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RESEARCH ARTICLE

Aquatic hyphomycetes as survivors and/or first colonizers after a red sludge disaster in the Torna stream, Hungary

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A dam of a red sludge reservoir had ruptured on October 4, 2010 in Hungary and a mixture of 1 million m^3 red sludge and water inundated two villages via the Torna stream. The industrial waste wiped out all detectable forms of life in the Torna stream, mainly due to its high pH value (pH 12.8). To study the fungal colonization leaves, *Fagus sylvatica*, *Quercus cerris*, *Populus nigra*, and *Salix alba* leaf litter were used as baits at two sites of the Torna stream. The leaves were sampled at seven occasions between February and June 2011 and a total of 26 aquatic hyphomycetes were revealed. *Tricladium* sp., *Heliscus lugdunensis*, and *Tetracladium marchalianum* were the dominant species at the impacted site. The total number of fungal species was lower at the impacted than at the reference site. The peak number of species associated with leaf litter was achieved later at the impacted site. Fungal species richness between leaf-species at the two sites was compared by Fisher's exact test. Gypsum, which was dispersed into the stream in order to reduce the alkalinity, formed a layer on the leaves. Its effect for fungal colonization is discussed.

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1 Introduction

Aquatic hyphomycetes or Ingoldian fungi are well-adapted to colonize [1, 2] and degrade plant litter in streams [3] and other freshwater ecosystems, like treeholes [4].

Ingold [5, 6] and other experts [7–10] of aquatic hyphomycetes found that these fungi are common and diverse in clean, unpolluted, well-oxygenated streams. Human activities modify these conditions [11] and only a few papers were dealing with Ingoldian fungi in polluted areas and/or habitats with extreme conditions [12–14].

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Several studies have suggested that stream water chemistry is of major importance in determining fungal species richness and species composition of hyphomycete communities. The most thoroughly studied factor is the pH/alkalinity/Ca^{2+} complex. Most field surveys suggest that species richness of aquatic hyphomycetes declines noticeably below pH 4.5 and above pH 8.0 [15–19]. However, little is known to which extent Ingoldian fungi are able to persist in chronically polluted habitats [12, 14].

It is known that aquatic hyphomycete species occur at some extremely polluted sites and their apparent resistance to extremely high levels of pollutants makes them promising candidates for bioremediation [13, 20].

A dam of a red sludge reservoir had ruptured on October 4th, 2010, in Hungary and approximately 1 million m^3 of industrial waste (produced during bauxite refining) of the Ajkai Timföldgyár alumina plant inundated the villages of Devecser and Kolontár via the Torna stream. The red sludge (mud) is a solid by-product of bauxite processing via the Bayer process which involves reaction with NaOH; consists mainly of Fe_2O_3, Al_2O_3, Na_2O, SiO_2, and TiO_2 in hydrated forms. Minor or trace elements in this material can be K, Cr, V, Ni, Ba, Cu, Mn, Pb, Zn, Zr, Y, Sc, Ga, etc. and even the radioactive U and Th. Currently, about 90 million tonnes of this highly alkaline waste material with pH 10–12.5 are produced globally [21]. The waste wiped out all detectable forms of life in the Torna stream, mainly due to its high pH level (12.8). This raises the question of how aquatic hyphomycete communities had been affected by this type of human interference.

Microscopical examinations of foam samples showed a few species of fungi (mainly aquatic hyphomycetes, e.g., *Tricladium angulatum*) and diatoms 1 month after the disaster. Then the pH level of the stream water lowered to 8.30 ± 0.67 and it fluctuated around the indicated values. The fluctuation (which prevailed during this study) was attributable to artificial neutralization (with cc. HCl) managed by local authorities.

Since February 2011, we have begun to investigate the impact of red sludge and the effect of absence of macro-invertebrates on fungal communities in the Torna stream. The objectives of the present study were to determine: (a) the succession of Ingoldian fungi and their diversity, to compare: (b) aquatic hyphomycete communities on leaf baits at the reference and impacted sites of the Torna stream.

