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MICROBIOLOGY OF AQUATIC SYSTEMS

Effects of Fungal Inocula and Habitat Conditions on Alder and Eucalyptus Leaf Litter Decomposition in Streams of Northern Spain

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Abstract We investigated how fungal decomposer (aquatic hyphomycetes) communities colonizing alder and eucalyptus leaf litter respond to changes in habitat characteristics (transplantation experiment). We examined the breakdown of leaf materials and the associated fungal communities at two contrasting sites, a headwater stream (H) and a midreach (M). Agroforestry increased from headwater to midreach. One month after the start of experiments at both sites, some leaf samples from the midreach site were transplanted to the headwater site (M–H treatment). Although both sites showed similar dissolved inorganic nutrient concentrations, eucalyptus leaves initially incubated at the midreach site (M, M–H) increased their breakdown rate compared to those incubated along the experiment at the headwater site (H). Alder breakdown rate was not enhanced, suggesting that their consumption was not limited by nutrient availability. Sporulation rates clearly differed between leaf types (alder > eucalyptus) and streams (H > M), but no transplantation effect was detected. When comparing conidial assemblages after transplantation, an inoculum effect (persistence of early colonizing species) was clear in both leaf species. Substrate preference and shifts in the relative importance of some fungal species along the process were also observed. Overall, our results support the determining role of the initial conditioning phase on the whole litter breakdown process, highlighting the importance of intrinsic leaf characteristics and those of the incubation habitat.

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Introduction

Allochthonous organic matter acts as a basal resource in forested headwater streams [50]; the detrital pathway is essential for the sustainability of these ecosystems, as primary production is very limited by nutrient-poor waters and forest shading. Terrestrial inputs, mainly in the form of leaf litter [1], represent the principal source of matter and energy for these systems [51]. The organisms responsible for detritus processing are microbial decomposers [7, 8] and invertebrate detritivores [24, 30], both of which mediate the transfer of energy to higher trophic levels [50]. In these ecosystems, microbial decomposers utilize dead leaves as carbon source while acquiring N and P from the water column [14, 48]. This process, usually defined as microbial conditioning, transforms litter inputs into a more suitable food resource for invertebrates in streams [8]. Inorganic nutrients in the stream water are reported to accelerate leaf decomposition through the stimulation of microbial activity [34, 39], even at low concentrations [37]. Aquatic hyphomycetes are considered to be among the major microbial decomposers of leaf litter [6, 11], although the contribution of bacteria may increase proportionally in eutrophic streams [29, 34]. A low specificity of aquatic hyphomycetes for leaf litter types is assumed because after a long incubation period in the same habitat, fungal assemblages tend to be similar regardless of substrate [7]. Nevertheless, some fungal selectivity has also been observed [27]. Knowledge of how the aquatic microbial community, particularly hyphomycetes, drives the breakdown process of leaf species of different quality is still limited, although very often the native riparian vegetation is substituted by tree plantations that can cause noticeable changes in the quality of leaf inputs into streams (e.g., [42]).

Monoculture plantations of eucalyptus (*Eucalyptus globulus* Labill.) have frequently replaced natural deciduous forests in the Iberian Peninsula in recent decades, causing an

alteration on the timing, quality, and quantity of litter entering the streams [32]. Leaf inputs show a different phenology under eucalyptus plantations than in native deciduous forest [42], with eucalyptus also being a poorer nutrient resource (lower N and P content) [25] than native species such as alder, a highly palatable resource for consumers [31]. It has been shown that eucalyptus leaves can be colonized by fungal assemblages similar to those found in alder [15], although both leaf species may show differences from site to site in terms of species richness and dominance [15, 37]; some clarification on the resulting fungal colonist assemblage on substrates of contrasting quality, and therefore on their decomposition processes, could be gained through transplantation experiments. Considering the enhancing effect of the dissolved nutrient availability on the fungal conditioning [35], especially in the early stages of the processing, when fungal colonization and leaf-quality maxima concur [37], we designed a transplantation experiment to determine the importance of the initial colonizers on the ulterior fungal assemblages and processes [19]. Our transplant was made from an anthropized site to a more oligotrophic one. Although this design is opposite to the more usual trend from oligotrophic to eutrophic conditions, works focusing on the effect of stream eutrophication on aquatic fungi associated with leaf litter breakdown (even without the necessity of transplant experiments) are relatively frequent in the literature, as illustrated above, but the reverse is not true. Our transplant could be, to a certain extent, reflecting conditions after stream restoration, but our knowledge on the effects of fluvial restoration on fungal communities is rather scarce. In this study, we assessed the activity and diversity of fungi on decomposing leaves of two species of different quality, alder (*Alnus glutinosa* (L.) Gaertn.) and eucalyptus (*E. globulus* Labill), incubated in a headwater (H) site and in a more anthropized midreach (M) site and compared them to those from leaves transplanted from the midreach to the headwater (M–H). We hypothesized that the anthropized site (M) would show faster leaf litter breakdown rates, led by a higher fungal activity. The hyphomycete community colonizing the leaf litter at this site would be able to facilitate leaf litter breakdown once transplanted into a pristine site. Finally, we also hypothesized that the leaf litter with lower initial quality, eucalyptus, would be more affected than alder by the fungal conditioning acquired prior to transplantation.

Materials and Methods

Study Sites

The sampling sites were in the Agüera river, which is located in the north of Spain in a rural area with a low population density. This river drains a watershed where forestry and agriculture are the main land uses and flows through small villages, maintaining a good status of conservation in the headwaters. We selected two sites with contrasting water chemistry as a result of increases in urban and agricultural runoff and changes in the catchment geology from one site to the other [17, 33, 40]. The headwater site (H) is located in the Agüera river headwaters in a 3-m-wide first-order stream, Salderrey, at 350 m a.s.l. The stream flows along a siliceous bottom consisting mainly of rocks, pebbles, and gravel, and the riparian vegetation includes *Quercus robur* L., *Alnus glutinosa* (L.) Gaertner, *Castanea sativa* Miller, *Corylus avellana* L., and *Crataegus monogyna* Jacq. The anthropized site (M), at 80 m a.s.l., is 15 km downstream from the spring and below a calcareous belt in the catchment and two urban areas located close to the main course of the Agüera river (Fig. 1). There, the Agüera is a third-order stream about 10 m wide. This midreach section flows over siliceous bedrock dominated by large rocks, with alternating riffles and pools, and is bordered by a mature deciduous forest, including alder, oak, and sycamore (*Platanus hybrida* Brot.) and surrounded by eucalyptus (*E. globulus* Labill) plantations. The geology and the impairment of the catchment from site H to site M cause increases in conductivity (from 109.9±1.8 to 241.6±4.1 μS cm^{-1}), pH (from 7.21±0.04 to 8.39±0.04), and dissolved phosphorus (from 5.1±0.4 to 16.1±1.3 μg P l^{-1}) (mean values ± 1 SE derived from different sampling dates (n = 62–130) spanning more than a decade prior to our study period (December 1988 to January 2001) and reported by the references above).

