ORIGINAL ARTICLE

Elevated Aluminium Concentration in Acidified Headwater Streams Lowers Aquatic Hyphomycete Diversity and Impairs Leaf-Litter Breakdown

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Abstract Aquatic hyphomycetes play an essential role in the decomposition of allochthonous organic matter which is a fundamental process driving the functioning of forested headwater streams. We studied the effect of anthropogenic acidification on aquatic hyphomycetes associated with decaying leaves of *Fagus sylvatica* in six forested headwater streams (pH range, 4.3–7.1). Non-metric multidimensional scaling revealed marked differences in aquatic hyphomycete assemblages between acidified and reference streams. We found strong relationships between aquatic hyphomycete richness and mean Al concentration ($r=-0.998$, $p<0.0001$) and mean pH ($r=0.962$, $p<0.002$), meaning that fungal diversity was severely depleted in acidified streams. By contrast, mean fungal biomass was not related to acidity. Leaf breakdown rate was drastically reduced under acidic conditions raising the issue of whether the functioning of headwater ecosystems could be impaired by a loss of aquatic hyphomycete species.

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Introduction

Running-water ecosystems that are subjected to anthropogenic acidification are typically first- to third-order streams, i.e. headwater streams often draining forested watersheds. Due to several ecological factors, such as light limitation by the riparian vegetation, the primary production in these streams is very low. Conversely, forested headwater streams receive by far most of their energy from the riparian vegetation. Therefore, the metabolism of headwater streams is generally heterotrophic [16, 67]. Leaf litter, which represents an important fraction of the allochthonous input entering the ecosystems, is used by decomposers and detritivores. Micro-organisms and leaf-shredding macro-invertebrates play an essential role in allochthonous organic matter processing and its incorporation into the trophic webs [31, 68]. Aquatic fungi, particularly aquatic hypho-mycetes which form a phylogenetically diverse group of anamorphous species (mainly ascomycetes and few basi-diomycetes), contribute the most to the microbial biomass associated with leaves in streams [26, 38, 69]. These filamentous fungi commonly occur in freshwaters and produce tetraradiate, variously branched or sigmoid asexual spores (i.e. conidia) adapted to dispersal in flowing water and adhesion to decaying plant tissues [7]. A rapid conidial germination and mycelial invasion of the leaf matrix, typically within a few weeks, allow aquatic hyphomycetes to quickly colonize their ephemeral resource [32]. In addition, the mycelial development coincides with or is closely followed by a very high release of conidia which constitutes a major part of the fungal production [32]. Aquatic hyphomycetes are responsible for the initial breakdown of leaves and for enhancing the palatability of leaf detritus, thus providing a more suitable food source for shredders which further contribute to the conversion of leaf

litter into fine particles and other decomposition products [3, 4, 8, 10, 15, 31, 34, 61]. Consequently, due to their function, aquatic hyphomycetes occupy an essential place in the functional role that leaf breakdown plays in forested headwater streams.

Several studies have clearly shown that chemical alteration of aquatic ecosystems can have deleterious effects on fungal communities. A decline in aquatic hyphomycete diversity has been reported in streams contaminated with either heavy metals [9, 22] or organic compounds [5, 59]. Similarly, leaf breakdown rates are often affected in rivers polluted with either mineral or organic pollutants (for review, see [29]). In particular, several studies have clearly demonstrated that leaf-litter breakdown is severely reduced in acidified ecosystems [19, 53].

During the last three decades, a large number of studies have focused on the effects of acidification on aquatic biota (fish, macroinvertebrates, zooplankton, macrophytes, algae). Most of them have revealed deleterious effects leading to the loss of biodiversity. In particular, increased concentrations of aqueous aluminium arising from freshwater acidification are considered a major environmental problem due to its high toxicity to aquatic organisms. Aqueous aluminium has been recognized as a main toxicant for aquatic animals [27], and several studies have also suggested a direct effect on microbial metabolism [12, 55, 57]. Surprisingly and despite the key role they play in headwater stream functioning, little is known about the impact of anthropogenically acidified running waters on the diversity of microorganisms in general and on aquatic hyphomycetes in particular. Results from the few studies dealing with acidic water and the diversity of aquatic hyphomycetes are contradictory. In some studies, aquatic hyphomycetes have been shown to benefit from lower pH [6, 70], whereas in other studies, the richness was lower [44, 60] or not related to pH [10]. Obviously, additional data are needed to more fully understand the effects of low-pH waters on fungi colonizing leaves in streams [62].

