The effects of eucalypt plantations on plant litter decomposition and macroinvertebrate communities in Iberian streams

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ARTICLE INFO

ABSTRACT

Eucalypt plantations cover over 1.5 million ha in the Iberian Peninsula. The effects of the replacement of native deciduous forests by exotic plantations on stream communities and litter decomposition, a key ecosystem process in forest streams, are poorly understood. We compared microbially driven and total (microbes + invertebrates) decomposition of alder and oak leaf litter (high and low quality resource, respectively) as well as macroinvertebrate communities associated with decomposing litter and in the benthos, in five streams flowing through native deciduous broad-leaved forests and five streams flowing through eucalypt plantations in central Portugal and northern Spain (20 streams total). Total decomposition rate of alder leaf litter was slower in eucalypt than in deciduous streams, which was attributed to lower macroinvertebrate (and also shredder) colonization. No major effects of eucalypt plantations were found on macroinvertebrate colonization and total decomposition of oak litter, likely due to the low contribution of invertebrates to the decomposition of nutrient-poor litter. Microbially driven litter decomposition was generally not affected by forest change, likely due to high functional redundancy among microbes. Eucalypt streams had fewer invertebrates in Portugal than in Spain, which might be attributed to summer droughts in Portugal and the absence of deciduous riparian corridors in eucalypt plantations. In northern Spain, the relatively wet climate allows streams to flow year-round and eucalypt plantations have riparian deciduous trees that mitigate the effects of plantations. This study highlights the need to consider regional differences in climate, native vegetation, and the importance of macroinvertebrates, when assessing the effects of plantations on stream ecosystem processes such as carbon cycling. It also suggests that preservation of native riparian corridors, especially in drier areas, where the native vegetation provides high quality litter to the streams, and where invertebrates play an important role in aquatic processes, may mitigate the effects of plantations on stream communities and processes.

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1. Introduction

Planting of trees for timber, charcoal, and pulp is an important economic activity worldwide (Forest Resource Assessment (FRA), 2010). *Eucalyptus* is the second most common genus used in plantations due to its high adaptability, fast growth, and high economic value. It is now present in over 90 countries, mostly between 35° S and 35° N, covering a global area of over 20 million ha (Iglesias-Trabado and Wilstermann, 2009). *Eucalyptus globulus* Labill.

plantations cover over 1.5 million ha in the Iberian Peninsula and have replaced native mixed broad-leaved deciduous forests over large areas (Tercer Inventario Forestal Nacional (IFN3), 2007, Instituto de Conservação da Natureza e das Florestas (ICNF), 2013). These areas are typically drained by small streams, which derive most of their energy and carbon from litter provided by the surrounding vegetation (Fisher and Likens, 1973; Wallace et al., 1997).

Streams flowing through native temperate deciduous broad-leaved forests receive most of their litter input in autumn, and this input is often a diverse mixture of litter species that vary in physical and chemical characteristics (Abelho and Graça, 1996; Pozo et al., 1997; Molinero and Pozo, 2004; Swan and Palmer, 2004). In contrast, streams flowing through eucalypt plantations in the Iberian Peninsula receive their litter input year round (in some

areas with higher summer intensity), and this input is dominated by eucalypt leaves (Abelho and Graça, 1996; Pozo et al., 1997; Molinero and Pozo, 2004). Leaves of *E. globulus* have low nutrient concentrations, high concentration of secondary compounds (e.g. essential oils and polyphenolics), and a waxy cuticle (Canhoto and Graça, 1999). Annual litter input is also generally lower in eucalypt than in deciduous streams (Pozo et al., 1997; Molinero and Pozo, 2004). However, due to differences in seasonality of litter fall between deciduous broad-leaved forests and eucalypt plantations, and higher stream discharge in autumn/winter than in summer, benthic organic matter storage is generally higher in eucalypt than in deciduous streams (Abelho and Graça, 1996; Molinero and Pozo, 2004). These changes in seasonality, diversity, composition, and quantity of litter inputs to streams have the potential to affect aquatic communities and ecosystem processes but studies tend to provide conflicting results (see below).

Both fungi and invertebrate shredders are key players in the decomposition of allochthonous organic matter in streams (Hieber and Gessner, 2002). This is a key ecosystem process that leads to mineralization of nutrients, incorporation of organic carbon into decomposer biomass, and transfer of nutrients and energy to higher trophic levels (Gessner et al., 1999). Therefore, changes in fungal and invertebrate communities and activity due to eucalypt plantations may have important repercussions for stream ecosystem functioning. The replacement of mixed deciduous broad-leaved forests by eucalypt plantations was found to reduce aquatic invertebrate species richness, diversity, density, and biomass (Basaguren and Pozo, 1994; Abelho and Graça, 1996; Larrañaga et al., 2009a, 2009b). On the other hand, some studies reported an impact on aquatic fungal species richness and conidial production in Portuguese streams, but not in Spanish ones (Chauvet et al., 1997; Bärlocher and Graça, 2002; Ferreira et al., 2006a).

The majority of studies that addressed the effects of eucalypt plantations on streams have been done in the Iberian Peninsula. An exception is Laćan et al. (2010) where litter decomposition rates and macroinvertebrate communities were compared between stream reaches lined by native vegetation or *E. globulus* in California. No strong effects of eucalypt were found in this study, likely due to eucalypt trees forming isolated groves of mostly old trees rather than plantations. Another study (Masese et al., 2014) compared streams flowing through mainly forest, mainly agricultural (with riparian zones dominated by *E. globulus*) or mixed catchments in the Kenian Rift Valley; however, the results were confounded by simultaneous occurrence of eucalypt trees and other types of agricultural impact. In general, some studies from the Iberian Peninsula reported reduced litter decomposition rates in eucalypt when compared to deciduous streams (Pozo, 1993; Abelho and Graça, 1996), while others reported no major change (Pozo et al., 1998; Bärlocher and Graça, 2002; Ferreira et al., 2006a), even though aquatic communities were strongly affected (Bärlocher and Graça, 2002; Ferreira et al., 2006a). These results suggest that (i) the structure of aquatic communities may be more sensitive to eucalypt plantations than ecosystem processes, and (ii) that responses of aquatic communities and processes to forest change may depend on other factors such as identity/quality of litter, type of decomposer community, and region.

