Ecosystems (2021) 24: 1436–1449
https://doi.org/10.1007/s10021-020-00592-7



# Loss of Key Riparian Plant Species Impacts Stream Ecosystem Functioning

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## Abstract

Leaf litter of alder (*Alnus glutinosa*) is a key resource to detrital stream food webs. Due to its high quality and palatability, it is readily colonised by microorganisms and consumed by detritivores, contributing significantly to carbon and nutrient cycling and to ecosystem functioning. Given that this species has declined due to the spread of the pathogen *Phytophthora alni*, we investigated how its loss would alter leaf litter decomposition and associated stream assemblages of aquatic hyphomycetes and invertebrates, in a field experiment conducted in three streams. We compared litter mixtures containing alder plus three other species (*Corylus avellana*, *Quercus robur* and *Salix atrocinerea*; that is, 4-species treatments) with mixtures that excluded alder (3-species treatments) and all the monocultures (1-species treatments). The loss of alder reduced decomposition rates, despite the existence of an overall negative diversity effect after 3 weeks of exposure (that is, monocultures decomposed faster than mixtures) and no diversity effect after 6 weeks. Aquatic hyphomycete and detritivore assemblage structure in the mixture without alder differed from those of the mixture with alder and the monocultures, and the former had lower fungal sporulation rate and taxon richness. Our results suggest that alder loss from the riparian vegetation can significantly slow down the processing of organic matter in streams and produce shifts in stream assemblages, with potential consequences on overall ecosystem functioning. We highlight the importance of assessing the ecological consequences of losing single species, particularly those especially vulnerable to stressors, to complement the multiple studies that have assessed the effects of random species loss.

**Key words:** Litter decomposition; Alder; Non-random species loss; Aquatic hyphomycetes; Detritivores; Macroinvertebrates; Net diversity effect; Plant diversity.

Received 9 October 2020; accepted 4 December 2020;
published online 28 January 2021

**Supplementary Information:** The online version of this article (https://doi.org/10.1007/s10021-020-00592-7) contains supplementary material, which is available to authorised users.

Authors' Contribution: LB and JB designed the study with feedback from JP and NLR; AA, JP, SM, NLR and AB conducted fieldwork; AA, JP, SM and NLR analysed the samples in the laboratory; AA analysed the data with substantial contributions from NLR and feedback from all other authors; AA and LB wrote the manuscript with feedback from all other authors.

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## Highlights

- The loss of alder reduced decomposition.
- It also reduced aquatic hyphomycete sporulation

rate and taxon richness.
- Stream assemblages differed when alder was absent in mixtures.

## Introduction

Biodiversity loss as a result of anthropogenic impact is known to be a major driver of change in the functioning of ecosystems (Hooper and others 2012). However, most available knowledge has emerged from experiments simulating random species loss (Gross and Cardinale 2005; Wardle 2016), with fewer studies addressing more realistic scenarios of species extinction as a result of particular stressors or their combination (Kominoski and others 2013). Among these, empirical studies have relied on information about the vulnerability of different species to a given stressor, finding that the consequences of ordered species loss can differ from those of random loss (Larsen and others 2005; García-Valdés and others 2018), an outcome supported by mathematical models (Ives and Cardinale 2004; Gross and Cardinale 2005). The relative scarcity of studies considering ordered extinctions could be due to lack of knowledge on species sensitivity to different stressors, as this requires information about their biological traits that is often unavailable. However, this may not be the case for some stressors such as the spread of pathogens, which usually affect particular taxa and thus facilitate the prediction of species loss scenarios (Harvell and others 2002).

Plant diseases caused by fungal pathogens are poorly known beyond the context of agriculture (Almeida and others 2019), but they are widespread and cause declines in species abundance and richness that can alter the functioning of terrestrial and aquatic ecosystems (Bjelke and others 2016). Streams are among the ecosystems most severely affected by plant diversity loss (Kominoski and others 2013), as they often rely on the allochthonous organic matter inputs from the riparian forest for their functioning, given that primary production is largely limited by riparian shading and low nutrient availability (Fisher and Likens 1973; Wallace and others 1997). Leaf litter accounts for a high proportion of plant biomass entering streams, with some species providing litter resources that are particularly nutrient-rich and palatable for consumers, such as alder (that is, *Alnus*; Graça and others 2015). Alder species are nitrogen (N)-fixing trees that produce leaves with high concentration of N and low concentration of refractory carbon (C) compounds (Waring and Running 2010). The loss of key species such as alder could be expected to cause notable impact on the functioning of streams, but this remains unexplored, despite its likelihood as a result of the spread of the pathogen *Phytophthora alni* across Europe (Brasier and others 2004; Bjelke and others 2016).

*Phytophthora alni* is a species complex of oomycetes composed by *Phytophthora* × *alni* (Brasier & S.A. Kirk) Husson, Ioos & Marçais, *Phytophthora uniformis* (Brasier & S.A. Kirk) Husson, Ioos & Aguayo, and *Phytophthora* × *multiformis* (Brasier & S.A. Kirk) Husson, Ioos & P. Frey (Husson and others 2015). It induces alder dieback, whose main symptom is a decrease in tree vitality until its death (Jung and Blaschke 2004; Bjelke and others 2016). The *P. alni* dieback can affect nearly 50% of alder trees in some European regions (Bjelke and others 2016) and, in the Iberian peninsula, *Alnus glutinosa* (L.) Gaertn. is infected by the three species of the *P. alni* complex. To date, it has been reported in the northwest of the peninsula (Solla and others 2010; Pintos-Varela and others 2017), but it is expected to expand its distribution area due to the rise in temperature caused by climate change (Thoirain and others 2007). This is especially true for *P.* × *alni*, which is the most pathogenic and frost-sensitive species (Bjelke and others 2016). The expansion of *P. alni* will most likely affect *A. glutinosa* populations in Atlantic areas, where this riparian species is dominant and provides a major resource for stream assemblages (Douda and others 2016).

