Environmental concentrations of the fungicide tebuconazole alter microbial biodiversity and trigger biofilm-released transformation products

Citation for the original published paper (version of record):
Gómez-Martínez, D., Selvin, M., Nilsson, A. et al (2024). Environmental concentrations of the fungicide tebuconazole alter microbial biodiversity and
trigger biofilm-released transformation products. Chemosphere, 369.
http://dx.doi.org/10.1016/j.chemosphere.2024.143854

Chemosphere 369 (2024) 143854

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Environmental concentrations of the fungicide tebuconazole alter microbial biodiversity and trigger biofilm-released transformation products

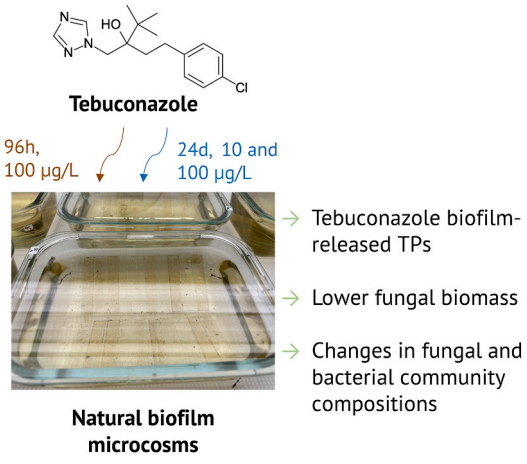
Daniela Gómez-Martínez [a,f,*], Mary A. Selvin [a], Anders K. Nilsson [b], Eric Carmona [c], Judith Sorel Ngou [a], Erik Kristiansson [d,f], R Henrik Nilsson [e], Natàlia Corcoll [f,**]

[a] Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[b] Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[c] Atmospheric Chemistry Department, Leibniz Institute for Tropospheric Research, Leipzig, Germany
[d] Department of Mathematical Sciences, Chalmers University of Technology and University of Gothenburg, Gothenburg, Sweden
[e] Department of Biological and Environmental Sciences, Gothenburg Global Biodiversity Center, University of Gothenburg, Gothenburg, Sweden
[f] Department of Biological and Environmental Sciences, Centre for Future Chemical Risk Assessment and Management Strategies (FRAM) and Gothenburg Global Biodiversity Center (GGBC), University of Gothenburg, Gothenburg, Sweden

HIGHLIGHTS

- The ecotoxicological impact of chronic (24 days) and acute (3 days) tebuconazole exposures at high but environmental levels on the biomass and biodiversity of the active community of fungi and bacteria in river biofilm was assessed using an RNA metabarcoding approach.
- After 24 days of chronic exposure to 100 μg/L of tebuconazole, biofilms demonstrated the ability to biotransform the fungicide.
- Tebuconazole exposure reduced fungal biomass by 40% at a concentration of 10 μg/L and by 60% at 100 μg/L after 24 days of exposure.
- Chronic exposure to tebuconazole led to a shift in community composition. One fungal species was found to be significantly more abundant (*Lecanoromycetes* sp.), and one fungal species proved to be significantly less abundant (*Malasseziomycetes* sp.) after tebuconazole exposures.

GRAPHICAL ABSTRACT



* Corresponding author. Postal address: Box 463, 40530, Gothenburg, Sweden.
** Corresponding author. Postal address: Box 463, 40530, Gothenburg, Sweden.
E-mail addresses: daniela.gomez.martinez@bioenv.gu.se (D. Gómez-Martínez), maryprincessthilaga@gmail.com (M.A. Selvin), anders.k.nilsson@gu.se (A.K. Nilsson), eric-carmona.martinez@ufz.de (E. Carmona), judithngou@gmail.com (J.S. Ngou), erik.kristiansson@chalmers.se (E. Kristiansson), henrik.nilsson@bioenv.gu.se (R.H. Nilsson), natalia.corcoll@bioenv.gu.se (N. Corcoll).

https://doi.org/10.1016/j.chemosphere.2024.143854
Received 20 May 2024; Received in revised form 13 October 2024; Accepted 27 November 2024
Available online 4 December 2024

ARTICLE INFO

Keywords:
Tebuconazole
Biofilm
Ergosterol
Fungi
RNA metabarcoding

ABSTRACT

Freshwater microbial communities are integral components of riverine biodiversity. The ecological effects of toxic chemical pollutants, such as fungicides (e.g., tebuconazole), on microbial abundance and diversity are needed for risk assessment and regulation. The emergence of RNA metabarcoding approaches allow us to describe at unprecedented resolution the microbial diversity of the active part of a microbial community. Our study assesses the ecotoxicological impact of chronic and acute tebuconazole exposures on fungal, bacterial, and algal biomass and biodiversity of aquatic fungi and bacteria in stream biofilms using an RNA metabarcoding approach. In addition, the study uses HPLC-MS to evaluate the capability of biofilms to metabolize tebuconazole. Natural biofilm communities from a Swedish stream were exposed chronically (24 days) and acutely (96 h) to environmental concentrations of tebuconazole (10 and 100 μg/L) in microcosms conditions. The diversity and community structure of fungi and bacteria was assessed by ITS2 and 16S cDNA amplicon-sequencing, respectively. Biofilms chronically exposed to tebuconazole produced and released unidentified transformation products into the water column, suggesting a biotransformation capability following 24 days of uninterrupted exposure. The fungal biomass markedly decreased by a biomass loss of 40% when chronically exposed to 10 μg/L, and 60% when chronically exposed to 100 μg/L. Bacterial and algal biomass remained comparable with the controls in all tebuconazole treatments. Fungal and bacterial alpha diversity metrics were not significantly impacted, although a decreasing trend in fungal richness was observed with the treatments. However, beta diversity was significantly impacted in both fungal and bacterial compartments. Chronic exposures resulted in a shift in community composition, where taxa potentially more tolerant to tebuconazole (i.e. *Lecanoromycetes*) replaced more sensitive taxa (i.e. *Malasseziomycetes*). This study indicates that tebuconazole at environmental concentrations might pose a risk to freshwater systems, mainly due to its high toxicity to fungi.

1. Introduction

Tebuconazole is a widely used triazole fungicide used to prevent the growth of pathogenic fungi in crops. The extensive use of tebuconazole, together with its aqueous solubility, makes this compound one of the most frequently recovered micropollutant fungicides in the aquatic environment (Bernabò et al., 2020; Glinski et al., 2018). Tebuconazole has been increasingly detected in many regions of the world (De Gerónimo et al., 2014; Glinski et al., 2018; Necibi et al., 2021; Wang et al., 2021), for instance in concentrations ranging from 9.1 μg/L–200 μg/L in European surface waters (Berenzen et al., 2005; Finckh et al., 2024; Lefrancq et al., 2017). The mode of action of tebuconazole includes the inhibition of the ergosterol synthesis pathway which ultimately results in the inhibition of fungal growth. Aquatic fungi in freshwater environments are directly affected by tebuconazole as a micropollutant (Campos et al., 2015; Kramer et al., 2024). The increasing evidence of an ubiquitous presence of tebuconazole in surface water calls for the assessment of its effects on freshwater biofilms, and in particular their fungal compartment.

Fungi are abundant and diverse, yet poorly known, organisms that are found in nearly all kinds of environments, including terrestrial, marine, and freshwater habitats (Nilsson et al., 2019a,b). Most of the fungi in aquatic biofilms of European rivers with deciduous riparian forests colonize a diversity of substrates, including leaves, stones and pebbles, wood, and sediment. Fungi in biofilms live together with bacteria and algae, embedded in an organic polymer matrix (Battin et al., 2016), on substrata, and they provide important ecosystem services such as decomposition of organic matter, mineralization, and nutrient immobilization. These microbial communities interact with the water column and possess a highly reactive surface area for the adsorption and metabolism of contaminants (Bonnineau et al., 2021). In freshwater environments, fungal biomass can account for more than 99% of the total microbial biomass on decomposing plant detritus (Kuehn et al., 2000). Due to their significant presence, fungi play key roles in several ecosystem functions, notably decomposing organic matter, nutrient cycling, and feeding higher trophic levels (Kagami et al., 2014). Despite the high prevalence of fungicides in river ecosystems, little is known about their impact on natural aquatic microbial communities, including fungi. Research on the effects of fungicides on aquatic fungi and microbes has remained limited, likely due to technical challenges in assessing their diversity (Grossart et al., 2019; Jobard et al., 2010). The last two decades have seen increasing use of DNA and RNA-based metabarcoding (using for fungi the nuclear ribosomal internal transcribed spacer (ITS) region and for bacteria the 16S ribosomal RNA gene) as a means to explore and characterize microbial communities in a previously unachievable way. However, the path to straightforward characterization of aquatic fungal species remains long, one reason being that high-quality reference ITS sequences are available for a modest 25% of the ~150,000 formally described species of fungi, which in turn represent less than 2% of the estimated 2.3–6 million extant species of fungi (Abarenkov et al., 2022a). Metabarcoding analysis based on RNA allows targeting only the active part of the community. While some studies demonstrate that RNA outperforms DNA-based metabarcoding in assessing biodiversity changes to environemnatal variables (Kong et al., 2023), its use is still not common in environmental studies, including ecotoxicology. The metabolization of organic xenobiotics by aquatic fungi has been previously reported (Augustin et al., 2006a; Junghanns et al., 2005; Krauss et al., 2011a; Martin et al., 2007). Fungi have developed different strategies to increase their tolerance to fungicides, notably by interacting with these chemical compounds and by modifying the fungicides' original molecular structure (Augustin et al., 2006). Some fungal taxa may detoxify fungicides through metabolic pathways otherwise used for breaking down organic chemicals, while other taxa acquire or increase this ability as a form of detoxification (Krauss et al., 2011b).

The level of protection of the European Union's Environmental Risk Assessment (ERA) for fungi and the ecosystem functions they provide is still low (Zubrod et al., 2015). Most of the previous efforts to explore the effects and fate of tebuconazole in microbial communities have focused on assessing impacts in terms of total biomass, the eukaryotic community structure (mainly algal community), or the bacterial community composition using DNA-based approaches or enzymatic activities related to nutrient turnover (Artigas et al., 2012; Baudy et al., 2021; Bertrans-Tubau et al., 2023; Tlili et al., 2011a; Wijntjes et al., 2022). However, to our knowledge, no studies have addressed the effects of environmental concentrations of tebuconazole on active fungal communities using RNA metabarcoding.