In this study, we assessed the effect of the replacement of native deciduous broad-leaved forests by eucalypt plantations on total and microbially driven decomposition of leaf litter of two common tree species, alder and oak, and on the density and family richness of litter-associated and benthic macroinvertebrates by comparing five streams flowing through native forests and five streams flowing through eucalypt plantations in each of central Portugal and northern Spain (20 streams total). This is the largest comparison of deciduous and eucalypt streams using a common methodology (Graça et al., 2002) that allows us to assess if the effects of eucalypt

plantations depend on the region (Portugal vs. Spain), litter identity (alder vs. oak), and type of decomposer community (microbes only vs. microbes and invertebrates).

2. Materials and methods

2.1. Study sites

Five streams flowing through deciduous broad-leaved forests and five streams flowing through eucalypt plantations were selected in central Portugal (Vouga and Mondego River basins) and northern Spain (Agüera and Sámano River basins) (Table 1). Climate in central Portugal is temperate with mean annual air temperature of 15.0 °C and precipitation of ca. 1000 mm, mostly occurring in winter (Miranda et al., 2002), while climate in northern Spain is humid oceanic, with mean annual air temperature of 14.3 °C and precipitation of ca. 1500 mm evenly distributed over the year (Elosegi et al., 2002). Deciduous forests in the study areas were dominated by oak (*Quercus* spp.) and chestnut (*Castanea sativa* Miller) in both regions; alder (*Alnus glutinosa* (L.) Gaertner) was also present along the stream banks. Eucalypt plantations differed between regions; in Portugal, *E. globulus* was present up to the stream banks (i.e. mostly with no native deciduous trees along the riparian area), while in Spain, some deciduous trees were found along the stream (e.g. oak, alder, hazelnut (*Corylus avellana* L.), ash (*Fraxinus excelsior* L.)). No human impacts besides eucalypt plantations were apparent at the study sites. More information on the Spanish streams can be found in Larrañaga et al. (2009a).

2.2. Stream characterization

Water temperature was recorded every hour during litter incubation with data loggers (Onset Optic StowAway or ACR SmartButton). Electrical conductivity (WTW LF 330) and pH (Jenway 3310 in Portugal and Hanna HI9025 in Spain) were measured 3–8 times. On the same occasions, water was collected and transported cold to the laboratory. Unfiltered water was used to determine ammonium concentration and alkalinity within 48 h of collection, while filtered (Millipore APFF in Portugal and Whatman GF/C in Spain) water was frozen for later analyses of nitrate, nitrite and soluble reactive phosphorus (SRP) concentrations. Ammonium, nitrate, and nitrite concentrations were determined with automatic analyzers (Dionex DX-120 in Portugal and Traacs 800 in Spain). Nitrite concentration was always below the detection limit and thus dissolved inorganic nitrogen (DIN) concentration was calculated as $NO_3\text{-}N + NH_4\text{-}N$. SRP concentration was determined by the ascorbic acid method (APHA, 1995). Alkalinity was estimated by titration with H_2SO_4 to an end point pH of 4.5 (APHA, 1995).

2.3. Litter decomposition

Freshly fallen alder (*A. glutinosa* (L.) Gaertn.) and oak (*Quercus robur* L.) leaves were collected at a single site in each region, air-dried at room temperature, and stored in the dark. These species were selected because they are common along streams flowing through native forests in the Iberian Peninsula, have contrasting litter characteristics (lower nutrient concentration, higher polyphenolics concentration, and higher toughness in oak than alder), have distinct palatability to shredders (higher palatability of alder than oak), and decompose at different rates (faster for alder than oak) (Ferreira et al. 2006a, 2006b, 2012; Gulis et al., 2006). Decomposition of these leaf species has also been used for the assessment of stream health throughout Europe (Pascoal et al., 2003; Gulis et al., 2006; Castela et al., 2008; Riipinen et al., 2009; Woodward et al., 2012). Leaves were weighed (4.75–5.25 g), moistened with

Table 1
Location and size characteristics of deciduous and eucalypt streams in central Portugal and northern Spain.

Stream	Latitude (N)	Longitude (W)	Elevation (m a.s.l.)	Drainage area (km^2)	Distance from source (km)	Stream order
Portugal						
Deciduous streams						
Candal 2	40°04′44″	8°12′30″	640	0.3	0.7	2
Catarredor 2	40°04′07″	8°13′18″	785	0.2	0.5	1
Catarredor 1	40°04′10″	8°12′39″	720	0.5	0.4	2
Tábuas	40°03′49″	8°17′59″	295	2.1	2.8	3
Foz	40°30′57″	8°13′23″	105	3.8	3.3	4
Eucalypt streams						
Lázaro 2	40°30′57″	8°18′23″	215	2.0	1.5	3
Vale da Mó	40°27′11″	8°22′13″	165	1.2	1.0	3
Lázaro 3	40°30′52″	8°17′59″	230	0.4	0.3	2
Boialvo	40°30′10″	8°20′44″	160	0.4	0.8	3
Lázaro 1	40°30′52″	8°17′59″	250	1.6	1.3	3
Spain						
Deciduous streams						
Remendón 1	43°19′30″	3°20′05″	110	4.1	4.0	2
Peñalar	43°18′38″	3°15′28″	120	1.9	1.0	1
Perea	43°17′32″	3°14′02″	310	1.3	1.0	1
Salderrey	43°12′32″	3°15′40″	350	1.8	1.5	1
Antonilla	43°12′50″	3°16′10″	300	1.8	1.0	1
Eucalypt streams						
Remendón 2	43°19′52″	3°19′37″	90	5.7	4.8	2
Jerguerón	43°19′03″	3°16′03″	110	0.8	0.7	1
Tabernilla	43°20′02″	3°13′43″	120	3.6	1.5	2
Gamonal	43°21′12″	3°15′94″	120	1.3	1.0	1
Cabañaperaza	43°20′25″	3°15′86″	120	1.3	0.8	2

