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Functional repercussions of riparian biodiversity loss in streams

Multifunctionality, diversity metrics and underlying mechanisms

Naiara López-Rojo

PhD Thesis

December 2020

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supervised by

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Después de 4 años… ¡*habemus tesis*! Han pasado muchas cosas estos últimos años de mi vida, unas mejores y otras peores, mi vida ha estado patas arriba en algunos momentos… si hasta hemos vivido (mejor dicho, estamos viviendo) una pandemia mundial! A pesar de todo, estoy muy contenta y orgullosa del resultado, mi yo de hace unos años jamás se hubiese creído capaz de lograrlo. Y no habría sido posible sin la ayuda de personas a las que tengo mucho que agradecer.

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CONTENTS

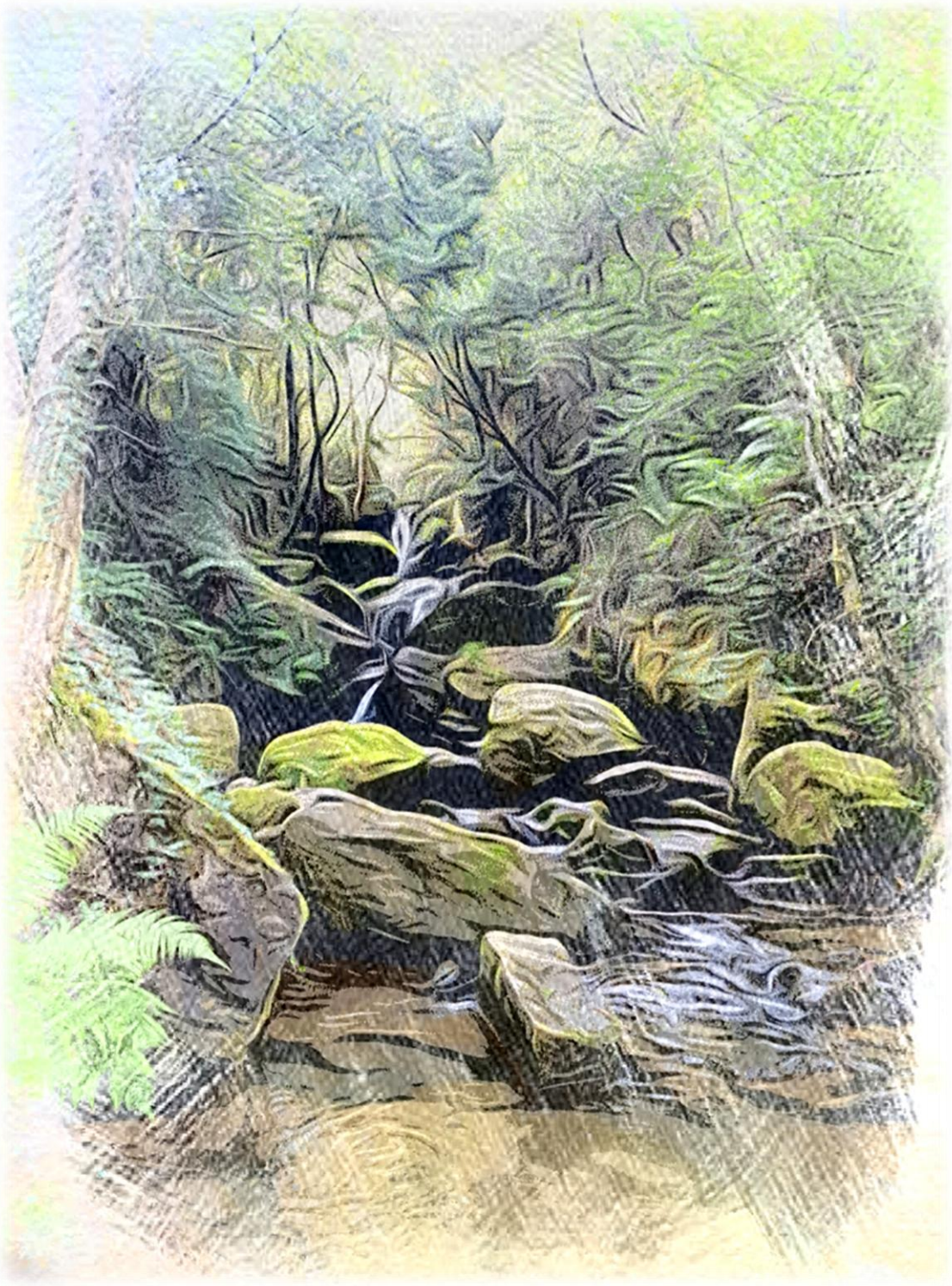
Summary

The objective of this thesis is to shed light on several important gaps that exist in biodiversity–ecosystem functioning (BEF) research, by addressing key questions about how riparian plant litter diversity loss can alter stream ecosystem functioning. In the first 2 chapters, I explore the issue of how plant litter diversity loss can simultaneously impact multiple processes that are fundamental to streams. While most relevant studies have focused on litter decomposition, I consider other processes that are intimately linked to decomposition and occur simultaneously, namely the cycling of major nutrients and secondary biomass production. My results provide novel evidence of simultaneous effects on multiple processes and demonstrate that, despite the usefulness of multifunctionality metrics to assess overall impacts on the ecosystem, the separate analysis of different processes is essential to fully understand how organisms, food webs and, ultimately, ecosystems, are altered. I found the largest and most consistent effects of litter diversity loss on nutrient cycling, suggesting that studies focused solely on decomposition may underestimate the consequences of plant diversity loss on streams. In chapter 3, I show the existence of only weak interactions between the detrital and the autotrophic pathways within stream food webs, with no complex relationships between biodiversity and ecosystem processes across pathways. In chapters 4 and 5, I examine the relevance of different biodiversity metrics (species richness, phylogenetic distance and litter trait variability) within a BEF context, using both field and microcosm experiments. I reveal important differences among these metrics, and highlight the major role of particular litter traits (mostly the concentrations of major nutrients) as drivers of BEF relationships. Finally, throughout the thesis I improve our mechanistic understanding of BEF relationships by exploring the relative role of complementarity and selection effects. I demonstrate that complementarity is often dominant, which highlights the relevance of interspecific interactions as drivers of biodiversity effects on ecosystem functioning. This thesis provides a major step towards our understanding of how the loss and replacement of riparian plant species can cause significant alterations in multiple processes that are central to the functioning of many stream ecosystems. Moreover, the finding of large and consistent effects on nutrient cycling suggests that the consequences of plant diversity loss go beyond the functioning of stream ecosystems, with the potential to alter global biogeochemical cycles.

Resumen

El objetivo de esta tesis es esclarecer varias lagunas de conocimiento, relativas al papel que juega la biodiversidad en el funcionamiento de los ecosistemas ('BEF', por sus siglas en inglés). En concreto, se abordan cuestiones clave sobre cómo la pérdida de diversidad del bosque ripario puede alterar el funcionamiento del ecosistema fluvial. En los primeros 2 capítulos, se explora cómo la pérdida de diversidad de hojarasca puede afectar de manera simultánea a múltiples procesos fundamentales para los arroyos de cabecera. Mientras que los estudios más relevantes se han centrado en la descomposición de la hojarasca, en esta tesis se consideran también otros procesos que están íntimamente relacionados y ocurren de manera conjunta, concretamente el reciclado de nutrientes y la producción secundaria. Los resultados evidencian efectos en múltiples procesos y demuestran que, a pesar de la utilidad de las métricas de multifuncionalidad para evaluar los impactos generales en el ecosistema, el análisis individual de diferentes procesos es esencial para comprender completamente cómo los organismos, las redes tróficas y, en última instancia, los ecosistemas, se ven alterados por la pérdida de diversidad de hojarasca. Los efectos más marcados y consistentes se detectaron sobre el reciclado de nutrientes, lo que sugiere que los muchos estudios centrados únicamente en descomposición pueden subestimar las consecuencias que tiene la pérdida de diversidad de especies riparias para el ecosistema fluvial. El capítulo 3 evidencia la existencia de interacciones débiles entre las vías detrítica y autotrófica dentro de la red trófica fluvial, sin relaciones complejas entre la biodiversidad y los procesos de los ecosistemas a través de ambas vías. En los capítulos 4 y 5, examino la relevancia de diferentes métricas de biodiversidad (riqueza de especies, distancia filogenética y variabilidad de rasgos biológicos) dentro de un contexto BEF, utilizando tanto experimentos de campo como de microcosmos. Revelo diferencias importantes entre estas métricas, destacando el papel principal de rasgos concretos de la hojarasca (principalmente las concentraciones de los nutrientes principales) como promotores de las relaciones BEF. Finalmente, a lo largo de la tesis, con el objetivo de mejorar nuestra comprensión de los mecanismos subyacentes a las relaciones BEF, se explora la importancia relativa de los efectos de complementariedad y selección. La complementariedad resultó ser, en la mayoría de los casos, el mecanismo dominante, lo que destaca la relevancia de las interacciones interespecíficas como mediadoras de los efectos de la biodiversidad en el funcionamiento de los ecosistemas. Esta tesis proporciona un avance importante en la comprensión de cómo la pérdida y el reemplazo de especies de plantas riparias puede causar alteraciones significativas en múltiples procesos que son fundamentales para el funcionamiento de los ecosistemas fluviales. Además, el hallazgo de efectos importantes y consistentes en el reciclado de nutrientes sugiere que las consecuencias de la pérdida de diversidad vegetal van más allá del funcionamiento de éstos ecosistemas, con el potencial de alterar los ciclos biogeoquímicos globales.

GENERAL INTRODUCTION



GENERAL INTRODUCTION

GLOBAL BIODIVERSITY LOSS AND STREAM ECOSYSTEMS

Biodiversity is rapidly decreasing at the global scale, to an extent that the occurrence of a 6th mass extinction has been advocated (Barnosky et al. 2011, Ceballos et al. 2017). The main causes of such decline are related to multiple anthropogenic activities including habitat fragmentation (Tamisier and Grillas 1994, Light and Marchetti 2007), the introduction of exotic species and pathogens into novel areas (Clavero et al. 2009, Hermoso et al. 2011, Doherty et al. 2016), and climate change (Midgley et al. 2002, Colossi Brustolin et al. 2019), among others. Importantly, while the loss of biodiversity is in itself worrying due to its intrinsic value (Ghilarov 2000), the repercussions of such loss extend to the functioning of ecosystems (Naeem et al. 1994) and the services they provide to humans, which are often altered (Cardinale et al. 2006). Studies addressing biodiversity-ecosystem functioning (hereafter, BEF) relationships emerged in the 1990's and have been so profuse since then that a new ecological discipline, known as BEF research (Box 1), has developed (Schulze and Mooney 1993, Tilman et al. 2014, Gonzalez et al. 2020). Still, the majority of BEF studies have focused on the process of primary production in terrestrial ecosystems (Cardinale et al. 2011, Maestre et al. 2012b), while other types of processes and ecosystems have received considerably less attention (Hooper et al. 2005, van der Plas 2019).

Freshwater ecosystems contribute substantially to global biodiversity, as they hold about the 6% of all described species despite the fact that they occupy only the 0.8% of the Earth's surface and contain 0.01% of the World's water (Dudgeon et al. 2006). However, these ecosystems are also profoundly altered by human activities (Ormerod et al. 2010, Vörösmarty et al. 2010), and their biodiversity loss rates far exceed those of the most affected terrestrial ecosystems (Harding et al. 1992, Allan and Flecker 1993, Dudgeon et al. 2006). Of particular concern are running waters, which play a fundamental role in the water cycle and global fluxes of nutrients and carbon (C), and provide fundamental ecosystem services to humans (e.g., drinking water, food, waste removal and renewable energy; Millenium Ecosystem Assessment 2005). Within river networks, headwater streams (i.e., first and second order streams) represent over two thirds of total channel length (Leopold et al. 2020) and hold a large and unique habitat and biological diversity (Meyer et al. 2007, Finn et al. 2011, Richardson 2019). Moreover, these streams are strongly connected with the surrounding terrestrial ecosystem, especially with the riparian forest (Wallace et al. 1997), so they can be affected by both aquatic and terrestrial biodiversity loss (Kominoski et al. 2013).

Box 1. Glossary of main terms pertaining to BEF research

BEF research: body of theory and collection of scientific studies that focus on the relationship between biodiversity and ecosystem functioning (Schulze and Mooney 1993).

Biodiversity: contraction of the term 'biological diversity', which refers to variety among living organisms. It can refer to diversity of species ('species richness') or taxa in general ('taxonomic diversity'), genes ('genetic diversity), or biological traits ('trait diversity'), including functional traits ('functional diversity') (Swingland 2013).

Ecosystem functioning: array of processes that sustain an ecosystem (Díaz and Cabido 2001).

Ecosystem process: flow of energy and matter over time and/or space driven by the interplay of abiotic (physical and chemical) and biotic factors. Examples include primary and secondary production, resource consumption, decomposition, respiration, denitrification and nutrient uptake (Reiss et al. 2009, Von Schiller et al. 2017).

Ecosystem service: value or good provided by an ecosystem process to humans. Examples are food production, water provision, detoxification, weather control, carbon sequestration and recreation (Díaz and Cabido 2001, Isbell et al. 2017).

Functional trait: morphological, physiological or phenological feature of an organism's phenotype, measurable at the individual level, which determines its effect on processes and its response to environmental factors (Petchey and Gaston 2006, Violle et al. 2007).

Phylogenetic distance: estimate of the amount of time since the most recent common ancestor of two species, which can be used as proxy for their differences in functional traits (i.e., functional diversity) (Darwin 1859, Harvey and Pagel 1991).

Most headwater streams are detritus-based systems where primary production is limited by riparian shading. Inputs of terrestrial detritus, mostly in the form of leaf litter, are the basis of the aquatic detrital food web, where detritivorous invertebrates play a key role (Wallace et al. 1997). When litter enters the stream, it is rapidly colonized by microbial decomposers, mainly aquatic hyphomycetes (Abelho and Graça 2006), which enhance the litter nutritional content and make it a suitable food source for litter-feeding detritivores (Wallace and Webster 1996, Graça and Cressa 2010, Tank et al. 2010). Litter is thus decomposed and fragmented by the joint action of microorganims, litter-feeding detritivores and physicochemical processes, leading to the production of fine particulate organic matter (FPOM) that feeds other detritivorous invertebrates such as collectors and filterers (Cummins and Klug 1979, Bundschuh and McKie 2016), and releasing nutrients to the water that are used by primary producers. The latter exude labile C that can be used by fungi and stimulate litter decomposition (Soares et al. 2017), and they serve as food for grazers (Cummins 1974). Finally, invertebrate and vertebrate predators are on top of the stream food web (Wallace et al. 1997) (Fig 1).

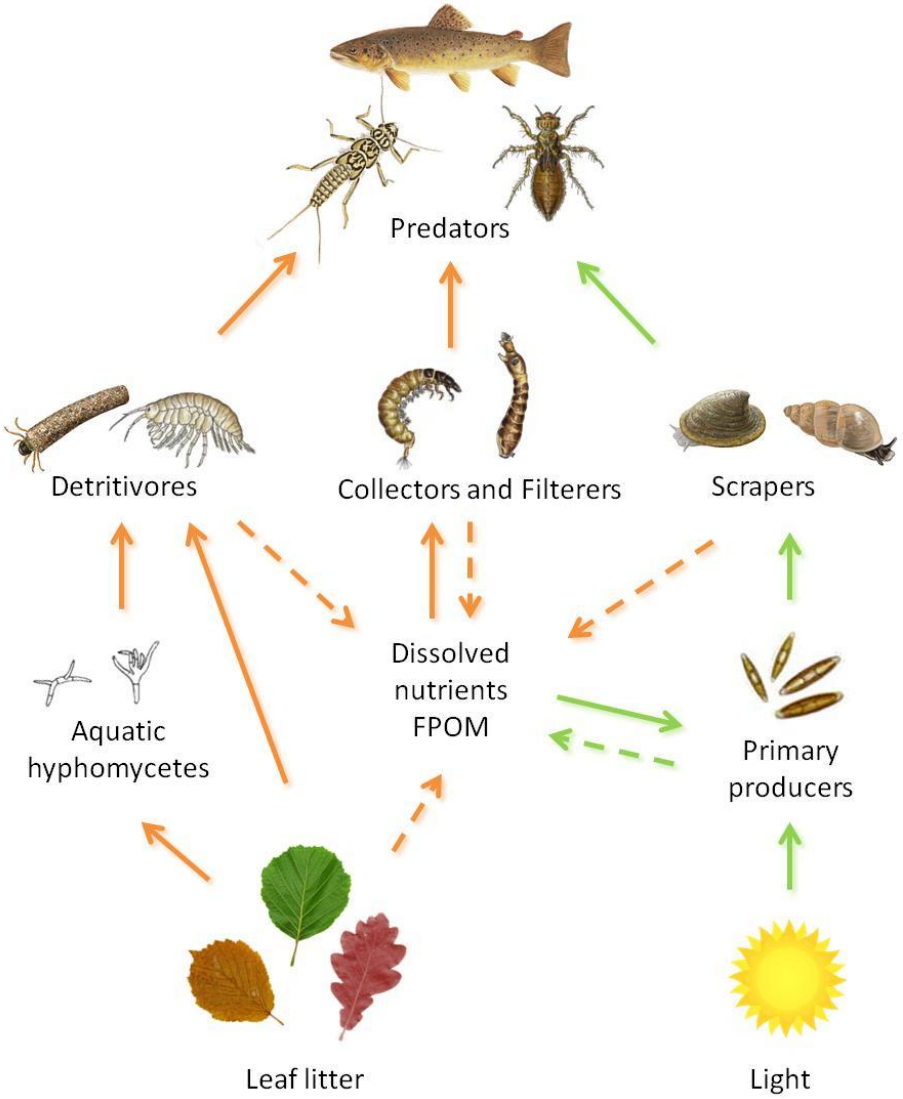


Fig 1. Main interactions occurring in the stream food web. Brown and green arrows refer to detrital and autotrophic pathways, respectively. Solid arrows represent feeding relationships, and dotted arrows represent the release of substances to the water column.

BEF STUDIES ON STREAM ECOSYSTEMS

As shown above, the decomposition of terrestrial litter and the concomitant recycling of nutrients and production of detritivore biomass are central processes to the functioning of headwater streams and the maintenance of the aquatic food web. It could thus be expected that changes in biodiversity of any of the trophic levels involved in the above processes (i.e., leaf litter, detritivores and aquatic hyphomycetes; Fig 1) would lead to alterations in stream ecosystem functioning (Gessner et al. 2010) and, ultimately, in biogeochemical cycles (Battin et al. 2009, Raymond et al. 2013). While microbial diversity has received relatively little attention in this context (but see Duarte et al. 2006, Bell et al. 2009), many studies have

examined the effects of plant litter or detritivore diversity (i.e., bottom-up or top-down diversity effects, respectively) on decomposition (Gessner et al. 2010) and, to a lesser extent, on associated processes (see below). However, there is still little consensus about BEF relationships in streams, because different studies have reported contrasting results. This is particularly true for bottom-up effects, which are generally weaker than top-down effects (Srivastava et al. 2009) and highly inconsistent across studies (Cardinale et al. 2011). This important gap of knowledge (see also Daam et al. 2019) has motivated this thesis, which focuses on the relationship between plant litter diversity (hereafter litter diversity) and stream ecosystem functioning.

BIODIVERSITY AND ECOSYSTEM MULTIFUNCIONALITY

Ecosystems are often valued for their capacity to maintain multiple processes or functions simultaneously, a concept termed 'multifunctionality' (Hector and Bagchi 2007). Importantly, the number of species (or traits) needed to maintain ecosystem functioning increases with the number of processes measured, because progressively more species (or traits) are required to sustain multiple processes (Reiss et al. 2009). While most studies assessing BEF relationships have examined single processes in isolation, the study of multifunctionality has recently gained importance (Byrnes et al. 2014), and there is now ample evidence that biodiversity affects multifunctionality in terrestrial ecosystems (Maestre et al. 2012b, Delgado-Baquerizo et al. 2016, Mori et al. 2016). In contrast, there is little information available for streams, with very few exceptions (e.g., Perkins et al. 2015, who focused on effects of invertebrate diversity). The detrital stream food web is supported by several associated processes and complex interactions between resources and organisms at different trophic levels (Fig. 1). Therefore, it seems relevant to examine these processes and interactions together in order to reveal effects of plant diversity loss on the ecosystem. However, stream BEF studies have focused almost solely on the process of litter decomposition, ignoring associated processes such us the production of FPOM and animal biomass, or nutrient cycling. The consequences of biodiversity loss on stream ecosystem functioning may thus have been underestimated, and this thesis addresses this issue by examining multifunctionality.

In addition to considering multiple processes, an interesting and underexplored question is whether BEF relationships occur across different compartments of the stream food web, namely heterotrophic and autotrophic pathways (Fig. 1). Even if the former is dominant in low-order streams, primary producers are also present and thus both pathways generally coexist in the food web. Moreover, part of the leaf litter that enters low-order streams is not processed *in situ*, but rather transported downstream to mid reaches, where primary production becomes dominant (Vannote et al. 1980). It

thus seems likely that litter diversity may influence primary production and associated algal assemblages and, to a lesser extent, that algal diversity may have an influence on decomposition and associated processes. This thesis introduces this multi-trophic perspective and examines the reciprocal effects of biodiversity on ecosystem functioning between the brown and green pathways of the stream food web.

HOW TO MEASURE BIODIVERSITY

Another important drawback of many BEF studies is the way biodiversity is usually measured (Box 1). While the most often used metric is species richness, it has been argued that functional diversity of biological assemblages is probably a better predictor of ecosystem functioning than taxonomic diversity in general or species richness in particular (Buchmann and Roy 2002). This means that it is important to understand what organisms do and which role they play in the ecosystem, rather than simply knowing how many species are present. This change in perspective has motivated an increase in the number of studies using biodiversity measures based on functional traits (Petchey and Gaston 2002). Still, there is no simple o standard measure of functional diversity (Díaz and Cabido 2001), with organisms being usually categorized based on a set of traits that are considered relevant for ecosystem functioning based on existing knowledge. An alternative to this is using phylogenetic distance as proxy for functional diversity, as it does not rely on an *a priori* selection of traits, but it rather is an integrative measure that includes all species traits (Swenson 2013). Although phylogenetic distance has shown relationships with ecosystem processes such as primary production (Cadotte et al. 2008) and litter decomposition (Boyero et al. 2016), the relevance of this measure compared to others such as functional diversity and species richness has not been explicitly addressed, and this is one of the issues I explore in this thesis.

BIOLOGICAL MECHANISMS UNDERLYING BEF RELATIONSHIPS

A further limitation of many BEF studies is the lack of examination of the biological mechanisms underlying the described patterns. One procedure is to partition the diversity effects on ecosystem functioning in two different types of effect: complementarity and selection (Box 2). Within this context, the overall effect is called net diversity effect, and the two components (which can be positive or negative) can serve as an indication of the biological mechanisms the underlie such effects (Loreau and Hector 2001). Positive complementarity often indicates the existence of resource partitioning and/or facilitation between species (Tonin et al. 2018), with negative

complementarity suggesting that negative interspecific interactions (e.g., competition) occur (Creed et al. 2009). Positive (or negative) selection indicates that there is a species with particularly high (or low) contribution to a given function (Fox 2005, Jiang et al. 2008). Exploring the biological mechanisms that cause BEF relationships is essential to understand their relevance for the ecosystem, so I have used this approach in several chapters of this thesis.

Box 2. Partitioning biodiversity effects (based on Loreau and Hector 2001)

The net diversity effect is calculated as the difference between the observed value of a given process in a species mixture and its expected value, the latter being calculated from the performance of each species in monoculture and averaged by its relative abundance in the mixture:

$$\text{Net} = \sum_i (\text{Value}_{\text{Observed}} - \text{Value}_{\text{Expected}}) = \sum_i [(\text{Value}_{\text{Mixture}} - \text{Value}_{\text{Monoculture}}) * \text{Abundance}_{\text{Mixture}}]$$

This net diversity effect results from the addition of positive and/or negative complementarity and/or selection effects. The complementarity effect is calculated as the average deviation from the expected performance of each species in the mixture, multiplied by the mean performance of species in monoculture and the number of species (n) in the mixture. The selection effect is calculated as the covariance between the deviation from the expected performance in the mixture and the performance in monoculture, multiplied by the number of species:

$$\text{Complementarity} = \text{mean}\,(\text{Value}_{\text{Mixture}} - \text{Value}_{\text{Monoculture}}) \times \text{mean Value}_{\text{Monoculture}} \times n$$

$$\text{Selection} = \text{cov}\,[(\text{Value}_{\text{Mixture}} - \text{Value}_{\text{Monoculture}}), \text{Value}_{\text{Monoculture}}] \times n$$

OBJECTIVES AND OUTLINE OF THE THESIS

This dissertation intends to improve our knowledge on how the loss of diversity of terrestrial plant litter affects stream ecosystem functioning, by examining the research questions outlined above using several experimental laboratory and field approaches (Fig. 2). My first general objective is to examine how changes in litter diversity affect multiple stream ecosystem processes (decomposition, FPOM production, nutrient cycling and secondary production), separately and together (*i.e.,* multifuncionality). My second general objective is to study the connection between different stream food web compartments (brown and green) within a BEF context. My third general objective is to assess the adequacy of different biodiversity measures (species richness, functional diversity and phylogenetic distance) to explore BEF (i.e., litter diversity–stream ecosystem functioning) relationships. My fourth general objective is to explore the biological mechanisms underlying BEF relationships in streams by partitioning diversity effects into complementarity and selection effects, for different processes

and in different contexts. To address these general objectives, I have structured the thesis in five chapters, which are listed below.

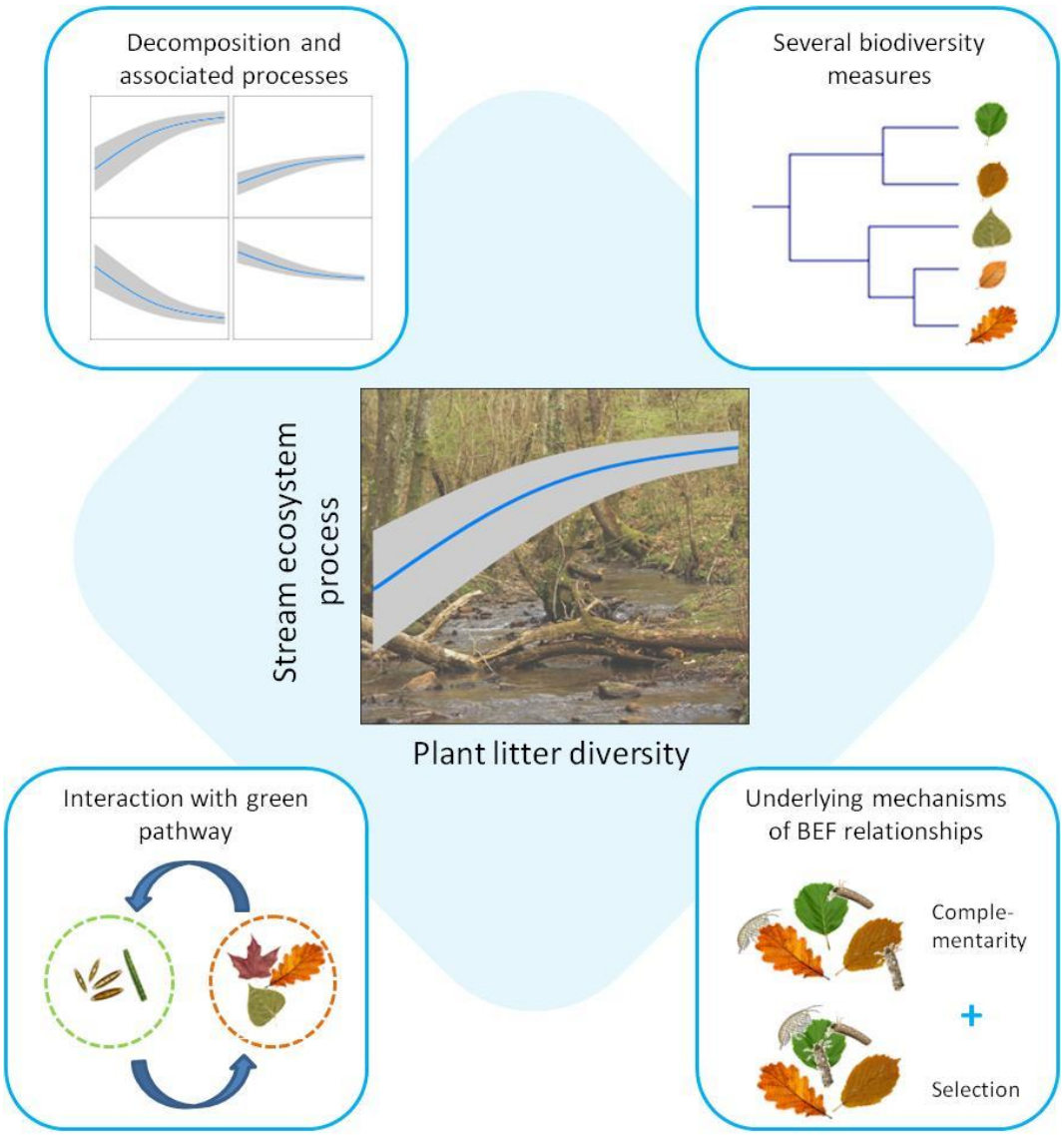


Fig 2. Main questions addressed in this thesis.