This study aims to investigate how acute and chronic exposures to tebuconazole affect the abundance of aquatic fungi in river biofilms (along with associated bacteria and algae) and the diversity of fungi and bacteria. Additionally, it examines the role of biofilms in removing tebuconazole from the environment. To achieve this, natural biofilm communities were collected from a Swedish stream and exposed in laboratory conditions to environmental concentrations of tebuconazole

(i.e 10 and 100 μg/L) over a period of 96 h (acute exposure), or a period of 24 days (chronic exposure). Metabarcoding analysis based on RNA was used to assess ecotoxicological impacts on the structure and diversity of active fungal and bacterial communities. The capacity of biofilms to dissipate tebuconazole was studied using 96 h tebuconazole kinetics tests, where tebuconazole, its main metabolite hydroxy-tebuconazole, and other transformation products were identified using high-performance liquid chromatography in tandem with a mass spectrometer (HPLC-MS). Overall, we hypothesized that environmental concentrations of tebuconazole would reduce fungal biomass and alter community composition in a dose- and time-dependent manner. Specifically, it was expected that chronic exposure to tebuconazole at 100 μg/L would cause a shift in the composition of the fungal communities, where sensitive taxa would be replaced by tolerant ones, a process that in turn would negatively affect fungal biomass and diversity. We also anticipated that biofilms tolerant to tebuconazole exposure would be able to metabolize tebuconazole, releasing its transformation products into the water as a coping mechanism to high concentrations. For bacteria and algae, we predicted changes in their biomass and bacterial diversity, driven by the reduction in fungal biomass due to tebuconazole exposure.

2. Materials and methods

2.1. Biofilm colonization of glass slides in a Swedish river

Glass slides were used as artificial substrata for biofilm colonization. These were held in a total of six polyethylene-sampling racks (Corcoll et al., 2017) that were left hanging in the water column at 0.5 m below the surface. The racks were held in a vertical position in the river with long wooden sticks that were embedded in the sediment floor and ropes that were tied to the trees along the bank of the river (Supplementary Fig. 2). These racks were deployed in the upstream waters of the river Mölndalsån in a location close to the river's source (57°40′ 58.2″N 12°12′ 59.7″E) for one week during September 2021. The river Mölndalsån, a tributary of the river Göta Älv, is an approximately 40 km long water system with a catchment area of approximately 266 km^2. It originates from the Lake Rådasjön, and it is of great importance as a migration route for fish, including salmon and sea trout (*Nulägesbeskrivning Över Mölndalsåns Vattensystem Regional Samverkan Inom*, 2022). The rack-deployment site is surrounded by a deciduous forest, in addition to a deciduous swamp forest that is wet for all or most of the year (*Nulägesbeskrivning Över Mölndalsåns Vattensystem Regional Samverkan Inom*, 2022). The annual precipitation for 2021 in the region was 906 mm, and the average air temperature during September 2021 ranged from 11.1 to 18.3 °C (data retrieved from Sveriges meteorologiska och hydrologiska institute; SMHI).

Each rack contained 34 glass slides (1.7 × 1.4 cm each). The glass slides were washed, dried, and sterilized in 70% ethanol before use. After a week of deployment, when biofilm growth was observed on the slides, the racks were collected from the river and brought to the laboratory.

About 250 L of surface river water were collected from the river, brought to the laboratory, sterilized (filtration and subsequent autoclaving, see section 2.2), and stored at 4 °C prior to conducting the microcosm experiment.

2.2. Microcosms set up and experimental design

The experiment was conducted in a thermo-constant room indoors, using glass vessels (microcosms) from October 1st to November 1st, 2021. It was divided into two phases (Supplementary Fig. 1): phase 1) acclimation of the biofilms to the microcosms conditions (10 days), and phase 2) exposure to tebuconazole (24 days). The experiment was performed at 18 °C using a light cycle of 12/12 h of light/dark with a light intensity at the surface of the microcosms of 120 μmol photons m^{-2} s^{-1}. In total, the experiment consisted of 18 independent microcosms (Supplementary Fig. 1). Each microscosm was a rectangular glass vessel (22.5 × 16 × 7 cm) with a holding volume of 1.5 L covered with a glass lid. Each vessel contained a final volume of 1.2 L of enriched river water (see below). The vessels were designed with a tubular glass rod fixed to the bottom so that 15 slides could be submerged and placed semi-vertically in the laterals of the vessel (Supplementary Fig. 3). Biofilms were cultivated with enriched river water that was filtered twice using GF/D (2.7 μm) and GF/F (0.7 μm) filters, autoclaved, and amended with nitrate ($NaNO_3$; 0.2 mg/L) and phosphate (KH_2PO_4; 0.02 mg/L) to avoid nutrient depletion. The water of each microcosm was renewed every 3–4 days. The microcosms were kept in constant agitation by using horizontal shakers at 47 rpm to mimic the river flow.

After the acclimation phase, the exposure phase (24 days) started, in which the 18 microcosms were divided into four tebuconazole treatments and negative controls. These were, in triplicates, 1) river biofilm not exposed to tebuconazole for 24 days (Control 0 μg/L, n = 3), 2) river biofilm exposed to 10 μg/L of tebuconazole for 24 days (Chronic 10 μg/L, n = 3), 3) river biofilm exposed to 10 μg/L of tebuconazole during the first 10 days, and to 100 μg/L of tebuconazole for the remaining 14 days (Chronic 100 μg/L, n = 3), and 4) river biofilm exposed to 100 μg/L only for the last 96 h of the exposure phase (Acute 100 μg/L, n = 3). In addition, as a negative control, microcosms without biofilms (non-biofilm control microcosms) and spiked with 10 and 100 μg/L of tebuconazole, in triplicates, were prepared in the same way as the microcosms containing biofilm to monitor the potential abiotic loss of tebuconazole due to the test system. The microcosms were spiked with the corresponding concentration of tebuconazole every 96 h.

The tebuconazole stock solution (40 g/L in 96 % MeOH) was prepared from powdered tebuconazole (CAS nr107534-96-3; Sigma-Aldrich). From this solution, an intermediate solution of 0.08 g/L of tebuconazole in MilliQ water was prepared and stored at 4 °C until use. A total of 8 L was prepared for each treatment every time the water was replaced in the microcosms. The treatments that contained tebuconazole were spiked with the tebuconazole stock solution of 0.08 g/L to reach the final concentrations of 10 and 100 μg/L in the water.

Water samples were collected weekly during the entire experiment to monitor the nutrient and tebuconazole levels of each microcosm before and after water renewal. For nutrient analysis, 10 mL of water was collected, filtered through 0.45 μm filters (VWR, 0.45 μm PES), and preserved at −20 °C until further analysis. For the analysis of tebuconazole and its transformation products, 3 mL of filtered water samples (VWR, 0.45 μm PES) were taken and stored in 4 mL glass chromatographic vials at −20 °C until further analysis.

At the end of the exposure phase, biofilms were sampled to analyze several biological parameters (biomass of fungi, bacteria, and algae plus microbial composition of fungi and bacteria (ITS2 and 16S metabarcoding; see section 2.7). Biofilms were scraped using a sterile cell scrapper into sterile Petri dishes and homogenized to a slurry. For each biological measurement, a volume of 1 mL of the slurry was aliquoted into 1.5 mL Eppendorf tubes, centrifuged at 4500 g for 10 min, and stored at −80 °C until further analysis.

2.3. Water analyses

The physicochemical parameters (pH, oxygen, conductivity, and temperature) of the enriched river water used in the microcosms were measured using a multiparameter instrument (HANNA instruments). Nutrients analyses were performed by Eurofins Water Testing Sweden using standard spectrophotometric methods (phosphate: ISO 15923–1:2013 Annex F, nitrate: ISO 15923–1:2013 Annex F, and dissolved organic carbon (DOC): SSEN 1484:1997).

Tebuconazole and hydroxytebuconazole in water samples were extracted in a solid phase extraction (SPE) and were identified and quantified using HPLC-MS. A volume of 625 μL of 40 ng/mL of deuterated tebuconazole (d9-tebuconazole, CAS:1246818-83-6; Sigma-

Aldrich) in MeOH was added to 2.5 mL of each water sample (previously thawed) as internal standard, reaching a final concentration of 10 ng/mL. The sample was then passed through an SPE cartridge (Oasis PRiME HLB cartridges, 3 cc/60 mg) according to the manufacturer's instructions. The column was allowed to dry and then eluted in 2 mL acetonitrile:methanol (9:1). The solutions containing the analytes were evaporated until dry using an air compressed speed vac (SpeedVac SVC100, Savant), and then resuspended in 200 μL of formic acid 0.1% in MeOH: formic acid 0.1% in water (30:70, v/v). The resuspended contents were transferred to a 200 μL fixed insert vial (Chromacol 03-FISV) and stored at 4 °C until injection in an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA) coupled to an Agilent 6470 triple-quadrupole mass spectrometer (QqQ). The HPLC analysis was performed using a Kinetex 2.6 μm EVO C18 100 Å, 2.1 × 50 mm analytical column for chemical separation. Tebuconazole (parent compound, CAS: 107534-96-3; Sigma-Aldrich) and hydroxytebuconazole (main transformation product of tebuconazole, CAS: 212267-64-6; Sigma-Aldrich) were identified and quantified following (Carmona et al., 2017). Formic acid 0.1% in MeOH (A) and formic acid 0.1% in water (B) were used as HPLC mobile phases for electrospray ionization in positive mode. Mobile phases were at a flow 0.3 mL/min in a linear increase from 30 to 95% mobile phase A during 7 min, followed by an isocratic elution for 5 min. The injection volume was set to 30 μL. Source parameters for the Agilent 6470 were for ES (+) gas temperature 250 °C, gas flow 4L/min, nebulizer 30 psi, and capillary 4000 V. Both Q1 and Q3 were set to unit resolution for all transitions. Compound-specific settings were optimized for tebuconazole, tebuconazole-d9, and hydroxytebuconazole (Supplementary Table 1). For the quantification of tebuconazole and its transformation products, the obtained peaks were integrated using MassHunter Workstation Software Quantitative Analysis for QQQ (version B.10.00, Agilent Technologies). The detection limit for tebuconazole was 0.25 ng/L and for hydroxytebuconazole 0.01 ng/L. The working calibration of the standards' concentrations ranged between 0.5 μg/L and 250 μg/L for tebuconazole, and between 0.1 and 250 μg/L for hydroxytebuconazole. Other putative transformation products from tebuconazole were also identified as these peaks did not appear either in the corresponding standard sample or in the control samples corresponding to water in contact with biofilm without tebuconazole treatment. These peaks were integrated for quantification in MassHunter Workstation Software Quantitative Analysis for QQQ (version B.10.00, Agilent Technologies) and normalized to the internal standard d9-tebuconazole.

2.4. Tebuconazole kinetics test

A tebuconazole kinetics test on the water column was performed on day 20 -when biofilms were mature and 96 h prior to the end of the experiment-of the exposure phase for the control, 100 μg/L tebuconazole chronic and acute, and non-biofilm (abiotic) control treatments, in triplicates. This test involved the exposure phase for the control, 100 μg/L tebuconazole chronic and acute, and non-biofilm (abiotic) control treatments, in triplicates. The kinetics test lasted for 96 h (h). Tebuconazole was spiked at a concentration of 100 μg/L, and water samples were taken at 0, 24, 48, 72, and 96 h after the onset of exposure. Water samples were analyzed to monitor the fate of the parent compound, tebuconazole, the transformation compound hydroxy-tebuconazole, and unidentified transformation products using HPLC-MS (see section 2.3).

