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Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams

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\textbf{A B S T R A C T}

Anthropogenic acidification has deleterious effects on both structure and functioning of surface water ecosystems. This study examined how it may affect the leaf decomposition rate and the community structure and activity of decomposers in both benthic and hyporheic zones of five headwater streams along an acidification gradient from highly acidic (pH 4.6) to circumneutral (pH 7.4). Overall, responses to acidification in hyporheic zones were less pronounced, but followed the same pattern as in their benthic counterparts. Leaf decomposition was much faster in the circumneutral stream, both in the hyporheic and benthic zones (\(k = 0.0068\) and \(0.0534 \text{ d}^{-1}\), respectively), than in the most acidic one (\(k = 0.0016\) and \(0.0055 \text{ d}^{-1}\), respectively), and correlated well with the acidic gradient in both compartments. Interestingly, leaf litter decomposition was less affected by acidification in hyporheic compared to benthic compartments, likely due to the relatively low sensitivity of fungi, which were the main decomposers of buried coarse particulate organic matter. These results argue in favour of conserving hyporheic habitats in acidified streams as they can maintain matter and species fluxes that are essential to the ecosystem.

1. Introduction

Over the last two decades there has been an increased recognition of the role of the hyporheic compartment – i.e., the permeable sediments through which there is significant exchange of water with surface zones – in the ecological functioning of stream systems (Brunke and Gonser, 1997; Boulton et al., 1998; Hancock et al., 2005; Boulton et al., 2010). It is now clearly demonstrated that as water flows through the hyporheic zone, the physical and chemical characteristics prevailing in this latter can affect a wide range of ecological processes, including nutrient (Duff and Triska, 1990; Triska et al., 1993; McKnight et al., 2004) and carbon cycling (Metzler and Smock, 1990; Naegeli et al., 1995; Jones et al., 2000; McElroy et al., 2001).

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Acidification of ecosystems resulting from atmospheric pollution is one of the most demonstrative illustrations that human activities can strongly impair terrestrial and aquatic ecosystems even in remote areas. During the last three decades, international efforts aiming at reducing atmospheric emissions have led to a marked decreased of sulphate deposition, both in North America and Europe (EMEP, 1999; Evans et al., 2001; Likens et al., 2001), while nitrate and ammonia remained stable (Cooper, 2005) or decreased (Barrett et al., 2000; Vuorenmaa, 2004) depending on the regions. Despite these abatements, acidification remains a major threat in numerous areas, notably to headwater streams (Alewell et al., 2001; Evans et al., 2001; Baudoin et al., 2008). In addition, acid rain is now being reported from Asia where economies and populations are expanding (Tang et al., 2001; Larssen et al., 2006) leading to an acidification of freshwater ecosystems in the near future.

Acidification has been shown to induce severe deleterious effects on both structure and functioning of surface water ecosystems (Mulholland et al., 1992; Dangles and Guerold, 2001; Dangles et al., 2004; Baudoin et al., 2008; Simon et al., 2009), with low-order streams being particularly impacted. Leaf litter decomposition is a key process driving the flows of energy and nutrients in woodland stream ecosystems (Cummins et al., 1989). Several studies have shown the extent to which this ecological process is depressed by atmospheric acidification (Burton et al., 1985; Chamier, 1987; Groom and Hildrew, 1989; Mulholland et al., 1992; Griffith and Perry, 1993; Dangles and Guerold, 2001; Dangles et al., 2004; Baudoin et al., 2008). However, a substantial portion of leaf litter entering running waters is subject to burial into the streambed as a consequence of flood events (Herbst, 1980) and sediment movement (Metzler and Smock, 1990; Naegeli et al., 1995). For the streams in which both benthic and hyporheic coarse particulate organic matter (CPOM) amounts have been measured, the latter accounts for 25–90% of total stored organic matter (Cummins et al., 1983; Metzler and Smock, 1990). In the same line, a recent study from Cornut et al. (2012) reported that the total CPOM content in the interstitial zone of headwater streams can be up to one order of magnitude higher than that stored at the sediment surface. Under the control of abiotic processes and decomposers’ activity, the hyporheic zone may thus act as a sink or a source of detrital organic matter to surface waters. We have recently shown that, compared to the benthic habitat, leaf litter decomposition in the hyporheic zone tends to be slower with the decomposer community being dominated by aquatic fungi (Cornut et al., 2010). The extent to which this major process and the associated biota in the hyporheic zone are affected by an anthropogenic stressor like acidification is however unknown, even though this is essential to encompass both benthic and hyporheic compartments when assessing stream ecosystem impairment and elaborating appropriate management measures in accordance with the Water Framework Directive (Smith, 2005).

The aim of this study was to assess the effects of acidification on the leaf-associated decomposer communities and on leaf litter decomposition according to the location of leaf litter within the streamed (i.e., on the surface or buried). On one hand, burial within the substratum generally reduces the access of decomposers to leaves with the small interstices of the gravely sediment acting as a physical constraint, especially for the largest shredders. On the other hand, numerous studies have emphasized the deleterious effects of freshwater acidification on macroinvertebrate communities. In particular the high sensitivity of numerous macroinvertebrate species including efficient shredders such as gammaridae, has been repeatedly demonstrated in North America and Europe, while Trichoptera showed a more varied response to acidification (Guéroud et al., 2000). In contrast, based on numerous studies that have investigated fungal growth, richness and activity of aquatic hyphomycete species within a relatively broad pH region, Krauss et al. (2011) in their review concluded that low pH values per se do not appear to be harmful to aquatic hyphomycetes. Therefore, if shredders are more sensitive to acidity compared to aquatic fungi, the impact of acidification will be more pronounced in the benthic than in the hyporheic zone. As a consequence, we expected the discrepancy in decomposition rates between the two stream compartments to be lowest at the lower end and highest at the higher end of the pH range. We tested these hypotheses by conducting an experimental study where leaf-associated microbial and macroinvertebrate communities, as well as leaf decomposition rates and the activity of microbial decomposers, were compared in the benthic and hyporheic zones of five low-order streams along a pronounced acidification gradient.