2 Materials and methods

2.1 Study sites

The study was conducted at two sampling sites (reference: N 47°5′33.6″, E 17°32′3.3″; impacted: N 47°6′36.7″, E 17° 26′9.9″) of the Torna stream (Fig. 1). It is a right tributary of the Marcal river, and has its source near a small village of Csehbánya located in the area of Bakony Mountains. The length of Torna stream is 51 km and the catchment area is about 498 km^2. The two study sites were hydro-morphologically similar (average depth: 30–35 cm, velocity: 0.98 $m\ s^{-1}$ at reference site; average depth: 35–40 cm, velocity: 1.05 $m\ s^{-1}$ at impacted site) but one was impacted by the red sludge disaster and the other was not. The latter served as reference site.

2.2 Field procedures

To determine fungal colonization, air-dried leaves of *Salix alba*, *Quercus cerris*, *Fagus sylvatica*, and *Populus nigra* were collected in fall of 2010. They were put into big paper boxes to avoid breaking and transported to the laboratory. The leaves were dried to constant mass at 70°C. On February 17, 2011, they were exposed at each site in coarse litter-bags (10 cm × 10 cm, mesh size: 5 mm × 2 mm) for 125 days. Eighty-four litter-bags (three replicates) received 10 g of each litter type. The water temperature, pH, conductivity, dissolved oxygen, oxygen saturation were measured (HQ40d multimeter) at the time of each sampling occasion.

2.3 Laboratory procedures

Four randomly collected bags (one of each type of litter) were carried to the laboratory on the following dates: February 24, March 30, April 13, May 11, May 25, June 8, and June 22. One-one leaf of each leaf-species was taken from the bags randomly and washed in tap water and

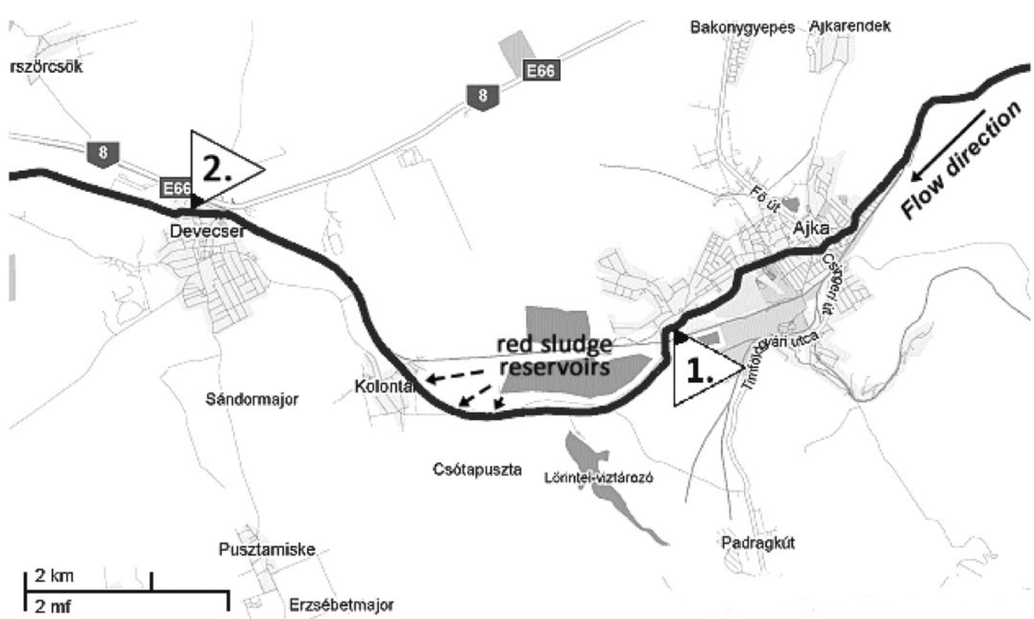

Figure 1. Map showing the two sampling sites. Site 1: the reference site. Site 2: the impacted site of the stream.

