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Comparative sensitivity to the fungicide tebuconazole of biofilm and plankton microbial communities in freshwater ecosystems

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HIGHLIGHTS

- Biofilm and plankton microbial communities were exposed to Tebuconazole (TBZ).
- TBZ had effects on biofilm function including respiration and photosynthetic activity.
- Biofilm communities from polluted sites exhibited induced tolerance to TBZ.
- Plankton communities responded to TBZ by increasing bacterial cell densities.
- TBZ toxicity depends on the nature of microbial communities and pre-exposure to TBZ.

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ABSTRACT

Stream and lake ecosystems in agricultural watersheds are exposed to fungicide inputs that can threaten the structure and functioning of aquatic microbial communities. This research analyzes the impact of the triazole fungicide tebuconazole (TBZ) on natural biofilm and plankton microbial communities from sites presenting different degrees of agricultural contamination. Biofilm and plankton communities from less-polluted (LP) and polluted (P) sites were exposed to nominal concentrations of 0 (control), 2 and 20 μg TBZ L^{-1} in 3-week microcosm experiments. Descriptors of microbial community structure (bacterial density and chlorophyll-*a* concentration) and function (bacterial respiration and production and photosynthesis) were analyzed to chart the effects of TBZ and the kinetics of TBZ attenuation in water during the experiments. The results showed TBZ-induced effects on biofilm function (inhibition of substrate-induced respiration and photosynthetic activity), especially in LP-site communities, whereas plankton communities experienced a transitory stimulation of bacterial densities in communities from both LP and P sites. TBZ attenuation was stronger in biofilm (60–75%) than plankton (15–18%) experiments, probably due to greater adsorption on biofilms. The differences between biofilm and plankton responses to TBZ were likely explained by differences in community structure (presence of extracellular polymeric substances (EPS) matrix) and microbial composition. Biofilm communities also exhibited different sensitivity levels according to their in-field pre-exposure to fungicide, with P-site communities demonstrating adaptation capacities to TBZ. This study indicates that TBZ toxicity to non-targeted aquatic microbial communities essentially composed by microalgae and bacteria was moderate, and that its effects varied between stream and lake microbial communities.

1. Introduction

Freshwater ecosystems in agricultural watersheds are widely exposed to multiple pesticidecontamination events, including fungicides which pose major ecotoxicological threats to aquatic microbial community biodiversity and function (*e.g.* Montuelle et al., 2010; Rasmussen et al., 2012a). The intensive use of modern azole fungicides like

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fluconazole, propiconazole and tebuconazole (TBZ) (Richardson, 2009) has prompted studies aiming to understand their effects on aquatic detritivores and microbial decomposers (Bundschuh et al., 2011; Rasmussen et al., 2012b). TBZ has been found in numerous freshwater ecosystems in Europe, including rivers and lakes, and is reported as relatively persistent even after wastewater treatment (Berenzen et al., 2005; Kahle et al., 2008).

The main aquatic microbial communities potentially threatened by TBZ are fungi. TBZ affects fungal activity-related processes such as litter decomposition (Rasmussen et al., 2012b; Bundschuh et al., 2011) and inhibits ergosterol biosynthesis, which hampers fungal mycelium growth (Copping and Hewitt, 1998). Exposing submerged leaves to TBZ led to lower fungal biomass accumulation and reduced the ability of microorganisms to decompose cellulose and hemicellulose compounds (Artigas et al., 2012). Important bacterial groups such as nitrogen-fixing and nitrifying bacteria can also be directly or indirectly affected by fungicides (Johnsen et al., 2001). Specifically, TBZ has been reported to stimulate ammonifying bacteria populations at early exposure in soils and nitrifying bacteria in later exposure phases (Chen et al., 2001; Cycoń et al., 2006; Muñoz-Leoz et al., 2011). This stimulating effect on bacterial groups could be associated with increased levels of nutrients and energy sources released from dead fungal hyphae killed by TBZ (Cycoń et al., 2006) or by chemicals present in the fungicide formulation becoming available to bacteria (Muñoz-Leoz et al., 2011). Although such results suggest that similar effects will be found in aquatic ecosystems, with consequences on microbial processes, only a few studies have explored the effects of TBZ on aquatic microbial communities other than fungi in biofilms and plankton (Tlili et al., 2011a; Artigas et al., 2012).

Microbial community exposure to TBZ varies between stream and lake ecosystems. Pesticide mobilization in agricultural watersheds mostly depends on drift, run-off and drainage processes from the watershed (Kolpin et al., 1996), and often coincides with fungicide applications in conjunction with cultural practices (Rabiet et al., 2010). However, the arrival of fungicides into surface water depends on watershed topography (especially field slope), soil characteristics (*i.e.* organic carbon-water binding coefficient), and the half-life of the molecule (49–610 days for TBZ in aerobic soils; Strickland et al., 2004). Once in the aquatic ecosystem, hydrological conditions in lotic ecosystems mostly generate peak exposure scenarios (lasting a few hours to days) at high contaminant concentrations, whereas lentic ecosystems establish a more chronic exposure scenario (several months) at lower contaminant concentration (Simmons and Wallschlager, 2005). Consistently with this pattern, TBZ concentrations recorded in rivers (often at μg L^{-1}) exceed those found in lakes (often at ng L^{-1}; Richardson, 2009; Rabiet et al., 2010; Kahle et al., 2008). However, TBZ concentrations in rivers are highly variable over time (Rabiet et al., 2010) and chronic exposure scenarios to low fungicide concentrations can also be detected (*i.e.* Reilly et al., 2012). Knowing that level and duration of exposure of microbial communities to the pesticide are important factors determining the adaptation processes of microorganisms (Le Jeune et al., 2007; Pesce et al., 2009), microbial communities from stream biofilms and lakes are likely to display contrasted susceptibility to this fungicide.