To answer the question of whether acidified soft waters with high aluminium concentrations affect the diversity of aquatic hyphomycete assemblages associated with decaying leaves, a litter-breakdown experiment was conducted in six streams characterized by different acidification levels. After immersion in the streams, the leaf mass remaining was determined, and the sporulation of the leaf detritus was induced in the laboratory, allowing the identification of fungal species through released conidia. The relationship between diversity and breakdown rate was investigated to link the potential effect of species loss on leaf-litter processing in headwater streams.

Materials and Methods

Study Sites

The study was conducted in the Vosges Mountains (Northeastern France) where anthropogenic acidification has adversely affected surface waters (Fig. 1) [17, 58]. Based on previous studies [19, 20, 25, 66], six headwater streams showing different levels of acidity (Table 1) but similar hy-

Figure 1 Map showing the location of the six headwater streams studied in the Vosges Mountains
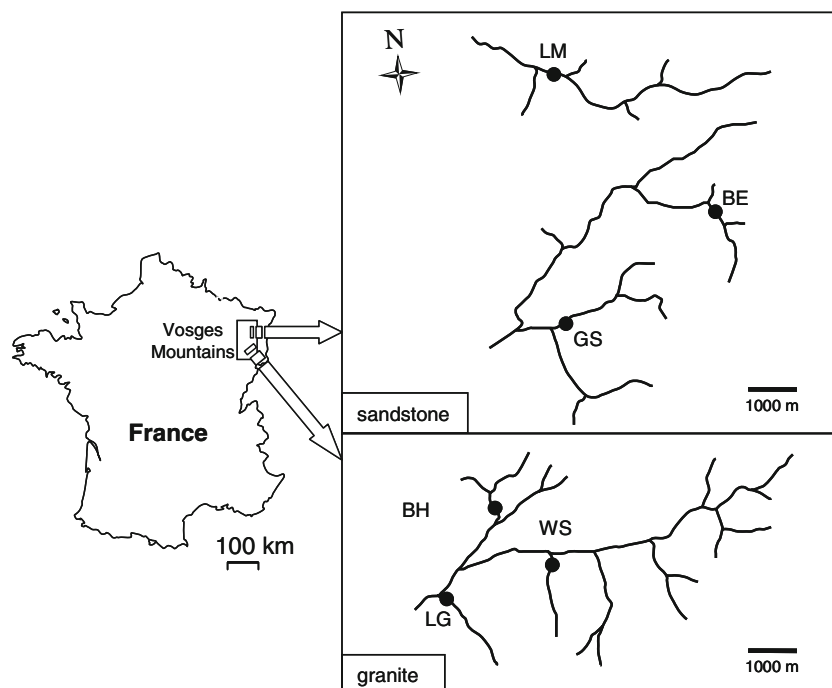
Table 1 Mean values (min–max) of the physico-chemical variables for the six streams over the study period ($n=9$)

Stream	Acronym	Latitude N	Longitude E	pH	Conductivity (μS cm^{-1})	ANC (μeq l^{-1})	Cl$^-$	NO$_3^-$	SO$_4^{2-}$	Ca^{2+} (mg l^{-1})	Mg^{2+}	Na$^+$	K$^+$	Al$_{tot}$ (μg l^{-1})
La Maix	LM	48°29′24″	7°03′17″	7.1 (6.4–7.7)	81 (73–100)	379 (260–590)	1.36 (1.27–1.48)	4.04 (3.51–4.35)	6.53 (5.55–7.33)	6.98 (6.15–8.24)	3.73 (3.13–4.41)	1.04 (0.98–1.17)	1.60 (1.50–1.67)	55 (10–148)
Bihet	BH	47°57′56″	6°52′32″	6.6 (5.8–7.6)	51 (29–75)	89 (32–194)	5.92 (1.37–12.5)	2.50 (1.11–3.44)	2.78 (2.52–3.15)	2.74 (1.73–3.52)	1.37 (0.81–2.25)	3.73 (1.79–6.64)	0.43 (0.29–0.68)	49 (10–150)
Longfoigneux	LG	47°57′56″	6°51′57″	5.1 (4.2–5.8)	15 (13–18)	−6 (−14–3)	0.78 (0.73–0.84)	0.89 (0.26–1.46)	2.76 (2.58–3.05)	0.56 (0.38–1.00)	0.22 (0.19–0.29)	1.16 (0.98–1.41)	0.11 (0.08–0.15)	221 (124–376)
Wassongoutte	WA	47°58′23″	6°53′13″	4.9 (4.0–5.4)	15 (13–16)	−7 (−13–0)	0.78 (0.66–0.90)	0.99 (0.35–1.42)	2.69 (2.60–2.82)	0.52 (0.41–0.70)	0.29 (0.19–0.94)	1.32 (1.07–1.84)	0.19 (0.10–0.50)	212 (92–407)
Gentil Sapin	GS	48°26′58″	7°03′45″	4.6 (3.9–4.9)	35 (31–39)	−18 (−48–8)	1.09 (0.99–1.19)	5.28 (4.79–5.83)	4.81 (4.23–5.06)	1.35 (0.90–1.60)	0.61 (0.43–0.68)	0.77 (0.65–0.85)	1.08 (0.90–1.14)	379 (102–540)
Basse Escaliers	BE	48°27′59″	7°05′45″	4.3 (3.8–4.5)	33 (28–39)	−33 (−58–21)	0.77 (0.69–0.92)	3.15 (2.30–3.63)	5.15 (4.33–5.51)	0.68 (0.61–0.75)	0.26 (0.22–0.27)	0.61 (0.52–0.73)	0.76 (0.67–0.91)	652 (316–960)