CHAPTER 1 examines the effects of litter diversity loss (measured through species richness) on three ecosystem processes (litter decomposition, FPOM production and detritivore biomass production), with emphasis on the identity of the species lost, through a stream microcosm experiment.

CHAPTER 2 tests the effects of litter diversity loss (measured through species richness) on nine key ecosystem processes [litter decomposition, nitrogen (N) and phosphorus

(P) loss from litter, FPOM production, N and P release to the water, detritivore biomass production, and detritivore N and P gain] separately and together (through multifunctionality indexes), through a stream microcosm experiment.

CHAPTER 3 explores the effects of litter and primary producer (periphytic algae) diversity loss (measured through species richness) on ecosystem processes of both brown and green stream food webs (decomposition, fungal sporulation, algal growth and carrying capacity, and net primary production), through a stream mesocosm experiment.

CHAPTER 4 explores the effects of different measures of plant litter diversity (species richness, functional diversity and phylogenetic distance) on litter decomposition and the associated invertebrate assemblages, through a stream field experiment.

CHAPTER 5 examines the effects of different measures of litter diversity (species richness, functional diversity and phylogenetic distance) on seven ecosystem processes (litter decomposition, N and P loss from litter, fungal sporulation, detritivore growth, and detritivore N and P gain), through a stream microcosm experiment.

CHAPTER 1

Leaf traits drive plant diversity effects on litter decomposition and FPOM production in streams

This chapter is published with the following reference:

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ABSTRACT

Biodiversity loss in riparian forests has the potential to alter rates of leaf litter decomposition in stream ecosystems. However, studies have reported the full range of positive, negative and no effects of plant diversity loss on decomposition, and there is currently no explanation for such inconsistent results. Furthermore, it is uncertain whether plant diversity loss affects other ecological processes related to decomposition, such as fine particulate organic matter production or detritivore growth, which precludes a thorough understanding of how detrital stream food webs are impacted by plant diversity loss. We used a microcosm experiment to examine the effects of plant diversity loss on litter decomposition, fine particulate organic matter production, and growth of a dominant leaf-shredding detritivore, using litter mixtures varying in species composition. We hypothesized that plant diversity loss would decrease the rates of all studied processes, but such effects would depend on the leaf traits present in litter mixtures (both their average values and their variability). Our findings partly supported our hypotheses, showing that plant diversity loss had a consistently negative effect on litter decomposition and fine particulate organic matter production (but not on detritivore growth) across litter mixtures, which was mediated by detritivores. Importantly, the magnitude of the diversity effect and the relative importance of different mechanisms underlying this effect (i.e., complementarity vs. selection) varied depending on the species composition of litter mixtures, mainly because of differences in litter nutritional quality and trait variability. Complementarity was prevalent but varied in size, with positive selection effects also occurring in some mixtures. Our results support the notion that loss of riparian plant species is detrimental to key stream ecosystem processes that drive detrital food webs, but that the magnitude of such effects largely depends on the the order of species loss.

KEY WORDS: ecosystem processes, leaf litter, nutrititional quality, detritivores, complementarity, selection

INTRODUCTION

Human activities are altering freshwater ecosystems worlwide, with significant impacts on biological communities and ecosystem processes (Carpenter et al. 2011). The removal of riparian vegetation, which often occurs in association with agriculture, deforestation or afforestation with exotic species, can affect key stream ecosystem processes such as leaf litter decomposition (Graça et al. 2002). This is particularly evident in forested streams, where inputs of dead organic matter from the surrounding vegetation constitute the basis of a detrital food web (Wallace et al. 1997). Leaf litter is used by microbial decomposers and by invertebrate leaf-shredding

detritivores, which incorporate the plant material into animal biomass (Wallace and Webster 1996). Microbial and detritivore activity, together with mechanical fragmentation, also lead to the production of fine particulate organic matter (FPOM), which provides an important resource for other consumers such as collector-gatherers and filter-feeders (Cummins and Klug 1979, Bundschuh and McKie 2016), ultimately supporting invertebrate and vertebrate predators (Wallace et al. 1997). Understanding how these key processes are affected by biodiversity loss is important to predict alterations in stream ecosystem functioning (Dudgeon et al. 2006), given the current high rates of extinction (Barnosky et al. 2011).

The consequences of riparian plant diversity loss for decomposition in streams have been extensively studied. However, this relationship is still unclear because studies have found the full range of positive, negative and no effects of plant diversity loss on decomposition (Gessner et al. 2010). Furthermore, we know little about the potential effects of plant diversity loss on processes associated with decomposition, such as detritivore growth and FPOM production, which are likely to have top-down and bottom-up effects for the detrital food web, respectively (Rosemond et al. 2001, Bundschuh and McKie 2016). Thus, our understanding of how detrital stream food webs are impacted by the loss of riparian plant diversity is limited.

We examined the effects of plant diversity loss on the rates of litter decomposition, FPOM production and detritivore growth in streams, using a microcosm experiment with four riparian plant species (*Alnus glutinosa* (L.) Gaertner, *Corylus avellana* L., *Quercus robur* L. and *Ilex aquifolium* L.; hereafter *Alnus*, *Corylus*, *Quercus* and *Ilex*) and a detritivore species (*Sericostoma pyrenaicum* Pictet), all of which are common in our study area. Firstly, we hypothesized that plant diversity loss would lead to a general decrease in litter decomposition (Cardinale et al. 2011), but the magnitude of such effect would vary depending on the leaf traits present initially in litter mixtures. Thus, we examined the effects of losing plant diversity on decomposition not only from the 4-species mixture composed of *Alnus* (A), *Corylus* (C), *Quercus* (Q) and *Ilex* (I), but also from the four different possible 3-species mixtures (i.e., ACQ, ACI, AQI and CQI), which differed in their average leaf traits and variability.

Secondly, we hypothesized that the effect of plant diversity on decomposition would be due to a combination of positive complementarity (effects resulting from synergistic interactions) and positive selection (effects due to the presence of a species with particularly high decomposition rates) (Handa et al. 2014), but the relative importance of both mechanisms would vary depending on the species present in the mixture. Specifically, we expected that selection effects would become more important in litter mixtures containing *Alnus*, because litter from this species decomposes faster than many other species and is often preferred by detritivores (Graça et al. 2001, Tonin et al. 2017). Finally, we hypothesized that effects of plant

diversity loss on FPOM production and detritivore growth would be similar to those on decomposition, as these threee processes are intimately related, although our experimental design did not allow exploring the relative role of complementarity and selection effects on FPOM production and detritivore growth because the contribution of different plant species to these proceses cannot be distinguished in polycultures.

MATERIALS AND METHODS

We selected four plant species that were present in the riparian forest and belonged to different plant functional types: a nitrogen (N) fixer (*Alnus*), a deciduous fast decomposer (*Corylus*), a deciduous slow decomposer (*Quercus*) and an evergreen species (*Ilex*). Although *Ilex* was less abundant than the other species, its relative contribution to stream leaf litter was considerable in seasons other than autumn (pers. obs.). The species differed in several leaf traits (Table 1): N and phosphorus (P) concentrations [% dry mass (DM)]; specific leaf area [SLA; ratio of disc area (mm^2) to leaf DM (mg)]; leaf toughness [measured as the pressure required to pierce the leave tissue using a steel rod (kPa)]; and ash concentration [(% DM remaining after high-temperature combustion), which is a surrogate of leaf defense (Moles et al. 2013)]. We collected leaves from the Agüera stream catchment in northern Spain (43.20 ^{o}N, 3.26 ^{o}W) in the autumn of 2015. Leaves of deciduous species were collected from the forest floor immediately after natural abscission. For the evergreen *Ilex*, as there is no peak of abscission that allows the collection of leaves at one time, we followed Handa et al.'s procedure (Handa et al. 2014) and collected branches, which were stored in the laboratory until the leaves were dry. Leaf discs of 12-mm diameter were cut with a cork borer avoiding the basal midrib, air dried, and weighed to the nearest 0.01 mg using a precision balance in groups of 12, 16, 24 or 48 discs.

The experiment was carried out in May 2016 in 150 microcosms placed in a temperature-controlled room kept at 10 °C (in order to mimic natural conditions in this region and minimize evaporation), with constant aeration and a light:dark regime of 12:12 h. The microcosms consisted of 500-mL glass jars containing 400 mL of stream water (soluble reactive P: 10.0± 0.9 µg P L^{-1}; dissolved inorganic N: 453.6 ± 30.4 µg N L^{-1}; n = 4) filtered through 100-µm mesh, which allowed the entrance of microorganisms. Each microcosm received 48 leaf discs that belonged to 1 plant species (monocultures) or to 2, 3 or 4 species (i.e., litter mixtures containing 24, 16 or 12 discs per species, respectively), with 10 microcosms per treatment. Leaf discs were incubated for 48 h to allow the leaching of soluble compounds and initial microbial conditioning, and after that the water was replaced with filtered (100 µm) stream water, and detritivores were added to half of the microcosms; the other half remained without detritivores, which allowed separating effects mediated by detritivores and microorganisms.

Microcosms with detritivores received 3 larvae of the caddisfly *Sericostoma pyrenaicum* (hereafter *Sericostoma*), which had been starved for 48 h prior to the experiment. Initial detritivore dry mass (DM) was estimated from a case length (CL) (mm) / DM (mg) relationship, using 45 additional larvae collected simultaneously as experimental individuals and with a similar case length range ($DM = 0.0043 \times CL^{2.8041}$; $r^2 = 0.79$). Case length was measured under a binocular microscope with an accuracy of 0.5 mm, and then individuals were uncased, freeze-dried and weighed. Detritivore initial biomass per microcosm was on average (± SE) 17.82 ± 0.36 mg.

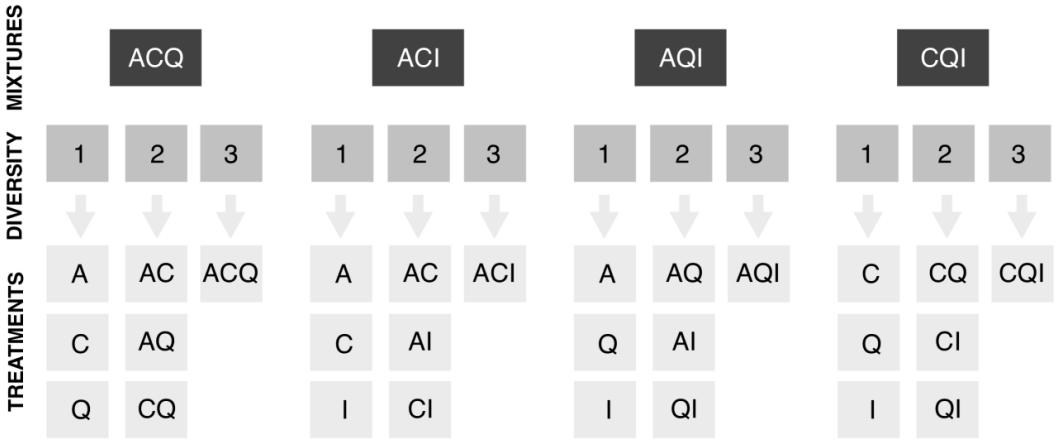

Fig 1. Experimental treatments for each litter mixture and plant diversity level. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robut*; I: *Ilex aquifolium*.

During the experiment, water was again replaced on days 7 and 14, and the experiment finished on day 21. On each occasion, water from microcosms was firstly filtered through 100-µm mesh to retain the invertebrates and leaf discs and fragments, and water was replaced with filtered (100 µm) stream water. The outgoing water was filtered through preweighed glass fibre filters (Whatman GF/F; pore size: 0.7-µm), and filters were oven-dried (70 °C, 72 h), ashed (550 °C, 4 h) and weighed (mg) to estimate FPOM production (water entering the microcosms on each occasion had no FPOM, which was determined as explained for outgoing water). On day 21 all the leaf material was separated by species, oven-dried and weighed to determine final DM, and ashed and re-weighed to determine final AFDM. Detritivores from each microcosm were uncased, freeze-dried and weighed to calculate their final DM.

Twenty extra microcosms (5 per species, each containing 48 pre-weighed leaf discs) were used to estimate initial (post-leaching) AFDM of leaf discs, as well as leaf traits (N, P and ash concentrations and SLA). Leaf discs were removed after 48 h, oven-dried (70 °C, 72 h), weighed (mg), and SLA was estimated by dividing total leaf disc area (mm^2) by DM (mg). Discs from each microcosm were then divided into two subsamples; one was ashed (550 °C, 4h), and re-weighed to estimate initial AFDM and ash concentration (% DM). The other subsample was used to measure N and P

concentrations: N concentration (% DM) was determined using a Perkin Elmer series II CHNS/O elemental analyser, and P concentration (% DM) was measured spectrophotometrically after autoclave-assisted extraction (APHA 1998). Additionally, four microcosms were used to leach 25 leaf discs per species for 48 h and to determine leaf toughness using a penetrometer with a 0.79-mm diameter steel rod (Boyero et al. 2011a); for each species we calculated the average for each set of 5 measurements, resulting in 5 final values.

Data analysis

We quantified litter decomposition through litter mass loss, calculated as the difference between initial and final litter AFDM; initial AFDM was corrected for mass loss due to leaching using the correction factor obtained from extra microcosms (0.753 for *Alnus*, 0.843 for *Corylus*, 0.844 for *Quercus* and 0.767 for *Ilex*). In microcosms with detritivores, we divided litter mass loss by detritivore initial DM in order to remove possible effects due to slight differences in detritivore size across microcosms. FPOM production was calculated as the accumulated FPOM collected in successive water replacements for each microcosm, divided by detritivore initial DM in microcosms with detritivores. Detritivore growth was quantified as the % change in detritivore DM: [(final DM – initial DM) / initial DM] ×100.

We examined the differences in initial leaf traits (N and P concentrations, SLA, toughness and ash concentration) across plant species and across 3-spp litter mixtures with linear models using the gls function (generalized least squares) and restricted maximum likelihood (REML) method in the 'nlme' R package (version 3.2.5; R Development Core Team 2016), with plant species as a fixed factor, followed by Tukey pairwise multiple comparisons using the glht function of the 'multcomp' package (Hothorn et al. 2008).

We explored whether litter decomposition, FPOM production and detritivore growth decreased with diversity loss from 4 to 1 species (categorical variable) in the ACQI litter mixture, with linear models followed by pairwise multiple comparisons (as above). For decomposition and FPOM production we did separate analyses for microcosms with and without detritivores. Subsequently, we split the dataset in 4 subsets consisting of 3-species litter mixtures (ACQ, ACI, AQI, CQI; Fig. 1) by removing one species each time (e.g., *Ilex* was removed in ACQ). We examined effects of diversity loss from 3 to 1 species in the ACQ, ACI, AQI and CQI litter mixtures using linear models followed by pairwise multiple comparisons (e.g., the ACQ 3-spp mixture was compared with the AC, AQ and CQ 2-spp mixtures and the A, C and Q monocultures). Multi-panel plots for each model showed that the homogeneity of variances assumption was violated, requiring the use of a variance structure that takes

these differences into account [VarIdent function of 'nlme' R package (Pinheiro et al. 2018)].

In cases where diversity loss had a significant effect on decomposition, we partitioned the net diversity effect into complementarity and selection effects using the additive partitioning method (Loreau and Hector 2001). The net diversity effect is the difference between observed and expected decomposition (the latter being estimated based on monocultures); the complementarity effect is the average deviation from expected decomposition in a mixture multiplied by mean decomposition in monocultures and by the number of species in the mixture; and the selection effect is 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 (Loreau and Hector 2001). Thus, complementarity results from synergistic or antagonistic interactions, while selection arises when the presence of a particular species with high or low process rates dominates the mixture (Handa et al. 2014). Additive partitioning was not applied to FPOM production or detritivore growth because it was not possible to separate the contribution of different plant species to these processes in litter mixtures.

We compared the net diversity, complementarity and selection effects on litter decomposition, and the net diversity effect on FPOM production (calculated as the difference between observed and expected values, as described for litter decomposition), across 3-spp litter mixtures (ACQ, ACI, AQI and CQI) with linear models followed by pairwise multiple comparisons. Further, we explored whether the net diversity, complementarity and selection effects on litter decomposition and the net diversity effect on FPOM production (all continuous variables) were related to average leaf trait values within a mixture (corrected by the DM of each species in the mixture) and their varaibility using linear models; data were log-transformed to comply with linear model assumptions. Leaf trait variability was estimated as the mean distance between species pairs in a mixture; the distance between species pairs was calculated using cluster analysis on standardized data based on all the measured leaf traits using JMP 9.0.1 software (www.jmp.com).

RESULTS

Plant species differed in all the leaf traits examined: N concentration was higher in *Alnus* than in the other species; P concentration was highest in *Alnus* and lowest in *Quercus*; SLA was highest in *Corylus* and lowest in *Ilex*; toughness was highest in *Ilex* and lowest in *Alnus* and *Corylus*; and ash concentration was higher in *Alnus* and *Corylus* than in the other two species (Table 1). The ACQ mixture had the highest N

Table 1. Mean (± SE) of nitrogen (N) and phosphorus (P) concentrations (% DM), specific leaf area (SLA; $mm^2\ mg^{-1}$), leaf toughness (kPa) and ash concentration (% DM) for each plant species (based on measurements of five replicates) and litter mixture, and trait distance in each litter mixture based on cluster analysis. Different letters indicate significant differences ($p < 0.05$) across single species and 3-spps litter mixtures, on the basis of linear models followed by pairwise multiple comparisons. *N concentration of *Ilex aquifolium* leaves used in the experiment (which were collected from branches) did not significantly differ from a sample of senescent leaves collected from the ground (1.62 ± 0.13 % DM; t-test, t = -0.28, df = 2.54, p = 0.601).

	N	P	SLA	Toughness	Ash	Trait distance
Plant species						
***Alnus glutinosa* (A)**	3.30 ± 0.16^{a}	0.08 ± 4e-3^{a}	13.28 ± 0.34^{b}	1397 ± 75^{c}	5.64 ± 0.31^{a}	
***Corylus avellana* (C)**	1.48 ± 0.03^{b}	0.05 ± 1e-3^{c}	21.90 ± 1.04^{a}	1016 ± 44^{c}	5.66 ± 0.18^{a}	
Quercus robur (Q)	1.23 ± 0.07^{b}	0.04 ± 4e-3^{d}	11.50 ± 0.15^{b}	2793 ± 158^{b}	4.68 ± 0.19^{b}	
***Ilex aquifolium* (I)**	1.58 ± 0.05^{b} *	0.07 ± 2e-3^{b}	6.57 ± 0.26^{c}	7715 ± 100^{a}	3.93 ± 0.05^{b}	
Litter mixtures						
ACQI	1.80 ± 7e-3	0.06 ± 2e-4	13.31 ± 1.29	4751 ± 43	4.64 ± 0.01	3.43 ± 1e-3
ACQ	2.09 ± 0.01^{a}	0.06 ± 2e-4^{c}	15.56 ± 1.26^{a}	2263 ± 9^{c}	5.26 ± 0.01^{a}	3.30 ± 8e-3^{c}
ACI	2.02 ± 0.02^{b}	0.07 ± 2e-4^{a}	13.91 ± 1.71^{b}	5246 ± 84^{b}	4.66 ± 0.02^{b}	3.69± 5e-3^{a}
AQI	1.88 ± 5e-3^{c}	0.06 ± 2e-3^{b}	10.44 ± 0.77^{c}	5059 ± 69^{b}	4.49 ± 0.01^{c}	3.56± 3e-3^{b}
CQI	1.46 ± 2e-3^{d}	0.06 ± 2e-3^{d}	13.32 ± 1.74^{b}	5521 ± 53^{a}	4.42 ± 0.01^{d}	3.27 ± 5e-3^{c}

concentration and SLA and the lowest toughness; P concentration was highest in ACI; ash concentration was highest in ACQ and lowest in CQI; and trait variability was highest in ACI followed by AQI (Table 1).

Table 2. Results of linear models exploring effects of plant diversity loss (from 4 to 1 species in ACQI, or from 3 to 1 species in ACQ, ACI, AQI and CQI) on litter decomposition (mg mg detritivore^{-1}), FPOM production (mg mg detritivore^{-1}) and detritivore growth (percentage) for different litter mixtures in microcosms with detritivores (df = degrees of freedom; F = F-statistic; p = p-value). A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robur*; I: *Ilex aquifolium*.

Litter mixture	df	F	p
Litter decomposition			
ACQI	3	6.95	< 0.001
ACQ	2	4.42	0.021
ACI	2	11.97	< 0.001
AQI	2	5.98	0.006
CQI	2	10.94	< 0.001
FPOM production			
ACQI	3	7.55	< 0.001
ACQ	2	5.48	0.009
ACI	2	9.12	< 0.001
AQI	2	5.37	0.030
CQI	2	5.59	0.008
Detritivore growth			
ACQI	3	1.62	0.192
ACQ	2	2.23	0.124
ACI	2	0.94	0.399
AQI	2	3.02	0.063
CQI	2	1.54	0.229

Plant diversity loss from 4 to 1 species in ACQI reduced decomposition and FPOM production in microcosms with detritivores, but not in microcosms without detritivores, and there was no effect on detritivore growth (Table 2, Table S1; Fig 2). When 3-species litter mixtures were examined separately, the negative effect of diversity loss from 3 to 1 species on decomposition and FPOM production was significant in all mixtures; diversity loss had no effect on detritivore growth, or on any process in microcosms without detritivores (Table 2, Table S1, Fig 3).

Net diversity effects on litter decomposition in the 4-species litter mixture were due to a combination of complementarity and selection effects, which contributed on average 66% and 34%, respectively (Table S2, Fig 4). When 3-species litter mixtures were examined separately, the relative contribution of complementarity and selection effects was more balanced in AQI (56% and 44%, respectively), while complementarity effects had considerably higher contributions than selection effects in the other mixtures, ranging from 67% in ACQ to 99% in CQI (Fig 4). The net diversity effect on decomposition varied across litter mixtures, being significantly higher in ACI than in CQI; the complementarity effect did not vary across litter mixtures; the selecion effect

was lower (and negative) in CQI than in ACI and AQI; and the net diversity effect on FPOM production was higher in ACI than in CQI (Fig 5).

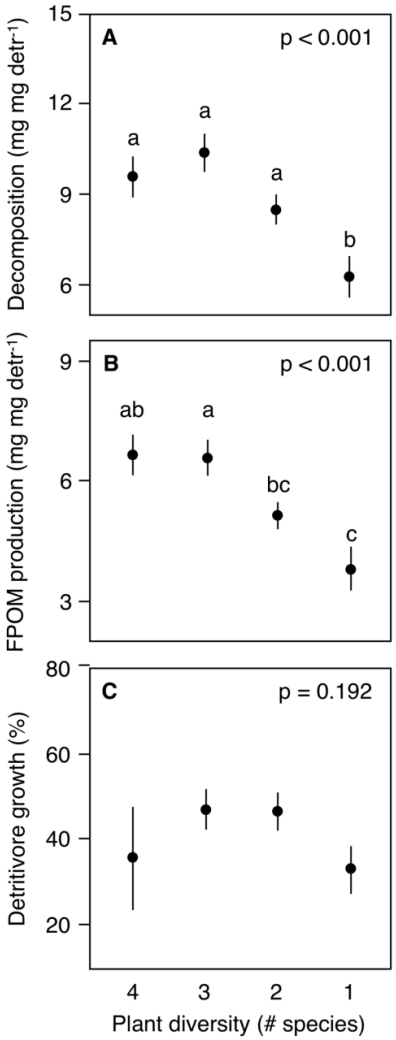

Fig 2. Changes in mean (± SE) (A) litter decomposition (mg leaf mg detritivore^{-1}), (B) FPOM production (mg FPOM mg detritivore^{-1}) and (C) detritivore growth (%) with plant diversity loss from 4 to 1 species, in microcosms with detritivores. Different lower-case letters represent significant differences across treatments ($p < 0.05$).

The net diversity effect on decomposition was positively related to the initial average N and P concentration and leaf trait variability in litter mixtures (Table 3); thus, the highest effect occurred in the ACI mixture (Fig. 5A), which had the highest P concentration and trait variability and the second highest N concentration and SLA (Table 1), and the lowest effect occurred in CQI (Fig. 5A), which had the lowest N and P concentrations and trait variability and the highest toughness (Table 1).The complementarity effect on decomposition was not related to any trait or their variability (Table 3). The selection effect on decomposition was related to N and P

concentration and trait variability (Table 3), with the highest effect occurring in AQI and the lowest effect in CQI (Fig. 5C). The net diversity effect on FPOM production was related to P concentration and trait variability (Table 3), with the highest effect occurring in ACI and the lowest effect in CQI (Fig. 5D).

