ALTERATION OF LEAF DECOMPOSITION IN COPPER-CONTAMINATED FRESHWATER MESOCOSMS

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Abstract—The influence of copper on leaf litter decomposition was examined in experimental streams. Controls and three levels of contamination (5, 25, and 75 μg/L) were tested in triplicate in 20-m-long mesocosms. Equal quantities of alder, maple, and oak leaves were enclosed in mesh bags and placed in the upper and lower mesocosm sections that exhibited different habitat characteristics (shallow with pebble substrate or deep with fine substrate and macrophytes, respectively). Decomposition rate in the upper section of the 75 μg/L mesocosms was significantly reduced to 28% of control values. No significant reductions in the decomposition rate were seen in the lower section. No differences in the leaf-associated mycelial biomass and sporulation rates were observed between treatments, but they were consistently higher in the upper sections. The aquatic hyphomycete community was not affected by the contamination. The abundances of total leaf-associated macroinvertebrates and the dominant shredder (*Gammarus pulex*) were considerably reduced in the upper sections at 75 μg/L. Therefore, the reduced leaf decomposition probably resulted from a reduction in the abundance of macroinvertebrate detritivores. In addition, local variations in physical and biological characteristics of the habitat along the mesocosms significantly influenced the effects of copper on leaf-associated decomposers.

Keywords—Copper Aquatic mesocosms Leaf litter decomposition Aquatic hyphomycetes Macroinvertebrates

INTRODUCTION

The decomposition of leaves shed from the riparian vegetation plays a major role in carbon and nutrient cycling in streams. It is a central part of the trophic dynamics and biogeochemistry of these ecosystems [1–3]. The decomposition process has been subdivided into three subprocesses—leaching, colonization by fungi and bacteria, and invertebrate feeding—that partly overlap and interact [4,5]. Aquatic fungi produce a wide array of enzymes, leading to the partial degradation of refractory plant material into compounds that can be digested by detrital feeders [2]. They convert nonpalatable material into microbial tissue of high dietary value [6]. Because of its crucial role in carbon and nutrient cycling and the involvement of organisms at different organizational levels, leaf litter decomposition has been proposed as a measure of the functional integrity of stream ecosystems [4]. Decomposer communities were suspected to be highly sensitive to copper pollution. Copper sulfate has fungicidal properties and has been widely used in the form of the Bordeaux mixture for more than a century to prevent mildew on grape vines. The runoff from vine parcels is one of the major sources of copper pollution in freshwater streams in addition to the contamination from industrial, urban, and other agricultural activities. Total copper concentrations in surface waters have been shown to range mostly from 0.2 to 30 μg/L [7].

Coal mine effluents with high concentrations of iron, manganese, magnesium, calcium, and nickel were found to strongly affect both the structure and activity of aquatic hyphomycete communities and leaf decomposition [8], but Abel and Barlöcher [9] showed that the in vitro growth and reproduction of these fungi were inhibited by low concentrations of a mixture of cadmium, copper, and zinc. Moreover, Schultheis et al. [10] reported a strong reduction in the leaf decomposition rate of streams receiving acid mine effluents with moderately high copper concentrations.

The present study aimed to evaluate the effects of copper on leaf litter decomposition and the associated decomposer communities at three environmentally realistic concentrations. The litter bag technique was used to assess the functional and structural status of the ecosystem. It also permitted us to compare the impact of copper on two habitats with different characteristics (i.e., pebbles or fine sediments). The present study was conducted using experimental streams (i.e., mesocosms) that are an ideal tool to investigate the long-term effects of copper on leaf litter decomposition. Although smaller and less complex than real-world ecosystems, mesocosms allow the investigation of ecological problems through properly replicated experiments that are more easily managed in terms of costs and logistics [11,12].

MATERIALS AND METHODS

Mesocosms

The experiment was performed in 12 outdoor mesocosms located at the INERIS Institute (Verneuil-en-Halatte, France)

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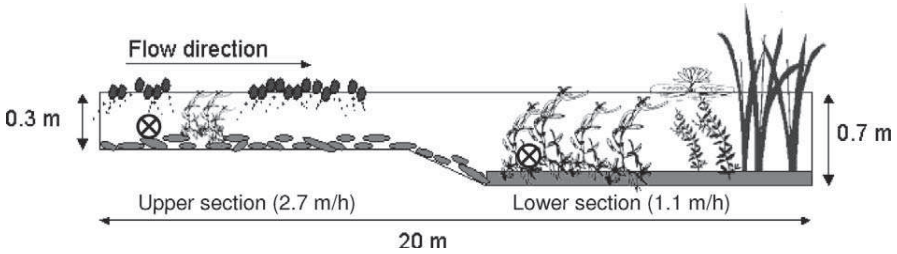

Fig. 1. Schematic view of one mesocosm, current speed, and location of litter bags in the upper and lower sections. The upper section is characterized by pebbles and floating macrophytes and the lower section by fine sediment and rooted, submerged macrophytes.