drological and morphological characteristics were selected. Soils of all catchments range from acid brown to podzolic and are underlain by granite or sandstone bedrock. Mixed forests dominated by silver fir (*Abies alba*), Norway spruce (*Picea abies*) and common beech (*Fagus sylvatica*) form the riparian zones. All sampling sites were located upstream of any direct anthropogenic pressures (housing, farming or industries).

Physical and Chemical Analyses

At each sampling date, water samples were collected from each stream into polyethylene bottles and kept cold (4°C) until laboratory and analysed within 48 h. Stream pH was measured in the laboratory using a microprocessor pH meter (pH 3000, WTW), and acid-neutralizing capacity (ANC) was determined by Gran's titration. Conductivity was measured with a Metrohm Herisau Conductometer E518 (Herisau, Switzerland) at 25°C. Concentrations of Ca^{2+}, Mg^{2+}, Na$^+$, K$^+$ and total aluminium (after acidification with HNO$_3$) were determined by atomic absorption spectrophotometry (Aanalyst 100; Perkin Elmer and Varian SpectrAA-300) and concentrations of Cl$^-$, SO$_4^{2-}$ and NO$_3^-$ by ion chromatography (Dionex 1500i with a AS 4 A SC column; Sunnyvale, USA).

Leaf-Litter Breakdown

We used beech (*F. sylvatica*) leaves because this species is by far the most common deciduous riparian tree species and provides almost all leaf-litter input. Beech leaves were collected from trees just before abscission in fall 2002. Leaf bags were created by placing 5 g (±0.05) of air-dried leaves in 10 mm plastic mesh bags (15×20 cm). Each exact mass of leaf material was recorded and each bag identified with a plastic label. Weighed leaves were moistened with distilled water to avoid breakage during placement into mesh bags. Leaf bags were closed with nylon line so that they took the shape of a tetrahedron thus ensuring a good circulation flow and a natural arrangement of leaves. About 24 bags were submerged at each study site in zones expected to naturally accumulate leaves. They were individually secured to the bank with plastic-coated wire, which was anchored to the stream bottom with large boulders. Four replicate bags were randomly retrieved from the six streams after 23, 35, 46, 63, 83 and 124 days of exposure, immediately placed in individual sealed plastic bags with stream water and transported to the laboratory in a cool box. For each sampling date, 7 1 of stream water was collected for laboratory processing of the leaf bags.

The leaves were rinsed individually with water from the respective stream to remove attached fine particles and invertebrates. Two leaf disks were cut avoiding the central

vein with a 12 mm diameter cork borer from five randomly selected leaves in each bag. Five of them were stored at −80°C in a zip-lock plastic bag until ergosterol determination, and the five others were immediately processed to allow sporulation. The remaining leaf material was oven-dried to constant mass (105°C, 48 h) and weighed to the nearest 0.1 mg, and subsamples (500 mg) were ignited in a muffle furnace (550°C, 4 h) to relate dry mass to ash-free dry mass (AFDM). Ten additional leaf bags were kept in the laboratory before the beginning of the experiment. Four replicates were used to determine leaching loss by submerging them in a bowl under a slow flow of dechlorinated water for 24 h and six others to estimate the initial oven-dried mass and AFDM of all leaf bags.

Fungal Biomass

Ergosterol was extracted from leaf disks and determined as previously described [33]. Briefly, the leaf disks were lyophilised, weighed to the nearest 0.1 mg and heated in 5 ml hot alkaline ethanol (KOH, 8 g l^{-1}) for 30 min at 80°C. The extract was purified by solid-phase extraction on cartridges (Waters Oasis HLB, 60 mg, 3 cc). Ergosterol was separated by reversed phase high performance liquid chromatography on C$_{18}$ and quantified by measuring absorbance at 282 nm. Ergosterol was converted to fungal biomass using a conversion factor of 5.5 mg ergosterol g^{-1} mycelial dry mass [30].