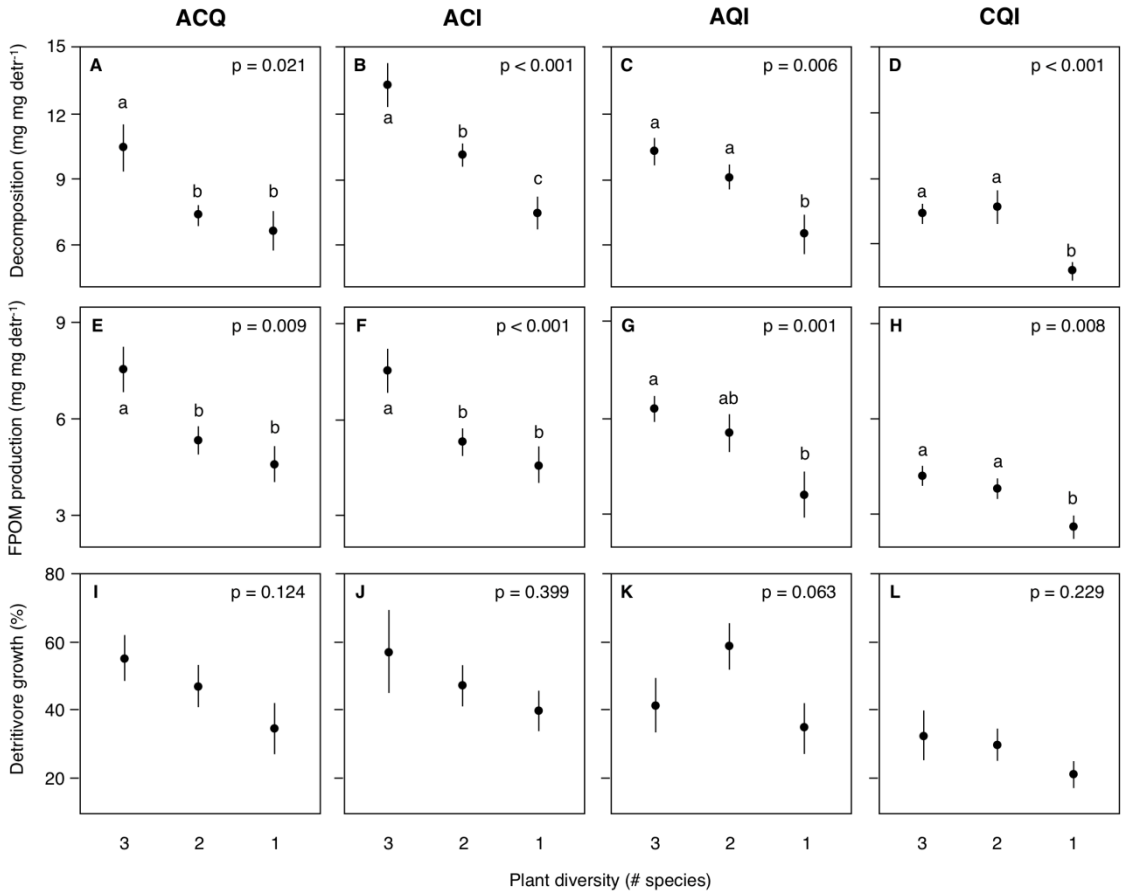

Fig 3. Changes in mean (± SE) (A-D) litter decomposition (mg leaf mg detritivore^{-1}), (E-H) FPOM production (mg FPOM mg detritivore^{-1}) and (I-L) detritivore growth (%) with plant diversity loss from 3 to 1 species in the different 3-species litter mixtures. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robur*; I: *Ilex aquifolium*), in microcosms with detritivores. Different lower-case letters represent significant differences across treatments ($p < 0.05$).

DISCUSSION

The results of our experiment showed that the loss of plant diversity negatively affected litter decomposition in all the litter mixtures examined. This is in agreement with a synthesis of 39 stream studies (Cardinale et al. 2011), but contrasts with several individual studies reporting negative or no effects of increasing plant diversity on decomposition (e.g., Lecerf et al. 2007, Taylor et al. 2007, Ferreira et al. 2012). It is noteworthy that most studies examining litter diversity effects on decomposition have been conducted in streams, in contrast with experiments testing for effects of fungal

or detritivore diversity, which have been mostly performed in microcosms (e.g., Jonsson et al. 2002, Jonsson and Malmqvist 2003, Dang et al. 2005) and have generally found stronger diversity effects on decomposition (Srivastava et al. 2009). Field experiments have greater realism than microcosm experiments, but environmental variation often constrains the capacity for disentangling diversity-decomposition relationships, which are context-dependent (Leroy and Marks 2006, Lecerf and Richardson 2010). The choice of plant species may also have influenced the results of different studies: by selecting species belonging to different functional groups, our study and others (Handa et al. 2014) could have maximized the potential for observing effects of diversity loss on decomposition.

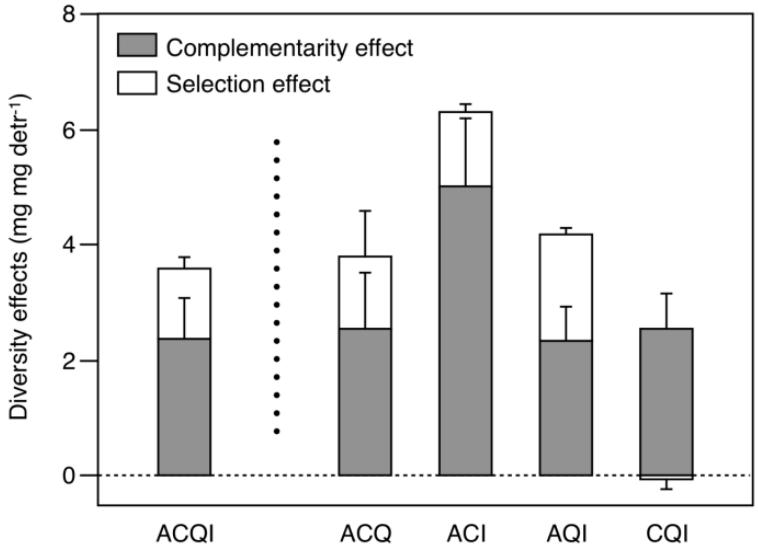

Fig 4. Mean (± SE) complementarity and selection effects on litter decomposition for different litter mixtures. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robut*; I: *Ilex aquifolium*. Whole bars represent the net diversity effect (i.e., the sum of complementarity and selection effects), except for the CQI mixture where the selection effect is negative.

In our experiment, the plant diversity effect on decomposition was mediated by detritivores. Others have reported effects of plant diversity on decomposition mediated by microorganisms, but effects were more than 10 times stronger when detritivores were present (Tonin et al. 2017). Importantly, the effect of plant diversity on decomposition was highest in those mixtures with higher leaf quality and a higher variety of leaf traits, supporting our first hypothesis. Thus, the highest diversity effect on decomposition occurred in the mixture with the highest leaf quality and trait variability, and the lowest effect occurred in the mixture with the lowest leaf quality and trait variability. This result is noteworthy because previous studies had found weak or no evidence of the importance of leaf trait variability in mediating litter diversity effects on decomposition (Schindler and Gessner 2009, Lecerf et al. 2011).

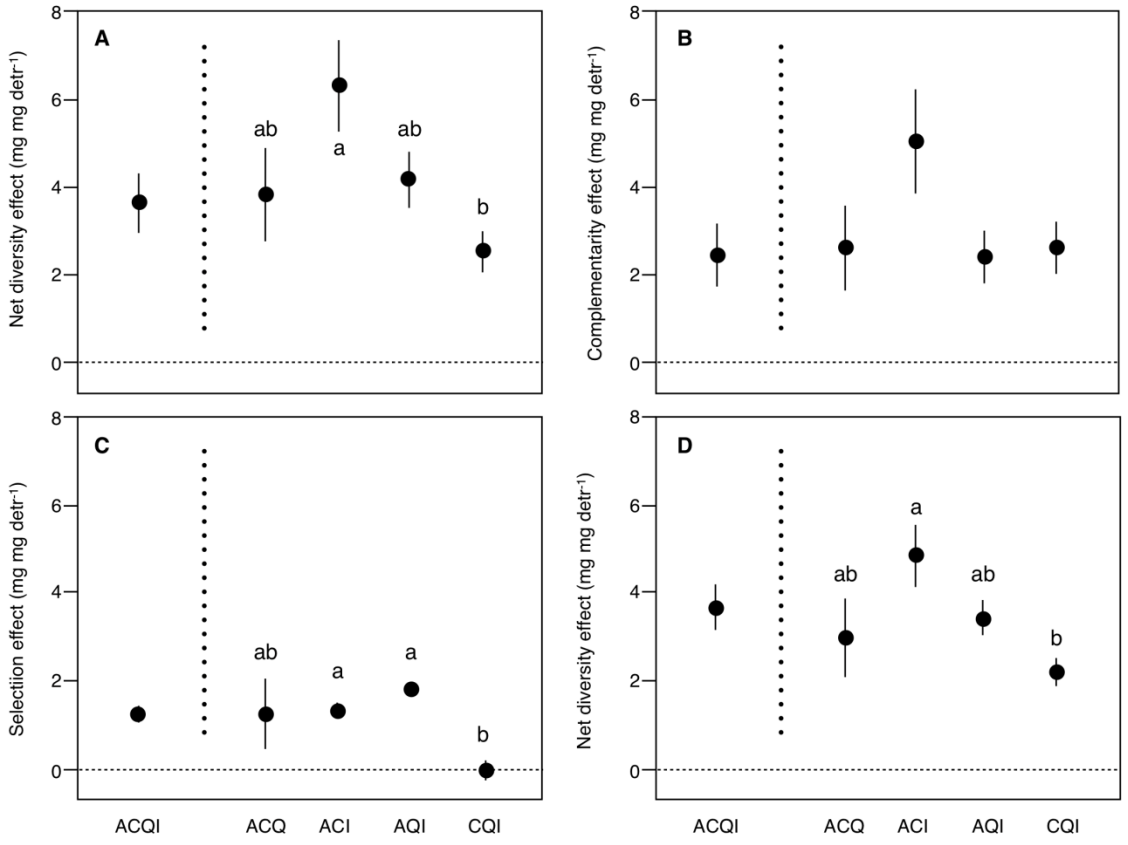

Fig 5. Mean (± SE) (A) net diversity, (B) complementarity and (C) selection effects on litter decomposition and (D) net diversity effects on FPOM production for different litter mixtures in microcosms with detritivores. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robut*; I: *Ilex aquifolium*. Different lower-case letters represent significant differences across treatments ($p < 0.05$).

Plant diversity loss affected decomposition through a combination of complementarity and selection effects, but the relative contribution of both mechanisms varied, as predicted by our second hypothesis. A positive complementarity effect was dominant in most cases (56-99% of net diversity effects), which indicated the prevalence of resource partitioning or facilitation (Loreau and Hector 2001). Thus, leaves from different species may provide complementary resoruces for detritivores (e.g., different nutrients), or the presence of some species may facilitate the use of other species by detritivores. For example, Handa et al.'s (2014) findings suggested the existence of nutrient transfer among species through fungal decomposers: N seemed to be transferred from leaves of N-fixer plants to leaves of non-fixers. Others have found that the presence of refractory leaves can enhance the decomposition of labile leaves (Sanpera-Calbet et al. 2009), possibly due to a reduction in negative density-dependent effects resulting from the aggregation of detritivores in higher-quality leaves (Gessner et al. 2010).

Nevertheless, a positive selection effect was also important in our study (21%-44% of net diversity effects), except for the CQI mixture (the only mixture not

containing *Alnus*), where the selection effect was negligible. Thus, as expected, a selection effect only appeared in the initial presence of *Alnus*, which is consistently preferred by detritivores over other species in experiments (e.g., Friberg and Jacobsen 1994, Cristina and Graça 1995, Graça et al. 2001, Tonin et al. 2017). *Alnus* leaves are very rich in N and P and highly palatable, so the loss of this species from litter mixtures seems to have a large effect on decomposition. Others have also shown that diversity effects on decomposition can depend on whether the remaining species are more or less preferred by detritivores (Bastian et al. 2008), but our study gives further light on the mechanisms underlying such effects. Thus, when *Alnus* leaves are present in a mixture, this species comes to dominate the decomposition process, even if present in equal abundances to other species; this differs from primary productivity, where the selection effect is often associated with numerical abundance of the dominant species (Loreau and Hector 2001).

We found that plant diversity loss negatively affected FPOM production, so our third hypothesis was partly supported. Again, the effect of plant diversity on FPOM production was mediated by detritivores, as there was no effect in microcosms without detritivores. FPOM production is tightly linked to detritivore-mediated litter decomposition, as FPOM is composed of their faeces (Joyce et al. 2007) and small litter fragments produced as a result of their feeding activity (Wallace and Webster 1996). Another experiment also found that FPOM production was reduced when 3-species litter mixtures lost one or two species (Fernandes et al. 2015). Here we further showed that the effect of plant diversity on FPOM production occurred across different litter mixtures but its magnitude varied, being stronger in ACI and weaker in CQI. This variation was again related to P concentration and trait variability, both of which were highest in ACI and lowest in CQI.

Surprisingly, even if plant diversity effects on decomposition and FPOM production were mediated by detritivores, detritivore growth was not affected. This lack of effect could be to the fact that all litter mixtures might have offered sufficient resources for maximum growth (Frainer et al. 2016, Tonin et al. 2017). However, there was a trend for detritivore growth to decrease with plant diversity loss in most mixtures (ACQ, ACI and CQI), even if the trend was not significant; this suggests that detritivore growth responds similarly to decomposition and FPOM production, but we may have not been able to detect a significant effect because of the higher data variability. Such variability could be due to the fact that detritivore initial biomass was not measured directly, but rather estimated from caddisfly case length; or to the relatively short experimental time, although this is unlikely because mean growth was 42% during the experiment. Another plausible explanation for the lack of effect of diversity on growth is that detritivores can modulate their growth efficiency (Sterner and Elser 2002) – that is, change their assimilation and eggestion efficiencies in order to balance their stoichiometric demands (Evans-White and Halvorson 2017). For

example, detritivores may be able to modify the composition of their fecal pelets in order to maintain their body composition regardless of their diet (Balseiro and Albariño 2006), or reduce their carbon use eficiency when resources are nutrient limited (Manzoni et al. 2010).

Table 3. Results of linear models exploring the relationship between diversity effects (i.e., net diversity, complementarity or selection effect on decomposition and net diversity effect on FPOM production) and initial average leaf traits (N and P concentrations, SLA, leaf toughness and ash concentration) or trait variability in 3-species litter mixtures, in microcosms with detritivores (F = F-statistic; p = p-value).

Variable	F	p
Net diversity effect on litter decomposition		
N	5.02	0.039
P	10.22	0.005
SLA	0.06	0.800
Toughness	0.02	0.875
Ash	0.09	0.760
Trait variability	10.10	0.005
Complementarity effect on litter decomposition		
N	0.14	0.707
P	1.85	0.191
SLA	0.12	0.730
Toughness	0.56	0.463
Ash	0.12	0.737
Trait variability	1.80	0.197
Selection effect on litter decomposition		
N	8.02	0.012
P	4.70	0.044
SLA	0.02	0.888
Toughness	0.51	0.483
Ash	0.59	0.450
Trait variability	4.97	0.039
Net diversity effect on FPOM production		
N	3.75	0.069
P	11.39	0.004
SLA	0.42	0.523
Toughness	0.27	0.606
Ash	5 e-3	0.945
Trait variability	11.99	0.003

Our results support the existence of widespread effects of riparian plant species loss on key stream ecosystem processes driving detrital food webs, such as litter decomposition and FPOM production, both of which are slowed as a result of species loss. This is in agreement with a synthesis reporting a negative effect of plant diversity loss on litter decomposition (Cardinale et al. 2011) and with the only available study reporting a negative effect of plant diversity loss on FPOM production to our knowledge (Fernandes et al. 2015). However, our results further suggest that plant diversity effects on these processes can be stronger or weaker depending on which

riparian species are present originally in litter mixtures. Thus, litter mixtures that initially are of higher quality (i.e., with higher N and/or P concentrations) are strongly affected by plant diversity loss, as are litter mixtures with higher varaibility of leaf traits. A key outcome of our experiment is that the risk of species loss to stream ecosystem functioning was largely due to a loss of complementarity, but selection effects were also important in mixtures containing *Alnus*. This highlights the importance of riparian species such as *Alnus glutinosa*, which provide litter of high quality, their loss being likely to have substantial detrimental effects on stream ecosystem functioning, particularly when other riparian species are of lower quality.

ACKNOWLEDGMENTS

We thank Richard G. Pearson for his comments on the manuscript and Alan M. Tonin for statistical advice.

SUPPORTING INFORMATION

Table S1. Effects of plant diversity loss on litter decomposition (mg) and FPOM production (mg) for the 4-species litter mixture (ACQI) and the different 3-species mixtures (ACQ, ACI, AQI and CQI) in microcosms without detritivores, examined with linear models. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robut*; I: *Ilex aquifolium*.

	df	MS	F	p
Litter decomposition				
ACQI	3	26.88	0.06	0.978
ACQ	2	8.51	0.17	0.843
ACI	2	14.82	0.02	0.977
AQI	2	60.22	0.11	0.895
CQI	2	10.89	0.02	0.984
FPOM production				
ACQI	3	1.25	1.27	0.291
ACQ	2	0.67	0.51	0.606
ACI	2	1.29	1.05	0.363
AQI	2	0.50	0.67	0.518
CQI	2	0.64	0.54	0.586

Table S2. Mean (± SE) net diversity, complementarity and selection effects on litter decomposition, and net diversity effect on FPOM production, for the 4-species litter mixture (ACQI) and the different 3-species mixtures (ACQ, ACI, AQI and CQI) in microcosms with detritivores. A: *Alnus glutinosa*; C: *Corylus avellana*; Q: *Quercus robut*; I: *Ilex aquifolium*

	Net diversity effect	Complementarity effect	Selection effect
Litter decomposition			
ACQI	3.64 ± 0.68	2.39 ± 0.73	1.25 ± 0.20
ACQ	3.81 ± 1.06	2.57 ± 0.96	1.25 ± 0.78
ACI	6.31 ± 1.05	5.01 ± 1.19	1.30 ± 0.18
AQI	4.16 ± 0.64	2.34 ± 0.59	1.82 ± 0.13
CQI	2.53 ± 0.48	2.56 ± 0.61	-0.03 ± 0.21
FPOM production			
ACQI	3.62 ± 0.51		
ACQ	2.92 ± 0.89		
ACI	4.81 ± 0.71		
AQI	3.36 ± 0.39		
CQI	2.13 ± 0.30		

CHAPTER 2

Plant diversity loss affects stream ecosystem multifunctionality

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ABSTRACT

Biodiversity loss is occurring globally at unprecedented rates, altering the functioning of the Earth's ecosystems. Multiple processes are often key components of ecosystem functioning, but it is unclear how biodiversity loss affects ecosystem multifunctionality (i.e., the ability of ecosystems to maintain multiple processes simultaneously). This is particularly true for some ecosystem types such as streams, which have been understudied, despite their key role in global biogeochemical cycles and their serious impairment by the widespread loss of riparian vegetation as a result of global change. Using a microcosm experiment we tested whether losing riparian plant diversity affected stream multifunctionality, taking into account 9 key processes related to litter decomposition, animal biomass production and nutrient cycling, and simulating plant species loss from 4 to 1 in the presence or absence of litter-feeding detritivores. Multifunctionality increased with plant diversity in the presence of detritivores and decreased in their absence, evidencing a key role of detritivores in biodiversity-ecosystem functioning (BEF) relationships. Moreover, by exploring effects of plant diversity on each process individually we were able to reveal potential mechanisms underlying BEF relationships – for example, effects of plant diversity on nutrient cycling occurred at least partly via indirect nutrient transfer, and were possibly accompanied by changes in microbial stoichiometry. Such mechanisms were unnoticeable when examining multifunctionality metrics, suggesting that individual processes provide crucial information to understand how stream ecosystem functioning is impaired by biodiversity loss.

KEY WORDS: Detritivore growth, detritus-based streams, ecosystem functioning, FPOM production, litter decomposition, multiple processes, nutrient cycling, plant diversity

INTRODUCTION

Ecosystem functioning is being increasingly affected by human impacts such as climate warming (Woodward et al. 2010), pollution (Woodward et al. 2012) or habitat loss (Haddad et al. 2015), as well as by the associated loss of biological diversity (Cardinale et al. 2012, Hooper et al. 2012, Sánchez-Bayo and Wyckhuys 2019). Biodiversity is decreasing globally at rates as high as those reported for mass extinction periods (Barnosky et al. 2011, Ceballos et al. 2017), so understanding how this decrease might alter key ecological processes is critical in predicting future scenarios of the functioning

of ecosystems and their provision of goods and services such as carbon (C) sequestration, wood production and water purification (Cardinale et al. 2012).

Importantly, ecosystems maintain multiple processes simultaneously which has been termed as multifunctionality (Hector and Bagchi 2007) or, more specifically, ecosystem function multifunctionality (Manning et al. 2018). Ecosystems are often valued for such capacity to maintain multiple processes, yet most studies assessing biodiversity-ecosystem functioning (BEF) relationships have examined single processes in isolation (Hector and Bagchi 2007). However, there is now evidence that biodiversity effects on multifunctionality can be different from effects on single processes (Byrnes et al. 2014). Species loss has been shown to negatively impact multifunctionality in plant (Maestre et al. 2012b), microbial (Delgado-Baquerizo et al. 2016, Mori et al. 2016), animal (Lefcheck et al. 2015) and whole soil communities (Wagg et al. 2014). In contrast, some ecosystem types, such as streams, have been underexplored in this respect, although they experience biodiversity declines far greater than those of the most affected terrestrial ecosystems (Dudgeon et al. 2006) and are key components of the global C cycle (Battin et al. 2009, Raymond et al. 2013).

Many streams are detritus-based systems where the major basal resource is terrestrial plant litter (Vannote et al. 1980, Wallace et al. 1997). Litter entering the stream is decomposed by abiotic (leaching) and biotic agents (microorganisms and litter-feeding detritivores – hereafter detritivores), fueling the food web (Tank et al. 2010). Hence, plant litter decomposition is a pivotal component of stream ecosystem functioning (Gessner and Chauvet 2002, Von Schiller et al. 2017), and most BEF studies in streams have focused on this process. Unfortunately, decomposition has often been considered in isolation, neglecting other related and fundamental processes such as the production of fine particulate organic matter (FPOM), which results from litter processing by detritivores and is subsequently consumed by collectors; the production of new detritivore biomass, which is subsequently consumed by predators; or the release of nutrients from litter to the water, where they are used by microorganisms and primary producers and thus recycled (Cummins 1974).

Given that all the above processes (i.e. decomposition, FPOM production, biomass production and nutrient cycling) occur simultaneously and are key components of stream multifunctionality, we explored how their rates varied in response to changes in plant diversity (1-4 species) within stream microcosms. We predicted that plant diversity loss would reduce stream multifunctionality, as shown for other ecosystem types (Maestre et al. 2012a, Maestre et al. 2012b) (hypothesis 1). We used metrics proposed to measure multifunctionality (Byrnes et al. 2014), but also examined each process separately, as we expected varied responses to plant diversity loss (hypothesis 2). Given that processes represent different aspects of ecosystem functioning, their separate analysis could provide clues to identify mechanisms driving

BEF relationships, a major need of this research field (Cardinale et al. 2009, Gamfeldt and Roger 2017). Lastly, we investigated whether effects of plant diversity on the studied processes were mainly driven by microorganisms or detritivores, by manipulating the presence of detritivores in microcosms. We expected that plant diversity effects on litter decomposition and its conversion to FPOM would occur mostly in the presence of detritivores, which are key drivers of these processes (Graça 2001), while effects on nutrient cycling (in which microorganisms play a crucial role; Gessner et al. 2010) would also be evident (but not necessarily equal) in the absence of detritivores (hypothesis 3).

MATERIAL AND METHODS

Leaf litter and detritivore collection

We collected litter and detritivores in the Agüera stream catchment in northern Spain (43.21 °N, 3.27 °W). The climate is humid oceanic, with annual mean precipitation of 1,650 mm distributed regularly through the year and mean annual temperature of 11.0 °C (monthly averages ranging from 5.8 to 17.0 °C). Vegetation of the catchment consists of mixed native forest dominated by *Quercus robur* L. (Fagaceae), *Alnus glutinosa* (L.) Gaertner (Betulaceae), *Castanea sativa* L. (Fagaceae) and *Corylus avellana* L. (Betulaceae).

In autumn 2015 we collected leaves of four plant species selected based on their different C allocation strategies (deciduous vs. evergreen), nitrogen (N) acquisition strategies (N-fixing vs. non-N-fixing) and litter recalcitrance (rapidly decomposing vs. slowly decomposing). These species thus represented different plant functional types (sensu Handa et al. 2014) and a broad spectrum of litter inputs to streams in the study region: *A. glutinosa* (deciduous, N-fixing, rapidly decomposing species); *C. avellana* (deciduous, non-N-fixing, rapidly decomposing species); *Q. robur* (deciduous, non-N-fixing, slowly decomposing species); and *Ilex aquifolium* Walter (Aquifoliaceae; evergreen, non-N-fixing, slowly decomposing species) – hereafter *Alnus*, *Corylus*, *Quercus* and *Ilex*. Leaves of deciduous species were collected from the forest floor immediately after natural abscission and air dried in the laboratory. For the perennial *Ilex*, branches were collected and stored in the laboratory until they were dry to simulate senescence (Handa et al. 2014). In the laboratory, discs of 12 mm diameter were cut using a cork borer, avoiding the widest part of the central nerve next to the petiole; discs were weighed to the nearest 0.01 mg and used as described below.

Detritivores were larvae of *Sericostoma pyrenaicum* Pictet, 1865 (Trichoptera: Sericostomatidae), one of the most common detritivores in streams of the Agüera

catchment (Martínez et al. 2016). In May 2016, detritivores were manually picked from litter in the riverbed of one stream in this catchment (Perea stream: 43.296 ºN, 3.254 ºW), and placed in a container with aeration and litter from the same riverbed. They were acclimated for 72 h, placing the container within a controlled-temperature room set at 10 ºC, which was at the lower end of the stream temperature range at the season when detritivores were collected (10-15 ºC), but which significantly reduced evaporation. After that, litter was removed and detritivores were starved for the next 48 h, just before the experiment started.

Experimental procedure

In May-June 2016, we conducted an experiment in 150 microcosms (580-mL, 8 cm-diameter glass cups) that were constantly aerated. These were placed within the above-mentioned controlled-temperature room under a light/dark regime of 12:12 h. Each microcosm contained 400 mL of filtered (100µm) stream water (dissolved inorganic nitrogen: 453.61 ± 30.36 µg L^{-1}; soluble reactive phosphorus: 9.98 ± 0.92 µg L^{-1}; measured during the experiment before each water change, n = 4) and 48 litter discs (air-dried and pre-weighed to the nearest 0.01 mg). The discs belonged to 1 species (monocultures) or to 2, 3 or 4 species (polycultures with all possible species combinations, containing 24, 16 or 12 discs per species, respectively). Within each plant combination there were 5 replicates with detritivores (3 larvae per microcosm) and 5 without detritivores.

The experiment was run for 24 days. Initially, only the litter discs were added to the microcosms to allow the leaching of soluble compounds and initial microbial conditioning (Findlay and Arsuffi 1989). Water was replaced on day 3, and detritivores (previously measured under a binocular microscope with an accuracy of 0.5 mm) were added. Water was again replaced on days 11 and 18, using a 100-µm-mesh filter to avoid losing litter fragments. From each water replacement we collected 2 water samples from each microcosm: 1 sample (100 mL) was filtered through a 0.7-µm pre-weighed glass fiber filter (Whatman GF/F), which was incinerated (550 ºC, 4 h) and re-weighed to estimate FPOM production in terms of ash-free dry mass (AFDM); the other sample (45 mL) was frozen and stored at –20 ºC for analyses of total N [by catalytic combustion and NDIR detection using a TOC/TN analyzer (TOC-L_{CSH}/TNM-L, Shimadzu)] and total P [using autoclave-assisted extraction (APHA 1998)].

At the end of the experiment, all the remaining litter material in each microcosm was separated by species, oven dried (60 ºC, 72 h) and weighed to estimate final dry mass (DM). We separated each litter sample into 2 subsamples, which were either incinerated (550 ºC, 4 h) and used to estimate final AFDM, or ground into powder (1-mm screen) and used to determine N [using a Perkin Elmer series II CHNS/O elemental analyzer (Perkin Elmer, Norwalk, CT, USA)] and P contents (as above).