distilled water, and enclosed in mesh bags (15 × 10 cm). Two mesh sizes were used: fine mesh bags (0.5 mm mesh, FM) that prevent access of most invertebrates to the litter and so decomposition is mainly driven by the microbial community, and coarse mesh bags (10 mm mesh, CM) that allow invertebrates to enter and, thus, litter decomposition is driven by both microbes and invertebrates. Six litter bags of each leaf species and mesh size (24 total) were tied to iron bars driven into the substrate in each stream in riffles <30 cm deep. Litter bags were deployed in the streams on 26 November 2002 (alder) and 26 January 2003 (oak) in Portugal, and on 10 December 2002 (both species) in Spain. Additionally, six extra bags per leaf species and mesh size were taken to the field in each region, submerged for ∼10 min, and returned to the laboratory to determine initial air-dry mass to ash-free dry mass (AFDM) conversion factors.

We aimed to retrieve litter bags (both mesh sizes) of each species when ∼50% initial litter mass had been lost. To estimate sampling dates, additional sets of CM bags were incubated in a deciduous stream in each region (Candal 2 in Portugal and Remendón 1 in Spain). These extra sets consisted of 22 bags of each species that were retrieved periodically over 42 (Portugal) and 43 (Spain) days for alder, and over 74 (Portugal) and 104 (Spain) days for oak (Ferreira et al., 2006a). Based on the litter decomposition dynamics in these streams, alder bags from all other streams were retrieved after 26 (Portugal) and 31 (Spain) days, while oak bags were retrieved after 57 (Portugal) and 104 (Spain) days. During sampling, a hand net (0.5 mm mesh) was placed downstream of the bags to minimize the loss of macroinvertebrates associated with the litter. Litter bags were enclosed individually in zip-lock bags and transported to the laboratory in a cooler. Litter was rinsed with tap water onto a 0.5 mm mesh sieve to remove sediments while retaining litter fragments and macroinvertebrates. The litter material was dried (105 °C, 24–48 h), weighed, combusted (550 °C, 4 h), and reweighed to determine AFDM remaining. Exponential decomposition rates (k, dd^{-1}) were calculated as the slope of linear regressions of ln-transformed fraction of remaining AFDM over the accumulated mean daily temperature by the sampling date (degree-days, °C), with the intercept fixed at 0, i.e. Ln(1) at 0 degree-days. Decomposition rates were expressed per degree-day to standardize for differences in temperature among streams (Boulton and Boom, 1991).

2.4. Macroinvertebrates

Macroinvertebrates from litter bags were sorted and preserved with 70% ethanol. Individuals were identified to family level, counted, and assigned to shredders and non-shredders following Tachet et al. (2000). Results were expressed as total macroinvertebrate and shredder richness (no. taxa sample^{-1}) and density (no. individuals g^{-1} AFDM).

Benthic macroinvertebrates were sampled once during litter incubation. In Portugal (4 December 2002), a kick-net (0.3 × 0.3 m opening, 0.5 mm mesh) was used and sampling was standardized by time (1 min kicking) and area (∼0.3 × 1 m). In each stream, a benthic sample was composed of six subsamples (kicks) from different stream microhabitats that were pooled in situ. In Spain (25 March 2003), a Surber sampler (0.3 × 0.3 m, 0.5 mm mesh) was used to collect five benthic subsamples per stream. Benthic samples were preserved with 4% formalin (Portugal) or 70% ethanol (Spain). Macroinvertebrates were then sorted, identified and counted as described above. Results were expressed as total macroinvertebrate and shredder richness (no. taxa sample^{-1}) and density (no. individuals sample^{-1}).

2.5. Data analysis

Hourly values of temperature were averaged to produce daily means. Physicochemical characteristics of stream water, decomposition rates, and macroinvertebrate and shredder richness and density associated with decomposing litter were compared between regions (Portugal vs. Spain), stream types (deciduous vs. eucalypt), litter species (alder vs. oak), and mesh sizes (fine vs. coarse) by linear mixed models. Parameters were estimated using restricted maximum likelihood (REML). Since benthic macroinvertebrates

were sampled on different occasions and using different methods in Spain and Portugal, we used average values per stream to compare benthic total macroinvertebrate and shredder richness and density between stream types within each region by *t*-test. Normal distribution of residuals was assessed by Shapiro–Wilk test, and data were log-transformed (or $\log_{10}(x+1)$ transformed in case of macroinvertebrate variables) when non-normal distribution was detected. Ratios of litter decomposition rates in eucalypt streams to decomposition rates in deciduous streams ($k_{eucalypt}/k_{deciduous}$) were also calculated as an index of functional stream integrity (Gessner and Chauvet, 2002). Analyses were performed in RStudio (RStudio, 2012) with the nlme package (Pinheiro et al., 2013).

3. Results

3.1. Stream characterization

Streams drained small siliceous catchments, and the water had circumneutral pH, low conductivity, alkalinity, and nutrient concentrations (Table 2). These variables were significantly higher in Spain than in Portugal, except for SRP concentrations that did not significantly differ between regions (Table 3), while no significant effect of stream type was found for either region (Tables 2 and 3). Water temperature was significantly higher in Portugal than in Spain; in Portugal, water temperature was ca. 2 °C higher in eucalypt than in deciduous streams, differences being statistically significant (Tables 2 and 3).