2. Materials and methods

2.1. Study sites

The experiment was conducted in the Vosges Mountains (North-eastern France) where anthropogenic deposition of acidifying substances has adversely affected surface waters during the last decades (Dambrine et al., 1998; Probst et al., 1999). Such impacts of acidification have been observed in many areas of the world since the end of the 19th century, due to long-range atmospheric transport and subsequent deposition of acidifying sulphur and nitrogen compounds (Schindler, 1988; Driscoll et al., 2001). Forestry was the only other potential anthropogenic disturbance, although limited within the study area. Five first and second-order streams were selected along an acidification gradient. They differed in pH and total Al concentration, but exhibited similar hydrological and morphological characteristics. Courbeligne (CL, 48°26’24"N; 7°03’54"E) was the most acidified stream. Ravines (RV, 48°24’47"N; 6°55’22"E) and Gravelle (GV, 48°16’22"N; 6°49’17"E) were two moderately acidic streams, differing by their concentrations in total Al and NO₃, and acid neutralising capacities (ANC). Two non-acidified streams, Ménoumbre (MB, 48°29’05"N; 7°02’48”E) and La Maix (LM, 48°29’00”N; 7°04’13”E), showed a circumneutral pH. These two streams exhibited rather similar physical and chemical characteristics, but strongly differed regarding their ANC and total Al.
mean values of the physico-chemical parameters of water of the five streams from November 2008 to January 2010. Vegetation in the selected catchments was dominated by silver fir Abies alba Mill., Norway spruce Picea abies L. and beech Fagus sylvatica L. Beech and alder (Alnus glutinosa (L.) Gaertn.) were by far the most common deciduous tree species adjacent to streams. The five streams had similar substratum, which was unconsolidated and mostly made of gravel and coarse/fine sand sediments.

2.2. Stream physico-chemistry

Water chemistry was determined on the seven dates when leaf bags were introduced into or retrieved from the streams. Samples of surface and interstitial water were collected from each stream, stored in pre-rinsed polyethylene bottles, and placed in an icebox until they were returned to the laboratory to be analysed within 48 h. Interstitial water was pumped from plexiglas minipiezometers using a hand-held vacuum pump. 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\(^{2+}\) and total Al (after acidification with HNO\(_3\)) were determined by atomic absorption spectrophotometry (Aanalyst 100; Perkin Elmer and Varian SpectrAA-300) and concentrations of NO\(_3^-\) by ion chromatography (Dionex 1500i with a AS 4 A SC column; Sunnyvale, USA). During the leaf litter decomposition study, the temperature of surface and interstitial waters was recorded every 4 h (SmartButton, ACR System Inc., BC, Canada).

2.3. Leaf decomposition

We selected alder as one of the commonest riparian tree species across stream sites. In October 2008, leaves were collected from trees at abscission and air-dried for 15 days at 20–22 °C. Leaf bags consisted of 3.00 g (mean air-dry mass ± 0.03 g) of leaves enclosed in plastic net bags (15 × 10 cm, 5 mm mesh) to simulate natural accumulations of leaf detritus in the stream. Before being enclosed into bags, the leaves were moistened with distilled water from a vaporizer to prevent breakage during handling and transport. A total of 48 leaf bags per stream were introduced at the head of riffles (downwelling zones) on 18th November 2008. The upstream end of riffles was chosen i) to circumvent the local spatial variability in surface and subsurface hydrology within riffle scale affecting leaf litter breakdown rates and ii) because it represents a downwelling area (infiltration of surface water into the hyporheic zone, Hendricks and White, 1991) fed with oxygen and nutrients, resulting in a higher breakdown rate (Tillman et al., 2003). Leaf bags were subject to two treatments: benthic and hyporheic (i.e., buried in the sediment). Benthic leaf bags were placed on the sediment surface and secured to the bank with plastic-coated wire, which was anchored to the stream bottom with large boulders. Hyporheic leaf bags were embedded approximately 15–20 cm below the sediment surface using a small shovel, with a coloured plastic wire attached to facilitate localization and retrieval (Cornut et al., 2010). Four replicate bags were randomly retrieved from each zone of the five streams after 7, 14, 21, 28, 49, and 70 days of exposure, immediately placed and stored individually in plastic zip-lock bags with stream water and transported to the laboratory in a cool box. During leaf bag sampling, a Surber net (500 µm mesh size) was used to minimize invertebrate loss due to passive or active drift. In the laboratory, leaves were washed individually with water from the respective stream to remove sediments, exogenous organic matter and macro-invertebrates, which were collected in a 500 µm screen sieve and then preserved in 70% ethanol until processing. One set of five 12 mm diameter discs and another of ten were cut from leaves of each bag retrieved, avoiding the central vein. The set of five leaf discs was promptly frozen at −20 °C until processing for ergosterol extraction, and the set of ten was immediately used for incubation in aerated microcosms (see below). The remaining leaf litter was dried at 105 °C to