Here, we assess how the loss of *A. glutinosa* (hereafter alder) from the riparian vegetation affects a fundamental stream ecosystem process, litter decomposition, as well as the composition and structure of associated macroinvertebrate and microbial assemblages, through a field experiment conducted in three streams in northern Spain. We incubated litter mixtures with and without alder for 3- and 6-week periods, as well as monocultures (that is, single-species treatments) that allowed exploring the mechanisms underlying any diversity effects. For this purpose, we studied the net diversity effect (that is, the deviation between the observed value in the mixture and that expected from the single-species treatments), which we partitioned into complementarity and selection effects. The complementarity effect arises from synergistic or antagonistic interactions among species, which can facilitate (positive complementarity) or inhibit (negative complementarity) the consumption of the others, for example through the leaching of nutrients, tannins or refractory compounds (Loreau and Hector 2001; López-Rojo and others 2020). The selection effect is caused by the presence of a

species with particularly high or low decomposition rate, which can increase (positive selection) or reduce (negative selection) the overall consumption of the mixture due to its strong influence (Loreau and Hector 2001; Handa and others 2014). Although both effects play a role in diversity–decomposition relationships, complementarity is often dominant when random species loss in litter mixtures is assessed (Handa and others 2014; Tonin and others 2017), but selection could be expected to gain importance when dealing with the loss of key species since they are preferred by detritivores and microbial decomposers over other plant species, and in consequence, they decompose faster (López-Rojo and others 2018).

Given alder declines due to the pathogen *P. alni*, we hypothesised that (i) the loss of alder would reduce the decomposition of litter mixtures; (ii) the selection effect would be the main mechanism for reduced decomposition, because of the strong preference of detritivores and microbial decomposers for this species (Graça and others 2001; Gulis 2001; López-Rojo and others 2018); and (iii) the loss of alder would reduce the diversity and abundance of microbial decomposers (aquatic hyphomycetes) and invertebrate detritivores, thus altering the composition and structure of both assemblages.

## Materials and Methods

### Study Area

The study was conducted in three low-order streams of the Agüera stream catchment in northern Spain (43.20º N, 3.26º W), from January to March 2020. The climate is temperate oceanic with an annual mean temperature of 11 ºC and annual mean precipitation of 1650 mm regularly distributed (López-Rojo and others 2019). Streams mostly have siliceous substrate and drain mixed Atlantic forests composed of *Quercus robur* L. (Fagaceae; hereafter oak), *A. glutinosa* (Betulaceae), *Castanea sativa* L. (Fagaceae), *Corylus avellana* L. (Betulaceae; hereafter hazel) and *Salix atrocinerea* Brot. (Salicaceae; hereafter willow). The studied streams had well-oxygenated water, circumneutral pH, low conductivity, low nutrient concentration and temperatures of approximately 7.5 °C at the time of the study (Table 1). Anthropogenic activity in the study area is negligible.

### Stream Water Characterisation

Water temperature, conductivity, pH, oxygen concentration and saturation, discharge, dissolved inorganic N (DIN = nitrate + nitrite + ammonium) and soluble reactive phosphorus (SRP) were measured six times at each stream during the study period. Temperature, conductivity, pH and oxygen were determined with a multiparametric probe (WTW Multi 3630 IDS; WTW, Weilheim, Germany) and discharge was estimated from water velocity obtained with a current meter (MiniAir 20; Schiltknecht Co, Gossau, Switzerland). Nutrients were determined from water samples that were filtered (glass fibre filters, Whatman GF/F; pore size: 0.7 μm) and frozen until analysis by capillary ion electrophoresis (nitrate; Agilent CE, Agilent Technologies, Waldbronn, Germany), the sulphanilamide method (nitrite), the salicylate method (ammonium) and the molybdate method (SRP; APHA 1998).

### Decomposition Experiment

In autumn 2019, leaves of alder, hazel, oak and willow were collected from the forest floor immediately after their natural abscission. These species were chosen in order to represent litter inputs in forested streams in the study region, and differed in several leaf traits (Table S1). Leaves of alder, hazel and oak were collected along the Agüera catchment, whereas willow leaves were collected next to the University of the Basque Country (43.32º N, 2.97º W) due to its more gradual abscission and hence lower availability at the study site. Leaves were air-dried in the laboratory, weighed and enclosed in coarse-mesh litterbags (20 × 25 cm, 5-mm mesh). Each of these litterbags received 4.0 ± 0.2 g of litter in total, comprising 1, 3 or 4 species depending on the treatment. There were six treatments: one litter mixture with all species (that is, including alder; 1 ± 0.05 g per species), one mixture containing hazel, oak and willow (that is, excluding alder; 1.33 ± 0.07 g per species), and the four monocultures (alder, hazel, oak or willow). We also used fine-mesh litterbags (12 × 15 cm, 0.5-mm mesh) containing the monocultures (1 ± 0.05 g) to examine differences in microbial decomposition among plant species. We used less litter mass in fine-mesh than in coarse-mesh litterbags to facilitate the observation of differences across treatments (because of the lower pace of microbial decomposition), as done elsewhere (for example, Kreutzweiser 2008; Schindler and Gessner 2009).

Sixty coarse-mesh and 40 fine-mesh litterbags were deployed in each stream, with ten replicates per treatment. Deployment (and subsequent collection) was done on two separate days in order to

**Table 1.** Physicochemical Characteristics of the Selected Streams During the Study Period

| Variable | Stream 1 | Stream 2 | Stream 3 |
|---|---|---|---|
| Latitude (º N) | 43.208 | 43.207 | 43.213 |
| Longitude (º W) | 3.267 | 3.263 | 3.271 |
| Altitude (m a.s.l.) | 325 | 350 | 305 |
| Temperature (ºC) | 7.57 ± 0.54 | 7.80 ± 0.53 | 7.65 ± 0.54 |
| Conductivity (µS $cm^{-1}$) | 143.70 ± 5.70 | 105.87 ± 2.06 | 95.90 ± 3.46 |
| pH | 7.61 ± 0.06 | 7.72 ± 0.07 | 7.40 ± 0.15 |
| Dissolved $O_2$ (mg $l^{-1}$) | 11.83 ± 0.21 | 11.69 ± 0.21 | 11.69 ± 0.20 |
| % Saturation $O_2$ | 101.45 ± 0.30 | 100.75 ± 0.40 | 100.33 ± 0.50 |
| Flow (l $s^{-1}$) | 27.85 ± 9.89 | 20.43 ± 6.75 | 26.33 ± 10.09 |
| DIN (µg $l^{-1}$) | 528.86 ± 46.37 | 613.58 ± 59.99 | 289.97 ± 34.39 |
| SRP (µg $l^{-1}$) | 3.48 ± 1.01 | 5.60 ± 1.62 | 8.51 ± 2.03 |

