

# Eucalyptus plantations affect fungal communities associated with leaf-litter decomposition in Iberian streams

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With 7 figures and 3 tables

**Abstract:** The replacement of diverse deciduous forests by eucalyptus plantations changes the timing, quality and quantity of litter inputs to streams, which has the potential to affect the activity of decomposers and thus ecosystem functioning. Here, we compared (a) the decomposition rate of alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal, (b) the activity (fungal biomass and sporulation) and diversity (species richness and Pielou's evenness index) of the associated fungal communities and (c) changes in N and P content of leaves. Alder and oak leaves decomposed at similar rates in both stream types and countries, with the exception of oak leaves in the Spanish eucalyptus stream, which decomposed faster than in the corresponding deciduous stream or in the Portuguese eucalyptus stream. This difference was attributed to physical fragmentation due to flooding and not to forest cover. Higher nitrogen and phosphorus content and higher fungal biomass and sporulation were generally found on leaves from eucalyptus rather than from deciduous streams. The higher fungal activity in eucalyptus streams was attributed to higher water temperature and benthic organic matter storage. The Spanish eucalyptus stream had higher species richness of aquatic hyphomycetes than the deciduous one (27 vs. 20) while in Portugal the opposite was true (16 vs. 20). Fungal community evenness was significantly higher on alder leaves in eucalyptus than in deciduous streams. The community structure (MDS analysis) discriminated both stream types in Portugal much better than it did in Spain. At least for Portugal, differences between stream types can be explained by higher litter diversity in deciduous than in eucalyptus streams. In

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conclusion, stream fungal communities in Portugal were more affected by eucalyptus plantations than in Spain. In both countries, fungal diversity and activity were more affected by eucalyptus plantations than decomposition rates of submerged litter. We suggest therefore that, to mitigate the effect of eucalyptus plantations, deciduous trees could be planted on the river banks or, preferably, riparian strips of native vegetation should be left unmodified.

**Key words:** litter decomposition, alder leaves, fungal diversity, fungal community, river banks.

## Introduction

Coarse particulate organic matter (CPOM) provided by riparian trees is the primary source of energy and carbon for aquatic communities in small woodland streams (FISHER & LIKENS 1973, VANNOTE et al. 1980). Aquatic fungi and invertebrate shredders are the key players in decomposition of this organic matter and its incorporation into aquatic food webs (HIEBER & GESSNER 2002). As these organisms can be sensitive to the amount (WALLACE et al. 1999, LAITUNG et al. 2002) and quality (CANHOTO & GRAÇA 1995, 1999, BÄRLOCHER & GRAÇA 2002) of organic matter entering the streams, changes in the composition of the riparian forest can potentially affect both community structure and function of aquatic ecosystems.

Vast areas of deciduous forests have been converted into *Eucalyptus globulus* LABILL. plantations in the Iberian Peninsula. This substitution is highly profitable since *Eucalyptus globulus* has fast growth rates allowing prompt reforestation of burned areas and short rotation while timber is used by pulp and paper industry (reviewed by CANHOTO et al. 2004). Eucalyptus plantations occupy 21 % of the forested area in Portugal (Direcção Geral Florestas 2005); data from Spain are less reliable because the Third National Forest Inventory is still in progress. However, eucalyptus plantations are known to represent around 12 % of total forest cover in the Atlantic regions of northern Spain (Ministerio de Medio Ambiente 2003). The replacement of diverse deciduous forests by evergreen eucalyptus trees changes the timing, quality and quantity of litter input to streams. In eucalyptus plantations, litter input occurs throughout the year, with a peak in summer, litter is less diverse and total input may be smaller and of lower nutritional quality (lower N and P content) than in native deciduous forests (reviewed by GRAÇA et al. 2002, see also ABELHO & GRAÇA 1996, POZO et al. 1997 a, b, MOLINERO & POZO 2003, 2004). As a result, the inputs of nitrogen and phosphorus are also lower in these streams (POZO et al. 1997 a, b, MOLINERO & POZO 2003). Despite the lower litter input, which peaks in summer during a period of low discharge, eucalyptus streams tend to store larger amounts of CPOM than deciduous ones

(POZO et al. 1997b, CANHOTO & GRAÇA 1998, MOLINERO & POZO 2003, 2004).

Comparisons of microbial communities between deciduous and eucalyptus streams in Spain and Portugal yielded contrasting results. BÄRLOCHER & GRAÇA (2002) reported that streams running through eucalyptus plantations in Portugal had lower diversity of aquatic hyphomycetes than streams running through deciduous forests. This could be explained in part by the lower diversity of CPOM. Some species of aquatic hyphomycetes have substrate preferences (GULIS 2001), so more diverse resources may support a higher number of species. However, in Spain, similar number of species was found in both stream types (CHAUVET et al. 1997). Also, no differences in conidia concentration in water (BÄRLOCHER & GRAÇA 2002), fungal biomass and nitrogen and phosphorus content of decomposing substrates were found between streams running through eucalyptus plantations and deciduous forests (MOLINERO et al. 1996, POZO et al. 1998, DíEZ et al. 2002). Differences in diversity of aquatic hyphomycete communities may translate into differences in richness and diversity of invertebrates (ABELHO & GRAÇA 1996) since shredders can discriminate among leaf patches colonized by different fungal species (AR-SUFFI & SUBERKROPP 1985, 1986, 1989, reviewed by SUBERKROPP 1992).

Changes in litter seasonality, quantity and diversity, and their effect on invertebrates and aquatic hyphomycetes can potentially affect litter decomposition. However, the available information on this subject is contradictory. ABELHO & GRAÇA (1996) reported lower decomposition rates of *Castanea sativa* MILL. leaves in eucalyptus than in deciduous streams while no differences in decomposition of native and exotic leaf species and wood between both stream types were found in other studies (MOLINERO et al. 1996, POZO et al. 1998, BÄRLOCHER & GRAÇA 2002, DíEZ et al. 2002). If species richness and diversity of decomposers decline in eucalyptus streams without corresponding decrease in decomposition rates, then decomposers would be characterized by functional redundancy (WALKER 1992).

There may be some differences in the effect of eucalyptus plantations on aquatic communities in Portugal and Spain, potentially explained by differences in climate (reviewed by GRAÇA et al. 2002): precipitation is highly seasonal in Central Portugal whereas in Northern Spain it is more evenly distributed along the year. In this study, we investigated the decomposition of leaves of two native species (alder, *Alnus glutinosa* (L.) GAERTNER, and oak, *Quercus robur* L.) and associated fungal communities in two streams running through eucalyptus plantations and two streams running through deciduous forests, one pair in Portugal and the other in Spain. Alder and oak were selected because (a) they are close to the extremes of the decomposition rates continuum and (b) are common in the riparian area of most deciduous streams in both countries.

## Methods

### Study sites

One stream running through a deciduous forest (deciduous stream) and one stream running through a eucalyptus plantation (eucalyptus stream) were selected in Central Portugal (Lousã and Caramulo mountains, respectively) and in Northern Spain (Cantabria) (Table 1). Chestnut (*Castanea sativa* MILL.) and oak (*Quercus* spp.) trees shaded the stream running through the deciduous forest in Portugal while in the eucalyptus plantation *Eucalyptus globulus* was the dominant species along the stream banks. Oak (*Quercus robur*) and alder (*Alnus glutinosa*) dominated the riparian corridor of the deciduous stream in Spain while *E. globulus* was dominant along the eucalyptus stream but accompanied by young deciduous trees (oak, alder, hazelnut *Corylus avellana* L., ash *Fraxinus excelsior* L.) in the understory. Except for riparian vegetation, pairs of streams in both countries were chosen to be as similar as possible with respect to morphology, geology, substrate and physico-chemical characteristics (Table 1).

### Water parameters

During the study period and in all streams, water temperature was recorded every hour (Portugal) or two hours (Spain) with temperature probes (Onset Optic stowAway in Portugal and ACR SmartButton in Spain), and conductivity (WTW LF 330) and pH (JENWAY 3310 in Portugal and Hanna HI9025 in Spain) were measured 4–9 times. From each stream, 1L of stream water was collected (4–9 times) in acid washed glass bottles (Portugal) or polyethylene flasks (Spain). Water was filtered through glass fiber filters in the field (Portugal, Millipore APFF) or transported in ice chests to the laboratory and then filtered (Spain, Whatman GF/F). An unfiltered subsample was promptly analyzed for ammonia while filtered subsamples for nitrate and soluble reactive phosphorus (SRP) analyses were frozen. Nitrate and ammonia concentrations were determined by ion chromatography (Dionex DX-120 in Portugal) or colorimetry (Autoanalyzer TRAACS 800 in Spain). SRP was determined by the ascorbic acid method (APHA 1995). Alkalinity was measured on 3–9 occasions by titration to an end pH of 4.5 (APHA 1995).