<table>
<thead>
<tr>
<th>Streams</th>
<th>LM</th>
<th>MB</th>
<th>GV</th>
<th>RV</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Benthic</td>
<td>7.4 (7.3/7.4)</td>
<td>7.0 (6.8/7.2)</td>
<td>6.4 (6.2/6.5)</td>
<td>6.1 (5.9/6.3)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>7.4 (7.3/7.6)</td>
<td>6.9 (6.6/7.1)</td>
<td>6.4 (6.3/6.5)</td>
<td>6.3 (6.2/6.7)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Benthic</td>
<td>6.0 (4.7/7.0)</td>
<td>5.8 (4.3/7.3)</td>
<td>6.6 (5.3/7.6)</td>
<td>5.6 (3.8/6.8)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>5.9 (4.7/6.7)</td>
<td>5.8 (4.5/7.1)</td>
<td>6.6 (5.3/7.6)</td>
<td>5.5 (3.8/6.7)</td>
</tr>
<tr>
<td>[DO] (mg/L)</td>
<td>Benthic</td>
<td>11.8 (11.3/12.4)</td>
<td>11.8 (10.1/12.5)</td>
<td>11.3 (10.8/11.8)</td>
<td>12.1 (11.7/12.8)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>10.3 (9.4/10.9)</td>
<td>10.1 (9.2/11.1)</td>
<td>9.9 (9.4/10.4)</td>
<td>10.2 (9.9/10.6)</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>Benthic</td>
<td>71.3 (60.0/76.0)</td>
<td>52.7 (41.5/66.0)</td>
<td>35.5 (34.9/36.0)</td>
<td>37.0 (36.5/37.8)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>75.9 (62.5/83.0)</td>
<td>61.7 (48.5/75.5)</td>
<td>36.2 (35.0/37.0)</td>
<td>39.0 (37.5/40.0)</td>
</tr>
<tr>
<td>ANC (µEq/L)</td>
<td>Benthic</td>
<td>448 (348/512)</td>
<td>204 (113/286)</td>
<td>53 (44/63)</td>
<td>25 (15/33)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>486 (372/548)</td>
<td>302 (198/509)</td>
<td>60 (48/72)</td>
<td>43 (31/76)</td>
</tr>
<tr>
<td>[Total Al] (µg/L)</td>
<td>Benthic</td>
<td>26 (11/67)</td>
<td>93 (72/110)</td>
<td>32 (23/53)</td>
<td>105 (44/310)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>140 (29/237)</td>
<td>249 (145/427)</td>
<td>67 (32/119)</td>
<td>360 (70/688)</td>
</tr>
<tr>
<td>[Ca(^{2+})] (mg/L)</td>
<td>Benthic</td>
<td>6.7 (5.8/8.4)</td>
<td>4.0 (3.0/4.7)</td>
<td>2.4 (2.3/2.5)</td>
<td>2.2 (2.2/2.3)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>7.4 (5.8/8.4)</td>
<td>5.4 (4.0/7.4)</td>
<td>2.4 (2.3/2.6)</td>
<td>2.4 (2.2/2.6)</td>
</tr>
<tr>
<td>[NO(_3^-)] (mg/L)</td>
<td>Benthic</td>
<td>3.2 (3.0/3.4)</td>
<td>2.8 (2.5/2.9)</td>
<td>1.6 (1.6/1.7)</td>
<td>2.6 (2.5/2.8)</td>
</tr>
<tr>
<td></td>
<td>Hyporheic</td>
<td>3.2 (3.0/3.4)</td>
<td>2.3 (1.0/2.8)</td>
<td>1.6 (1.5/1.6)</td>
<td>2.6 (2.5/2.7)</td>
</tr>
</tbody>
</table>
constant mass and weighed to the nearest 0.01 g. The leaf material was ground using a micro hammer mill (Culatti, Zurich, Switzerland) with a 0.5 mm mesh. Portions of leaf material of about 500 mg were ashed at 550 °C for 4 h in a muffle furnace and weighed to determine the organic matter content. The leaf mass remaining in each bag was determined by adding the mass of oven-dried litter to those of leaf discs (see below). The leaf mass remaining was expressed as the ratio of the ash-free dry mass (AFDM) between the final and initial leaf litter. Four unexposed batches of leaf litter were used to determine the initial AFDM and oven-dried mass to air-dried mass ratio of leaves according to the procedures above.

2.4. Fungal diversity

Once cut, the batches of ten fresh leaf discs were quickly placed in aerated microcosms (Suberkropp, 1991) filled with 40 mL filtered (Glass fibre GF/F, Whatman) water from the respective stream and incubated at 10 °C. After 48 h, a 20 mL aliquot of spore suspension from each microcosm was transferred into a 30 mL glass tube and preserved with 1.5 mL of 37% formalin. The leaf discs were then lyophilized and weighed to the nearest 0.1 mg. 0.5 mL of Triton X-100 (0.5% solution) was added to each spore suspension, which was stirred gently to ensure uniform distribution of spores. A 5 mL aliquot was then filtered through a membrane filter (5 μm pore size, diameter 25 mm; Millipore Corporation, Bedford, MA, USA), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal and Webster, 1973). Spores were counted and identified under the microscope (>200).

2.5. Fungal biomass

Mycelial biomass in leaves was assessed through the content in ergosterol (Gessner and Chauvet, 1993). Leaf material was lyophilised and weighed to the nearest 0.1 mg, and then lipids were extracted with alkaline methanol heated at 80 °C for 30 min. Extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc, Waters, Milford, MA, USA) and ergosterol was quantified by high-performance liquid chromatography (procedure slightly modified from Gessner (2005)). The ergosterol amount was corrected for the extraction efficiency (87–100%), which was measured for each sample series on controls to which known amounts of ergosterol were added. Ergosterol was converted into fungal biomass using a conversion factor of 5.5 mg ergosterol g⁻¹ fungal dry mass (Gessner and Chauvet, 1993).

2.6. Macroinvertebrates

Macroinvertebrates retained over a 500-μm mesh sieve were counted and identified to the lowest practicable level. Identification was to the genus/species level whenever possible, except for Oligochaeta and Diptera (family and sub-family or tribe, respectively), and individuals were assigned to shredders, grazers or others (Tachet et al., 2000). For each leaf sample, the biomass of macroinvertebrates assigned to the shredder group was determined to the nearest 0.01 mg after drying to constant mass at 60 °C.