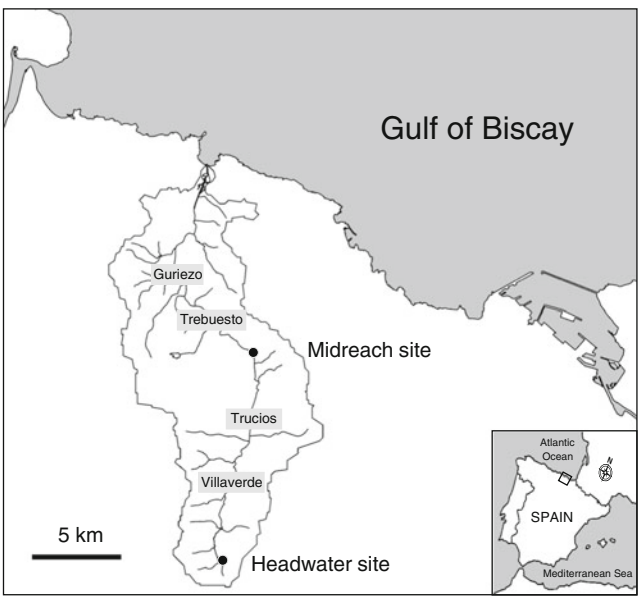

Fig. 1 Location of the headwater reach and the midreach site (below two small villages, Villaverde and Trucios) in the Agüera river catchment (north of the Iberian Peninsula)

Field Procedures and Transplantation Experiment

Leaves of *Alnus glutinosa* and *E. globulus* were collected from the soil just after natural abscission, and portions of 10–12 g were placed into leaf bags (5-mm mesh size; 20×25 cm). This mesh size was chosen in order to allow the leaf consumption by almost the complete detritivore community, as our aim was to assess the whole decomposition process. Forty-four and 28 leaf bags of each leaf litter species were immersed at M and H, respectively. After 31 days, 16 bags of each species were transplanted from site M to site H (M–H). Four replicate bags of each leaf type were collected from each site after 6, 14, 31, 37, 49, 70, and 103 days of immersion, between 25 October 2003 and 5 February 2004. Furthermore, on the last four sampling dates, four bags of each species of the transplanted materials were taken from site H (M-H). On each sampling date, the retrieved bags were placed in individual ziplock plastic bags and transported to the laboratory in coolers with ice.

The leaf material from each bag was washed with deionized water to remove sediments, and two alder leaves and two fragments of two different eucalyptus leaves were separated for aquatic hyphomycete sporulation (see below). The remaining material was oven-dried (70 °C, 72 h) and weighed (±0.001 g). A portion of the dry material was stored (−20 °C) for later nutrient and phenol content determination (see below). The remaining material was combusted (500 °C, 8 h) and weighed (±0.01 g) to estimate the ash-free dry mass (AFDM). Water temperature was recorded every hour with ACR Smart-Button data loggers. On each sampling date, dissolved oxygen, pH, and conductivity were measured in the field (WTW equipment). Stream water samples were taken for later determination of alkalinity (titration to an end pH of 4.5) and dissolved inorganic nitrogen (DIN, nitrate + nitrite + ammonium) and soluble reactive phosphorus (SRP) concentrations [4].

Sporulation of Aquatic Hyphomycetes

The leaf fragments taken from each bag for fungal sporulation (see above) were incubated in 100-ml Erlenmeyer flasks containing 75 ml of filtered stream water (glass fiber Whatman GF/F filters) on an orbital shaker (60 rpm) for 48 h at 10 °C. The resulting conidial suspensions were transferred into 500-ml plastic storage bottles. Erlenmeyers were rinsed twice with distilled water, and conidia were fixed with 25 ml 37 % formalin; the final volume of 250 ml was achieved by adding distilled water. In order to ensure a uniform conidial distribution, 250 μl of 0.5 % Triton-X-100 was added. An aliquot (2–50 ml, estimated depending on the conidial concentration) was filtered (Millipore nitrocellulose SMWP 5-μm pore size filters) with a manual pump, and the filters were stained with Trypan Blue in lactic acid (final concentration ca. 0.05 %) for identification and counting. Two filters from each sample were studied, and at least 100 conidia per filter were counted. Conidia were scanned under a bright-field microscope at a magnification of ×200 to ×400. The leaf mass used for sporulation (0.21±0.01 and 0.43±0.01 g leaf DM for alder and eucalyptus, respectively; mean ± 1 SE) was determined as described above for the bulk leaf material. Sporulation rates were expressed as number of conidia μg leaf DM^{-1} day^{-1}.

Leaf Nutrient and Phenol Contents

Concentrations of carbon (C), nitrogen (N), phosphorus (P), and phenolic compounds were measured throughout the process. Leaf material was ground to a fine powder (1-mm pore sieve) for chemical composition analyses. Carbon and nitrogen were determined using a Perkin Elmer series II CHNS/O elemental analyser. Phosphorus was determined spectrophotometrically after mixed acid digestion (molybdenum blue method [3]), and phenol compounds were determined following Folin–Ciocalteau methodology [44]. Results were expressed as percentage leaf dry mass.