*Mean ± SE; n = 6. DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus.*

be able to handle all sporulation analyses upon collection (see below). One replicate per treatment was tied to one of ten iron bars, which were anchored at random locations within riffle sections of the streambed. An extra set of 20 fine-mesh litterbags (five per species, containing 2 ± 0.1 g of litter) was incubated for 92 h in one of the streams. Half of this litter was used to estimate the initial leaching of soluble compounds and calculate air-dry mass (DM) to oven DM (72 h at 70 °C) and to ash-free DM (AFDM; 4 h at 500 °C) conversion factors. The other half was used to measure leaf toughness (in fresh), specific leaf area (SLA), N and phosphorus (P) concentrations, which were used as surrogates for litter quality. The proportion of mass loss due to leaching was calculated as the difference between AFDM of non-incubated and incubated litter divided by AFDM of non-incubated litter. Leaf toughness was determined using a penetrometer with a 1.55-mm diameter steel rod (Boyero and others 2011), measured in 5 fragments per sample (fresh litter, after leaching). SLA was calculated as leaf area ($mm^2$) divided by dry mass (mg) measured in five 12-mm diameter leaf discs per sample. N concentration was measured with a PerkinElmer series II CHNS/O elemental analyser (PerkinElmer, Norwalk, Connecticut, USA). P concentration was measured spectrophotometrically after autoclave-assisted extraction (APHA 1998).

Half of the litterbags were collected after 21 days of instream incubation, enclosed individually in zip-lock bags and transported in a refrigerated cooler to the laboratory. The remaining litterbags were retrieved 42 days after their deployment and processed the same way. Litter material from each litterbag was rinsed with filtered (100 µm) stream water on a 500-µm sieve to remove sediment and associated macroinvertebrates. Five 12-mm diameter leaf discs were obtained from each species in each replicate coarse-mesh litterbag collected at day 21 (randomly selected among litter fragments, with no more than one disc per fragment) to induce fungal sporulation (see below); the remaining material was oven-dried (70 °C, 72 h), weighed to determine final DM, incinerated (500 °C, 4 h) and weighed to determine final AFDM.

## Aquatic Hyphomycetes and Macroinvertebrates

To measure fungal sporulation rate, litter discs were placed in Erlenmeyer flasks filled with 25 mL of filtered stream water (glass fibre filters, Whatman GF/F; pore size: 0.7 µm). Flasks were incubated for 48 ± 2 h on a shaker at 80 rpm and 10 °C. Conidial suspensions were poured into 50-mL Falcon tubes, pre-stained with 2 drops of 0.05% trypan blue in 60% lactic acid, preserved with 2 mL of 35% formalin and adjusted to 40 mL with distilled water. In order to identify and count conidia, 150 µL of Triton X-100 (0.5%) were added to each sample and mixed with a magnetic stirring bar to ensure a uniform distribution of conidia. Subsequently, an aliquot of 10 mL of the conidial suspension was filtered (25-mm diameter, pore size 5 µm, Millipore SMWP, Millipore Corporation) with gentle vacuum. Finally, filters were stained with trypan blue and spores were identified and counted at 200× magnification (Gulis and others 2005). Sporulation rate (number of conidia g litter $DM^{-1}$ $d^{-1}$) and taxon richness were calculated for each replicate.

Macroinvertebrates collected from coarse-mesh litterbags retrieved at day 21 were preserved in 70% ethanol for subsequent identification. They were identified to the lowest taxonomic level pos-

sible (typically genus) and classified into litter-consuming detritivores and other macroinvertebrates using Tachet and others (2010). For each sample, abundance and taxon richness (number of individuals and taxa per litterbag, respectively) were calculated for detritivores and all macroinvertebrates.

## Data Analysis

We quantified total decomposition (in litter mixtures and monocultures) and microbial decomposition (in monocultures only) through proportional litter mass loss [LML = (initial AFDM − final AFDM)/initial AFDM]. Initial AFDM was previously corrected for mass loss due to leaching (using the extra fine-mesh litterbags), which is a common procedure that allows removing differences among species in terms of rapid mass loss as a consequence of litter drying (Bärlocher 2020). We examined differences in decomposition between mixtures with and without alder (for total decomposition) and among plant species (alder, hazel, oak and willow; for total and microbial decomposition) using linear mixed-effect models (''lm'' function in the nlme R package; Pinheiro and others 2009). Litter mixture or plant species was a fixed factor in the model, and stream was a random factor (Zuur and others 2009). When significant differences were found ($\alpha = 0.05$; significance level used for all tests), Tukey tests were used to identify differences among plant species. We first ran an analysis that included sampling date (3 and 6 weeks) as fixed factor and its interaction with mixture or species. As the interaction was significant (see Results), we then ran separate analyses for both sampling dates to reduce model complexity.

Net diversity, complementarity and selection effects on LML were calculated in mixtures with and without alder following Loreau and Hector (2001). The net diversity effect was calculated as the difference between observed and expected decomposition, the latter based on decomposition of monocultures and the proportion of each of them in the mixture: Net diversity effect = $\Sigma i$ ($Value_{Obs} - Value_{Exp}$) = Complementarity effect + Selection effect. The complementarity effect was calculated as the average deviation from the expected decomposition in a mixture, multiplied by the number of species in the mixture and the mean decomposition in monocultures: Complementarity effect = mean ($Value_{Mixture} - Value_{Monoculture}$) × mean $Value_{Monoculture} \times n$. The selection effect was calculated as the covariance between decomposition of species in monoculture and the average deviation from expected decomposition of species in the mixture, multiplied by the number of species in the mixture: Selection effect = cov [($Value_{Mixture} - Value_{Monoculture}$), $Value_{Monoculture}$] $\times n$. Differences between the mixtures were examined with linear mixed-effect models using stream as random effect, followed by Tukey tests when significant differences were found, again separately for each sampling date.

We examined differences in fungal sporulation rate and taxon richness separately for each species, depending on whether it was incubated in the mixture with alder (4 species), the mixture without alder (3 species) or in monoculture (1 species). We used a linear mixed-effect model, with litter diversity (4, 3 or 1 species) and plant species identity as fixed factors, and stream as random factor, followed by Tukey tests when significant differences were found (Zar 1999). Fungal sporulation rate was previously log-transformed to meet the requirements of parametric analyses. The net diversity effect on aquatic hyphomycete variables (that is, sporulation rate and taxon richness) was calculated as the difference between the weighted mean of the observed values of species in each mixture and the expected value based on the respective monocultures, and were tested with linear mixed-effect models, with net diversity effect as fixed factor and stream as random factor. Fungal assemblage structure was analysed with non-metric dimensional scaling (NMDS) based on the Bray Curtis similarity index using conidial abundance data (''metaMDS'' function of the *vegan* R package), followed by permutational multivariate analysis of variance (''adonis'' function of the *vegan* R package) to test if the assemblage varied depending on plant species (alder, hazel, oak and willow) and on whether they were in mixtures with alder, in mixtures without alder or in monocultures. The most representative taxa of each assemblage were determined using an indicator value index (''multipatt'' function of the indicspecies R package; De Cáceres 2013).