2.7. Fine particulate organic matter production

After removal of the 20 mL aliquot for spore production and fungal diversity determinations (see above), the contents of the microcosms were filtered through a 1-mm mesh screen to retain coarse particles. The 20 mL remaining were then filtered through a membrane filter (0.45 μm pore size, diameter 25 mm; Millipore Corporation), washed three times beforehand with 10 mL of pure water, dried at 80 °C and pre-weighted to the nearest μg. The fine particulate organic matter (FPOM) release due to microbial activity was determined to the nearest μg after drying to constant mass at 80 °C.

2.8. Statistical 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, DO, Ca²⁺ and NO₃⁻. Leaf decomposition rates (k) for each treatment were estimated by using the exponential decay model, \( M_t = M_0 e^{-kt} \), where \( M_0 \) is the initial AFDM, \( M_t \) is the AFDM remaining at time \( t \), and \( t \) is the time in days (Petersen and Cummins, 1974). The \( k \) values were determined from linear regression of the log-transformed relationship. An analysis of covariance (ANCOVA) was used to compare \( k \) values among the five streams and the two exposure treatments, followed by a multiple-comparison (Tukey HSD test). A three-way factorial ANOVA was used to assess differences in microbial and macroinvertebrate parameters with stream, treatment (i.e., benthic or hyporheic) and exposure time as the main effects. Physicochemical differences among streams and between the hyporheic and benthic zone of streams, and the potential interactions between these factors were examined using a two-way factorial ANOVA, with stream and treatment as categorical predictors. Data were log transformed to improve homoscedasticity when necessary. When significant differences were detected between treatments, Tukey HSD tests were carried out for post hoc pairwise comparisons.

The Simpson’s dominance index and the Simpson’s diversity index were computed for fungal communities associated with decomposing leaves from each compartment of the five streams. Non-metric multidimensional scaling (NMDS) analyses of sporulation data and shredder abundances were used to assess differences in species composition among sites. This ordination method is a robust procedure for analysing ecological data (Minchin, 1987). The Bray–Curtis coefficient was used to quantify the dissimilarity among sites based on joint occurrence and abundance of taxa. NMDS attempts to maximise the fit between measured dissimilarities and distance between resulting data points within a predefined number of spatial dimensions (Legendre and Legendre, 1998; Legendre and Marti, 1999). Stress function for each NMDS plot indicates the fitness of representation of differences among sites. Stress values range 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-
cyte and shredder assemblages (Hawkins et al., 1997; Baudoin et al., 2008). PCA was performed using R software 2.6.0 (R development Core Team, 2007). NMDS analysis was performed with PRIMER 6 (Clarke and Gorley, 2001). STATISTICA 6.0 (StatSoft Inc., 2001) was used for all other statistical analyses. Differences were considered significant when \( P < 0.05 \).

3. Results

3.1. Physico-chemical conditions in benthic and hyporheic zones

The five headwater streams ranged from circumneutral (mean \( pH = 7.4 \), in both benthic and hyporheic zones of LM) to strongly acidified (mean \( pH = 4.6 \) and 4.7 in CL benthic and hyporheic zones, respectively). The factorial plane defined by axes 1 and 3 of the PCA explained 72.2% of the total variance (Fig. 1). The first axis of the PCA was primarily defined by the ANC, \( Ca^{2+} \), \( pH \) and conductivity (Fig. 1a). It explained 57.5% of the total variance and strongly separated the five streams along the acidification gradient (Fig. 1b). An additional 14.7% of the total variance was explained by the third axis, which discriminated among benthic and hyporheic zones of streams. Hyporheic zones of the five streams were mainly influenced by CL, the stream showing the highest NO\(_3\) concentration. All five streams significantly varied in acidity (\( F_{4,60} = 756.8, P < 10^{-3} \) and HSD test) and each stream exhibited quite similar values in benthic and hyporheic zones (mean \( pH \) range = 4.6–7.4 and 4.7–7.4, respectively), except RV whose \( pH \) was slightly higher in hyporheic than in benthic zone (\( F_{4,60} = 2.6, P = 0.04 \) and HSD test; Table 1). Not surprisingly, the most acidified stream (CL) and the two moderately acidic streams (RV and GV) presented typical chemical patterns of acid-stressed headwater streams, i.e., low conductivity, negative or low positive ANC and low \( Ca^{2+} \) concentration (\( F_{4,60} = 229.30, P < 10^{-3} \), \( F_{4,60} = 287.68, P < 10^{-3} \), \( F_{4,60} = 3011.11, P < 10^{-3} \) and HSD test, respectively). As a result of high acid deposition in the region during the last decades, total Al concentration strongly varied between streams (\( F_{4,60} = 19.70, P < 10^{-3} \)) and showed a trend, although non-significant, of being higher in the hyporheic zone than in the benthic one (\( F_{4,60} = 0.82, P = 0.52 \); Table 1). The concentration in atmospheric-derived nitrates differed greatly among streams (\( F_{4,60} = 259.88, P < 10^{-3} \) and HSD test) with the most acidic stream exhibiting higher concentrations (Table 1), but was quite similar between the benthic and hyporheic zones (\( F_{4,60} = 2.40, P = 0.06 \)). Water temperature was not different between the two stream compartments (\( F_{4,60} = 0.024, P = 0.99 \)), but significantly varied among streams mainly due to the lower temperature of the most acidic stream (CL; \( F_{4,60} = 14.19, P < 10^{-3} \) and HSD test; Table 1). The five streams were always well oxygenated throughout the experiment and presented similar values of dissolved oxygen (\( F_{4,60} = 1.66, P = 0.17 \)) while these latter were significantly lower in the hyporheic than in the benthic zones, showing a reduction by ca. 12% (\( F_{4,60} = 132.28, P < 10^{-3} \); Table 1).