3.2. Litter decomposition

In general, leaf litter decomposition was slower in eucalypt than in deciduous streams, in Spain than in Portugal, in fine than in coarse mesh bags, and for oak than for alder (Fig. 1, Tables 4 and A). The effects of eucalypt plantations on litter decomposition were similar for both regions (non significant Region × Stream type interaction; Table 4), but were stronger in coarse than in fine mesh bags (significant Stream type × Mesh size interaction; Table 4) and stronger for alder than for oak (significant Stream type × Litter species interaction; Table 4). The effects of region were stronger for fine than for coarse mesh bags (significant Region × Mesh size interaction; Table 4). There were, however, some exceptions as indicated by the significant 3-factor and 4-factor interactions (Table 4).

The ratios of litter decomposition rates in eucalypt streams to decomposition rates in deciduous streams ($k_{eucalypt}/k_{deciduous}$) indicated that litter decomposition rates in fine mesh bags were not sensitive to forest change (Table 5). The ratios of litter decomposition rates in coarse mesh bags generally classified the eucalypt streams as impaired (Table 5).

Table 3
Summary table of the linear mixed model analysis performed on water variables in deciduous and eucalypt streams (Stream type) in central Portugal and northern Spain (Region) during litter incubation.

Source of variation	numdf	dendf	F	P
Temperature				
Intercept	1	1786	5236.591	<0.0001
Region	1	16	67.375	<0.0001
Stream type	1	16	19.372	<0.001
Region × Stream type	1	16	5.915	0.027
Conductivity[a]				
Intercept	1	102	2772.982	<0.0001
Region	1	16	36.497	<0.0001
Stream type	1	16	3.320	0.087
Region × Stream type	1	16	0.666	0.426
pH				
Intercept	1	92	6880.119	<0.0001
Region	1	16	6.134	0.025
Stream type	1	16	0.547	0.470
Region × Stream type	1	16	0.198	0.662
Alkalinity[a]				
Intercept	1	90	341.527	<0.0001
Region	1	16	21.460	<0.001
Stream type	1	16	0.020	0.889
Region × Stream type	1	16	<0.001	0.998
DIN[a]				
Intercept	1	106	2940.202	<0.0001
Region	1	16	24.312	<0.001
Stream type	1	16	0.140	0.713
Region × Stream type	1	16	0.543	0.472
SRP[a]				
Intercept	1	104	326.702	<0.001
Region	1	17	<0.001	0.978
Stream type	1	17	0.071	0.790
Region × Stream type	1	17	<0.001	0.989

[a] $\log_{10}$-transformed.

3.3. Benthic macroinvertebrates

A total of 72 macroinvertebrate families were found in the benthos across all 20 streams; 56 families in Portugal and 55 in Spain. The most abundant taxa were Baetidae, Heptageniidae, Nemouridae, Elminthidae, Chironomidae, Simuliidae and Gammaridae (only in Spain), as well as Oligochaeta (Table B). Total macroinvertebrate richness did not significantly differ between stream types in either region (*t*-test, $P = 0.378$ for Portugal and 0.218 for Spain), while total density was significantly lower in eucalypt than in deciduous streams in Portugal ($P = 0.042$) but not in Spain ($P = 0.397$) (Fig. 2).

Fifteen shredder families were found across all 20 streams: 10 in Portugal and 11 in Spain (Table B). Shredders accounted for 15–28% of total macroinvertebrate richness, and 7–57% of total density in the benthos. The most abundant shredders in the benthos were Leptophlebiidae mayflies, Leuctridae and Nemouridae stoneflies, Limnephilidae caddisflies, Gammaridae amphipods,
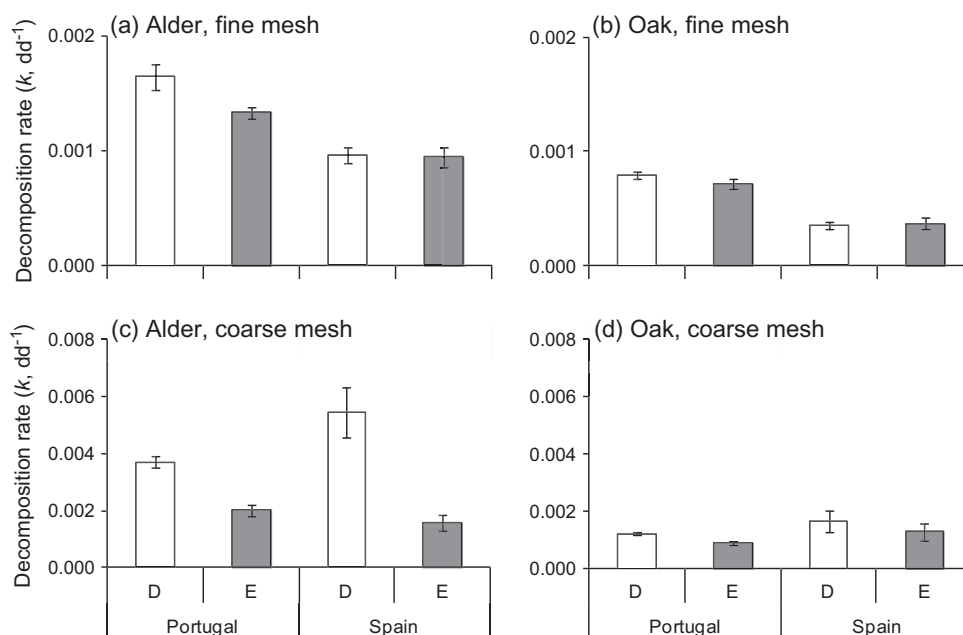
Table 2
Water characteristics of the deciduous and eucalypt streams in central Portugal and northern Spain during the experiments. Means of five streams ± 1 SE.