Differences of macroinvertebrate abundance and richness between mixtures with and without alder and among plant species (alder, hazel, oak and willow) were examined using linear mixed-effect models, with plant species as fixed factor and stream as random factor, followed by Tukey tests to identify the differences among plant species when significant differences were found. We calculated the net diversity effect of macroinvertebrate abundance and richness as the difference between the observed value in each mixture and the expected value based on the respective monocultures.

Differences in net diversity between mixtures with and without alder were analysed with linear mixed-effect models, with net diversity effect as fixed factor and stream as random factor. We used NMDS, permutational multivariate analysis of variance and indicator value index (as above) to examine differences among treatments and indicator taxa of macroinvertebrate assemblage structure. These analyses were performed for detritivores and all macroinvertebrates.

## Results

### Litter Decomposition

LML was significantly affected by litter mixture, but the effect depended on sampling date (significant interaction between litter mixture and sampling date, $F = 13.816$, $p < 0.001$). Therefore, analyses were done separately for each sampling date. Litter mixtures with alder decomposed faster than those without alder at both sampling dates, and decomposition varied among plant species, being lowest for oak, highest for alder and with intermediate mass loss for hazel and willow, again at both sampling dates (Table 2, Figure 1). Microbial decomposition also differed among plant species at both dates, being higher for alder and hazel than for willow and oak (Table 2, Figure 1). We observed differences in diversity effects between sampling dates. At day 21, both mixtures presented negative net and complementarity effects and a positive selection effect, with no differences between mixtures (Table 2, Figure 2). At day 42, the mixture with alder showed positive complementarity and negative selection effects, resulting in the absence of a net diversity effect, whereas the mixture without alder presented negative net, complementarity and selection effects. Net and complementarity effects differed between mixtures, but there were no differences in the selection effect (Table 2, Figure 2).

### Aquatic Hyphomycetes

Sporulation rate varied with plant species, with higher values for alder and willow, intermediate for hazel and lower for oak and, for each species, it was higher in the monoculture and the mixture with alder than in the mixture without alder (Table 3, Figure 3A). Taxon richness also differed among species, being lower in oak than in the other species, and was again higher in the monoculture and the mixture with alder than in the mixture without alder (Table 3, Figure 3C). The net diversity effect for sporulation rate (Figure 3C) and taxon richness (Figure 3D) was higher in the presence of alder, although differences were only significant for taxon richness.

Aquatic hyphomycete assemblage structure was influenced by plant species, with differences between all species pairs except alder and willow, and mixtures with alder and monocultures were similar but differed from mixtures without alder (Table 3, Table S2). Mixtures with alder were associated with *Alatospora acuminata* Ingold, *Alatospora pulchella* Marvanová, *Lunulospora curvula* Ingold and *Tetrachaetum elegans* Ingold, and *Tetracladium mar-*

**Table 2.** Results of Linear Mixed-Effects Models Testing the Effects of Mixture (With and Without Alder) and Plant Species (Alder, Hazel, Oak and Willow) on Decomposition (Total and Microbial) and Net, Complementarity and Selection Effects in Each Sampling Date (21 and 42 days)

| Sampling date | Response variable | Factor | num df | den df | *F* | *p* |
|---|---|---|---|---|---|---|
| 21 d | Total decomposition | Mixture | 1 | 26 | 16.140 | < 0.001 |
| | Total decomposition | Species | 3 | 51 | 79.259 | < 0.001 |
| | Microbial decomposition | Species | 3 | 51 | 12.438 | < 0.001 |
| | Net diversity effect | Mixture | 1 | 26 | 0.490 | 0.490 |
| | Selection effect | Mixture | 1 | 26 | 0.410 | 0.528 |
| | Complementarity effect | Mixture | 1 | 26 | 1.014 | 0.323 |
| 42 d | Total decomposition | Mixture | 1 | 26 | 56.730 | < 0.001 |
| | Total decomposition | Species | 3 | 52 | 58.973 | < 0.001 |
| | Microbial decomposition | Species | 3 | 54 | 26.373 | < 0.001 |
| | Net diversity effect | Mixture | 1 | 26 | 11.959 | 0.002 |
| | Selection effect | Mixture | 3 | 26 | 2.821 | 0.105 |
| | Complementarity effect | Mixture | 1 | 26 | 8.046 | 0.009 |

*num df = degrees of freedom of numerator, den df = degrees of freedom of denominator, F = F-statistic; p = p-value.*