incubated in aerated Milli-Q water (150 mL) for 3–4 days in separate beakers at room temperature. The suspensions were passed through membrane filters (8 μm pore size, 25 mm diameter, Whatman PC) using a pneumatic pump (Antlia Pressure Filtration System, Schleicher and Schuell, Dassel, Germany). The conidia trapped on the filters were stained with cotton blue in lactophenol. The entire surface of each filter was thoroughly scanned at 400× magnification (Nikon Eclipse E400) and the conidia were identified and counted.

2.4 Data analyses

Species diversity was calculated using Shannon's index:

$$H = -\sum_{i=1}^{S}(p_i)\log_2 p_i$$

where H is the index of species diversity, S is the total number of species, and p_i is the proportion of the ith species [22].

Fisher's exact test was used with STATISTICA 6.0 to compare fungal species richness between leaf (*S. alba*, *Q. cerris*, *F. sylvatica*, *P. nigra*) species at the two sites (impacted and reference site) on February 24 to June 22. Fisher's exact test is a statistical test used in the analysis of contingency tables where sample sizes (at our case the cumulative no. of species) are small [23].

3 Results

Physical and chemical parameters of the two stream sites are summarized in Table 1 and Fig. 2. Conductivity was slightly higher at the impacted site. Dissolved oxygen was 7.54–14.5 and 8.69–11.61 mg L^{-1} at the reference and the impacted site, respectively. Table 2 lists the aquatic hyphomycete species and their diversities associated with leaves collected on seven dates. A total of 26 taxa of aquatic hyphomycetes were detected: 17 were identified to the species level, 4 were assigned to the genus level, and 5 remained unidentified. Values of fungal species diversity were higher at the reference site. Fungal species diversity

Table 1. Conductivity, dissolved oxygen, and oxygen saturation of the two sites

Site	Reference	Impacted
Conductivity (μS cm^{-1})	736–1018	830–1264
O_2 (mg L^{-1})	7.54–14.5	8.69–11.61
O_2 sat. (%)	81.7–129	91.9–108.6

Numbers describe range of values between February 17 and June 22, 2011.

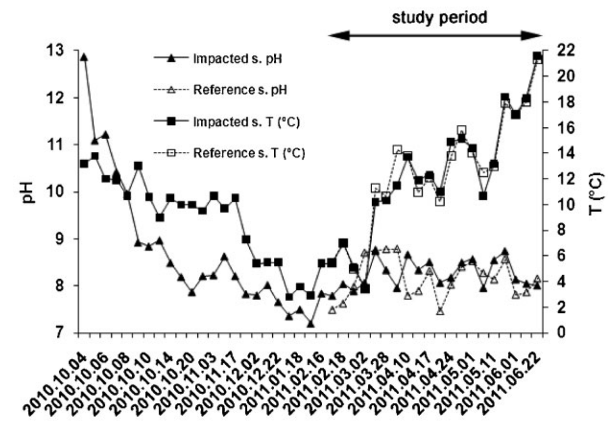

Figure 2. Stream water temperature (°C) and pH of the Torna stream between October 4, 2010 and June 22, 2011.

peaked earlier at the reference site (April 13) than at the impacted site (May 25). At the beginning of fungal colonization, there were differences in succession and cumulative number of species between the two sampling sites, but in the case of diversity and species evenness differences have only occurred after 4–6 wk of intensive decomposition. Thenceforth the diversity and evenness of fungal species decreased substantially (at the reference site) while at the impacted site these parameters prevailed for longer time (till June).

Anguillospora mediocris, *Cylindrocarpon* sp., *Tetracladium marchalianum*, *Tricladium* sp., and an unidentified sigmoid form were the most common species during the whole study.

