



## ECOSYSTEMS

# The effect of arsenic on the structure and composition of stream hyphomycetes assemblages

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**Abstract:** Aquatic hyphomycetes are fungi with a fundamental ecological role in forested streams. These organisms are responsible for cycling of nutrients in aquatic environments. However, their structure and composition can be affected when exposed to certain pollutants. Arsenic (As) is a trace element with high toxicity for the aquatic biota. Here we evaluated the effects of different concentrations of Arsenite ( $As^{III}$ ) and Arsenate ( $As^V$ ) on aquatic hyphomycetes assemblages. To test As toxicity, we conditioned *Nectandra megapotamica* leaves in a stream and after this period, we incubated leaf discs with stream water and different concentrations of  $As^{III}$  and  $As^V$ . Species richness was negatively affected by both As form. Likewise, the hyphomycetes assemblages presented variation in the composition of species. However, the sporulation rates were not influenced by As. The As showed toxicity on species of hyphomycetes more sensitive, remaining only in species tolerant to its toxicity. In this way, As generated a change in the aquatic hyphomycetes composition. We observed that As had a negative effect on the aquatic hyphomycetes assemblages, regardless of the chemical form. Our results point to the toxicity of this element and its effects on a group that is fundamental to the streams ecosystems functioning.

**Key words:** chemical speciation, trace elements, ecological process, ecotoxicology, fungi, water quality.

## INTRODUCTION

Anthropogenic activities can contaminate aquatic environments in different ways, however, the release of chemicals is the most common and aggressive practice (Hepp & Santos 2009, Wang et al. 2011). Among the chemical substances released in untreated water bodies, trace elements are frequent, since they form part of the formulation of numerous wastes (e.g. pesticides, sewage, industrial waste) (Ahmed et al. 2011). Although trace elements can be found at low concentrations in environments, they exert relevant toxic effects on aquatic ecosystems (Schaller et al. 2011). At high concentrations, the

trace elements have the potential to generate losses or alteration of ecosystem services, such as the decrease of the organic matter processing, punctually affecting nutrient cycling and biogeochemical cycles (Foust et al. 2016).

Arsenic (As) is a trace element found naturally or associated with anthropogenic sources (Phillips 1990). The main natural source of As is the rock weathering, which is stimulated by the reactivity of the elements with  $CO_2/H_2O$  (Alonso et al. 2014). Anthropogenic sources of As are agricultural inputs, mining, smelting of nonferrous metals, manufacture and use of organoarsenic herbicides and fossil fuels,

characterizing diffuse and point sources of pollution (Phillips 1990). As can be described as a metalloid, capable of forming covalent compounds or anionic species, having oxidation states arsenate ( $\text{As}^{\text{V}}$ ), arsenite ( $\text{As}^{\text{III}}$ ), arsenic ( $\text{As}^0$ ), and arsine ( $\text{As}^{\text{III}}$ ) (Sarmiente et al. 2009, Sharma & Sohn 2009). In aquatic environments, inorganic forms arsenate ( $\text{As}^{\text{V}}$ ) and arsenite ( $\text{As}^{\text{III}}$ ) are the predominant. Speciation of As may result in different levels of toxicity to aquatic organisms. In general, Arsenate ( $\text{As}^{\text{V}}$ ) is much less toxic than Arsenite ( $\text{As}^{\text{III}}$ ) (Singh et al. 2015).

The As can cause changes in aquatic communities (Foust et al. 2016) compromising ecosystem services (Ferreira et al. 2016). Several studies have reported that the survival, growth and reproduction of aquatic organisms are reduced when As is present (Tisler & Zagorc-Koncan 2002, Chaffin et al. 2005, Sole et al. 2008, Hepp et al. 2017). In addition to biological aspects observed directly in the individuals, studies have shown that the presence of As reduces diversity and alters the composition of aquatic communities (Chi et al. 2017, Hepp et al. 2017) and ecological process (Chaffin et al. 2005). However, these studies disregard the effects of the different forms of As available in the environment on biological communities, especially communities directly linked to ecosystem processes.