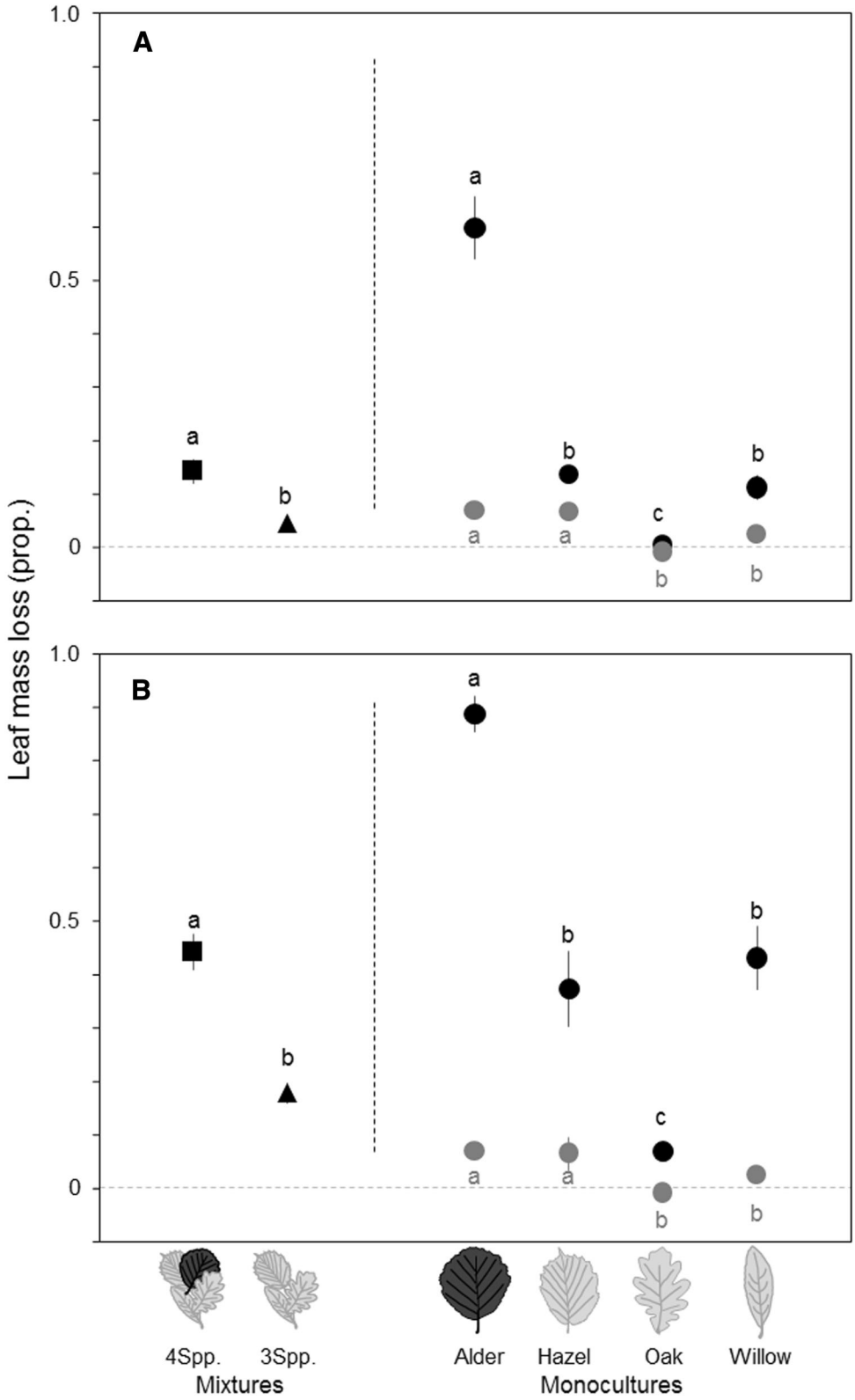


**Figure 1.** Total (black) and microbial (grey) decomposition (proportion of litter mass loss) at 21 (A) and 42 days (B). Symbols are means (squares represent the mixture with alder, triangles represent the mixture without alder, and circles represent monocultures) and whiskers are standard errors. Different letters indicate significant differences.

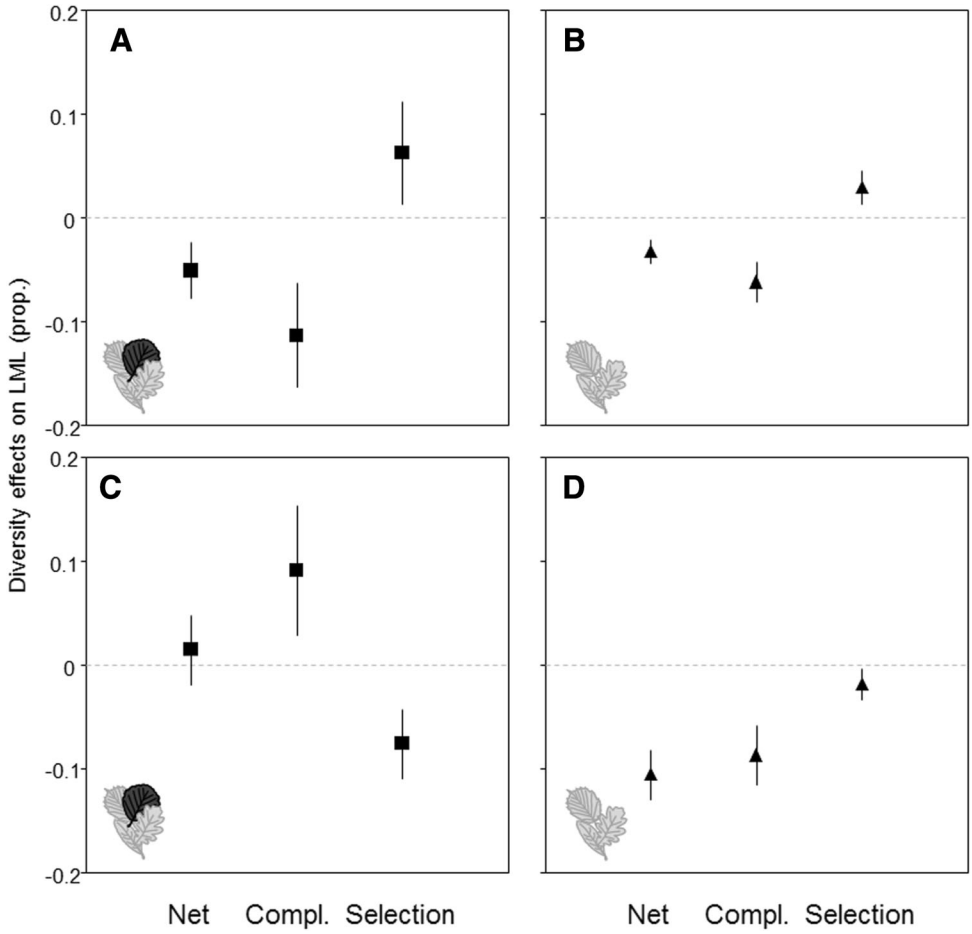


**Figure 2.** Net diversity, complementarity and selection effects on decomposition (measured as proportion of litter mass loss) at 21 days in the mixture with alder (A) and in the mixture without alder (B), and at 42 days in the mixture with alder (C) and in the mixture without alder (D). Symbols are means (squares represent the mixture with alder and triangles represent the mixture without alder) and whiskers are standard errors.