3.2. Litter decomposition

In both benthic and hyporheic zones, a significant decrease in leaf decomposition rates was observed under acidic conditions. After 10 weeks, the percentage of remaining AFDM in the benthic zone was less than 2% in the circumneutral LM and MB, while it was more than 30% in the GV and RV and 60%

![Fig. 1](image-url)
in the most acidic stream CL. As a result, leaf decomposition rates at the sediment surface in LM and MB were significantly higher than in the other streams \( (F_{9,229} = 74.08, P < 10^{-3}) \) and HSD test, all comparisons, \( P < 10^{-3} \).

The differences among streams in the hyporheic zone were less pronounced (Fig. 2), with percentages of remaining AFDM varying between 55 and 79% for the circumneutral LM and the most acidic CL streams, respectively. As a consequence, the ratio of circumneutral-to-acidic decomposition rates (LM:CL) for the hyporheic zone was 4.34 while it was 9.76 in the benthic counterpart.

Since water temperature of the most acidic stream was significantly lower than the four other ones, leaf litter decomposition rates were normalized for temperature by referring to degree-days (sum of daily mean water temperatures over the decomposition time) instead of days. Leaf litter decomposition rate patterns among streams and compartments however remained unchanged \( (F_{9,229} = 75.89, P < 10^{-3}) \) and HSD test, all comparisons, \( P < 10^{-3} \) demonstrating the pre-eminence of the acidic stress over temperature effect.

### 3.3. Fungal diversity and biomass

A total of 34 sporulating species of aquatic hyphomycetes was detected during leaf decomposition (Table 2). The highest fungal diversity (cumulated number of species from benthic and hyporheic zones) was found in the circumneutral stream LM (24 species) and the lowest (15 species) in the two most acidic ones, RV and CL. Whatever the stream, the fungal species richness associated with decomposing leaf-litter was always lower in the hyporheic than in the benthic zone (Table 2). This difference appeared relatively small for the circumneutral stream (i.e., 18 and 17 species for the benthic and hyporheic zones, respectively). In contrast, the leaf-associated species richness from the hyporheic zone of the most acidic stream was less than half of that from the benthic zone (6 vs. 13 species). Throughout the experiment, the most striking difference among aquatic hyphomycetes assemblages from decomposing leaves was the dominance of Flagellospora curvula in the most acidic stream CL, both in the benthic and hyporheic zones (accounting for 96% and 81% of the pool of conidia produced, respectively). In contrast, four to six species (i.e., F. curvula, Heliscus lugdunensis, Tetrachaetum elegans, Lembombre cornuta, Alatospora pulchella, Alatospora acuminata) were codominant on leaves from the circumneutral stream LM in both the hyporheic and benthic zones. The NMDS ordination highlighted the overall differences in aquatic hyphomycete assemblages between the five streams and the two compartments (Fig. 3). It revealed marked differences in species assemblages along the acidification gradient: Axis 2 strongly correlated with pH \( (r = 0.83, P = 0.001) \). The position along this axis reflected the acidification status of the streams and distinctly separated the acidic streams with lower fungal community richness and evenness from circumneutral streams (Table 2). The NMDS ordination also separated well aquatic hyphomycetes from the benthic and hyporheic zones, with Axis 1 correlating with the concentration in dissolved oxygen \( (r = 0.80, P = 0.003) \).

Freshly collected leaves contained minute amounts of ergosterol, indicating that fungal colonization was negligible at the beginning of the experiment. Fig. 4 shows that fungal biomass associated with decomposing leaves at the sediment surface increased rapidly within the first 7 weeks, except for the circumneutral stream LM, which reached a maximum of 28 mg g\(^{-1}\) AFDM after 3 weeks, then rapidly declined. Leaves exposed at the sediment surface in the most acidic stream CL differed from this general pattern in that mycelial biomass continuously increased to reach a maximum of 91 mg g\(^{-1}\) AFDM after 10 weeks, whereas leaves in the four other streams were decomposed almost entirely before the last sampling date. Consequently, biomass associated with decomposing leaves in these streams was determined from the first five sampling dates for MB, GV and RV and from the first four sampling dates only for LM (Fig. 4).

The mycelial biomass showed no particular trend regarding the acidification gradient, except that maxima were higher in the most acidic stream, both in benthic and hyporheic zones. The maximum reached during the course of the experiment differed significantly between streams in the benthic \( (F_{4,12} = 5.38, P < 10^{-3}) \) and hyporheic zones \( (F_{4,15} = 7.99, P < 10^{-3}) \). The mycelial biomass from the first four sampling dates differed significantly between compartments \( (F_{1,149} = 25.12, P < 10^{-3}) \) and among streams \( (F_{4,149} = 2.60, P < 10^{-3}) \). The maximum biomass on leaves exposed in the hyporheic zone, except for the most acidic stream CL, was delayed by 3–7 weeks in comparison with the benthic zone. Surprisingly, the most acidic stream CL, exhibiting also the highest total Al concentrations both in benthic and hyporheic zones (means of 697 and 682 µg/L, respectively), showed similar or higher fungal biomass compared to the other streams.