The total number of Ingoldian fungi was lower at the impacted than at the reference site (Fig. 3). The increase in species number was slower and the peak number of species was achieved later at the impacted site. Figure 4 displays changes in the number of fungal species on each leaf-species. At first sight, there were no notable differences between the two sites, except for *Salix alba*. Using Fisher's exact test, we found significant differences between fungal species richness of the same leaf-species at the two sites. Species richness on *Salix alba* was significantly higher at the reference than at the impacted site on February 24 ($p = 0.0049$), but the opposite was found on May 25 ($p = 0.0497$). Furthermore, on *Fagus sylvatica* a similar difference (lower fungal species richness at the reference site) was found on June 8 ($p = 0.0248$). In the other cases, there were no significant differences between sites.

The succession of Ingoldian fungi is shown in Fig. 5. *Heliscus lugdunensis* and *Tricladium angulatum* occurred frequently and had larger conidia at the impacted site. Typically, *Tricladium* species were present at higher numbers at the impacted site. The early colonist *Tetracladium marchalianum* showed higher relative

Table 2. Aquatic hyphomycete species detected and their diversities (average of fungal communities' diversity on each leaves) at two sites of Torna stream

Species	Reference site							Impacted site						
	February 24	March 30	April 13	May 11	May 25	June 08	June 22	February 24	March 30	April 13	May 11	May 25	June 08	June 22
Alatospora acuminata Ingold		FQ	FQPS	Q	Q	Q			FQPS	FQPS	FQP	P	F	
Anguillospora longissima (Sacc. and Syd) Ingold	P		F	FQP		Q	Q		F				F	
Anguillospora mediocris Gönczöl and Marvanová		FP	FQPS	FQPS	FQP	FQP	FQP	Q	FQPS	FQPS	FP	Q	F	FQ
Anguillospora sp.	PS		FQS	FPS	FPS	QPS	FPS			FQS	FQPS	Q	Q	P
Clavariopsis aquatica De Wild	F	FQP	FQPS	P	P	P	P			FPS	FP	P		
Cylindrocarpon sp.	FQ	FQPS	QPS	FPS	Q	QPS	PS	Q	Q	Q	FQ	FS	QPS	FQP
Dactyella submersa (Ingold) Nilsson		P	FQP							F			FQ	P
Flabellospora sp.		F							S					
Heliscus lugdunensis Sacc. and Therry	F	QP	QP					F	Q	FQ	FS		F	
Lemmoniera aquatica De Wild.		FQP	Q						FQP	QP	FS	S		
Retiarius bovicornutus D.L. Olivier		Q	Q	Q					FS	QP				
Tetrachaetum elegans Ingold		F												
Tetradadium marchalianum De Wild	FQPS	FQPS	FQPS		Q			FP	FQPS	FQPS	FS	FPS	FP	FP
Tetracladium setigerum (Grove) Ingold										P				
Tricladium angulatum Ingold	S	FQP	FQPS	Q	Q	Q			FQPS	FQP	FQS	FPS		
Tricladium caudatum Kuzuha													F	
Tricladium gracile Ingold		FP	FQ									FP	Q	
Tricladium sp.	S	FQP	Q							F	FS	FPS		
Trinacrium subtile Fresen.				Q								P		
Tripospernum camelopardus Ingold, Dann and McDougall			Q											
Varicosporium elodeae Kegel			S											
Sigmoid 1	QS	QPS	FQS			QPS	QS	FP	Q	Q		QS	QS	FQ
Sigmoid2	F			F					Q					
Unknown 1	S											F		
Unknown 2	S	S									Q			
Unknown 3			Q	Q										
Diversity (H)	0.979	2.066	2.490	2.105	1.041	1.447	1.327	0.886	2.060	1.731	1.929	1.959	1.448	0.865
Evenness (E)	0.208	0.440	0.530	0.448	0.221	0.308	0.282	0.188	0.438	0.368	0.410	0.417	0.308	0.184

