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Combined effects of drought and the fungicide tebuconazole on aquatic leaf litter decomposition

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ABSTRACT

Loss of biodiversity and altered ecosystem functioning are driven by the cumulative effects of multiple natural and anthropogenic stressors affecting both quantity and quality of water resources. Here we performed a 40-day laboratory microcosm experiment to assess the individual and combined effects of drought and the model fungicide tebuconazole (TBZ) on leaf litter decomposition (LLD), a fundamental biogeochemical process in freshwater ecosystems. Starting out from a worst-case scenario perspective, leaf-associated microbial communities were exposed to severe drought conditions (four 5-day drought periods alternated with 4-day immersion periods) and/or a chronic exposure to TBZ (nominal concentration of 20 $\mu g\,L^{-1}$). We assessed the direct effects of drought and fungicide on the structure (biomass, diversity) and activity (extracellular enzymatic potential) of fungal and bacterial assemblages colonizing leaves. We also investigated indirect effects on the feeding rates of the amphipod *Gammarus fossarum* on leaves previously exposed to drought and/or TBZ contamination. Results indicate a stronger effect of drought stress than fungicide contamination under the experimental conditions applied. Indeed, the drought stress strongly impacted microbial community structure and activities, inhibiting the LLD process and leading to cascading effects on macroinvertebrate feeding. However, despite the lack of significant effect of TBZ applied alone, the effects of drought on microbial functions (i.e., decrease in LLD and in enzymatic activities) and on *Gammarus* feeding rates were more pronounced when drought and TBZ stresses were applied together. In a perspective of ecological risk assessment and ecosystem management for sustainability, these findings stress the need for deeper insight into how multiple stressors can affect the functioning of aquatic ecosystems and associated services.

1. Introduction

Among ecological processes, the decomposition of allochthonous organic matter in the form of leaves is central to the functioning of lotic ecosystems and their associated services (Webster and Meyer, 1997). Leaf litter decomposition (LLD) is mediated by the combined action of microbial and invertebrate communities and contributes to the recycling of organic carbon and nutrients originating from the riparian vegetation (Graça, 2001; Romaní et al., 2006). Through their extracellular enzymatic activities, fungi, particularly aquatic hyphomycetes, and bacteria drive the first steps of LLD and regulate the transfer of matter and energy between particulate organic matter and upper trophic levels through conversion into microbial biomass (Baldy et al., 1995; Romaní et al., 2006). Moreover, microorganisms stimulate leaf consumption by macroinvertebrate shredders through their enzymatic activities and subsequent softening of leaf tissues, and by increasing the nutritional quality of leaf (Suberkropp et al., 1983). Due to its fundamental role in ecosystem metabolism, LLD is a relevant tool to assess the functional integrity of streams (Gessner and Chauvet, 2002; Zubrod et al., 2015a).

Among other stressors, the occurrence, frequency and severity of drying events are increasing in rivers worldwide, leading to an increase in number and length of intermittent rivers (Datry

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et al., 2014). Drought events can profoundly affect the LLD process by reducing decomposition activity during dry phases (Langhans and Tockner, 2006; Corti et al., 2011), with legacy effects persisting in invertebrate communities for several months after flow has resumed (Datry et al., 2011). Such effects are partly explained by changes in abiotic processes driving LLD, including leaching of soluble compounds and physical abrasion (Bärlocher, 1992). However, the most critical aspect of emersion periods lies in the physiological stress and spatial confinement (i.e., humid sediment patches and leaf litter refuges (Amalfitano et al., 2007; Ylla et al., 2010)) imposed on aquatic organisms that results in marked changes in the community structure and activity dynamics of microbial decomposers and invertebrate detritivores (Larned et al., 2007; Datry, 2012; Foulquier et al., 2015). Moreover, terrestrial invertebrates colonizing the river during droughts (Adis and Junk, 2002; Corti and Datry, 2016) can eventually outcompete strictly aquatic organisms and modify the decomposition of leaf litter accumulated in dry river beds. Increasing emersion duration thus decreases LLD rates via a reduction in microbial biomass and activity, and in shredder densities or feeding activities (Langhans and Tockner, 2006; Datry et al., 2011; Foulquier et al., 2015).

Chemical contamination also counts as a widespread stressor in freshwater ecosystems (Segner et al., 2014). This is especially the case in rivers draining agricultural areas, where the diffuse pollution of pesticides to freshwaters can reduce the LLD process (Piscart et al., 2011; Rasmussen et al., 2012a). Recent studies showed that the fungicide tebuconazole (TBZ), which belongs to the intensively used class of azoles (Richardson and Ternes, 2009), can significantly inhibit LLD through a reduction in fungal and bacterial biomass on leaves, changes in microbial community structure, and a decrease in microbial extracellular enzyme activities responsible for cellulose and hemicellulose degradation (Bundschuh et al., 2011; Zubrod et al., 2011, 2015a; Artigas et al., 2012). The mode of action of TBZ relies on the inhibition of the biosynthesis of ergosterol, a sterol essential for the functioning of fungal cells due to its role in cell membrane permeability and fluidity. The reported impacts of TBZ on microbial communities can also induce indirect effects on leaf shredding invertebrates due to the reduction in palatability and nutritional quality of leaf resources (Bundschuh et al., 2011; Zubrod et al., 2011). TBZ can also have direct sublethal effects on shredders, especially inhibition of feeding activity (Zubrod et al., 2014). Fungicides may thus potentially have cascading impacts on aquatic food webs, and strongly influence the organic matter dynamics in river ecosystems.