[13]. The mesocosms channels were 20 m in length, 1 m in width, and 30 cm in depth in the first half (upper section) and 70 cm in the remaining half (lower section) (Fig. 1). The substratum of the upper and lower sections was made of pebbles and fine sediment, respectively. Initially, each mesocosm was set up with equal amounts of natural sediment, aquatic plants, phytoplankton, periphyton, benthic and pelagic invertebrates, and fish (*Gasterosteus aculeatus* L.) collected from a nearby, unpolluted stream. Care was taken to ensure equal stocking densities in each mesocosm. The fine sediment layer was made with a mixture of clay (65%), sand (14%), and sediment collected from the stream. To ensure a natural microbial inoculum, approximately 10 L of decomposing leaf litter collected from the same stream were introduced into each mesocosm within the first 2 m of the upper section. The mesocosms were set up gradually from September to December of 2001 and left undisturbed until the beginning of the decomposition assay (March 25, 2002). Mesocosms were sampled before the start of the present study, and the animal, plant, and microbial assemblages were compared across mesocosms to check for similarity [13]. The upper section was covered with a few floating macrophytes (*Nasturtium officinale* R. Br.), whereas the vegetation in the lower section was dense and consisted of *Callitriche platycarpa* Kütz., *Myriophyllum verticillatum* L., *Nymphaea alba* L. and *Iris pseudacorus* L.

Each mesocosm was fed by dechlorinated water from a drinking water treatment plant continuously supplemented with a copper solution via a peristaltic pump and an automatic control device that provided good oxygenation and continuous exposure to copper throughout the experiment. The flow of each individual mesocosm was 800 L/h. Copper sulfate pentahydrate ($CuSO_4$, $5H_2O$; Acros Organics, Noisy le Grand, France) was added to give three copper concentrations (5, 25, and 75 μg/L) in triplicate, whereas three control mesocosms remained uncontaminated. Copper treatments were randomly assigned within each of three blocks of four mesocosms. Copper contamination started on April 15, 2002, which was 10 weeks after the immersion of litter bags, and was kept constant until the end of the decomposition experiment.

Water and sediment quality

Concentrations of dissolved copper and other elements were determined every week for the first month and then every two weeks for the remainder of the experiment. For 8 of 12 mesocosms (i.e., two per treatment), water samples were taken at 5, 10, 15, and 19 m at a depth of 20 cm and then pooled. For the four remaining mesocosms (i.e., one per treatment), water samples taken at 5, 10, 15, and 19 m were analyzed separately to evaluate the spatial distribution of contamination.

Dissolved copper was analyzed by graphite furnace atomic absorption spectrometry (Varian SpectrAA 220 Zeeman, Les Ulis, France) following the norm NF EN ISO 15586.

Average effective concentration was calculated for each mesocosm using the integration method from Van Wijngaarden et al. [14]. Copper concentrations were determined at six sampling occasions.

Water quality was followed in each mesocosm from pooled samples (as for copper determination) every week during the first month of contamination. Because the water quality was relatively constant, the sampling frequency was extended to every two weeks up to the end of the decomposition experiment. Determinations included pH, temperature, conductivity, dissolved oxygen, aluminum, chlorine, iron, silicon, calcium, magnesium, sodium, potassium, NH_4^+, $CaCO_3$, NO_3^-, PO_4^{3-}, SO_4^{2-}, suspended matter, and total and dissolved organic carbon. Temperature, pH, conductivity, and dissolved oxygen concentration were determined separately in the upper and lower mesocosm sections.

Before contamination and after three months of exposure, sediment cores were sampled at 11, 13, 15, 17, and 19 m in each mesocosm and then pooled to analyze the total copper concentration by inductively coupled plasma–atomic emission spectrometry after mineralization by acid digestion (Jobin Yvon Horiba, Ultima, France) following the norm NF EN ISO 11885.

Litter bags

To evaluate the effect of copper and habitat type on leaf decomposition and decomposer activity, the litter bag technique was used, and two main end points were assessed [4]. Leaf decomposition rate was used as an indicator for the functioning of the ecosystem, whereas qualitative information regarding the leaf-associated fungal and invertebrate communities provided an index of the ecosystem structure [4].

Leaves of alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus robur* L.), and maple (*Acer campestre* L. and *Acer pseudoplatanus* L.) were collected in December 2001 from an uncontaminated nearby forest (Forêt de Lavergny, France). They were gently rinsed and dried for 3 d at 60°C. Leaves were introduced into bags made of a coarse mesh (1 cm) net allowing access to macroinvertebrates at the top and a fine mesh (250 μm) net retaining leaf particles at the bottom. Each bag contained 3 g of an equal mix of dry leaves from the four species. Two sets of six litter bags were placed in each mesocosm. The first was placed in the upper section, 2 m from the inlet and at a depth of 30 cm, with pebbles and scarce vegetation, and the second was placed in the lower section, 13 m from the inlet and at a depth of 70 cm, with fine sediment and dense vegetation. This was done to evaluate the influence of the physical characteristics of the habitat.

