi associated with individual litter species. We predicted that (a) fungal activity and decomposition rates would be faster at the N enriched site than at the reference site, due to a stimulation of fungal activity by nutrient enrichment, (b) stimulation of fungal activity and litter decomposition by N enrichment would be stronger for the N poor (chestnut and poplar) than for the N rich litter species (alder), (c) at the reference site, the 2-species mixtures whose component species differ the most in initial N concentrations would decompose faster than predicted by the decomposition rate of the component species individually (non-additive effects), (d) at the N enriched site, the decomposition rate of the 2-species mixtures would not differ from that predicted by the decomposition rate of the component species individually, as mass loss of N poor litter would not be N limited (additive effects), and (e) aquatic hyphomycete community structure would be sensitive to nutrient concentration, litter species and mixing. Since component litter species in mixtures may respond in opposite ways to mixing and lead to apparent additive effects ('counterbalance hypothesis'; Schindler and Gessner, 2009), the response of component litter species to N enrichment and mixing was assessed.

Table 1
Water physical and chemical variables (mean ± SE) at sites R (reference) and N (nutrient enriched) during the litter decomposition experiment (9 June – 14 July, 2008). Comparisons between sites were done by *t*-tests and p values are shown.

Water variables	n	Site R	Site N	p
Temperature (°C) at ~9 am	4	12.55 ± 0.27	12.65 ± 0.31	0.816
Conductivity (μS/cm)	4	63.75 ± 0.32	65.03 ± 0.78	0.183
Alkalinity (mg $CaCO_3$/L)	4	17.75 ± 0.59	17.10 ± 0.26	0.354
pH	4	6.86 ± 0.04	7.10 ± 0.03	0.003
O_2 (mg/L)	4	10.12 ± 0.06	10.35 ± 0.03	0.027
NO_3-N (μg/L)	6	186.77 ± 49.88	1681.25 ± 162.76	<0.001
SRP (μg/L)	4	62.38 ± 1.64	60.72 ± 1.77	0.516

SRP, soluble reactive phosphorus.
NO_2 and NH_4 were below the detection limit (<100 μg/L and <50 μg/L, respectively).

2. Methods

2.1. Study stream and experimental N enrichment

The experiment took place in a first-order stream located at Margaraça Forest, a protected area with low human activity in Açor Mountain (Central Portugal; 40°13′N, 7°56′W, 600 m a.s.l.). The stream flows over schist bedrock, across a mixed deciduous forest dominated by chestnuts (*Castanea sativa* Mill.) and oaks (*Quercus robur* L.)(Paiva, 1981). The stream is N limited (7–197 μg NO_3-N/L, 43–216 μg PO_4-P/L; range over autumn 2003 and 2004) and has low discharge (0.7–3.0 L/s; range over autumn 2003 and 2004), which makes it suitable to study the effects of experimental N enrichment on litter decomposition (Ferreira et al., 2006). More information on the stream and surrounding forest can be found in Paiva (1981), Abelho and Graça (1998) and Ferreira et al. (2006).

Two sites with similar characteristics were selected within the stream, with the upstream site acting as a reference (site R) and the downstream site being experimentally N enriched (site N) (Table 1). The study reach was ~1.5 m wide, ~10 cm deep and had a discharge of 4.4 ± 1.5 L/s (mean ± SE); sites were apart by ~3 m that coincided with a pronounced discontinuity in slope (abrupt fall by ~50 cm). Experimental N enrichment at site N began one month before the start of the litter decomposition experiment and was carried out continuously until the experiment ended by using $CaNO_3$ concentrated solution (550 g/L), dripping from ten 5-L glass Mariotte bottles (Fig. A1 in Appendix A). Bottles were refilled weekly and dripping rates set according with discharge, measured by chloride release (see below). This resulted in a ~9 fold increase in N concentration at site N over the reference concentration (Table 1). N concentration at site N was within the values found for streams affected by agricultural activities (Gulis et al., 2006).

2.2. Water variables

Conductivity, water temperature (WTW LF 330, WTW, Weilheim, Germany), dissolved oxygen concentration (WTW OXI 92, WTW, Weilheim, Germany) and pH (pH 3110, WTW, Weilheim,