### 3.4. Shredders

The shredder biomass differed significantly among streams \( (F_{4,210} = 5.58, P < 10^{-3}) \) and between location within the
Fig. 3 – NMDS plot of sites based on (a) aquatic hyphomycete and (b) shredder species associated with leaves decomposing in the benthic and hyporheic zones of five streams along an acidification gradient. LM: La Maix, MB: Ménonbru, GV: Gravelle, RV: Ravines, CL: Courbeline.
stream \((F_{1,210} = 34.74, P < 10^{-3})\). The highest biomasses on benthic leaves were found in the two circumneutral streams LM (mean of 20.2 mg g\(^{-1}\) AFDM) and MB (15.0 mg g\(^{-1}\) AFDM), where they differed significantly from their hyporheic counterpart (0.004 and 0.007 mg g\(^{-1}\), respectively, Fig. 5). A considerable reduction in shredder biomass on benthic leaves was observed along the acidification gradient, with values from the two circumneutral streams LM and MB being significantly higher than in the three acidic streams (CL: 0.145, RV: 7.060 and GV: 2.297 mg g\(^{-1}\) AFDM, Fig. 5). In the hyporheic zone, shredder biomass was similar among streams and mostly dominated by Leuctra spp. and Amphinemura sulcicollis (Fig. 5 and Table 3). The peaks in shredder biomass observed on benthic leaves at T49 only occurred in the two circumneutral streams LM and MB and were mostly attributable to individuals of Gammarus fossarum and Trichoptera (Sericostoma personatum, Potamophylax sp. and unidentified Limnephilinae), i.e., the largest dominant shredder taxa in the stream (Fig. 5 and Table 3). However, these taxa were generally found at low densities. In acidic streams GV and RV, shredders were represented by stoneflies, mainly Leuctra spp., A. sulcicollis, Protonemura sp. and Nemoura sp., often in abundance (Table 3). In the most acidic stream CL, shredder assemblages in both benthic and hyporheic zones were dominated, in biomass and numbers, by Leuctra spp (Table 3). Shredder assemblages among the five streams were well discriminated on Axis 2 of the NMDS analysis (Fig. 3), which was correlated with pH \((r = 0.75, P = 0.007)\). Similarly, shredder assemblages associated with leaves from the benthic and hyporheic zones were distinguished along Axis 1, which was well correlated with the concentration in dissolved oxygen \((r = -0.79, P = 0.003)\). The discrimination between macroinvertebrate communities along the acidification gradient held for both benthic and hyporheic zones of the streams. In particular, communities from benthic and hyporheic zones were found to converge in the most acidic stream whereas they diverged the most in the two circumneutral streams. This overall pattern was remarkably similar to that of fungal assemblages (Fig. 3).
3.5. Microbial FPOM

The temporal pattern of microbial FPOM production from decomposing leaves was comparable across streams and treatments, however with contrasted dynamics (Fig. 6). Overall, the release of FPOM by microbial activity increased significantly with time ($F_{4,150} = 315.42, P < 10^{-3}$) and decreased in a consistent trend along the acidification gradient ($F_{4,150} = 116.65, P < 10^{-3}$) in both compartments ($F_{4,150} = 4.13, P < 10^{-3}$). At any date and for any stream, the FPOM release in the benthic zone exceeded that from the hyporheic zone. The mean FPOM production in the benthic zone varied from 11.0 to 50.2 mg g$^{-1}$ litter d$^{-1}$ in the most acidic stream CL and the circumneutral one LM, respectively, and from 8.5 to 42.3 mg g$^{-1}$ litter d$^{-1}$ for the hyporheic zone of the same streams.

### Table 3 – Relative occurrence (%) of shredder genera associated with alder leaves in the two compartments of the five streams (all sampling dates and replicates combined).

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Fig. 6 – Rate of microbial production of fine particulate organic matter from leaves decomposing in the benthic and hyporheic zones of five streams along an acidification gradient at six incubation dates (mean $\pm$ SE, $n = 4$). LM: La Maix, MB: Ménonbru, GV: Gravelle, RV: Ravines, CL: Courbeline.

4. Discussion

4.1. Acidic stress and ecosystem function in benthic and hyporheic zones

To our knowledge, the present study provides the first comparative data set on benthic and hyporheic leaf-decomposer communities and litter decomposition in streams affected by anthropogenic acidification. This study presents evidence that the anthropogenic acidification reduced decomposition of leaves both in benthic and hyporheic zones of headwater streams. However, the overall response to acidification in the hyporheic zones was less pronounced than in their benthic counterparts, which fully supports our initial hypothesis. Although a substantial decrease in decomposition rates of
leaves was observed under acidic conditions, this effect was not primarily due to changes in fungal biomass (Fig. 4). Fungal biomass has generally been shown to increase to a maximum and then decline during leaf decomposition (Gessner and Chauvet, 1994). In contrast, fungal biomass associated with leaves decomposing in both benthic and hyporheic zones generally increased over the whole experiment for the five streams whatever the level of constraint generated by acidic deposition. As already reported by Dangles and Chauvet (2003), a significant decline following a peak only occurred in the benthic zone of the circumneutral stream (Fig. 4), which was the least affected by acidic deposition and exhibited the highest decomposition rates both in benthic and hyporheic zones. Our findings thus corroborated the results from Dangles and Chauvet (2003), Baudoin et al. (2008) and Simon et al. (2009) showing that fungal biomass was unresponsive to pH for streams of the Vosges mountains in France and of the Western Virginia in USA, but contrasted markedly with the substantial reduction in fungal biomass on oak and maple leaves in an acidified West Virginia stream, while the extent of acidic stress was quite similar (Griffith and Perry, 1994).

Moreover, studies that have attempted to link aquatic hyphomycete richness and activity and pH have reported ambiguous or contradictory patterns, while Krauss et al. (2011) suggested that aquatic hyphomycetes do not appear to be sensitive to low pH values per se. Bärlocher (1987), based on an investigation in ten streams of Nova Scotia and New Brunswick combined with an earlier study, revealed a significant negative correlation between aquatic hyphomycete species richness and stream water pH, and even 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”. In the same line, Wood-Eggenchwiler and Bärlocher (1983) found a clear decline in aquatic hyphomycete species richness with increasing pH in various European streams.