The leaf bags were immersed on February 4, 2002, and collection started seven weeks later and continued every two weeks until week 17. Copper contamination started 10 weeks after leaf immersion to verify that no deviation in decomposition rates and associated parameters occurred among mesocosms before contamination.

At each sampling date, one bag was collected at the upper and lower sections of each mesocosm. The bag contents were then transferred into white trays to separate macroinvertebrates from the leaf litter and gently wash sediment particles adhering to leaves. Macroinvertebrates from each bag were sorted under a dissecting microscope; identified at the species, family, class, or order levels; and preserved in 10% formaldehyde (Acros Organics, Noisy-le-Grand, France). The leaf material was separated into four visually equal portions. The first portion was preserved in case it was needed for further analyses. The second portion was frozen (−20°C) and stored for later fungal biomass determination. The third portion was dried at 105°C for 7 d, weighed, and then placed into a muffle furnace at 550°C for 3 h to determine its organic content. The last portion was incubated in 500-ml Erlenmeyer flasks containing 150 ml of uncontaminated filtered water (pore size, 1.6 μm; Whatman GF/A, Maidstone, Kent, UK) over 48 h at ambient temperature (20 ± 2°C). Samples (2 ml) of the incubated medium were then collected and filtered through Whatman cellulose-acetate membrane filters (pore size, 5 μm). The filters were stained with 0.01% trypan blue in 60% lactic acid and stored at 4°C until microscopic identification and counting of conidia (i.e., asexual fungal spores). The incubated leaves were removed from the flasks, dried, and weighed. The surface areas of leaves from the first, second, and fourth leaf portions were scanned and measured using specially designed software (Mesurim 2.4 for Windows®; Académie d'Amiens, Amiens, France). The ratio of leaf surface to dry mass from the fourth portion was calculated to determine the dry mass of the first and second portions. The leaf ash-free dry mass remaining was used to calculate the exponential decay coefficient (k; i.e., decomposition rate) using nonlinear regression analysis [15] with the calculations based on the four sampling dates occurring after the beginning of the contamination (t_0). Because no difference was found in the loss of leaf mass between the controls and copper treatments at both t_{-1} and t_1, the mass at t_0 was calculated as the average of the values determined at t_{-1} (1 week before the beginning of contamination) and t_1 (1 week following contamination).

Ergosterol was used as an index of mycelial biomass [16]. Determinations were performed on lyophilized leaf samples by high-performance liquid chromatography following the procedure described by Gessner et al. [17]. Fungal community structure and reproductive output were evaluated from the conidia released from the incubated leaves. Only aquatic hyphomycetes were identified to the species level using specialized literature. Conidial production relied on spore counts in microscope fields (magnification, ×100–200), the mass of incubated leaves, and the duration of incubation.

Statistical analysis

Differences in the spatial distribution of dissolved copper during the experiment were tested by using a two-way analysis of variance (ANOVA) with copper treatment and location within mesocosm as factors. Differences in the physical and chemical characteristics of the water between control and treated mesocosms were analyzed using one-way ANOVA per sampling date.

The null hypothesis of no difference in leaf decomposition rate between the four contamination treatments and between the two locations within mesocosms was tested by analysis of covariance (ANCOVA) with time as continuous predictor in the log-transformed relationships. The Bonferroni post hoc test was used to determine which treatment differed from the others.

Sporulation rate, mycelial biomass, and abundance of macroinvertebrates were compared using two-way, split-plot ANOVAs with mesocosm (i.e., copper concentration) and habitat type (i.e., upper and lower mesocosm sections) as the main plot and the subplot factors, respectively.

Data were $\ln(ax + 1)$ transformed, where x stands for abundance numbers [18]. The value of a was selected in such a way that when the lowest value greater than zero was chosen for x, ax yields a value of two. This transformation that provided an approximately normal distribution of the data was especially appropriate, because some of the observed values were equal or close to zero [19].

All the above analyses were done using Statistica® software (Ver 6.0; Statsoft, Tulsa, OK, USA).

The Williams test, which was performed on the sporulation rate, mycelial biomass, and abundance of macroinvertebrates, was used to compare treated and control mesocosms at each date and per habitat [20]. This test assumes that the mean response of the variable is a monotonic function of dose (i.e., an increasing effect for an increasing dose) [21], and the results were considered to be valid when the same treatment effect was observed for at least two consecutive sampling dates [20]. The test was performed using Toxstat® software (release 3.0) [22]. To satisfy the normality and homogeneity of variance assumptions, data were $\ln(ax + 1)$ transformed, as previously explained. All end points were checked for similarity among mesocosms in the pretreatment period (t_{-3} [3 weeks before the beginning of contamination] and t_{-1}). For all analyses, differences were considered to be significant at $p < 0.05$.