Data Analysis

Individual *t* tests were used to determine differences in physicochemical variables (temperature, pH, alkalinity, conductivity, percentage of O_2 saturation, and dissolved nutrient concentrations) between the two stream reaches. Breakdown rates of alder and eucalyptus were calculated by linear regression of the remaining AFDM against the incubation period ($M_t = M_0 - bt$), where b is the linear breakdown rate, t is the incubation period in days, and M_0 is the initial mass. A linear model was chosen because it produced better fits than an exponential one (more frequent in literature) when the initial values are not included in the regression due to the high leaching losses in the first days [41]. As there were temperature differences between streams, processing rates were also calculated using degree-days (dd being the sum of mean daily temperatures over the time period considered) in order to take into account the possible effects of water temperature in the process [38]. Breakdown rates of the transplanted materials were determined for the whole series of data (pre-trans at site M + post-trans at site H). Statistical differences in breakdown rates were tested using ANCOVA, either between leaf species for each treatment (M, H, and M–H) or between treatments for each leaf species (comparisons M vs H or H vs M–H, in the last case indicated by the subscript "Trans"). We studied the community of aquatic hyphomycetes (i.e., conidial assemblage) associated with decomposing alder and eucalyptus leaf litter at each sampling date in order to observe taxa richness, diversity (Shannon index), and sporulation rate temporal dynamics. To search for general patterns in associated fungal community structure along the in-stream decomposing process, non-metric multidimensional scaling (NMDS) analyses

were performed based on the Bray–Curtis dissimilarity matrix of mean taxa densities found in each sampling date, alder, and eucalyptus separately. Leaf quality changes and aquatic hyphomycete community performance were compared between the two studied sites (H vs M) to detect any differences between stream reaches or between H and M–H (Trans), in order to determine whether there was a transplantation experiment effect, tested by two-way ANOVA (site or treatment × sampling date). Subsequent pair-wise comparisons were performed using Tukey's tests [52]. Relationships between variables (water parameters, decomposition rates, quality descriptors, and aquatic hyphomycetes) were tested using linear regression analyses. Statistical calculations were performed with SPSS 19.0 (IBM-SPSS) and R statistical program (version 2.13.2 [43]).

Results

Stream Water Characteristics

Analysis of stream water variables during the experiment showed that pH (6.8 vs 7.8), temperature (9.7 vs 11.2 °C), and conductivity (90.3 vs 187.6 μS cm^{-1}) were lower at the headwater site (H) than at the midreach (M) (Table 1). This trend was not found for inorganic nutrient concentrations, which resulted in very similar values (DIN, 869 vs 831 μgN l^{-1}; SRP, 9.7 vs 7.9 μg P l^{-1}).

Leaf Litter Breakdown and Dynamics of Chemical Compounds

Mass loss of alder and eucalyptus leaves fitted a linear decay model over 103 days of leaf immersion in both streams. Alder decomposed faster than eucalyptus at the headwater site (ANCOVA, $F_{1,55}$=57.2, p<0.001) and in M–H treatment (ANCOVA, $F_{1,55}$=11.2, p<0.01), but not at the M site (ANCOVA, $F_{1,55}$=0.7, n.s.) (Fig. 2). There were no significant differences in alder leaf breakdown rate between the two stream reaches (ANCOVA $F_{1,55}$=0.4, n.s.). No transplant effect was detected for alder leaf litter ($ANCOVA_{Trans}$ $F_{1,55}$=1.7, n.s.), the breakdown rates of both treatments (H and M–H) being similar regardless of the incubation site at the initial phases. In contrast, eucalyptus processing was slower at the headwater site (ANCOVA $F_{1,55}$=29.3, p<0.001). The transplanted eucalyptus leaves (M–H) were processed faster than those incubated only at the headwater site ($ANCOVA_{Trans}$ $F_{1,55}$=27.2, p<0.001). When considering the incubation period in terms of degree-days instead of days, all these patterns and differences persisted (data not shown).

At the first sampling (after 6 days in the stream), alder and eucalyptus leaf litter showed slight differences in the studied leaf compound concentrations between sites (Table 2). Along the incubation, there was an overall reduction in carbon and phenolic compound concentrations for both species (Fig. 3). In contrast, leaf N concentration tended to increase, showing statistical differences between incubation sites only for eucalyptus leaves (ANOVA $F_{1,55}$=9.1, p=0.004), with the midreach showing higher N concentrations. These increases in N concentration also represented net immobilization of this element in this material at both sites before the transplantation date, with net mineralization thereafter (data not shown). The changes in leaf phosphorus were more variable between sites, although both species showed higher P concentrations at the midreach site (ANOVA $F_{1,43}$=22.3, p<0.001; ANOVA $F_{1,55}$=62.5, p<0.001, respectively, for alder and eucalyptus). Net immobilization of P only occurred for eucalyptus at site M before the transplantation, with net mineralization thereafter (data not shown). In short, and for both species during the experiment, C and phenolics tended to decrease, N tended to increase, and P tended to increase only at the midreach. In terms of a transplantation effect in leaf compound dynamics, the responses on eucalyptus were clearer than on alder, but there was not a general trend for all variables. Thus, the C concentration of transplanted eucalyptus leaves tended to follow the headwater pattern of this species, whereas phenolic compounds and N followed the midreach trend (Fig. 3). When considering leaf P concentrations, transplanted leaves tended to approach the dynamics of headwater materials, the magnitude of the differences being reduced between sites both for

Table 1 Physical and chemical characteristics of the stream water (Mean ± 1 SE, *n* number of samples) at the sampling sites (H and M) throughout the study period

Parameter	H	M	*n*	*t* test	*p* value
pH	6.85±0.07	7.76±0.02	8	15.5	<0.001
Temperature (°C)	9.71±0.40	11.19±0.36	7	2.8	0.017
O_2 saturation (%)	92.0±2.3	89.3±3.6	6	0.6	n.s.
Conductivity (μS cm^{-1})	90.2±1.1	187.6±4.5	8	21.1	<0.001
Nitrate (μg N l^{-1})	842.3±100.1	810.7±51.6	4	0.3	n.s.
Nitrite (μg N l^{-1})	2.85±0.79	3.50±0.60	4	0.7	n.s.
Ammonium (μg N l^{-1})	26.2±7.2	26.4±8.0	4	0.0	n.s.
Phosphate (μg P l^{-1})	9.72±2.15	7.88±0.78	5	0.8	n.s.