Water characteristics	Portugal		Spain	
	Deciduous streams	Eucalypt streams	Deciduous streams	Eucalypt streams
Temperature (°C)	10.1 ± 0.4	11.9 ± 0.2	8.5 ± 0.3	9.0 ± 0.1
Conductivity (μS/cm)	31 ± 4	48 ± 9	92 ± 13	116 ± 26
pH	6.5 ± 0.1	6.5 ± 0.1	7.0 ± 0.2	6.8 ± 0.2
Alkalinity (mg CaCO$_3$/L)	5.3 ± 0.5	6.0 ± 1.0	21.4 ± 7.2	22.0 ± 5.0
DIN (μg N/L)	231 ± 40	250 ± 42	900 ± 239	631 ± 71
SRP (μg P/L)	3.7 ± 0.2	3.3 ± 0.3	6.0 ± 0.8	7.5 ± 1.1

DIN, dissolved inorganic nitrogen (DIN = NO_3-N + NH_4-N; NO_2-N was always below the detection limit); SRP, soluble reactive phosphorus.

Fig. 1. Decomposition rates of alder (a, c) and oak (b, d) litter in fine (a, b) and coarse (c, d) mesh bags incubated in deciduous (D) and eucalypt (E) streams in central Portugal and northern Spain. Values are means of five streams ± 1 SE.

Table 4
Summary table of the linear mixed model analysis performed on the decomposition rate (k, dd^{-1}; $\log_{10}$-transformed) of alder and oak litter (Litter species) in fine and coarse mesh bags (Mesh size) incubated in deciduous and eucalypt streams (Stream type) in central Portugal and northern Spain (Region).

Source of variation	numdf	dendf	F	P
Intercept	1	427	15770.832	<0.0001
Stream type	1	16	9.954	0.006
Region	1	16	6.355	0.023
Mesh size	1	427	515.235	<0.0001
Litter species	1	427	688.988	<0.0001
Stream type × Region	1	16	0.181	0.676
Stream type × Mesh size	1	427	65.470	<0.0001
Stream type × Litter species	1	427	16.024	<0.001
Region × Mesh size	1	427	104.900	<0.0001
Region × Litter species	1	427	3.574	0.059
Mesh size × Litter species	1	427	0.128	0.720
Stream type × Region × Mesh size	1	427	15.176	<0.001
Stream type × Region × Litter species	1	427	3.001	0.084
Stream type × Mesh size × Litter species	1	427	9.652	0.002
Region × Mesh size × Litter species	1	427	11.999	<0.001
Stream type × Region × Mesh size × Litter species	1	427	3.475	0.063

and Scirtidae beetles. Similar to patterns observed for total macro-invertebrates, benthic shredder density was significantly lower in eucalypt than in deciduous streams in Portugal (*t*-test, *P* = 0.022), while in Spain the tendency was not significant (*P* = 0.091) (Fig. 2). This was due to the significantly lower density of Nemouri-dae in eucalypt than in deciduous streams (*t*-test, *P* = 0.039), and due to the absence of Limnephilidae and Tipulidae from eucalypt streams in Portugal. In Spain, no significant difference in shredder richness (*t*-test, *P* = 0.218) and density (*P* = 0.397) was found between stream types. However, shredder communities in Spanish deciduous streams were dominated by Gammaridae (31–78%), while their contribution decreased to 0–14% in most eucalypt streams (except at Remendón 2 where Gammaridae still accounted for 75% of benthic shredder density) (*t*-test, *P* = 0.126).

3.4. Litter macroinvertebrates

In general, total macroinvertebrate richness and density associated with decomposing litter were lower in eucalypt than in deciduous streams and in Portugal than in Spain, while no signifi-cant differences were found between litter species (Fig. 3, Tables 6 and C). The effects of eucalypt plantations were stronger for total macroinvertebrate density associated with alder than with oak lit-ter (significant Stream type × Litter species interaction; Table 6). Total macroinvertebrate richness tended to be higher in alder than in oak in Spain (significant Region × Litter species interactions; Table 6), while total density tended to be lower in oak than in alder in deciduous streams in Spain and higher in oak than in alder in eucalypt streams in Portugal (significant Stream type × Re-gion × Litter species interactions; Table 6).

Shredders represented 18–100% (alder) and 14–52% (oak) of total macroinvertebrate richness, and 5–100% (alder) and 4–74% (oak) of total macroinvertebrate density in litter bags. In general, shredder richness and density associated with decomposing litter were lower in eucalypt than in deciduous streams, in Portugal than in Spain, and in alder than in oak (Fig. 4, Tables 6 and D). There were some exceptions for shredder richness as indicated by the significant Stream type × Region × Litter type interaction (Fig. 4,

Table 5
Ratios of litter decomposition rates in eucalypt streams to decomposition rates in deciduous streams ($k_{eucalypt}/k_{deciduous}$) for alder and oak in coarse and fine mesh bags in central Portugal and northern Spain. The score was given following Gessner and Chauvet (2002): 2, ratio = 0.75–1.33; 1, ratio = 0.50–0.75 or 1.33–2.00; 0, ratio = <0.50 or >2.00.

	$k_{eucalypt}/k_{deciduous}$	Score[a]
Portugal		
Alder		
Fine mesh	0.81	2
Coarse mesh	0.54	1
Oak		
Fine mesh	0.90	2
Coarse mesh	0.72	1
Spain		
Alder		
Fine mesh	0.98	2
Coarse mesh	0.29	0
Oak		
Fine mesh	1.05	2
Coarse mesh	0.78	2

[a] Interpretation of ratios: 0, severely compromised stream functioning; 1, compromised stream functioning; 2, no clear evidence of impaired stream functioning (Gessner and Chauvet, 2002).

Table 6). Shredder density associated with oak litter also did not significantly differ between eucalypt and deciduous streams (significant Stream type × Litter species interaction; Fig. 4, Table 6).

