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The breakdown of Blue Gum (*Eucalyptus globulus* Labill.) bark in a Portuguese stream

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With 5 figures and 3 tables

Abstract: Eucalypt forests produce large amounts of bark, which potentially accumulate in streams, constituting an important carbon and nutrient source for benthic food webs. In this study, we compared the breakdown (and associated microbial activity and diversity and invertebrate abundance) of *Eucalyptus globulus* bark and leaves, enclosed in coarse and fine mesh bags, in a 3rd order stream of central Portugal. Biofilm development on bark was also analyzed with scanning electronic microscopy and respiration rates quantified. After 90 days of incubation, bark lost 21–51 % of its initial mass while leaves lost 48–57 %. Fungal biomass (as determined from ergosterol concentrations) increased over time and was higher in leaves than in bark (79 vs. 50 mg [g AFDM]$^{-1}$ at day 90). Sporulation by aquatic hyphomycetes was only observed after 2 weeks (leaves and bark in coarse mesh bags) or 2 months (bark in fine mesh bags). The initial litter mass converted into conidia in leaves was 7–45 fold the values found in bark. Fungal communities were dissimilar in the two substrates with bark presenting the lowest number of species. *Lunulospora curvula* and *Anguillospora crassa* dominated the fungal communities in bark, while *L. curvula* and *Tetrachaetum elegans* were the dominant species in leaves. Respiration rates, as a measurement of microbial activity, were lower in bark than in leaves (0.10 vs. 0.25 mg O_2 [g AFDM]$^{-1}$ h^{-1} in fine mesh and 0.11 vs. 0.39 mg O_2 [g^{-1}AFDM]$^{-1}$ h^{-1} in coarse mesh). Biofilms in bark clearly increased after 15 days of immersion and contributed to 6–85 % of total oxygen consumption. Overall, the results suggest that microbial decomposition pathways dominate the processing of eucalyptus leaves and bark, although leaching and physical fragmentation may stimulate and facilitate the breakdown of bark.

Key words: Eucalyptus, decay, biofilm, hyphomycetes, fungal biomass, sporulation.

Introduction

Eucalyptus plantations are an important economic activity in Portugal and occupy at present ca. 21 % of the Portuguese forested area. Environmental impacts following the replacement of native deciduous forests by *Eucalyptus globulus* monocultures in stream ecosystems have been previously assessed (reviewed by Graça et al. 2002 and Graça & Canhoto 2006). In eucalyptus plantations litter input to streams occurs throughout the year, with a peak in summer; this litter is less diverse and of lower nutritional quality when compared with mixed deciduous forests. These changes have the potential to affect both the structure of aquatic communities and ecosystem functioning (Abelho & Graça 1996, Molinero & Pozo 2004).

Biotic communities of small streams running through forests derive most of their energetic requirements from senescent leaves produced by riparian trees (Vannote et al. 1980). Therefore, studies assessing the

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direct impact of eucalyptus plantations in aquatic systems have focused on the decomposition of eucalyptus leaves (Abelho & Graça 1996, Pozo et al. 1998) and to a lesser extent, of eucalyptus wood (Díez et al. 2002). *Eucalyptus globulus* also produce large amounts of bark and, as far as we know, the importance of this material to stream metabolism has not been evaluated in exotic eucalyptus streams.

In eucalyptus plantations we measured up to 900 g m^{-2} (pers. obs.) of bark on soil and Molinero & Pozo (2004) reported that twigs and bark may constitute up to 21 % of the litter input to streams running through eucalyptus plantations. Bark strips accumulate in streambeds especially in summer and early autumn when bark sloughing is intense (Penfold & Willis 1961) and stream flow is low.

In terrestrial systems the rates of turnover of the eucalyptus bark are intermediate between leaves and wood (Jones et al. 1999). Assuming a similar processing rate after immersion, we can expect bark to constitute an additional energy source to the biota and a suitable substratum for the development of biofilm. Aquatic hyphomycetes are known to degrade wood, as well as leaves (Díez et al. 2002, Spänhoff & Gessner 2004, Ferreira et al. 2006) and some invertebrates are also able to feed (Schulte et al. 2003) and colonize (Hax & Golladay 1993) wood. Biofilms are associations of microorganisms connected by a hydrated extra cellular polymer on a solid surface and may include algae, fungi and bacteria (Harrison et al. 2005). These organic microlayers may degrade submerged woody substrates (Clausen 1996, Ryder 2004) and constitute the main food source for invertebrate grazers (and shredders; Franken et al. 2005). It has been suggested that they may also constitute a supplementary carbon resource for shredders that depend on recalcitrant nutrient-poor substrata such as eucalyptus leaves (Canhoto 2001).