Against this background, there is now strong evidence that drought and fungicide contamination can affect LLD through both direct and indirect effects on decomposer communities. However, the combined effects of these two stressors on aquatic ecosystem functioning remain to be determined. Current approaches to hazard and risk assessment mainly focus on single stressor effects, yet there is a pressing need for better knowledge on how multiple stressors can affect aquatic biota and functioning (Segner et al., 2014). Here we performed a 40-day laboratory microcosm experiment to assess the individual and combined effects of drought and fungicide (TBZ) on leaf-associated microbial (fungi and bacteria) communities responsible for LLD, including subsequent modification of leaf palatability for invertebrate shredders. Starting out from a worst-case scenario perspective, the microbial communities were exposed to severe drought conditions (four 5-day drought periods preceded and followed by 4-day immersion periods) and/or a chronic exposure to TBZ (nominal concentration of 20 $\mu g\,L^{-1}$). We assessed the direct effects of drought and fungicide on the structure (biomass, diversity) and activity (LLD, extracellular enzyme activity) of fungal and bacterial assemblages colonizing leaves. We also investigated indirect effects on the feeding rates of the invertebrate crustacean *Gammarus fossarum* (a shredder amphipod which represents the dominant macroinvertebrate species, in terms of biomass, in many lotic ecosystems; Macneil et al., 1997) by using leaves previously exposed to drought and/or TBZ contamination.

We hypothesized that each stress applied individually would impair microbial community diversity and activity by modifying community composition, reducing microbial biomasses and enzymatic activities, and consequently decreasing LLD rates. We also addressed the question of combined effects. Ergosterol was recently found to be a key membrane component involved in the resistance of fungi to air-drying through its role of antioxidant and mechanical stabilizer in the plasma membrane (Dupont et al., 2012). The inhibition of fungal ergosterol biosynthesis under TBZ exposure could thus increase the stress induced by drying events on fungal communities. Conversely, the physiological stress imposed on microbial communities during drying events could weaken microorganisms and increase their sensitivity toward TBZ. Accordingly, we made the hypothesis of a synergistic effect of the two stressors on LLD. We expected that these direct effects would indirectly affect *G. fossarum* feeding activity, either by reducing leaf consumption rates due to a decrease in palatability or, on the contrary, by triggering an increase in feeding rates to compensate for the decrease in leaf nutritional quality (mechanism of compensatory feeding (Agatz et al., 2014).

2. Materials and methods

2.1. Microbial colonization of leaves

Recently-fallen leaves of *Alnus glutinosa* were collected in November 2013, transported to the lab and allowed to dry at room temperature. The leaves were rehydrated, cut into 1 and 2 cm-diameter circles, and oven-dried for 48 h at 70 °C. These leaf disks were weighed and respectively placed in small litter bags ($l \times w = 9 \times 5.5$ cm) containing 20 leaf discs (1 cm in diameter) and large litter bags ($l \times w = 9 \times 12$ cm) containing 12 leaf disks (2 cm in diameter). Bags were placed in fine-meshed nylon bags (0.25 mm) to prevent invertebrate colonization and immersed for one week (3–10 March, 2014) in the upstream section of the Ardières River (Beaujolais vineyard area, Eastern France) draining a grassland/forest area to allow microbial colonization prior to the microcosm experiment. Colonized leaves were collected and transported to the lab, and used for the subsequent microcosm experiments.

2.2. Experimental design

The experiment was performed in glass indoor channels (Length × Width × Height = 83 cm × 11 cm × 10 cm) simulating four different conditions (Fig. 1): (i) "Ic' (immersed control), where leaves were permanently immersed (40 days) without TBZ exposure, (ii) "Itbz' (immersed TBZ), where leaves were permanently immersed with TBZ exposure (nominal concentration of 20 $\mu g\,L^{-1}$), (iii) 'Dc' (dried control), where leaves were temporarily immersed (4×5 days) without TBZ exposure, and (iv) "Dtbz" (dried TBZ), where leaves were temporarily immersed with TBZ exposure (4×5 days, nominal concentration of 20 $\mu g\,L^{-1}$). Each treatment was replicated in three independent channels, giving 12 channels in total. Each channel was connected to a 20 L glass tank (i.e., one independent tank per channel) through an aquarium pump (NEWA MJ 750) for water recirculation with a flow velocity of 1.5 L min^{-1}.

The study was led from March 10 to April 20, 2014 under controlled temperature (19.9 °C ± 0.1) and light (14 h/10 h light/dark cycle) conditions. Each independent channel was filled with 5 L recirculating water. The water was drilled groundwater stored in two 150 L glass tanks and homogenized using recirculating pumps before being used to fill each independent 20 L glass tank at each