F, *Fagus sylvatica*; Q, *Quercus cerris*; P, *Populus nigra*; S: *Salix alba.*

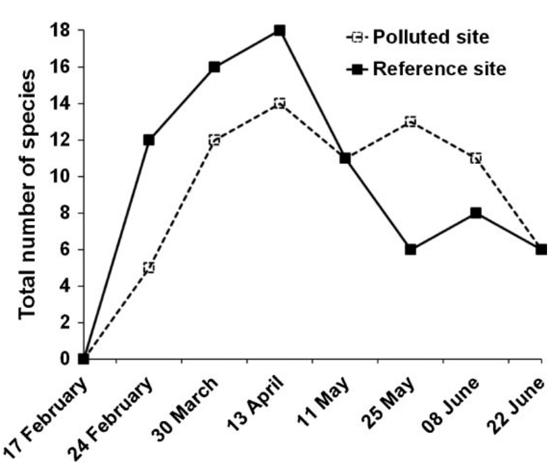

Figure 3. Variation of the total number of aquatic hyphomycete species associated with leaf litter during the study period.

proportion on *Salix* and *Populus* leaves than in the reference site. The maximal differences in species composition and succession of fungi were found on oak leaves.

4 Discussion

The number of conidia in the stream water and the structure of fungal communities are affected by a number of factors, among which water chemistry and the quantity and quality of substratum were suggested to be the most relevant [15–17, 24, 25]. Environmental pollution commonly results in a decrease of fungal diversity [20]. Those human activities that change the quantity of available substrata for colonization of fungi in streams may also decrease the number of fungal species.

A few days after the disaster, large amounts of gypsum were dispersed into the impacted site of the Torna stream by the local authorities in order to reduce the alkalinity. It was not a successful management strategy to regain the natural hydro-chemical balance of stream water, but had substantial side effects. Gypsum layers were formed on

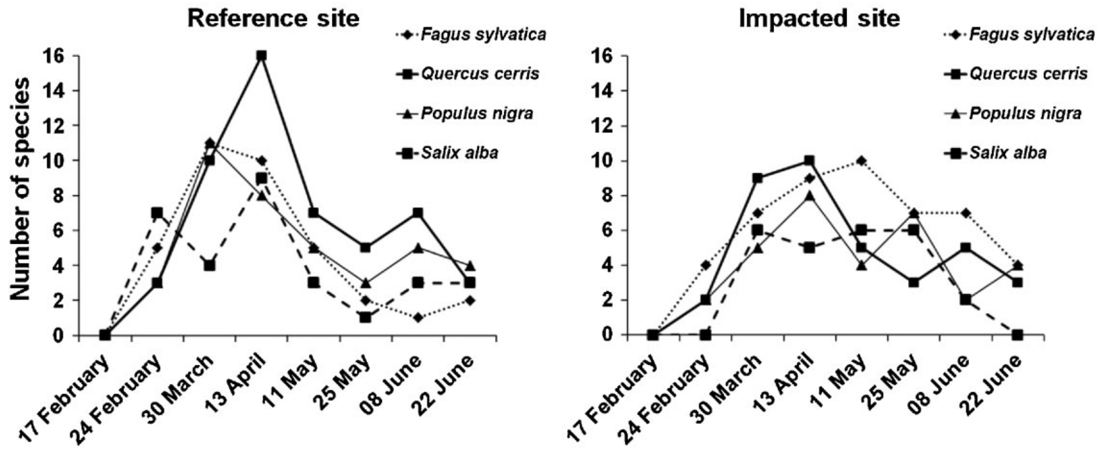

Figure 4. Number of aquatic hyphomycete species associated with the four leaf-species at the reference and the impacted site during the study period.