The aim of this study was to document the breakdown of eucalyptus bark, associated microbial activity and invertebrate colonization, in comparison with eucalyptus leaves. Litter was exposed to, and protected from, invertebrate feeding in a lowland stream running through eucalyptus plantations.

Methods

Study stream and water parameters

The study was carried out in a lowland third order stream (Ribeira do Botão, Coimbra, Portugal, 40°18′22″N, 8°23′37″ W) running through eucalyptus plantations. The stream was 0.2 m deep, 2.6 m wide and had a mean discharge of 0.34 m^3 s^{-1}. Stream water was well oxygenated (10.8 mg L^{-1}), circumneutral (pH = 7.1) and nutrient rich (NO_3^- = 8.81 mg L^{-1}; soluble reactive phosphorus = 5.82 µg L^{-1}). At the study site the riparian vegetation included alder (*Alnus glutinosa* (L.) Gaertn).

Litter bags and decomposition

Eucalyptus leaves were collected from the same stand just before abscission while recently shed bark was collected from the ground, in September 2005. Litter was air dried in the dark and stored until needed. Initial phosphorus and nitrogen (Graça et al. 2005), total phenols (Graça et al. 2005), lignin (Goering & Van Soest 1970) and energetic content (Paiva et al. 2006) were determined for leaves and bark. Fine mesh (FM; 10 × 15 cm, 0.5 mm mesh) and coarse mesh (CM; 10 × 15 cm, 10 mm mesh) bags were prepared with 4 leaves (3.22–7.64 g initial air-dry mass) or 4 bark rectangles (10 × 2 × 0.1 cm, 4.50–10.88 g initial air-dry mass). Six groups of 8 bags of each substrate (4 CM + 4 FM) were tied to a rope and fixed to the stream bed on November 15, 2005. Four extra bags of each litter and mesh type were taken to the stream and brought back to the laboratory the same day to determine initial ash free dry mass (AFDM) allowing for losses due to handling. Four bags of each litter and mesh type were retrieved from the stream after 2, 7, 13, 28, 62 and 90 days emersion, placed in individual zip lock bags and immediately transported to the laboratory in a cooler for processing (on the same day). Each sample was gently rinsed with distilled water into a 500 µm mesh sieve to retain invertebrates (see below) and three (leaves) or four (bark) sets of five litter disks were punched out with a cork borer (12 mm diam.) for microbial determinations (see below). The remaining mass was oven dried at 105 ºC, for 24 h, weighed, ashed at 550 ºC for 6 h, and reweighed to determine AFDM remaining.

Microbial parameters

One set of leaf or bark disks was used for ergosterol determination, as a measure of fungal biomass (Graça et al. 2005). Ergosterol was converted into fungal biomass with a conversion factor of 5.5 µg ergosterol mg^{-1} fungi dry mass (Gessner & Chauvet 1993). Results were expressed as mg fungal biomass $[g^{-1}AFDM]^{-1}$. A second set of leaf or bark disks was incubated with filtered stream water on an orbital shaker (48 h; 15 °C) to induce sporulation by aquatic hyphomycetes. The conidia suspensions were fixed with formalin for later counting and identification. Aliquots of the suspension were then filtered and filters stained with cotton blue in lactic acid; spores were identified (Graça et al. 2005) and counted under a compound microscope at 200×. Leaf disks were then used to calculate remaining AFDM (as above). Sporulation rates were expressed as number of conidia released $[mg\ AFDM]^{-1}\ day^{-1}$. A third set of litter disks was used to assess microbial respiration rates associated with decomposing leaves and bark (Graça et al. 2005). Oxygen consumption was expressed as mg O_2 $[g\ AFDM]^{-1}\ h^{-1}$. A fourth set of bark disks (days 7–28) was used to assess biofilm respiration rates by measuring O_2 consumption of disks (Graça et al. 2005) before and after the biofilm was scraped away. Biofilm was removed from both surfaces of bark disks under a stereoscopic microscope with a blade. Bark disks from each mesh type and sampling date were also prepared for microscopical observations according to Canhoto & Graça (1999). Observations were performed with a – T330 Scanning Electron Microscope.