**Table 3.** Results of Linear Models Testing the Effects of Mixture (Monoculture, Mixture With and Without Alder) and Plant Species (Alder, Hazel, Oak and Willow) on Aquatic Hyphomycete Sporulation Rate (Conidia $g^{-1}$ DM $d^{-1}$) and Taxon Richness, and Results of PERMANOVA Testing the Effects of Mixture, Plant Species and the Interaction Between Them and With Stream, on Aquatic Hyphomycete Assemblage Structure

| Variable | Factor | df | $R^2$ | $F$ | $p$ |
|---|---|---|---|---|---|
| Sporulation rate | Species | 3, 156 | | 85.068 | < 0.001 |
| | Mixture | 2, 156 | | 20.143 | < 0.001 |
| Taxon richness | Species | 3, 156 | | 15.370 | < 0.001 |
| | Mixture | 2, 156 | | 9.680 | < 0.001 |
| Community structure | Mixture | 2 | 0.039 | 4.612 | < 0.001 |
| | Species | 3 | 0.273 | 21.319 | < 0.001 |
| | Mixture: Species | 5 | 0.024 | 1.115 | 0.312 |
| | Mixture: Species(Stream) | 22 | 0.103 | 1.097 | 0.262 |

*df = degrees of freedom; F = F-statistic; $R^2$ = adjusted R squared; p = p-value.*

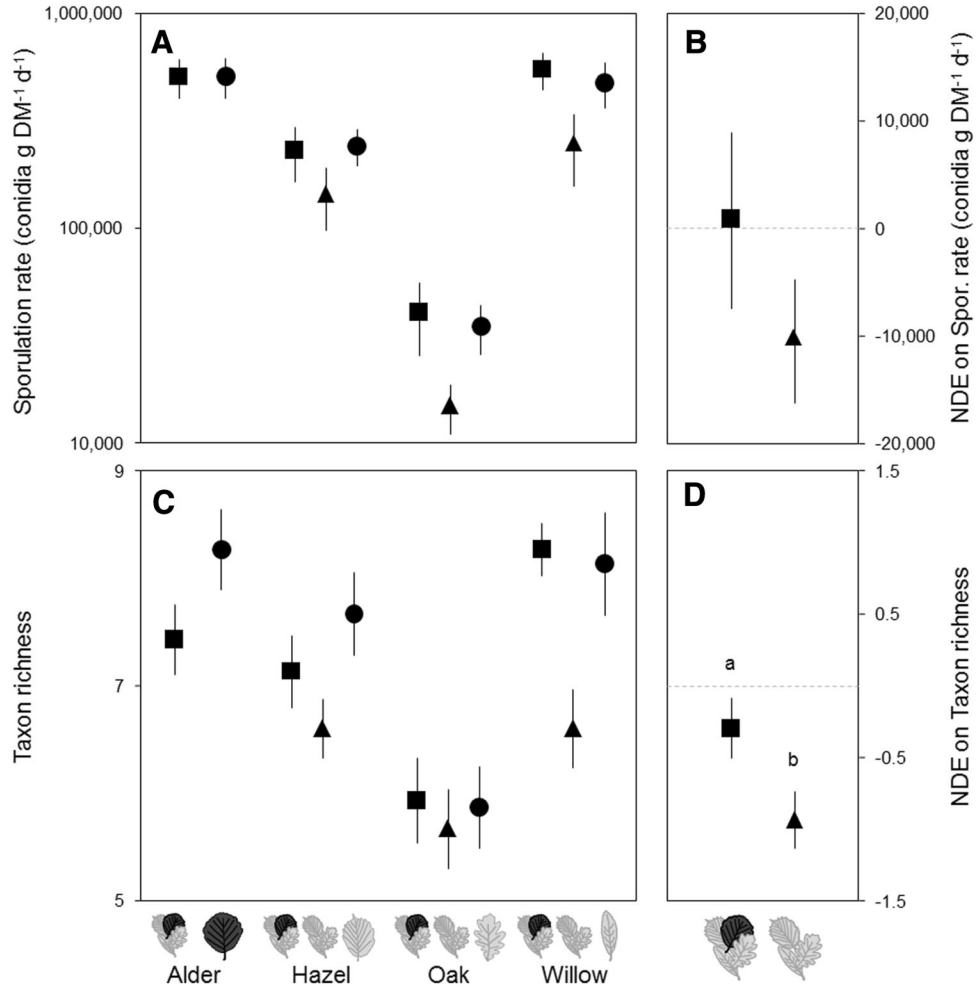


**Figure 3.** Aquatic hyphomycete sporulation rate (conidia g $DM^{-1}$ $d^{-1}$; **A**), net diversity effect on sporulation rate (**B**), taxon richness (**C**), and net diversity effect on taxon richness (**D**), on alder, hazel, oak and willow on mixtures with alder (squares), mixtures without alder (triangles) and monocultures (circles) at 21 days. Symbols are means and whiskers are standard errors.

*chalianum* De Wild., which was also characteristic of monocultures.

Assemblages found in alder and willow were characterised by *Articulospora tetracladia* Ingold, *T. elegans*, *Flagellospora curvula* Ingold, *Heliscus lugdunensis* Sacc. & Therry, *Anguillospora filiformis* Greath., *A. pulchella* and *A. acuminata*; *L. curvula* was also important in alder. There were no indicator taxa that were specific of hazel, which shared *A. filiformis*, *A. pulchella* and *A. acuminata* with alder and willow. Assemblages in oak were characterised by *Taeniospora gracilis* Marvanová (Table S3).

## Macroinvertebrates

Detritivore abundance did not differ between mixtures but varied with plant species, being higher in hazel than in oak and willow (Table 4, Figure 4A). Detritivore richness did not vary between mixtures or plant species (Table 4, Figure 4C). The net diversity effect did not differ between mixtures for abundance and richness (Figure 4). Detritivore assemblage structure varied among treatments (Table 4); the mixture with alder differed from that without alder, hazel differed from oak, willow and the mixture without alder, and oak differed from the mixture with alder (Table S4). The assemblage in hazel was characterised by Leuctridae (Plecoptera), and *Amphinemura* (Plecoptera) was characteristic of alder, hazel and the mixture with alder, both taxa being (litter-consuming) detritivores (Table S5).