Caddisfly larvae were removed from their cases, freeze-dried, weighed and ground to powder, and their final N and P contents were determined as for litter samples. Forty-five extra larvae were used to estimate the relationship between case length (CL, mm) and body dry mass (DM, mg) ($DM = 0.0043\ CL^{2.8041}$; $r^2 = 0.79$) and to estimate initial body N and P contents.

Twenty extra microcosms were used to estimate the initial (post-leaching) AFDM and N and P contents of litter discs. Each microcosm contained 48 air-dried, pre-weighed litter discs of one of the 4 species (n = 5), which were collected on day 3 and separated into 2 subsamples. One subsample was oven dried (60 °C, 72 h) and weighed to determine initial N and P contents. The other subsample was incinerated (550 °C, 4h) and weighed to estimate AFDM. We used these data to correct the initial DM data of litter discs in experimental microcosms.

Data analyses

We examined 9 ecosystem processes which are key in detritus-based streams. Despite moderate to high correlations among some of these processes (examined with Pearson correlation coefficients; average: 0.63; range: 0.48-0.83; Table S1], we considered that each process would provide unique information that would help identify mechanisms underlying BEF relationships, as stated above. The first 3 processes examined were related to mass and nutrient losses in leaf litter: (1) litter mass loss = initial – final AFDM (mg); (2) litter N loss = initial – final N content (mg); and (3) litter P loss = initial – final P content (mg). Another 3 processes were related to the amounts of organic matter and nutrients in the water, which were used as proxies for organic matter and nutrient cycling, as has been done elsewhere [e.g., Maestre et al. (2012b)]: (4) FPOM production (mg), calculated as the accumulated FPOM collected in the successive water replacements for each microcosm; (5) N release to the water (mg), calculated as the accumulated amount of N in the successive water replacements corrected by initial water N content; and (6) P release to the water (mg), calculated as for N release. Finally, 3 processes were related to detritivore growth and the increase in their nutrient contents: (7) detritivore relative growth = (final – initial DM)/initial DM (mg); (8) detritivore N gain = (final – initial N)/initial N (mg); and (9) detritivore P gain = (final – initial P)/initial P (mg). While processes 7–9 were examined only in microcosms with detritivores for obvious reasons, processes 1–6 were examined both in microcosms with and without detritivores. In microcosms with detritivores, all processes were standarized by mean detritivore DM to remove possible effects of differences in detritivore size among microcosms.

We tested our 1st hypothesis (i.e., that multifunctionality is positively related to plant diversity) by calculating a multifunctionality index based on the averaging approach, as elsewhere (e.g., Maestre et al. 2012b, Delgado-Baquerizo et al. 2016).

The index was the average of all processes standardized by their maximum observed value (Wagg et al. 2014). As the measured values of some variables were negative (e.g., water N release in the absence of detritivores, Table S2), we standardized them by accounting for the range of values in the dataset using the formula: $(x\text{-}z)/(a\text{-}z)$, where x was the observed value and z and a were the lowest and highest observed value in the data set, respectively (e.g., Perkins et al. 2015).

We explored the relationship between plant diversity and multifunctionality using mixed effects models, with plant diversity as fixed factor (1, 2, 3 and 4 species) and species combination as random factor. We initially ran both linear and additive mixed models, as we did not have an *a priori* expectation about the shape of the relationship. Linear models were fitted using the lme function using restricted maximum likelihood (REML) estimation in the 'nlme' package (Pinheiro et al. 2018) in R software (version 3.2.5; R Core Team 2018), and additive models were fitted using the gamm function (generalized additive mixed model) in the 'mgcv' R package (Wood 2011). As linear and additive models showed similar results, but estimated degrees of freedom of additive models equaled 1, indicating linear relationships (Wood 2017), we only show the results of linear mixed effects models. We examined separately microcosms with and without detritivores because data exploration revealed clear differences in the variance of each response variable between both treatments, and thus potential violation of the homogeneity of variances assumption for linear models (Fig. S1), as well as to avoid very complex models with many interactions (cf., Tonin et al. 2017). We tested the significance of the models calculating ordinary nonparametric bootstrapped 95% confidence intervals [BCa method using the boot function on 'boot' R package, based on 999 bootstrap replicates (Davison and Hinkley 1997, Canty and Ripley 2016) for the slope coefficient (Fox 2016)]. We determined whether or not those intervals contained the value of zero – i.e., the null expectation that plant diversity had no effect on multifunctionality.

Additionally, we used the multiple threshold approach for further exploration of the relationship between plant diversity and multifunctionality. This approach describes a relationship between diversity and the total number of processes performing at or above a given threshold (i.e., a given proportion of the maximum observed rate for each process), and had been considered as the most comprehensive description of multifunctionality (Byrnes et al. 2014), although recent research has suggested that it should be interpreted with caution (Gamfeldt and Roger 2017), as discussed below. Methods and results of this method are provided as supporting information.

We explored our 2nd hypothesis (i.e., that different ecosystem processes would respond differently to plant diversity loss despite an overall effect on multifunctionality) using mixed effects models – which again fitted a linear relationship

– and nonparametric bootstrapped 95% confidence intervals for each individual process. Initial data exploration using Cleveland dot- and boxplots revealed 2 outliers (1 for litter mass loss in the presence of detritivores and 1 for FPOM production in the absence of detritivores), which were removed prior to analyses (Ieno and Zuur 2015). Additionally, for processes showing a significant relationship with plant diversity, we examined the ratio between (1) the performance of the 4-spp polyculture and the average of the performances of monocultures, testing for a net diversity effect; and (2) the ratio between the polyculture and the best performing monoculture, testing for a max diversity effect (cf., Allen et al. 2016). We calculated nonparametric bootstrapped 95% confidence intervals (as above) for these ratios to determine whether or not these intervals contained the value of 1 – i.e., the null expectation that the performance of the polyculture was not different from the mean or the best monoculture.

To test our 3rd hypothesis (i.e., that plant diversity effects on ecosystem processes would differ depending on the presence or absence of detritivores), we examined whether bootstrapped 95% confidence intervals for the slope of linear models exploring the relationship between plant diversity and stream processes (each single process and the multifunctionality index, with and without detritivores), overlapped; i.e., the null expectation that diversity effects on ecosystem functioning did not differ.

RESULTS

Plant diversity had an effect on multifunctionality, supporting our 1st hypothesis; the multifunctionality index increased with plant diversity in the presence of detritivores and decreased in the absence of detritivores (Fig 1, Table S3). The multiple threshold approach also showed a positive effect on multifunctionality (i.e., a positive slope of the diversity-multifunctionality relationship) in the presence of detritivores, across thresholds up to 93%, with a maximum effect at the 44% threshold; and a negative effect in the absence of detritivores, for thresholds below 5%, with no significant effect for the rest of thresholds (Fig. S2).

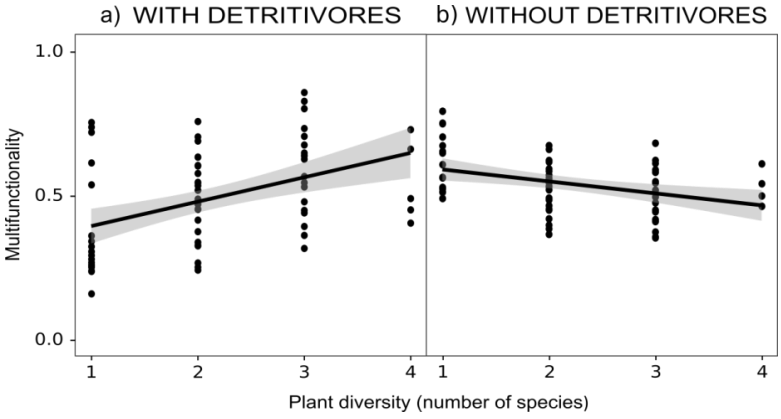

Fig 1. Linear relationship between plant diversity (1–4 species) and the multifunctionality index (averaging approach) in the presence and absence of detritivores. Dots represent replicates, lines represent the fit of significant (p < 0.05) linear models, and grey areas represent 95% confidence intervals from linear models.

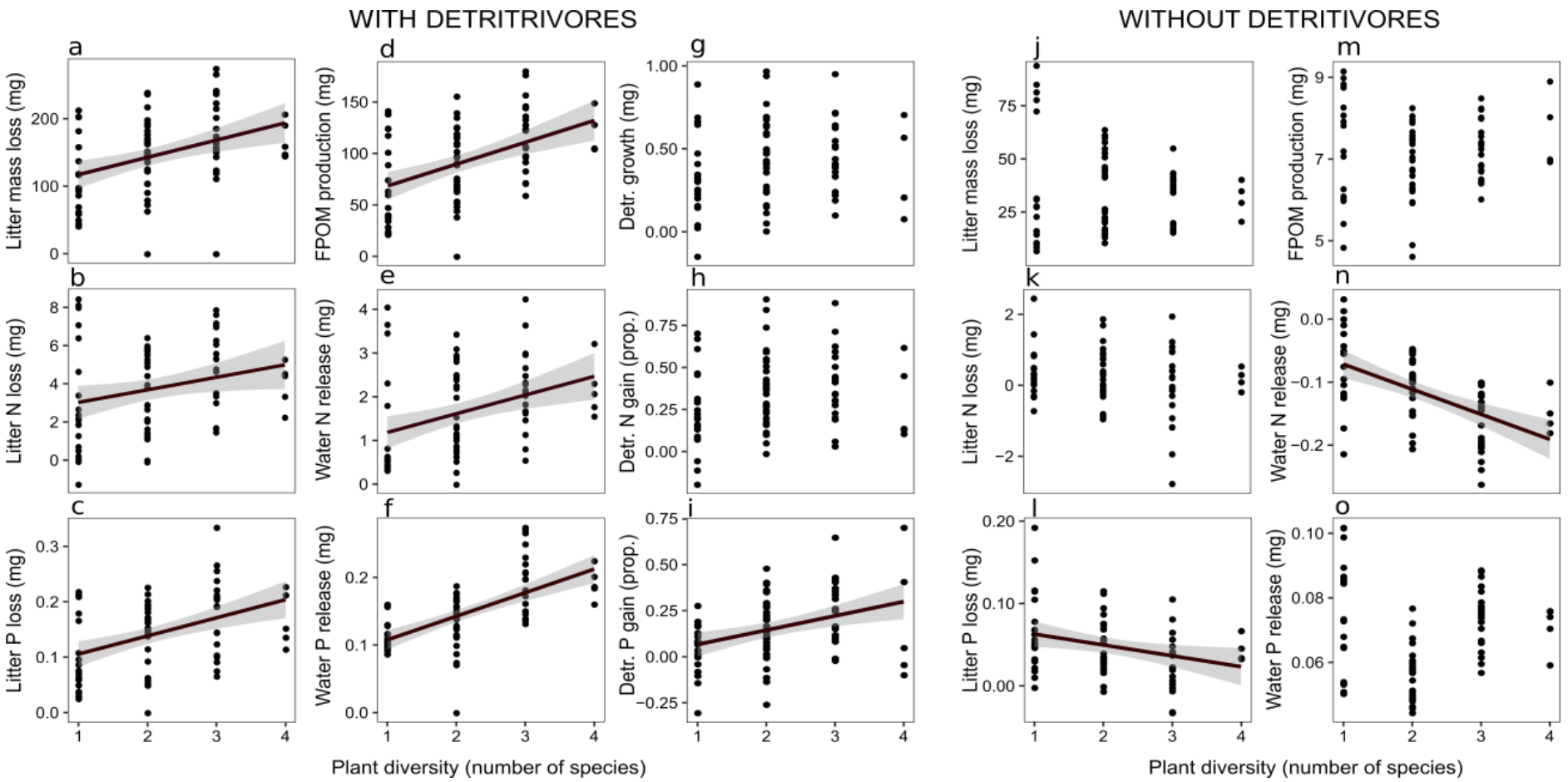

Fig 2. Linear relationships between plant diversity (1–4 species) and stream processes: litter mass loss (panels A, J), litter N loss (B, K), litter P loss (C, L), FPOM production (D, M), N release (E, N) and P release (F, O) to the water, all of them in the presence or absence of detritivores; and growth (G), N gain (H) and P gain (I) in detritivores. Dots represent replicates, lines represent the fit of significant ($p < 0.05$) linear models, and grey areas represent 95% confidence intervals from linear models.

When processes were examined separately, we found different responses to changes in plant diversity, in support of our 2nd hypothesis (Fig. 2). In the presence of detritivores, most processes (7 out of 9: litter mass loss, litter N loss, litter P loss, FPOM production, water N release, water P release and detritivore P gain) increased with plant diversity. We found a net diversity effect (i.e., the performance of the 4-spp polyculture was higher than the average performance of monocultures) for litter mass loss, litter P loss, FPOM production and water N and P release; and a max diversity effect (i.e., the performance of the 4-spp polyculture was higher than that of the best performing monoculture, *Alnus*) for water P release (Fig. 3). In the absence of detritivores, a lower number of processes were affected by plant diversity (2 out of 6: litter P loss and water N release) and the relationship was negative in both cases (Fig. 2; Table S4).

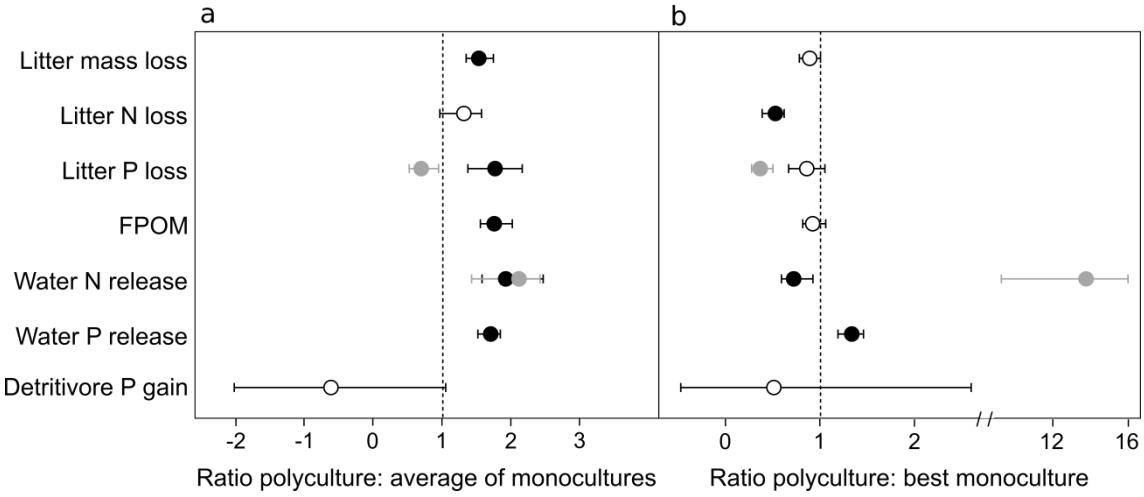

Fig 3. Ratios of the performance of the 4spp-polyculture against the average of monocultures (left-hand panel) or against the best-performing monoculture (right-hand panel) for stream processes significantly related to plant diversity. The dashed line denotes the value of 1 (i.e., the null expectation that performances of polyculture and monocultures do not differ). Circles are means (black and grey represent treatments with and without detritivores, respectively) and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis (i.e., do not contain the value of one) and open circles represent intervals that do not reject the null hypothesis.

Bootstrapped intervals of models with and without detritivores did not overlap for any single process or the multifunctionality index, indicating different effects depending on the presence or absence of detritivores, as predicted by our 3rd hypothesis. Effects were higher with than without detritivores in all cases, except for the effect on water N release, which was greater (and negative – indicating N uptake rather than release) without detritivores (Fig 4).

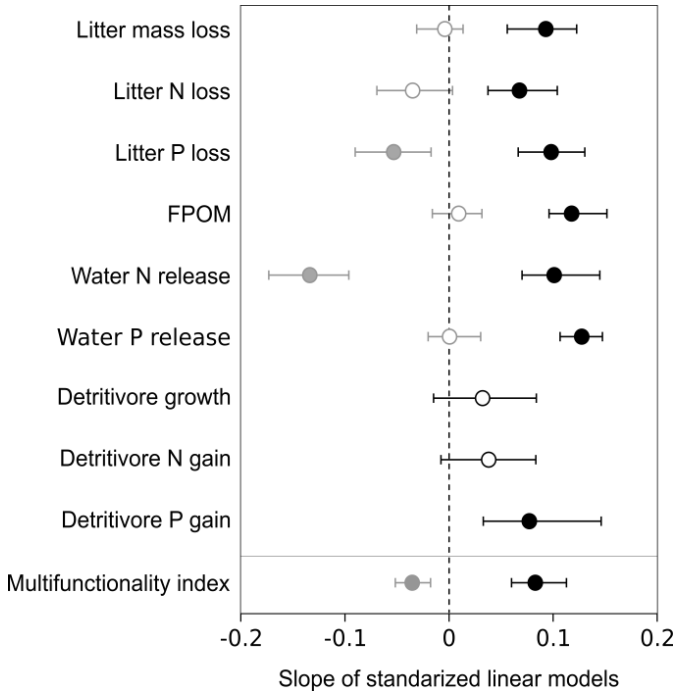

Fig 4. Slope and confidence intervals (BCa) of linear models (i.e., the change in process rates and their variability with plant diversity, respectively) shown in Fig. 1, with data standardized by their maximum value. The dashed line denotes the value of 0 (i.e., the null expectation that there is no effect of plant diversity). Circles are means (black and grey represent treatments with and without detritivores, respectively) and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis (i.e., do not contain the value of zero) and open circles represent intervals that do not reject the null hypothesis.

DISCUSSION

Riparian plant diversity loss alters stream ecosystem multifunctionality

Our study demonstrates that riparian plant diversity loss alters stream ecosystem multifunctionality, as shown elsewhere for plant and microbial diversity in terrestrial ecosystems (Maestre et al. 2012b, Delgado-Baquerizo et al. 2016, Mori et al. 2016). Importantly, multifunctionality decreased as a result of plant diversity loss in the presence of detritivores, while it showed the opposite pattern in their absence, indicating a major role of detritivores as drivers of BEF relationships, which we discuss below.

Both the averaging approach and the multiple threshold approach evidenced similar effects of plant diversity on multifunctionality. However, the usefulness of these approaches is controversial. Several studies have claimed that an average standardized index cannot identify tradeoffs among processes (e.g., it cannot distinguish between scenarios where two processes are performing at their opposite extremes vs. two processes performing at intermediate values; Gamfeldt et al. 2008, Lefcheck et al. 2015). Others, in contrast, have warned that the multiple threshold

approach is highly sensitive to the number of species, the number of processes considered, their original distributions, or the method of standardization, impeding direct comparison across studies and suggesting that this approach should be used with caution (Gamfeldt and Roger 2017).

Moreover, we highlight that, even if these two methods can evidence an effect of diversity on multifunctionality, neither of them provides a clue about the underlying mechanisms, which should be a priority in the biodiversity–multifunctionality field (Cardinale et al. 2009, Gamfeldt and Roger 2017). Our study thus goes one step further, compared to previous studies, by analyzing plant diversity effects on single processes and suggesting mechanisms that could underlie such effects, as discussed below.

Detritivores are key drivers of BEF relationships

A key finding of our experiment was that stream multifunctionality and most single processes had their rates altered as a result of changed plant diversity in the presence of detritivores, while only two processes were affected when detritivores were absent. This suggests that detritivores are key drivers of BEF relationships. We should note, however, that microbial processing generally occurs at a slower pace than detritivore feeding (Hieber and Gessner 2002), suggesting that further effects of plant diversity mediated by microorganisms might occur at later stages of decomposition (Fernandes et al. 2013). However, this can only be tested in longer experiments, which are difficult to conduct in microcosms and may require other approaches. In any case, this observation does not invalidate the fact that detritivores played a key role in the BEF relationships found in this study.

Processes affected by plant diversity in the presence of detritivores all linearly increased their rates with increasing diversity. Two of these processes were litter mass loss and FPOM production, both of which are intimately related. FPOM is produced in streams as a result of detritivore feeding and mechanical fragmentation by flow (Patrick 2013), but the latter is negligible in microcosms, where FPOM results from detritivore feeding only. Thus, it is not surprising that these two processes showed similar patterns, as shown in another experiment (Fernandes et al. 2015). While most stream BEF studies have focused on litter mass loss, effects on FPOM production are likely to be more relevant for stream invertebrate communities, because FPOM is the main food source for collectors (Cummins 1973), which often are the predominant functional group of invertebrates in streams (Wallace et al. 1997, Cheshire et al. 2005).

Both litter mass loss and FPOM production showed a net diversity effect (Allen et al. 2016) in the presence of detritivores, meaning that the litter mixture showed higher process rates than the average of single species. Such an effect is important,

despite the absence of a max diversity effect (i.e., the litter mixture showing higher process rates than the best-performing species; Allen et al. 2016), as best-performing species could change along time in relation to phenology and environmental changes.

Despite the crucial role of detritivores in BEF relationships, detritivore growth was unaffected by plant diversity. This matches the findings of previous experiments, which suggested that all litter combinations could provide sufficient resources for maximum detritivore growth (Tonin et al. 2017). Our data support this hypothesis, as we found high rates of detritivore growth (average of 2.00% day^{-1}) compared to other studies (e.g., 0.75% day^{-1} for S*ericostoma personatum* feeding on *Alnus*; Friberg and Jacobsen 1999). Moreover, litter-feeding detritivores – especially caddisflies – show high feeding plasticity and can grow using alternative food sources such as FPOM (Carvalho and Graça 2007).

Plant diversity loss alters nutrient dynamics via detritivores and microorganisms

We found that N and P dynamics were affected by plant diversity in the presence of detritivores. At higher levels of diversity, more N and P were lost from litter and released to the water, and more P was incorporated by detritivores. Moreover, we observed a max diversity effect for water P release, which means that the polyculture outperformed the best-performing species, *Alnus*. Such an effect, also called transgressive overyielding, is rarely found in experiments, where the most efficient species usually outperforms the polyculture (Cardinale et al. 2011).

We thus found that plant diversity affected the transfer of nutrients between different compartments of the ecosystem (litter, water and detritivores), with the strongest effect found for P release from litter to water. This suggests that nutrient transfer between litter of different species, a mechanism proposed to drive BEF relationships (Gessner et al. 2010), can occur at least partly due to initial leaching from litter of certain species and subsequent capture by fungi colonizing litter of other species, although direct nutrient transfer between species fungal hyphae has been proposed elsewhere (Handa et al. 2014). Besides, we should note that indirect nutrient transfer might be more important in microcosms than in streams, where nutrients may not be so readily available due to the action of flow.

When detritivores were absent and thus only microorganisms contributed to litter processing, N concentration did not increase as expected, but it rather decreased throughout the experiment. Microorganisms most likely used N from the water when it was not abundant enough in litter (Tonin et al. 2017), and N did not return to the water as it does through excretion by detritivores when these are present (Díaz-Villanueva et al. 2012). Moreover, N uptake increased with plant diversity, which could be due to higher demand of nutrients related to higher fungal biomass (Fernandes et

al. 2013). However, this contrasted with the decrease in litter P loss with diversity, which suggested possible changes in microbial stoichiometry, possibly in relation to changes in community composition depending on P availability (Heuck et al. 2015).

Conclusions and insights

Riparian species loss is a widespread phenomenon as a result of human activities (Nilsson and Berggren 2000), the expansion of fungal infections (Bjelke et al. 2016) and climate change (Kominoski et al. 2013), hence the importance of our results. We provide evidence for a significant reduction in stream multifunctionality and the rate of multiple key processes, meaning that stream functioning is likely to be impaired as a result of riparian plant diversity loss. Our findings support previous research on effects of plant diversity loss on several processes (Handa et al. 2014, Fernandes et al. 2015, López-Rojo et al. 2018) and provides evidence of similar effects for multiple processes, shedding light on potential mechanisms underlying BEF relationships in streams (e.g., indirect nutrient transfer or changes in microbial stoichiometry) and on the crucial role of detritivores, and showing the usefulness of a comprehensive analysis of single processes when exploring multifunctionality. As a result of altered ecosystem multifunctionality, the capacity of streams to provide necessary goods and services is likely to be compromised (e.g., impaired capacity for water purification as a result of reduced nutrient cycling), which entails major consequences for human wellbeing (Cardinale et al. 2012).

Although there are limitations inherent to microcosm experiments (e.g., the short duration, number of species that can be manipulated, low environmental complexity, or absence of flow), our results suggest likely patterns that should ideally be further examined within a real-world context. Our inclusion of 4 plant species may seem too limited to draw conclusions about the number of species required to sustain multiple processes; however, our experiment represented a plausible scenario of plant species diversity in natural litter mixtures in temperate streams, often composed of few species (Swan and Palmer 2004, Boyero et al. 2017). Moreover, most studies examining effects of diversity loss on decomposition have based their conclusions on microcosm or field experiments using similar numbers of species of plants (e.g., 4 species in Swan et al. 2009, 3 species in Bruder et al. 2014) or detritivores (e.g., 3 species in Jonsson and Malmqvist 2000, 4 species in Perkins et al. 2015), partly due to the inherent limitation of experiments in terms of numbers of experimental units that can be managed. The fact that field studies have often failed to find effects of plant diversity on decomposition (e.g., Lecerf et al. 2007, Schindler and Gessner 2009), in contrast to microcosm experiments (e.g., Fernandes et al. 2015, Tonin et al. 2017), suggests that diversity effects are masked by the very high environmental and biological complexity of streams. Future experiments should try to better mimic natural conditions (e.g., using stream mesocosms and longer experiments); examine

microbial processes, such as nutrient immobilization and mineralization, and changes in microbial biomass, community composition and stoichiometry; and further explore the role that detritivores play in BEF relationships, including the potential consequences of concomitant changes in the diversity of riparian plants and detritivores.

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SUPPORTING INFORMATION

The multiple threshold approach to explore multifunctionality

Although we based our calculation of multifunctionality on the averaging approach, we used the multiple threshold approach for further, visual exploration of the relationship between plant diversity and multifunctionality. This approach describes a relationship between diversity and the total number of processes performing at or above a given threshold (i.e., a given proportion of the maximum observed rate for each process).

We calculated the maximum value for each process using the mean of the $n + 1$ highest measurements of a process, n being the smallest sample size of a single diversity level (i.e., $n + 1 = 6$ in our case) (Byrnes et al. 2014). The inverse value of the multifunctionality metric estimates the proportional increase in multifunctionality per addition of a species (e.g., a value of 0.25 indicates that 4 additional species are needed to bring an extra process above a given threshold) (Perkins et al. 2015).

We first used selected thresholds (20, 40, 60 and 80%) to examine the relationship between plant diversity and multifunctionality with linear and additive models (as described in the main text). Then we explored how the diversity effect on multifunctionality (i.e., the slope of each linear model) changed with the threshold (from 1% to 99%), which indicated the change in the number of processes per species added. We used the multifunc R package (Byrnes 2015) to compute the number of processes performing at or above the full range of thresholds from 1% to 99% (Byrnes et al. 2014, Delgado-Baquerizo et al. 2016).

From the latter relationship we extracted several metrics that are used to characterize multifunctionality (Byrnes et al. 2014): the minimum and maximum thresholds (T_{min} and T_{max}; i.e., the lower and upper thresholds beyond which diversity has no effect on multifunctionality); the threshold of maximum diversity effect (T_{mde}; i.e, the threshold at which the diversity effect on multifunctionality is strongest); and the realized maximum diversity effect (R_{mde}; i.e., the strength of the relationship at T_{mde}). These metrics were calculated using the 'getIndices' function in the multifunc R package, and plots were drawn using the ggplot2 (Wickham 2016) and gridExtra (Auguie et al. 2016) R packages.