In lotic environments, aquatic hyphomycetes are important group of organisms for the functioning of these environments. The aquatic hyphomycetes are primary fungi in the conditioning and degradation of organic matter (Graça et al. 2015, 2016). These organisms act in the cycling of nutrients through the release of extracellular enzymes that degrade proteins, polysaccharides, pectin, cellulose and recalcitrant compounds, as well as through carbon mineralization through respiration (Suberkropp 1998, Chung & Suberkropp 2008, Krauss et

al. 2011). In addition, aquatic hyphomycetes improve the performance of detritivores (Jabiol & Chauvet 2012) and may serve as a source of energy for shredders invertebrates (Chung & Suberkropp 2008). However, degradation of aquatic environments by anthropic activities leads to reduced biomass, reproductive activity (i.e., conidia production) and diversity of aquatic hyphomycetes (Lecerf & Chauvet 2008).

In this study we evaluated the effects of As on reproductive rates, richness and composition of aquatic hyphomycetes assemblages associated with leaf litter in a microcosm approach experiment. We hypothesized (i) that As will have an effect on the reproductive rates and richness of hyphomycetes, as well as (ii) change the composition of hyphomycetes assemblages. Our predictions are that (i) the highest concentrations of As in both chemical forms will reduce the richness and reproductive rates of hyphomycetes. In addition, (ii) we expect the composition of the hyphomycetes assemblages to be different between the As and control treatments, showing less variability in the control treatment compared to the As treatments.

## MATERIALS AND METHODS

### Leaf conditioning

We incubated 10 litter bags (16 × 14 cm, 0.5 mm mesh; n = 24) in stream, containing 5 g/bag of senescent leaves of *Nectandra megapotamica* (Spreng) Mez. (Lauraceae) to microbial conditioning of the detritus. Microbial conditioning consists of the transfer and colonization of microorganisms from the aquatic environment to a fresh substrate. The stream is 2nd order (27°36'44"S, 52°14'9"W) with ~0.9 m wide, an average depth of ~0.12 m and a flow rate of ~0.02 m<sup>3</sup> s<sup>-1</sup>. The stream waters are well oxygenated (>9mg L<sup>-1</sup>), with low electrical

conductivity ( $58 \mu\text{S cm}^{-1}$ ) and slightly basic waters (pH  $\sim 7.5$ ). We used *N. megapotamica* leaves as a model of detritus because it is a common species in the streams riparian forests of study area, especially in the stream used to condition the detritus (Tonin et al. 2018). The litter bags were fixed in moderate stream flow, which allow the appropriate deposit of leaves and colonization of the microbial community.

After  $\sim 20$  days of immersion, we removed litter bags from the stream, packaged in iceboxes and transferred the material to the laboratory, where the leaves were gently washed in running water to remove shedding. This conditioning period is considered sufficient for microbial conditioning (especially hyphomycete fungi) (Wright & Covich 2005, Biasi et al. 2017).

### Laboratory experiment

From the conditioned leaves we cut 168 leaf discs ( $12 \text{ mm } \varnothing$ ), with the aid of cork-borer, avoiding primary veins. A set of eight leaf discs was placed in erlenmeyers filled with 35 mL of stream water and different concentrations of As. We organized a control treatment, containing only conditioned leaf discs and stream water. In addition to this treatment, we prepared six other treatments, three of them containing different concentrations of Arsenate ( $\text{As}^{\text{V}}$ ,  $\text{Na}_3\text{AsO}_4$ ) and three others using Arsenite ( $\text{As}^{\text{III}}$ ,  $\text{NaAsO}_2$ ). The concentrations used for the respective ionic forms of As were  $0.005 \mu\text{g g}^{-1}$ ,  $0.01 \mu\text{g g}^{-1}$  and  $0.05 \mu\text{g g}^{-1}$ . For each treatment, we set up three replicates. As concentrations were based on Brazilian Legislation (Law #430/2011 of the National Environmental Council) which defines  $0.01 \mu\text{g g}^{-1}$  of As as the maximum concentration allowed for lotic environments. After the preparation of the seven sets of treatments (control, 3 concentrations of  $\text{As}^{\text{V}}$  and 3  $\text{As}^{\text{III}}$  concentrations) we incubated the erlenmeyers on automatic shaker for 48 hours at a temperature of  $18 \pm 1^\circ\text{C}$

with constant agitation of 90 rpm to stimulate the fungal sporulation process which allows the identification and counting of the reproductive rate of fungi (Graça et al. 2005).