Invertebrates in litter bags

Invertebrates from coarse mesh bags retained over a 500 µm mesh sieve were preserved in 95 % ethanol for later counting and identification. Identification was taken to the genus/species level when possible, except for Oligochaeta and Diptera (family and sub-family or tribe, respectively), and individuals classified as shredders, grazers and others (Tachet et al. 2000).

Data analysis

Decomposition rates (k) were estimated by linear regression of mass (ln transformed) over time, assuming a negative exponential model $M_t = M_o \times e^{-kt}$, where M_o is the initial mass, M_t is the remaining mass at time t and k is the decomposition rate. Slopes of regressions were compared by ANCOVA with litter and mesh type as categorical variables and time as the continuous variable, followed by Tukey's test (Zar 1999).

Fungal biomass (log transformed), species richness, and microbial oxygen consumption were compared among treatments by 3-way ANOVA (litter type, mesh type and time as categorical variables) followed by Tukey's test when necessary. Maximum sporulation rates by aquatic hyphomycetes (log transformed) were compared among treatments by 2-way ANOVA (litter type and mesh type as categorical variables) followed by Tukey's test. Cumulative conidial production at the last sampling date was calculated by summing up values of daily production at each sampling date and linearly approximated values for each day between sampling dates. Percentage of initial litter AFDM converted into conidia was calculated assuming a mean conidia mass of 8.17×10^{-7} mg (calculated from Bärlocher & Schweizer (1983) and Chauvet & Suberkropp (1998)) and initial disks AFDM of 98 and 260 mg for leaves and bark, respectively.

Shannon's diversity index (log transformed; days 28–90) and Pielou's evenness index (arcsine square root transformed; days 28–90) of aquatic hyphomycete communities colonizing decomposing substrates in coarse mesh bags were calculated from conidial abundances (PRIMER 6) and compared between litter types by 2-way ANOVA (litter type and time as categorical variables). Ordination of samples (days 28–90) of both litter types in coarse mesh bags was done by multidimensional scaling (MDS) based on Bray-Curtis similarity matrix of relative abundances of aquatic hyphomycete conidia (PRIMER 6; Clarke & Gorley 2001). Differences in overall aquatic hyphomycete communities between litter types (days 28–90) were assessed by analysis of similarities (ANOSIM, PRIMER 6; Clarke & Gorley 2001). Data on conidial abundances from before day 28 and fine mesh bags were excluded from indices, MDS and ANOSIM calculations because in most bark sample no conidia production had occurred.

Table 1. Mean (SD) initial lignin, nitrogen, phosphorus, phenols and energetic content of eucalyptus leaves and bark (n = number of measurements). DM = dry mass.

	n	Leaves	Bark
Lignin (%DM)	2	13.4	17.1
Phenols (%DM)	4	5.6 (0.3)	4.4 (0.2)
Nitrogen (%DM)	3	1.4 (0.1)	1.5 (0.2)
Phosphorus (%DM)	3	0.118 (0.004)	0.058 (0.015)
Energy (J/mg)	4	28.5 (0.6)	18.9 (0.1)

Microbial oxygen consumption in bark disks (days 7–28), before and after biofilm removal, was compared by a paired t test. Total invertebrate abundance (log (x+1) transformed) in coarse mesh bags were compared between leaf types by 2-way ANOVA with litter type and time as categorical variables. Data was transformed when necessary to achieve normality (Zar 1999) and analyses were performed with STATISTICA 6 software, unless otherwise indicated.

Results

Initial litter quality and decomposition

Initial content of lignin was lower in leaves than in bark, while the opposite was observed for total phenols, phosphorus and energy (Table 1). Mass loss due to leaching was higher in leaves than in bark (20 vs. 8 % in the first 2 days; Fig. 1). After 90 days incubation, leaves lost 48 (FM) – 57 (CM) % of their initial mass while bark lost 21 (FM) – 50 (CM) % (Fig. 1). Decomposition of leaves (k = 0.0084 d^{-1} – 0.0103^{-1}d; k CM: k FM = 1.2) was significantly faster than that of bark (k = 0.0030 – 0.0066 d^{-1}; k CM: k FM = 2.2) for both mesh types (ANCOVA, $p < 0.001$). Decomposition of bark was significantly faster in coarse than in fine mesh bags (ANCOVA, $p = 0.005$) (Table 2).