Fungal Diversity

The sporulation rate and species composition of the leaf-associated fungal assemblages were determined in the laboratory [28]. Five leaf discs from each replicate bag were placed in 100-ml Erlenmeyer flasks with 25 ml filtered water (Whatman glass fiber filter GF/F) from the stream in which the leaves were retrieved. Then, the flasks were placed on an orbital shaker (100 rpm) for 48 h at 10°C. After incubation, the suspension was poured into 50-ml centrifuge tube without the discs, which were rinsed in the flask with distilled water (3×2 ml) to dislodge remaining attached conidia. Rinse water was combined in the tube, and the volume was adjusted to 35 ml with distilled water and 2 ml of 37% formalin. Conidial suspensions were stored in the dark until analysis. The five leaf discs from each leaf pack were oven-dried (105°C, 48 h) and weighed to the nearest 0.1 mg, and AFDM was determined (550°C, 4 h).

The conidial suspension was transferred to a beaker with 1 ml Triton X-100 solution (0.5%) and shaken on a magnetic stirrer for 10 min, and an aliquot (4–8 ml) was filtered (membrane filter, 5-mm pore size, 25 mm diameter), stained with 0.1% Trypan blue in 60% lactic acid and examined microscopically. Conidia were identified by relying on specific literature [e.g. 39] and counted by scanning the entire filter. For each species, the sporulation rate (conidia mg^{-1} AFDM day^{-1}) was determined.

Data Analysis

Principal components analysis (PCA) was carried out to ordinate streams with respect to physical and chemical variables. PCA was performed using the following variables: pH, ANC, conductivity, total Al, [NO$_3$+SO$_4$] and [Ca+Mg].

Non-metric multidimensional scaling (NMDS) analysis of sporulation data was used to assess differences among sites in aquatic hyphomycete assemblages. This ordination method is a robust procedure for analysing ecological data [52]. We used the Bray-Curtis coefficient to quantify the dissimilarity among sites based on joint occurrence and abundance of taxa [14]. NMDS attempts to maximise the fit between measured dissimilarities and distance between resulting data points within a predefined number of spatial dimensions [46, 47]. The goodness of fit was estimated with a stress function, which ranges from 0 to 1, with values close to zero indicating a good fit. Axes from the NMDS analysis were correlated (Spearman rank correlation) with physical and chemical data to identify variables most strongly corresponding to among-site differences in aquatic hypho-mycete assemblages [41].

Leaf breakdown rates (k) were estimated by fitting the AFDM data with the linear model $m_t = m_0 − kt$ where m_t is the AFDM remaining at time t, and m_0 is the initial AFDM at the beginning of the experiment. The linear model was used because it fitted the data better than the exponential model proposed by Petersen and Cummins [56]. Daily decay rates were compared using analysis of covariance (ANCOVA) followed by a multiple-comparison (Tukey's test, [72]).

A one-way analysis of variance (ANOVA) was used to test for differences in fungal biomass, and Pearson correlations were performed to investigate possible relationships between diversity and physical and chemical parameters.

Statistical analyses were performed using ADE4 (PCA, [63]), SPSS for windows (NMDS) and Statistica (ANOVA, ANCOVA, Spearman rank and Pearson correlations).

Results

Water Chemistry

The six headwater streams ranged from near neutral [mean pH=7.1 and 6.6 in La Maix (LM) and Bihet (BH), respectively] to strongly acidified (mean pH=4.6 and 4.3 in Gentil Sapin (GS) and Basse Escaliers (BE), respectively).

The first factorial plane of the PCA explained 91.4% of the total variance (Fig. 2). The first axis of the PCA was defined primarily by the ANC and Ca + Mg (Fig. 2a). It explained 68.6% of the total variance and strongly separated the circumneutral streams (LM and BH) from the acidified ones [GS, BE, Wassongoutte (WA), Longfoigneux (LG); Fig. 2b]. An additional 22.8% of the total variance was explained by the second axis, which discriminated among streams draining catchments underlain by sandstone vs granite bedrock. Streams draining granite catchments (BH, WA, LG) were mainly characterized by lower concentrations of strong acid anions. In addition, acidified streams draining sandstone (BE, GS) exhibited higher total Al concentrations than acidified streams draining granite (WA, LG).

Fungal Diversity and Mycelial Biomass

A total of 37 species of aquatic hyphomycetes was observed during the leaf-litter breakdown experiment (Table 2). The higher fungal diversity was found in the two circumneutral streams BH and LM (27 and 25 species, respectively) and the lowest in BE (seven taxa) which was also the most acidic one. Approximately 17 species were never recorded in acidic streams. Among these species, eight were shared by the two circumneutral streams BH and LM: *Alatospora pulchella*, *Culicidospora gravida*, *Lemonniera aquatica*, *Tricladium chaetocladium*, *Tricladium splendens*, *Tumularia aquatica*, *Tumularia tuberculata* and *Ypsilina graminea*. The nine other species were observed only in one of the circumneutral streams, LM or BH. On the other hand, only one species,

Casaresia sphagnorum, was recorded in the four acidic streams and never in the circumneutral ones.