### Litter bags and decomposition

In each country, alder (*Alnus glutinosa*) and oak (*Quercus robur*) leaves were collected from the same stand just after abscission in autumn 2002. Leaves were air-dried and stored dry until needed. On November 26 (Portugal) and December 10 (Spain), 2002, batches of  $5.0 \pm 0.25$  g of alder or oak leaves were weighed, rehydrated, enclosed into tetrahedral coarse mesh bags (15 × 10 cm, 10 mm mesh) and deployed in streams the following day. A flood in early January 2003 washed away most oak bags in Portugal, so new batches were prepared and deployed in the same streams on January 26, 2003, and the results from these bags will be presented here. A flood was also registered in Spain on February 4, 2003, affecting only oak bags (alder experiment was already fin-

**Table 1.** Location, geology and physico-chemical characteristics (mean and range) of deciduous and eucalyptus streams in Spain and Portugal. n, number of measurements.

	Spain			Portugal		
	n	Deciduous	Eucalyptus	n	Deciduous	Eucalyptus
Latitude, N		43° 19' 30"	43° 19' 52"		40° 4' 44"	40° 30' 57"
Longitude, W		3° 20' 05"	3° 19' 37"		8° 12' 30"	8° 18' 23"
Altitude (m a. s. l.)		135	75		640	215
Catchment area (km <sup>2</sup> )		4.1	5.7		0.3	2.0
Distance to source (km)		4.0	4.8		0.7	1.5
Geology		siliceous	siliceous		siliceous	siliceous
Dominant substrate type (cm)		20–40	6–40		20–40	6–20
Temperature (°C)	1248	8.8 (2.4–13.2)	8.7 (2.9–15.8)	2278	9.2 (5.4–11.5)	11.7 (8.7–14.1)
Conductivity (µS/cm)	9	84 (54–113)	83 (54–116)	5	23 (22–23)	34 (32–38)
Alkalinity (mg CaCO <sub>3</sub> /L)	9	25.1 (15.0–34.5)	22.8 (10.0–32.0)	3	4.5 (4.1–5.1)	4.4 (4.1–4.7)
NO <sub>3</sub> -N (µg/L)	9	461.5 (198.0–652.0)	425.6 (237.0–570.0)	7	110.1 (79.5–153.8)	137.0 (98.3–204.4)
NH <sub>4</sub> -N (µg/L)	9	42.2 (3.0–142.0)	52.5 (6.0–139.0)	4	<10.0–14.0	<10.0
SRP (µg/L)	9	9.4 (2.0–23.0)	<1.0–49.0	7	<2.0–7.0	3.4 (2.0–4.0)
pH	9	7.3 (6.6–7.6)	7.2 (6.9–7.5)	4	6.4 (6.3–6.6)	6.3 (6.2–6.5)

ished). This flood, however, had less dramatic effects than that in Portugal allowing for two samplings after the event.

Leaf bags (22 per leaf species per stream) were tied by nylon lines (2 per line) to iron rebars driven into the stream bed along a 200 m reach. Alder leaf bags were retrieved randomly after 7, 13, 20, 26 and 42 days of incubation in Portugal, and after 7, 23, 28, 31 and 43 days in Spain, whereas oak leaves were retrieved after 14, 26, 43, 57 and 74 days in Portugal, and 23, 41, 65 and 104 days in Spain (4–6 replicates of each substrate type each time). Extra sets of leaves were prepared to calculate corrections for leaching and air dry mass to ash free dry mass (AFDM) conversion factors. After retrieval, bags were placed in individual zip lock bags and transported in ice chests to the laboratory where they were processed within 24 h. In the laboratory, leaf material from each bag was gently rinsed with distilled water and 2 sets of 5 leaf disks were cut out with a cork borer (12 mm diameter, see below). The remaining material was oven dried at 105 °C for 24 h and weighed. A subsample was then taken, weighed, ashed at 550 °C for 4 h and reweighed to calculate % ash and AFDM.

### **Nitrogen and phosphorus in leaves**

A subsample of leaf material was ground into a fine powder (Retsch ZN100 or Culatti DFH48 mills, 1 mm screen) and analyzed for N and P in the same laboratory. Nitrogen was determined using a Perkin Elmer Series II CHNS/O analyzer. Phosphorus was determined spectrophotometrically at 700 nm, after mixed acid digestion (15 min. at 325 °C; ALLEN 1989). Results were expressed as % N and % P of the remaining leaf litter AFDM.

### **Fungal biomass**

One set of leaf disks was used for ergosterol determination (GESSNER & SCHMITT 1996, GESSNER 2005), which is a measure of fungal biomass. Leaf disks were frozen and later freeze-dried just before extraction. For ergosterol extraction, leaf disks were placed in tightly closed tubes with 10 mL of KOH/methanol in a water bath (80 °C) for 30 min., with stirring. The extract was then purified by solid-phase extraction (Waters Sep-Pak Vac RC tC<sub>18</sub> cartridges) as described by GESSNER (2005). Ergosterol was quantified by high performance liquid chromatography (HPLC) by measuring absorbance at 282 nm. The HPLC system (Dionex DX-120) was equipped with the reverse phase C<sub>18</sub> column (Brownlee SPHERI-5RP-18, Applied Biosystems) maintained at 33 °C. The mobile phase was 100% methanol and the flow rate was set at 1.5 mL/min. Ergosterol was converted into fungal biomass with a conversion factor of 5.5 µg ergosterol/mg fungal dry mass (GESSNER & CHAUVET 1993). Ergosterol analyses were done in Portugal and results were expressed as mg fungal biomass/g AFDM.

### **Sporulation of aquatic hyphomycete**

The 2<sup>nd</sup> set of leaf disks was used to induce sporulation of aquatic hyphomycetes (BÄRLOCHER 2005). Disks were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (glass fiber filter, Millipore APFF) on a shaker (100 rpm) for 48 h

at 10 °C. The conidia suspensions were decanted into 50 mL centrifuge tubes, flasks rinsed twice, and conidia fixed with 2 mL of 37 % formalin to be later counted and identified. When preparing slides for conidia identification in Portugal, 100 µL of Triton X-100 solution (0.5 %) were added to the suspension to ensure a uniform distribution of conidia, stirred and an aliquot of the suspension was filtered (Millipore SMWP, 5 µm pore size). Filters were stained with trypan blue in lactic acid (0.05 %), and spores were identified and counted with a compound microscope at 200 ×. Leaf disk AFDM was determined as described above for bulk leaf material. Sporulation rates were expressed as number of conidia released mg<sup>-1</sup> AFDM day<sup>-1</sup>.

## Statistical analysis

After correcting leaf litter initial mass for leaching, decomposition rates,  $k$ , were calculated by linear regression of ln transformed data (negative exponential model  $M_t = M_0 \cdot e^{-kt}$ , where  $M_0$  is the initial mass,  $M_t$  is the remaining mass at time  $t$ , and  $k$  is the decomposition rate). As there were differences in temperature between streams (Table 1), decomposition rates were expressed in terms of /degree days by replacing time ( $t$ ) by the sum of mean daily temperatures accumulated by the sampling day. Decomposition rates were compared by ANCOVA followed by Tukey's test (ZAR 1999).