### Fungi identification

After 48 hours of stirring, we filtered the conidial suspension with a membrane filter ( $5 \mu\text{m}$  porosity, Millipore®) and stained with a 0.05% Trypan Blue solution in 60% Lactic Acid. The fungi were counted and identified up to species level using a microscope ( $400\times$  increase) according to the key proposed by Graça et al. (2005) and Fiuza et al. (2017). We set the counts for the number of conidia per mg dry mass of leaf discs, total sample volume, filter volume used and filter area.

### Data analysis

We evaluated the differences between the rates of sporulation and the richness of the aquatic hyphomycetes in the different treatments from a Variance Analysis (one way ANOVA) with Tukey Test a posteriori. To evaluate the composition of the hyphomycetes assemblages, we used non-metric multidimensional scaling (NMDS; Bray-Curtis distance) performed from a matrix composed of hyphomycetes species and their respective abundance. After ordination analysis, we tested the differences in the composition of hyphomycetes assemblages between the different treatments using a Multivariate Analysis of Permutational Variance (PerMANOVA, 9999 permutations). Statistical analyses were performed by the statistical program R (R Core Team 2018).

## RESULTS

We identified 21 species of hyphomycetes, being 18 species in the control treatment, 12 species in the treatments with  $\text{As}^{\text{III}}$  and 10 species in

the treatments with As<sup>V</sup> (Table I). The mean of hyphomycetes richness in the control treatment was  $12.3 \pm 2.6$  (mean  $\pm$  SE). For As<sup>III</sup> the richness decreased 2.6 $\times$  at the concentration  $0.05 \mu\text{g g}^{-1}$  compared to the control treatment (Fig. 1). On the other hand, for As<sup>V</sup> the richness decreased by 2.7 $\times$  in the concentration  $0.05 \mu\text{g g}^{-1}$  compared to the control treatment (Fig. 1).

Hypomycetes richness was negatively affected by As<sup>III</sup> (ANOVA,  $F_{3,18} = 33.7$ ,  $p < 0.001$ ) and As<sup>V</sup> (ANOVA,  $F_{3,18} = 38.3$ ,  $p < 0.001$ ) comparing with

the control treatment (Fig. 1). The hyphomycetes richness in all As<sup>III</sup> concentrations were similar to each other. However, the species richness at the concentration of  $0.005 \mu\text{g g}^{-1}$  of As<sup>V</sup> was different from the other concentrations (i.e. 0.01 and  $0.05 \mu\text{g g}^{-1}$ ) (Fig. 1).

Sporulation rates in As<sup>III</sup> and As<sup>V</sup> treatments were similar to control treatments (ANOVA,  $F_{3,18} = 0.7$ ,  $p = 0.54$  and  $F_{3,18} = 1.3$ ,  $p = 0.32$ , respectively) (Fig. 1). The mean sporulation in the control treatment was  $1.5 \pm 3.3$  conidia  $\text{mg}^{-1}$ , while in As<sup>III</sup>

**Table I. Aquatic hyphomycetes species identified in the control, Arsenite (As<sup>III</sup>), and Arsenate (As<sup>V</sup>) treatments. X = occurrence.**

Species	Control	As <sup>III</sup>	As <sup>V</sup>
<i>Alatospora acuminata</i> Ingold, 1942	X	X	
<i>Anguillospora furtiva</i> J. Webster & Descal, 1999	X	X	
<i>Amniculicolalongissima</i> (Sacc. & P. Syd.) Nadeeshan & K.D. Hyde, 2016 )	X		
<i>Aquanectria submersa</i> (H.J. Huds.) L. Lombard & Crous, 2015	X		
<i>Campylospora chaetocladia</i> Ranzoni, 1953	X	X	X
<i>Campylospora parvula</i> Kuzuha, 1973		X	X
<i>Filosporella</i> sp.	X	X	X
<i>Flagellospora curvula</i> Ingold, 1942	X	X	X
<i>Flagellospora penicillioides</i> Ingold, 1944	X		
<i>Goniopila monticola</i> (Dyko) Marvanová & Descals, 1985		X	X
<i>Heliscus tentaculus</i> Umphlett, 1959	X		
<i>Lunullospora curvula</i> Ingold, 1942	X	X	X
<i>Margaritospora aquatica</i> Ingold, 1942	X		
<i>Mycocentrospora acerina</i> (R. Hartig) Deighton, 1972			X
<i>Mycocentrospora aquatica</i> (S.H. Iqbal) S.H. Iqbal, 1974	X		
<i>Mycocentrospora clavata</i> S.H. Iqbal, 1974	X	X	X
<i>Neonectria lugdunensis</i> (Sacc. & Therry) L. Lombard & Crous, 2014	X	X	
<i>Pleuropedium multiseptatum</i> Marvanová & Descals, 1996	X		
<i>Tetrachaetum elegans</i> Ingold, 1942	X	X	X
<i>Triscelophorus acuminatus</i> Nawawi, 1975	X		
<i>Triscelophorus monosporus</i> Ingold, 1943	X	X	X
Total species richness	18	12	10