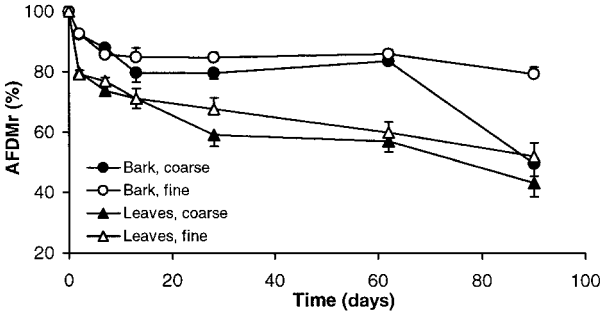

Fig. 1. Remaining ash free dry mass (AFDMr; mean ± 1SE) of eucalyptus bark and leaves in coarse (CM) and fine (FM) mesh bags.

Table 2. Decomposition rates (k (d^{-1})) of eucalyptus leaves and bark in coarse and fine mesh bags, 95 % confidence limits (95 % CL) and the regression coefficient (R^2). Comparisons among litter and mesh types by ANCOVA (different letters indicate significant differences at $p < 0.05$ among slopes).

Litter	Mesh	k (d^{-1})	95 % CL	R^2	ANCOVA
Leaves	Coarse	0.0103	0.0015	0.84	a
	Fine	0.0084	0.0009	0.79	a
Bark	Coarse	0.0066	0.0006	0.81	b
	Fine	0.0030	0.0005	0.65	c

Microbial parameters

Fungal biomass

Fungal biomass increased through time in all treatments (48–82 mg [g AFDM]$^{-1}$ by day 90), although biomass build up was faster in coarse mesh bags where it reached an initial peak by day 28 (Fig. 2). Fungal biomass was significantly higher in leaves (up to 79 mg [g AFDM]$^{-1}$) than in bark (up to 50 mg [g AFDM]$^{-1}$) for both mesh types (3-way ANOVA, $p < 0.001$) and no significant differences were found between mesh types for either leaves or bark (3-way ANOVA, $p = 0.072$).

Aquatic hyphomycete sporulation

Sporulation by aquatic hyphomycetes commenced after 2 weeks in all substrates, except in bark, in fine mesh bags, where it commenced after 2 months (Fig. 2). In leaves sporulation peaked by day 28 in both coarse (3226 conidia [mg AFDM]$^{-1}$ d^{-1}) and fine (1810 conidia [mg AFDM]$^{-1}$ d^{-1}) mesh bags. In bark sporulation reached its maxima by day 62 in coarse mesh bags (264 conidia [mg AFDM]$^{-1}$ d^{-1}) and it continued to increase until the last sampling date in fine mesh bags (122 conidia [mg AFDM]$^{-1}$ d^{-1}) (Fig. 2). Maximum sporulation rates were significantly higher in leaves than in bark (2-way ANOVA, $p < 0.001$), but no significant differences were found between mesh types for each substrate (2-way ANOVA, $p = 0.310$). Conversion of initial AFDM into conidia varied between 0.2 % (bark, FM) and 9.8 % (leaves, CM) and was 7 (CM) to 45 (FM) times higher for leaves than for bark.