During beech-leaf processing, *Clavariopsis aquatica* was the dominant species in the two circumneutral streams (i.e. 83.4% of the conidia produced in LM and 49.5% in BH), and *Flagellospora curvula* was dominant in the four acidic streams (i.e. from 83.1% in WA to 98.9% in BE).

The NMDS ordination described well the overall differences in aquatic hyphomycete assemblages between the six streams (Fig. 3). Stress was very low (0.003), indicating a high degree of representation. Axis 1 of the NMDS was strongly correlated (Spearman rank correlation) with pH ($r = 0.89$; $p < 0.05$), ANC ($r = 0.89$; $p < 0.05$) and total aluminium concentration ($r = -0.89$; $p < 0.05$). Position along this axis reflected the acidification status of the streams and strongly separated the acidic streams with low fungal richness (Table 2) from the circumneutral streams with markedly higher richness. Axis 2 correlated best with $[SO_4^{2-} + NO_3^-]$ concentrations ($r = 0.83$; $p < 0.05$) and separated streams according to the bedrock of their catchments.

Pearson correlation analyses between physical and chemical parameters and fungal species richness revealed significant relationships (Fig. 4) only with mean total aluminium concentration ($\log_{10}$ transformed; $r = -0.998$, $p < 0.0001$) and mean pH ($r = 0.962$; $p < 0.002$).

When considering fungal biomass, no clear tendency was observed, and mean values did not differ significantly between streams (one-way ANOVA; $F = 1.352$, $p = 0.271$), demonstrating that fungal biomass associated with decaying leaves was not affected by acidification (Fig. 5).
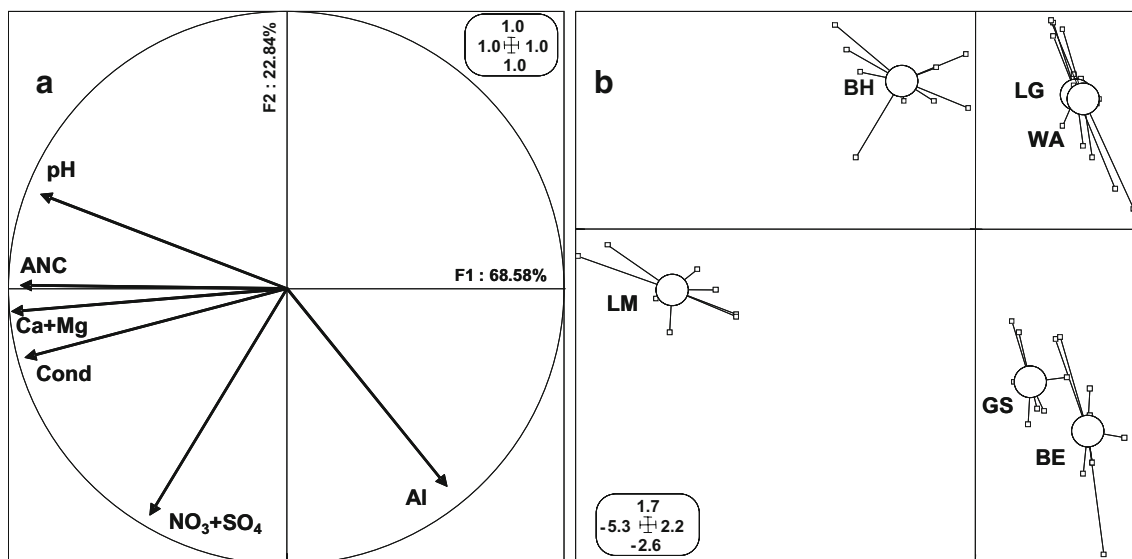
Figure 2 PCA performed on the physico-chemical variables in the six headwater streams during the leaf breakdown experiment. **a** Correlation circle on the F1×F2 factorial plane. **b** Ordination of the six sites on the F1×F2 factorial plane. *Small squares* represent the sample position at each sampling date. *Circles* denote the weighted average of all samples taken from a given stream. *Lines* link samples to their weighted average

Table 2 List of aquatic hyphomycete species associated with decaying beech leaves in each stream