For all macroinvertebrates, abundance varied with plant species, being higher in hazel than in alder, oak and willow, but not between mixtures (Table 4, Figure 4A). There were no differences for

**Table 4.** Results of Linear Models Testing the Effects of Mixture (With and Without Alder) and Plant Species (Alder, Hazel, Oak and Willow) on Abundance (ind. $bag^{-1}$) and Taxon Richness, and Results of PERMANOVA Testing the Effects of Treatment (Mixture With Alder and Mixture Without Alder, Alder, Hazel, Oak, Willow) and Their Interaction with Stream, on Detritivore Assemblage Structure and Macroinvertebrate Structure, of Detritivores and Total Macroinvertebrates

| | Variable | Factor | df | $R^2$ | $F$ | $p$ |
|---|---|---|---|---|---|---|
| Detritivore | Abundance | Mixture | 1, 26 | | 1.208 | 0.282 |
| | | Species | 3, 54 | | 6.687 | < 0.001 |
| | Taxon richness | Mixture | 1, 26 | | 1.366 | 0.253 |
| | | Species | 3, 54 | | 1.300 | 0.284 |
| | Community structure | Treatment | 5 | 0.113 | 2.346 | < 0.001 |
| | | Treatment: Stream | 12 | 0.193 | 1.668 | 0.001 |
| Macroinvertebrate | Abundance | Mixture | 1, 26 | | 0.931 | 0.344 |
| | | Species | 3, 54 | | 5.648 | 0.002 |
| | Taxon richness | Mixture | 1, 26 | | 0.470 | 0.499 |
| | | Species | 3, 54 | | 1.922 | 0.137 |
| | Community structure | Treatment | 5 | 0.104 | 2.197 | < 0.001 |
| | | Treatment: Stream | 12 | 0.215 | 1.890 | < 0.001 |

*df = degrees of freedom; F = F-statistic; $R^2$ = adjusted $R^2$; p = p-value.*

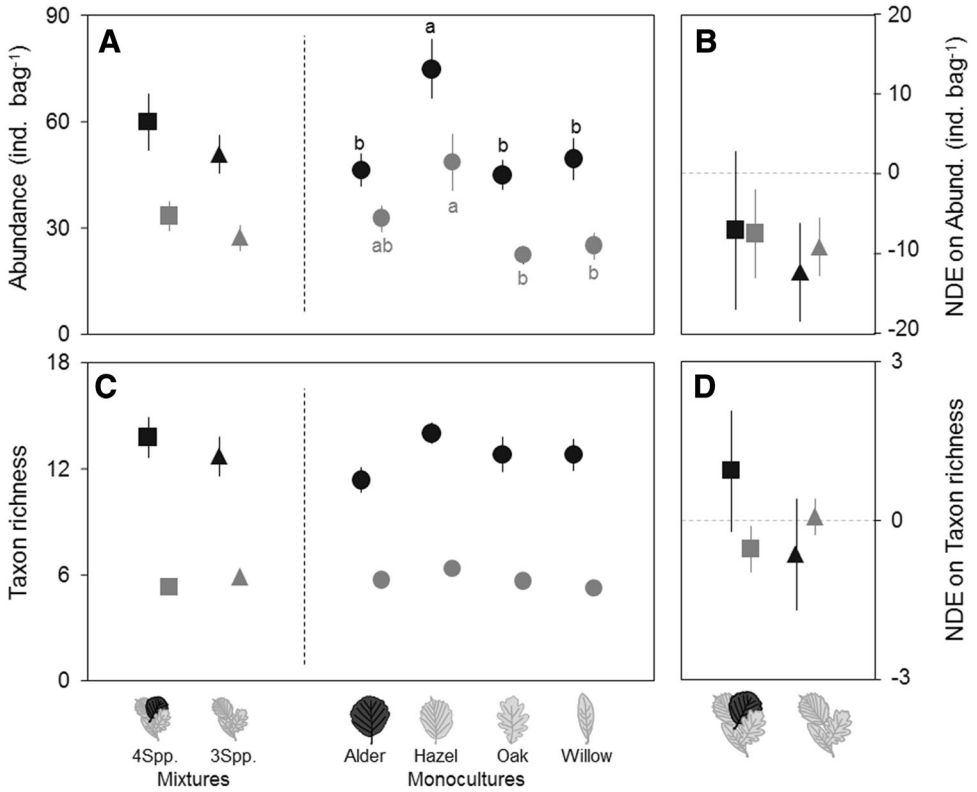


**Figure 4.** Macroinvertebrate abundance (individuals per bag; **A**), net diversity effect on abundance (**B**), taxon richness (**C**), and net diversity effect on taxon richness (**D**), for detritivores (grey) and all macroinvertebrates (black) at 21 days. Symbols are means (squares represent the mixture with alder, triangles represent the mixture without alder, and circles represent monocultures) and whiskers are standard error. Different letters indicate significant differences.

taxon richness (Table 4, Figure 4C) and net diversity effects (Figure 4). Assemblage structure varied with treatment (Table 4), with differences between alder and all others; between hazel and the other monocultures; and between the mixture with alder and alder and oak monocultures (Table S4). The

indicative species were the same as in detritivore assemblages, with the addition of the collector-gatherer *Habroleptoides* (Ephemeroptera), which was important in all assemblages except those of alder (Table S5).

## Discussion

Effects of plant litter diversity loss on decomposition have been widely studied in both terrestrial and aquatic ecosystems, but most experiments have focused on random species losses and have rendered contradictory evidence (that is, positive, negative or no effects). Thus, even if some studies have revealed general patterns and drivers of litter mixing effects on decomposition (for example, Liu and others 2020), the fact that random species loss is unrealistic (Wardle 2016) has been seldom taken into account (Berg and others 2015). Here, we simulated the loss of alder trees as a result of infection by *P. alni* (Bjelke and others 2016) and thus examined a realistic extinction scenario and its consequences on litter decomposition in streams and the associated assemblages.

### The Presence of Alder Increased Decomposition Despite an Overall Negative Diversity Effect

Our experiment, conducted in three streams, showed that the presence or absence of alder strongly influenced stream ecosystem functioning. As predicted, litter decomposition was increased in its presence, with mixtures containing alder decomposing faster than mixtures without alder, regardless of incubation time (that is, 3 or 6 weeks). Importantly, this difference could not be explained by the increase in diversity per se in mixtures (from 3 to 4 species), because the examination of monocultures revealed a negative net diversity effect in most cases. Thus, the most likely explanation for the effect of alder was related to its particular characteristics, as discussed below.

The negative net diversity effect was mainly explained by a negative complementarity effect, which consisted in a reduction in decomposition due to the interaction among the plant species in the mixture, and possibly indicated the existence of physical or chemical interference among traits of different plant species. For example, the presence of tannins or other inhibitory compounds in one species may limit fungal colonisation and/or detritivore consumption of higher-quality species (that is, those with high nutrient content), thus reducing overall decomposition in the mixture (McArthur and others 1994; Graça and others 2001; López-Rojo and others 2020). Although we did not measure condensed tannins in litter from this experiment, a previous study found highly variable concentrations in our studied species (17.4% in *Salix*, 11.4% in *Quercus*, 2.3% in *Corylus* and 1.7% in *Alnus*; L.B., unpubl. data from Boyero and others 2017). In contrast, the positive selection effect found at week 3 indicated a preference of consumers towards alder (or hazel in the mixture without alder). Preference for alder over other species has been described elsewhere for both microbial decomposers and detritivores and it has been attributed to its high concentrations of nutrients (N and P) and low concentration of secondary compounds, which increase its decomposability and palatability (Friberg and Jacobsen 1994; Graça and others 2001; Gulis 2001; López-Rojo and others 2018). The positive selection effect was nevertheless smaller than the negative complementarity effect, and hence not of sufficient magnitude to increase decomposition in the mixture compared to the average monoculture.

