



Effects of an antihistamine on carbon and nutrient recycling in streams



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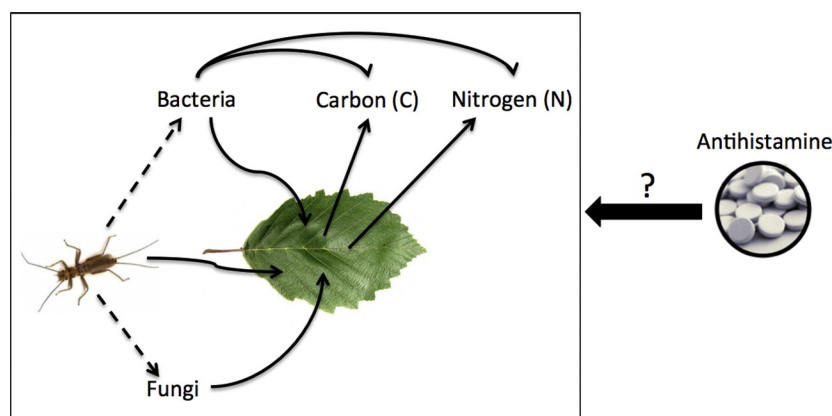
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HIGHLIGHTS

- Antihistamine contamination had no detectable effect on insect larvae.
- Low levels of an antihistamine altered carbon and nitrogen uptake from water.
- Microbial respiration rates increased in response to antihistamine exposure.
- Low levels of antihistamines may impact resource recycling in streams.

GRAPHICAL ABSTRACT



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ABSTRACT

In stream ecosystems, microbes and macroinvertebrates consume leaf litter deposited from the riparian vegetation, and thereby recycle resources tied up in the litter. Several environmental variables influence rates of this recycling, but it is not well known if common pharmaceuticals, such as antihistamines, originating from wastewater effluent, have additional impacts. Exposure to dilute concentrations of antihistamines may adversely influence aquatic detritivorous invertebrates, because invertebrates use histamines for neurotransmission, resulting in hampered recycling of resource tied up in leaf detritus. In this study, we therefore investigated if the antihistamine fexofenadine, at a concentration of 2000 ng l⁻¹, alters rates of leaf litter decomposition in stream microcosms. Stonefly larvae (n = 10, per microcosm), together with natural microbial communities, served as main decomposer organisms on alder leaf litter. First, we used 30 microcosms containing fexofenadine, while the other 30 served as non-contaminated controls, and of each 30 microcosms, 14 contained stonefly larvae and microbes, while the remaining 16 contained only microbes. We found, in contrast to our hypothesis, that fexofenadine had no effect on leaf litter decomposition via impacts on the stonefly larvae. However, independent on if stoneflies were present or not, concentrations of organic carbon (TOC) and nitrogen (N) were strongly affected, with 20–26 and 24–31% lower concentrations of TOC and N, respectively, in the presence of fexofenadine. Second, in a scaled down follow-up experiment, we found that microbial activity increased by 85%, resulting in a 10% decrease in pH, in the presence of fexofenadine. While the antihistamine concentration we used is higher than those thus far found in the field (1–10 ng l⁻¹), it is still 100 times lower than the predicted no-effect concentration for fexofenadine. As such, our results indicate that low µg l⁻¹ levels of antihistamines can have an effect on carbon and nutrient recycling in aquatic system.

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1. Introduction

Decomposition of detritus is an important process that generates the major flow of energy in all types of ecosystems (Polis and Strong, 1996). Stream ecosystems often receive large quantities of detrital matter, primarily in the form of plant material from adjacent riparian vegetation. This litter input constitutes an important consumer food resource, in particular in small streams where primary production is often low due to light limitation (Vannote et al., 1980; Richardson, 1991). Soon after the plant litter enters streams, up to 30% of its initial biomass is lost via leaching of soluble compounds (Petersen and Cummins, 1974), and the leaf litter is colonized by microbes (i.e. bacteria and fungi) that feed on carbon (C) and nutrients in the leaves (Graça, 2001). In boreal and temperate streams, fungi are the primary microbial decomposers of plant litter, and in this process they break down complex nutritional compounds into less complex structures, making them more available for detritivorous macroinvertebrates (Bärlocher, 1985; Gulis and Suberkropp, 2003). The microbes also assimilate C and nutrients directly from the water column, and previous studies suggest that bacteria are better at taking up nutrients from the water, while fungi rely more on nutrients from leaf litter as a source of energy (e.g. Hall and Meyer, 1998; Manning et al., in press) – at least as long as the litter is of relatively high quality.

Some stream macroinvertebrates (i.e. ‘shredders’) specialize in feeding on plant litter, but they depend heavily on microbial colonization of the leaf surface to increase the nutritional value of the litter (Bärlocher, 1985). When shredders feed on leaf litter, they produce particles (i.e. frass and feces), facilitate further leaching of soluble compounds, and hence produce food resources for other organisms (e.g. filter feeders and microbes) (Short and Maslin, 1977; Jonsson and Malmqvist, 2005).

Several environmental factors, such as water chemistry and stream physical characteristics, influence decomposer organisms and, hence, the rate at which plant litter is decomposed in streams, i.e. the rates at which C and nutrients are recycled (Webster and Benfield, 1986). However, in addition to these environmental drivers, human-induced modifications of natural environments have increasingly severe impacts on aquatic ecosystems (Malmqvist and Rundle, 2002), and a potential threat is the input of pharmaceuticals entering streams via wastewater effluent (Rosi-Marshall and Royer, 2012). Pharmaceuticals represent a wide range of chemicals that are often inefficiently removed in wastewater treatment processes (Nikolaou et al., 2007; Verlicchi et al., 2012). Hence, biologically active forms of pharmaceuticals end up in aquatic systems that receive wastewater effluent, but their ecological impacts are still poorly understood (Rosi-Marshall and Royer, 2012; Boxall et al., 2012; Brodin et al., 2014).