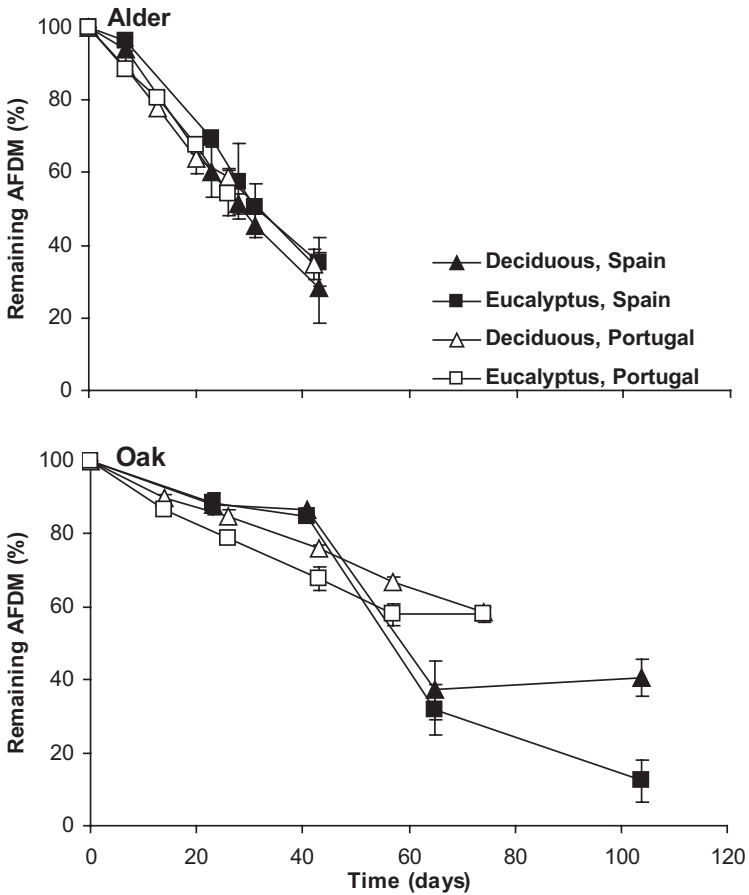
Evenness (Pielou's index,  $J'$ ) of fungal communities from decomposing leaves was calculated from conidial abundances (PRIMER 6) (WASHINGTON 1984). Comparisons of N and P content of litter, fungal biomass and sporulation, aquatic hyphomycete species richness and evenness of fungal communities associated with decomposing leaves between deciduous and eucalyptus streams, within each country, were done by 2-way ANOVA with stream type and time as factors (STATISTICA 6). Comparisons between deciduous or eucalyptus streams between countries were done by t test of mean values over time (N and P content of leaves) or peak values (fungal biomass and sporulation, species richness and Pielou's index). Initial values of fungal biomass and N and P content of alder and oak leaves were also compared between countries by t test.

Multidimensional scaling (MDS) based on Bray-Curtis similarity matrix of relative abundances of aquatic hyphomycetes in both stream types (all samples considered) for each leaf species and country was performed with PRIMER 6. Differences in aquatic hyphomycete communities between stream types were assessed by analysis of similarities (ANOSIM, PRIMER 6; CLARKE & GORLEY 2001).

## Results

### Decomposition

Alder leaves decomposed fast in both countries with 28–54 % of AFDM remaining after 4–6 weeks (Fig. 1). Oak leaves decomposed more slowly with 58 % of AFDM remaining after 11 weeks of incubation in Portugal and 12–41 % after 15 weeks of incubation in Spain. Generally, mass loss patterns were similar in both countries, and no significant differences in decomposition rates were found between stream types or between countries for alder (ANCOVA,



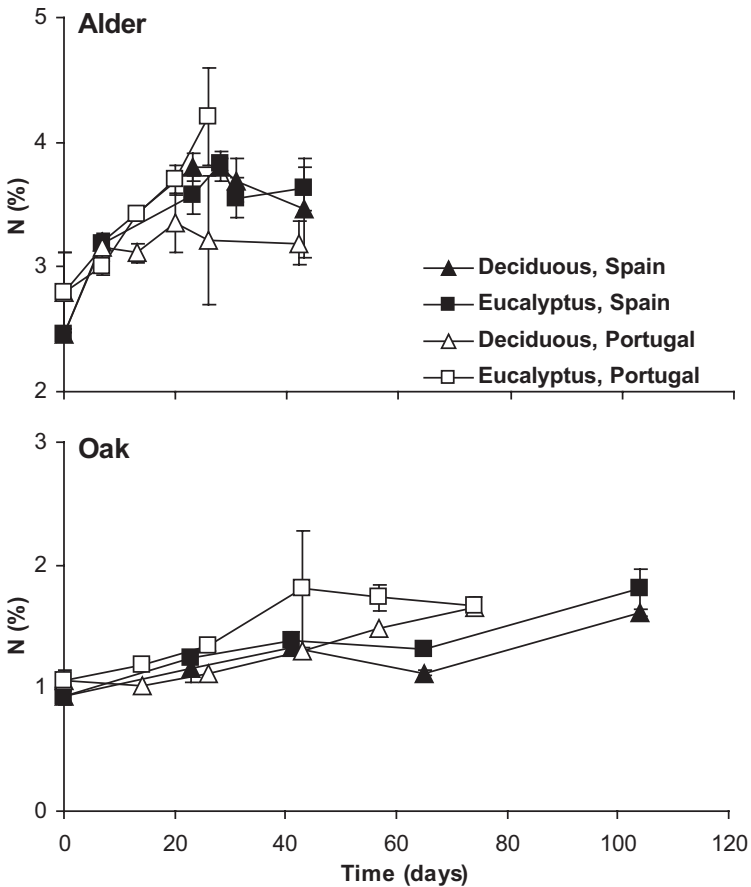
**Fig. 1.** Remaining mass (mean  $\pm$  1 SE) of alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.

**Table 2.** Decomposition rates ( $k$ /degree-day, 1 SE in parenthesis) of alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal. For each leaf species, decomposition rates with the same letter were not significantly different (ANCOVA followed by Tukey’s test,  $p > 0.05$ ).

		Alder		Oak	
Spain	Deciduous	0.0032 (0.0004)	a	0.0012 (0.0002)	a
	Eucalyptus	0.0024 (0.0002)	a	0.0025 (0.0003)	b
Portugal	Deciduous	0.0024 (0.0001)	a	0.0008 (<0.0001)	a
	Eucalyptus	0.0019 (0.0002)	a	0.0008 (<0.0001)	a

$R^2$  varied between 0.53 and 0.97



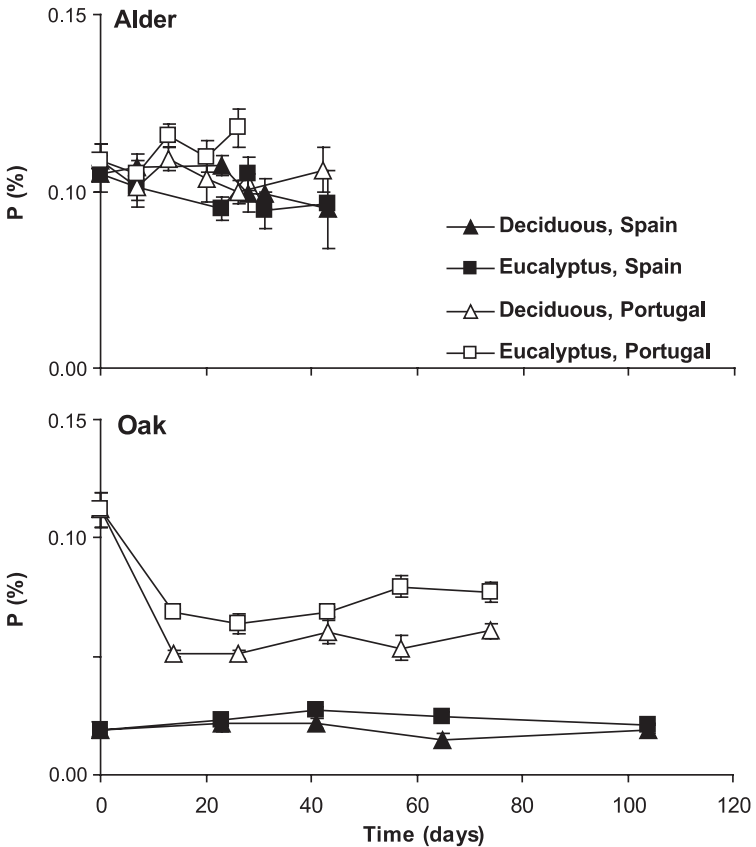


**Fig. 2.** Nitrogen content (mean  $\pm$  1 SE) of alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.

$F_{81,4} = 1.58$ ,  $p = 0.199$ ). For oak leaves decomposition was faster in the eucalyptus stream in Spain than in the corresponding deciduous stream or in the eucalyptus stream in Portugal (ANCOVA,  $F_{77,3} = 15.69$ ,  $p < 0.001$ ; Table 2).