Statistical comparisons between sites (Student's *t*) are given

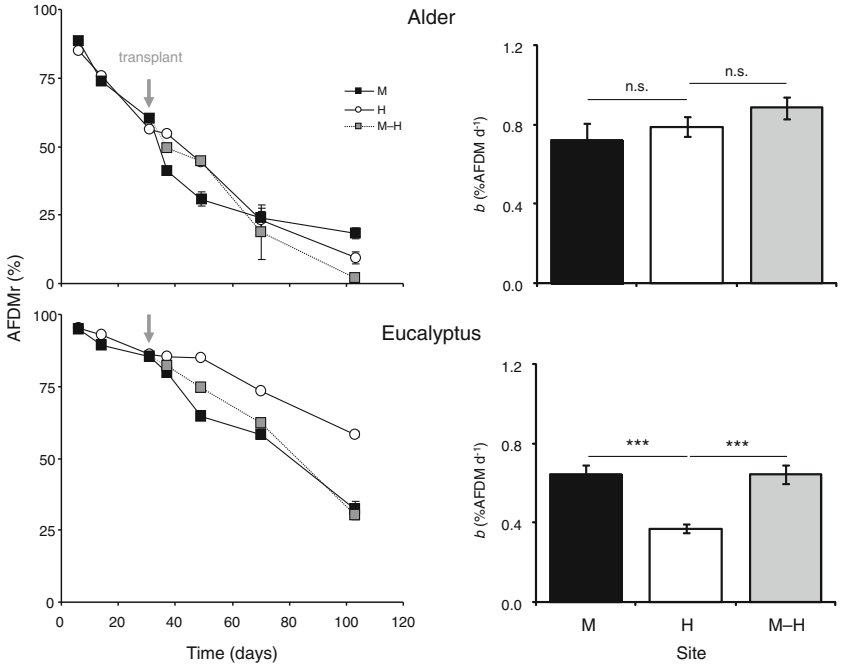

Fig. 2 Ash-free dry mass remaining (AFDMr) of decomposing alder and eucalyptus leaves (mean ± 1 SE, $n=4$) at the midreach site (M), the headwater site (H), and transplanted from M to H (M–H). The *vertical gray arrow* points to the bags' transplantation from the midreach site to the headwater site. Breakdown rates ± 1 SE considering the complete process at the sites and transplant experiment ($n=28$). The statistical results of the breakdown rate comparisons (ANCOVA) between sites (M vs H) and between treatments (H vs M–H) are given in the plot (n.s.: $p>0.05$; ***$p<0.001$)

alder ($ANOVA_{Trans}$ $F_{1,44}=11.9$, $p=0.002$) and eucalyptus ($ANOVA_{Trans}$ $F_{1,55}=5.0$, $p=0.030$). A significantly lower leaf P concentration was, however, always found in leaves maintained at the headwater site (Fig. 3).

Aquatic Hyphomycete Communities

A total of 29 taxa of aquatic hyphomycetes were found in sporulation samples during the study (Table 3), 26 on alder (8 exclusive) and 21 on eucalyptus (3 exclusive). The mean number of taxa identified in the first sampling (after 1 week of incubation) was low, ranging from 0.8 (at H) to 5.5 (at M) per sample, both values being for eucalyptus leaves (Fig. 4). Taxa richness increased until the third to fourth samplings in all cases, leveling off thereafter with the highest taxa richness for alder after 1 month. There were differences between sites (ANOVA $F_{1,55}=13.3$, $p=0.001$; ANOVA $F_{6,55}=39.4$, $p<0.001$, for alder and eucalyptus, respectively), with leaves incubated in the headwater reach having fewer taxa than those incubated throughout the process in the midreach. Transplanted materials tended to approach taxa numbers at the headwater site, although statistically these materials maintained a higher richness than leaves processed in the headwater reach for the whole period (Fig. 4; $ANOVA_{Trans}$ $F_{1,55}=4.6$, $p=0.039$; $ANOVA_{Trans}$ $F_{1,55}=7.9$, $p=0.007$, for alder and eucalyptus, respectively). Significant differences along the process were detected in the diversity of the fungal community associated with alder (ANOVA $F_{6,55}=26.3$, $p<0.001$) and eucalyptus (ANOVA $F_{6,55}=21.5$, $p<0.001$). The conidial assemblages featured very low diversity values in the initial phases of the process (for the pre-trans period, first month of incubation). Thereafter, the diversity index leveled off at high values, coinciding with the higher taxa richness (Fig. 4). Conidial assemblages of both leaf species showed higher diversity values at the midreach than at the headwater site (ANOVA $F_{1,55}=9.5$, $p=0.004$; ANOVA $F_{1,55}=28.7$, $p<0.001$, for alder and eucalyptus, respectively). There were no statistical differences when comparing the diversity of transplanted vs non-transplanted materials in the headwater reach ($ANOVA_{Trans}$ $F_{1,55}=2.5$, $p=0.129$; $ANOVA_{Trans}$ $F_{1,55}=2.3$, $p=0.138$, for alder and eucalyptus, respectively). Especially in eucalyptus, it was clear that the conidial assemblage diversity of the transplanted material followed the same trend as the material kept in the headwaters (Fig. 4). There were major differences in sporulation rates between alder and eucalyptus, although both leaf species responded similarly to habitat conditions (Fig. 4). Fungi showed

Table 2 Leaf litter composition of alder and eucalyptus after the first week of in-stream incubation

Concentrations are given in relation to leaf DM (mean±1*SE*, $n=4$)

Leaf	Site	% Carbon	% Phenolic compounds	% Nitrogen	% Phosphorus
Alder	H	50.4±0.2	5.4±0.5	4.00±0.10	0.099±0.003
	M	48.9±2.3	6.7±1.3	3.85±0.21	0.095±0.004
Eucalyptus	H	54.1±0.3	7.4±0.3	0.88±0.03	0.035±0.004
	M	55.7±0.5	6.2±0.5	0.86±0.06	0.027±0.007