At week 6, the complementarity effect remained negative in the absence of alder but became positive in its presence, with a negative selection effect in both cases. The increase in complementarity effect through time has been observed elsewhere (Cardinale and others 2007) and could be due to an increase in the palatability of low-quality litter (that is, litter with low nutrient contents) caused by nutrient transfer from high-quality litter (alder in our study), possibly through fungal hyphae (Handa and others 2014). The change in the selection effect from positive to negative could be related to the depletion of high-quality litter through time, causing low-quality litter to dominate the mixture, which could make decomposers avoid it (Loreau and Hector 2001; Larrañaga and others 2020). However, litter traits and detritivore preferences can vary through time (Compson and others 2018); so this issue merits further attention.

### Aquatic Hyphomycete Assemblages Varied with Litter Identity and Mixture Composition

Fungal sporulation rate and taxon richness varied mainly depending on litter identity, in agreement with many other studies (Kominoski and others 2007; Fernandes and others 2012; Ferreira and others 2012; Jabiol and Chauvet 2012). A positive relationship between fungal richness and litter diversity has been reported elsewhere (Laitung and Chauvet 2005), but this was not the case in our

experiment. We found the highest sporulation rates and taxon richness (although the latter did not differ statistically from hazel) in alder and willow, and the lowest in oak, which matches the results of other studies (Gessner and Chauvet 1994; Gulis 2001; Ferreira and others 2012; Cornejo and others 2020). This is most likely related to differences in concentrations of nutrients (high in alder and low in oak), which favour the activity of aquatic hyphomycetes, and those of lignin and phenolic compounds (low in alder and high in oak), which inhibit it (McArthur and others 1994; Ferreira and others 2012). Willow litter also has high nutrient concentration, similar to alder and other Betulaceae (Webster and Benfield 1986; Gulis 2001), although this species has been seldom used in decomposition stream experiments.

When fungal sporulation rate and taxon richness were examined for hazel, oak and willow in monocultures vs. mixtures, we observed a negative diversity effect when alder was absent (that is, lower values for the three-species mixture than for the monoculture in all cases), but no effect when it was present (that is, similar values in the four-species mixture and the monocultures). Thus, alder seemed to compensate for any decrease in the fungal assemblage of a given species introduced by the addition of other species, which could possibly occur through increased competition (Jabiol and Chauvet 2012). Competition could be due to harder conditions caused by the presence of condensed tannins or other inhibitory compounds (Kominoski and others 2007; Ferreira and others 2012), and would be indicated by the reduction in taxon richness and sporulation rate in the mixture without alder. Tannin concentrations were 8 and 5 times greater in *Salix* and *Quercus*, respectively, than in *Corylus*, and 10 and 7 times greater than in *Alnus* (L.B., unpubl. data from Boyero and others 2017). All species of aquatic hyphomycetes decreased in abundance, but the reduction was greater in non-dominant taxa (*Alatospora pulchella, Alatospora acuminata* and *Tetrachaetum elegans*) than in dominant taxa (*Articulospora tetracladia* and *Anguillospora filiformis*). The inclusion of alder in the mixture could increase the nutrient content and hence reduce competition compared to the mixture without alder, allowing fungal assemblages similar to those of monocultures (Kominoski and others 2007); this may not occur when N is readily available in the water and used by aquatic hyphomycetes (Tonin and others 2017), but N concentration was low in our study streams (Table 1).

### The Presence of Alder in Mixtures Affected Detritivore Assemblage Structure

Detritivore and total macroinvertebrate abundances were the highest in hazel, as observed elsewhere (Sanpera-Calbet and others 2009). Although higher abundances could be expected in alder, the faster decomposition of this species resulted in large biomass reduction by the end of the experiment, which most likely caused the higher abundances in hazel compared to alder. Hazel litter did not have high nutrient content, but it did have high specific leaf area, thus offering greater surface availability and stability (Dobson 1994; Kominoski and Pringle 2009; Sanpera-Calbet and others 2009). Hazel and alder shared detritivore indicative taxa (for example, *Amphinemura*), which suggests that detritivores feeding on alder may shift to hazel when the former is no longer available. Macroinvertebrates that do not feed on leaf litter (for example, *Habroleptoides*) were less common in alder than in other treatments, possibly because its lower usefulness as substrate due to its faster decomposition (Dobson 1994; Kominoski and Pringle 2009).

## Conclusions

The overall loss of riparian plant diversity is known to have important consequences on stream ecosystem functioning by altering several key processes such us decomposition rates, nutrient dynamics, fine particulate organic matter production and invertebrate growth (López-Rojo and others 2019). However, not all species are equally vulnerable to extinction, so it is important to establish the most likely scenarios of species loss and assess how losing these particular species will affect the ecosystem. We have shown how the loss of alder, which is caused by the expansion of *P. alni* across Europe (Bjelke and others 2016), can inhibit litter decomposition and aquatic hyphomycete sporulation and modify stream assemblages, hence seriously altering stream ecosystem functioning and structure. Similar results have been obtained in studies testing the effects of the loss of North American ashes due to the invasive insect *Agrilus planipennis* Fairmaire, where the absence of this species reduced decomposition and modified invertebrate community (Kreutzweiser and others 2019). Therefore, we suggest that similar studies be conducted with other species such as oak, which is affected by *P. cinnamomi* Rand (Hernández-Lambraño and others 2019), in order to improve our

predictions about how stream ecosystems can be affected by species loss in the near future.

## ACKNOWLEDGEMENTS

This study was funded by the Spanish Ministry for Science, Innovation and Universities and FEDER (BioLoss project, Ref. RTI2018-095023-B- I00 to LB) and the Basque Government (Ref. IT951-16 to the Stream Ecology Group at the UPV/EHU). AA and NLR were supported by UPV/EHU and Basque Government predoctoral fellowships, respectively.

## Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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