### Nitrogen and phosphorus in leaves

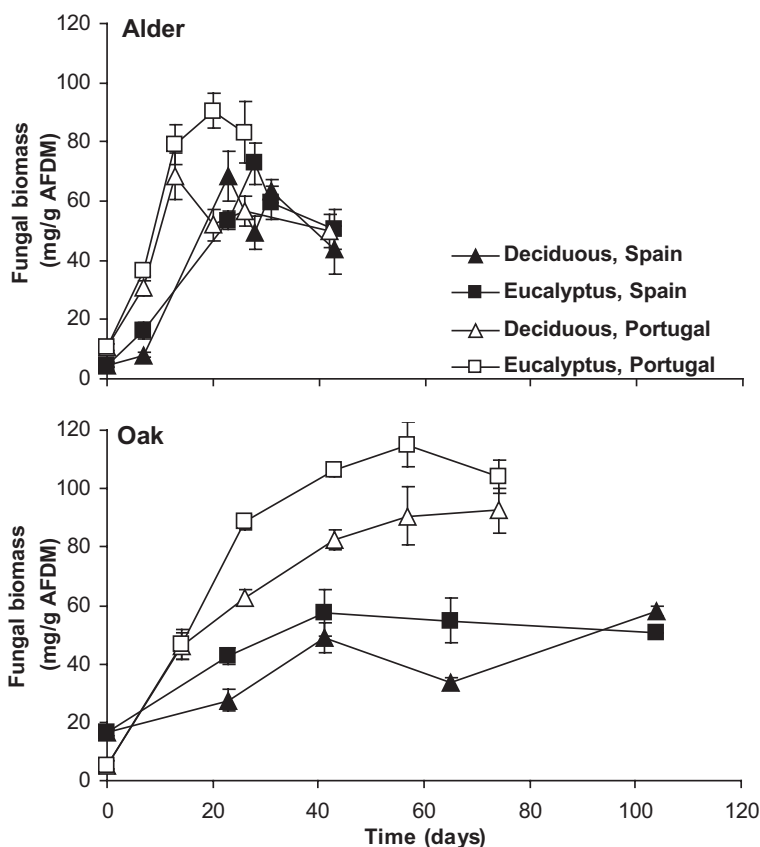
Initial leaf litter N content was similar between countries (t test,  $p = 0.314$  and  $0.284$ , for alder and oak, respectively). Nitrogen content of leaves generally increased through time in both alder and oak (Fig. 2), suggesting microbial N immobilization. Leaves incubated in the eucalyptus stream had significantly higher N content than leaves in the deciduous stream in both countries (2-way ANOVA,  $p < 0.038$ ), except for alder leaves in Spain (2-way ANOVA,  $p =$



**Fig. 3.** Phosphorus content (mean  $\pm$  1 SE) of alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.

0.749). Nitrogen content of alder leaves incubated in the deciduous stream was higher in Spain than in Portugal (*t* test,  $p = 0.013$ ).

The dynamics of phosphorus in leaves was not consistent between streams or countries (Fig. 3). P content of alder leaves decreased (Spain), remained constant (Portugal, deciduous stream) or increased (Portugal, eucalyptus stream) through time, being significantly higher in the eucalyptus than in the deciduous stream in Portugal (2-way ANOVA,  $p = 0.012$ ). Oak leaves in Portugal had 6 times higher initial P content than in Spain (*t* test,  $p < 0.001$ ), and after the initial decrease, P content remained relatively constant until the end of the experiment, while in Spain it remained constant throughout the experiment. In both countries, P content of oak leaves was significantly higher in the eucalyptus than in the deciduous stream (2-way ANOVA,  $p < 0.018$ ). Phosphorus content of leaves was higher in Portugal than in Spain (*t* test,  $p < 0.005$ ),

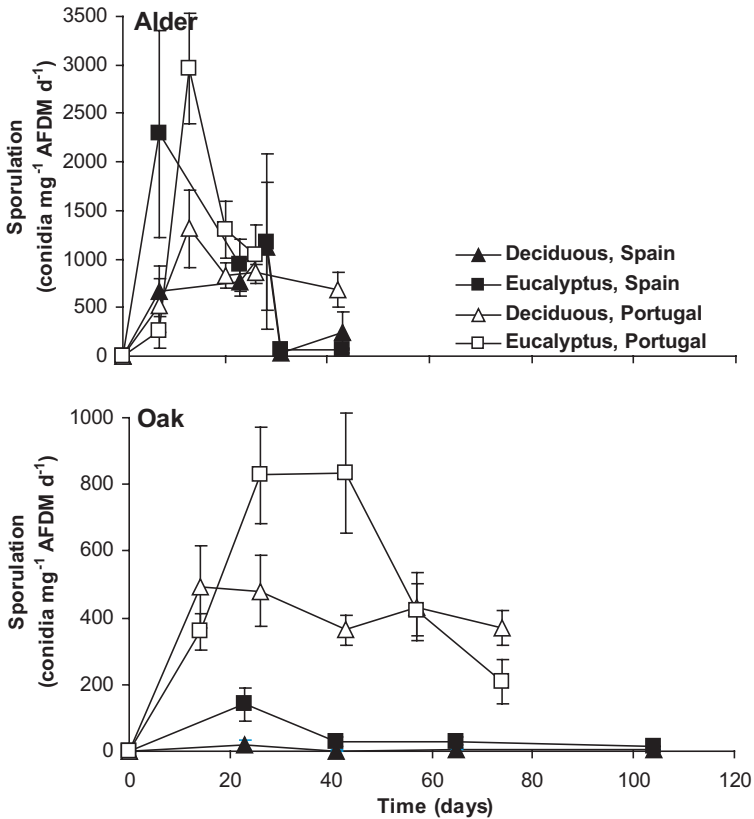


**Fig. 4.** Fungal biomass (mean  $\pm$  1 SE) associated with alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.

except for alder leaves in the deciduous streams where there were no significant differences (t test,  $p = 0.403$ ).

### Fungal biomass

The difference in initial fungal biomass between countries was significant for both leaf species (t test,  $p = 0.029$  for alder and  $0.008$  for oak), being higher in Portugal than in Spain for alder while the opposite was true for oak. Fungal biomass associated with alder leaves peaked earlier in the deciduous than in the eucalyptus stream, in both countries (day 14 vs. 20 in Portugal and day 23 vs. 28 in Spain; Fig. 4), however, eucalyptus streams had significantly higher fungal biomass than deciduous ones (2-way ANOVA,  $p < 0.017$ ). Although the fungal biomass peaked earlier in Portugal than in Spain, no significant differences were found in peak values between countries (t test,  $p > 0.099$ ).



**Fig. 5.** Sporulation rate (mean  $\pm$  1 SE) of aquatic hyphomycetes associated with alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.

Fungal biomass associated with oak leaves in eucalyptus streams peaked by days 41–57 while in deciduous streams it increased through time (Fig. 4). Leaves incubated in the eucalyptus stream had higher fungal biomass than leaves in the deciduous stream in both countries (2-way ANOVA,  $p < 0.001$ ). Maximum fungal biomass associated with decomposing oak was higher in Portugal than in Spain (t test,  $p < 0.014$ ).