2.5. Biomass parameters

2.5.1. Fungal biomass

Ergosterol, a mycosterol found in cell membranes of fungi (Voshall et al., 2021), was used as a proxy for fungal biomass. Ergosterol was extracted from the biofilm samples using an adaptation of the three-phase extraction method following Vale et al. (2019). This three-phase lipid extraction (3PLE) allowed extraction and fractionation of lipids by polarity, obtaining one aqueous, upper organic phase enriched in neutral lipids and two organic phases containing the major glycerophospholipids. Briefly, 5 μL of 1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine (1 μg/μL) and 5 μL of β-cholestanol (1 μg/μL) were added to a 12 × 75 mm glass tube and dried under N_2. Once dry, the lyophilized biofilm samples were added together with 1.5 mL of boiling isopropanol. The mixture was dried under N_2, after which 1 mL of hexane with 0.025 % butylated hydroxytoluene (BHT), 1 mL of methyl acetate, 0.75 mL of acetonitrile, and 1 mL of water were added. After extraction in an ultrasonic bath for 30 min, the tubes were centrifuged at 2500 g for 5 min. As a result, three phases (i.e. upper, middle, and lower) were obtained. Ergosterol was contained in the upper fraction (non-polar lipid). This fraction was isolated in a new tube, dried under N_2, and dissolved in 100 μL of isopropanol with 0.025% BHT. This mixture was preserved at −20 °C until further analysis.

Ergosterol was identified and quantified via HPLC-MS (Agilent 1260 Infinity HPLC system (Agilent Technologies) coupled to an Agilent 6470 triple-quadrupole mass spectrometer (Agilent Technologies), using a Kinetex 2.6 μm EVO C18 100 Å, 2.1 × 50 mm analytical column. Analytical grade ergosterol (CAS: 57-87-4; Sigma-Aldrich) was used as authentic reference, and β-cholestanol (CAS: 80-97-7; Sigma-Aldrich) was used as the internal standard. Formic acid 0.1% in water and formic acid 0.1% in MeOH: Isopropanol (3:1, v/v) were used as HPLC mobile phases A and B, respectively. Mobile phases were at a constant flow of 0.3 mL/min, starting at 40 % (B) with a linear increase to 100% (B) in 8 min, and kept for 3 min. They were then decreased to 40% B for 1 min, and reconditioning of the column was done for 5 min before the next injection. The injection volume was set to 5 μL. Source parameters for the Agilent 6470 were the same as described in section 2.5. Compound-specific settings were optimized (Supplementary Table 2). The obtained peak areas were normalized to the internal standard β-cholestanol, and the peaks were integrated using Mass Hunter Workstation Software Quantitative Analysis for QQQ. The working calibration of the standard concentrations ranged between 0.5 μg/μL and 20 μg/μL for ergosterol. Ergosterol is expressed in μg/g of dry weight. For dry weight measurements, a total of 1 mL of fresh slurry was transferred into previously weighed 20 mL glass scintillation vials. The vials were covered with perforated aluminum foil and left in an oven at 60 °C until completely dry. The dried weight of the vial was determined by extracting the weight of the empty vials from the weight of the vials containing the dry biofilms.

2.5.2. Bacterial abundance

Quantitative polymerase chain reaction (qPCR) was used to amplify and quantify the bacterial marker gene rpoB (1 copy per bacterial cell) as a proxy of bacterial abundance in the biofilms (Dahllof et al., 2000). RNA was extracted using Quick-RNA Fecal/Soil Microbe Microprep Kit (Zymo Research, CA), following the manufacturer's instructions. Biofilm samples were preserved at −80 °C prior to RNA extraction. DNA contamination was removed using PureLink™ DNase Set (Thermo Fisher Scientific, Waltham, MA, USA). The extracted RNA was used as a template for cDNA synthesis using iScript Select cDNA Synthesis Kit (Bio-Rad, USA) following the reaction setup with random primers provided by the manufacturers. The concentration and purity of the synthesized cDNA were determined using a Qubit fluorometer and a Nanodrop Spectrophotometer, respectively. The qPCR reaction was performed using SYBR green detection chemistry on a CFX96 Touch real-time PCR machine using the CFX Maestro software (Bio-Rad, USA). The target gene, rpoB, was amplified using the forward primer rpoB1698f (5′-AACATCGGTTTGATCAAC-3′) and the reverse primer rpoB2041r (5′-CGTTGCATGTTGGTACCCAT-3′) from Dahllof et al. (2000). The qPCR conditions were optimized for the gene of interest (98 °C for 3 min [1 cycle]; 98 °C for 15 s, 50 °C for 90 s, and 72 °C for 90 s [30 cycles]; 72 °C for 10 min [1 cycle]). The obtained standard curve (7 points, 5-fold dilutions of rpoB amplicons of known concentrations) yielded an efficiency of 94.7% and a correlation coefficient of 0.957. The

identity of the rpoB amplicons was confirmed with Sanger sequencing (Eurofins). The number of rpoB copies is used as a proxy for the number of bacterial cells (Dahllof et al., 2000) and is expressed in the number of copies of rpoB/cm^2.

2.5.3. Algal biomass

Chlorophyll-a was extracted and identified following Gómez-Martínez et al. (2023). Briefly, the frozen pellets were thawed in ice, resuspended in 1 mL of solvent (80/20 acetone/methanol v/v), and incubated for 1 h at −20 °C in dark conditions. Then, ultrasonication was performed at 4 °C for 3 min, followed by incubation overnight at −20 °C, to ensure total pigment extraction. The extracts were filtered through 0.45 μm Target2™ Nylon Syringe 4 mm diameter filters (Thermo Scientific™, cat n° F2504-1) and stored at −20 °C in dark glass vials. The extracts were analyzed via high-performance liquid chromatography (Shimadze Prominence HPLC Systems) following Corcoll et al. (2019). Chlorophyll-a was identified using an internal standard, and its relative abundance was estimated following Wright and Jeffrey (2006). The content of chlorophyll-a (Chl-*a*) was used as a proxy for the total algal biomass and is expressed in ng of chlorophyll-a/dry weight.

2.6. Univariate statistical analysis

Statistical differences in ergosterol, abundance of rpoB gene (bacterial biomass), content of chlorophyll-a (algal biomass), and alpha diveristy measurementes for ITS2 and 16S (see below) among treatments were assessed by one-way analysis of variance (ANOVA), followed by a Tukey post-hoc test for multiple comparisons using the R package multcompView (Spencer Graves, Hans- Peter Piepho, 2024). Approximate normality was confirmed through quantile-quantile plots of the residuals. No model had residuals that deviated substantially from a normal distribution (Supplementary Fig. 6). In all tests, p-values <0.05 were considered statistically significant.

2.7. Fungal and bacterial community composition analysis

Fungal and bacterial community composition in the biofilms was evaluated by RNA metabarcoding (amplicon sequencing). While DNA metabarcoding reflects the genomic content from the total microbial community, RNA metabarcoding reflects expression and, thus, active organisms in microbial communities. It excludes organisms that are dead or dormant as well as propagules and free DNA (Adamo et al., 2021). RNA was extracted and used as template to synthesize cDNA, as described in section 2.7.2. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the forward primer Bakt 341F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer Bakt 805R (5′-GACTACHVGGGTATCTAATCC-3′) following Andersson et al. (2010). The fungal nuclear ribosomal internal transcribed spacer 2 was amplified using the forward primer ITS3-Mix1,2.

(5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-AAC-caWC-GATGAAGAACGCAg-3′) and the reverse primer mix ITS4-cwmix1 (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-AA-TCCTCCGCTTAyTgATAtGc) together with ITS4-cwmix2 (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-AA-TCCTCCGCTTAtTrATAtGc-3′) following Wurzbacher et al. (2020).

The sequencing was done by CD Genomics (New York, USA). One sequencing library from each amplicon was prepared using the 600 cycle Amplicon kit with PhiX library preparation kit (Illumina Inc.). Sequencing was performed in a NovaSeq 6000, PE250 (Illumina Inc.), producing 300 bp paired-end fragments. The raw sequences were deposited in the NCBI sequence read archive (SRA) with BioProject accession number PRJNA1092544 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1092544).

For the 16S amplicons, the obtained raw reads were processed with QIIME2 (v. 2023.07). Once the sequences were loaded into QIIME2 (v. 2023.07), the primer sequences were trimmed using cutadapt. The denoising was performed with DADA2 using the following parameters: -*p*-trunc-len-f 270 –*p*-trunc-len-r 220 –p-max-ee-f 2.0 –p-max-ee-r 2.0. RNA sequencing of the 16S rRNA gene yielded, on average, 30,304 reads per sample (min: 26,712; max: 35,602). Filtering resulted in the removal of 40% of the reads. Next, QIIME2 was used to group the reads into amplicon sequence variants (ASVs; Callahan et al., 2017). The number of ASVs present in each sample was calculated after randomly rarefying each sample down to 853 sequences (corresponding to the lowest sequencing depth) to minimize any bias due to differences in number of sequences between the samples. For 16S amplicons, taxonomic annotation was performed by assigning the sequences to the silva-138-99-515-806-nb-classifier reference dataset (Quast et al., 2013).

Raw reads for ITS2 amplicons were processed in QIIME2 (v. 2023.07) and clustered to operational taxonomic units (OTUs (Blaxter et al., 2005);). For the ITS2 sequences, we chose to cluster our sequences into OTUs due to the high level of intraspecific variation of the ITS2 marker (Kauserud, 2023). RNA sequencing of the ITS2 region yielded, on average, 110,899 reads per sample (min: 82,805; max:135,153). Briefly, primer trimming was performed using cutadapt, and sequence quality filtering was carried out using the "quality-filter q-score" QIIME2 plugin with a minimum acceptable PHRED score of 20. Sequences were first dereplicated and subsequently merged to operational taxonomic units using the VSEARCH plugin with a sequence similarity cut-off of 97%. The number of OTUs present in each sample was calculated after randomly rarefying each sample down to 5070 sequences (corresponding to the lowest sequencing depth) to minimize any bias due to differences in number of sequences between the samples. Taxonomic annotation was performed by assigning the OTUs to the UNITE database (v. 2023.07.25; Nilsson et al. (2019)). OTUs that were assigned to non-fungal species were manually removed from the resulting OTU table, since the aim of this part of the study was to investigate the biofilms' fungal component. Moreover, the 50 most abundant OTUs were manually verified for veracity and taxonomic affiliation using BLAST (GenBank; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Only 43 OTUs from the initial 5070 OTU remained as OTUs assigned to fungi, since most of the rest were identified as algae or animals.

For both 16S and ITS2, sequences with poorly resolved (i.e. Fungi incertae sedis) or non-fungal/non-bacterial taxonomic annotations, respectively, were removed prior to further analysis. Furthermore, an additional filtering of the data was perfomed in order to avoid false positives with low counts and single presence coming from PCR artifacts (rt-PCR and region-specific PCR), where all ASVs or OTUs that were not present in at least 3 samples and contained fewer than 5 reads were excluded from further analysis, resulting in 379 ASVs for 16S and 30 OTUs for ITS2 after filtering.