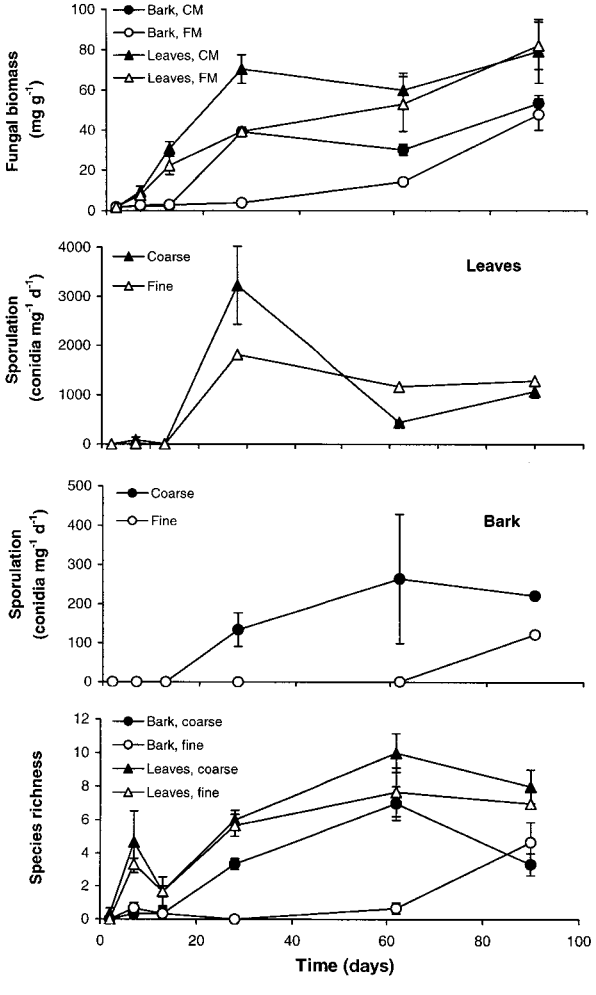

Fig. 2. Mean (± 1SE) fungal biomass (per g AFDM) and sporulation rates (per mg AFDM), and species richness of aquatic hyphomycetes in eucalyptus bark and leaves in coarse (CM) and fine (FM) mesh bags.

Aquatic hyphomycete communities

Aquatic hyphomycete species richness peaked by day 62 (7–10 species) in all treatments, except in bark in fine mesh bags, where it increased through time (Fig. 2). Species richness was significantly higher in leaves than in bark (3-way ANOVA, $p < 0.001$). Species richness in bark was significantly higher in coarse than in fine mesh bags (3-way ANOVA, $p = 0.001$). The to-

Table 3. Mean relative abundances (%, days 28–90) of aquatic hyphomycete conidia from eucalyptus leaves and bark in coarse and fine mesh bags. Total number of species, mean Shannon's diversity index and mean Pielou's evenness index are given. Comparisons were made between litter types (2-way ANOVA, different letters indicate significant differences ($p < 0.050$)).

	Leaves		Bark	
	Coarse	Fine	Coarse	Fine
Alatospora acuminata	7.32	0.53	2.64	
Alatospora pulchella	0.15	0.28		
Anguillospora crassa	0.18	0.10	17.67	52.18
Anguillospora filiformis	0.43	0.40	0.04	
Articulospora tetracladia	0.26	0.13	0.06	0.23
Clavariopsis aquatica	0.12	0.03	1.69	0.64
Heliscus submersus	9.58	1.37	0.09	
Heliscus lugdunensis	0.02	4.87	0.04	0.08
Lunulospora curvula	57.07	54.55	76.65	46.07
Tetracladium marchalianum	3.25	0.44	0.51	0.31
Tetrachaetum elegans	11.21	34.57	0.16	0.23
Tricladium chaetocladium	1.42	0.83	0.03	
Triscelophorus acuminatus	6.35	1.33	0.14	0.05
Triscelophorus monosporus	2.57	0.46	0.25	
Varicosporium sp.	0.06			
Unidentified tetraradiate		0.08	0.03	0.23
small sigmoid (< 60 µm)		0.03		
Total no. species	15	16	14	9
Pielou's evenness, J	0.57[a]		0.32[b]	
Shannon's diversity, H'	1.20[a]		0.52[b]	

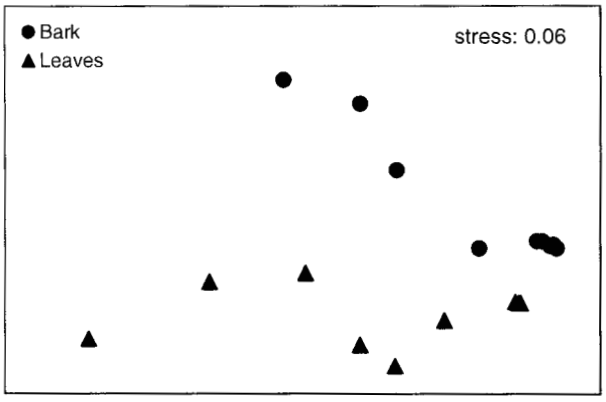

Fig. 3. Ordination (MDS) of samples (leaves and bark, CM) from days 28–90, based on Bray-Curtis similarity matrix of relative abundances of aquatic hyphomycete conidia.