The studies of Iqbal and Webster (1977) and Shearer and Webster (1985) contrasted with these conclusions, as these studies reported impoverished fungal diversity in upland acidic water compared to lowland circumneutral sites. However, this may be due to a confounding effect of altitude, as Chamier (1987) found more species of aquatic hyphomycetes at lowland than at upland sites, regardless of pH (4.9–6.8) in seven streams of the English Lake District. Through a series of experiment on the physiological requirements of aquatic hyphomycetes, Rosset and Bärlocher (1985) reported that 10 species grew the best on solid media at pH values between 4 and 5, compared to pH around 7. In addition, some species even ceased to grow completely at pH > 7. However, aquatic hyphomycete growth rates were always higher when grown on solid media amended with Ca$^{2+}$. In a laboratory stream experiment, Thompson and Bärlocher (1989) demonstrated that weight loss of maple leaves due to microbial activity peaked at a pH between 5.5 and 6.0. However, although these different examples indicate that lowered pH values are not necessarily detrimental to aquatic hyphomycetes, anthropogenic acidification can also raise Al concentration in stream water, which may severely depress fungal richness and activity (Krauss et al., 2011).

Surprisingly, the leaves exposed in the stream with the lowest pH in the present study (CL: 4.6 and 4.7 in benthic and hyporheic zones, respectively) and highest total Al concentrations (697 and 682 µg/L, respectively) showed the highest fungal biomass, both in benthic and hyporheic zones. As suggested by Dangles and Chauvet (2003), this pattern could be partly explained by the relatively high concentrations of atmospheric-derived nitrates in this stream (4.4 mg/L in the two zones), contrasting with the particularly low values reported by Griffith and Perry (1994), providing inorganic N to fungal production (Suberkropp, 1998) and thus compensating for the unfavourable acidic conditions. This difference might also result from discrepancies in substrate recalcitrance, which in the case of oak and maple (Griffith and Perry, 1994), led to a limited extent of development of fungal communities and therefore a substantial reduction in fungal biomass compared to alder (Danger et al., 2012).

Our results show that the peak of mycelial biomass in the hyporheic zone of the five streams was delayed by 7 weeks in comparison with the benthic zone, even though the latter also showed a strong increase at the final sampling date. Moreover, the amount of leaf-associated mycelial biomass was depressed in burial conditions, as reported from alder leaves in an analogous stream (Cornut et al., 2010) or from woody debris in a headwater mountain stream (Crenshaw et al., 2002). Surprisingly, in the present study, differences in dissolved oxygen between hyporheic and benthic zones remained low (from 1.0 to 1.9 mg/L) and the reduced fungal biomass in the hyporheic zone could only be partly explained by oxygen limitation, as supported by experimental data from hypoxic environments (Medeiros et al., 2009).

Also, we expect the number of conidia in water to drop from the surface to the deepest sediment layers. Even though fungal spores may disperse and allow small, short-lived colonies to develop on the substrates they encounter in the hyporheic zone (Bärlocher et al., 2006), the lower amount of conidia circulating in the sediment may have also contributed to the observed delay to reach a substantial accrual of mycelial biomass.

4.2. Evidence for microbiological processes

The contribution of shredders to leaf decomposition in the hyporheic zone of the present streams was rather low as reported in a recent study (Cornut et al., 2010). Shredders were rare on buried leaves whatever the level of acidification, although they were relatively abundant on leaf detritus at the surface of sediments. In addition, the only taxa with the appropriate morphology to penetrate the substratum in this study, i.e., Leuctra spp. and A. sulcicollis, were also the less efficient detritivores in leaf decomposition. The burial of leaves in the sandy substratum thus reduced the access of invertebrate decomposers to leaves due to the small interstices of the sediment and the subsequent bottom instability, particularly constraining the largest shredders. As a consequence, the contributions of fungi vs. shredders to leaf decomposition, as well as the level of their trophic interactions (Cornut et al., 2010), were contrasted in the two habitat types of the present acidified streams (Fig. 7). The absence of shredding invertebrates in the most acidic stream (CL) virtually suppressed the competition with fungi, and allowed the latter to grow to a higher extent than in the circumneutral stream (LM). This increased importance of
aquatic fungi in litter decomposition in the hyporheic environment is in accordance with recent findings (Cornut et al., 2010). While the extent of fungal development was possibly related to differential abundance of shredding invertebrates, trophic interactions in both compartments were also modified (Fig. 7). Overall, this is discernible when extrapolating data beyond the final sampling date in the hyporheic zone that leaf litter remaining in this habitat (i.e., more than 50% of initial mass in LM, the stream exhibiting the highest decomposition rates) can sustain further fungal growth in contrast to the benthic zone where the decomposition process is nearly completed after 70 days. Also, as suggested by Cornut et al. (2010), it is expected that fungal decomposers in the hyporheic zone compensate, if not fully, at least partly for the action of invertebrate detritivores in the benthic zone.

The acidification of freshwater ecosystems resulting from atmospheric pollution is often accompanied by increased concentrations of aqueous Al, which is considered a major environmental issue due to its high toxicity to aquatic organisms. Indeed, aqueous Al has been recognized as a main toxicant for aquatic animals (Gensemer and Playle, 1999), and several studies have also suggested a direct effect on microbial metabolism (Myrold and Nason, 1992; Pina and Cervantes, 1996; Chamier and Tipping, 1997) and diversity (Baudoin et al., 2008). The presence of aquatic hyphomycetes in our streams strongly contaminated by aqueous Al confirms that at least some species are resistant to this constraint, as already shown for other metals (Krauss et al., 2011).

Overall, our findings showed that the diversity of aquatic hyphomycetes on leaves in hyporheic zone, assessed through the release of conidia, was substantially lower than on the streambed. It is however important to note that these results were only based on conidia released during leaf decomposition. Thereby, non-sporeulating fungal species may have not been detected. Molecular techniques might be fruitfully applied to fungal communities on plant detritus in streams to circumvent such obstacles, although still subject to uncertainties and limitations (Bärlocher, 2010).