Species	LM	BH	LG	WA	GS	BE
Alatospora acuminata Ingold	+	+	+	+	+	+
Alatospora flagellata (Gönczöl) Marvanová	+	+	+			
Alatospora pulchella Marvanová	+	+				
Anguillospora filiformis Greathead	+	+		+		+
Anguillospora furtiva Webster and Descals		+				
Anguillospora longissima (Sacc. and Syd.) Inglod		+	+			
Angulillospora rosea Webster and Descals	+	+	+	+	+	+
Articulospora tetracladia Ingold	+	+	+	+	+	+
Casaresia sphagnorum Gonz. Frag.			+	+	+	+
Clavariopsis aquatica De Wild.	+	+	+	+		
Clavatospora longibrachiata (Ingold) Marvanová and Nilsson	+	+	+	+		
Culicidospora aquatica Petersen	+					
Culicidospora gravida Petersen	+	+				
Dendrospora tenella Descals and Webster				+	+	
Flagellospora curvula Ingold	+	+	+	+	+	+
Fontanospora eccentrica (Petersen) Dyco			+		+	
Fontanospora fusiramosa Marvanová			+			
Heliscella stellata (Ingold and Cox) Marvanová and Nilsson	+	+		+		
Heliscina campanulata Marvanová				+		
Lemonniera aquatica De Wild.	+	+				
Lemonniera terrestris Tubaki	+	+	+	+	+	
Lunulospora curvula Ingold		+				
Mycocentrospora angulata (Petersen) Iqbal		+				
Mycofalcella calcarata Marvanová, Om-Kalth. and Webster		+				
Pleuropedium multiseptatum Marvanová and Descals		+				
Stenocladiella neglecta (Marvanová and Descals) Marvanová and Descals	+		+			
Tetrachaetum elegans Ingold	+	+		+		+
Tetracladium marchalianum De Wild.	+	+	+	+	+	
Tricladium angulatum Ingold	+					
Tricladium chaetocladium Ingold	+	+				
Tricladium patulum Marvanová and Marvan	+					
Tricladium splendens Ingold	+	+				
Tripospermum camelopardus Ingold, Dann and McDougall		+	+	+	+	
Tumularia aquatica (Ingold) Descals and Marvanová	+	+				
Tumularia tuberculata (Gönczöl) Descals and Marvanová	+	+				
Varicosporium macrosporum Nawawi	+					
Ypsilina graminea Inglod, McDougall and Dann	+	+				

Breakdown Rates

Breakdown rates of beech leaves were significantly different among streams (ANCOVA; $F = 41.9$, $p < 0.001$) and ranged from 0.0045 day^{-1} in the most acidic site (BE) to 0.0285 day^{-1} in the circumneutral stream BH (Fig. 6; Table 3). The breakdown rate in the circumneutral stream draining granite (BH) was significantly faster than that observed in the circumneutral stream draining sandstone (LM). However, both streams exhibited faster breakdown rates than acidic streams, while no significant differences occurred among the four acidic streams. After 124 days of exposure in these acidic streams, the mass loss did not exceed 16%, indicating that leaf-litter breakdown in these headwater streams had virtually stopped.

Discussion

Acidification of ecosystems resulting from atmospheric pollution is one of the most revealing demonstrations that human activities can strongly impair terrestrial and aquatic ecosystems located in remote areas. Among the most striking consequences of freshwater acidification, the loss of biodiversity has been well documented for different taxonomic groups such as fish [42, 48], invertebrates [21, 37], algae [51, 54] and macrophytes [23]. By comparison, the effect of anthropogenic acidification of aquatic ecosystems on the diversity of micro-organisms in general and aquatic hyphomycetes in particular has received much less attention despite their importance at multiple ecological scales. The few studies that have attempted to link aquatic
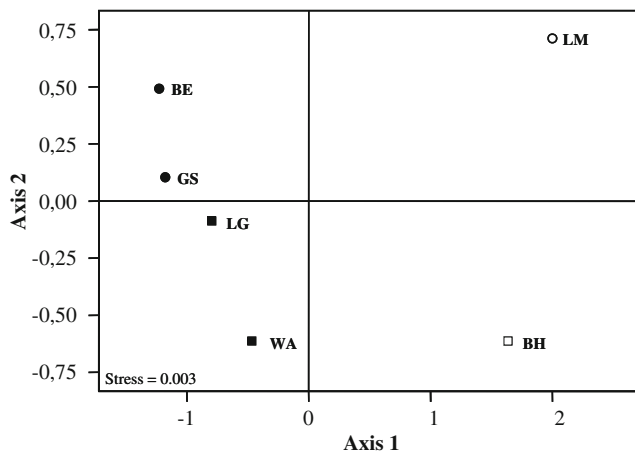
Figure 3 NMDS plot based on hyphomycete assemblages associated with decaying leaves in the six headwater streams. Circumneutral streams are shown in *white* and acidic streams in *black*. *Squares* represent streams draining granite bedrock and *circles* streams draining sandstone bedrock

hyphomycete richness and acidity have provided very contradictory results. For instance, in a study of aquatic hyphomycetes in ten streams of New Brunswick and Nova Scotia, Bärlocher [6] concluded that "their tolerance of low pH values makes them one of the rare groups of stream organisms that may actually benefit from the effects of acid rain". This conclusion contrasts markedly with the results of the previous studies of Iqbal and Webster [44] and Shearer and Webster [60] in Devon who reported impoverished fungal diversity in upland acidic water compared to lowland circumneutral sites, whereas Chamier [10] found low species richness in upland streams regardless of pH (4.9–6.8) in seven streams of the English Lake District.