Alpha diversity analysis, principal coordinates analyses (PCoA) to describe beta diversity, and relative abundance analysis were carried out with the phyloseq R package (v. 1.44.0; McMurdie and Holmes, 2013). The relative abundances at the phylum and class levels were plotted using the microbiome R package (v. 1.23.1) (https://bioconductor.org/packages/release/bioc/html/microbiome.html). Differentially abundant ASVs (16S) and OTUs (ITS2) were identified using the DESeq2 v.1.36.0 package, from Bioconductor v 3.15 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). The resulting p-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) algorithm. Differentially abundant taxa within treatments were selected with a cut-off of $|\log2 \text{ Fold Change (FC)}| > 2$ and adj p value < 0.05. PERMANOVA (adonis2) tests were performed using the vegan package v. 2.5–7 to test differences between the microcosm groupings.

3. Results

3.1. Experimental conditions

Physical and chemical conditions were comparable between

treatments. Average water conditions in the microcosms were: pH 6.39 ± 0.73, percentage of dissolved oxygen 79 ± 1.2 %, conductivity 57 ± 13.4 μS/cm, temperature 11.79 ± 0.13 °C, 0.025 ± 0.002 mg/L of PO_4^{3-}, 0.19 ± 0.017 mg/L of NO_3^{2-}, and 10.6 ± 0.57 mg/L of DOC. The tebuconazole concentrations in the exposed treatments were close to the nominal concentrations (5 and 10, and 25% below the nominal concentrations, in treatments chronic 10 and 100 μg/L, and acute 100 μg/L, respectively; Table 1). Tebuconazole was not detected in the microcosms not receiving pollutant (Control 0 μg/L).

3.2. Kinetics of tebuconazole and the release of transformation products

In the biotic treatments, tebuconazole kinetics tests showed a high persistence over 96 h, where only 24 % ± 8.5 % (n = 6) of the compound was dissipated from the water column (Fig. 1). Hydroxytebuconazole (OH-tebuconazole), its main transformation product, was detected at 0 h at a concentration of 0.05 ± 0.008 μg/L. After 96 h, a 2-fold increase in OH-tebuconazole concentrations had occurred in both the biotic and the abiotic treatments. Transformation products (TPs) were identified after 96 h in a 10-fold significantly higher presence (p = 0.0057, ANOVA & Tukey's HSD) in the samples from microcosms that contained biofilms (Fig. 1). The presence of TPs at the start of the tebuconazole kinetic test (0 h) was also significantly higher in the water coming from the 24-day chronic exposure to tebuconazole in comparison to the water coming from the abiotic test (p = 0.047, ANOVA & Tukey's HSD) and the one coming from the acute treatment (p = 0.01, ANOVA & Tukey's HSD). This indicates that the TPs are more abundant in the presence of biofilms that have already been exposed to tebuconazole and that these biofilms can release them to the water column.

3.3. Effects on fungal, bacterial and algal biomass

The concentration of ergosterol in biofilms decreased with increasing concentrations of tebuconazole (Fig. 2a). The biofilms chronically exposed to tebuconazole had significantly lower ergosterol content than control biofilms (Fig. 2a; Supplementary Table 3). Compared to the control, a 40% decrease was observed in the biofilms treated with chronic exposure to 10 μg/L, and a decrease of 60% was observed for the biofilms chronically exposed to 100 μg/L of tebuconazole. In addition, a 40% decrease of ergosterol was observed in the biofilms under the acute treatment of 100 μg/L of tebuconazole (Fig. 2a).

The number of bacteria in the biofilm (number of rpoB copies per cm^2) was not affected by the tebuconazole exposure, which was demonstrated by a similar number of rpoB copies between treatments (Fig. 2b). Similarly, the algal biomass (chlorophyll-a content) remained relatively constant between treatments (Fig. 2c).

3.4. Effects on fungal diversity and community composition

Both the richness and Shannon index (alpha diversity) showed non-significant differences between control and treatments (p = 0.132 (richness) and p = 0.051 (Shannon); ANOVA; Fig. 3a). For both indexes, the values showed a lower trend in biofilms exposed to both a 96 h acute and chronic exposure to 100 μg/L of tebuconazole compared to non-exposed biofilms. Biofilms exposed to 10 μg/L of tebuconazole showed values similar to the control biofilms. Principal coordinate analysis using the Bray-Curtis dissimilarity measure (beta diversity) showed that the

Table 1
Tebuconazole concentrations in microcosms during the exposure phase. Values are means and standard deviations for each treatment after water renewal.

Treatment	Control	Chronic 10 μg/L	Acute 100 μg/L	Chronic 100 μg/L
Tebuconazole (μg/L)	0 ± 0 (n = 12)	9.5 ± 0.51 (n = 22)	74.81 ± 3.72 (n = 6)	89.2 ± 0.95 (n = 18)

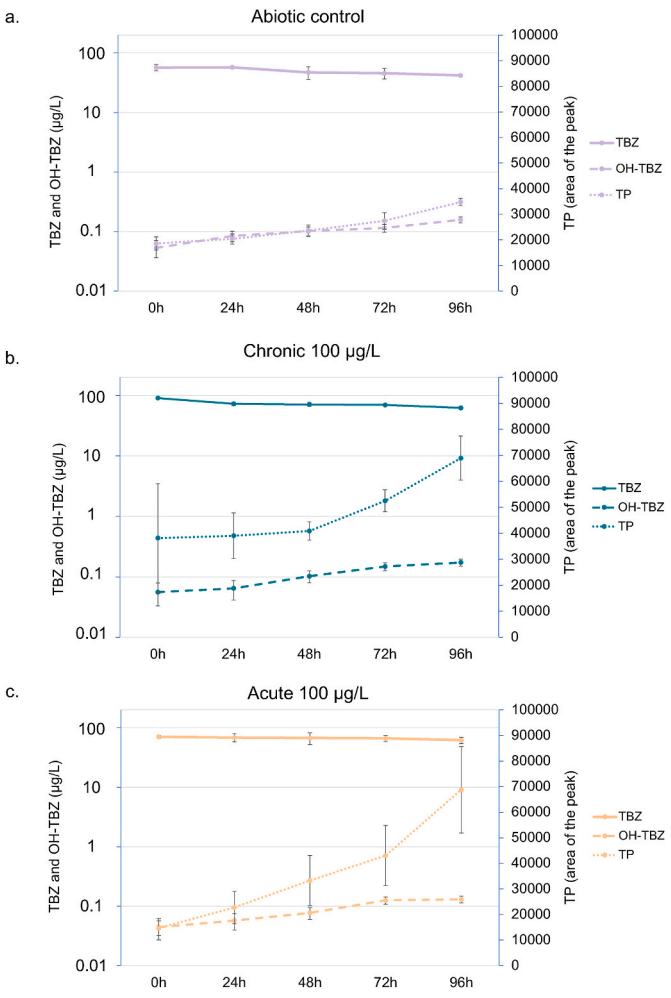


Fig. 1. Tebuconazole (TBZ), hydroxy-tebuconazole (OH-TBZ), and unidentified transformation products (TP) kinetics over a 96-h period after a tebuconazole spike (acute) of 100 μg/L (0h), in a) Abiotic control: microcosms without biofilm, b) Chronic 100 μg/L: biofilms exposed to 100 μg/L of tebuconazole chronically for 24 days, and c) Acute 100 μg/L: biofilms not pre-exposed to tebuconazole. Average and standard deviation are shown in dots for each time point. The concentration (normalized abundance) in μg/L of TBZ and TBZ-OH are represented in the left-y-axis (log-scale) and the sum of the peak areas from the TPs are shown in the right-y-axis.

fungal composition in biofilms exposed chronically to 100 μg/L of tebuconazole grouped differently from the fungal community of the control samples (PcoA1 = 35.8%; Fig. 3b). Even though the fungal communities exposed to 10 μg/L and those exposed in a acute manner to 100 μg/L of tebuconazole did not show any obvious different grouping, they presented a higher heterogeneity than did the control samples. Moreover, permutational multivariate analysis of variance (PERMANOVA) detected significant differences between the different microcosm groups (Adonis, p = 0.036).

The fungal phyla *Ascomycota*, *Basidiomycota*, *Rozellomycota*, and *Mucuromycota* were identified in similar proportions in all treatments (Fig. 3c and Supplementary Table 4). At the class level (Fig. 3d and Supplementary Table 5), almost 80% of the taxa remained unresolved. The fungal classes *Monoblepharidomycetes*, *Sordariomycetes*, *Malasseziomycetes*, *Eurotiomycetes*, *Dothideomycetes*, and *Lecanoromycetes* were identified in all samples in a similar proportion between treatments. Fungal species belonging to *Malasseziomycetes* and *Eurotiomycetes* were present in all treatments except in the chronic exposure to tebuconazole 100 μg/L treatment. Fungal species assigned to *Dothideomycetes* and *Lecanoromycetes* showed a higher abundance trend in exposed biofilms

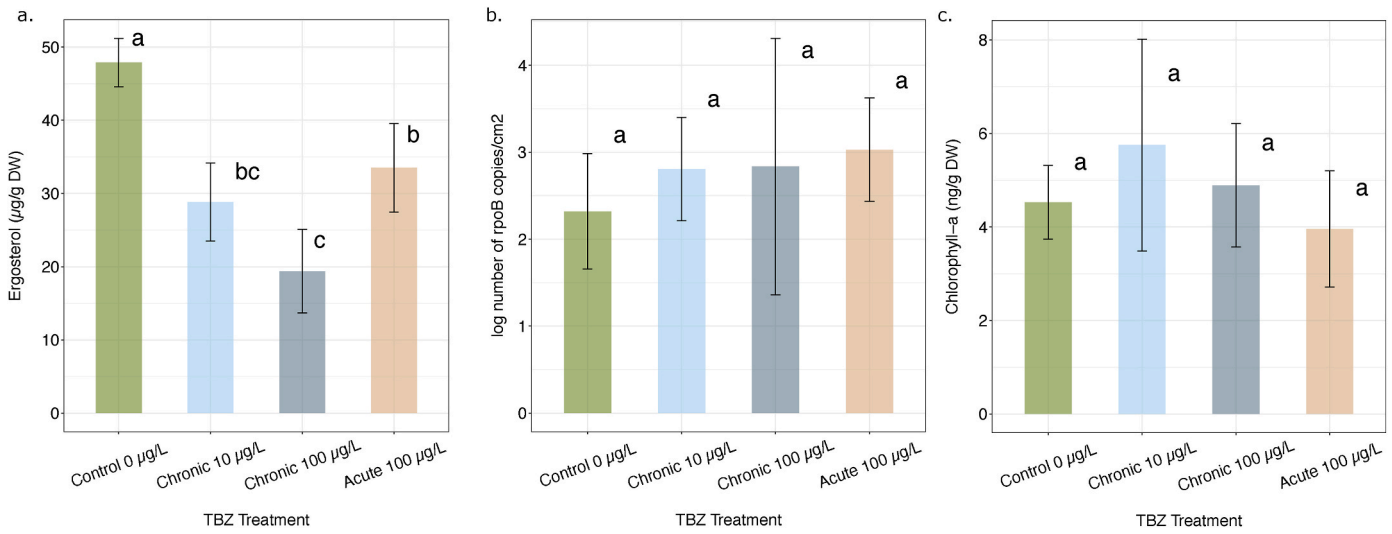


Fig. 2. Biofilm responses upon exposure to tebuconazole acute (96 h) and chronic (24 days) in terms of fungal biomass – ergosterol concentration (a), bacterial biomass – number of rpoB copies (b), and algal biomass – chlorophyll-a concentration (c). Colored bars represent average values, the error bars represent the standard deviation (n = 3), and the letters on top of the bars indicate a significant result between treatments obtained after Tukey's multiple comparisons post hoc test ($p < 0.005$).