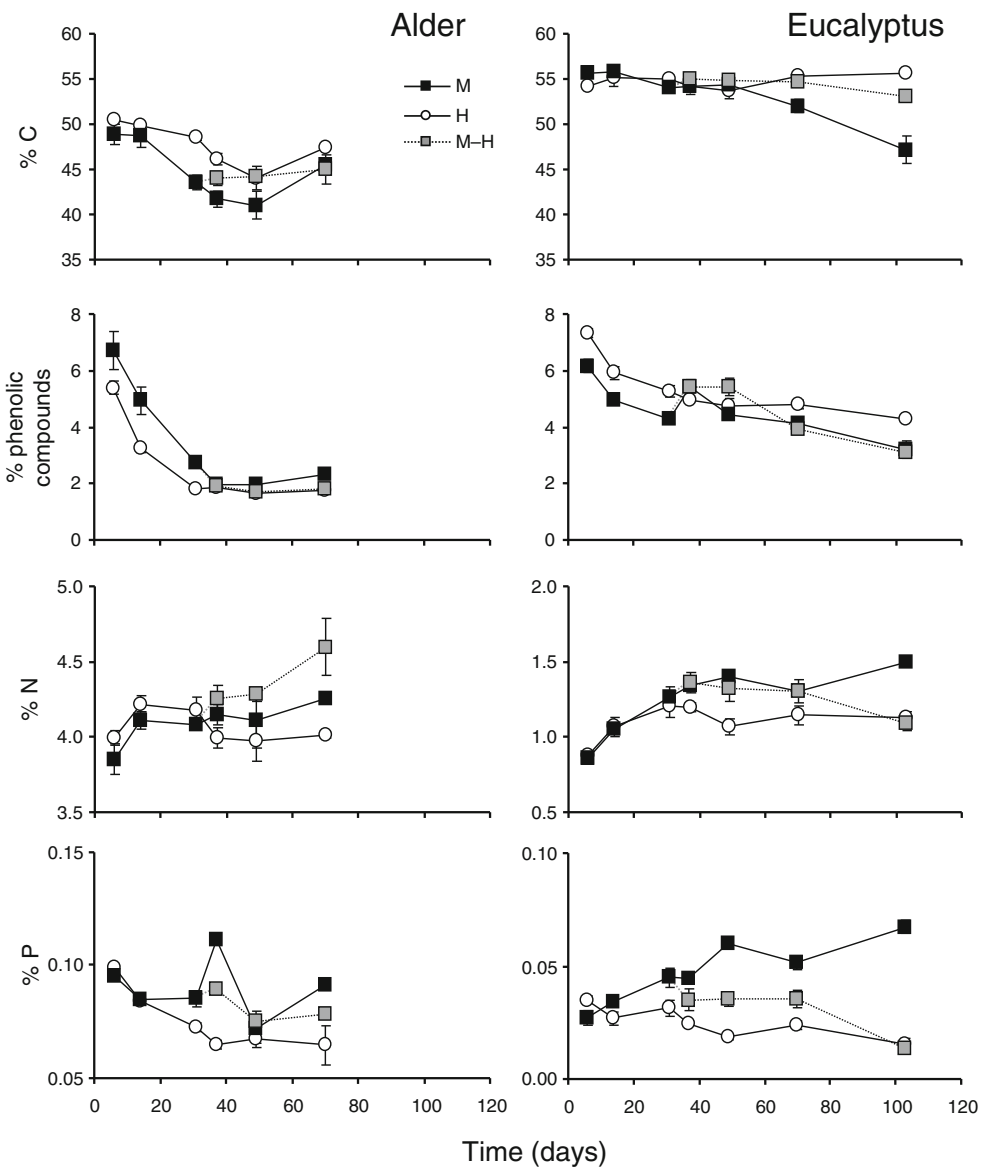

Fig. 3 Dynamics of carbon (C), phenolic compounds, nitrogen (N), and phosphorus (P) in alder and eucalyptus leaf litter (mean % of DM ± 1 SE, $n=4$) along the decomposition process. Note the scale difference for %N and %P between both leaf species

a clear sporulation peak after approximately 2 weeks of incubation at the headwater site (H), but differed in magnitude between alder (3.57 conidia μg DM^{-1}) and eucalyptus (0.74 conidia μg DM^{-1}). Sporulation peaks at the midreach site (M) for both leaf types were lower (0.80 conidia μg DM^{-1} and 0.20 conidia μg DM^{-1} for alder and eucalyptus, respectively) than at the H site and were reached after 2 (alder) or 4 (eucalyptus) weeks in the stream. The difference in mean sporulation rates between sites was close to the statistical significance only in alder (ANOVA $F_{1,55}=3.8$, $p=0.056$).

Fungal assemblages at the initial stages of the decomposition process were characterized by *Flagellospora curvula* Ingold which clearly dominated spore production in alder (>71 %) and in eucalyptus (>43 %) at both sites, co-dominating with *Tetracladium marchalianum* de Wildeman in eucalyptus leaves at the midreach site (Table 3). *Flagellospora curvula* continued dominating fungal assemblages at advanced stages of the process in alder at site H, but it was surpassed by *Alatospora acuminata* Ingold. in this leaf species at site M and by *Heliscus lugdunensis* Sacc. et Thérry in eucalyptus at both sites. Transplanted alder leaves (M–H) showed a conidial assemblage dominated by *Heliscella stellata* (Ingold et Cox) Marvanová et S. Nilsson. Meanwhile, transplanted eucalyptus leaves were dominated by *Tetracladium marchalianum*. All mentioned species represented individually more than 25 % of total conidia production at least in one given period (highlighted in Table 3). The performed NMDS on aquatic fungi reflects temporal dynamics and also differences between incubation sites on the associated conidial assemblages (Fig. 5). Marked changes in the conidial assemblage structure between the first 2 weeks of the processing were observed for both leaf species at both sites,

Table 3 Contribution of aquatic hyphomycete taxa to conidial production (%), taxa richness, and mean sporulation rate (no. of conidia μg DM^{-1} day^{-1}) in each period