4. Discussion

In this study we assessed the effects of the replacement of native deciduous forests by eucalypt plantations on stream ecosystems in the Iberian Peninsula, where eucalypts cover large areas. We found that (i) the response of litter decomposition to forest change depends on leaf litter species (stronger for alder) and presence of macroinvertebrates in litter bags, (ii) the response of litter-associated macroinvertebrates to forest change depends on litter species (stronger for alder), and (iii) the response of benthic

macroinvertebrates depends on the region (significant effect of eucalypt plantations only in Portugal).

The effect of the replacement of native deciduous forests by eucalypt plantations on litter decomposition depended on litter species and macroinvertebrate access to litter bags, as plantations resulted in slower total decomposition of alder litter. This can be explained by lower macroinvertebrate (especially shredder) colonization of the high quality alder litter in eucalypt than in deciduous streams in this study, while no significant effect of forest type was found on macroinvertebrate colonization of the nutrient-poor oak litter. The greater sensitivity of high quality litter to forest change may be attributed to higher contribution of shredders to its decomposition compared to low quality litter, where fungi could play a greater role (Hieber and Gessner, 2002; Gulis et al., 2006). As microbial communities tend to be more functionally redundant than invertebrate assemblages, the decomposition of low quality litter and the decomposition of litter in the absence of invertebrates should be less strongly affected by forest change. Overall, our results are consistent with previous studies. Kominoski et al. (2011) found a stimulation of the decomposition of the high-quality alder litter (*A. rubra*), but not of the nutrient-poor western hemlock (*Tsuga heterophylla*), with an increase in the percentage of deciduous riparian canopy in streams flowing through coniferous plantations, but only in the presence of macro-invertebrates. Martínez et al. (2013) reported an inhibition of total decomposition of the nutrient-rich alder (*A. glutinosa*) litter, but not of the recalcitrant pine (*Pinus radiata*), with the replacement of deciduous forests by pine plantations. In both studies, the response of litter decomposition to forest change was mediated by changes in macroinvertebrate colonization of the decomposing litter. Together, these results suggest that the sensitivity of litter decomposition to forest change is higher for high quality (low carbon:nutrient ratio and lignin concentration) than for low quality litter, and for total (microbes + invertebrates) than for microbially driven litter decomposition.

Microbially driven litter decomposition was generally not affected by forest change. This agrees with earlier studies that showed no differences in litter decomposition between eucalypt and deciduous streams (Pozo et al., 1998; Bärlocher and Graça,
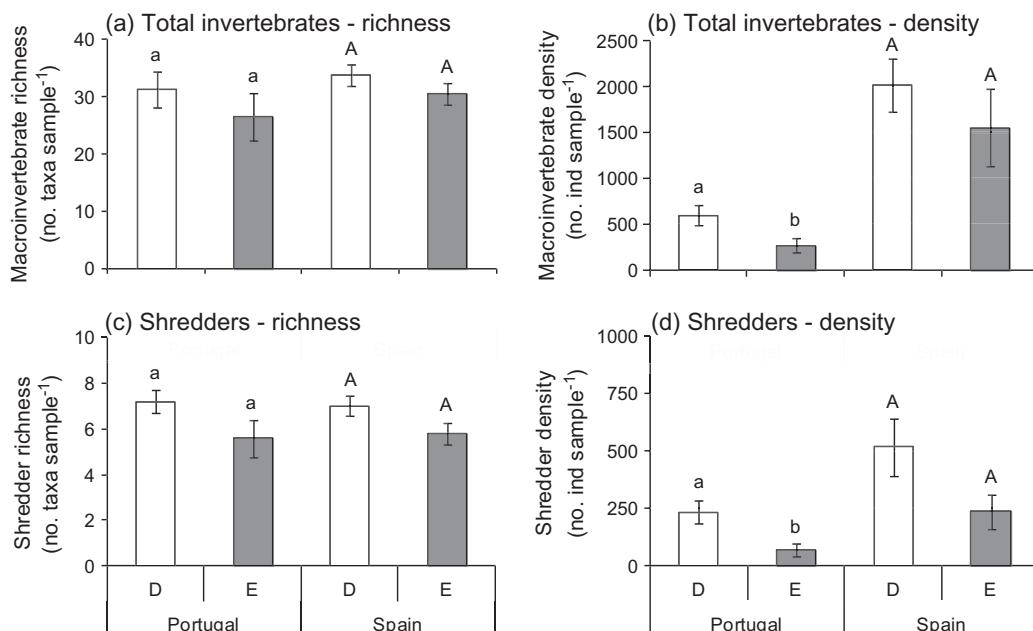

Fig. 2. Benthic total macroinvertebrate richness (a) and density (b) and shredder richness (c) and density (d) in deciduous (D) and eucalypt (E) streams in central Portugal and northern Spain. Values are means of five streams ± 1 SE.
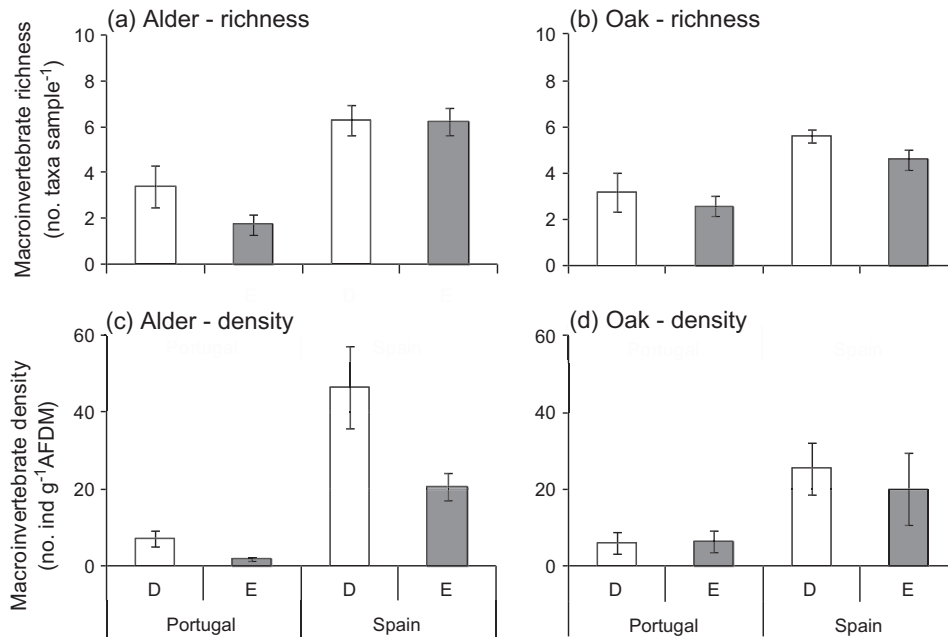
Fig. 3. Total macroinvertebrate richness (a, b) and density (c, d) associated with alder (a, c) and oak (b, d) litter in coarse mesh bags incubated in deciduous (D) and eucalypt (E) streams in central Portugal and northern Spain. Values are means of five streams ± 1 SE.