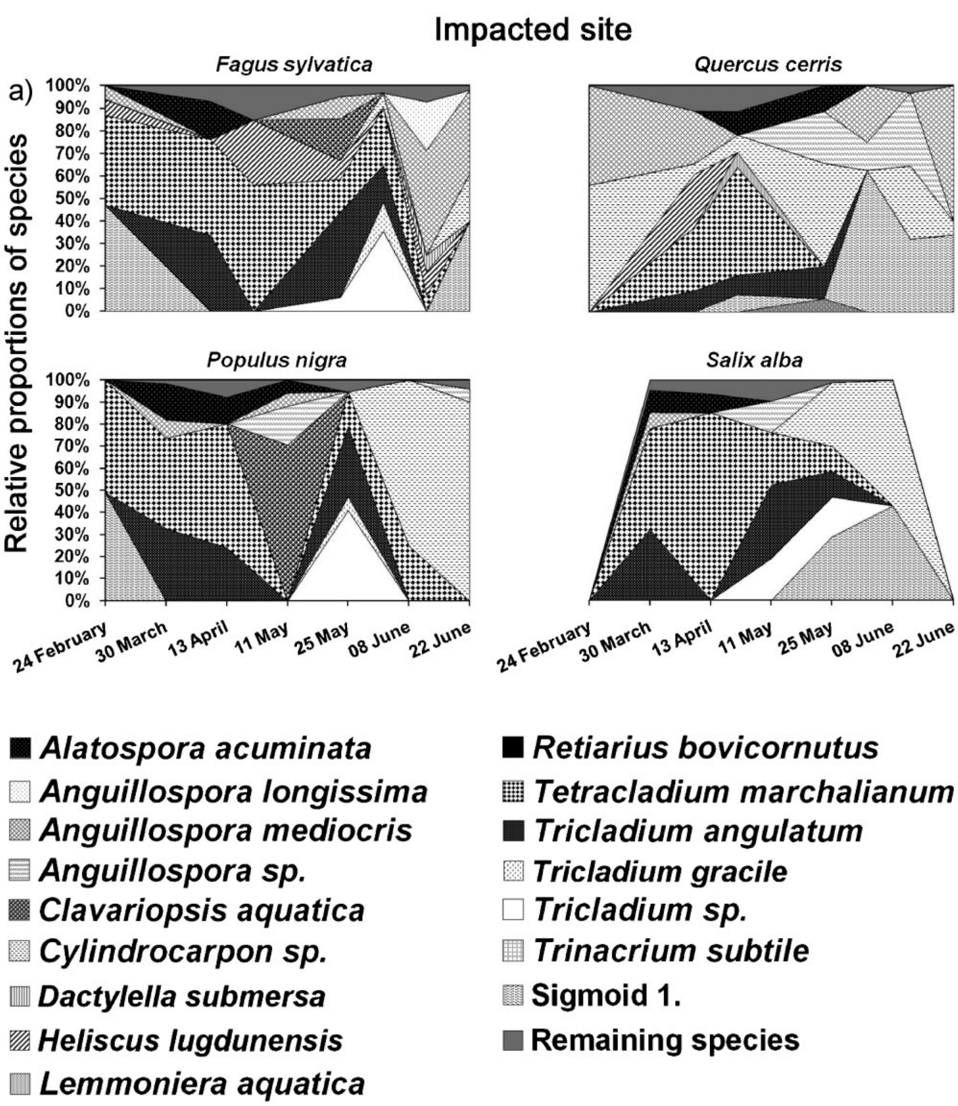

Figure 5.