Both aquatic hyphomycete and macroinvertebrate communities were strongly modified under acidic conditions, and they responded similarly to the acidification gradient across the benthic and hyporheic zones. However, communities differed much more between the two compartments of circumneutral streams than those of the most acidic one (Fig. 3a and b). For invertebrates, this pattern is more likely due to the converging effects of physical (hyporheic) and chemical (acidic) constraints, which led to drastic reduction in their diversity and density (Fig. 7). The same pattern observed in fungal assemblages means that fungi responded in a similar way to both physical and chemical barriers, even though individual responses, perhaps exacerbated by interspecific interactions, differed among species (cf. Table 2). Environmental filtering of shredder and aquatic hyphomycete species assemblages through acidity and habitat conditions may therefore act synergistically, leading to lower leaf litter decomposition. The observed reduction in leaf litter decomposition rate along the acidic gradient for both benthic and hyporheic zones supports the contention that stream acidification has a profound impact on leaf litter decomposition, as previously reported (Chamier, 1987; Mulholland et al., 1992; Griffith et al., 1995; Dangles and Guerold, 2001; Dangles et al., 2004; Baudoin et al., 2008). However, as stressed by Dangles et al. (2004), it is not well defined whether this effect is mainly due to changes in community structure and taxonomic diversity of aquatic hyphomycetes and shredders and/or to a lower microbial activity. Dangles et al. (2004) reported that the abundance and biomass of a single acid-sensitive macroinvertebrate species, G. fossarum, in the same or similar streams were good predictors of leaf litter decomposition, explaining 80% and 73%, respectively, of variations in litter decomposition rate among streams. There are no counter-part studies for aquatic hyphomycete taxa to our knowledge. Experimental findings by Dang et al. (2005) and Duarte et al. (2006) however suggest that specific traits of certain species may have a greater influence on leaf decomposition rate than species richness of fungal assemblages.

Fig. 7 – Conceptual scheme of how anthropogenic acidification affects trophic and structural relationships in stream food web both in the benthic and hyporheic zones. The figure displays a simplified food web for the benthos and hyporheos, consisting of five elements (symbolised by circles): fish, large and small predatory invertebrates, detritivores and herbivores, consisting of five elements (symbolised by circles): fish, large and small predatory invertebrates, detritivores and herbivores, algae, organic matter, fungi and bacteria. Trophic linkages within the web are indicated by lines and arrows between circles. The circle diameter is proportional to the abundance of each group and the thickness of lines and arrows to the intensity of interactions (adapted from Hildrew, 1996).
Despite the lower fungal diversity in acidic hyporheic environments, leaf decomposition was relatively less affected by acidification in this environment compared with the bentic one, which was supported by the maintenance of a substantial biomass (Fig. 4) and activity (Fig. 6) of fungal decomposers. This argues for a lower sensitivity of aquatic fungi to the present physical and chemical stressors, occurring at the functional but not structural level. A distinction must however be made between the metabolic activity oriented towards the maintenance of mycelial biomass, the reproductive activity (i.e., production of conidia) and the enzymatic activities leading to the production of FPOM and leaf decomposition (Suberkropp and Klug, 1980). All activities do not respond equally to different levels of stressors, with the maintenance of fungal biomass or reproductive activity being sometimes reported as insensitive to or even stimulated by certain stressor levels (e.g., acidification, Baudoin et al., 2008; copper contamination, Roussel et al., 2008). The less severe effects of moderate pollution on fungal biomass and degradational activity than on fungal diversity have been reported in a review (Krauss et al., 2011), suggesting the occurrence of some compensation by resistant strains or species. The subsistence of active fungal decomposers in environments unoccupied by invertebrate detritivores represents insurance for impaired stream ecosystems to maintain carbon fluxes, with the hyporheic compartment acting as an important source of organic matter and propagules to downstream. More importantly, this biodiversity potentially confers resilience of the stream ecosystem functions to natural and anthropogenic disturbances. Nonetheless, the biodiversity and ecosystem services from the hyporheic zone are generally under-estimated, jeopardising their effective protection and wise management (Boulton et al., 2010).

5. Conclusion

In both bentic and hyporheic zones, a significant decrease in leaf decomposition rates was observed under acidic conditions. Our study provides evidence that this reduced decomposition of leaves in acidified streams was not primarily due to changes in fungal biomass. Indeed, fungal biomass showed no particular trend regarding the acidification gradient, except that maxima were higher in the most acidic stream, likely resulting from the depressed competition and predation by shredders. In parallel, the structure of aquatic hyphomycetes and leaf shredding macroinvertebrate communities was strongly modified in acidic conditions. Interestingly, leaf litter decomposition was less affected by acidification in the hyporheic zone compared with the bentic one, probably due to the lower sensitivity of at least some fungal decomposer taxa. The subsistence of active fungal decomposers in environments unoccupied by invertebrate detritivores is a strong insurance for impaired stream ecosystems to maintain carbon fluxes. Despite widespread interest in the concept of stream health (Meyer, 1997), the notion of hyporheic health has received little attention (Boulton et al., 2008). If an intact hyporheic zone may underpin stream health in some streams, assessment of ecological health of hyporheic zone of reaches where the streams are impacted by human activities is a logical direction for management applications (Boulton et al., 2010). Typically, river restoration focused on surface systems and their longitudinal and lateral connections, whereas the vertical dimension was largely ignored (Ward et al., 2001; Boulton, 2007). Within the Water Framework Directive, subsurface water is identified as a resource that requires specific management attention. In the light of our findings and in line with a new direction emerging since the last decade, we think that assessing the ecological integrity of streams by considering the health and good functioning of the hyporheic zone, in a manner equivalent to that already practiced or proposed for surface waters, is needed in a whole-ecosystem management perspective.

Statement of authorship

J.C., F.G., E.C., A.E. and C.P. designed the experiments, J.C. and H.C. carried out the experiments, and J.C., A.E., E.C. and F.G. wrote the manuscript.

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