Plant diversity was positively correlated with the number of processes exceeding threshold values of 20%, 40% and 60% when detritivores were present, and the slope of the diversity-multifunctionality relationship across the full range of thresholds revealed that plant diversity had a positive effect on multifunctionality up to a T_{max} of 93%, with no T_{min}; T_{mde} was 44% and R_{mde} was 1.73. In the absence of detritivores, plant diversity was negatively correlated with the number of processes exceeding the threshold value of 20%, and the slope of the diversity–multifunctionality relationship across the full range of thresholds did not show a clear pattern; T_{min} was 5% and there was no T_{max}, T_{mde} or R_{mde} (Fig. S2).

Table S1. Pearson´s pairwise correlations between stream processes.

	Litter N loss	Litter P loss	FPOM	Water N rel.	Water P rel.	Detr. Gr.	Detr. N gain	Detr P gain
Litter mass loss	0.68	0.82	0.74	0.59	0.74	0.57	0.54	0.56
Litter N loss		0.81	0.81	0.83	0.64	0.66	0.51	0.46
Litter P loss			0.78	0.71	0.77	0.63	0.50	0.50
FPOM				0.78	0.77	0.69	0.53	0.56
Water N rel.					0.62	0.59	0.48	0.39
Water P rel.						0.49	0.40	0.50
Detr. Gr.							0.59	0.49
Detr. N gain								0.78

Table S2. Mean and standard error (SE) for each stream process at each plant diversity level, in treatments with and without detritivores. Units are mg except for growth, N and P gain, which are expressed in proportion.

	Plant diversity (number of species)			
	1	**2**	**3**	**4**
Variable	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**
With Detritivores				
Litter mass loss	111.47 ± 12.24	151.14 ± 8.35	183.47 ± 11.11	169.85 ± 12.31
Litter N loss	3.04 ± 0.67	3.61 ± 0.36	4.74 ± 0.49	3.97 ± 0.53
Litter P loss	0.09 ± 0.01	0.15 ± 0.01	0.18 ± 0.02	0.17 ± 0.02
FPOM production	67.74 ± 9.54	91.35 ± 5.91	116.91 ± 7.61	118.41 ± 8.92
Water N release	1.14 ± 0.27	1.67 ± 0.17	2.13 ± 0.19	2.19 ± 0.28
Water P release	0.11 ± 5e-3	0.13 ± 6e-3	0.19 ± 0.01	0.19 ± 0.01
Detritivore growth	0.33 ± 0.05	0.46 ± 0.04	0.47 ± 0.04	0.35 ± 0.12
Detritivore N gain	0.25 ± 0.05	0.36 ± 0.03	0.39 ± 0.04	0.29 ± 0.10
Detritivore P gain	0.05 ± 0.03	0.15 ± 0.03	0.25 ± 0.03	0.20 ± 0.15
Without Detritivores				
Litter mass loss	35.71 ± 7.29	35.58 ± 3.32	34.22 ± 2.56	31.43 ± 4.20
Litter N loss	0.41 ± 0.17	0.28 ± 0.14	-0.07 ± 0.26	0.19 ± 0.15
Litter P loss	0.06 ± 0.01	0.04 ± 6e-3	3e-3 ± 9e-3	0.04 ± 7e-3
FPOM production	7.31 ± 0.32	6.90 ± 0.16	7.26 ± 0.15	7.72 ± 0.47
Water N release	-0.05 ± 0.01	-0.10 ± 8e-3	-0.17 ± 0.01	-0.14 ± 0.01
Water P release	0.07 ± 4e-3	0.05 ± 1e-3	0.07 ± 2e-3	0.07 ± 3e-3

Table S3. Estimated regression parameters, slope and bootstrapped 95% confidence intervals of the slope for linear models testing the effects of plant diversity on the multifunctionality index and several multifunctionality thresholds (multiple threshold approach), with and without detritivores.

Variable	Estimate	Slope	Confidence interval
With detritivores			
Multifunc. index	0.313	0.082	(0.059, 0.112)
80% threshold	0.054	0.543	(0.182, 0.958)
60% threshold	0.563	1.117	(0.863, 1.339)
40% threshold	1.691	1.188	(1.007, 1.410)
20% threshold	4.036	0.595	(0.425, 0.756)
Without detritivores			
Multifunc. index	0.614	-0.035	(-0.051, -0.017)
80% threshold	1.629	-0.259	(-0.448, -0.028)
60% threshold	2.396	-0.077	(-0.244, 0.103)
40% threshold	3.416	-0.083	(-0.324, 0.090)
20% threshold	4.028	-0.031	(-0.231, 0.171)

Table S4. Estimated regression parameters, slope and bootstrapped 95% confidence intervals of the slope for linear models testing the effects of plant diversity on single stream processes in microcosms, with and without detritivores.

Variable	Estimate	Slope	Confidence interval
With detritivores			
Litter mass loss	0.333	0.092	(0.055, 0.122)
Litter N loss	0.373	0.067	(0.037, 0.103)
Litter P loss	0.217	0.098	(0.066, 0.130)
FPOM	0.262	0.117	(0.096, 0.151)
Water N release	0.178	0.101	(0.070, 0.144)
Water P release	0.265	0.127	(0.106, 0.147)
Detr. Growth	0.443	0.032	(-0.015, 0.083)
Detr. N gain	0.404	0.038	(-0.007, 0.083)
Detr. P gain	0.290	0.076	(0.032, 0.146)
Without detritivores			
Litter mass loss	0.374	-0.004	(-0.031, 0.013)
Litter N loss	0.648	-0.034	(-0.069, 0.003)
Litter P loss	0.469	-0.053	(-0.090, -0.017)
FPOM	0.761	0.009	(-0.016, 0.031)
Water N release	0.783	-0.133	(-0.173, -0.096)
Water P release	0.659	4e-3	(-0.020, 0.030)

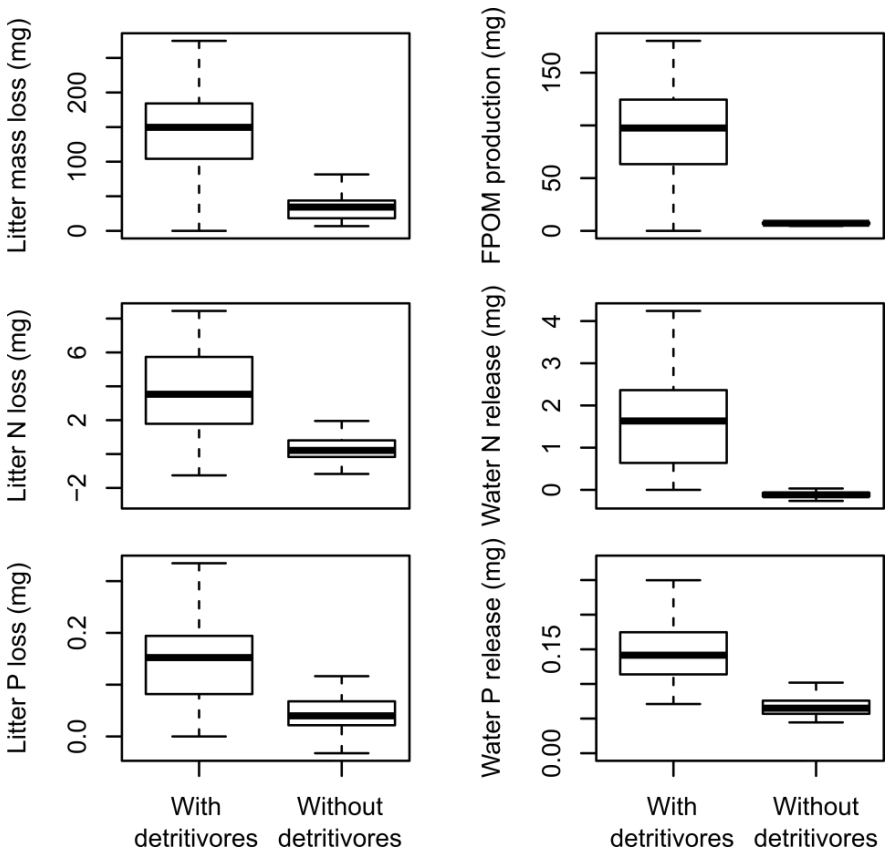

Fig. S1. Boxplots showing variation in process rates measured in the presence and absence of detritivores.

CHAPTER 3

No evidence for biodiversity effects on stream ecosystem functioning across green and brown stream food web pathways

This chapter has been submitted to Freshwater Biology

López-Rojo N., Boyero L., Pérez V., Basaguren A. & Cardinale B.J. No evidence for biodiversity effects on stream ecosystem functioning across green and brown stream food web pathways. Freshwater biology. Under review

ABSTRACT

Biodiversity loss is known to affect the two fundamental and opposite processes controlling carbon and nutrient cycles globally, primary production and decomposition, which are driven by green and brown pathways of food webs, respectively. However, these two pathways of food webs have been rarely studied together, and their potential reciprocal effects on ecosystem processes as a result of biodiversity loss remain unclear. We conducted a 35-day stream mesocosm experiment with two levels of algal diversity (natural and diluted periphyton communities) and three levels of litter diversity (no litter, monocultures of poplar, maple and oak, and the 3-spp mixture) to simulate changes in biodiversity in both the green and brown pathways of an aquatic food web. We then measured multiple ecosystem processes pertaining to carbon cycling. We predicted that algal diversity would enhance decomposition and sporulation of fungal decomposers, while litter diversity would enhance algal growth and net primary production, due to the more diverse algal exudates or litter nutrients being released from more diverse mixtures. In contrast to this hypothesis, we only found biodiversity effects on an ecosystem process within the green pathway: there was a relationship between algal diversity and carrying capacity. Nevertheless, we found that this relationship was influenced by the presence or absence of litter, as it was positive in its presence and negative in its absence. Litter presence and identity also influenced the algal community structure. Our results suggest a lack of complex relationships between biodiversity and ecosystem processes in different parts of the food web, which may facilitate the prediction of the impacts of biodiversity loss on ecosystems.

KEY WORDS: algae, aquatic hyphomycetes, biofilm metabolism, leaf litter decomposition, mesocosms, species richness

INTRODUCTION

Studies focused on the relationship between biodiversity and ecosystem functioning (B-EF) have increased in the last decades due to concerns about the potential ecological consequences of biodiversity loss (Naeem et al. 1994, Cardinale et al. 2012, Tilman et al. 2014). Ecosystems are often valued for their capacity to maintain multiple processes, yet most studies assessing biodiversity–ecosystem-functioning relationships have examined single processes in isolation (Hector and Bagchi 2007). Among the different components of ecosystem functioning, B-EF studies have mostly focused on two opposite and fundamental processes controlling carbon and nutrient cycles globally (Field et al. 1998, Gessner et al. 2010): primary production, and how it is affected by the diversity of primary producers in the 'green pathway' (Cardinale et al.

2011); and leaf litter decomposition, and how it is affected by the diversity of litter or consumers in the 'brown pathway' (Sanpera-Calbet et al. 2009, López-Rojo et al. 2019). These processes have mostly been considered separately, possibly because they are often dominant in different parts of the river network (Vannote et al. 1980). However, both processes generally co-occur, and studies have neglected how biodiversity in both food web compartments and the processes occurring within them may interact to influence one another.

Microbial decomposers (mainly aquatic hyphomycetes) secrete extracellular enzymes that allow the decomposition of litter recalcitrant organic compounds (Marks 2019). This process can be favored by the presence of periphytic algae, which exude fresh, labile carbon (C) that can be used by fungi to invest in growth and enzyme production (Soares et al. 2017), in a phenomenon known as 'priming effect' (Löhnis 1926, Guenet et al. 2010). There is also evidence that algal accumulation in the epilithic biofilm increases the amount of organic substrates available for bacteria, and thus can enhance the use of organic matter by heterotrophic assemblages (Roman and Sabater 1999). Similarly, when litter enters the stream, soluble compounds are released to the water column by leaching, including dissolved organic matter mainly in the form of carbohydrates and nutrients (Bärlocher 2005), which can enhance algal nutrient uptake and growth (Elser et al. 2007) and increase the C:nutrient ratios of algae (Stelzer and Lamberti 2001). Some studies have addressed the complex interactions between primary producers and heterotrophic decomposers (Harte and Kinzig 1993, Daufresne and Loreau 2001, Danger et al. 2007), but there is no evidence of whether such interactions across food web compartments are magnified by biodiversity, which could occur through the same mechanisms that operate within compartments. For example, different algal taxa often produce chemically distinct exudates (Widrig et al. 1996, Hamels et al. 2004) that might be used more efficiently by microbial decomposers (i.e., a complementarity effect); and the presence of more litter types could increase the chance that a nutrient-rich species might be present, with higher nutrient concentrations enhancing algal activity (i.e., a selection effect).

Here, we studied reciprocal effects between biodiversity and ecosystem processes between green and brown food web compartments (i.e., how biodiversity in the green compartment affected processes in the brown compartment, and vice versa). To do so, we completed a stream mesocosm experiment with two levels of both algal and litter diversity, where we examined rates of litter decomposition, fungal sporulation, algal growth, and net primary production. We hypothesized that: (1) algal diversity would enhance litter decomposition and fungal sporulation and (2) the presence of litter and its diversity would promote algal growth, carrying capacity and net primary production. In both cases, (3) we predicted shifts in (fungal and algal) taxon richness and assemblage composition, mediated by the differences in resource use (Frost et al. 2007).

MATERIALS AND METHODS

Collection site and stream mesocosms

We collected the leaf litter, microbial inoculum, and periphytic algae from a section of the Huron River that runs through the University of Michigan's Nichols Arboretum in Ann Arbor, Michigan (42.283 °N, 83.724 °W). At this location, the Huron River is a 5^{th}-order stream that drains 1,888 km^2 in southeast Michigan, with a mean annual discharge of 13.31 m^3 s^{-1}. Conductivity was 716 µS cm^{-1}, dissolved oxygen concentration 10.40 mg L^{-1}, nitrogen (N) concentration 365 µg L^{-1} and phosphorus (P) concentration 19 µg L^{-1} (USGS Station #04174500).

The experiment was conducted at the experimental flume facility of the University of Michigan, which is equipped with recirculating streams called flumes. Each flume was 0.6 m long × 0.1 m wide × 0.1 m deep, held 13.3 L of water, and had a 7-cm diameter propeller controlled by a DC motor attached to a TechPower HY3020E 3-amp voltage regulator that maintains water flow [set at 20 cm s^{-1} (SD = 0.02)]. Temperature was maintained at 13 ± 1 °C by coolers, and lighting was provided by Coralife Aqualight T5 light fixtures (containing two 9-watt, 10K daylight spectrum fluorescent lamps) set to a 14:10 h light:dark cycle. These flumes are too small to be realistic depictions of stream systems, but they are useful as laboratory 'mesocosms' that can be used for experiments with high degree of control and replication.

Experimental design

The experiment included 2 algal treatments (low and high diversity) and 5 litter treatments (no litter, 3 monocultures and the 3-spp mixture), resulting in 10 treatments. Combinations with litter were replicated 5 times (n=5; 40 flumes) while those without litter were replicated 3 times (n=3; 6 flumes), for a total of 46 experimental flumes, which were randomly assigned to treatment. We added 0.5 L of inorganic sediment, 400 mL of gravel-sized rocks (4 ± 1 cm Ø) and 100 mL of pea-sized gravel (1 ± 0.5 cm Ø), to a 220-cm2 working section at the bottom of each flume, thus creating a heterogeneous substrate for colonization and growth of periphytic algae that was consistent across all flumes. We also added 4 round ceramic tiles (1.9-cm Ø) to the working section as a substrate with a standardized area (2.84 cm^2) to simplify algal sampling and quantification. We filled the flumes with dechlorinated Ann Arbor city water (which comes from the Huron River) that was kept in an opaque holding tank recirculating through an ultraviolet sterilizer (Aqua Ultraviolet, USA) for 72 h before its use. Immediately before the experiment, we added $NaNO_3$ and KH_2PO_4 to the water to achieve the ambient concentrations of nutrients in the Huron River (USGS Station #04174500).

Algal communities

On October 27, 2019, we collected ca. 14 L of cobbles that were evenly spaced along transects placed in both riffle and run habitats of the Huron River. We transported the cobbles to the laboratory in a cooler immersed in stream water, and then gently removed their biofilm with a soft toothbrush. We filtered the resulting biofilm slurry (7 L) through a 250-µm sieve to remove macroinvertebrates and large detritus. A 15-mL subsample of the slurry was preserved in 3% formalin for later determination of algal cell density and community composition using a Neubauer-improved hemocytometer in a binocular microscope at 400× magnification. The remaining slurry was used to prepare two solutions representing the two treatments of algal diversity: the initial slurry (i.e., the natural community, composed of 20 morphospecies) was used as the high algal diversity treatment, which was used to inoculate half of the flumes; and a 6-fold dilution of the initial slurry [which eliminated less abundant species, reducing diversity to 6 morphospecies, thus simulating extinction of rare species (Costello et al. 2018)] was used as the low diversity treatment, which was inoculated to the other half of the flumes. All mesocosms were inoculated with the same number of algal cells (ca. 30,000 per flume).

Leaf litter and microbial inoculum

In October 2019 we collected recently abscised litter of three of the most common species in riparian habitat along the Huron river in south eastern Michigan: *Populus deltoides* W. Bartram ex Marshall (hereafter poplar), *Acer saccharum* Marshall (hereafter maple) and *Quercus rubra* L. (hereafter oak). Litter was transported to the laboratory, air dried to constant mass, and leaf discs (1.27-cm Ø) were cut using a cork borer. Sets of 48 discs (belonging to one or three species; 16 discs per species in the latter case) were weighed to the nearest 0.0001 g and enclosed in 2-mm mesh bags. Each flume received five litter bags belonging to one of four treatments (i.e., monocultures of poplar, maple or oak, or the 3-spp polyculture), while others received no litter.

Additionally, we collected litter from natural leaf packs from the bed of the Huron river (DM = 37.80 g; 43.56% *Acer* spp., 27.83% *Quercus* spp., 7.14% *Platanus occidentalis*, 4.77% *Ulmus americana*, 4.20% *Populus deltoides*, 3.15% *Tilia americana,* and 9.35% unrecognized fragments and seeds) and associated natural foam (i.e. natural foam-like aggregates containing high density of aquatic hyphomycete conidia) to obtain a representative inoculum of the microbial decomposer community (Descals 2005). We transported the material (litter and foam) to the laboratory within ziplock bags filled with stream water in a cooler, and incubated it for 5 d in a plastic container with 7 L of water (the same used to fill the flumes) and constant aeration. Water was replaced every 24 h until the start of the experiment (to ensure freshly detached

conidia), at which, we added 125 mL of this microbial inoculum (ca. $3x10^3$ conidia) to each flume. We also collected 8 subsamples of this inoculum and preserved them in 2% formalin to characterize the initial fungal community. For this purpose we added 150 µL of 0.5% Triton X-100 to each preserved sample, which was mixed with a magnetic stirrer in order to ensure a uniform distribution of conidia; 10-15 mL were filtered (25 mm diameter, pore size 5 µm, Millipore SMWP, Millipore Corporation; Descals 2005) and stained with 0.05% trypan blue in 60% lactic acid, and conidia were identified and counted at 200× magnification (Gulis et al. 2005).

Experimental procedure

On day 1 of the experiment (October 30, 2019), litter bags were introduced in the flumes and attached to floating styrofoam squares that suspended them in the water column and maintained separation from the sampling section in order to avoid shading. We then we added algal and microbial inocula, as described in the previous section. For the first two days, flow in the flumes was kept at a low velocity of 10 cm s^{-1} (half of the flow velocity during the rest of the experiment) to facilitate algal settling and colonization of the substrates. We replaced 50% of the water in each flume weekly in order to minimize nutrient depletion, and to maintain water pH. We collected one litter bag per flume on days 3, 6, 10, 16 and 32 to measure decomposition via mass loss, and one ceramic tile per flume on days 7, 11, 17 and 33 to measure algal biomass per unit area. Collection of litter bags and tiles was separated by one day to allow sample processing within the first 24 h. We did not collect tiles on day 4 because it was too early to detect algal biomass accrual.

Upon collection, litter from each bag was dried (60 °C, 72 h), weighed, incinerated (550 °C, 4 h) and reweighed to measure the remaining ash-free dry mass (AFDM). On day 3 (hereafter post-leaching), litter from each species was processed separately and, before incineration, we divided each sample in two subsamples; one was preserved to analyze nitrogen (N; Perkin Elmer series II CHNS/O elemental analyzer) and phosphorus (spectrometer after autoclave-assisted extraction; APHA 1998) contents (% DM) in order to calculate the amount of N and P leached. Specific leaf area (SLA; mm^2 mg^{-1}) was also measured as a proxy of leaf toughness. We used extra non-incubated leaf discs (3-10 replicates per species) to calculate % moisture, initial ash and N and P contents and initial SLA (as above). Decomposition rate for each litter treatment was estimated using the single-phase exponential decay model $M_r = M_i \times e^{-kt}$, where M_r = remaining mass at time t, M_i = initial mass, and k = decomposition rate. This model proved to be a good fit to mass loss, explaining an average 63% of the variation.

On day 32, we separated 6 discs from each bag (2 per species in mixtures) before being dried to measure fungal sporulation rate, following 48 h incubation of the

discs in 25 mL of water placed on a shaker table that was set at 100 rpm at the same temperature as the flumes. We preserved the resulting conidial suspension to characterize the fungal community (as above) and processed the leaf discs and determined the final SLA (as above). Tiles were scratched with a soft tooth-brush in ca. 10 mL of water in order to obtain the biofilm. The resulting solution was filtered (pre-dried and weighed 0.7 µm GF/F glass fiber filters) and filters were oven-dried (60 °C, 72 h) and reweighed to calculate biofilm DM. Biofilm growth rate and carrying capacity were estimated using the logistic growth model $dB/dt = r \times B \times (1 - (B/c))$, *where* B = biomass at time t, r = growth rate, and c = carrying capacity.

On days 34-35, after all bags and tiles had been collected, and only periphyton growing on the natural substrate remained in the flumes, we measured the change in oxygen concentration in 40 flumes; one replicate flume was excluded in treatments with litter monocultures due to equipment limitations. Flumes were deployed with an oxymeter (miniDOT Logger, PME, US), totally filled with water removing air bubbles, and hermetically closed. We then recorded oxygen concentration every minute over the course of a 8-h light, and 12-h dark period. We calculated respiration rate (*R*) as the slope of the decrease in oxygen concentration (mg $0_2\, L^{-1}\, h^{-1}$) during the dark period, and net primary production (*NPP*) as the slope of the increase in oxygen concentration; we then calculated gross primary production (*GPP*) as the difference between *GPP* and *R.*

Data analyses

We examined the effect of algal and litter diversity on decomposition and sporulation rates, algal growth rate, carrying capacity and NPP with linear mixed effects (LME) models [lme function, 'nlme' R package (Pinheiro et al. 2018)]. All models included litter and algal diversity as fixed effects (fitted as an interaction to test whether algal diversity effects varied depending on litter diversity treatments and vice versa), litter species as a random effect, and the variance function structure varIdent, which allowed different variances for each algal diversity level (low or high); the need for this term was identified in initial data exploration and confirmed by comparison of the Akaike Information Criterion (AIC) of models with and without this component. Additionally, we examined whether algal diversity effects differed between litter of different species (litter species as fixed factor) with lineal models as above (lm functions, *nlme* R packages). When necessary, response variables where log-transformed to comply with model assumptions (Ieno and Zuur 2015).

We analyzed the effect of initial algal and litter diversity on the final fungal conidial and algal diversity with LME models (as above), and on taxonomic structure of fungal conidial and algal assemblages with non-metric multidimensional scaling (NMDS) and permutational analysis of variance (PERMANOVA) based on a Bray–Curtis

dissimilarity matrix of Hellinger transformed data, (adonis function, 'vegan' package); we determined the most representative taxa or morpho-species (simper, 'vegan' package).

Table 1. Results of lineal effects models testing for the effect of algal diversity and litter species (*Acer, Populus* or *Quercus*) and their interaction on decomposition and sporulation rate, biofilm growth rate and carrying capacity and net primary production (NPP). Df: degrees of freedom, F: F statistic value, p: p-value.

Variable	Factor	df	F	p
Decomposition rate	Algal div	1	1.578	0.222
	Litter species	2	108.091	<0.001
	Algal div: Litter species	2	2.695	0.089
Sporulation rate	Algal div	1	0.325	0.574
	Litter species	2	24.070	<0.001
	Algal div: Litter species	2	0.770	0.474
Growth rate	Algal div	1	1.136	0.298
	Litter species	2	0.144	0.866
	Algal div: Litter species	2	0.771	0.474
Carrying capacity	Algal div	1	21.682	<0.001
	Litter species	2	3.299	0.056
	Algal div: Litter species	2	5.163	0.015
NPP	Algal div	1	0.829	0.374
	Litter species	2	0.753	0.485
	Algal div: Litter species	2	1.633	0.223
Aquatic hyphomycete diversity	Algal div	1	0.708	0.408
	Litter species	2	3.285	0.055
	Algal div: Litter species	2	0.322	0.727
Algal final diversity	Algal div	1	9.062	0.006
	Litter species	2	0.498	0.614
	Algal div: Litter species	2	4.359	0.027

Lastly, given that litter and algal diversity did not explain variation in the studied variables (excepting algal community structure; see Results), we explored how the variables were affected by the leaf litter characteristics and the amount of leached N and P during the first 72h of incubation. We constructed linear models (lm function in the 'nlme' package with initial and post-leaching N, P, ash (%) and SLA, final SLA and amount of leached N and P as response variables and then selected the model with the lowest Akaike information criterion (AIC) using the step function ('stats' package).

Table 2. Results of lineal-mixed effects models testing for the effect of algal diversity, litter diversity and their interaction on decomposition and sporulation rate, biofilm growth rate and carrying capacity, net primary production (NPP) and aquatic hyphomycete and algal richness and community structure. Df: degrees of freedom, F: F statistic value, p: p-value.

Variable	Factor	df	F	p
Decomposition rate	Algal div	1,32	0.141	0.709
	Litter div	1,2	0.002	0.964
	Algal div: Litter div	1,32	0.127	0.724
Sporulation rate	Algal div	1,33	0.456	0.503
	Litter div	1,2	0.004	0.954
	Algal div: Litter div	1,33	0.337	0.565
Growth rate	Algal div	1,35	3.524	0.068
	Litter div	1,3	2.374	0.221
	Algal div: Litter div	1,35	1.757	0.139
Carrying capacity	Algal div	1,36	8.652	0.005
	Litter div	1,3	0.134	0.738
	Algal div: Litter div	1,36	0.032	0.859
NPP	Algal div	1,30	0.390	0.536
	Litter div	1,3	1.815	0.271
	Algal div: Litter div	1,30	1.416	0.243
Aquatic hyphomycete conidial diversity	Algal div	1,33	0.062	0.804
	Litter div	1,2	0.699	0.491
	Algal div: Litter div	1,33	1.975	0.169
Final algal diversity	Algal div	1,36	10.108	0.003
	Litter div	1,3	3.239	0.169
	Algal div: Litter div	1,36	0.576	0.453
Aquatic hyphomycete community	Algal div		0.859	0.519
	Litter div		1.468	0.188
	Algal div: Litter div		1.461	0188
Algal community	Algal div		2.011	0.018
	Litter div		2.130	0.013
	Algal div: Litter div		0.682	0.777

RESULTS

Litter identity, but not diversity, had an effect on processes of the brown pathway. Thus, decomposition and sporulation rates and fungal conidial diversity varied among litter types (Fig. 1A, 1B, 3A, Table 1). Decomposition rates ranged from -0.001 to

0.017d^{-1}, being highest for maple and lowest for oak litter. Sporulation rates varied from 205.84 to 1.67 conidia mg^{-1} d^{-1}, being highest for poplar and maple and again, lowest for oak (Table S1). Litter samples presented an average of 5 ± 0.32 (mean ± SE) fungal conidial species. All litter types released N and P to the water during the first 72 h (leaching period), with the exception of oak, which immobilized N. Poplar was the species which released more N, while oak leached the highest amount of P (Tables 3 and 4).