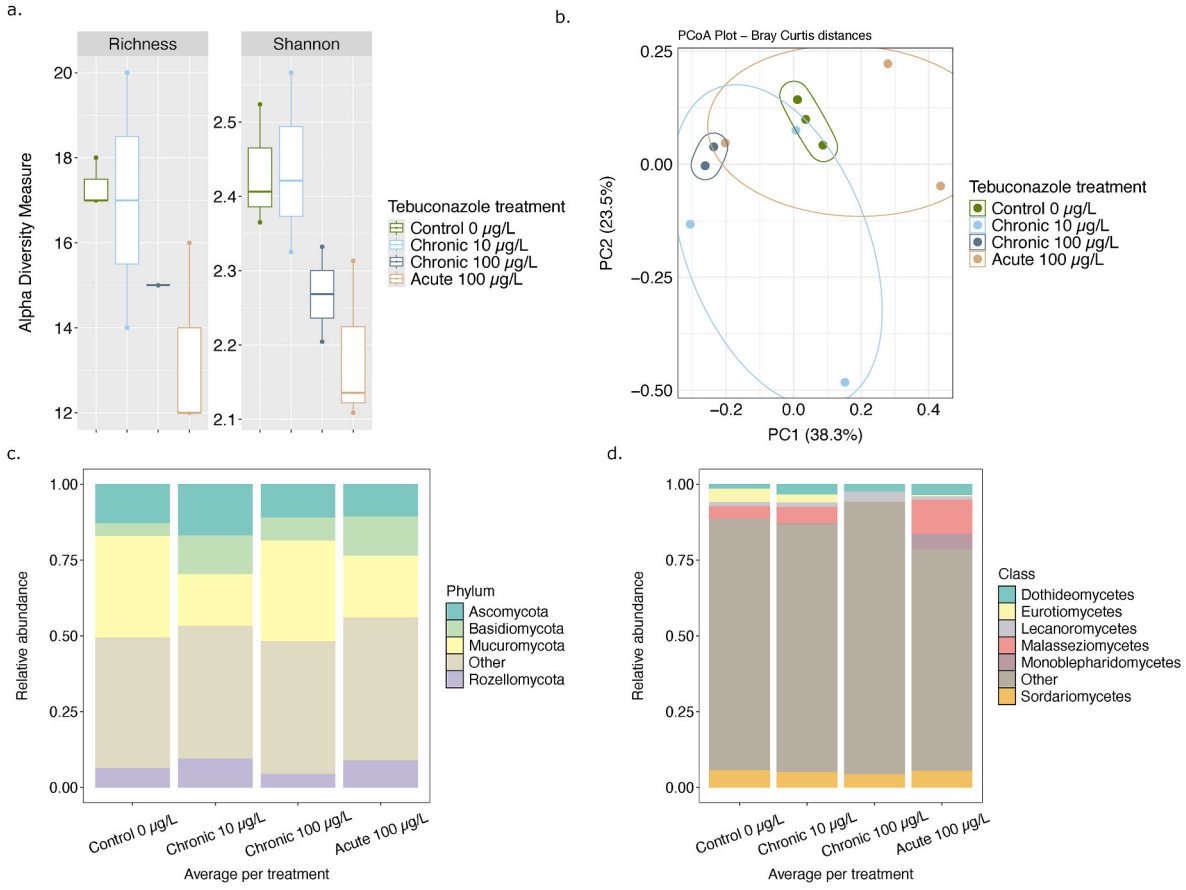


Fig. 3. Fungal richness and Shannon alpha diversity indexes (a), PCoA plot based on Bray-Curtis distances (b), and fungal taxonomic profiles at the phylum (c) and class (d) levels, resulting from the RNA metabarcoding (ITS2) from the control biofilms, biofilms exposed for 24 days chronically to 10 and 100 µg/L of tebuconazole, and biofilms exposed in a 96 h acute pulse to 100 µg/L of tebuconazole, respectively.

compared to the controls.

Among the 30 OTUs analyzed for differential abundance, three OTUs exhibited significant differences for both biofilms exposed to 10 and 100 µg/L of tebuconazole for 24 days, respectively, using a cutoff of |log2 Fold Change (FC)| > 2 and adj p-value <0.05 (Table 2). All treatments were individually compared to the control samples. Five OTUs were differentially abundant in the acute-treated biofilms. Species belonging to the classes *Lecanoromycetes*, *Malasseziomycetes*, and *Eurotiomycetes* as

Table 2
Differentially abundant fungal OTUs after DESeq2. All exposed biofilms were compared to control biofilms.

Phylum	Class	log2FoldChange	padj
Chronic 10 μg/L *vs. Control*			
Chytridiomycota	*Monoblepharidomycetes*	−5.42	4.7e-07
Ascomycota	*Lecanoromycetes*	5.51	4.2e-07
Basidiomycota	Other	−6.67	6.1e-10
Chronic 100 μg/L *vs. Control*			
Basidiomycota	*Malasseziomycetes*	2.78	0.04
Ascomycota	*Eurotiomycetes*	5.17	2.8e-06
Basidiomycota	Other	−6.32	6.2e-09
Acute 100 μg/L *vs. Control*			
Rozellomycota	Other	2.60	0.04
Other	Other	2.77	0.03
Other	Other	2.96	0.02
Chytridiomycota	*Monoblepharidomycetes*	−5.74	1.1e-07
Basidiomycota	Other	−6.49	2.1e-09

well as one species belonging to the phylum *Rozellomycota* were more abundant in tebuconazole treated biofilms when compared with the controls. Differentially abundant OTUs in the phylum *Basiodiomycota* and species of the fungal class *Monoblepharidomycetes* were less abundant in tebuconazole-treated samples compared to the controls (see Table 2).

3.5. Effects on bacterial diversity and community composition

No significant differences were found between treatments in terms of richness or Shannon index ($p = 0.06$ (richness); $p = 0.07$ (Shannon); ANOVA). Despite this lack of significance, the richness and Shannon index showed a noticeable upward trend in all tebuconazole-treated biofilms (Fig. 4a). Principal coordinate analysis utilizing the Bray-Curtis dissimilarity measure indicated that the bacterial composition of biofilms chronically exposed to both 10 and 100 μg/L of tebuconazole for 24 days differed from the control samples (PcoA1 = 23.01%; Fig. 4b). The bacterial community composition of biofilms exposed to 100 μg/L of tebuconazole in an acute manner did not exhibit distinct clustering. However, permutational multivariate analysis of variance (PERMANOVA) detected significant differences between the different microcosm groups (Adonis, $p = 0.001$, $r^2 = 0.41$).

Taxonomic analysis revealed that the bacterial biofilm communities were dominated by the phyla Bacteroidota, Cyanobacteria, Planctomycetota, and Pseudomonadota (Fig. 4c and Supplementary Table 6). At the genus level, the relative abundance of species belonging to the genus *Pseudanabaena*_PCC-7429 decreased in biofilms exposed to tebuconazole. Inversely, species belonging to the genus SM1A02 (family Phycisphaeraceae) were higher in biofilms exposed to tebuconazole compared to the controls (Fig. 4d and Supplementary Table 7). Out of the 379 ASVs subjected to differential abundance analysis, three and five ASVs were differentially abundant in biofilms exposed to 10 and 100 μg/L of tebuconazole for 24 days, using a cutoff of |log2 Fold Change (FC)| > 2 and adj p-value <0.05, respectively (Table 3). The abundance of ASVs belonging to the families Phycisphaerales and Chitinophagales were higher in tebuconazole-treated samples.

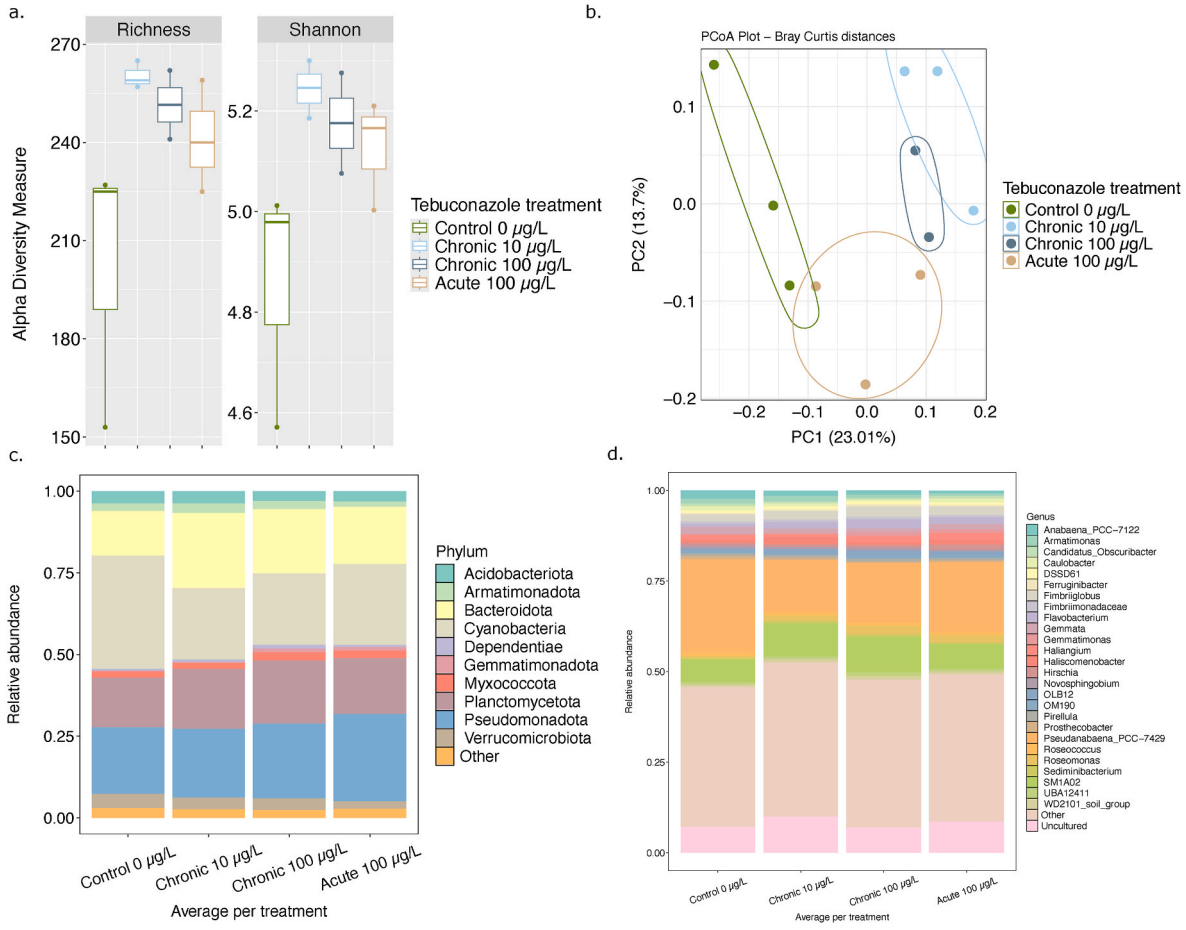


Fig. 4. Bacterial richness and Shannon alpha diversity indexes (a), PCoA plot based on Bray-Curtis distances (b), and bacterial taxonomic profiles at the phylum (c) and genus (d) levels, resulting from the RNA metabarcoding (16S rRNA gene) from the control biofilms, biofilms exposed for 24 days chronically to 10 and 100 μg/L of tebuconazole, and biofilms exposed in a 96 h acute pulse to 100 μg/L of tebuconazole, respectively.