In our studies, we observed a severe depletion of species in acidified streams (from 44 to 75%), and more than 45%

of the species associated with decaying leaves in circumneutral streams were never recorded in acidified streams. The species richness was strongly correlated with aluminium concentration and pH. However, because mineral acidity is the main factor regulating Al concentrations in acid surface waters, Al is more likely to control the diversity of aquatic hyphomycetes than H^+. In experiments to test for the effect of Al on aquatic hyphomycetes, Chamier and Tipping [12] found that deleterious effect of Al depend on the species considered, the effects being more marked on species common in circumneutral streams such as *T. splendens*. Similarly, differences in sensitivity to other metals among aquatic hyphomycete species have also been reported from experiments performed to assess the toxicity of Cd [1].

Several hypotheses can be proposed to explain these discrepancies. Chemical parameters such as Al were not analysed in earlier studies, and the type of acidity (natural, i.e. organic vs anthropogenic) was not considered. Therefore, the potential toxicity of water can markedly differ even in streams with similar pH. Furthermore, the results provided by these previous studies of species richness in relation to pH are difficult to compare as several of them have been performed in upland and lowland streams and/or in forested streams and streams without riparian trees.

In our study, the acidity of headwater streams did not originate from organic acidity (DOC < 2.3 mg l^{-1}), all the streams were forested (mainly *A. alba*, *P. abies* and *F. sylvatica*) and exhibited very similar environmental characteristics, allowing us to compare fungal diversity and species composition and to investigate the effects of anthropogenic acidification. Our results show that in the Vosges mountains, aquatic hyphomycete communities are severely impoverished, as are macroinvertebrate communities, as established in previous studies [37, 66]. In these streams, a combined toxic effect of high protons and aluminium concentrations
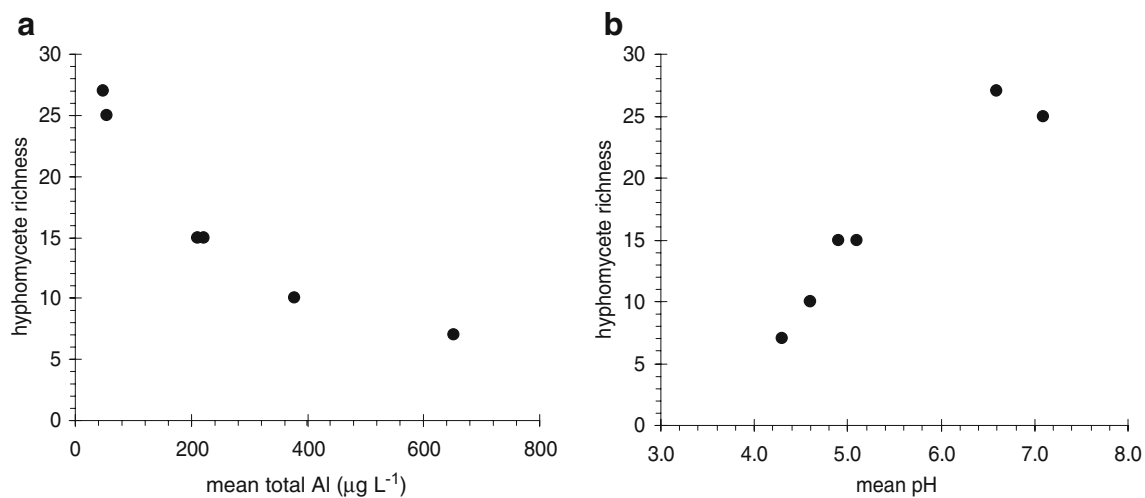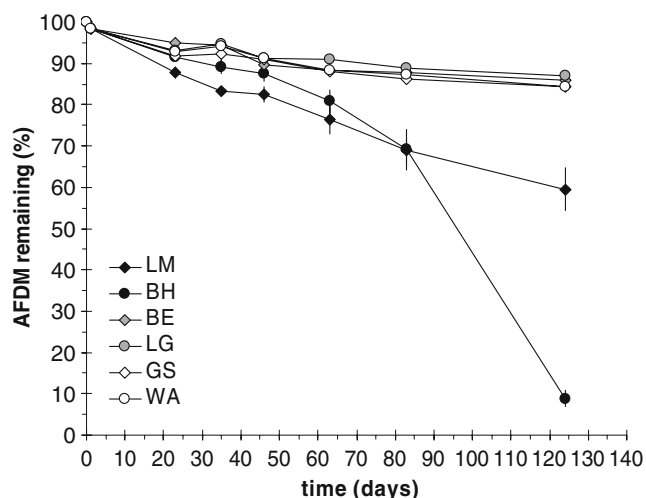