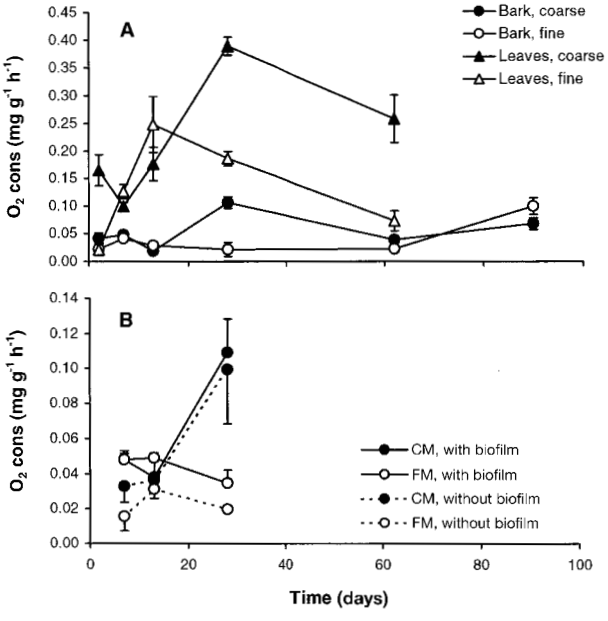

Fig. 4. Total microbial respiration (mean ± 1SE) per g AFDM in coarse (CM) and fine (FM) mesh bags. **A:** In eucalyptus bark and leaves. **B:** In bark with and without biofilm.

tal number of species identified in a treatment varied between 9 (bark, FM) and 16 (leaves, FM) (Table 3). The most common species of aquatic hyphomycetes were *Tetrachaetum elegans* (leaves) and *Anguillospora crassa* (bark), which were initial colonizers and *Lunulospora curvula* (all treatments) that dominated in the last sampling dates (Table 3). The communities of aquatic hyphomycetes in leaves had significantly higher diversity and evenness than those in bark (2-way ANOVA, $p < 0.001$ and 0.001, respectively; Table 3). Fungal communities in leaves and bark (CM), from day 28 on, were clearly separated by litter type (ANOSIM, $p = 0.002$ and $R^2 = 0.99$) (Fig. 3).

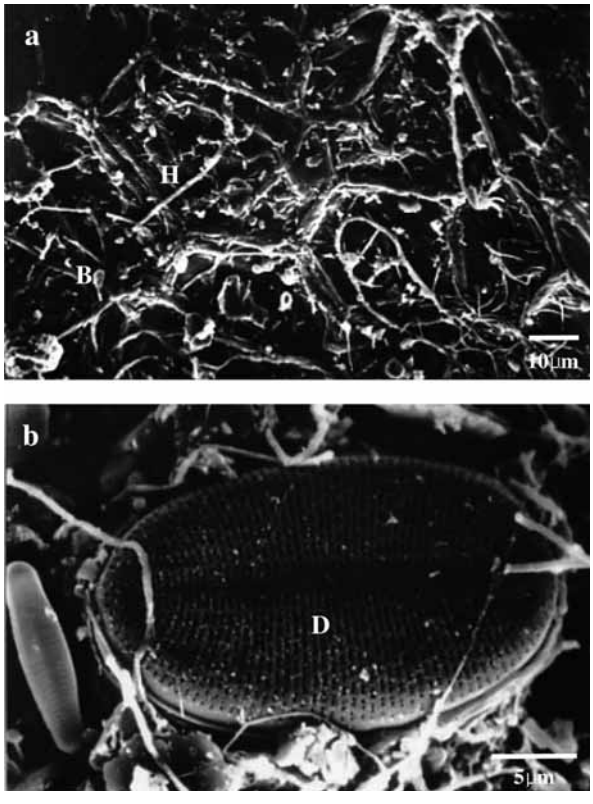

Fig. 5. Scanning micrographs of a section of eucalyptus bark. **a)** Hyphae (H) and bacteria (B) on the bark surface after 13 days of immersion; **b)** Diatoms (D) encrusted on the bark surface after 28 days of immersion.