Table 6
Summary table of the linear mixed model analysis performed on total macro-invertebrate and shredder richness and density ($\log_{10}(x + 1)$ transformed) associated with alder and oak litter (Litter species) in coarse mesh bags incubated in deciduous and eucalypt streams (Stream type) in central Portugal and northern Spain (Region).

Source of variation	numdf	dendf	F	P
Total macroinvertebrate richness				
Intercept	1	201	816.815	<0.0001
Stream type	1	201	5.044	0.026
Region	1	18	53.426	<0.0001
Litter species	1	201	0.097	0.755
Stream type × Region	1	201	1.611	0.206
Stream type × Litter species	1	201	1.507	0.221
Region × Litter species	1	201	5.657	0.018
Stream type × Region × Litter species	1	201	5.486	0.020
Total macroinvertebrate density				
Intercept	1	201	386.940	<0.0001
Stream type	1	201	6.838	0.010
Region	1	18	50.819	<0.0001
Litter species	1	201	1.299	0.256
Stream type × Region	1	201	0.204	0.652
Stream type × Litter species	1	201	7.642	0.006
Region × Litter species	1	201	16.752	<0.001
Stream type × Region × Litter species	1	201	5.686	0.018
Shredder richness				
Intercept	1	203	252.533	<0.0001
Stream type	1	16	6.585	0.021
Region	1	16	19.152	<0.001
Litter species	1	203	6.846	0.010
Stream type × Region	1	16	0.406	0.533
Stream type × Litter species	1	203	2.627	0.107
Region × Litter species	1	203	0.482	0.488
Stream type × Region × Litter species	1	203	10.860	0.001
Shredder density				
Intercept	1	203	107.555	<0.0001
Stream type	1	16	13.009	0.002
Region	1	16	23.738	<0.001
Litter species	1	203	4.260	0.040
Stream type × Region	1	16	2.968	0.104
Stream type × Litter species	1	203	16.934	<0.001
Region × Litter species	1	203	2.827	0.094
Stream type × Region × Litter species	1	203	2.602	0.108

2002; Ferreira et al., 2006a), despite often reported changes in microbial variables. For instance, Ferreira et al. (2006a) reported higher conidial production and faster fungal biomass accrual in eucalypt streams in both Spain and Portugal while Bärlocher and Graça (2002) found lower fungal species richness in eucalypt streams in central Portugal. On the other hand, Chauvet et al. (1997) found no significant differences in conidial production and fungal species richness between eucalypt and deciduous streams in northern Spain. Decomposition studies that dealt with other types of human impact also found lower sensitivity of microbially driven than of total litter decomposition to environmental change (Gulis et al., 2006; Riipinen et al., 2009; Kominoski et al., 2011). Thus, due to high level of redundancy within microbial communities (Dang et al., 2005; Duarte et al., 2006; Ferreira and Chauvet, 2012), microbially driven processes seem to be more resistant to environmental change than those driven to large extent by macroinvertebrate consumers.

The magnitude and direction of the effect of eucalypt plantations on litter decomposition in streams ($k_{eucalypt}/k_{deciduous}$ = 0.29–1.05; i.e. strong inhibition to no effect) is comparable with the effect of some heavy metals, chemical pollutants, and vegetation changes, but the changes are less severe and in the opposite direction from those elicited by nutrient enrichment or logging (Gessner and Chauvet, 2002).