### Sporulation of aquatic hyphomycetes

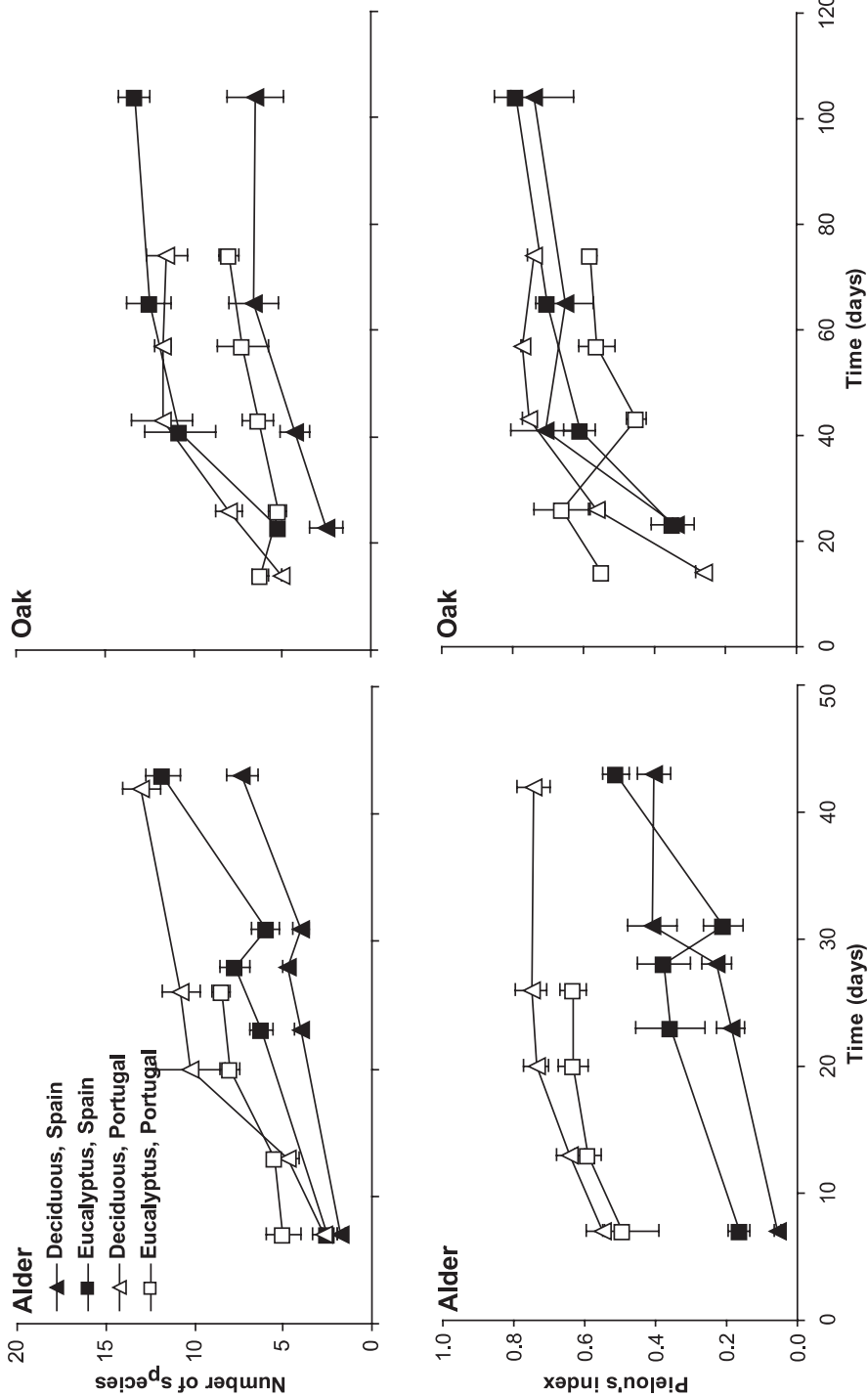
Maximum sporulation rates of aquatic hyphomycetes associated with alder leaves tended to be higher in eucalyptus than in deciduous streams (2288 vs. 1128 conidia  $\text{mg}^{-1}$  AFDM  $\text{d}^{-1}$  in Spain and 2969 vs. 1311 conidia  $\text{mg}^{-1}$  AFDM  $\text{d}^{-1}$  in Portugal; Fig. 5). However, no overall significant differences were found between stream types in both countries (2-way ANOVA,  $p > 0.239$ ). No signif-

**Table 3.** Mean relative abundances (% , over 4–5 sampling dates) of aquatic hyphomycete conidia from alder and oak leaves incubated in deciduous (D) and eucalyptus (E) streams in Spain and Portugal. Total numbers of species and mean evenness index are also given. +, mean relative abundance <0.1%.

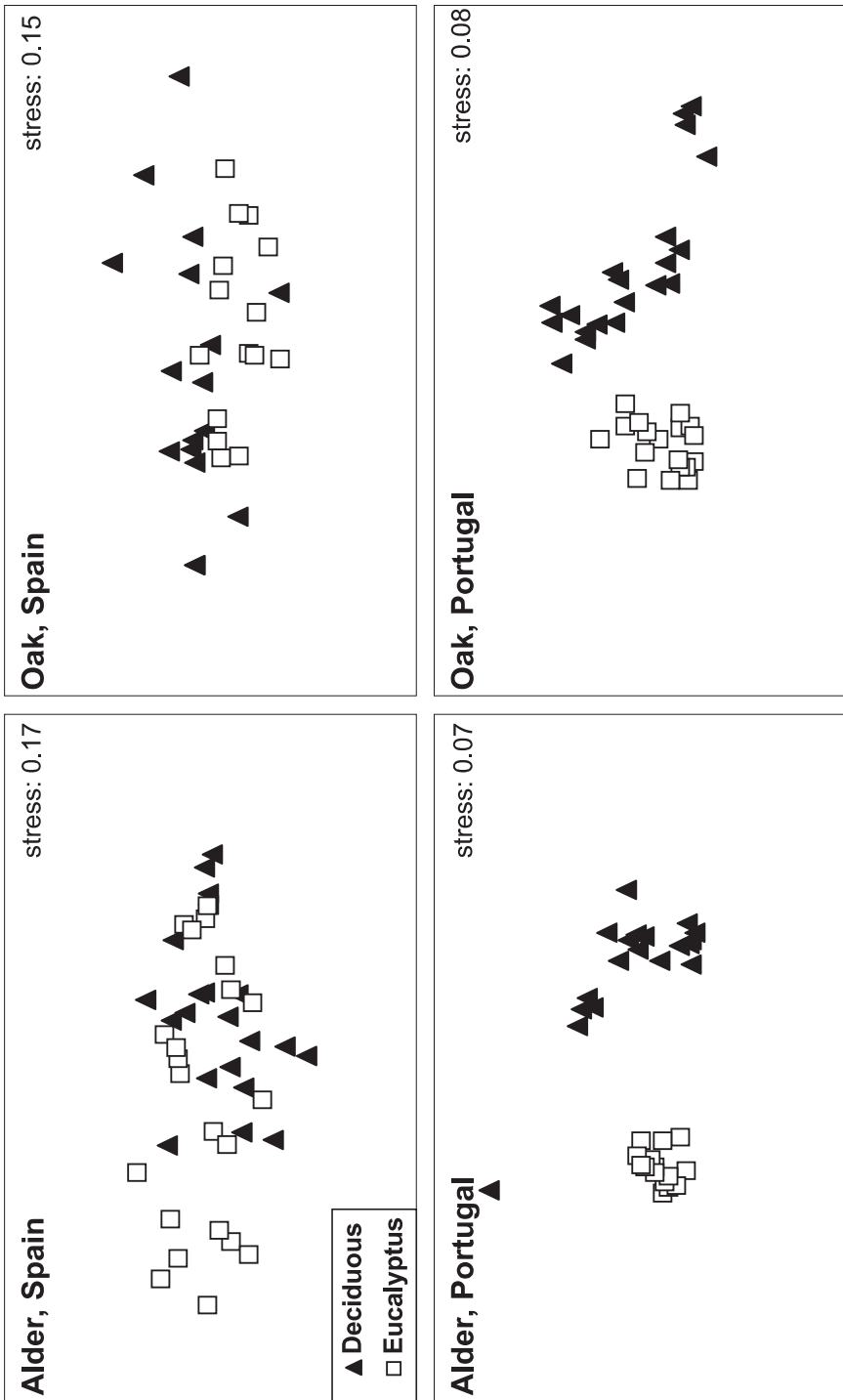
Aquatic Hyphomycete	Alder				Oak			
	Spain		Portugal		Spain		Portugal	
	D	E	D	E	D	E	D	E
<i>Alatospora acuminata</i> INGOLD s. l.	0.8	1.1	1.5	0.5	6.3	5.1	2.2	0.2
<i>Alatospora flagellata</i> (J. GÖNCZÖL) MARVANOVÁ			+		0.2	+	0.1	
<i>Alatospora pulchella</i> MARVANOVÁ			0.1	+	0.1		4.0	0.3
<i>Anguillospora crassa</i> INGOLD						0.3		
<i>Anguillospora filiformis</i> GREATH.	0.5	0.9	9.2	51.3	0.5	1.1	10.7	38.5
<i>Anguillospora</i> cf. <i>furtiva</i> J. WEBSTER & DESCALS						+		
<i>Anguillospora</i> cf. <i>longissima</i> (SACC. & P. SYD.) INGOLD		0.1						
<i>Articulospora tetracladia</i> INGOLD	6.0	4.9	1.7	1.1	14.9	8.7	1.9	1.2
? <i>Calcarispora hiemalis</i> MARVANOVÁ & MARVAN		+		0.1	0.3			
<i>Clavariopsis aquatica</i> DE WILD.	0.1	0.3			2.3	2.9	+	
<i>Clavatospora longibrachiata</i> (INGOLD) MARVANOVÁ & SV. NILSSON	0.1	1.4	8.3	0.5	1.9	2.3	11.1	2.0
<i>Crucella subtilis</i> MARVANOVÁ & SUBERKR.		+	11.0			0.1	2.3	
<i>Flagellospora curvula</i> INGOLD	89.2	82.9	14.4	0.1	53.6	44.1	4.2	0.4
<i>Goniopila monticola</i> (DYKO) MARVANOVÁ & DESCALS		+	0.5			0.2	0.1	
<i>Heliscella stellata</i> (INGOLD & V. J. COX) MARVANOVÁ		0.3	3.4	+	4.7	1.5	8.3	0.1
<i>Heliscus lugdunensis</i> SACC. & THERRY		0.1						
<i>Lemonniera aquatica</i> DE WILD.				0.7			+	0.3
<i>Lemonniera terrestris</i> TUBAKI								0.1
<i>Lumulospora curvula</i> INGOLD	1.4	4.4		29.6	7.3	19.4		5.9
<i>Stenocladia neglecta</i> (MARVANOVÁ & DESCALS) MARVANOVÁ & DESCALS	+	0.4	0.8		+	2.1	0.3	
<i>Taeniospora gracilis</i> var. <i>enecta</i> MARVANOVÁ & STALPERS			0.3			0.1	0.4	
<i>Tetrachaetum elegans</i> INGOLD	1.4	2.6	32.9	0.4	4.7	3.3	36.6	0.5
<i>Tetracladium marchalianum</i> DE WILD.	+	+						
<i>Tricladium chaetocladium</i> INGOLD		0.3	1.4	12.0		2.9	16.3	48.7
<i>Tricladium splendens</i> INGOLD							+	
<i>Triscelophorus acuminatus</i> NAWAWI		+	8.9	0.9		0.3	0.1	0.3
<i>Triscelophorus monosporus</i> INGOLD		+			1.0	4.0		
<i>Variocladium giganteum</i> (S. H. IQBAL) DESCALS & MARVANOVÁ					1.2	0.2		
Sigmoid conidia (<60µm long)	0.1	0.1	5.5	2.7	0.6	0.1	1.3	1.4
Sigmoid conidia (60–120µm long)					0.2			
Sigmoid conidia (>120µm long)					0.1	0.1		+
Unidentified tetrastrate conidia	0.3	0.1	0.1	0.2	0.2	0.9	0.2	0.1
Total number of species	12	21	17	14	19	24	20	16
Pielou's evenness index	0.28	0.33	0.70	0.59	0.65	0.60	0.62	0.57