RESULTS

Water and sediment analysis

Copper concentration in all mesocosms before contamination was less than the detection limit (<0.5 μg/L). No differences occurred in the spatial distribution of copper along the mesocosms (treatment × spatial distribution: $df = 6, 36$; $F = 0.44$; $p = 0.85$). The average effective copper concentrations (mean ± standard error) over the duration of the experiment were 4.7 ± 0.1, 21.2 ± 0.2, and 72.8 ± 3.5 μg/L for the expected concentrations of 5, 25, and 75 μg/L, respectively. Copper concentrations in control mesocosms were less than the detection limit (<0.5 μg/L). For simplicity, treatments will be referred to as nominal copper concentrations.

The chemical and physical characteristics of the mesocosm water during the experimental period are presented in Table 1. No effect of the copper treatments was detected on any of the water-quality variables at any sampling date (one-way ANOVA: $df = 3, 8$; $F < 0.7$; $p > 0.05$). The rather wide range observed for sulfate resulted from the treatment by the supplying water plant. It was, however, consistent among the mesocosms.

The chemical and physical characteristics measured at the upper and lower sections were similar with the exception of the dissolved oxygen concentration, which was higher in the

Table 1. Physical and chemical characteristics of water in all mesocosms over the experimental period (April 15–June 3, 2002)[a]

Quality parameter	Mean	Standard deviation
Temperature (°C)	12.1	2.0
Dissolved oxygen (mg/L)	11.1	1.8
pH	7.8	0.2
Conductivity (μS/cm)	699	35
$CaCO_3$ (mg/L)	245	25
PO_4^{3-} (mg/L)	<0.05	
NH_4^+ (mg/L)	0.10	0.17
SO_4^{2-} (mg/L)	4.57	6.21
NO_3^- (mg/L)	1.11	1.46
Al (mg/L)	<0.01	
Fe (mg/L)	<0.02	
Si (mg/L)	5.0	0.2
Ca (mg/L)	112	3.7
Na (mg/L)	10.4	0.8
Mg (mg/L)	11.2	0.3
K (mg/L)	3.0	0.6
Cl (mg/L)	66.0	18.0
Suspended matter (mg/L)	<4	
DOC (mg/L)	2.1	1.3
TOC (mg/L)	2.3	1.4

[a] DOC = dissolved organic carbon; TOC = total organic carbon.

upper sections (12.2 mg/L) than in the lower ones (10 mg/L; one-way ANOVA: $df = 1, 22$; $F = 209$; $p < 0.001$).

The background copper concentration in the sediment was 17 ± 1.2 mg/kg dry mass and was similar for all the mesocosms before contamination. After three months of contamination, the sediment copper concentrations were 15.6 ± 0.5, 20.2 ± 0.2, 37.2 ± 1.1, and 64.3 ± 10.6 mg/kg for the control and the 5, 25, and 75 μg/L treatments, respectively.

Decomposition rate

No variation in decomposition rates occurred between treatments before contamination (ANCOVA: $df = 3, 19$; upper section, $F = 1.66$, $p = 0.21$; lower section, $F = 0.61$, $p = 0.62$). After contamination, the ANCOVA for the upper mesocosm sections showed a significant effect of copper on the leaf decomposition rate in the 75 μg/L treatment, which differed from both the control and 5 μg/L treatment (ANCOVA: Bonferroni post-hoc test; $df = 3, 43$; $p < 0.05$) (Table 2). Decomposition rates for the 25 and 75 μg/L treatments were 61 and 28%, respectively, of the control values (Table 2). By contrast, no significant effect was found in the lower mesocosm sections, although a slight, but not significant, increase in k was observed in the 5 and 25 μg/L treatment mesocosms. A slight, but not significant, decrease was observed at the highest copper concentration in the lower sections, with the decomposition rate representing 73% of the control value (Table 2). Before contamination, decomposition rates were significantly lower in the lower sections of the mesocosms (ANCOVA: $df = 1, 9$; $F > 19$; $p < 0.002$; for each of the four treatments). After contamination, the decomposition rate in the lower sections also was lower than in the upper sections for control and the low and medium treatments (ANCOVA: $df = 1, 21$; $F > 39$; $p < 0.001$). For example, in the control mesocosms, the ratio of k in the lower versus upper sections was 47%. By contrast, the decomposition rate in the lower section for the 75 μg/L treatment was 20% higher than that in the upper section (ANCOVA: $df = 1, 21$; $F = 45.2$; $p < 0.001$). In general, the effect of copper in reducing the decomposition rate was much greater in the upper than in the lower mesocosm sections.