Taxa	ID	Alder					Eucalyptus				
		Pre-trans		Post-trans			Pre-trans		Post-trans		
		M	H	M	H	M–H	M	H	M	H	M–H
Alatospora acuminata Ingold	**Aac**	2.2	0.4	**25.0**	10.4	13.7	3.3	+	2.6	9.9	6.7
Anguillospora filiformis Greathead	Afi	+	0.7	+	2.1	0.3	0.1	0.6	0.1	2.4	
Anguillospora longissima (Sacc. et Sydow) Ingold	Alo	0.1		1.4	0.1	0.3	0.4	+	0.7	0.1	9.5
Articulospora tetracladia Ingold	Ate	1.7	0.8	1.1	10.1	2.6	+	+	0.1	3.5	
Campylospora chaetocladia Ranzoni	Cch			0.1		0.5			+		+
Clavariopsis aquatica de Wildeman	Caq	1.6	+	3.6	0.2	1.8	0.2	+	1.2	0.5	3.6
Clavatospora longibrachiata (Ingold) Marvanová et Nilsson	Clo	1.5	+	3.2	0.5	4.1			0.1	+	0.9
Culicidospora aquatica R.H. Petersen	Caq'									0.1	
Flagellospora curvula Ingold	**Fcu**	**71.3**	**89.3**	14.5	**34.7**	20.5	**43.7**	**98.8**	13.3	10.0	12.6
Goniopila/Margaritospora	GM		0.2		0.8						
Heliscella stellata (Ingold et Cox) Marvanová et Nilsson	**Hst**	2.7	0.2	14.1	20.1	**30.4**	+			0.2	2.4
Heliscus lugdunensis Sacc. et Thérry	**Hlu**	0.5	0.4	1.1	3.4	0.5	3.5	0.1	**30.2**	**43.0**	6.1
Heliscus tentaculus Umphlett	Hte	0.2		0.1		0.1	1.4	+	0.3	0.4	0.4
Lunulospora curvula Ingold	Lcu	1.2	0.5	6.0	3.1	2.7	3.8	+	8.8	3.1	5.4
Mycofalcella calcarata	Mca				+						
Stenocladiella neglecta Marvanová et Descals	Sne	1.5		2.1		0.8					
Tetrachaetum elegans Ingold	Tel	2.4	5.0	1.4	1.4	3.1	0.5	0.1	0.7	0.4	0.3
Tetracladium marchalianum de Wildeman	**Tma**	6.7	+	19.5	0.1	8.7	**39.5**	+	10.3	3.8	**30.0**
Tricladium angulatum Ingold	Tan		+	0.2							
Tricladium chaetocladium Ingold	Tch	+	0.1	0.3	1.0	0.3	+	+	1.2	0.2	5.8
Tricladium patulum Marvanová et Marvan	Tpa		+		+						
Tricladium splendens Ingold	Tsp						+		+		+
Triscelosphorus monosporus Ingold	Tmo	0.6		0.9	+	1.0	0.4		0.5	0.1	7.9
Variocladium giganteum (Iqbal) Descals et Marvanová	Vgi									0.1	
Unidentified cylindrical A	UCA	5.1	1.5	4.5	7.0	6.0	2.2	0.2	24.3	6.8	5.8
Unidentified cylindrical B	UCB	0.1	+	0.1	0.4	0.5	1.0		5.5	15.3	2.7
Unidentified sigmoid A	USA	0.3	1.1	0.7	4.3	1.8					
Unidentified sigmoid B	USB				0.1						
Unidentified tetraradiate	UT	0.2	+	+		0.3					
Taxa richness		20	19	22	21	21	17	13	18	19	17
Mean sporulation rate (conidia μg DM^{-1} day^{-1})		0.34	1.36	0.29	0.11	0.21	0.13	0.26	0.06	0.06	0.07

Data for the five dominant taxa (>25 %) on any of the streams or treatments are highlighted in bold. Low densities (<0.1%) are replaced by + symbol

reflecting the sporulation peaks (Fig. 4). The main responsible species for this shift was *Flagellospora curvula* (Fig. 5, second sampling displayed to the left side). Thereafter, conidial assemblages showed in general a more even distribution, reflected in a lower dispersion of samplings in the NMDS plots. The transplanted materials showed a higher similarity with leaves at the last stages of the decomposition at site M than with the corresponding at site H. In fact, all the species recorded during the decomposition of transplanted materials (21 in alder and 17 in eucalyptus) were registered at site M (Table 3). *Alatospora acuminata* and *Heliscella stellata*, in alder, and *Heliscus lugdunensis*, in eucalyptus, would indicate fungal succession (last stages) on leaf decomposition, whereas *Tetracladium marchalianum* in eucalyptus would be a clear indicator of the initial microbial conditioning at site M.

Discussion

The contrasting water chemistry of the two studied sites, as described in previous studies [17, 33, 40], is the result of changes in geology and an increase in agricultural and