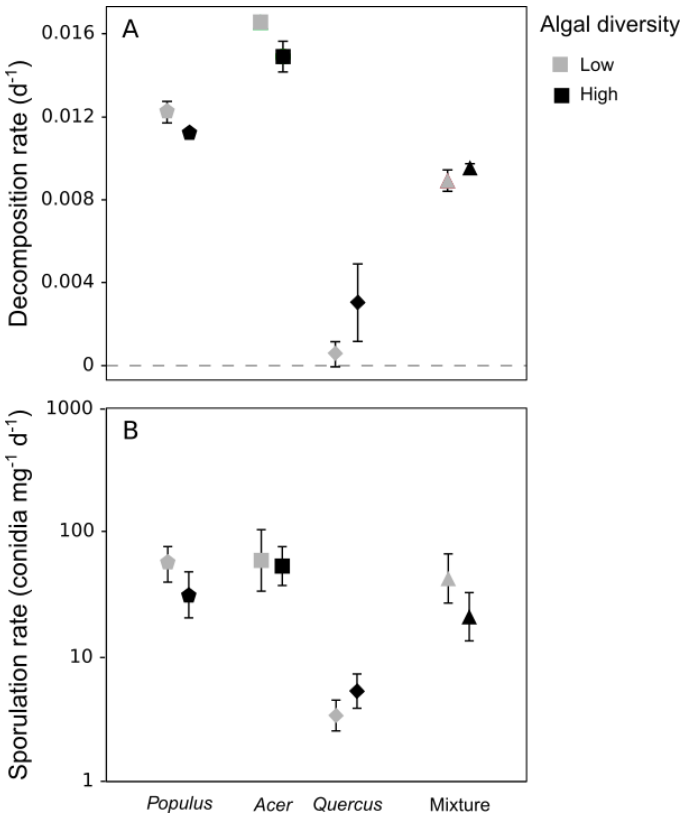

Fig 1. Litter decomposition rate (d^{-1}) and sporulation rate (conidia mg^{-1} d^{-1}, logarithmic scale) for treatment with low or high diversity of algae and with no litter, monocultures (*Acer, Populus* or *Quercus*) or the mixture (mean ± SE).

Initial algal diversity had a positive effect on algal carrying capacity and final diversity, but not on algal growth rate or primary production. The latter was in general low, ranging from -0.006 to 0.048 mgO_2 $L^{-1}d^{-1}$. At the end of the experiment, differences between algal diversity treatments were lower than initially but still significant (t = -3.007, df = 40.696, p = 0.004). Flumes corresponding to initial low and high algal diversity treatments presented 20.70 ± 0.70 and 23.85 ± 0.74 morphospecies respectively (Table S2).

We found no evidence that initial algal diversity enhanced rates of decomposition or fungal sporulation (Fig 1, Table 2), thus rejecting our first hypothesis. Similarly, neither litter presence nor diversity promoted algal growth or carrying capacity or primary production (Fig. 2, Table 2), rejecting our second hypothesis. However, we did find an interaction between the green and brown pathways: in the absence of litter, algal carrying capacity was higher at the low algal diversity treatment, and the pattern was opposite in its presence, regardless of diversity (Fig. 2B).

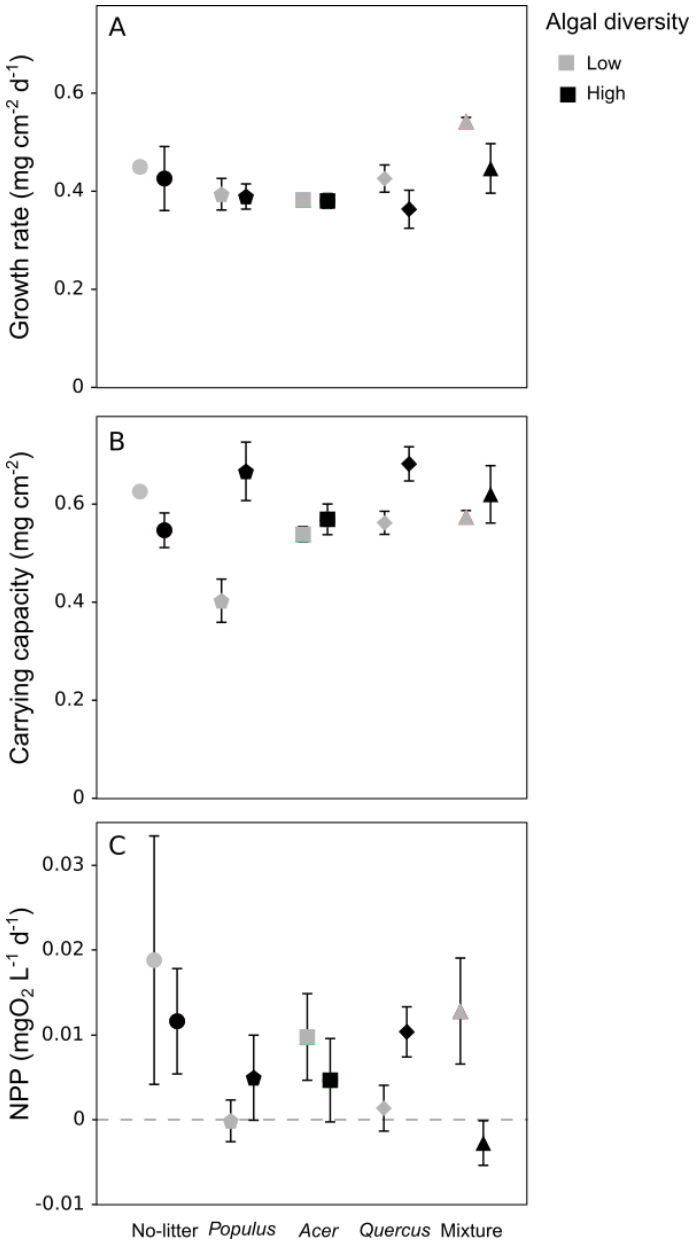

Fig 2. Biofilm growth rate (mg·cm^2·d^{-1}) and carrying capacity (mg·cm^2) and net primary production (NPP, mgO$_2$·L^{-1}·d^{-1}) for treatment with low or high diversity of algae and with no litter, monocultures (*Acer, Populus* or *Quercus*) or the mixture (mean ± SE).

We did not find an effect of algal diversity on fungal conidial richness or assemblage structure. The most abundant species were *Tetracladium marchalianum* and *Lemmoneira pseudofloscula* (Table S1). Litter diversity tended to increase final algal diversity, but the trend was not significant. However, algal assemblage structure varied depending on litter presence and identity; flumes without litter and with oak litter differed from others. Diatoms were abundant in all samples, but simper analysis revealed that flumes without litter were characterized by the abundance of *Limnothrix,* while flumes with litter were characterized by the presence of *Fragilaria, Synedra, Nitzchia* and diatom 4 (Fig 3D, Table S2).

Table 3. Amount of nitrogen (N) or phosphorous (P) leached to the water (mg per flume) in treatments with litter of poplar (*Populus),* maple (*Acer*), oak (*Quercus*) or the 3-spp mixture.

Litter treatment	Leached N (mg/flume)	Leached P (mg/flume)
Populus	4.99 ± 1.16	0.86 ± 0.05
Acer	2.24 ± 0.37	0.22 ± 0.02
Quercus	-1.70 ± 0.66	0.97 ± 0.02
Mixture	2.39 ± 0.24	0.68 ± 0.02

Table 4. Initial and post-leaching nitrogen (N), phosphorous (P) and ash percentage (mean ± SE) and specific leaf area (SLA, $mm^2\ mg^{-1}$) of litter species (*Populus, Acer* and *Quercus).*

Litter species		N	P	Ash	SLA
Populus	Initial	1.32 ± 0.08	0.08 ± 0.01	12.04 ± 0.16	11.06 ± 0.14
	Post-leaching	1.38 ± 0.04	0.07 ± 0.00	9.45 ± 0.31	13.12 ± 0.15
Acer	Initial	1.07 ± 0.02	0.06 ± 0.00	9.22 ± 0.22	21.28 ± 0.54
	Post-leaching	1.13 ± 0.05	0.06 ± 0.00	11.00 ± 0.18	23.61 ± 0.30
Quercus	Initial	0.90 ± 0.05	0.10 ± 0.01	5.23 ± 0.23	17.91 ± 0.33
	Post-leaching	1.00 ± 0.05	0.04 ± 0.00	5.37 ± 0.30	16.39 ± 0.32

The model selection procedure showed that decomposition rate was mainly explained by post-leaching ash content, sporulation rate was explained by final SLA and initial and post-leaching ash, and aquatic hyphomycete conidial diversity was explained by initial P. The model for algal growth rate included post-leaching N but its influence was non-significant (p= 0.173), and the same occurred for net primary production with final SLA and initial P (p= 0.06 and 0.07 respectively) and gross primary production by final SLA. None of measured litter characteristics explained the variation on algal carrying capacity or final algal diversity (Table 5).

DISCUSSION

Primary production and litter decomposition are key processes determining stream ecosystem functioning, and both can be altered by changes in biodiversity. However, studies have mainly focused on how primary producer diversity affects primary production (i.e., the green pathway of the food web) and how leaf litter diversity affects decomposition (i.e., the brown pathway), mostly ignoring the reciprocal interaction between both pathways. Here, we addressed this issue through a stream mesocosm experiment, finding that leaf litter presence and identity (but not diversity) affected the green pathway, but no effects in the other direction. Below we discuss these results, and suggest future research directions that may improve our understanding of biodiversity effects on stream ecosystem functioning.

Algal diversity did not affect the brown pathway

Our experiment did not reveal any effect of algal diversity on microbially-mediated litter decomposition or aquatic hyphomycete sporulation. This lack of effect was unexpected, as we had hypothesized that the algal priming intensity (i.e., the magnitude of the priming effect on heterotrophic activity; Halvorson et al. 2019) would increase with the variety of algal exudates, hence with algal diversity. However, the difference between our two algal diversity treatments at the end of the experiment (20 vs. 24 morphospecies), albeit significant, was not as large as it was initially (6 vs. 20), possibly due to unwanted colonization of additional algal species (mostly of the low-diversity flumes) through the microbial inoculum. This smaller-than-expected difference between our low and high algal diversity treatments may have precluded the occurrence of a diversity effect on the brown pathway, although further studies will be needed to confirm this hypothesis.

There are other possible explanations for the lack of effect of algal diversity on the brown pathway. The overall low net primary production rates measured at the end of the experiment suggests an important contribution of heterotrophic bacteria to the composition of biofilm in the flumes. These bacteria could monopolize the organic exudates excreted by algae (Marshall, 1989) and prevent their use by fungal decomposers. Also, microbial decomposers often show high functional redundancy (Gessner et al. 2010), which may have precluded any complementarity effect derived from the existence of a higher variety of algal exudates.

Leaf litter presence and identity (but not diversity) affected the green pathway

Litter diversity had no effect on algal growth, carrying capacity or primary production, which again contradicted our expectations of enhanced algal activity in the presence of a higher variety of nutrients in the water as a result of leaching. Strikingly, rates of the

above processes did not even differ between treatments with and without litter, despite the fact that most litter types released N and P to the water (except for oak, which immobilized N). Algal biomass production is usually enhanced as a result of N and P enrichment (Artigas et al. 2013), but here nutrient enhancement was due to leaching, which may be expected to be lower in magnitude than enrichment due to other sources. Despite a trend for algal growth to be higher in presence of the litter mixture (especially in treatments with low algal diversity), this trend was not

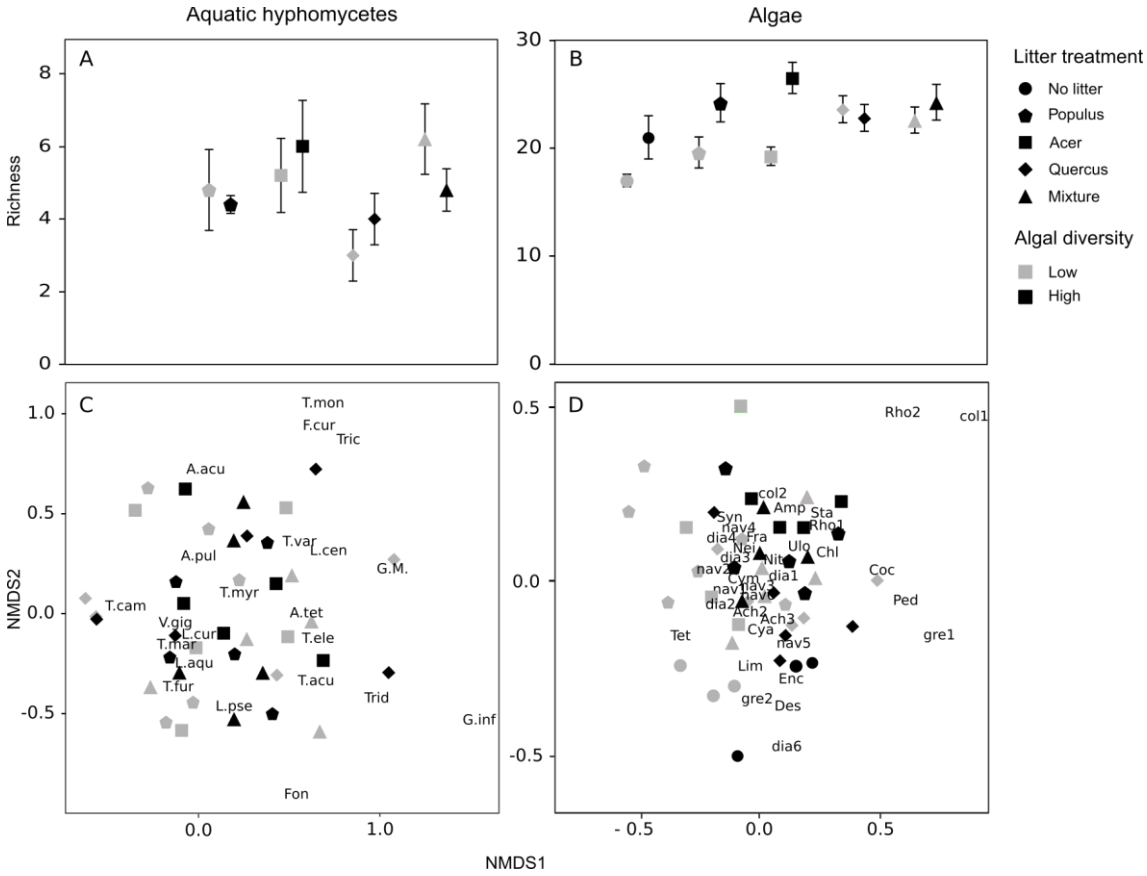

Fig 3. Aquatic hyphomycete and algal richness for treatment with low or high diversity of algae and with no litter, monocultures (*Acer, Populus* or *Quercus*) or the mixture (mean ± SE) and NMDS analysis of aquatic hyphomycete and algal community composition. A.acu: *Alatospora acuminata*; A.pul: *Alatospora pulchella*; A.tet: *Articulospora tetracladia*; F.cur: *Flagelospora curvula*; Fon: *Fontanella* sp.; G.inf: *Geniculospora inflata*; G/M: *Gonopila/Margaristospora*; L.aqu: *Lemmoneira aquatica*; L.cen: *Lemmoneira centrosphaera*; L.pse: *Lemmoneira pseudofloscula;* L.cur: *Lunulospora curvula;* T.ele: *Tetrachaetum elegans;* T.fur: *Tetracladium furcatum;* T.mar: *Tetracladium marchalianum*; Tric: *Tricladium* sp.; T.var: *Tricladium varium*; Trid: *Tridentaria* sp.*;* T.acu: *Triscelophorus monosphorus*; T.cam: *Trypospermun camelopardus;* T.myr: *Trypospermun myrti;* V.gig: *Variocladium giganteum.* Ulo: *Ullothrix;* Fra: *Fragillaria;* Ach1-3: morphospecies of Achanthidiaceae; col1-3: colonial algae; Enc: *Encyonema;* nav1-6: morphospecies of naviculoid diatoms; Nit: Nitzschioid diatom; Cym: *Cymbellonitzschia*; Nei: *Neidium;* dia1-7: morphospecies of diatoms; Syn: *Synedra;* Diat: *Diatoma;* gre1-2: morphospecies of green algae; Cya: cyanobacteria; Lim: *Limnothrix;* Sce: *Scenodesmus;* Chl: *Chlorococcum;* Ped: *Pediastrum;* Des: *Desmodesmus;* Rho1-2: morphospecies of *Rhoicospenia*; Coc: *Cocconeis;* Amp: *Amphora;* Tet: *Tetraedron;* Clo: *Closterium.*

significant and thus did not indicate an effect of litter diversity. Again, the lack of effects could be related to the low rates of net primary production found in the flumes. This situation might be similar to that of many detritus-based streams, where algal production is generally low (Fisher and Likens 1973).

Table 5. Results of linear models examining the variability of decomposition and sporulation rates, algal growth rate and carrying capacity, net and gross primary production (NPP and GPP) and aquatic hyphomycete conidial diversity based on initial and post-leaching N, P and ash (%) and SLA, final SLA and amount of leached N and P (mg per flume). Retained variance: percentage of the variance retained by each factor. (+) and (–) indicate positive and negative relationships, respectively

Response variable	Selected factors	Retained variance	F value	p-value
Decomposition rate	Post-leaching ash (+)	84.64%	305.11	<0.001
	Leached N (+)	2.35%	8.46	0.006
	Final SLA (+)	2.05%	7.39	0.010
	Initial P (-)	1.19%	4.28	0.046
	Initial ash (+)	0.89%	3.22	0.081
Sporulation rate	Final SLA (+)	20.99%	15.32	<0.001
	Initial ash (+)	15.97%	11.66	0.001
	Post-leaching ash (-)	15.11%	11.03	0.002
Growth rate	Post-leaching N (+)	9.52%	1.99	0.173
NPP	Initial P (-)	13.99%	3.82	0.067
	Final SLA (-)	13.37%	3.65	0.072
	Post-leaching SLA (+)	10.48%	2.86	0.108
GPP	Final SLA (+)	19.11%	5.11	0.036
	Post-leaching SLA (-)	13.60%	3.63	0.072
Aquatic hyphomycete diversity	Initial P (-)	12.04%	5.06	0.030

Interestingly, the presence of litter modulated the relationship between algal diversity and algal carrying capacity. This relationship was negative in the absence of litter (i.e., carrying capacity was higher in less diverse algal communities, which were mainly characterized by high numbers of green algae and lower numbers of diatoms) and was positive in the presence of litter (i.e., carrying capacity was higher in more diverse algal assemblages). This different may be related to the fact that more diverse algal assemblages can take greater advantage of nutrients leached from litter (trough

resource partitioning), allowing the coexistence of more species at higher population sizes (Chapin et al. 1997, Cardinale 2011).

Finally, we found differences in algal assemblage composition depending on litter identity. The presence of diatoms like *Fragillaria* on treatments with poplar, maple or the liiter mixture agrees with other studies that related the presence of these species with high contents of water N (Costello et al. 2018). Others have related higher abundances of other diatoms (i.e. *Rhoicosphenia* and *Nitzchia*, which in our case were more abundant in flumes with litter) with nutrient enriched conditions (Artigas et al. 2013). In contrast, the dominance of green algae in flumes without litter may be related to the scarcity of silicon in the water, as this element is necessary for diatom growth and is common in structural compounds of leaf litter. Measurements of inorganic content and micronutrients of leaf litter could provide a better understanding of litter identity effects on algal assemblage composition, as observed for microbial decomposers (Purahong et al. 2016).

Conclusions and insights

Our results revealed notable effects of litter presence and identity on algal assemblages, although we did not see any change in biomass and primary production, which was low overall. Similarly, algal diversity had no effects in the brown pathway, which agrees with other field and laboratory studies finding no or little evidence of algal priming effects on decomposition (Bengtsson et al. 2014, Elosegi et al. 2018). As a whole, these results suggest that biodiversity–ecosystem functioning relationships in streams occur mostly within food web compartments, and are thus less complex than we expected, which may facilitate the prediction of the impacts of biodiversity loss on these ecosystems; however, this result should be taken with caution due to the experimental drawbacks discussed above. An important result of our research is that nutrients leached from leaf litter can modify the composition of algal assemblages, which could drive further changes in the green pathway in the longer term. Given that algae and microbial decomposers compete for the same inorganic nutrients, and that algae are often worse competitors (Currie and Kalff 1984) and seasonality dependent (Francoeur et al. 1999), interactions between both types of organisms may vary with light and nutrient availability and thus change throughout the year, which merits further attention.

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SUPPORTING IMFORMATION

Table S1. Sporulation rate (mean ± SE, conidia mg^{-1} d^{-1}) for each litter and algal diversity treatment. Fungal species code: see Figure 3.

	Algal low diversity				**Algal high diversity**			
	Populus	***Acer***	***Quercus***	**Mixture**	***Populus***	***Acer***	***Quercus***	**Mixture**
A.acu	0.77 ± 0.39	0.41 ± 0.26		0.11 ± 0.11	0.21 ± 0.13	1.14 ± 0.55	0.28 ± 0.17	0.37 ± 0.24
A.pul	0.13 ± 0.13			0.28 ± 0.17	0.10 ± 0.10			
A.tet	0.83 ± 0.83	0.37 ± 0.23	0.43 ± 0.27	0.97 ± 0.49	0.52 ± 0.28	1.29 ± 0.83	0.49 ± 0.21	0.64 ± 0.40
F.cur		0.35 ± 0.35						
Fon					0.11 ± 0.11			
G.inf							0.10 ± 0.10	
G/M		0.26 ± 0.26	0.45 ± 0.45					
L.aqu	0.11 ± 0.11	0.51 ± 0.51			0.11 ± 0.11	0.77 ± 0.56	0.11 ± 0.11	0.82 ± 0.25
L.cen				0.11 ± 0.11				
L.pse	2.22 ± 0.94	2.69 ± 1.94	0.14 ± 0.14	6.89 ± 2.93	0.77 ± 0.29	5.68 ± 2.21	0.22 ± 0.13	1.76 ± 0.99
L.cur		1.81 ± 0.92		3.44 ± 2.83	0.11 ± 0.11			
T.ele		0.20 ± 0.20				0.19 ± 0.19		
T.fur	0.87 ± 0.27	1.54 ± 1.54		0.11 ± 0.11			0.11 ± 0.11	0.11 ± 0.11
T.mar	52.62 ± 18.27	58.26 ± 31.12	1.54 ± 0.62	31.97 ± 19.31	31.89 ± 13.65	41.74 ± 19.44	3.14 ± 1.52	18.22 ± 9.62
Tric				0.68 ± 0.68			0.15 ± 0.15	
T.var	0.13 ± 0.13	0.18 ± 0.18	0.30 ± 0.30	0.24 ± 0.15		0.18 ± 0.18		0.23 ± 0.23
Trid						0.18 ± 0.18		
T.acu	0.11 ± 0.11		0.15 ± 0.15	0.15 ± 0.15	0.11 ± 0.11	0.19 ± 0.19		0.12 ± 0.12
T.mon						0.19 ± 0.19	0.15 ± 0.15	0.11 ± 0.11
T.cam	0.22 ± 0.22	2.11 ± 1.45	0.52 ± 0.35	0.97 ± 0.39	0.51 ± 0.23	4.71 ± 2.18	0.47 ± 0.33	0.78 ± 0.37
T.myr	0.11 ± 0.11	0.87 ± 0.55		0.13 ± 0.13		0.77 ± 0.37	0.36 ± 0.23	
V.gig	0.34 ± 0.34			1.16 ± 1.03		0.19 ± 0.19		
Total	**58.48 ± 18.57**	**69.57 ± 35.81**	**2.82 ± 1.03**	**47.2 ± 20.09**	**34.45 ± 13.84**	**57.23 ± 19.71**	**5.58 ± 1.72**	**23.16 ± 21.21**

Table S2 (1/2). Algal morphospecies abundance (mean ± SE, number of cell mL^{-1}) for each litter and algal diversity treatment. Algal species code: see Figure 3

	Algal low diversity					Algal high diversity				
	none	***Populus***	***Acer***	***Quercus***	**Mixture**	**none**	***Populus***	***Acer***	***Quercus***	**Mixture**
col1							1.00 ± 1.00			
Ulo		6.89 ± 4.80		6.67 ± 3.12	6.40 ± 2.18		24.28 ± 13.11	24.44 ± 21.88	5.44 ± 3.04	0.83 ± 0.83
Fra	1.85 ± 1.85	31.60 ± 14.76	10.14 ± 4.20	9.78 ± 4.96	27.64 ± 8.29	4.07 ± 4.07	32.06 ± 15.05	26.25 ± 9.80	24.83 ± 23.20	63.61 ± 42.25
Ach1		2.00 ± 2.00	6.67 ± 6.67		1.11 ± 1.11		3.33 ± 2.11	1.39 ± 1.39	2.22 ± 2.22	0.56 ± 0.56
col2		12.98 ± 6.04	6.27 ± 3.94	22.00 ± 7.98	11.56 ± 9.73	2.22 ± 2.22	76.28 ± 25.20	42.78 ± 32.03	28.22 ± 18.36	19.72 ± 11.58
Enc	1.11 ± 0.64	1.78 ± 0.90	0.36 ± 0.36	1.78 ± 0.57	2.13 ± 0.73	4.13 ± 2.08	2.72 ± 1.14	1.81 ± 1.19	2.17 ± 1.35	0.28 ± 0.28
Ach3	3.33 ± 0.64	7.07 ± 4.08	3.15 ± 2.00	4.89 ± 2.01	12.53 ± 4.89	5.24 ± 0.99	12.28 ± 5.62	8.06 ± 0.95	11.56 ± 7.31	8.61 ± 3.81
nav1	13.33 ± 0.64	33.11 ± 19.28	15.89 ± 7.22	16.44 ± 4.53	49.20 ± 18.67	14.29 ± 4.33	40.00 ± 20.28	34.44 ± 4.23	12.17 ± 6.49	35.56 ± 7.56
nav2	13.70 ± 1.96	22.89 ± 9.29	33.00 ± 10.07	15.11 ± 3.73	38.80 ± 17.23	14.92 ± 3.27	31.94 ± 13.23	22.64 ± 3.36	18.78 ± 7.30	31.81 ± 8.36
nav3	8.52 ± 4.55	10.76 ± 8.90	11.83 ± 6.35	8.00 ± 2.00	21.07 ± 9.75	13.23 ± 2.33	19.28 ± 4.78	27.78 ± 9.27	9.44 ± 4.09	35.56 ± 21.63
Nit	2.96 ± 1.96	7.42 ± 2.93	7.60 ± 1.90	6.89 ± 0.65	16.13 ± 5.47	4.76 ± 2.90	27.83 ± 12.95	12.08 ± 4.50	9.06 ± 3.19	30.00 ± 10.49
Cym	1.11 ± 0.64			0.44 ± 0.27		0.74 ± 0.74	0.72 ± 0.49	2.08 ± 1.21	0.22 ± 0.22	1.11 ± 0.45
nav4	1.11 ± 0.64	9.91 ± 8.55	2.08 ± 1.58	0.67 ± 0.44	6.84 ± 2.62	1.90 ± 1.90	8.67 ± 2.21	16.81 ± 5.17	2.78 ± 0.90	8.61 ± 4.04
nav5	0.37 ± 0.37		2.44 ± 1.99	1.78 ± 0.90	8.40 ± 4.36	3.76 ± 1.03	4.78 ± 2.94	2.92 ± 1.05	4.83 ± 3.22	3.33 ± 2.36
nav6	0.37 ± 0.37	1.02 ± 0.77	0.83 ± 0.83		0.67 ± 0.44	0.37 ± 0.37	0.67 ± 0.44	3.75 ± 1.78	0.72 ± 0.49	0.56 ± 0.32
dia1	7.41 ± 3.70	16.58 ± 6.57	49.27 ± 22.76	32.67 ± 4.94	53.78 ± 23.26	35.61 ± 15.33	82.17 ± 33.09	48.19 ± 7.57	56.67 ± 31.04	111.39 ± 73.06
dia2	62.96 ± 18.60	81.47 ± 41.17	148.95 ± 56.48	136.22 ± 36.20	192.27 ± 98.18	189.37 ± 38.25	165.11 ± 58.07	144.17 ± 9.83	143.33 ± 82.25	218.19 ± 119.89
Nei	7.78 ± 1.92	38.22 ± 13.55	20.40 ± 4.95	20.22 ± 4.73	61.56 ± 28.46	20.05 ± 13.49	61.28 ± 29.37	42.08 ± 10.00	21.22 ± 7.28	38.33 ± 2.59
dia3	6.67 ± 2.22	27.07 ± 19.22	11.09 ± 3.51	11.56 ± 2.37	48.84 ± 19.00	18.99 ± 1.38	28.33 ± 10.11	42.22 ± 20.38	10.72 ± 3.77	34.44 ± 22.07
dia4	0.37 ± 0.37	7.69 ± 2.07	5.16 ± 2.62	2.22 ± 0.86	9.73 ± 2.88		10.89 ± 4.85	3.47 ± 1.25	6.72 ± 4.03	6.81 ± 1.12
Syn		15.64 ± 3.97	13.06 ± 6.47	8.44 ± 1.30	19.29 ± 5.51	5.03 ± 1.45	35.50 ± 8.19	18.06 ± 5.00	11.44 ± 5.00	28.89 ± 8.91