Fungi

A total of 12 hyphomycetes species were identified (Table 3). The dominant species were *Alatospora acuminata* Ingold, *Tetracladium marchalianum* de Wildeman, and *Anguillospora longissima* (Saccardo & Sydow) Ingold.

Before contamination, the mycelial biomass, expressed as μg ergosterol/mg leaf dry mass, and the sporulation rate showed no difference between mesocosm treatments (split-plot ANOVA and Williams test).

Before contamination, the sporulation rate was significantly higher in the upper mesocosm sections (split-plot ANOVA: $df = 1, 32$; $F = 28.9$; $p < 0.001$). After contamination started, the sporulation rate was significantly affected by the habitat type (split-plot ANOVA: $df = 1, 78$; $F = 58.9$; $p < 0.001$), with lower rates occurring in litter bags placed in the lower sections. The latter represented 14, 10, 11, and 21% of the values determined in the upper sections for the control and the 5, 25, and 75 μg/L treatments, respectively. Leaves from the 75 μg/L treatment mesocosms exhibited significantly higher sporulation rates at t_1 and t_3 (3 weeks following contamination) in the upper section (Williams test) (Fig. 2).

Before contamination, the mycelial biomass was not affected by the habitat type (split-plot ANOVA). After contamination started, the mycelial biomass was significantly affected by the habitat type, with the lower sections exhibiting lower biomass (split-plot ANOVA: $df = 1, 78$; $F = 4.2$; $p = 0.04$). The interaction between treatment and habitat type was significant (split-plot ANOVA: $df = 3, 78$; $F = 3.1$; $p = 0.03$), with the controls exhibiting a low mycelial biomass in the lower sections and the 75 μg/L treatment a low mycelial biomass in the upper sections. Mycelial biomass was not significantly affected by copper contamination in the upper and lower sections at any sampling date (Williams test) (Fig. 3).

Macroinvertebrates

A total of seven leaf-associated macroinvertebrate taxa were identified. The most abundant were *Gammarus pulex* L.,

Table 2. Leaf decomposition rate (k) determined for each treatment at the upper and lower mesocosm sections[a]

Treatment (μg Cu/L)	Upper section		Lower section	
	k (1/d)	$k_{treated}/k_{control}$	k (1/d)	$k_{treated}/k_{control}$
0	0.0086 ± 0.0014 A	100	0.0040 ± 0.0007 A	100
5	0.0095 ± 0.0018 A	110	0.0044 ± 0.0012 A	109
25	0.0053 ± 0.0011 AB	61	0.0045 ± 0.0013 A	112
75	0.0024 ± 0.0011 B	28	0.0029 ± 0.0010 A	73

[a] Values are presented as the mean ± asymptotic standard error. Ratio (%) of rates between the treated ($k_{treated}$) and control ($k_{control}$) mesocosms also are shown. For each section, rates with different uppercase letters are significantly different ($p < 0.05$).

Table 3. Mean sporulation rate and relative abundance of aquatic hyphomycete species for each treatment in the upper and lower sections over the four sampling dates after the beginning of contamination

	Upper section				Lower section			
Treatment (μg Cu/L)	0	5	25	75	0	5	25	75
Sporulation rate (conidia produced/mg leaf dry mass/h)[a]	6.5 ± 6.1	12.0 ± 11.6	12.0 ± 10.8	20.0 ± 22.4	1.0 ± 1.6	1.6 ± 2.2	1.3 ± 2.2	4.5 ± 4.8
Relative abundance (%)								
Alatospora acuminata Ingold	41	33.7	57.9	42.3	40.7	27.8	72.2	41.8
Anguillospora longissima (Saccardo & Sydow) Ingold	26	23.6	9	13.3	42.4	55.7	14.9	41.3
Tetracladium marchalianum de Wildeman	23.6	11.9	27.7	35.8	6.2	11.8	7.1	15.6
Unidentified filiform	7.4	28.9	4.4	5.3	8.8	4.5	4.9	0.8
Tricladium angulatum Ingold	0.7	1.2	0.8	2.2	0	0	0	0.4
Clavariopsis aquatica de Wildeman	0.5	0.1	0.1	0.1	0	0.2	0	0
Tetracladium setigerum (Grove) Ingold	0.2	0.1	0.2	0	0	0	0	0.2
Lemonniera aquatica de Wildeman	0.2	0	0.1	0.1	0	0	0	0
Tripospermum myrti (Lind.) Hughes	0.2	0	0	0	0	0	0.2	0
Articulospora tetracladia Ingold	0.1	0	0	0.1	0	0	0.2	0
Tricladium chaetocladium Ingold	0	0	0	1	0	0	0	0
Unidentified triradiate	0	0	0	0	1.9	0	0.4	0

[a] Values are presented as the mean ± standard deviation.

chironomids, and gastropods (Order Basommatophora: *Lymnaea peregra* (Müller) and *Physa* sp.) (Table 4). Before contamination, no differences in the abundance of macroinvertebrates associated with leaf litter were found between the mesocosm treatments (split-plot ANOVA: $df = 3, 32$; $F = 0.3$; $p = 0.8$), but higher abundances were observed in the upper sections (split-plot ANOVA: $df = 1, 32$; $F = 38.6$; $p < 0.001$).