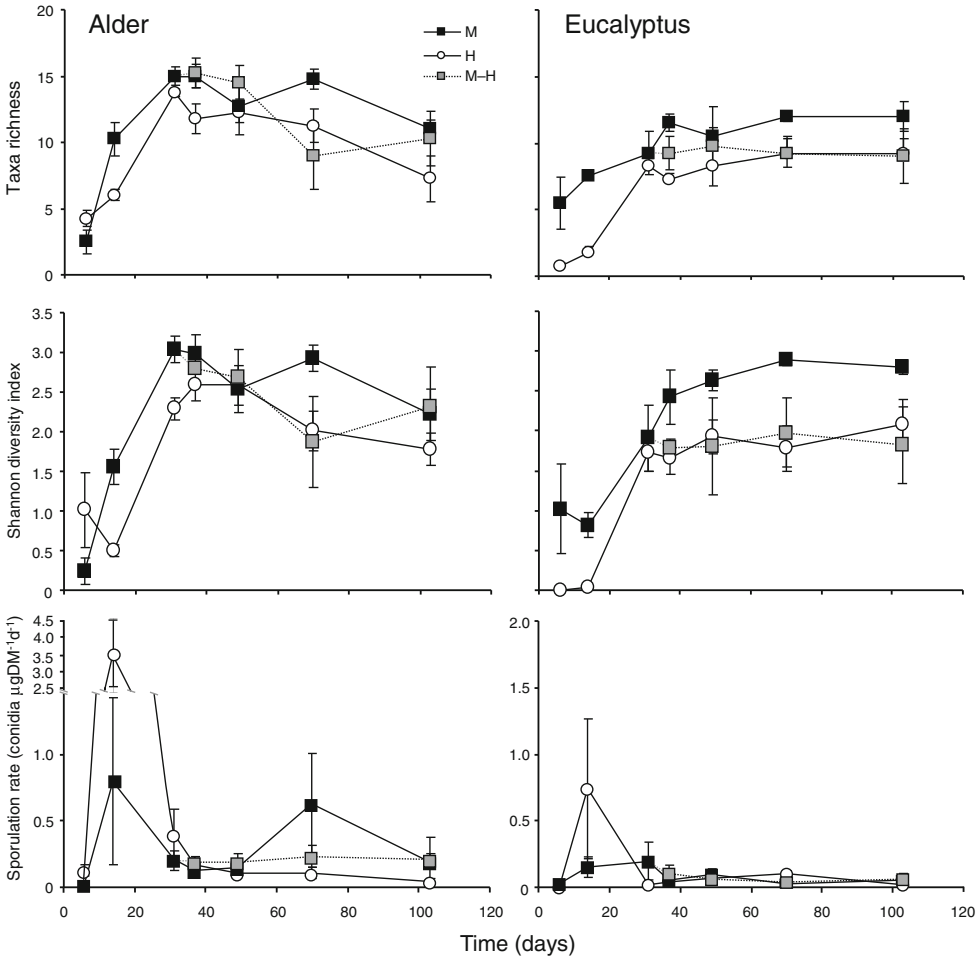

Fig. 4 Aquatic hyphomycete taxa richness, Shannon diversity index, and sporulation rates (conidia µg DM^{-1} day^{-1}) throughout the decomposition process for alder and eucalyptus leaf litter (mean ± 1 SE, n = 4)

afforestation land uses in the catchment, from pristine headwaters to sites in the midland reaches below small urban areas, where conductivity, pH, and dissolved phosphorus tend to increase. During the trials for the present study, both sites showed similar values for dissolved inorganic nutrients, which must be interpreted as a consequence of the high self-purification already reported for the midreach [21]. The higher nutrient enrichment of leaf litter throughout decomposition at the midreach is compared to that at the headwater site in this direction and has been shown in previous studies [33, 40]. Other variables, however, maintained differences between both sites (e.g., pH, conductivity).

In this work, we investigated how decomposing alder and eucalyptus leaf litter and their associated fungal communities (aquatic hyphomycetes) from an anthropized site respond to a sudden change to oligotrophic conditions, by transplanting colonized leaves from one (midreach) to a second (headwater) site, in order to look for the importance of the initial colonizers on the ulterior fungal assemblages and on the whole breakdown process. A previous study [40] has observed that the headwater site had a higher detritivore activity than the midreach, where the microbial role was more evident [15], reflecting some common changes along the river gradient. According to the previous results [10, 40], shredders were more abundant in headwaters than in the midreach and were represented mostly by plecopteran *Leuctra*, *Nemoura*, and *Protonemura* and crustacean *Echinogammarus*, whereas downstream, the invertebrates associated to both litters reflected benthic communities more clearly dominated by collector–gatherers, *Echinogammarus* being the main shredder.

In the present study, we found similar breakdown rates for alder at both sites, whereas eucalyptus leaf litter was processed faster at midreach, where this material showed higher values of leaf nutrient and conidial assemblage diversity along the process. As we hypothesized, the effects of nutrient enrichment on leaf litter processing were more evident in eucalyptus, supporting previous observations in this stream [33] where the dissolved nutrient only enhanced the processing rate of low-quality litter. There were no differences in alder processing rates between transplanted leaves and those incubated throughout the whole experiment at the headwater site, whereas transplanted eucalyptus leaves were processed faster than those maintained in the headwater site.

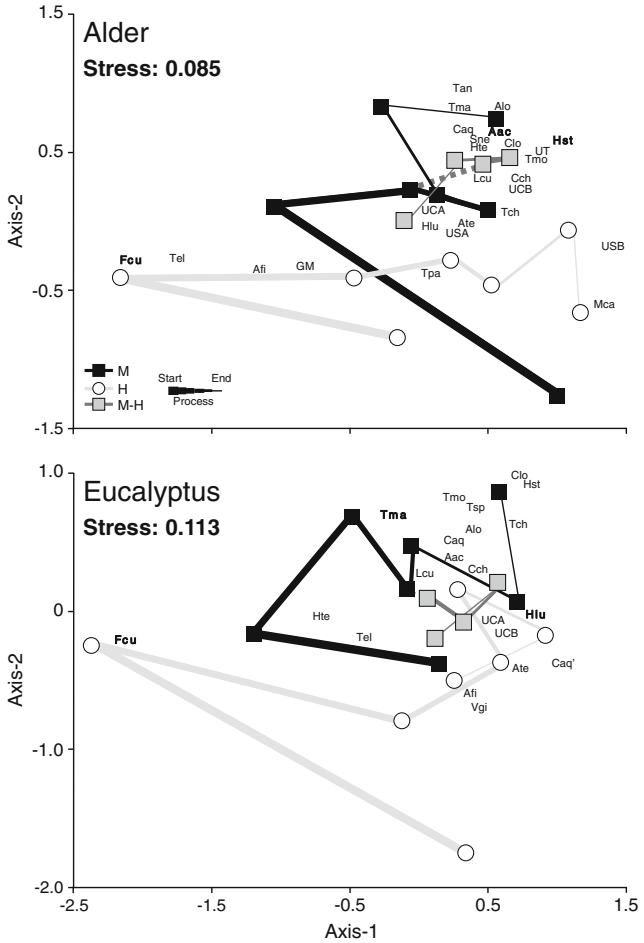

Fig. 5 NMDS ordination based on Bray–Curtis similarity matrices on aquatic hyphomycete mean conidial assemblages associated to alder and eucalyptus leaf litter incubated in the studied reaches (*different symbols*) in each sampling date; *line width reduction* represents the process forward. The stress values of the ordination and the relative position of each species in the analyses (see aquatic hyphomycetes complete names in Table 3) are shown. Fungal dominant taxa are highlighted in *bold*

Eucalyptus leaf litter initially had four times less nitrogen than alder leaves, but conditioning at the midreach produced an increase in leaf N concentration of up to 170 % at the date of transplantation; higher leaf N presumably means higher fungal biomass, therefore higher microbial decomposition but also stronger attraction for detritivores of the transplanted materials. Thus, the experiment detected differences in the breakdown rate between leaf types following differences in its initial quality, with the alder leaves being less influenced by the incubation site than were the nutrient-poor eucalyptus leaves, which were strongly affected by their initial microbial conditioning. This explanation agrees with previous results from the same sites by Pozo et al. [40] based on eucalyptus leaf litter: leaves incubated at the midreach site had a higher ergosterol concentration (a measure of total fungal biomass) than alder leaves, therefore explaining the stronger leaf N increase. The increase in leaf palatability for shredders through the microbial conditioning at the midreach prior to transplantation might determine overall breakdown rates, as other authors suggest [23, 26, 30].