Comparative studies assessing pollutant toxicity between lotic (stream) and lentic (lake) ecosystems are rare in the literature (Munawar et al., 1999; Simmons and Wallschlager, 2005), despite their potential utility for better understanding how the ecological, hydrological and biogeochemical characteristics of each type of ecosystem can modulate pollutant ecotoxicity. In this global framework, we adopted an experimental approach to investigate the effects of TBZ on non-targeted microalgal and bacterial communities living in stream biofilms and lake plankton samples collected in natural ecosystems. For each ecosystem (stream and lake), samples were collected in sites qualified as "less polluted" and "polluted" based on previous *in situ* measurements in order to assess the influence of pre-exposure to TBZ on their susceptibility to the fungicide. The responses of all these communities to TBZ (at two doses, 2 and 20 μg TBZ L^{-1}) were examined using a range of structural and functional descriptors. This experimental scheme was thus designed to test the respective effects of community lifestyle (benthic *versus* planktonic) and pre-exposure to the pollutant on the responses of non-targeted aquatic microbial communities to TBZ.

2. Materials and methods

2.1. Biological communities

Biofilm and plankton microbial communities from less-polluted (LP) and polluted (P) sites were obtained from stream and lake ecosystems in eastern France. Biofilms were obtained from two sites (upstream in Saint Joseph and downstream in Saint Ennemond) of Morcille River (Beaujolais vineyard region, 46.15°N 4.60°E), a siliceous stream composed by three nested sub-watersheds that experiences an upstream-to-downstream gradient of agricultural contamination (Rabiet et al., 2010; Montuelle et al., 2010). This pollution gradient is characterized by increases in dissolved organic carbon, phosphate (125% to 300% increase between upstream and downstream sites, respectively) and copper, arsenic, herbicide (diuron and its breakdown products) and fungicide (azoxystrobin, carbendazim, tebuconazole, procymidone and dimetomorph) concentrations (Montuelle et al., 2010). Previous surveys have shown that TBZ concentrations ranged from 0.001–0.1 μg L^{-1} at the upstream LP site of Saint Joseph to 0.01–4 μg L^{-1} at the downstream P site of Saint Ennemond (Margoum C., pers. comm.). Artificial glass substrata (20 cm^2 tiles) were left to colonize for one month at both study sites. Biofilm colonization was conducted during late summer, when the highest TBZ contamination peaks had been detected at the P site (Montuelle et al., 2010). The resulting biofilms from LP and P sites were collected, transported cold (4 °C) to the laboratory, and placed in indoor stream channels for TBZ experiments.

Plankton communities were obtained from two different perialpine lakes experiencing contrasting degrees of agricultural contamination — one less polluted (Lake Aiguebelette, mean TBZ concentration <0.001 μg L^{-1}) and one polluted (Lake Geneva, mean TBZ concentration = 0.001 μg L^{-1}). Lake Aiguebelette (LP site) is situated in a small forested watershed (70 km^2) bearing low anthropogenic pressure, whereas Lake Geneva (P site) has a much larger watershed (7395 km^2) bearing high pressure due to urbanization and agriculture. Samples (400 L) from each lake were collected from the 0–50 cm layer and filtered through a 50 μm pore-size filter to remove zooplankton. Samples were collected at a central part in each lake (Lake Aiguebelette: 5°47′56″, 45°33′02″, Lake Geneva: 6°35′34″, 46°27′22″). Samples with plankton communities from LP and P sites were transported in plastic containers, stored overnight at 4 °C, and used for subsequent TBZ experiments in indoor microcosms.

2.2. Laboratory experiments

The experiments were performed in glass indoor stream channels (L × W × D = 63 cm × 11 cm × 4 cm) for biofilms and in transparent polycarbonate bottles (20 L) for plankton. Each type of microbial community (LP-site biofilm, P-site biofilm, LP-site plankton, P-site plankton) was subjected to three different treatments consisting of control (non-contaminated), low-TBZ dose (2 μg L^{-1}) and high-TBZ dose (20 μg L^{-1}) exposure.

For biofilms, each microbial community (LP and P sites) and treatment was tested in three independent channel replicates (18 channels in total), each connected to a separate 5 L reservoir (Pyrex, USA) by means of an aquarium pump (NEWA MJ 750). Water temperature was set to 20 °C and flow conditions were set to 0.19 L s^{-1}, all under a 12 h/12 h light/dark cycle. Before TBZ contamination, stream biofilms were allowed to adapt for one week to the indoor channels by recirculating tap water (previously dechlorinated using an active