The differences between deciduous and eucalypt streams in macroinvertebrate colonization of litter bags reflected differences in benthic communities between stream types, which were more pronounced for Portuguese than Spanish streams. The stronger effect of forest change on benthic macroinvertebrates found in central Portugal could be due to differences in hydrology between eucalypt and deciduous streams. Soil hydrophobicity is higher in eucalypt plantations than in deciduous forests, which translates into higher intra-annual variation in discharge (Abelho and Graça, 1996), with occurrence of spates in winter and droughts in summer, sometimes resulting in intermittent flow. Intermittent streams tend to have lower density and richness of macroinvertebrates than perennial streams (Datry et al., 2011). Additionally, in Portugal, eucalypt streams flow through pure eucalypt monocultures, which are often almost completely devoid of other tree spe-
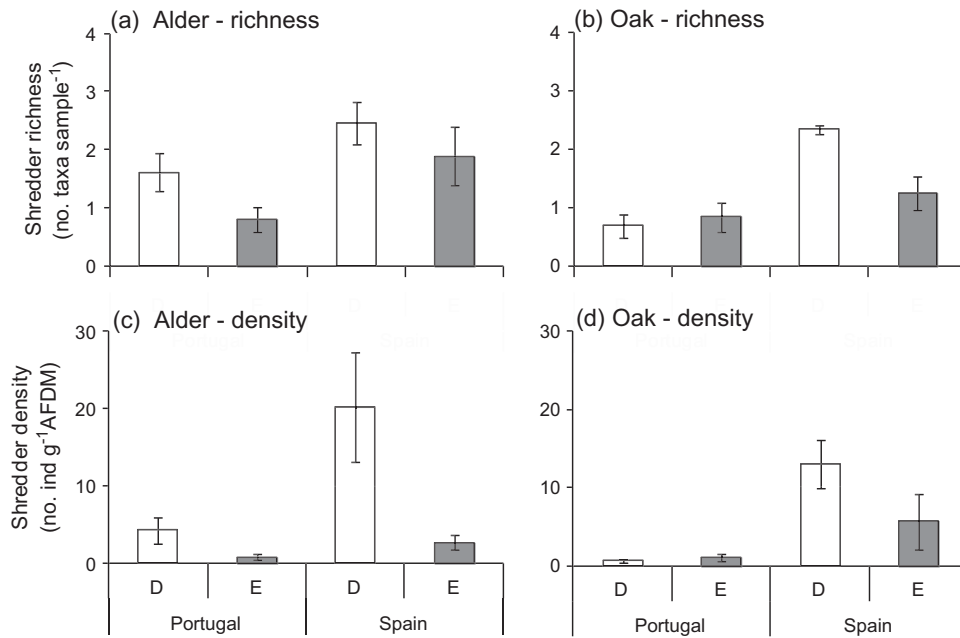
Fig. 4. Shredder richness (a, b) and density (c, d) associated with alder (a, c) and oak (b, d) litter in coarse mesh bags incubated in deciduous (D) and eucalypt (E) streams in central Portugal and northern Spain. Values are means of five streams ± 1 SE.

cies, and, therefore, litter inputs to streams consist of a single, recalcitrant leaf type. It is known that eucalypt litter is of poor quality to invertebrates (Pozo et al., 1998; Canhoto and Graça, 1999) that can lead to lower macroinvertebrate richness and density in streams where it dominates the litter input (Abelho and Graça, 1996; Larrañaga et al., 2009a, 2009b). In northern Spain, the relatively wet climate allows for the presence of alder trees on the stream banks in eucalypt plantations (Pozo et al., 1997; Molinero and Pozo, 2006), which increases the diversity and overall quality of litter and may explain the weaker effect of forest change on benthic macroinvertebrates in Spain.

Water variables were similar in deciduous and eucalypt streams within each region, with the only exception of temperature in Portugal, which was significantly higher in eucalypt than in deciduous streams due to lower elevations of the former since eucalypt trees are sensitive to cold temperatures (Close et al., 2000). However, we believe that temperature-driven differences in litter decomposition were accounted for by our choice of expressing decomposition rates in degree-days accumulated. Also, in the absence of human disturbance, litter decomposition has been shown to the quite uniform within a region, even across gradients of stream size, elevation, and temperature (Tiegs et al., 2009). On the other hand, Portuguese and Spanish streams differed in most water variables, which may explain somewhat different absolute rates of decomposition and macroinvertebrate parameters. Also, alder and oak litter incubated in Portuguese streams had higher initial nutrient concentration (nitrogen (N): 2.8% for alder and 1.1% for oak; phosphorus (P): 0.06% for alder and 0.11% for oak) than that used in Spanish streams (N: 2.5% for alder and 0.9% for oak; P: 0.05% for alder and 0.02% for oak) (Ferreira et al., 2006a; Gulis et al., 2006), which can help explaining the faster microbially driven decomposition in Portugal in this study that had probably resulted from higher microbial activity associated with decomposing litter in central Portugal than in northern Spain (Ferreira et al., 2006a). Several studies have shown that intraspecific variability in litter characteristics can affect microbial colonization and litter decomposition similarly to interspecific differences, with litter having higher nutrient concentrations decomposing faster than litter with lower nutrient

concentrations (LeRoy et al., 2007; Lecerf and Chauvet, 2008; Graça and Poquet, 2014). Thus, in future studies aimed at comparisons among regions, litter of the same origin should be used to alleviate the possible effects of differences in litter quality.

Total litter decomposition, driven by both microorganisms and invertebrates, however, did not significantly differ between regions. This can be attributed to higher invertebrate and shredder richness and density in litter bags in Spain than Portugal, which may have counteracted the effect of higher substrate nutrients or other factors that resulted in faster microbially driven decomposition in Portuguese streams. The difference in invertebrate numbers between the two regions could be due to differences in climate, hydrology or biogeographic patterns. The relatively wet climate in northern Spain usually maintains stream flow throughout the year, while in central Portugal, small streams, especially those flowing through eucalypt plantations, can dry out during the warmer months.

5. Conclusions

We found that replacement of native deciduous forests by eucalypt plantations affects both aquatic communities and processes. However, the effects of forest change on benthic macroinvertebrate communities and plant litter decomposition are complex as they depend on the identity/quality of the litter and in-stream biological communities such as aquatic macroinvertebrates, which interact with environmental conditions. Therefore, generalizations may be difficult due to regional differences in climate, vegetation, and in the importance of invertebrates in ecological processes, such as carbon cycling. In addition, the majority of studies addressing the effects of the replacement of native forests by eucalypt plantations on stream functioning or communities have been done in the Iberian Peninsula, despite eucalypt plantations covering over 20 million ha worldwide. Nevertheless, our findings suggest that the effects of eucalypt plantations on stream ecosystems may be more pronounced in drier climates, where streams experience seasonal drought and where riparian corridors of native tree species are absent, in streams where the native vegetation provide high qual-

ity litter to aquatic food webs, and in streams where macroinvertebrates are important players in ecosystem processes. Thus, the preservation of native riparian areas, especially in streams with the above mentioned characteristics, may mitigate the effects of eucalypt plantations on stream communities and processes.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2014.09.013.

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