Microbial oxygen consumption

Microbial oxygen consumption increased rapidly in leaves, peaking by days 13 or 28 while in bark it increased more slowly until days 28 or 90 (Fig. 4a). Oxygen consumption was significantly higher in leaves than in bark (3-way ANOVA, $p < 0.001$). For leaves it was also significantly higher in coarse than in fine mesh bags (3-way ANOVA, $p < 0.001$).

Bark biofilms

Oxygen consumption was significantly higher in bark disks with biofilm than in those without (paired t test, $p < 0.001$). The biofilm was responsible for 6–85 % of total oxygen consumption, being the difference attributed to respiration by mycelium inside the bark matrix (Fig. 4b). Microscopical observation of bark disks confirmed the increased in microbial flora (especially fungal hyphae) on the bark surface, in both mesh bags, after day 13 (Fig. 5).

Invertebrates in litter bags

The number of invertebrates colonizing decomposing litter was initially higher in bark than in leaves, but this tendency was reversed after day 28. Simulidae (filterers) were the most common invertebrates found in litter bags, followed by Leuctridae (shredders) in bark and Planorbidae (grazers) in leaves. However, invertebrate abundance was always low (< 10 ind g^{-1}AFDM) and no significant differences were found between litter types (2-way ANOVA, p = 0.183).

Discussion

Our data indicates that bark may play an important role in energy flow and nutrient cycling in streams where eucalyptus forms the dominant riparian vegetation. Bark may be quantitatively important in these water courses and its incorporation into secondary production may occur, according to our results, in a reasonable time span (~50 % loss within 90 days).

Breakdown rates of eucalyptus leaves were intermediate between those observed previously in the Iberian Peninsula and Australia for the same species (Boulton 1991, Pozo et al. 1998). As expected, eucalyptus bark degradation was slower than leaves but faster than reported for other natural woody substrates (reviewed by Spänhoff & Meyer 2004).

The slower breakdown rates of bark when compared with leaves may be related to the chemical and physical differences between the two substrates. Higher lignin contents usually result in slower microbial colonization, reduced invertebrate feeding and lower decomposition rates (e.g. Mellilo et al. 1983, Wright & Covich 2005). On the other hand, the higher surface : volume ratio of bark when compared to sticks, twigs and branches (Golladay & Webster 1987), and its fibrous fissured structure (Quilhó et al. 1999) in comparison with the tough woody material (Fahn 1990, Brooker 2002) may facilitate degradation.

Leaching was slower in bark than in leaves inhibiting microbial colonization and biofilm development for about 2 weeks (vs. 2 days in leaves). It is generally accepted that fungal colonization usually follows a period of abiotic release of soluble inorganic and organic compounds into the water (Gessner et al. 1999) and that the presence of *E. globulus* leachates may inhibit algal growth (Friberg & Winterbourn 1996). The prolonged presence of defensive compounds such as polyphenols and terpenes in bark (Sakai 2001) may have also contributed to retard aquatic hyphomycetes colonization (see also Canhoto & Graça 1999). However, both substrata presented a similar decrease of ca. 20 % of initial mass within this period which is in the range reported for eucalyptus leaves (Boulton 1991, Canhoto & Graça 1996) and for the bark of other species (France et al. 1997).

Breakdown rates were also influenced by flow and litter structure: after 90 days immersion, the mass loss by eucalyptus bark, in coarse mesh bags, was comparable to that of leaves. This can be explained by increased physical abrasion promoted by current and suspended sediments resulting from heavy rains that occurred after day 60. Bark in fine mesh bags was probably protected from this physical abrasion, which resulted in a breakdown rate twice as high in coarse mesh than in fine mesh bags. Leaves were less affected by increased water flow events, probably because their resilient thick cuticular envelope maintains leaf integrity for a long time (Canhoto & Graça 1999). The patterns of bark degradation seem to include an elongated phase of leaching and microbial digestion of tissues followed by a faster, dominantly longitudinal, disintegration phase highly influenced by current velocity. Under natural conditions, the importance of this fragmentation process as a source of Fine Particulate Organic Matter (FPOM) and Dissolved Organic Matter (DOM) is still not known, but is likely to be relevant for the metabolism of the eucalyptus streams where invertebrate communities are impoverished (Naiman & Sedell 1979, Bunn 1988, Abelho & Graça 1996). Traditionally, invertebrates (mainly shredders) play the major role in the breakdown of coarse particulate organic matter (CPOM) into FPOM.