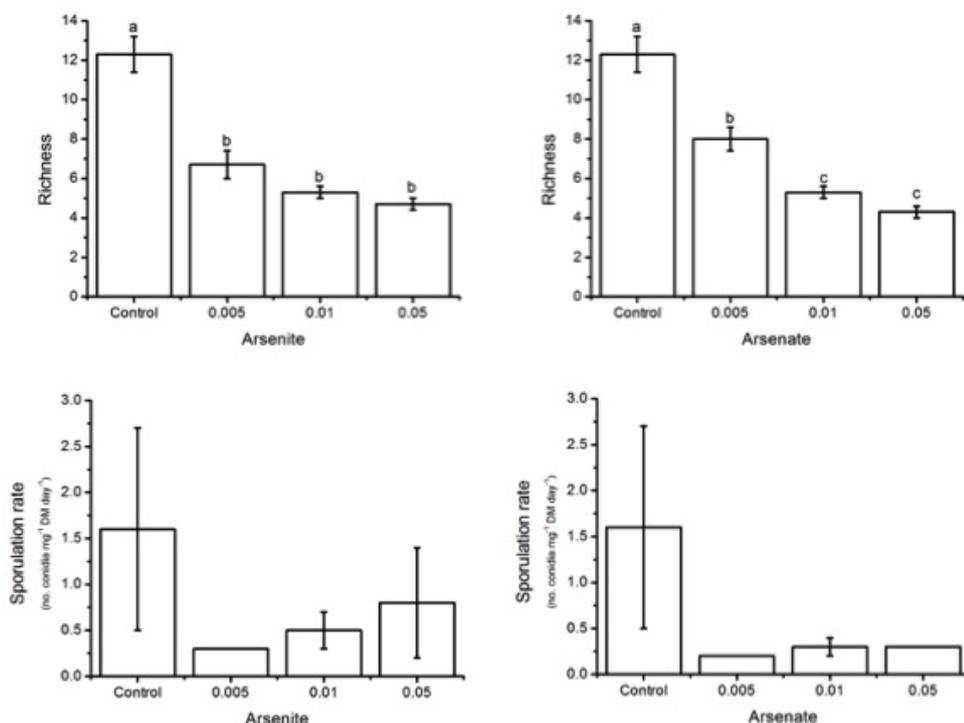
it was  $0.5 \pm 1.0$  conidia  $\text{mg}^{-1}$  and in  $\text{As}^{\text{V}}$  it was  $0.3 \pm 0.2$  conidia  $\text{mg}^{-1}$  (Fig. 1).

The species composition of the hyphomycetes assemblages varied between the control treatment and the treatments with  $\text{As}^{\text{III}}$  and  $\text{As}^{\text{V}}$  (PerANOVA,  $F_{2,18} = 2.1$ ,  $p = 0.008$ ). This pattern is evident from the NMDS ordering, which demonstrates that, in addition to the segregation of the treatment in relation to the others, it is possible to observe less dispersion of the sampling units in relation to the centroid (Fig. 2). The species *Amniculicola longissima*, *Flagellospora penicilioides*, *Aquanectria submersa*, *H. tentaculus*, *Margaritispora aquatica*, *Mycocentrospora aquatica*, *Pleuropedium multiseptatum*, and *Triscelophorus acuminatus* were exclusive to the control treatment. Only *Campylospora parvula* and *Goniopila monticola*, were exclusive to  $\text{As}^{\text{III}}$  and  $\text{As}^{\text{V}}$  treatments, while *Mycocentrospora acerina* was exclusive to  $\text{As}^{\text{V}}$ .