The abundance of macroinvertebrates was significantly modified by the copper treatments (split-plot ANOVA: $df = 3, 6$; $F = 14.5$; $p = 0.004$), and the interaction between treatment and habitat was significant (split-plot ANOVA: $df = 3, 80$; $F = 3.8$, $p = 0.01$). In the upper mesocosm sections, the highest copper concentration (75 μg/L) showed a significantly lower abundance than the control at t_3 and t_5 (5 weeks following contamination; Williams test) (Fig. 4). The abundance of macroinvertebrates in the lower mesocosm sections fluctuated through time, and no significant differences between treatments were detected at consecutive sampling dates. In the lower sections, a higher abundance of chironomid larvae coincided with lower densities of *G. pulex* (Table 4). The gammarid shredders (*G. pulex*) and the gastropod scrapers (Order Basommatophora: *L. peregra* and *Physa* sp.) were considerably reduced in the upper sections at the 75 μg/L treatment. By contrast, the chironomids and oligochaetes increased in the 75 μg/L treatment in the upper sections. *Asellus aquaticus* L. dominated the shredder assemblage at the highest copper concentration in the lower sections. *Asellus aquaticus*, chironomids, and oligochaetes were more abundant in the bags placed in the lower mesocosm sections.

DISCUSSION

The present study aimed to assess the effects of environmentally realistic concentrations of copper on leaf litter decomposition in two different habitats. Sediment and water quality, other than the copper concentrations, did not differ between the mesocosms; thus, the observed effects can be attributed to direct or indirect copper toxicity. In addition, the average effective concentration were remarkably close to the nominal concentrations.

Decomposition rate

The present results showed a pronounced decrease in the decomposition rate of the upper mesocosm sections at the copper concentration of 75 μg/L. Similarly, Schultheis et al. [10] reported a decomposition rate decrease of 45 to 55% for copper concentrations ranging from 12 to 32 μg/L. A direct effect of copper toxicity was partially involved because of the acidic conditions (pH ≈5.6) prevailing in their studied stream, a fact that has been reported to substantially increase copper toxicity [23]. Because the pH was close to 7.9 in the present study, such an effect was not expected at these concentrations, whereas a decrease in the decomposition rate of up to 72% occurred in the upper mesocosm sections after only seven weeks of exposure to copper at 75 μg/L. These findings generally are consistent with those reported by Niyogi et al. [24] for mine drainage, showing sustained community functions (production and decomposition) at low to moderate stress levels and a substantial decline at high levels.

Fungi

Other studies of sites exposed to heavy metals [25,26] have attributed the reduction in leaf decomposition rates to a lower

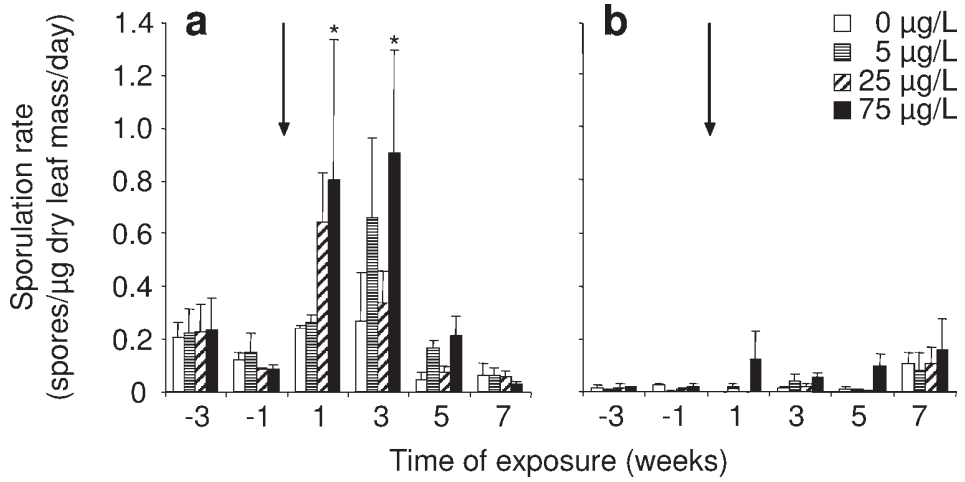

Fig. 2. Sporulation rate (mean ± standard error of mean) from leaves located in the upper mesocosm (**a**) and lower mesocosm (**b**) sections ($n = 3$). Arrows indicate copper contamination. Asterisks indicate significant differences from the controls (Williams test, $p < 0.05$).

activity of microbial decomposers, in particular aquatic hyphomycetes. In our experiment, copper did not affect the leaf-associated aquatic hyphomycete biomass and community structure. This may result from the short time of exposure (seven weeks) and the relatively low chronic copper concentrations. Whether aquatic hyphomycetes adapted to copper exposure or copper was present at nontoxic doses remains questionable. Fungi, including aquatic hyphomycetes, develop tolerance to heavy metals by synthesizing sulfur-rich compounds and peptides derived from glutathione (phytochelatine) [27,28]. Some extracellular copper-complexing ligands help to detoxify copper in the fungal environment and, thereby, to regulate the concentration of copper to biologically optimal levels [29].