The present results indicate that decomposition of leaves was carried out primarily by microbes (*k* ratio CM:FM ≈ 1) which agrees with Canhoto & Graça (1999), who found that aquatic hyphomycetes had a major role in the decomposition of eucalyptus leaves. Fungal colonization of eucalyptus leaves was relatively fast and increased over time as reported by Pozo et al. (1998) in a comparable stream. Fungal biomass in bark reached 5 % of detritus mass suggesting that bark is a suitable substrate for aquatic hyphomycetes (although colonization and growth may be slow). In fact, fungal biomass in bark was within the values observed in the leaves (8 % of the total detritus mass, Pozo et al. 1998), higher than previously reported for eucalyptus branches (Díez et al. 2002) and pine bark (Spänhoff & Gessner 2004) but lower than that for oak veneers (Gulis et al. 2004).

As expected, fungal sporulation in eucalyptus leaves did not occur until 2 weeks after immersion (Bärlocher et al. 1995, Canhoto & Graça 1996, Chau-

vet et al. 1997) whereas in bark it did not occur until after 2 weeks (CM) or 2 months (FM). For bark, investment in conidial production was lower than on leaves, but sporulation rates were within the values found for birch (Maharning & Bärlocher 1996) and balsa (Ferreira et al. 2006) woody substrates. This variation between studies was probably due to differences in surface: volume ratio, litter quality between substrata and/or differences in water chemistry. Methodological constraints may have also contributed to the reduced fungal activity in fine mesh bags as oxygenation (Raviraja et al. 1998), nutrients (Suberkropp & Chauvet 1995, Gulis & Suberkropp 2003) and flow (Ferreira & Graça 2006) may have been reduced inside fine mesh bags because of clogging by suspended sediments.

Fungal assemblages differed between leaves and bark. Although leaves always supported a higher number of species, 14 species found bark to be a suitable substrate. Of the common species to both substrates, *Anguillospora crassa* was the most successful in bark, which agrees with a previous study where this species dominated in woody substrates rather than in leaves (Ferreira et al. 2006). Our results support the idea that the decomposition of bark is carried out by a unique suite of aquatic hyphomycetes species, possibly reflecting substratum specificity (see also Ferreira et al. 2006).

Overall microbial activity, assessed through oxygen consumption, followed a pattern similar to that of sporulation for leaves supporting the notion of a high contribution of fungi to the total microbial oxygen consumption. In leaves, oxygen consumption was in the range previously reported for other leaf species (Stelzer et al. 2003, Gulis & Suberkropp 2003). In bark, values were comparable to that of some recalcitrant leaves (Gulis & Suberkropp 2003) and woody veneers (Stelzer et al. 2003, Gulis et al. 2004) which could be attributed to their similar surface : volume ratio.

In conclusion, the breakdown of bark was intermediate between that of leaves and wood and was influenced by abiotic processes such as leaching and physical fragmentation. Fungal colonization of bark was slower and its activity, although significant, was lower than that in leaves. Nevertheless, bark was a suitable substrate for most fungal species. In contrast with leaves, where the decomposition process occurs mainly from the inside (Canhoto & Graça 1999), the microbial decomposition of bark seems to be largely superficial and carried out by a biofilm community similar to the ones reported for wood (Collier et al. 2004). The development of biofilm on bark did not increase its consumption by invertebrates (Hax & Golladay 1993, Friberg & Jacobsen 1994). Whether this was a result of the substrata (bark + biofilm) poor quality, toxicity (Canhoto & Graça 1999, McKie & Cranston 2001) or/and a consequence of the presence of a high quality resource in the stream – alder leaves – is unclear (Canhoto & Graça 1995). Nonetheless, present evidence suggests a dominant physical and microbial degradative pathway for both eucalyptus leaves and bark, with macroinvertebrate processing playing a minor role.

We hypothesize that the contribution of bark to the energy flow and nutrient cycling in eucalyptus streams may be higher than previously thought. Bark constitutes an abundant carbon resource accessible to the microbial food webs in a reasonable time span after immersion. Its degradation maybe further hastened by the spate events characteristic of eucalyptus streams.

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