A substantial number of aquatic hyphomycete species were associated with leaf litter on both alder and eucalyptus leaves, as shown in previous studies [15], but the colonization dynamics was slower in eucalyptus leaves, probably due to physical (cereous cuticle) and chemical (oils and polyphenols) barriers [9, 13]. The diversity of associated hyphomycetes was slightly higher at midreach. In a recent paper [36], we have shown an increase of fungal diversity along a eutrophication gradient in low-order streams in the region. Although the observed concentrations of dissolved inorganic nutrients during our study period cannot explain this increase in diversity, it could be related to the change in the trophic status of the waters (sensu [18, 20]) below agricultural and urban areas. In the transplanted leaves, diversity was reduced only in eucalyptus, becoming more similar to those of the community at the headwater site than to those found at the midreach. Sridhar et al. [46] obtained similar results with alder in a cross-transplantation experiment, with no evidence of habitat effects on fungal diversity on transplanted leaves.

Total conidial production was higher on alder than on eucalyptus, as expected from the work by Gessner and Chauvet [23]. It might be related with the lower quality and higher concentration of inhibitory compounds in eucalyptus in comparison with alder [13]. Chauvet et al. [15] found no differences in sporulation rates between alder and eucalyptus in the same sites of the present study, pointing out that conidial production does not always respond in the same way to leaf litter quality. Sporulation rates of both leaf types differed slightly between stream reaches, but contrary to our expectations to find higher values at the site where leaf nutrient enrichment was more elevated, as other studies indicate [2, 15, 28, 37], peaks were higher at the headwater site. As both reaches showed dissolved nitrogen availability over fungal N requirements for sporulation (nitrate levels higher than 300 μg N l^{-1} [5]) and similar soluble reactive phosphorus concentrations, differences in sporulation rate between sites could not be explained by dissolved nutrient availability. Water temperature, slightly higher at the midreach, neither explained the higher sporulation rates at the headwater site, as fungal decomposer activity tends to increase with water temperature [11]. The low conidial production of transplanted materials follows the same pattern of no transplanted materials, i.e., later stages of the decomposition were characterized by low spore production. Suberkropp and Chauvet [48] found that the conidial production of transplanted material tended to follow the same dynamics as that of the reception habitat, but they carried out transplantation early during the experiment. However, Sridhar et al. [46] found that early hyphomycete colonizers sustain their sporulation rate dynamics after transplantation. Sporulation peaks on leaf litter decomposition both

in alder and eucalyptus are common after 2–4 weeks of incubation [15, 25], and the present results are within this range but cannot discuss effects of transplantation on sporulation peaks because they occurred before the exchange. As has already been suggested [37], sporulation peaks must be taken into account when looking for quantitative differences in conidial production, but we consider that conidial assemblages of the late decompositional stages give useful information for qualitative comparisons.

Regarding community structure, when studying early conidial assemblages, both leaf types were hierarchically structured around a single dominant species, *Flagellospora curvula*. This species is considered a pioneer or fast colonizer of decomposing litter [49]. It is usually the dominant hyphomycete fungi in alder sporulation peaks in the streams of our study area [15, 37]. In a previous study [15], eucalyptus leaves incubated at the same midreach site also had a conidial assemblage dominated by *Flagellospora curvula*, but with *Lunulospora curvula* instead of *Tetracladium marchalianum* as the co-dominant species. More than 75 % of species colonizing either alder or eucalyptus whatever the site were found in both studies. In the later stages of incubation, both species tended to reflect a more even conidial assemblage. The differences found in the hyphomycete community between alder and eucalyptus leaf litter support previous observations on the low specificity for substrate of common species [15, 22, 27]. When comparing the conidial assemblages after transplantation, we found some examples of an inoculum effect (persistence of early colonizers, sensu [46]) and also a replacement or enhancement of individual taxa in relation to substrate and succession. The initial inoculum effect was clear in both leaf species; *Tetracladium marchalianum*, for example, with noticeable abundance in the first stages of the decomposition at site M, persisted after transplantation and dominated fungal assemblage of transplanted eucalyptus leaves. *Tetracladium marchalianum* tends to be associated with decomposing alder litter in low densities in oligotrophic streams [37], increasing in moderately eutrophized sites of our study area [36], and has been described as common in many of the moderately to severely contaminated water bodies in Germany [47]. Furthermore, it seems to be an effective early colonizer, limiting the establishment of other species once it is fixed to a substratum [45], being able to dominate the conidial assemblage in alder [16]. In our study, *Heliscus lugdunensis* was the dominant species at late stages of the experiment in eucalyptus leaves at both sites and could be considered an example of substrate preference and succession. It has been previously found with certain frequency in alder [15, 37] and especially in eucalyptus leaves [12, 15]. *Heliscella stellata* would be an equivalent example for alder.

Our overall results suggest that the anthropized site led to an enhancement of nutrient enrichment and decomposition rate of a poor-quality material such as eucalyptus, with alder less influenced by the site, making diminished differences in processing velocity between species. The number of fungal colonizing species and sporulation rate seems to be dependent on the quality of material, independently of site. The composition and structure of the fungal community associated with decomposing leaf litter were determined by the initial inoculum and the persistence of early colonizers, an indication of the importance of the transplant direction. Thus, the results from our transplantation experiment support the determining role of the initial conditioning phase on the whole litter breakdown process, highlighting the importance of intrinsic leaf characteristics and those of the incubation habitat.

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