Table 3
Differentially abundant bacterial ASVs after DESeq2. Biofilms chronically exposed to 10 and 100 μg/L of tebuconazole were compared to control biofilms.

Phylum	Family	log2FoldChange	padj
Chronic 10 μg/L *vs. Control*			
Planctomycetota	Phycisphaeraceae	23.71	3.17e-05
Bacteroidota	Saprospiraceae	8.89	0.003
Planctomycetota	Phycisphaeraceae	6.86	2.39e-06
Other	Other	−24.01	8.31e-08
Other	Other	26.16	2.39e-06
Chronic 100 μg/L *vs. Control*			
Planctomycetota	Phycisphaeraceae	23.87	6.08e-07
Other	Other	−23.41	3.06e-09
Other	Other	26.09	2.99e-08

4. Discussion

In this study, natural biofilm communities from a Swedish river were exposed in microcosms to two environmental concentrations of the fungicide tebuconazole in both a chronic (24 days at 10 or 100 μg/L) and an acute manner (96 h at 100 μg/L). Our results show that biofilms chronically exposed to tebuconazole can partly metabolize this compound and release its transformation products to the water column. The biofilms exposed chronically to 10 and 100 μg/L of tebuconazole were the most affected in terms of fungal biomass, although this decrease in fungal biomass was not associated with any changes in bacterial or algae biomass. Both fungal and bacterial community composition were influenced by tebuconazole exposures. Chronic exposure to tebuconazole led to a drastic reduction of the fungal biomass accompanied by changes in the community composition, where taxa potentially more tolerant to tebuconazole (i.e. *Lecanoromycetes* sp.) replaced the more sensitive taxa (i.e. *Malasseziomycetes* sp).

Biofilms chronically exposed to tebuconazole dissipated around 20% of the compound, resulting in a 10-fold increase of tebuconazole transformation products after 96 h. Despite the very low mineralization potential of tebuconazole mainly due to the general recalcitrance of the triazole ring to microbial degradation (El Azhari et al., 2018), biodegradation of tebuconazole has been reported previously (Wang et al., 2018). Although studies that specifically target the biodegradation capacity of aquatic biofilms towards tebuconazole are lacking, our results are similar to those of Bertrans-Tubau et al. (2023), who reported that aquatic biofilms in microcosms were able to dissipate a pesticide mixture composed of tebuconazole, terbuthylazine, glyphosate and its metabolite aminomethylphosphonic acid, and imidacloprid at rates ranging from 2 to 35% in a period of 12 days.

Studies performed in soil microbiomes have previously reported up to 22 transformation products originating from tebuconazole (El Azhari et al., 2018; Storck et al., 2017). We were not able to identify the molecular structures of the detected transformation products. However, our study revealed that the release of transformation products occurs not only in soil, but also in aquatic environments. This observation calls for studies that elucidate the molecular structure of the transformation products; such studies are required to gain a better understanding of the environmental fate of tebuconazole and its ecotoxicological impact.

The fungal biomass decreased as a function of the concentration and exposure time, confirming that tebuconazole is toxic to non-target aquatic fungi. Chronic exposure of tebuconazole at 10 or 100 μg/L led to a 40 and 60% reduction in fungal biomass, respectively. Biofilms acutely exposed (96h) of 100 μg/L showed a 40% loss of fungal biomass. Previous studies have also reported the toxicity of tebuconazole to aquatic fungi. For instance, Artigas et al. (2012) reported a decrease of 29% in aquatic fungal biomass when aquatic fungal communities were exposed to 33 μg/L of tebuconazole. Similarly, Donnadieu et al. (2016) reported a 20 % decrease when aquatic microbial communities were exposed to 10 μg/L. To our knowledge, our study is the first to report the inhibition of aquatic fungal biomass by 100 μg/L of tebuconazole, a concentration that has been previously reported in agricultural streams when frequent fungicide applications and runoff during rainfall episodes co-occurred (Artigas et al., 2012). In our study, the decrease in fungal biomass did not indirectly affect neither the bacterial nor the algal biomass of the biofilms, however, indirect effects on bacterial community composition (beta diversiy) were observed. Antagonistic and synergistic interactions have been documented between aquatic bacteria and fungi (Dimitrov et al., 2014a; Gulis and Suberkropp, 2003). Consequently, a reduction of fungal biomass due to the effects of tebuconazole could be expected to induce changes in bacterial biomass. For instance, Mille-Lindblom and Tranvik (2003) reported strong antagonistic effects from fungi on bacteria and vice versa. Artigas et al. (2012) reported that bacterial biomass temporally decreased under tebuconazole exposure (20 μg/L), followed by full recovery. Our study also attempted to correlate the loss of fungal biomass to changes in bacterial and algal biomass, but they remained comparable to the control biofilms. The results corroborate the findings from Zubrod et al. (2011), where the number of bacterial cells remained stable when biofilms were exposed to 65 μg/L of tebuconazole. Heterotrophic fungi and autotrophic algae share a long history of ecological and evolutionary interactions, including parasitism and various forms of nutritional symbiosis (Bonito, 2024). Therefore, changes in algal biomass as a consequence of a loss of fungal biomass were expected. However, our results showed that tebuconazole does not influence total algal biomass. It could be hypothesized that under chronic exposures, the algal biomass could start to decrease, as shown by Tlili et al. (2011), who found that algal biomass decreased after 35 days of exposure to tebuconazole.

To our knowledge, our study is the first one to describe the microbial diversity of the active aquatic fungal community using RNA metabarcoding under exposure to tebuconazole in a microcosm setup. We observed a great loss of diversity from the field samples (Supplementary Figs. 4 and 5) in the fungal community we obtained in the microcosms. The glass slides were deployed in the field for 7 days to allow biofilm colonization. It could be argued that a longer colonization period may have increased biofilm maturity and diversity. However, since freshwater biofilms often undergo colonization, formation, and detachment phases within a scale of weeks (Villanueva et al., 2011), we aimed to allow colonization while preventing detachment during the microcosm experiment. Yet, we were able to study how the fungal community changes in the presence of tebuconazole under highly controlled conditions. The biofilms exposed acutely to 100 μg/L of tebuconazole showed a downward trend in diversity concerning both the controls and the chronic treatments. These diversity values were lower than those found in biofilms exposed to 100 μg/L of tebuconazole for 24 days, even though biofilms exposed to 100 μg/L of tebuconazole lost 60% of their fungal biomass after 24 days of chronic exposure. This indicates that some taxa were able to thrive under high concentrations of tebuconazole, and therefore might be more tolerant to tebuconazole. Two species - one belonging to the fungal class *Malasseziomycetes* and the other to *Eurotiomycetes* - proved to be more abundant in biofilms exposed chronically to 100 μg/L of tebuconazole. Some of the *Malasseziomycetes* fungal species are well characterized as human skin associates. However, *Malasseziomycetes* spp. are widespread and have been detected in a wide variety of both aquatic and terrestrial environments (Amend, 2014). Similarly, *Eurotiomycetes* species are widely distributed across various environments and have been documented in numerous terrestrial and aquatic habitats (Sandberg et al., 2014). Previous studies have reported variations in ergosterol content between different species. For instance, Gessner and Chauvet (1993) described significant differences in ergosterol concentrations between two strains of *Alatospora acuminata* and *Flagellospora curvula*. Moreover, Charcosset and Chauvet (2001) reported a variation by a factor of 14 between four aquatic hyphomycete species and culture conditions. It can therefore be hypothesized that species with lower ergosterol content may be less sensitive to tebuconazole exposures compared to species with higher ergosterol levels.

Nevertheless, in this study we were not able to annotate the OTUs to

genus or species level, limiting the possibility to compare our findings with existing literature and infer potential metabolic functions of the differentially abundant species, since less than 50% of the reads could be classified to a taxonomic rank beyond the fungal kingdom level, presumably due to the lack of reference sequences from aquatic fungi. Abarenkov et al. (2022) reported that roughly 42% of the full-length Sanger-derived fungal ITS sequences in the INSDC lack a full species name, and 29% of these are not annotated beyond the kingdom level. Therefore, the lack of information in the reference databases has definitely affected the low taxonomic resolution of many of our OTUs.

Additionally, in this study, we used an RNA, rather than DNA, metabarcoding approach since it explicity seeks to exclude non-active or dead cells, and therefore offers a better characterization of the active microbial community (Adamo et al., 2021; Cristescu, 2019). We therefore argue that our approach comes closer to capturing the effect of tebuconazole on active aquatic fungal communities than previous DNA-based approaches. However, it is conceivable that we missed the detection of some taxa, and therefore we agree with Adamo et al. (2021) that the best approach is to produce RNA and DNA-based datasets from identical environmental samples, and define the taxa that are shared between them. These "shared taxa" would potentially be less likely to correspond to artifacts, and their use may reduce the impact of nucleic acids from dead or dormant organisms on perceived microbial community composition.

The two most abundant fungal identified phyla were *Ascomycota* and *Mucuromycota*. The phylum *Ascomycota* has been previously identified in freshwater mesocosms when exposed to 238 μg/L of tebuconazole Dimitrov et al. (2014). The phylum *Mucuromycota* is typically found in soil environments, however species belonging to this phylum have been isolated from aquatic environments (Seeber et al., 2022) but they have not been assessed in fungicide exposure experiments before.