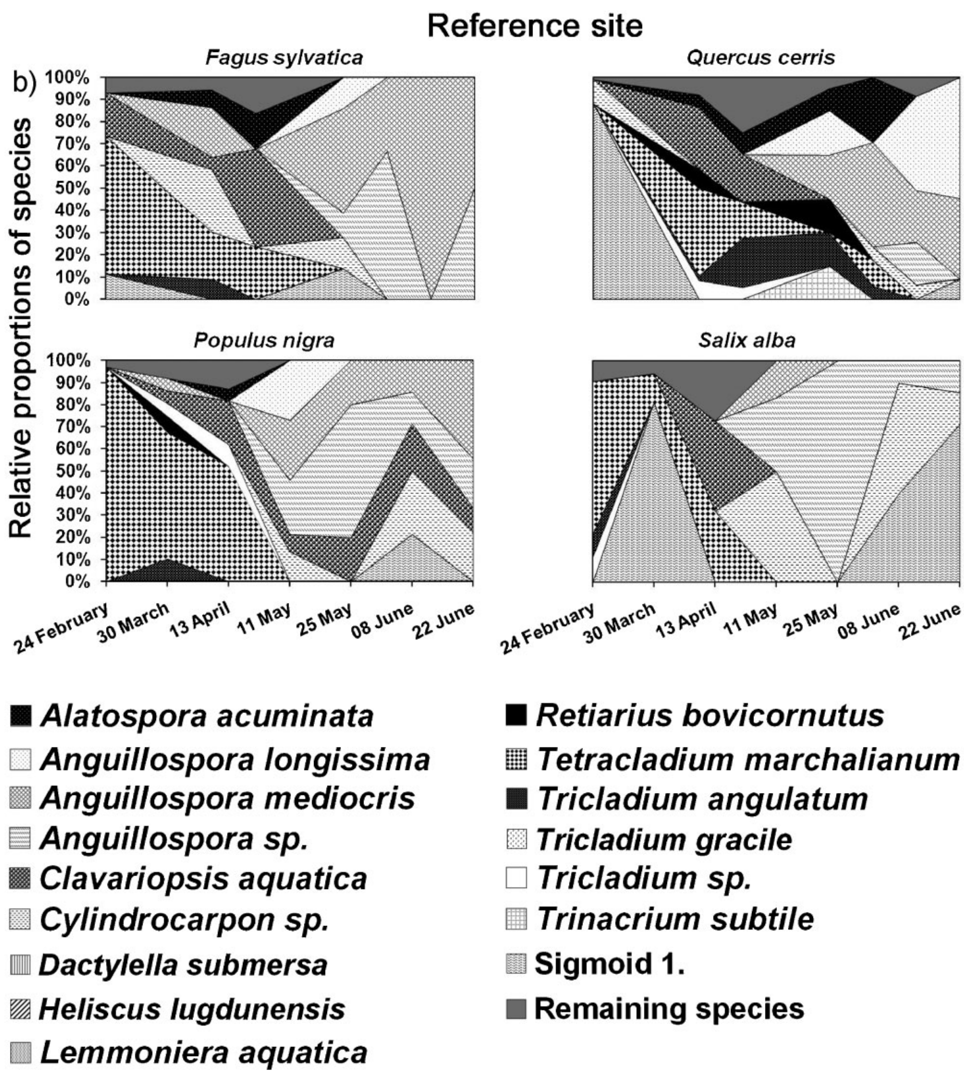

Figure 5. Temporal changes in the relative proportions of aquatic hyphomycete species (>5% of total) associated with different leaf-species at the two stream sites (a) Impacted site, (b) Reference site.

the leaves, which resulted in lower specific surface of leaves for saprophytic fungi as aquatic hyphomycetes. Large amounts of gypsum sedimented in the streambed by the progress of time at the impacted site. Later, the specific surface of leaves was getting higher, but there were no sufficient microhabitats for macroinvertebrates to colonize the impacted area of the watercourse [26]. The gypsum layer may be an explanation for why the exponential growth of these fungal species was slower and why the peak number of species prevailed for longer time at the contaminated section (Figs. 3 and 4). At the reference site, there were no such barriers to inhibit the rapid colonization and growth of fungal biomass on decaying leaves. However, saturation of the maximum number of species (Fig. 3), Shannon-diversity and evenness of fungal species were limited by the presence of macroinvertebrates, as well as a faster degradation rate [26].