The fungal sporulation rate showed an increase at 75 µg/L of copper in both sections. This phenomenon may be the indirect result of the decrease of macroinvertebrate predation [30], the lower intraspecific competition [31], or an experimental artefact (i.e., the induction of spore production by fungal mycelia in response to the contamination-free water used in the incubation). Invertebrates actually are shown to constrain fungal biomass and activity through competition for substrate and direct predation [32]. Therefore, the macroinvertebrates predation hypothesis seems to be plausible.

Macroinvertebrates

Evidence existed to support the view that the lower decomposition in the upper section exposed to 75 µg/L of copper was associated with a decrease in macroinvertebrates and changes in the community composition. Schultheis et al. [10] found that copper pollution greater than 12 µg/L interrupts the activity of leaf-shredding invertebrates, although the conditions in their acidic streams partly diverged from those prevailing in the present study. The decrease in decomposition rate observed in their study was related to direct copper toxicity affecting invertebrate abundance and community composition. A no-observed-effect concentration for copper of 10 µg/L was reported for the feeding rate of detritivorous invertebrates under conditions similar to those in the present study (lotic mesocosms, 35 d of exposure, pH 7.6–8.0) [33]. In addition, an experiment with *G. pulex* response showed a lowest-observed-effect concentration in terms of population density for copper of 14.6 µg/L [34]. These observations are consistent with the findings reported in the present study, and they suggest that a

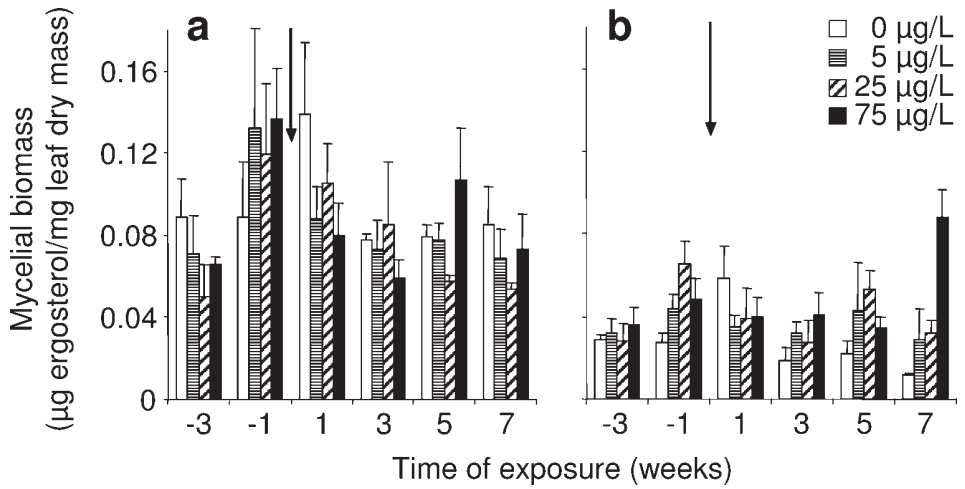

Fig. 3. Mycelial biomass (mean ± standard error of mean) associated with leaves located in the upper mesocosm (**a**) and lower mesocosm (**b**) sections ($n = 3$). Arrows indicate copper contamination.

Table 4. Mean density and relative abundance of invertebrate taxa for each treatment in the upper and lower sections over the four sampling dates after the beginning of contamination

	Upper section				Lower section			
Treatment (μg Cu/L)	0	5	25	75	0	5	25	75
Density (individuals/litter bag)[a]	47 ± 24	52 ± 26	56 ± 22	19 ± 13	46 ± 22	53 ± 31	56 ± 30	39 ± 15
Relative abundance (%)								
Family Chironomidae	45	26	31	77	74	71	60	49
Gammarus pulex L.	37	51	52	9	7	15	16	9
Order Basommatophora	11	17	9	4	10	6	2	4
Subclass Oligochaeta	5	3	5	7	5	4	3	12
Asellus aquaticus L.	1	2	2	2	4	4	18	26
Family Planariidae	1	2	0	0	0	0	1	0
Subclass Hirudinea	1	0	0	0	0	0	0	0

[a] Values are presented as the mean ± standard deviation.

deleterious effect on macroinvertebrates, particularly *G. pulex*, occurred above a threshold as low as 25 μg/L. The significant decrease in the leaf decomposition rate most probably was associated with the loss of this dominant shredder, a phenomenon that Dangles and Guérold [35] reported in acidic streams.