## DISCUSSION

Even though the literature reports several studies on the toxicological effects of As on aquatic organisms, our study is possibly the first record where the effects of this chemical element on aquatic fungi are evaluated. In this study our first hypothesis was partially corroborated, where the richness of hyphomycetes was reduced on the different forms of As, however, we did not find differences for sporulation. The second hypothesis was corroborated, where the increase of arsenic concentrations altered the composition of the assemblies of these organisms.

The richness of hyphomycetes decreased significantly when exposed to  $\text{As}^{\text{III}}$  and  $\text{As}^{\text{V}}$  concentrations, although the magnitude of the effect was different, since  $\text{As}^{\text{III}}$  showed a difference between its treatments. In general, the reduction in the number of species was similar demonstrating a toxicological potential of both As chemical forms for aquatic hyphomycetes,



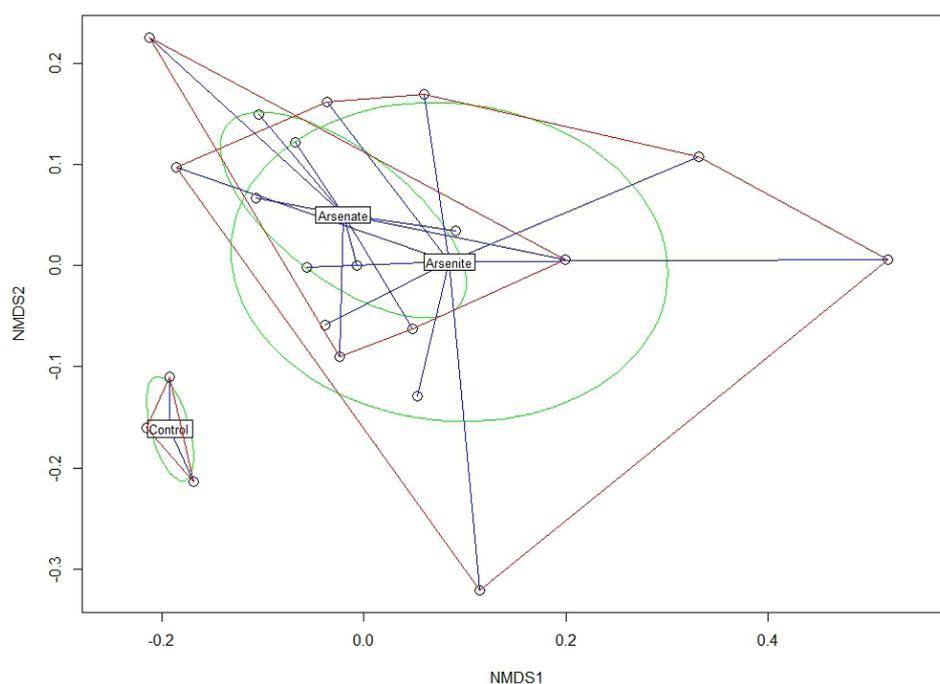
**Figure 1.** Species richness and sporulation rates of aquatic hyphomycetes in control, arsenite ( $\text{As}^{\text{III}}$ ) and arsenate ( $\text{As}^{\text{V}}$ ) treatments. Different letters (a, b, c) indicate significant difference ( $p < 0.05$ ).

although  $\text{As}^{\text{III}}$  was slightly more toxic. In its inorganic form, As exhibits high toxicity, as it competes with inorganic phosphate in oxidative phosphorylation, impacting organisms at biochemical levels (Phillips 1990). Generally,  $\text{As}^{\text{III}}$  is the most toxic because it reacts with sulphhydryl groups of cysteines in proteins causing protein inactivation (Sharma & Sohn 2009). In general, As causes physiological disturbances in exposed organisms, as it reacts with thiol groups of proteins and inhibits important metabolic pathways (Vala 2010). More specifically for the  $\text{As}^{\text{III}}$  form, its toxicity occurs from competition with the inorganic phosphate in the cellular energy matrix, reducing the competitive capacities of organisms (Tisler & Zagorc-Koncan 2002, Sharma & Sohn 2009).

In aquatic environments, loss of species by environmental stressors may alter the functioning of these ecosystems (Taniwaki et al. 2017), so As contamination can directly affect the processes and interactions these organisms exert in aquatic environments. In this study, the total richness in the different

chemical forms of As decreased by about 34% for  $\text{As}^{\text{III}}$  and about 45% for  $\text{As}^{\text{V}}$ . The decrease in aquatic hyphomycetes richness from pollutant contamination is generally not able to maintain ecosystem functioning compared to less polluted environments (Ferreira et al. 2012). The aquatic hyphomycetes play an important role in the leaf decomposition process acting as intermediates between leaf detritus and shredders (Bärlocher et al. 2010). The largest number of aquatic hyphomycetes species have been associated with increased leaf litter decomposition rates, improving litter quality for shredders (Lecerf & Chauvet 2008).