Table S2 (2/2). Algal morphospecies abundance (mean ± SE, number of cell mL^{-1}) for each litter and algal diversity treatment. Algal species code: see Figure 3

	Algal low diversity					Algal high diversity				
	none	***Populus***	***Acer***	***Quercus***	**Mixture**	**none**	***Populus***	***Acer***	***Quercus***	**Mixture**
gre1				0.67 ± 0.67	0.62 ± 0.41	0.48 ± 0.48	0.22 ± 0.22	0.83 ± 0.83	0.89 ± 0.65	
gre2	1.85 ± 0.98	2.40 ± 0.96	3.49 ± 3.13	6.89 ± 2.09	8.67 ± 7.84	2.22 ± 1.70	4.50 ± 3.31		4.61 ± 1.48	3.33 ± 2.27
Ach2	6.67 ± 2.22	13.11 ± 5.58	16.55 ± 3.53	14.22 ± 4.89	33.51 ± 17.91	14.97 ± 6.22	38.00 ± 20.48	15.14 ± 5.51	18.28 ± 9.40	38.33 ± 24.28
Cya	18.15 ± 7.44	23.56 ± 6.30	22.74 ± 6.15	24.67 ± 5.53	31.33 ± 9.91	48.84 ± 23.89	31.28 ± 9.56	17.78 ± 2.53	30.78 ± 7.30	58.47 ± 29.07
Lim	37.41 ± 15.53	11.78 ± 2.89	9.17 ± 2.10	28.67 ± 7.80	30.53 ± 17.43	66.46 ± 11.27	30.06 ± 6.10	11.53 ± 0.53	16.78 ± 3.25	58.06 ± 40.78
Sce						1.48 ± 1.48				
Chl		1.11 ± 0.70	0.71 ± 0.71	2.67 ± 2.40	4.00 ± 2.47	2.38 ± 2.38		1.11 ± 0.79	1.11 ± 0.70	19.58 ± 17.58
Ped				0.22 ± 0.22			0.33 ± 0.33			
col3				0.22 ± 0.22						
Des	2.96 ± 1.61	1.33 ± 1.08	0.36 ± 0.36	4.00 ± 2.55	9.78 ± 7.62	21.75 ± 6.43	8.11 ± 4.48	2.08 ± 0.92	9.78 ± 2.31	9.86 ± 3.53
Rho	0.74 ± 0.74	0.22 ± 0.22	0.28 ± 0.28	0.22 ± 0.22	2.00 ± 0.82	0.48 ± 0.48	2.28 ± 1.38	1.81 ± 1.19	0.44 ± 0.44	1.39 ± 0.83
Coc				2.22 ± 1.27	0.44 ± 0.44	1.59 ± 0.97	6.94 ± 3.46	2.50 ± 0.83	2.17 ± 1.40	2.22 ± 1.36
Sta		0.22 ± 0.22	0.28 ± 0.28	0.89 ± 0.42	0.80 ± 0.80	0.37 ± 0.37	3.83 ± 2.24	2.78 ± 1.32	0.22 ± 0.22	0.56 ± 0.56
Amp				1.33 ± 0.65		0.48 ± 0.48	0.67 ± 0.44	1.39 ± 0.53	0.44 ± 0.27	0.28 ± 0.28
Tet	1.48 ± 0.37	6.27 ± 2.43	9.82 ± 3.32	4.00 ± 2.24	0.89 ± 0.89	5.13 ± 3.24	2.22 ± 2.22	2.78 ± 0.96	2.22 ± 2.22	5.56 ± 4.84
Rho2				0.22 ± 0.22	0.44 ± 0.44		0.67 ± 0.67	0.83 ± 0.53		0.56 ± 0.32
dia5			0.56 ± 0.56				0.44 ± 0.44			
Clo								0.42 ± 0.42		
dia6					0.44 ± 0.44	0.37 ± 0.37				
dia7		0.22 ± 0.22						0.42 ± 0.42		
Total	**202.22 ± 52.65**	**394.76 ± 146.12**	**329.70 ± 113.96**	**396.89 ± 41.53**	**711.42 ± 298.15**	**505.66 ± 97.94**	**798.67 ± 265.93**	**469.44 ± 133.78**	**470.28 ± 215.87**	**701.11 ± 370.67**

CHAPTER 4

Shifts in key leaf litter traits can predict effects of plant diversity loss on decomposition in streams

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ABSTRACT

Plant biodiversity loss in riparian forests is known to alter key stream ecosystem processes such as leaf litter decomposition. One potential mechanism mediating this biodiversity-decomposition relationship is the increased variability of plant functional traits at higher levels of biodiversity, providing more varied resources for decomposers and thus improving their function. We explored this in a field experiment exposing litter from different assemblages with low or high trait variability (measured through phylogenetic distance, PD) to microbial decomposers and invertebrate detritivores within litterbags in a low-order stream. Litter assemblages generally lost less mass but more phosphorus (P) than expected from monocultures, and nitrogen (N) tended to increase in the absence of detritivores and decrease in their presence, with little effect of PD. In contrast, there were strong influences of mean values and variability of specific traits (mostly N, P and condensed tannins) on decomposition and on net diversity effects. The negative diversity effect on litter mass loss was mainly driven by negative complementarity (i.e., physical or chemical interference among species or traits), although there was positive selection (i.e., particular species or traits with large effects on decomposition) in high-PD assemblages with detritivores. High-PD assemblages tended to have more invertebrates and attracted more typical litter-consuming detritivores. Our study suggests that decomposition of litter assemblages is mainly driven by concentration and variability of several key litter traits, rather than overall trait heterogeneity (measured through PD). However, differences in invertebrates colonizing high-PD and low-PD assemblages pointed to potential long-term effects of PD on decomposition.

KEY WORDS: Complementarity effect, detritivores, ecosystem functioning, net diversity effect, riparian plants, selection effect.

INTRODUCTION

The first Earth Summit celebrated in 1992 led to the conclusion that species, genes and biological traits were being lost at alarming rates as a result of human actions (Cardinale et al. 2012). This gave rise to a new body of research, referred to as 'biodiversity and ecosystem functioning' or BEF (Schulze and Mooney 1993), with hundreds of studies exploring how biodiversity loss affected key ecosystem processes. The development of the BEF field has provided strong evidence that biodiversity loss reduces the efficiency of plant communities in capturing resources and producing biomass, but there is weaker evidence that plant litter diversity loss slows down decomposition and the recycling of elements (Cardinale et al. 2011, Handa et al. 2014). The latter effects are globally relevant because detritus is the major carbon pathway in

most ecosystems (Cebrian 1999), and of prime importance for headwater stream ecosystems, which are fuelled by detrital inputs from riparian forests due to the low availability of light and nutrients (Wallace et al. 1997).

Riparian forests are often altered due to forestry practices that replace native mixed vegetation by exotic monocultures (Pozo et al. 1998), fungal infections that cause dieback of riparian species (Bjelke et al. 2016), ornamental planting (Loewenstein and Loewenstein 2005), or plant invasions (Ferreira et al. 2016). Such alterations can change the diversity and composition of leaf litter (hereafter litter) that enters the stream and is decomposed through the activity of microorganisms (mainly fungi) and invertebrate detritivores specialized in shredding and consuming litter (Gessner et al. 2010). Understanding how changes in the diversity and composition of riparian vegetation affect decomposition rates is important, given the critical role of litter decomposition in stream ecosystem functioning (Gessner et al. 1999) and its significant contribution to global biogeochemical cycles (Battin et al. 2009).

Changes in the number of species present in litter have been shown to alter decomposition rates. However, the outcomes of different studies have differed: some experimental studies have shown a positive relationship between species richness and decomposition rate while others have shown a negative relationship or no effect (Gessner et al. 2010), and meta-analyses have found either no effect (Srivastava et al. 2009) or a positive but weak effect (Cardinale et al. 2011). This discrepancy across studies indicates that species richness might not be the most relevant feature of biodiversity influencing ecosystem functioning. Instead, the diversity of functional traits (i.e., characteristics of an organism's phenotype that affect its fitness and its effects on ecosystem processes; Truchy et al. 2015) is likely to have important functional repercussions (Schindler and Gessner 2009). However, measuring trait diversity is not as straightforward and consistent as measuring species richness, with an array of measures being used (Cadotte et al. 2009, Petchey et al. 2009).

We explored the relationship between litter trait diversity and decomposition [in terms of mass, nitrogen (N) and phosphorus (P) loss] using litter assemblages differing in their phylogenetic distance (PD). Species that are closer in the phylogeny often share a higher number of traits than more distant species (Cadotte et al. 2009, LeRoy et al. 2019), even if not all traits are phylogenetically conserved (Moles et al. 2013), and despite the existence of convergent evolution (Ackerly and Reich 1999) and phenotypic plasticity (Valladares et al. 2007). Thus, PD has been proposed as a useful proxy for trait diversity, as it generally contains more information than a few selected traits (Swenson 2013). We combined litter of 9 species in assemblages of 3 species that belonged to the same family (low PD) or to different families (high PD) in a field experiment, using fine-mesh and coarse-mesh litterbags that allowed quantifying

decomposition mediated by microorganisms and litter-consuming detritivores (hereafter detritivores) (Boyero et al. 2011b).

Besides comparing decomposition between low-PD and high-PD litter assemblages, we explored the proportional deviation between observed decomposition values in litter assemblages and the values expected from the corresponding monocultures (i.e., the net diversity effect). Additionally, we partitioned the net diversity effect into complementarity (which can occur through resource partitioning or from synergistic or antagonistic interactions) and selection effects (which arise when a species with particularly high or low decomposition rate dominates the mixture), as these give information about the potential mechanisms underlying diversity effects on decomposition (Truchy et al. 2015, Tonin et al. 2017, López-Rojo et al. 2018).

We predicted that PD would be a good proxy for litter trait diversity (hypothesis 1) and would influence decomposition, which would be faster in high-PD than in low-PD assemblages (hypothesis 2); we expected this effect to be evident both for decomposition and for net diversity effects. We further predicted that complementarity effects would be more important than selection effects (hypothesis 3), as shown elsewhere (Handa et al. 2014, Tonin et al. 2017). Lastly, we expected that some differences would arise between fine-mesh and coarse-mesh bags, with differences between low-PD and high-PD assemblages being more evident in coarse-mesh bags (hypothesis 4), due to different mechanisms operating in the absence and presence of detritivores. For example, a higher trait diversity in high-PD assemblages would benefit a wider variety of detritivore species with different morphological and behavioural characteristics (e.g., larger caddisflies are able to shred tougher leaves than smaller stoneflies; Tonin et al. 2018). In contrast, these differences might not be so evident for microorganisms, as fungal decomposers generally show high functional redundancy (Allison and Martiny 2008, Pérez et al. 2018, Martínez et al. 2019) and low resource specificity (Gulis 2001, Pérez et al. 2014).

MATERIALS AND METHODS

Plant species and study area

We selected 9 riparian tree species that belonged to 3 different families with wide distribution and an *a priori* high variety of litter traits: 3 species from the family Betulaceae [*Alnus acuminata* Kunth*., Alnus glutinosa* (L.) Gaertn*.* and *Alnus incana* L. Moench*],* 3 from the family Moraceae (*Ficus insipida* Willd*, Ficus natalensis* Hochst. and *Ficus dulciaria* Dugand) and 3 from the family Fagaceae (*Fagus sylvatica* L.*, Quercus prinus* L. and*, Castanea sativa* Mill.). These species were selected from a database containing data on several litter traits (Boyero et al. 2017) and were used in a

global-scale decomposition experiment conducted at ≈ 40 locations around the world (DecoDiv project, GLoBE network; www.globenetwork.es), of which the experiment detailed here is part. Litter with no visible signs of herbivory or decomposition was collected from the riparian forest floor or using vertical traps at different locations (Table S1) and air-dried in the laboratory.

We conducted the experiment in a permanent, first-order stream located within the Agüera river catchment in northern Spain (N 43° 12.745´, W 3° 16.256´; 350 m asl), between December 2017 and January 2018. The climate in the region is temperate oceanic, with mean temperature of 16 °C and annual precipitation of 1100 mm. Riparian vegetation in the catchment is composed of native mixed forest dominated by *Quercus robur* L. (Fagaceae), *A. glutinosa*, *Corylus avellana* L. (Betulaceae) and *C. sativa*.

Litter trait characterization

We measured several traits in litter, which was previously submerged in water in order to induce the leaching of soluble compounds. For that purpose, we introduced 3-g replicates (n = 6 per species) in glass jars with 400 mL of filtered (100 µm) stream water collected at the experimental site (see below). Jars were placed in a controlled-temperature room at 10 °C (close to the mean stream temperature at the time of the experiment) for 72 h, with water replacement every 24 h. Litter was then oven-dried (60 °C, 72 h), weighed to estimate the relationship between air dry mass (DM) and oven DM and divided into two subsamples. One was incinerated (550 °C, 4 h) and re-weighed to calculate a relationship between initial DM and post-leaching ash free dry mass (AFDM) and subsequently estimate litter mass loss due to leaching and the proportion of ash (i.e., the inorganic residue that remains after incineration) for each species. The other subsample was used to measure multiple traits, including the concentration of carbon (C), main nutrients (N and P), micronutrients and other elements [aluminium (Al), boron (B), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), potassium (K), silica (Si), sodium (Na), strontium (Sr) and titanium (Ti)], and secondary compounds (condensed tannins); and structural traits [concentrations of hemicellulose, cellulose and lignin; toughness; and specific leaf area (SLA)]. Methods for trait measurement are detailed in supporting Information.

Field work

Air-dried litter was introduced within fine-mesh (0.4 mm) and coarse-mesh (5 mm) bags (12 × 15 cm). Each bag contained 3 g of litter belonging to one species (monocultures, 9 treatments) or to 3 species (1 g each; mixtures, 6 treatments) of the same family or different families (Table 1), with 5 replicates per mesh type (fine and

coarse) and treatment (each of the monocultures or mixtures). Within each bag, litter of the same species was kept together using safety pins with attached coloured plastic rings, which facilitated species identification at the end of the experiment.

Table 1. Plant species and Rao´s quadratic diversity (RaoQ) of low-PD (I, II, III) and high-PD (IV, V, VI) litter assemblages.

Assemblages	Plant species			RaoQ
Low PD				
I	*Alnus acuminata*	*Alnus glutinosa*	*Alnus incana*	11.3
II	*Ficus insipida*	*Ficus natalensis*	*Ficus dulciaria*	20.8
III	*Fagus sylvatica*	*Quercus prinus*	*Castanea sativa*	8.5
High PD				
IV	*A. acuminata*	*F. insipida*	*F. sylvatica*	26.3
V	*A. glutinosa*	*F. natalensis*	*Q. prinus*	18.8
VI	*A. incana*	*F. dulciaria*	*C. sativa*	18.2

We selected 5 sites (run/pool habitats) within a 50-m long stream reach. At each site we tied one replicate per treatment to iron bars that were anchored randomly to the streambed. The bags were retrieved after 28 days (which equalled 235.4 degree days), enclosed individually in zip-lock bags and transported on ice to the laboratory. Litter from each bag was rinsed using filtered (100-µm) stream water on a 500-µm sieve to remove sediments and invertebrates. Then litter was sorted into species, oven-dried (70 °C, 72 h), weighed and divided in two sub-samples; one was incinerated (550 °C, 4 h) and re-weighed to estimate final AFDM, and the other was used to determine final N and P concentrations. Invertebrates collected from coarse-mesh bags were preserved in 70% ethanol, identified under a stereoscopic microscope to the lowest possible taxonomic level (genus for most taxa, with the exception of Diptera and Oligochaeta that were identified to family and class, respectively) and assigned to (litter-consuming) detritivore or non-detritivore categories using Tachet et al. (2000). They were subsequently oven-dried (70 °C for 72 h) and weighed to calculate biomass. We calculated taxon richness, abundance (number of individuals per bag) and biomass (mg per bag) for detritivores and total invertebrates in each bag.

Data analysis

We quantified decomposition through three variables: (i) the proportion of litter mass loss (LML), calculated as the difference between initial and final AFDM (g) divided by initial AFDM (g), with initial AFDM corrected by the proportion of LML due to leaching (calculated as above); (ii) the proportion of litter N loss (LNL), calculated as the difference between final and initial N contents (g) divided by initial N content (g); and (iii) the proportion of litter P loss (LPL), calculated as the difference between final and initial P contents (g) divided by initial P content (g). Potential outliers were identified

with Cleveland dot- and boxplots (Ieno and Zuur 2015) and were removed for subsequent analyses (<5% of the data). We calculated the net diversity effect as the difference between the observed value of LML, LNL or LPL in each assemblage and the expected value based on the respective monocultures. We further partitioned the net diversity effect into complementarity and selection effects using the additive partitioning method (Loreau and Hector 2001); this was done for LML only, as the existence of both positive and negative data precluded their calculation for LNL and LPL.

We examined hypothesis 1 (i.e., that PD is a good proxy for litter trait diversity) using two principal component analyses (PCAs), which were run using the prcomp function in the 'stats' package of R statistical software (R Core Team 2019; version 3.5.0). The first PCA explored the dispersion of the 9 plant species in multivariate trait space; the second one was based on the mean weighted value for each trait, and examined how the different litter assemblages varied in their trait composition. We explored trait variation in assemblages using Rao's quadratic diversity (RaoQ; dbFD function in the 'FD' package), which is the sum of pairwise functional distances between species weighted by their relative abundances (Rao 1982, Roscher et al. 2012).

In order to test hypothesis 2 (i.e., that decomposition and the net diversity effect on decomposition are higher in high-PD than in low-PD litter assemblages) we calculated ordinary nonparametric bootstrapped 95% confidence intervals for LML, LNL, LPL and diversity effects on LML, LNL and LPL, in low-PD and high-PD assemblages. We used the BCa method, based on 999 bootstrap replicates, using the boot function in the 'boot' R package (Davison and Hinkley 1997, Canty and Ripley 2016). We then determined whether the intervals contained the value of zero (i.e., the null expectation of no effect) and whether the intervals for low-PD and high-PD assemblages overlapped (i.e., the null expectation that effects did not differ depending on PD). We did so separately for fine-mesh and coarse-mesh bags (thus also partially exploring hypothesis 4).

We examined hypothesis 3 (i.e., that complementarity is more important than selection) through bootstrapped 95% confidence intervals for complementarity and selection effects. Again, we did this separately for fine-mesh and coarse-mesh bags to see if they overlapped (i.e., whether the effect differed depending on the presence of detritivores, thus partially examining hypothesis 4).

We further explored hypothesis 4 by comparing community descriptors (taxon richness, abundance and biomass) of all invertebrates and detritivores between low-PD and high-PD assemblages using BCa intervals, and we did the same for the net diversity effect on community descriptors (i.e., the difference between observed

values in assemblages and expected values based on monocultures). Additionally, we examined community structure using permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis dissimilarity matrix, comparing low-PD and high-PD assemblages (adonis function, 'vegan' package), and determining which were the most representative taxa in each assemblage (simper function, 'vegan' package).

Lastly, given that PD did not explain variation in the studied variables (except for selection effects on LML; see Results), we explored how all these variables were affected by the mean value and variability of individual litter traits. Mean values were measured through weighted trait means in each assemblage, and variability of each trait was calculated with RaoQ. We first excluded collinear traits (retaining the trait with the lowest variance inflation factor from each pair) using the vifcorr function in the 'usdm' package (Naimi et al. 2014); then constructed linear models (lm function in the 'nlme' package for variables and rda function in the 'vegan' package for community structure) with all possible combinations of traits; and finally selected the model with the lowest Akaike information criterion (AIC) using the step function ('stats' package).

RESULTS

Phylogenetic distance and trait diversity in litter assemblages

The two first axes of the first PCA explained 58.3% of variation in trait values among species (Fig. 1a). The first principal component (34.4%) mostly showed positive relationships with ash, Ca, Sr, Mg, hemicellulose and cellulose, and negative relationships with C, lignin, N:P, Pb and Cu. The second principal component (23.9%) mostly showed positive relationships with toughness, condensed tannins, B and Na and negative relationships with S, Fe, Ni and Li. The Betulaceae family was located in the lower left part of the two-dimensional space, while Fagaceae was located in the upper left part; Moraceae was the most diverse family and was located in the right part. The first two principal components of the second PCA explained 72.9% of variation in trait weighted means among treatments (Fig. 1b); high-PD assemblages were located near the centre of the axes, while low-PD assemblages were located further away from the centre. High-PD assemblages had higher RaoQ values than low-PD assemblages, with the exception of assemblage II, which RaoQ value was slightly higher than that of assemblages V and VI (Table 1).

Decomposition and diversity effects in low-PD and high-PD litter assemblages

Litter assemblages in fine-mesh bags lost, on average, 12% of their mass and 17% of P, and gained 13% of N; there was no influence of PD on LML and LPL; PD only

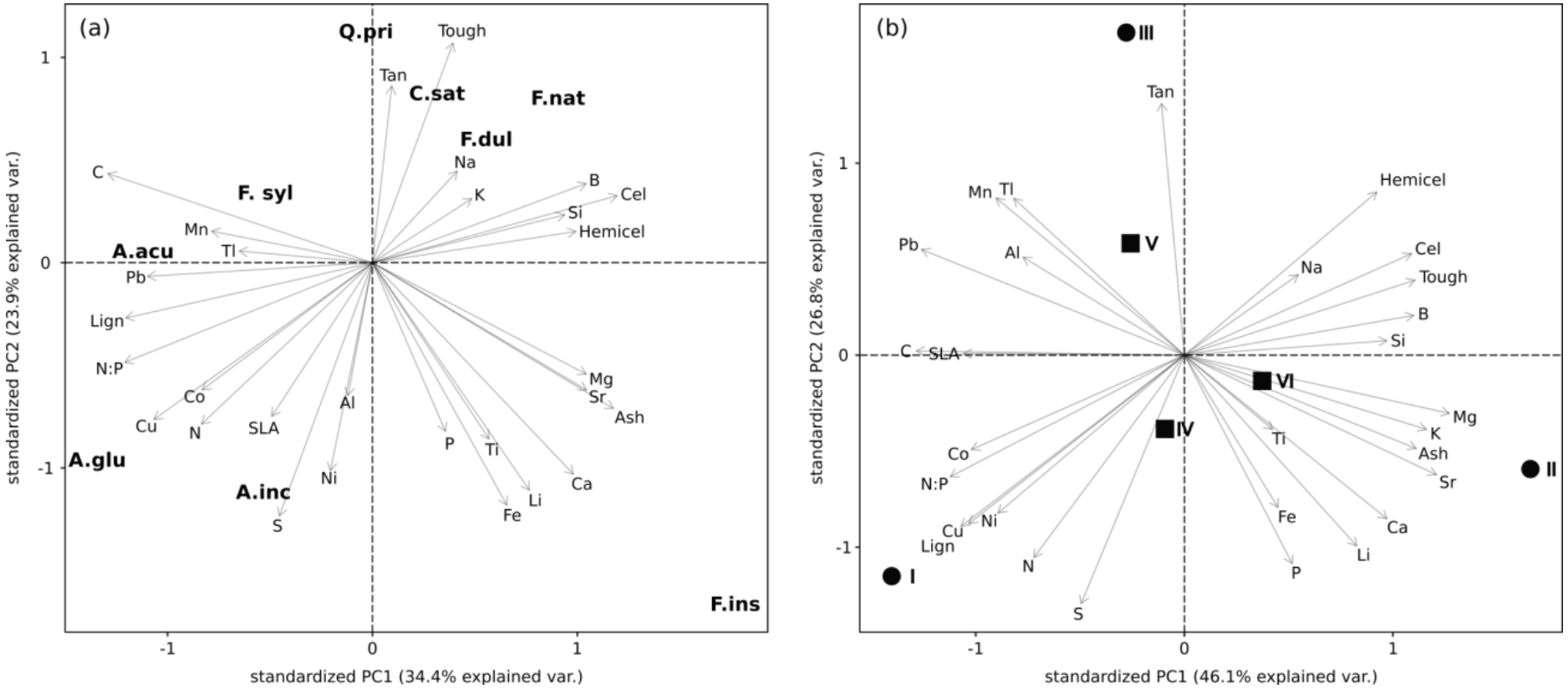

Fig. 1 Standardized Principal Components Analysis (PCA; first vs. second axes) of: a) the 9 plant species characterized by 29 litter traits, and b) the weighted means of trait values calculated for each assemblage. Species: A.acu, *Alnus acuminata;* A.glu*, Alnus glutinosa*; A.inc, *Alnus incan;* F.ins, *Ficus insipida*; F.dul, *Ficus dulciaria;* F.nat, *Ficus natalensis;* F.sylv, *Fagus sylvatica;* C.sat, *Castanea sativa;* Q.pri, *Quercus prinus.* Traits: C, carbon; N, nitrogen; P, phosphorous; Al, aluminium; B, boron; Ca, calcium; Cel, cellulose; Co, cobalt; Cu, copper; Fe, iron; Hemicel, hemicellulose; Li, lithium; Lign, lignin; Mg, magnesium; Mn, manganese; Ni, nickel; Pb, lead; K, potassium; Si, silica; Na, sodium; SLA: specific leaf area; Sr, strontium; Tan: condensed tannins; Ti, titanium; Tough: toughness. Assemblages: see Table 1.

determined LNL, which was negative (i.e., an increase in N content) in low-PD assemblages and null (i.e., no change in N content) in high-PD assemblages (Fig. 2a, Table S2). In coarse-mesh bags, litter assemblages lost, on average, 31% of their mass, 19% of N and 42% of P; again, PD did not affect LML, LNL or LPL (Fig. 2b, Table S2).