The higher frequencies of *Heliscus lugdunensis*, *Tricladium angulatum*, *Tetracladium marchalianum* (on *Salix* and *Populus* leaves) and the other *Tricladium* species at the impacted site (Fig. 5) could be explained in two ways. First by the absence of their fungal competitors at the impacted site. The other explanation is that these species might represent a group of fungi, which tolerate a broader range of ecological conditions and are affected less by fluctuating pH. The occurrence of two species (*H. lugdunensis* and *T. marchalianum*) was not surprising, because some papers mentioned their occurrence in polluted sites or in streams contaminated by heavy metals [12, 13, 27] and they appear to be resistant to Cd [28]. Typically, *T. marchalianum* is more common in alkaline streams [29]. Sridhar et al. [30] found that *T. marchalianum* contributed with 50.8% to all spores produced and *H. lugdunensis* with 28.0% in a heavily polluted site (the water column contained close to 2 g of dissolved Zn and several milligrams of other heavy metals per L, emerging as a spring from the mining and smelting waste dump). In another site (a channelized outlet from a lake where metal concentrations in the water column generally fall below 1 mg L^{-1}) of the same study, *T. marchalianum* contributed with 90.2% to all conidia released, followed by *Anguillospora* sp. (2.8%), *T. angulatum* (2.1%), and *H. lugdunensis* dropped to 7th place (0.3%). Their experiments were conducted in two small streams in the district of Mansfeld (Sachsen-Anhalt, central Germany). These species are apparent candidates when searching for specialized physiological adaptations to severe pollution [12]. In our study, *Cylindrocarpon* sp. and *Anguillospora* sp. were common species in all location as found by Krauss et al. [13, 31] in a hyperpolluted watercourse. In all the aforementioned studies there was a history of water pollution for a long period of time (at least 100 years).

Effects of alkaline conditions on fungal communities have been described only at pH 8.4–8.7, but strongly alkaline condition, as caused by red sludge, have not been described yet. The parameters of the dissolved oxygen and oxygen saturation (Table 1) did not inhibit the life functions of organisms. The increased concentration of ions (Na^{+}, K^{+}) from liquor of low solids containment red sludge resulted high conductivity (at the first day of disaster: 18.860 μS cm^{-1}, during the study: 830–1.264 μS cm^{-1}) and turbidity at the impacted site. After the strong alkaline load there were only some significant changes in frequencies of aquatic hyphomycete species (see above the results of Fischer's exact test). However, these significant differences may have reflected purely the physical effect by human activities and not by red sludge itself. In fact, the particles of gypsum ($\sim$50 μm) were bigger than of the red-sludge (1–4 μm) in fluid medium. Consequently, the particulates of gypsum formed a stable layer on the litter and also in the streambed while the small particles of red sludge moved downstream fast, and did not form a persistent layer in the streambed [26]. The accumulation of coagulated gypsum may have interfered with fungal activity on the leaves by inhibiting oxygen and nutrient uptake from the water column, or, the mycelium in the leaves may have been unable to produce conidiophores penetrating these layers [12]. Sridhar et al. [12] described that there is no obvious relationship between heavy metal concentration and the number of fungal species. Therefore, we conclude that the most severe effect in this case was the high level of gypsum on substrates. It resulted in lower fungal species richness and slower fungal growth on leaves at the impacted site of the Torna stream.

Apart from bacteria, aquatic hyphomycetes appeared to be the first group, which established successfully in the Torna stream after the red sludge disaster. However, the main question remains to be answered: are Ingoldian fungi the survivors or first colonizers in Torna stream after the red sludge catastrophe? Laboratory experiments (viability test on fungal cells affected by high pH (12.8)) would be helpful for clarification. However, results obtained here and also in another study [32] support that fungi show high resistance to environmental disasters, which are as destructive as red sludge catastrophe, or have high colonization capacity.

In conclusion, our study demonstrated that human activities may change characteristics of microhabitats in surface waters dramatically, but diverse fungal communities might survive and/or re-colonize fast and thus find a way to continue their life-cycles as main member of microbial decomposers. Investigation to what extent the changes in diversity of aquatic hyphomycete community will affect litter breakdown processes and the re-colonizer invertebrates are our future goals.

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The authors have declared no conflict of interest.

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