The sporulation rate is considered a fundamental measure for hyphomycete species, metals may affect reproduction, if species do not reproduce they may disappear (Azevedo & Cassio 2010). We observed that sporulation rates were not affected by As concentrations of both chemical forms. However, some dominant species may be responsible for most conidia, preventing significant differences. Maharning & Barlocher (1996) studying growth and



**Figure 2.** Non-metric Multidimensional Scaling ordination of aquatic hyphomycete assemblages composition in the control, arsenite ( $\text{As}^{\text{III}}$ ), and arsenate ( $\text{As}^{\text{V}}$ ) treatments generated by the data matrix of conidia production.

reproduction found that one species accounted for approximately 80% of the total conidia. However, evaluating sporulation rates is relevant to predict the long-term effects of metals on ecosystem functioning, as it assesses the effect on reproductive species that will colonize new substrates (Duarte et al. 2008).

The composition of the hyphomycetes assemblage varied between the control treatment and the other treatments with As. In addition, the variability of the composition of hyphomycetes assemblage species observed in the control treatment was low. This demonstrates that, in natural environments, the species of hyphomycetes remain in adequate survival conditions. More sensitive aquatic hyphomycetes species tend not to occur in locations with adverse conditions (Sole et al. 2008). On the other hand, the assemblages of hyphomycetes observed in As<sup>III</sup> and As<sup>V</sup> treatments presented high variability, corroborating Duarte et al. (2008). In our study, the variability in the composition of the hyphomycete assemblages occurred due to the negative effects of As concentrations on species richness. Approximately 28% of the species identified in this study were found exclusively in the control treatment, while, 9% for inorganic As treatments and 5% only As<sup>V</sup> treatments.

The change in the taxonomic composition of communities (beta diversity) is a structured mechanism of communities studied in aquatic environments (Hepp & Melo 2013). This pattern of biological diversity can be generated by two mechanisms (i) turnover, when species substitution occurs between two localities, or (ii) nestedness, when species loss occurs in one place, when compared to another (Baselga 2010). We observed that there was a turnover of species of hyphomycetes, comparing the As<sup>III</sup> and As<sup>V</sup> treatment, the abrupt decrease in species richness reminds us of a nestedness

mechanism. Thus, we can infer that the presence of pollutants in environment causes changes in aquatic communities' composition, based on the flow of species. However, when element toxicity is high, the effects on species composition occur by decreasing of species.

In this way, the toxicity of As may be selecting species that are resistant to their toxic effects and, consequently, altering the composition of the assemblies. Some species of hyphomycetes may be As-tolerant, as observed for cadmium and copper by Moreirinha et al. (2011). Species that have remained in the treatments with As may present detoxification mechanisms such as the formation of methylarsenic compounds which are less toxic products than the inorganic As (Cullen & Reimer 1989) and these mechanisms tend not to impair the reproduction of the remaining hyphomycete species. From the point of view of structuring communities, we can consider the effects of inter-specific relationships. The variability in the community can be understood by the opportunity generated by the decrease in competitive interactions, which may have allowed the reproductive activity of the chemical element, there may have been an inhibition of competitive species and its reduction facilitated the colonization of new species.

## CONCLUSIONS

In this study, As affected negatively the richness of hyphomycetes species, besides generating modifications in the composition of assemblages. Changes in species composition may be occurring by turnover, i.e. by the substitution of less tolerant species, by species more tolerant to As. This mechanism would explain the absence of As effect on sporulation rates. The toxicity of As regardless of chemical form for aquatic hyphomycetes, even at low

concentrations ( $0.005 \mu\text{g g}^{-1}$ ), demonstrates that the presence of this trace element affects the aquatic microbiota and has potential to alter the functioning of aquatic ecosystems. Although the analysis approach used may underestimate the diversity of hyphomycetes, the quantification of sporulation rates is an important indicator of long-term stream functioning. Thus, the hyphomycetes plays an important role in the trophic ecology of higher groups, such as shredders in streams.

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