An artefact was observed in the 25 μg/L treatment in which *G. pulex* abundance was 40% higher, but the decomposition rate was decreased by 40%, compared to the control. Such contradictory responses may have two explanations. First, the *G. pulex* population in the 25 μg/L treatment was dominated by juveniles, which, by far, do not present a per capita feeding efficiency as high as that of adults. Second, this population was shown to be lethally affected, with a marked decrease in abundance just after the last litter bag sampling date (data not shown), which argues for a copper sensitivity in this species at a level of 25 μg/L. Before this lethal effect was observed, however, chronic toxicity affecting their feeding rate could have occurred. Forrow and Maltby [36] have reported reductions in the processing rate of leaf litter by macroinvertebrates as an effect of decreases in their abundance or feeding activity because of toxic contamination. This suggests that copper at 25 μg/L probably has affected the feeding activity of *G. pulex* during the course of litter decomposition in our mesocosms.

Habitat

The effects of copper on the decomposition of organic matter varied according to the physical characteristics of the habitat, with the macrophyte-dominated fine sediment habitat (lower sections) having slower decomposition rates and communities that were less sensitive to copper pollution compared with the upper sections. The lower impact of copper in the lower sections may be linked to the macroinvertebrate community structure. In the lower sections, the numbers of leaf-associated chironomids, *A. aquaticus*, and oligochaetes were higher because of the presence of fine sediment and macrophytes. These species are more tolerant than *G. pulex* and basommatophora to copper. The 96-h median lethal concentration of copper for oligochaetes, chironomids, and asellids were reported to be 500, 630, and 9,200 μg/L, respectively [37–39]. Although more resistant, the macroinvertebrate community in the lower section habitat was less effective at leaf decomposition. A lower decomposition rate was measured in the lower sections. Graça et al. [40] showed that *A. aquaticus* scrapes the leaf surface but *G. pulex* bites through the leaf material, with the latter resulting in a higher loss of leaf mass. In addition, the lower sporulation rate and mycelial biomass in the lower section habitat can be explained by the preference of aquatic hyphomycetes for clear, turbulent, and well-aerated waters [2].

In conclusion, leaf decomposition was shown to respond to an increase in copper concentration and, thus, to provide an indication of the impairment in the functioning of the stream ecosystem. After a short-term exposure to copper, the no-ob-

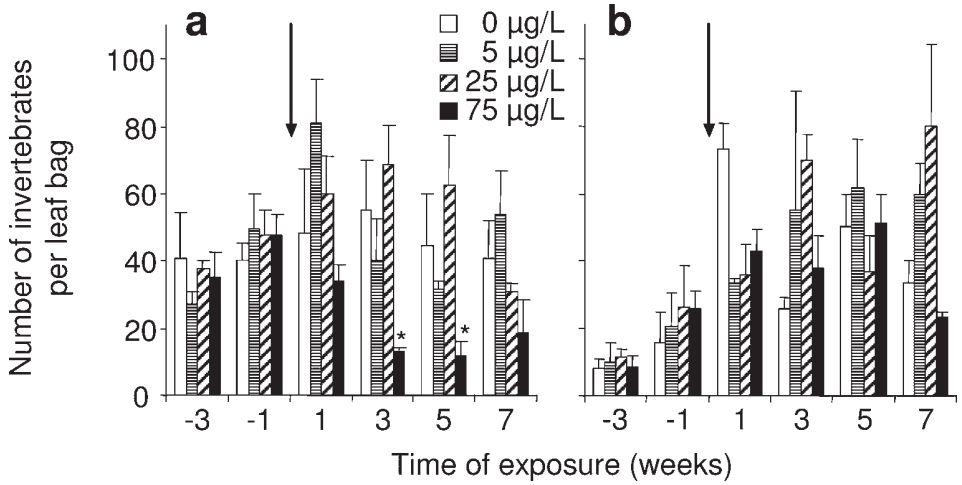

Fig. 4. Macroinvertebrate abundances (mean ± standard error of mean) on leaves located in the upper mesocosm (**a**) and lower mesocosm (**b**) sections ($n = 3$). Arrows indicate copper contamination. Asterisks indicate significant differences from the controls (Williams test, $p < 0.05$).

served-effect concentration for leaf decomposition was 25 μg/L according to the habitat and the associated community structure of the decomposers. Aquatic fungi and macroinvertebrates clearly exhibited different responses, suggesting that coarse and fine mesh bags, like those widely used in decomposition assays, may help to distinguish the effects on and the contribution from the two main decomposer types.

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