icant differences were found in the peak values between streams in Portugal and Spain (t test,  $p > 0.594$ ).

Maximum sporulation rates associated with oak leaves were higher in eucalyptus than in deciduous streams (828 vs. 492 conidia  $\text{mg}^{-1}$  AFDM  $\text{d}^{-1}$  in Portugal and 141 vs. 19 conidia  $\text{mg}^{-1}$  AFDM  $\text{d}^{-1}$  in Spain; Fig. 5). Sporulation in the Spanish eucalyptus stream was significantly higher than in the decidu-



**Fig. 6.** Species richness of aquatic hyphomycetes and Pielou's evenness of fungal communities (mean  $\pm$  1 SE) associated with alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal.



**Fig. 7.** MDS ordination of fungal assemblages colonizing alder and oak leaves incubated in deciduous and eucalyptus streams in Spain and Portugal. The stress was  $<0.20$ , the 2D ordination plot therefore faithfully represented the multi-dimensional relationships among the samples.

ous stream (2-way ANOVA,  $p < 0.001$ ). Streams in Portugal had significantly higher maximum sporulation rates than streams in Spain (t test,  $p < 0.009$ ).

### Aquatic hyphomycete communities

Thirty three species of aquatic hyphomycetes have been found in this study, 23 in Portugal and 29 in Spain (Table 3). Species richness was higher in the eucalyptus than in the deciduous stream in Spain (21 vs. 12 species on alder and 24 vs. 19 on oak) while in Portugal it was the opposite (14 vs. 17 on alder and 16 vs. 20 on oak). *Flagellospora curvula* dominated the fungal communities on both leaf species in both streams in Spain (mean relative abundance 82.9–89.2% on alder and 44.1–53.6% on oak). *F. curvula* was an early colonizer with its maximum spore production during the first weeks of litter decomposition that coincided with the overall sporulation peak. As its abundance declined during oak litter decomposition, *Lunulospora curvula* (7.3–19.4%) and *Articulospora tetracladia* (8.7–14.9%) became codominant while on alder the relative abundance of *F. curvula* never declined below 66%. *Tetrachaetum elegans* dominated on both alder and oak leaves in the deciduous stream in Portugal (32.9–36.6%) while *Anguillospora filiformis* (51.3%) and *L. curvula* (29.6%) dominated on alder leaves and *Tricladium chaetocladium* (48.7%) and *A. filiformis* (38.5%) on oak leaves in the eucalyptus stream.

Although there was a tendency for leaves incubated in Portugal to have higher fungal species richness and evenness in the deciduous than in the eucalyptus stream (Fig. 6, Table 3) differences between stream types were significant only for the Pielou's index for alder and species richness for oak leaves (2-way ANOVA,  $p < 0.028$ ). Species richness for both leaf species and Pielou's index for alder were higher in the eucalyptus than in the deciduous stream in Spain (2-way ANOVA,  $p < 0.019$ ). Alder leaves incubated in the deciduous stream in Portugal had higher maximum number of species and evenness than leaves incubated in Spain, (t test,  $p = 0.003$  and  $0.001$ , respectively), while the opposite was true for the maximum number of species for alder and oak leaves in the eucalyptus stream (t test,  $p = 0.031$  and  $0.006$ , respectively).

Fungal communities colonizing alder and oak leaves in Spain were different between the deciduous and the eucalyptus stream although there was some overlap (ANOSIM,  $p = 0.001$  and  $R = 0.52$  for alder and  $p = 0.002$  and  $R = 0.33$  for oak; Fig. 7) while in Portugal both stream types were completely separated based on their fungal communities (ANOSIM,  $p = 0.001$  and  $R = 0.92$  for alder and  $p = 0.001$  and  $R = 1.00$  for oak; Fig. 7).



## Discussion

Using identical protocols, this study assessed the effect of eucalyptus plantations on fungi associated with leaf litter decomposition in Portuguese and Spanish streams. The two streams in each country were selected to be similar with respect to geology, morphology, substrate and physico-chemical parameters and to differ only in riparian vegetation. However, there were some differences in water temperature and initial P content of oak litter between countries that may have confounded some comparisons. It is still difficult to generalize about the impact of eucalyptus plantations on aquatic fungal communities and associated processes since the eucalyptus stream in Portugal was apparently more affected than that in Spain. This differential effect of eucalyptus plantations probably resulted from differences in climate between the two regions.

## Decomposition

In agreement with previous studies using the same and other litter types (e. g. MOLINERO et al. 1996, POZO et al. 1998, DÍEZ et al. 2002, BÄRLOCHER & GRAÇA 2002), decomposition rates of alder and oak leaves in Portugal did not differ significantly between eucalyptus and deciduous forest streams. In Spain, however, oak leaves decomposed significantly faster in the eucalyptus than in the corresponding deciduous stream and than in the eucalyptus stream in Portugal. These differences were most likely brought about by physical abrasion caused by the flood in early February in Spain. Before this event decomposition dynamics of oak leaves were very similar in both the deciduous and the eucalyptus stream.

## Nitrogen and phosphorus in leaves

Litter nutrient dynamics has been reported to be closely associated with microbial dynamics (e. g. SAMPAIO et al. 2001, GULIS & SUBERKROPP 2003) as fungal biomass is nutrient rich. Nitrogen content of both leaf types increased through time as expected following fungal colonization (e. g. MOLINERO et al. 1996, POZO et al. 1998, SAMPAIO et al. 2001, GULIS & SUBERKROPP 2003) while phosphorus showed a more erratic pattern. The 6 times higher initial P content of oak leaves in Portugal than in Spain was a surprise (MOLINERO et al. 1996, SAMPAIO et al. 2001). Generally, there was higher nutrient content in leaves incubated in eucalyptus than in deciduous streams, which could be a result of higher fungal activity in the former ones. Our findings contradict some previous reports. MOLINERO et al. (1996) found no significant differences in the nutrient contents of oak, chestnut and eucalyptus leaves between stream

types and POZO et al. (1998) found higher N content in alder leaves incubated in a deciduous stream than in those incubated in an eucalyptus stream. Nutrient content was also higher in Portuguese than in Spanish streams, probably as a consequence of higher fungal activity in Portuguese streams.