Figure 4 Relationship between fungi richness and **a** mean total aluminium concentration, **b** mean pH
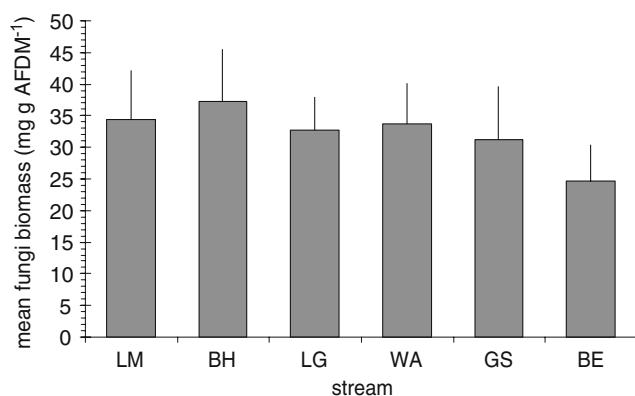
Figure 5 Mean (+SD) fungi biomass in each stream. Streams are arranged following decreasing pH from the *left* to the *right*

Table 3 Breakdown rates (day^{-1}) determined by fitting a linear model

Stream	$-k$	r^2
LM	0.0143[a]	0.979
BH	0.0285[c]	0.837
LG	0.0045[b]	0.873
WA	0.0054[b]	0.914
GS	0.0054[b]	0.884
BE	0.0051[b]	0.870

All regressions were significant ($p<0.005$). Rates with different superscript letters are significantly different (Tukey's test multiple comparisons of slopes, $p<0.005$)

has been demonstrated for different species of invertebrates [24, 25] as commonly reported for other aquatic organisms such as fish [50], crayfish [2], macrophytes [23] and algae [13, 40].

It is interesting to note that fungal biomass associated with decaying leaves was not affected by acidification, thus corroborating the results from Dangles and Chauvet [18] in five streams from the Vosges Mountains but contrasting markedly with the findings of Griffith and Perry [35], who found significantly reduced fungal biomass associated with decomposing oak leaves in a strongly acidified West Virginia stream. Such a discrepancy illustrates the fact that leaf-mass loss is no straightforward indicator of the fungal activities. A possible explanation for the significant differences in leaf breakdown rates despite similar fungal biomasses in our study lies in the sensitivity of pectin lyases to water chemistry. The enzymatic degradation of pectin represents a key process in leaf-mass loss [45], and different studies have shown that the activities of pectin

lyases are higher in neutral or alkaline waters than in acid or soft waters [11, 36, 45]. Moreover, Chamier and Tipping [12] have demonstrated that treatment with monomeric Al decreased pectinase production by four aquatic hyphomycete species.

Not surprisingly, our results showed that leaf-litter breakdown was severely depressed under acidic conditions, as previously reported in different studies [10, 19, 20, 36, 53]. In a recent study of 25 woodland first- and second-order streams along an acidification gradient in the Vosges Mountains (France), Dangles et al. [19] found that breakdown rates of beech (*F. sylvatica*) leaves varied more than 20-fold between the most acidified and circumneutral sites ($k=0.0002$–0.0055 day^{-1}) consistently with the associated fungal biomass and microbial respiration. Total abundance, biomass and species richness of leaf-shredding invertebrates associated with decomposing leaves were, however, not related to stream acidity (the diversity of aquatic hyphomycetes was unfortunately not investigated in this study) which suggests that fungi played a prominent role in acidified streams. That the slow breakdown rates we have measured in acidified headwater streams is linked to the decreased aquatic hyphomycete diversity is questionable. Several experimental studies have provided evidence that the loss of species can affect the efficiency with which resources are processed within an ecosystem [43, 49, 65] although the mechanisms underlying the response are not always well understood [71]. Whether diversity of aquatic hyphomycetes interferes with their functional role in leaf breakdown in the context of the present study should deserve special attention, e.g. through complementary in vitro and field experiments [e.g. 64]. Potential implications of such relationships are of major importance for higher trophic levels within the stream ecosystem.

Figure 6 Mean (±SD) percent of beech leaf remaining AFDM in the six streams

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