Bacterial diversity did not significantly change within treatments. The most common phyla were Bacteroidota, Cyanobacteria, Planctomycetota, and Pseudomonadota. A high abundance of Pseudomonadota has been found in mesocosms exposed to 238 μg/L of tebuconazole (Dimitrov et al., 2014). This phylum has been reported to increase after the introduction of leaf litter in limnic systems (Hutalle-Schmelzer et al., 2010), therefore it can be hypothesized that the abundance of Pseudomonadota could have been higher in our experiments since no leaves were introduced in the system. The presence of Cyanobacteria decreased by 20% in the biofilms from the 100 μg/L chronic tebuconazole treatment. Kumar et al. (2012) described the reduction of photosynthetic pigments as well as metabolic and enzymatic activities of cyanobacteria under exposure to tebuconazole, suggesting that cyanobacteria might be more sensitive to tebuconazole than other bacterial phyla. Finally, species belonging to the families Phycispheraceae (phylum Planctomycetota) and Saprosphyraceae (phylum Bacteroidota) were significantly more abundant in tebuconazole-exposed biofilms (Table 3). Both classes can be found in freshwater environments and express a wide variety of carbohydrate-active enzymes (CAZy) that allow them to degrade a wide spectrum of complex polysaccharides from freshwater particles (Chiriac et al., 2023). Therefore, these two classes may partially take over the ecological function of organic matter degradation from fungi during prolonged tebuconazole exposure.

5. Conclusions

This microcosm study allowed us to report the sensitivity of non-target fungal microbial communities at environmental concentrations of tebuconazole. Moreover, aquatic biofilms proved to release tebuconazole transformation products to the water column. The toxicity of these transformation products remains unknown and could potentially be higher than the toxicity of the parent compound. Tebuconazole exposures also triggered changes in the fungal community composition resulting in different beta diversity. This community shift could affect the ecological role of aquatic fungi in freshwater ecosystems, since the emerging species could be more tolerant to the fungicide at the expense of losing efficiency in other functions, such as decomposing organic matter. The bacterial and algal compartments of the biofilm remained stable in terms of biomass after being exposed to tebuconazole.

In conclusion, this study shows that environmental concentrations of tebuconazole are harmful to the fungal community of freshwater biofilms. Even though our ecotoxicological study in microcosm using natural communities such as biofilms represents a simplification of natural conditions, this approach provides the opportunity to understand the complex set of responses of biofilms to contaminants, which is necessary to establish more accurate environmental quality standards for contaminants such as the fungicide tebuconazole. It calls for improved assessment of the effects of environmental concentrations of this fungicide, ideally by combining molecular and analytical chemistry tools to gain a better understanding of microbial shifts and the significance of the release of transformation products to the water column. Our results provide new data that we hope will aid in defining appropriate environmental quality standards for tebuconazole.

CRediT authorship contribution statement

Daniela Gómez-Martínez: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mary A. Selvin:** Methodology, Investigation. **Anders K. Nilsson:** Validation, Methodology, Investigation. **Eric Carmona:** Writing – review & editing, Methodology, Formal analysis. **Judith Sorel Ngou:** Writing – review & editing, Methodology. **Erik Kristiansson:** Writing – review & editing, Validation, Supervision. **R Henrik Nilsson:** Writing – review & editing, Supervision, Resources, Methodology. **Natàlia Corcoll:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was financed by the Swedish Foundation Stiftelsen Brigit och Birger Wåhlströms minnesfond för den Bohuslänska havs-och insjömiljön.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143854.

Data availability

Data will be made available on request.

References

Abarenkov, K., Kristiansson, E., Ryberg, M., Nogal-Prata, S., Gómez-Martínez, D., Stüer-Patowsky, K., Jansson, T., Pölme, S., Ghobad-Nejhad, M., Corcoll, N., Scharn, R., Sánchez-García, M., Khomich, M., Wurzbacher, C., Nilsson, R.H., 2022. The curse of the uncultured fungus. MycoKeys 86, 177–194. https://doi.org/10.3897/MYCOKEYS.86.76053.

Adamo, M., Voyron, S., Chialva, M., Marmeisse, R., Girlanda, M., 2021. Metabarcoding on both environmental DNA and RNA highlights differences between fungal communities sampled in different habitats. PLoS One 15 (12), e0244682.

Amend, A., 2014. From dandruff to deep-sea vents: malassezia-like fungi are ecologically hyper-diverse. PLoS Pathog. 10 (8), 8–11. https://doi.org/10.1371/journal.ppat.1004277.

Andersson, A.F., Riemann, L., Bertilsson, S., 2010. Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. ISME J. 4 (2), 171–181. https://doi.org/10.1038/ismej.2009.108.

Artigas, J., Majerholc, J., Foulquier, A., Margoum, C., Volat, B., Neyra, M., Pesce, S., 2012. Effects of the fungicide tebuconazole on microbial capacities for litter breakdown in streams. Aquat. Toxicol. 122–123, 197–205. https://doi.org/10.1016/j.aquatox.2012.06.011.

Augustin, T., Schlosser, D., Baumbach, R., Schmidt, J., Grancharov, K., Krauss, G., Krauss, G.J., 2006. Biotransformation of 1-naphthol by a strictly aquatic fungus. Curr. Microbiol. 52 (3), 216–220. https://doi.org/10.1007/s00284-005-0239-z.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14 (4), 251–263. https://doi.org/10.1038/nrmicro.2016.15.

Baudy, P., Zubrod, J.P., Konschak, M., Röder, N., Nguyen, T.H., Schreiner, V.C., Baschien, C., Schulz, R., Bundschuh, M., 2021. Environmentally relevant fungicide levels modify fungal community composition and interactions but not functioning. Environ. Pollut. 285. https://doi.org/10.1016/j.envpol.2021.117234.

Berenzen, N., Lentzen-Godding, A., Probst, M., Schulz, H., Schulz, R., Liess, M., 2005. A comparison of predicted and measured levels of runoff-related pesticide concentrations in small lowland streams on a landscape level. Chemosphere 58 (5), 683–691. https://doi.org/10.1016/j.chemosphere.2004.05.009.

Bernabò, I., Guardia, A., Macirella, R., Sesti, S., Tripepi, S., Brunelli, E., 2020. Tissues injury and pathological changes in Hyla intermedia juveniles after chronic larval exposure to tebuconazole. Ecotoxicol. Environ. Saf. 205 (July). https://doi.org/10.1016/j.ecoenv.2020.111367.

Bertrans-Tubau, L., Menard, Y., Batisson, I., Creusot, N., Mazzella, N., Millan-Navarro, D., Moreira, A., Morin, S., Ponsá, S., Abril, M., Proia, L., Romaní, A.M., Artigas, J., 2023. Dissipation of pesticides by stream biofilms is influenced by hydrological histories. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 99 (9), 1–16. https://doi.org/10.1093/femsec/fiad083.

Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., Abebe, E., 2005. Defining operational taxonomic units using DNA barcode data. Phil. Trans. Biol. Sci. 360 (1462), 1935–1943. https://doi.org/10.1098/rstb.2005.1725.

Bonito, G., 2024. Ecology and evolution of algal–fungal symbioses. Curr. Opin. Microbiol. 79, 102452. https://doi.org/10.1016/j.mib.2024.102452.

Bonnineau, C., Artigas, J., Chaumet, B., Dabrin, A., Faburé, J., Ferrari, B.J.D., Lebrun, J. D., Margoum, C., Mazzella, N., Miège, C., Morin, S., Uher, E., Babut, M., Pesce, S., 2021. Role of biofilms in contaminant bioaccumulation and trophic transfer in aquatic ecosystems: current state of knowledge and future challenges. Rev. Environ. Contam. Toxicol. 253, 115–153. https://doi.org/10.1007/398_2019_39.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11 (12), 2639–2643. https://doi.org/10.1038/ismej.2017.119.

Campos, E.V.R., De Oliveira, J.L., Da Silva, C.M.G., Pascoli, M., Pasquoto, T., Lima, R., Abhilash, P.C., Fernandes Fraceto, L., 2015. Polymeric and solid lipid nanoparticles for sustained release of carbendazim and tebuconazole in agricultural applications. Sci. Rep. 5 (March), 1–14. https://doi.org/10.1038/srep13809.

Carmona, E., Andreu, V., Picó, Y., 2017. Multi-residue determination of 47 organic compounds in water, soil, sediment and fish—turia River as case study. J. Pharmaceut. Biomed. Anal. 146, 117–125. https://doi.org/10.1016/j.jpba.2017.08.014.

Charcosset, J.Y., Chauvet, E., 2001. Effect of culture conditions on ergosterol as an indicator of biomass in the aquatic hyphomycetes. Appl. Environ. Microbiol. 67 (5), 2051–2055. https://doi.org/10.1128/AEM.67.5.2051-2055.2001.

Chiriac, M.C., Haber, M., Salcher, M.M., 2023. Adaptive genetic traits in pelagic freshwater microbes. Environ. Microbiol. 25 (3), 606–641. https://doi.org/10.1111/1462-2920.16313.

Corcoll, N., Österlund, T., Sinclair, L., Eiler, A., Kristiansson, E., Backhaus, T., Martin Eriksson, K., 2017. Comparison of four DNA extraction methods for comprehensive assessment of 16S rRNA bacterial diversity in marine biofilms using high-throughput sequencing. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 364 (14), 1–9. https://doi.org/10.1093/femsle/fnx139.

Corcoll, N., Yang, J., Backhaus, T., Zhang, X., Eriksson, K.M., 2019. Copper affects composition and functioning of microbial communities in marine biofilms at environmentally relevant concentrations. Front. Microbiol. 10 (JAN), 1–15. https://doi.org/10.3389/fmicb.2018.03248.

Cristescu, M.E., 2019. Can environmental RNA revolutionize biodiversity science? Trends Ecol. Evol. 34 (8), 694–697. https://doi.org/10.1016/j.tree.2019.05.003.

Dahllof, I., Baillie, H., Kjelleberg, S., 2000. rpoB-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. Appl. Environ. Microbiol. 66 (8), 3376–3380. https://doi.org/10.1128/AEM.66.8.3376-3380.2000.

De Gerónimo, E., Aparicio, V.C., Bárbaro, S., Portocarrero, R., Jaime, S., Costa, J.L., 2014. Presence of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107, 423–431. https://doi.org/10.1016/j.chemosphere.2014.01.039.

Dimitrov, M.R., Kosol, S., Smidt, H., Buijse, L., Van den Brink, P.J., Van Wijngaarden, R. P.A., Brock, T.C.M., Maltby, L., 2014. Assessing effects of the fungicide tebuconazole to heterotrophic microbes in aquatic microcosms. Sci. Total Environ. 490, 1002–1011. https://doi.org/10.1016/j.scitotenv.2014.05.073.

Donnadieu, F., Besse-Hoggan, P., Forestier, C., Artigas, J., 2016. Influence of streambed substratum composition on stream microbial communities exposed to the fungicide tebuconazole. Freshw. Biol. 61 (12), 2026–2036. https://doi.org/10.1111/fwb.12679.

El Azhari, N., Dermou, E., Barnard, R.L., Storck, V., Tourna, M., Beguet, J., Karas, P.A., Lucini, L., Rouard, N., Botteri, L., Ferrari, F., Trevisan, M., Karpouzas, D.G., Martin-Laurent, F., 2018. The dissipation and microbial ecotoxicity of tebuconazole and its transformation products in soil under standard laboratory and simulated winter conditions. Sci. Total Environ. 637–638, 892–906. https://doi.org/10.1016/j.scitotenv.2018.05.088.