### **Fungal biomass and sporulation**

Fungal biomass and sporulation rates were generally higher on leaves incubated in eucalyptus than on leaves incubated in deciduous streams in both countries. In contrast, CHAUVET et al. (1997) and POZO et al. (1998) found no significant differences in these parameters between stream types. In this study, differences in fungal activity between stream types might have resulted not only from differences in the forest cover but also in stream water temperature. Higher water temperatures are known to stimulate the activity of some fungal species (CHAUVET & SUBERKROPP 1998, GRAÇA & FERREIRA 1995), and eucalyptus streams had higher water temperatures than deciduous streams. Higher temperatures in Portuguese than in Spanish streams could also explain higher sporulation rates and fungal biomass in the former ones, in spite of higher nutrient content in Spanish streams. The higher fungal activity in eucalyptus streams might also be a result of differences in litter dynamics. Although eucalyptus streams receive lower litter inputs, their benthic storage is higher than in deciduous streams as a result of the summer peak in litter input that coincides with a period of low discharge (POZO et al. 1997 a, b, MOLINERO & POZO 2003, 2004). Since aquatic hyphomycetes are sensitive to changes in the amount of CPOM in stream (LAITUNG et al. 2002), streams with higher benthic storage might be expected to support higher fungal activity.

### **Aquatic hyphomycete communities**

Although fungal activity was generally higher in the eucalyptus than in the deciduous stream in Portugal, the species richness and evenness of fungal communities were higher in the deciduous stream, which agrees with a previous report by BÄRLOCHER & GRAÇA (2002). This suggests a certain level of functional redundancy among species of aquatic hyphomycetes when loss of some species does not translate into appreciable decrease in ecosystem functioning (decomposition) (WALKER 1992, DANG et al. 2005). However, in Spain, we found higher species richness and higher evenness of fungal communities in the eucalyptus stream, which is difficult to explain unless the understory of young deciduous trees in the eucalyptus stream had a major effect (see below).

Stream types in Portugal were completely separated based on their fungal communities (MDS analysis) where 7 species were found only in the deciduous stream. Overlap in fungal communities was greater in Spain, even though

9 rare species were restricted to the eucalyptus stream. This suggests that the effect of eucalyptus plantation on fungal community structure was stronger in Portugal than in Spain. There are several possible explanations. (a) The eucalyptus plantation in Spain had young deciduous trees in the understory that increased the diversity of leaf litter entering the eucalyptus stream. This may support higher fungal diversity than in the eucalyptus stream in Portugal, which was bordered almost exclusively by eucalyptus trees. (b) Precipitation in northern Spain is higher and more evenly distributed throughout the year. In central Portugal, precipitation is highly seasonal with summer droughts affecting stream flow, benthic organic matter accumulation, water temperature and chemistry. All these effects are more pronounced in eucalyptus streams. Eucalyptus plantations have strongly hydrophobic soils, which have lower infiltration rates resulting in higher runoff in winter and lower stream water level in summer (streams can be reduced to ponds or completely dry out) (ABELHO & GRAÇA 1996). The tree canopy of eucalyptus plantations is more open than in deciduous forests because trees are generally young and regularly spaced, which allows for a greater water surface to be exposed to solar radiation (MOLINERO & POZO 2003). Reduced flow and higher water temperature (higher evaporation) results in increased ionic strength and concentration of phenolic compounds and decreased dissolved oxygen in eucalyptus streams.

Fungal communities colonizing alder and oak leaves were quite different between the two countries. *Flagellospora curvula* dominated fungal assemblages on both leaf species in both streams in Spain with *Lunulospora curvula* and *Articulospora tetracladia* as codominants. The dominance of *F. curvula* and *L. curvula* in Spanish streams was also reported by CHAUVET et al. (1997). *Tetrachaetum elegans*, *Anguillospora filiformis*, *Tricladium chaetocladium* and *L. curvula* were the most abundant species in Portugal. As observed before (BÄRLOCHER et al. 1995, CHAUVET et al. 1997, BÄRLOCHER & GRAÇA 2002, PASCOAL et al. 2005), *F. curvula* did not attain the same abundances in Portugal as it did in Spain (0.1–14.4% vs. 44.1–89.2%), which could be explained by the higher water temperatures in Portuguese streams and the preference of this species for colder waters (CHAUVET & SUBERKROPP 1998). Also, *L. curvula* was more abundant in Portuguese than in Spanish streams as previously reported (BÄRLOCHER et al. 1995, CHAUVET et al. 1997, BÄRLOCHER & GRAÇA 2002), which could again be explained by higher water temperature in Portuguese streams. The water temperature during the study period and across streams varied between 2.4 and 15.8 °C. The importance of *L. curvula* in this study therefore contrasts with previous reports that associated this species with warm waters (reviewed by BÄRLOCHER 1992). In a summer cool stream (< 15 °C), GESSNER et al. (1993) found a rather similar fungal community although species like *L. curvula* and *Triscelophorus monosporus*, considered warm water species, were not present.

The substitution of native forests by exotic monocultures has been associated with changes in sporulation dynamics of aquatic hyphomycetes (THOMAS et al. 1989) and decreases in fungal diversity and species richness (BÄRLOCHER & GRAÇA 2002). LAITUNG & CHAUVET (2005) also reported a strong effect of leaf litter abundance and diversity on conidia concentration in water, fungal species richness and diversity. However, in studies where leaf litter decomposition was simultaneously assessed, changes in fungal communities did not result in altered litter breakdown (BÄRLOCHER & GRAÇA 2002; this study). Changes in aquatic hyphomycete diversity but not in litter decomposition were also observed in 2 sites differing in water chemistry (PASCOAL et al. 2005) and in microcosm experiments (DUARTE et al. 2006). This could be explained by extensive functional redundancy among species of aquatic hyphomycetes (DANG et al. 2005), although BÄRLOCHER & CORKUM (2003) found that both species diversity and identity had significant effects on mass loss.

Nevertheless, as shredders can discriminate among leaf patches colonized by different fungal species (reviewed by SUBERKROPP 1992), changes in fungal diversity can indirectly affect litter decomposition in streams where shredders are abundant and diverse. This was not the case in our study where the abundance and species richness of shredders was low.

## Conclusions

Two main conclusions can be drawn from this study. First, stream fungal communities were more affected by riparian *Eucalyptus globulus* plantations in Portugal than in Spain. This is probably due to climatic differences. More humid conditions in Spain may facilitate survival of deciduous trees in the understory of eucalyptus plantations and, thus, litter inputs to eucalyptus streams may be less impoverished than in Portugal. Although there can be differences between stands, POZO et al. (1997 a, b) reported that 2% of leaves under eucalyptus plantations were deciduous and BAÑUELOS et al. (2004) found 8% of leaf material to be oak. Second, diversity and activity of aquatic hyphomycetes were more affected by eucalyptus plantations than leaf litter decomposition, probably as a result of functional redundancy among fungal species.

To mitigate the effect of eucalyptus plantations on aquatic communities, deciduous trees could be planted on the river banks or, preferably, riparian buffer strips of native vegetation should be kept. These would increase the diversity of litter input to streams and, hence, allow higher fungal diversity. Also, native buffer strips can regulate air and water temperature by reducing the amplitude of daily variations (MELEASON & QUINN 2004) as they may develop a closed canopy above small streams. The presence of these riparian

buffer strips is also of major importance when the plantations are harvested as they continue to provide shading and CPOM input to streams (BOOTHROY et al. 2004, QUINN et al. 2004) preventing the shift from heterotrophy to autotrophy and associated decreases in fungal diversity and abundance. The importance of native riparian buffer strips in alleviating the effects of eucalyptus plantations on aquatic ecosystems has also been emphasized in previous studies (POZO et al. 1997b, MOLINERO & POZO 2003, 2004).

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## References

- ABELHO, M. & GRAÇA, M. A. S. (1996): Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. – *Hydrobiologia* **324**: 195–204.
- ALLEN, S. E. (1989): *Chemical Analysis of Ecological Materials*. 2<sup>nd</sup> edn. – Blackwell Scientific Publications, Oxford, London.
- APHA (1995): *Standard Methods for the Examination of Water and Wastewater*, 19<sup>th</sup> edn. – EATON A. E., CLESCERI L. S. & GREENBERG A. E. (eds), Washington, D. C.
- ARSUFFI, T. L. & SUBERKROPP, K. (1985): Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. – *Oikos* **45**: 50–58.
- (1986): Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. – *J. N. Amer. Benthol. Soc.* **5**: 297–305.
- (1989): Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. – *Oecologia* **79**: 30–37.
- BAÑUELOS, R., LARRAÑAGA, S., ELOSEGI, A. & POZO, J. (2004): Effects of eucalyptus plantations on CPOM dynamics in headwater streams: a manipulative approach. – *Arch. Hydrobiol.* **159**: 211–228.
- BÄRLOCHER, F. (1992): Community organization. – In: BÄRLOCHER, F. (ed.): *The ecology of aquatic hyphomycetes*. – Springer-Verlag, Berlin, pp 38–76.
- (2005): Sporulation by aquatic hyphomycetes. – In: GRAÇA, M. A. S., BÄRLOCHER, F. & GESSNER, M. O. (eds): *Methods to Study Litter Decomposition. A Practical Guide*. – Springer, the Netherlands, pp 185–187.