One group of pharmaceuticals that has the potential to affect rates of leaf litter decomposition in streams is antihistamines, which primarily are used to treat allergies, and can be found at low concentrations (1–10 ng l⁻¹) in natural aquatic systems receiving wastewater effluent (Stackelberg et al., 2007; Kosonen and Kronberg, 2009; Gros et al., 2012; López-Serna et al., 2012). Invertebrates use histamines for neurotransmission (Hashemzadeh-Gargari and Freschi, 1992; Rosi-Marshall and Royer, 2012), and previous studies have found that growth (Hoppe et al., 2012) and behaviors (Jonsson et al., 2014) in aquatic invertebrates can be influenced by antihistamine contamination. It is therefore possible that shredder physiology and activity are impacted by antihistamine exposure, resulting in alterations of the litter decomposition process. It has also been shown that microbial communities can be adversely impacted by antihistamines (Rosi-Marshall et al., 2013). Aquatic macroinvertebrates – and in particular shredders – may therefore also be indirectly influenced by antihistamine contamination via effects on the microbial community (i.e. Bärlocher, 1985; Gulis and Suberkropp, 2003). Nevertheless, to date, few studies have investigated the ecological consequences (i.e. effects of ecosystem processes) of such direct or indirect effects (but see Zubrod et al., 2015a,b).

In this study, we investigate if an important stream ecosystem process, i.e. carbon (C) and nutrient recycling via plant litter decomposition, is altered by the presence of one antihistamine (fexofenadine) at a concentration of 2000 ng l⁻¹. To do this, we used aquatic microcosms containing plant litter, detritivorous insects (i.e. stonefly larvae), and microbial decomposers (i.e. bacteria and fungi). We chose an antihistamine concentration of 2000 ng l⁻¹, as this has previously been found to alter important behaviors in exposed aquatic macroinvertebrates (Jonsson et al., 2014). Hence, given the potential for behavioral alterations also in stonefly larvae, we hypothesized that antihistamine exposure would result in inhibited decomposition rates via negative impacts on the stonefly larvae.

2. Methods

2.1. Experimental set up

Both experiments in this study, investigating effects of an antihistamine on C and nutrient recycling in stream systems, were performed in climate rooms, at +12 °C and a 12:12 light:dark regime, which is similar to the natural temperature and light conditions during the study period (September to October). Leaves of alder (*Alnus incana*), which is a high-quality litter, were used as the decomposer resource. The leaves were collected from several alder trees, just before abscission (early September), and were dried in room temperature (+18 °C) to a constant biomass.

In the first experiment, 1.0-l aquaria were used. These aquaria were filled with aged tap water (0.7 l), and air supplied via aquaria stones (one per aquarium) created water circulation to mimic stream conditions (Jonsson and Malmqvist, 2000, 2003). To each aquarium, 2.0 g dry mass of alder leaves was added, together with an inoculum of water (100 ml per aquarium) from a pristine forest stream, to provide the aquaria with natural microbial communities (e.g. Jonsson and Malmqvist, 2000). In 30 of the aquaria, the antihistamine fexofenadine was added at a nominal concentration of 2000 ng l⁻¹, as this concentration of fexofenadine has been shown to influence behaviors of aquatic macroinvertebrates (Jonsson et al., 2014). This concentration is considerably higher than antihistamine concentrations found in the field (1–10 ng l⁻¹), but still much lower than the calculated predicted no-effect concentration (PNEC) for fexofenadine, which is 200,000 ng l⁻¹, based on the EC₅₀ 72 of the most sensitive toxicity endpoint (the green algae *Desmodesmus subspicatus*) (Fexofenadine, 2015). Fexofenadine has a pK_a of 4, and thus did not influence the pH at this concentration (data not shown). After adding fexofenadine, the aquaria were left for five days, to allow for microbial colonization of the leaves. In addition, five aquaria contained only leaves and tap water (i.e. no inoculum or insect larvae), to allow for estimation of pure (non-microbial) leaf leaching of soluble compounds.

In early September, stonefly larvae of the species *Protonemura meyeri* (Pictet) were collected from a pristine forest stream in the vicinity of Umeå (63°52'N, 20°11'E). *P. meyeri* is common in north European forest streams, and is an important decomposer of leaf litter that enters streams during autumn. A subsample (n = 7) of the collected individuals showed a per-capita biomass (mean ± 1 S.E.) of 1.4 ± 0.3 mg (wet weight) at the start of the study. This mean value was used to estimate average growth during the experiment. Before initiation of the experiment, the stonefly larvae were allowed to acclimatize to laboratory conditions for 10 days. At initiation, 10 individuals were introduced into each of the 14 aquaria containing fexofenadine and into 14 of the aquaria without fexofenadine. Thus, the entire setup contained 30 aquaria with fexofenadine, of which 14 contained stonefly larvae, 30 aquaria without fexofenadine, of which 14 contained stonefly larvae, and 5 aquaria containing only tap water and alder leaves.

After the introduction of the stonefly larvae, the experiment was left to run for 31 days before termination. Water samples, for analyses of fexofenadine concentrations, were taken day 1 and day 31 from all

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