Finckh, S., Carmona, E., Borchardt, D., Büttner, O., Krauss, M., Schulze, T., Yang, S., Brack, W., 2024. Mapping chemical footprints of organic micropollutants in European streams. Environ. Int. 183 (December 2023), 108371. https://doi.org/10.1016/j.envint.2023.108371.

Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. 59 (2), 502–507. https://doi.org/10.1128/aem.59.2.502-507.1993.

Glinski, D.A., Purucker, S.T., Van Meter, R.J., Black, M.C., Henderson, W.M., 2018. Analysis of pesticides in surface water, stemflow, and throughfall in an agricultural area in South Georgia, USA. Chemosphere 209, 496–507. https://doi.org/10.1016/j.chemosphere.2018.06.116.

Gómez-Martínez, D., Bengtson, J., Nilsson, A.K., Clarke, A.K., Nilsson, R.H., Kristiansson, E., Corcoll, N., 2023. Phenotypic and transcriptomic acclimation of the green microalga Raphidocelis subcapitata to high environmental levels of the herbicide diflufenican. Sci. Total Environ. 875 (February). https://doi.org/10.1016/j.scitotenv.2023.162604.

Grossart, H.-P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M., Rojas-Jimenez, K., 2019. Fungi in aquatic ecosystems. Nat. Rev. Microbiol. 17 (6), 339–354. https://doi.org/10.1038/s41579-019-0175-8.

Gulis and Suberkropp, 2003. Interactions between Stream Fungi and Bacteria, vol. 30, pp. 149–157. Bengtsson 1992.

Hutalle-Schmelzer, K.M.L., Zwirnmann, E., Krüger, A., Grossart, H.P., 2010. Changes in pelagic bacteria communities due to leaf litter addition. Microb. Ecol. 60 (2), 462–475. https://doi.org/10.1007/s00248-010-9639-0.

Jobard, M., Rasconi, S., Sime-Ngando, T., 2010. Diversity and functions of microscopic fungi: a missing component in pelagic food webs. Aquat. Sci. 72 (3), 255–268. https://doi.org/10.1007/s00027-010-0133-z.

Junghanns, C., Moeder, M., Krauss, G., Martin, C., Schlosser, D., 2005. Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151 (1), 45–57. https://doi.org/10.1099/mic.0.27431-0.

Kagami, M., Miki, T., Takimoto, G., 2014. Mycoloop: chytrids in aquatic food webs. Front. Microbiol. 5 (APR), 1–9. https://doi.org/10.3389/fmicb.2014.00166.

Kauserud, H., 2023. ITS alchemy: on the use of ITS as a DNA marker in fungal ecology. Fungal Ecology 65 (July), 101274. https://doi.org/10.1016/j.funeco.2023.101274.

Kong, H., Yang, E.J., Jiao, N., Lee, Y., Jung, J., Cho, K.H., Moon, J.K., Kim, J.H., Xu, D., 2023. RNA outperforms DNA-based metabarcoding in assessing the diversity and response of microeukaryotes to environmental variables in the Arctic Ocean. Sci. Total Environ. 876 (March), 162608. https://doi.org/10.1016/j.scitotenv.2023.162608.

Kramer, L., Schulze, T., Klüver, N., Altenburger, R., Hackermüller, J., Krauss, M., Busch, W., 2024. Curated mode-of-action data and effect concentrations for chemicals relevant for the aquatic environment. Sci. Data 11 (1), 1–14. https://doi.org/10.1038/s41597-023-02904-7.

Krauss, G.J., Solé, M., Krauss, G., Schlosser, D., Wesenberg, D., Bärlocher, F., 2011a. Fungi in freshwaters: ecology, physiology and biochemical potential. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 35 (4), 620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x.

Krauss, G.J., Solé, M., Krauss, G., Schlosser, D., Wesenberg, D., Bärlocher, F., 2011b. Fungi in freshwaters: ecology, physiology and biochemical potential. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 35 (4), 620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x.

Kuehn, K.A., Lemke, M.J., Suberkropp, K., Wetzel, R.G., 2000. Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte Juncus effusus. Limnol. Oceanogr. 45 (4), 862–870. https://doi.org/10.4319/lo.2000.45.4.0862.

Kumar, Nirmal, Bora, Anubhuti, Rita Kumar, M.K.A., 2012. Differential effects of agricultural pesticides endosulfan and tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, filamentous cyanobacteria. Biol. Environ. Sci 6 (16), 67–75.

Lefrancq, M., Jadas-Hécart, A., La Jeunesse, I., Landry, D., Payraudeau, S., 2017. High frequency monitoring of pesticides in runoff water to improve understanding of their transport and environmental impacts. Sci. Total Environ. 587–588, 75–86. https://doi.org/10.1016/j.scitotenv.2017.02.022.

Martin, C., Moeder, M., Daniel, X., Krauss, G., Schlosser, D., 2007. Biotransformation of the polycyclic musks HHCB and AHTN and metabolite formation by fungi occurring in freshwater environments. Environ. Sci. Technol. 41 (15), 5395–5402. https://doi.org/10.1021/es0711462.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4). https://doi.org/10.1371/journal.pone.0061217.

Mille-Lindblom, C., Tranvik, L.J., 2003. Antagonism between bacteria and fungi on decomposing aquatic plant litter. Microb. Ecol. 45 (2), 173–182. https://doi.org/10.1007/s00248-002-2030-z.

Necibi, M., Saadaoui, H., Atayat, A., Mzoughi, N., 2021. Determination of triazole pesticides in the surface water of the medjerda river, Tunisia. Anal. Lett. 54 (4), 742–759. https://doi.org/10.1080/00032719.2020.1780250.

Nilsson, R.H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., Tedersoo, L., 2019a. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat. Rev. Microbiol. 17 (2), 95–109. https://doi.org/10.1038/s41579-018-0116-y.

Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019b. The UNITE database for molecular identification of fungi:

handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47 (D1), D259–D264. https://doi.org/10.1093/nar/gky1022.
Nulägesbeskrivning över, 2022. Mölndalsåns Vattensystem Regional Samverkan Inom, pp. 1–134.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), 590–596. https://doi.org/10.1093/nar/gks1219.
Sandberg, D.C., Battista, L.J., Arnold, A.E., 2014. Fungal endophytes of aquatic macrophytes: diverse host-generalists characterized by tissue preferences and geographic structure. Microb. Ecol. 67 (4), 735–747. https://doi.org/10.1007/s00248-013-0324-y.
Seeber, P.A., von Hippel, B., Kauserud, H., Löber, U., Stoof-Leichsenring, K.R., Herzschuh, U., Epp, L.S., 2022. Evaluation of lake sedimentary ancient DNA metabarcoding to assess fungal biodiversity in Arctic paleoecosystems. Environmental DNA 4 (5), 1150–1163. https://doi.org/10.1002/edn3.315.
Spencer, Graves, Hans-Peter Piepho, L.S., 2024. Package ‘ multcompView .’.
Storck, V., Karpouzas, D.G., Martin-Laurent, F., 2017. Towards a better pesticide policy for the European Union. Sci. Total Environ. 575, 1027–1033. https://doi.org/10.1016/j.scitotenv.2016.09.167.
Tlili, A., Corcoll, N., Bonet, B., Morin, S., Montuelle, B., Bérard, A., Guasch, H., 2011. In situ spatio-temporal changes in pollution-induced community tolerance to zinc in autotrophic and heterotrophic biofilm communities. Ecotoxicology 20 (8), 1823–1839. https://doi.org/10.1007/s10646-011-0721-2.
Vale, G., Martin, S.A., Mitsche, M.A., Thompson, B.M., Eckert, K.M., McDonald, J.G., 2019. Three-phase liquid extraction: a simple and fast method for lipidomic workflows. JLR (J. Lipid Res.) 60 (3), 694–706. https://doi.org/10.1194/jlr.D090795.
Villanueva, V.D., Font, J., Schwartz, T., Romaní, A.M., 2011. Biofilm formation at warming temperature: acceleration of microbial colonization and microbial interactive effects. Biofouling 27 (1), 59–71. https://doi.org/10.1080/08927014.2010.538841.
Voshall, A., Christie, N.T.M., Rose, S.L., Khasin, M., Van Etten, J.L., Markham, J.E., Riekhof, W.R., Nickerson, K.W., 2021. Sterol biosynthesis in four green algae: a bioinformatic analysis of the ergosterol versus phytosterol decision point. J. Phycol. 57 (4), 1199–1211. https://doi.org/10.1111/jpy.13164.
Wang, X., Hou, X., Liang, S., Lu, Z., Hou, Z., Zhao, X., Sun, F., Zhang, H., 2018. Biodegradation of fungicide Tebuconazole by Serratia marcescens strain B1 and its application in bioremediation of contaminated soil. Int. Biodeterior. Biodegrad. 127 (December 2017), 185–191. https://doi.org/10.1016/j.ibiod.2017.12.001.
Wang, T., Zhong, M., Lu, M., Xu, D., Xue, Y., Huang, J., Blaney, L., Yu, G., 2021. Occurrence, spatiotemporal distribution, and risk assessment of current-use pesticides in surface water: a case study near Taihu Lake, China. Sci. Total Environ. 782. https://doi.org/10.1016/j.scitotenv.2021.146826.
Wijntjes, C., Weber, Y., Höger, S., Hollert, H., Schäffer, A., 2022. Effects of algae and fungicides on the fate of a sulfonylurea herbicide in a water-sediment system. Chemosphere 290 (October 2021). https://doi.org/10.1016/j.chemosphere.2021.133234.
Wright, S.W., Jeffrey, S.W., 2006. In: Volkman, J.K. (Ed.), Pigment Markers for Phytoplankton Production BT - Marine Organic Matter: Biomarkers, Isotopes and DNA, 104. Springer Berlin Heidelberg, p. 71. https://doi.org/10.1007/698_2_003.
Wurzbacher, C., Kreiling, A.K., Svantesson, S., Van den Wyngaert, S., Larsson, E., Heeger, F., Nilsson, H.R., Pálsson, S., 2020. Fungal communities in groundwater springs along the volcanic zone of Iceland. Inland Waters 10 (3), 418–427. https://doi.org/10.1080/20442041.2019.1689065.
Zubrod, J.P., Bundschuh, M., Feckler, A., Englert, D., Schulz, R., 2011. Ecotoxicological impact of the fungicide tebuconazole on an aquatic decomposer-detritivore system. Environ. Toxicol. Chem. 30 (12), 2718–2724. https://doi.org/10.1002/etc.679.
Zubrod, J.P., Englert, D., Feckler, A., Koksharova, N., Konschak, M., Bundschuh, R., Schnetzer, N., Englert, K., Schulz, R., Bundschuh, M., 2015. Does the current fungicide risk assessment provide sufficient protection for key drivers in aquatic ecosystem functioning? Environ. Sci. Technol. 49 (2), 1173–1181. https://doi.org/10.1021/es5050453.