- BÄRLOCHER, F., CANHOTO, C. & GRAÇA, M. A. S. (1995): Fungal colonization of alder and eucalypt leaves in two streams in Central Portugal. – Arch. Hydrobiol. **133**: 457–470.
- BÄRLOCHER, F. & CORKUM, M. (2003): Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. – Oikos **101**: 247–252.
- BÄRLOCHER, F. & GRAÇA, M. A. S. (2002): Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. – Freshwat. Biol. **47**: 1123–1135.
- BOOTHROY, I. K. G., QUINN, J. M., LANGER, E. R.(L.), COSTLEY, K. J. & STEWARD, G. (2004): Riparian buffers mitigate effects of pine plantation logging on New Zealand streams. 1. Riparian vegetation structure, stream geomorphology and periphyton. – Forest Ecol. & Manag. **194**: 199–213.
- CANHOTO, C., ABELHO, M. & GRAÇA, M. A. S. (2004): Efeitos das plantações de *Eucalyptus globulus* nos ribeiros de Portugal. – Recursos Hídricos **25**: 59–65.
- CANHOTO, C. & GRAÇA, M. A. S. (1995): Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis*. – Freshwat. Biol. **34**: 209–214.
- (1998): Leaf retention: a comparative study between two stream categories and leaf types. – Verh. Internat. Verein. Limnol. **26**: 990–993.
- (1999): Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. – Microb. Ecol. **37**: 1631–72.
- CHAUVET, E., FABRE, E., ELOSEGUI, A. & POZO, J. (1997): The impact of eucalypt on the leaf-associated aquatic hyphomycetes in Spanish streams. – Can. J. Bot. **75**: 880–887.
- CHAUVET, E. & SUBERKROPP, K. (1998): Temperature and sporulation of aquatic hyphomycetes. – Appl. Environ. Microbiol. **64**: 1522–1525.
- CLARKE, K. R. & GORLEY, R. N. (2001): Primer v5: User Manual/Tutorial. – Primer-E Ltd., Plymouth, UK.
- DANG, C. K., CHAUVET, E. & GESSNER, M. O. (2005): Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. – Ecol. Lett. **18**: 1129–1137.
- DÍEZ, J., ELOSEGI, A., CHAUVET, E. & POZO, J. (2002): Breakdown of wood in the Agüera stream. – Freshwat. Biol. **47**: 2205–2215.
- Direção Geral Florestas. (2005): <http://www.dgrf.min-agricultura.pt>
- DUARTE, S., PASCOAL, C., CÁSSIO, F. & BÄRLOCHER, F. (2006): Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. – Oecologia **147**: 658–666.
- FISHER, S. G. & LIKENS, G. E. (1973): Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. – Ecol. Monogr. **43**: 421–439.
- GESSNER, M. O. (2005): Ergosterol as a measure of fungal biomass. – In: GRAÇA, M. A. S., BÄRLOCHER, F. & GESSNER, M. O. (eds): *Methods to Study Litter Decomposition. A Practical Guide*. – Springer, the Netherlands, pp. 189–195.
- GESSNER, M. O. & CHAUVET, E. (1993): Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. – Appl. Environ. Microbiol. **59**: 502–507.
- GESSNER, M. O. & SCHMITT, A. L. (1996): Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi. – Appl. Environ. Microbiol. **62**: 415–419.

- GESSNER, M. O., THOMAS, M., JEAN-LOUIS, A.-M. & CHAUVET, E. (1993): Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. – *Mycol. Res.* **97**: 163–172.
- GRAÇA, M. A. S. & FERREIRA, R. C. F. (1995): The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater. – *Sydowia* **47**: 167–179.
- GRAÇA, M. A. S., POZO, J., CANHOTO, C. & ELOSEGI, A. (2002): Effects of Eucalyptus plantations on detritus, decomposers, and detritivores in streams. – *The Scientific World* **2**: 1173–1185.
- GULIS, V. (2001): Are there any substrate preferences in aquatic hyphomycetes? – *Mycol. Res.* **105**: 1088–1093.
- GULIS, V. & SUBERKROPP, K. (2003): Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. – *Freshwat. Biol.* **48**: 123–134.
- HIEBER, M. & GESSNER, M. O. (2002): Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. – *Ecology* **83**: 1026–1038.
- LAITUNG, B. & CHAUVET, E. (2005): Vegetation diversity increases species richness of leaf-decaying fungal communities in woodland streams. – *Arch. Hydrobiol.* **164**: 217–235.
- LAITUNG, B., PRETTY, J. L., CHAUVET, E. & DOBSON, M. (2002): Response of aquatic hyphomycete communities to enhanced stream retention in areas impacted by commercial forestry. – *Freshwat. Biol.* **47**: 313–323.
- MELEASON, M. A. & QUINN, J. M. (2004): Influence of riparian buffer width on air temperature at Whangapoua Forest, Coromandel Peninsula, New Zealand. – *Forest Ecol. & Manag.* **191**: 365–371.
- Ministerio de Medio Ambiente (2003): <http://www.mma.es/conservnat/inventarios/>
- MOLINERO, J. & POZO, J. (2003): Balances de hojarasca en dos arroyos forestados: impacto de las plantaciones de eucalipto en el funcionamiento ecológico de un sistema lótico. – *Limnetica* **22**: 63–70.
- (2004): Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation on the nutrient content and dynamics of coarse particulate organic matter (CPOM) in a small stream. – *Hydrobiologia* **528**: 143–165.
- MOLINERO, J., POZO, J. & GONZÁLEZ, E. (1996): Litter breakdown in streams of the Agüera catchment: influence of dissolved nutrients and land use. – *Freshwat. Biol.* **36**: 745–756.
- PASCOAL, C., CÁSSIO, F. & MARVANOVÁ, L. (2005): Anthropogenic stress may affect aquatic hyphomycetes diversity more than leaf decomposition in a low-order stream. – *Arch. Hydrobiol.* **162**: 481–496.
- POZO, J., BASAGUREN, A., ELÓSEGUI, A., MOLINERO, J., FABRE, E. & CHAUVET, E. (1998): Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. – *Hydrobiologia* **373/374**: 101–109.
- POZO, J., GONZÁLEZ, E., DÍEZ, J. & ELOSEGI, A. (1997 a): Leaf-litter budgets in two contrasting forested streams. – *Limnetica* **13**: 77–84.
- POZO, J., GONZÁLEZ, E., DÍEZ, J. R., MOLINERO, J. & ELOSEGUI, A. (1997 b): Inputs of particulate organic matter to streams with different riparian vegetation. – *J. N. Amer. Benthol. Soc.* **16**: 602–611.

- QUINN, J. M., BOOTHROY, I. K. G. & SMITH, B. J. (2004): Riparian buffers mitigate effects of pine plantation logging on New Zealand streams. 2. Invertebrate communities. – *Forest Ecol. & Manag.* **191**: 129–146.
- SAMPAIO, A., CORTES, R. & LEÃO, C. (2001): Invertebrate and microbial colonization in native and exotic leaf litter species in a mountain stream. – *Internat. Rev. Hydrobiol.* **86**: 527–540.
- SUBERKROPP, K. (1992): Interactions with invertebrates. In: BÄRLOCHER, F. (ed): *The ecology of aquatic hyphomycetes*. – Springer-Verlag, Berlin, pp 118–133.
- THOMAS, K., CHILVERS, G. A. & NORRIS, R. H. (1989): Seasonal occurrence of conidia of aquatic hyphomycetes (fungi) in Lees Creek, Australia Capital territory. – *Aust. J. Mar. Freshwat. Res.* **40**: 11–23.
- VANNOTE, R. L., MINSHALL, G. W., CUMMINS, K. W., SEDELL, J. R. & CUSHING, C. E. (1980): The river continuum concept. – *Can. J. Fish. Aquat. Sci.* **37**: 130–137.
- WALKER, B. H. (1992): Biodiversity and ecological redundancy. – *Conserv. Biol.* **6**: 18–23.
- WALLACE, J. B., EGGERT, S. L., MEYER, J. L. & WEBSTER, J. R. (1999): Effects of resource limitation on a detrital-based ecosystem. – *Ecol. Monogr.* **69**: 409–442.
- WASHINGTON, H. G. (1984): Diversity, biotic and similarity indices. A review with special relevance to aquatic systems. – *Water Res.* **18**: 653–694.
- ZAR, J. H. (1999): *Biostatistical Analysis*, 4th edn. – Prentice-Hall, Englewood Cliffs, NJ.

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