The net diversity effect on LML in fine-mesh bags was negative, with no effect of PD; the net diversity effect on LNL was not significant for low-PD and positive for high-PD assemblages; and the net diversity effect on LPL was positive and not affected by PD (Fig. 2c, Table S2). In coarse-mesh bags, the net diversity effect on LML was not significant for low-PD and negative for high-PD assemblages; the net diversity effect on LNL was not significant for either low-PD or high-PD assemblages; and the net diversity effect on LPL was positive, with no effect of PD (Fig. 2d, Table S2).

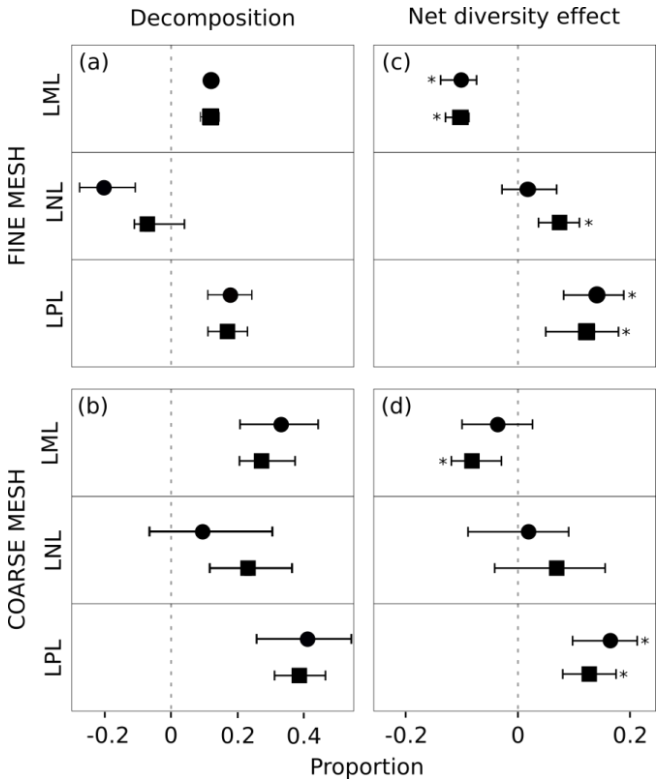

Fig. 2. Decomposition (proportion of litter mass loss, LML; litter nitrogen loss, LNL; and litter phosphorus loss, LPL) and net diversity effects (proportion) on LML, LNL and LPL, in low-PD (circles) and high-PD (squares) litter assemblages. Symbols are means, and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Significant net diversity effects (i.e., intervals that do not contain the value of zero) are marked with asterisks.

Complementarity effects on LML were negative in fine-mesh bags, did not vary with PD, and were, on average, 13 times greater than selection effects; the latter were negative for low-PD and not significant for high-PD assemblages (Fig. 3, Table S2). In

coarse-mesh bags, complementarity was not significant for low-PD and negative for high-PD assemblages, and selection was negative for low-PD and positive for high-PD assemblages; complementarity was, on average, 3 times greater in magnitude than selection (Fig. 3, Table S2).

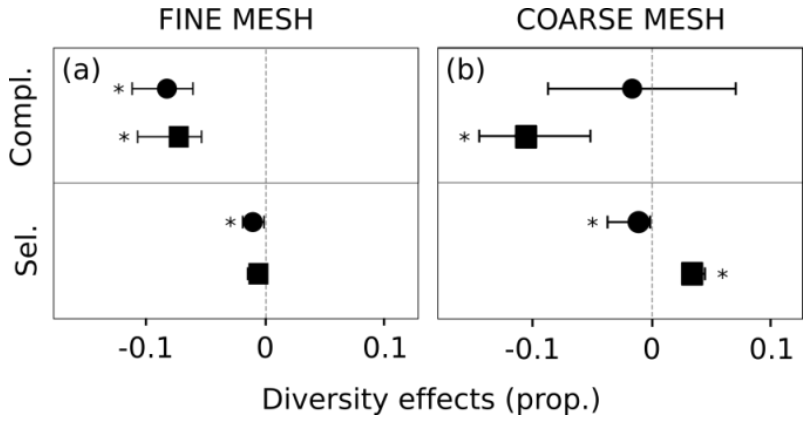

Fig. 3. Complementary and selection effects for litter mass loss in low-PD (circles) and high-PD (squares) assemblages. Symbols are means, and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Significant net diversity effects (i.e., intervals that do not contain the value of zero) are marked with asterisks.

Invertebrate communities

Low-PD assemblages within coarse-mesh bags contained, on average, 38 invertebrate individuals from 11 taxa and an average biomass of 34.09 mg; and 9 detritivore individuals of 4 taxa, with an average biomass of 19.46 mg (Fig. S3). High-PD assemblages within coarse-mesh bags had, on average, 49 invertebrate individuals from 14 taxa and an average biomass of 25.66 mg; and 14 detritivore individuals of 5 taxa, with an average biomass of 17.02 mg (Fig. S3). Thus, abundance and taxon richness tended to be higher in high-PD assemblages, while biomass tended to be higher in low-PD assemblages, although these differences were not significant, nor there were significant differences for net diversity effects on community descriptors (Fig. S3). The PERMANOVA reflected some differences between low-PD and high-PD assemblages in community structure ($p = 0.023$); taxa that most contributed to differences between both assemblages were Orthocladinae, Oligochaeta, Simuliidae, *Echinogammarus*, *Habroleptoides*, *Sericostoma*, *Leuctra* and *Amphinemura* (which were more abundant in high-PD assemblages), and Chironomini and Tanytarsini (which were more abundant in low-PD assemblages).

Mean trait values and trait variability in litter assemblages

We retained 15 traits to be included in the models; the others were excluded because of their high correlations with other traits (Fig. S1, S2). The model selection procedure showed that LML was mainly explained by mean P in fine-mesh bags, and mean P and N in coarse-mesh bags; LNL was explained by variability of Pb and P in fine-mesh bags, and mean N in coarse-mesh bags; and LPL was explained by mean condensed tannins in fine-mesh bags, and variability of P in coarse-mesh bags (Table S6). Net diversity effects on LML and complementarity were explained by mean C in fine-mesh bags, and mean N in coarse-mesh bags; selection was explained by mean condensed tannins in both fine-mesh and coarse-mesh bags; net diversity effects on LNL were explained by mean condensed tannins in fine-mesh bags and variability of C in coarse-mesh bags; and net diversity effects on LPL were explained by variability of P in fine-mesh bags and variability of C in coarse-mesh bags (Table S6). Taxon richness of detritivores was explained by mean P; taxon richness of all invertebrates was explained by variability of N; detritivore and invertebrate abundances were explained by variability of N; and detritivore and invertebrate biomass by mean N (Table S7).

DISCUSSION

Phylogenetic distance is not always a good proxy for litter trait diversity

The diversity of biological traits has been claimed as a more appropriate measure than species richness when assessing BEF relationships, due to the link between traits and the function or performance of species (Hillebrand and Matthiessen 2009), and to the often high intraspecific variation of traits, which is sometimes comparable to interspecific variation (LeRoy et al. 2006, Lecerf and Chauvet 2008). However, the lack of consensus on how to measure trait diversity (Petchey et al. 2009) has precluded a robust analysis of how it affects key ecosystem processes such as litter decomposition. We expected that PD would be a good proxy for trait diversity in litter assemblages and would predict decomposition (see Boyero et al. 2016), but this was not the case considering the set of traits that we measured. Even if species of the same family had more similar trait values than species of different families overall, there were exceptions (traits of *Ficus insipida* differed greatly from those of other *Ficus* species). However, we note that, despite the large number of traits measured, we may have excluded traits that would increase differences among families (hence differences between low-PD and high-PD assemblages). For example, *Ficus* species are characterized by the presence of latex (Lansky 2008, Konno 2011), which is not present in the Fagaceae or Betulaceae.

Low-PD and high-PD assemblages did not consistently differ in decomposition, although litter-consuming detritivores (i.e., *Echinogammarus*, *Sericostoma*, *Leuctra*

and *Amphinemura*) seemed to prefer high-PD assemblages, indicating potential effects on decomposition in the longer term that were not detected here. Moreover, a meta-analysis of 285 studies showed a key role of plant phylogenetic history in predicting decomposition (LeRoy et al. 2019), suggesting that the lack of relationship between PD and decomposition should be taken with caution.

Net diversity effects on decomposition

Our experimental design allowed us not only to examine effects of PD on decomposition, but also on net diversity effects, by comparing decomposition in litter assemblages vs. monocultures. Interestingly, we found mostly negative diversity effects on litter mass loss (i.e., litter of particular species lost more mass when in monoculture than in assemblages), but mostly positive diversity effects on litter P loss (i.e., litter lost more P in assemblages than in monocultures) and on litter N gain (i.e., high-PD assemblages gained or immobilized more N than monocultures). The fact that litter gained N when enclosed in fine-mesh bags but lost N in coarse-mesh bags points to different modes of resource acquisition by microorganisms and invertebrates, which may influence the remaining resource quality.

Our results contrasted with those of microcosm experiments finding positive diversity effects on litter mass loss in the presence and absence of detritivores, and on litter N and P loss in microcosms with detritivores (e.g., Tonin et al. 2017, López-Rojo et al. 2019). We suggest that these differences could be related to different experimental conditions; for example, nutrients lost from litter in microcosms can be recycled during the whole experiment, while in streams they can be rapidly lost downstream and thus reduce any potential diversity effect. Also, microcosms in the above-cited experiments contained one detritivore species, while our field study allowed litter to be colonized by multiple invertebrates; thus, in the field, feeding activities of different detritivores can be affected in opposite ways, and both negative and positive interspecific interactions can occur, resulting in masked or reversed litter diversity effects on litter mass loss (McKie et al. 2009). Furthermore, detritivores in the field can also feed on litter outside litterbags, and hence diversity effects can be diluted or modified (Tiegs et al. 2008). Another field study conducted across biomes (Handa et al. 2014) found overall positive, albeit small and inconsistent, diversity effects on litter mass loss and litter N loss, which also highlights the relevance of the environmental setting and local detritivore communities.

In our study, the negative diversity effect on litter mass loss was mainly driven by the complementarity effect, which was higher than the selection effect, particularly in fine-mesh bags. Negative complementarity, which occurred both in the presence and absence of invertebrates, suggested the existence of physical or chemical interference between species or litter traits. For example, the presence of litter with

higher tannin contents could have acted as a feeding deterrent for detritivores, limiting consumption of higher quality litter that would be consumed faster in monoculture (Graça et al. 2001). Similarly, the leaching and passive transfer of inhibitory compounds from some litter types could have limited microbial activity in others (Gessner et al. 2010).

Selection was considerably smaller than complementarity. However, interestingly, in coarse-mesh bags, selection was negative in low-PD assemblages, which contained more generalist taxa, and positive in high-PD assemblages, where more litter-feeding detritivores were found. This indicates that detritivores might be actively selecting higher-quality litter because of its nutritional quality, while other invertebrates could be avoiding litter with higher concentrations of toxic, secondary compounds. These compounds would be leached and affect these invertebrates even when they do not feed on litter, for example through the alteration of abiotic conditions such as dissolved oxygen (Chergui et al. 1997).

Decomposition is strongly influenced by a limited number of litter traits

In our experiment, the mean values of several traits influenced decomposition in litter assemblages, in agreement with previous studies (e.g., Fernandes et al. 2012, Ferreira et al. 2012, López-Rojo et al. 2018). Importantly, among the large number of litter traits examined (i.e., 29), we found that mean concentrations of N and P were the most relevant traits for decomposition and associated invertebrate communities, followed by concentration of condensed tannins and C. There is ample evidence for the positive effects of N and P on decomposition (Cornwell et al. 2008), and condensed tannins are known to delay decomposition because they are resistant and toxic to microorganisms (Graça and Bärlocher 2005). Moreover, tannins are generally inversely related to N and P (Boyero et al. 2017; r = –0.53 and r = –0.61, respectively, in this study), reinforcing differences in the quality of different litter types and hence in their decomposition.

Lignin, toughness and specific leaf area (SLA) can also be good indicators of litter quality (Ostrofsky 1997, Casas et al. 2013). However, in our study, these traits were excluded from models because of their high collinearity with other variables. Lignin is a recalcitrant compound that usually delays decomposition (Schindler and Gessner 2009), but in our study it was positively related to nutrients, mostly N (r = 0.90), and negatively related to toughness (r = –0.78). This may be due to the relatively high lignin concentration found in *Alnus* species (23.7-26.7%), compared to other studies (e.g., 3.9-18.7% for A. glutinosa; Lecerf and Chauvet 2008). Toughness and SLA are usually inversely related (Fugère et al. 2012; r = –0.84 in our study), and both were strongly related to C (r = –0.71 and r = 0.87, respectively), the latter affecting the structure of invertebrate communities colonizing litter assemblages. This could be

associated with differences in detritivore mouthparts, some of which are specialized for feeding on tougher litter while others allow feeding only on softer material (Tonin et al. 2018).

The role of many plant micronutrients and other elements is relatively unexplored, but some studies have revealed that decomposition can be affected by concentrations of Mg and Ca (Makkonen et al. 2012, García-Palacios et al. 2016). We did not find direct correlations between micronutrients and decomposition, but Fe, S and Ca were strongly and negatively related to tannins ($r = –0.81$, $r = –0.80$ and $r = –0.76$, respectively), A positive relationship between cations such as Ca, K, Mg and Na and litter decomposition has been reported in several terrestrial studies (Nicolai 1988, Cornelissen and Thompson 1997); our results indicate a possible positive influence of these micronutrients on decomposition also in stream ecosystems.

Trait variability can promote decomposition and mediate diversity effects on decomposition

In addition to mean trait values, we showed that the variability of some traits within litter assemblages also influenced decomposition. Particularly important was the variability of nutrients (N and P) and some micronutrients (Pb, Mn and Ti; and potentially S and Cu, which were strongly and positively related to N, $r = 0.97$ and $r = 0.93$, respectively), and possibly the variability of cellulose and SLA, also related to N ($r = 0.84$ and $r = 0.75$, respectively). Another study showed that, in the presence of detritivores, litter assemblages with intermediate quality but high trait heterogeneity decomposed faster than higher-quality assemblages (Landeira-Dabarca et al. 2018), possibly because the presence of more refractory litter enhanced overall consumption through a 'clutching at straws' effect (*sensu* Landeira-Dabarca et al. 2018; see also Sanpera-Calbet et al. 2009).

Importantly, the variability of some traits (i.e., hemicellulose – in fine-mesh bags only –, C, N, P and some micronutrients –mostly Pb, and possibly Ti, which was positively related to Pb) influenced litter diversity effects on decomposition (i.e., differences between observed decomposition in assemblages vs. expected decomposition based on monocultures). However, effects were positive only in some cases (hemicellulose and N), while in others the variability of the same trait had either positive or negative effects on different variables (C, P, Pb, and several other traits related to them). A laboratory experiment had shown that diversity effects on decomposition were higher in litter assemblages with higher overall trait heterogeneity (López-Rojo et al. 2018); we further show that diversity effects on LML could be mediated by a structural trait (hemicellulose) and a key nutrient (N). Furthermore, hemicellulose had an effect in fine-mesh bags, while N was relevant in coarse-mesh bags, suggesting a more important role of litter structure variability for

microorganisms and of nutrient variability for detritivores; this result seems counterintuitive and merits further exploration.

Conclusions and insights

Human-driven changes in riparian plant diversity involve changes in litter inputs to streams, which in turn can affect key processes such as litter decomposition (Pozo et al. 1998) and related ecosystem services such as C sequestration or water purification (Cardinale et al. 2012). We demonstrate that changes in litter decomposition can be largely predicted by considering how several key litter traits are altered, both in terms of mean values and their variability. We highlight the importance of considering nutrient dynamics when studying decomposition (see also Handa et al. 2014, López-Rojo et al. 2019), as these can differ from patterns of litter mass loss, which is often the only process considered in decomposition studies.

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SUPPORTING INFORMATION

Supplementary methodology

We measured C and N concentrations (% DM) using a Perkin Elmer series II CHNS/O elemental analyser; P concentration (% DM) with an spectrophotometer after autoclave-assisted extraction (APHA 1998); micronutrients and other elements with inductively coupled plasma optical emission spectroscopy (ICP-OES) using a THERMO ICAP 6500 DUO spectrometer; condensed tannins with a radial diffusion assay (Graça and Bärlocher 2005); hemicellulose, cellulose and lignin concentrations using an ANKOM 200/220 fibre analyser; toughness using a penetrometer, which measured the pressure (kPa) necessary to pierce the leaf tissue with a 1.55-mm diameter steel rod; and SLA as the ratio of leaf area (mm^2) to leaf DM (mg).

Table S1. Locations where leaf litter of each species was collected and most influential litter traits.

Family	Species	C (%)	N (%)	P (%)	Lignin (%)	Condensed tannins (%)	Ash (%)	Site of collection
Betulaceae	*Alnus acuminata*	56.16 ± 0.97	2.39 ± 0.08	0.05 ± 2e-3	26.72 ± 1.40	0.00 ± 0.00	2.50 ± 0.07	Ecuador (1.78ºS, 79.35ºW)
	Alnus glutinosa	52.46 ± 0.78	2.89 ± 0.01	0.05 ± 1e-3	21.22 ± 2.62	0.45 ± 0.10	4.86 ± 0.55	Spain (43.20ºN, 3.26ºW)
	Alnus incana	51.04 ± 0.57	3.55 ± 0.02	0.08 ± 3e-3	23.73 ± 2.03	0.00 ± 0.00	6.98 ± 0.20	Sweden (64.16ºN, 19.50ºE)
Moraceae	*Ficus insipida*	36.72 ± 1.05	1.09 ± 0.09	0.07 ± 3e-3	11.95 ± 0.61	0.00 ± 0.00	25.71 ± 1.03	Costa Rica (10.26ºN, 84.00ºW)
	Ficus natalensis	45.16 ± 0.68	1.32 ± 0.01	0.05 ± 2e-3	13.15 ± 1.38	0.42 ± 0.06	13.93 ± 0.60	Kenya (0.37ºS, 35.93ºE)
	Ficus dulciaria	47.59 ± 0.23	1.84 ± 0.07	0.08 ± 1e-3	19.34 ± 2.04	0.00 ± 0.00	7.31 ± 0.53	Ecuador (1.78ºS, 79.35ºW)
Fagaceae	*Fagus sylvatica*	49.82 ± 1.62	1.05 ± 0.07	0.04 ± 1e-3	20.11 ± 0.42	0.42 ± 0.06	3.70 ± 0.10	France (43.41ºN, 2.21ºE)
	Quercus prinus	48.61 ± 0.32	0.69 ± 0.02	0.03 ± 1e-3	14.28 ± 1.99	1.56 ± 0.02	5.74 ± 0.21	USA (39.23ºN, 76.74ºW)
	Castanea sativa	51.62 ± 0.60	1.05 ± 0.03	0.04 ± 2e-3	12.29 ± 0.96	2.26 ± 0.27	3.24 ± 0.17	Portugal (40.09ºN, 8.20ºW)

Table S2. Mean and standard error of variables measuring litter decomposition (litter mass loss, LML; litter nitrogen loss, LNL; and litter phosphorus loss, LPL) and diversity effects on decomposition (net, complementarity and selection effects) in the different litter assemblages (I-VI, see Table 1) within fine-mesh and coarse-mesh bags. Variables are expressed in proportion (i.e., difference between initial and final values divided by initial value).

		Litter decomposition			Diversity effects on litter decomposition				
Mesh	**Assemblage**	**LML**	**LNL**	**LPL**	**Net (LML)**	**Compl. (LML)**	**Sel. (LML)**	**Net (LNL)**	**Net (LPL)**
Fine	I	0.127 ± 0.008	-0.015 ± 0.057	0.230 ± 0.050	-0.058 ± 0.009	-0.045 ± 0.006	-0.013 ± 0.004	0.040 ± 0.057	0.060 ± 0.051
	II	0.147 ± 0.015	-0.227 ± 0.041	0.275 ± 0.028	-0.164 ± 0.015	-0.139 ± 0.012	-0.025 ± 0.004	-0.015 ± 0.040	0.184 ± 0.027
	III	0.087 ± 0.010	-0.329 ± 0.043	0.041 ± 0.043	-0.040 ± 0.009	-0.050 ± 0.007	0.010 ± 0.004	0.066 ± 0.044	0.201 ± 0.044
	IV	0.111 ± 0.003	-0.011 ± 0.039	0.311 ± 0.024	-0.062 ± 0.003	-0.054 ± 0.015	-0.008 ± 0.013	0.068 ± 0.039	0.210 ± 0.023
	V	0.076 ± 0.025	-0.114 ± 0.037	0.095 ± 0.035	-0.108 ± 0.025	-0.105 ± 0.027	-0.003 ± 0.003	0.124 ± 0.037	0.203 ± 0.035
	VI	0.182 ± 0.012	-0.097 ± 0.012	0.087 ± 0.022	-0.105 ± 0.011	-0.049 ± 0.012	-0.001 ± 0.001	0.065 ± 0.013	-0.040 ± 0.024
Coarse	I	0.567 ± 0.019	0.527 ± 0.026	0.699 ± 0.005	0.124 ± 0.018	0.159 ± 0.033	-0.035 ± 0.021	0.181 ± 0.026	0.218 ± 0.007
	II	0.391 ± 0.056	0.112 ± 0.084	0.476 ± 0.053	-0.076 ± 0.056	-0.074 ± 0.055	-0.002 ± 0.006	-0.063 ± 0.085	0.166 ± 0.053
	III	0.095 ± 0.014	-0.246 ± 0.038	0.131 ± 0.067	-0.088 ± 0.014	-0.092 ± 0.014	0.004 ± 0.001	0.011 ± 0.038	0.167 ± 0.068
	IV	0.153 ± 0.020	0.040 ± 0.066	0.330 ± 0.026	-0.111 ± 0.020	-0.165 ± 0.020	0.053 ± 0.006	-0.126 ± 0.066	0.067 ± 0.026
	V	0.197 ± 0.029	0.125 ± 0.054	0.273 ± 0.043	-0.100 ± 0.029	-0.130 ± 0.025	0.030 ± 0.005	0.152 ± 0.054	0.178 ± 0.044
	VI	0.485 ± 0.048	0.557 ± 0.044	0.584 ± 0.026	0.005 ± 0.047	-0.018 ± 0.047	0.022 ± 0.006	0.199 ± 0.039	0.170 ± 0.023

Table S3. Mean and standard error of variables measuring litter decomposition (litter mass loss, LML; litter nitrogen loss, LNL; and litter phosphorus loss, LPL) in monocultures within fine-mesh and coarse-mesh bags. Variables are expressed in proportion (i.e., difference between initial and final values divided by initial value).

Mesh	Species	LML	LNL	LPL
Fine	*Alnus acuminata*	0.092 ± 0.007	-0.204 ± 0.168	0.040 ± 0.108
	Alnus glutinosa	0.166 ± 0.014	0.427 ± 0.092	0.513 ± 0.057
	Alnus incana	0.351 ± 0.028	0.477 ± 0.117	0.590 ± 0.048
	Ficus natalensis	0.232 ± 0.007	-0.131 ± 0.016	-0.352 ± 0.078
	Ficus dulciaria	0.219 ± 0.015	-0.145 ± 0.070	-0.050 ± 0.118
	Ficus insipida	0.464 ± 0.008	-0.093 ± 0.147	0.235 ± 0.085
	Fagus sylvatica	0.056 ± 0.008	-0.585 ± 0.046	0.063 ± 0.025
	Castanea sativa	0.149 ± 0.013	-0.611 ± 0.095	0.035 ± 0.051
	Quercus prinus	0.145 ± 0.023	-0.565 ± 0.186	-0.649 ± 0.417
Coarse	*Alnus acuminata*	0.224 ± 0.056	0.040 ± 0.086	0.022 ± 0.085
	Alnus glutinosa	0.372 ± 0.058	-0.134 ± 0.083	-0.021 ± 0.096
	Alnus incana	0.858 ± 0.069	-0.054 ± 0.074	0.195 ± 0.093
	Ficus natalensis	0.353 ± 0.014	-0.016 ± 0.070	0.127 ± 0.068
	Ficus dulciaria	0.406 ± 0.060	0.491 ± 0.160	0.674 ± 0.094
	Ficus insipida	0.597 ± 0.034	0.415 ± 0.171	0.629 ± 0.106
	Fagus sylvatica	0.070 ± 0.013	-0.052 ± 0.064	0.216 ± 0.044
	Castanea sativa	0.277 ± 0.074	-0.115 ± 0.068	-0.106 ± 0.065
	Quercus prinus	0.164 ± 0.015	-0.255 ± 0.182	-0.041 ± 0.132

Table S4. Mean and bootstrapped 95% confidence intervals for variables measuring litter decomposition (litter mass loss, LML; litter nitrogen loss, LNL; and litter phosphorus loss, LPL) and diversity effects on decomposition (net, complementarity and selection effects) in assemblages with low and high phylogenetic distance (PD).

Mesh	Variable	PD	Mean	Confidence interval
Fine	LML	Low	0.120	(0.104, 0.140)
		High	0.119	(0.088, 0.145)
	LNL	Low	-0.203	(-0.278, -0.110)
		High	-0.072	(-0.114, -0.030)
	LPL	Low	0.179	(0.110, 0.244)
		High	0.170	(0.109, 0.232)
	Net (LML)	Low	-0.090	(-0.126, -0.062)
		High	-0.091	(-0.118, -0.076)
	Compl. (LML)	Low	-0.081	(-0.110, -0.059)
		High	-0.071	(-0.105, -0.052)
	Sel. (LML)	Low	-0.009	(-0.018, 0.000)
		High	-0.004	(-0.014, 0.003)
	Net (LNL)	Low	0.030	(-0.016, 0.081)
		High	0.087	(0.049, 0.122)
	Net (LPL)	Low	0.155	(0.094, 0.202)
		High	0.136	(0.062, 0.192)
Coarse	LML	Low	0.331	(0.205, 0.445)
		High	0.272	(0.203, 0.375)
	LNL	Low	0.095	(-0.069, 0.307)
		High	0.232	(0.113, 0.367)
	LPL	Low	0.411	(0.255, 0.546)
		High	0.386	(0.310, 0.467)
	Net (LML)	Low	-0.026	(-0.089, 0.037)
		High	-0.071	(-0.108, -0.019)
	Compl. (LML)	Low	-0.017	(-0.087, 0.070)
		High	-0.106	(-0.145, -0.052)
	Sel. (LML)	Low	-0.009	(-0.035, 0.000)
		High	0.035	(0.027, 0.045)
	Net (LNL)	Low	0.030	(-0.078, 0.102)
		High	0.081	(-0.031, 0.167)
	Net (LPL)	Low	0.181	(0.112, 0.227)
		High	0.141	(0.094, 0.189)

Table S5. Mean and bootstrapped 95% confidence intervals for abundance (individuals per bag), richness (taxa per bag) and biomass (mg per bag) of all invertebrates and litter-consuming detritivores in assemblages with low and high phylogenetic distance (PD).

Variable	PD	Mean	Confidence interval
Invertebrate richness	Low	11.25	(9.54, 12.18)
	High	13.54	(11.62, 15.23)
Detritivore richness	Low	3.83	(2.66, 4.58)
	High	4.92	(4.00, 5.53)
Invertebrate abundance	Low	38.33	(31.13, 47.31)
	High	48.62	(40.85, 55.85)
Detritivore abundance	Low	8.67	(6.00, 10.58)
	High	14.08	(10.46, 18.57)
Invertebrate biomass	Low	34.10	(19.45, 54.33)
	High	25.67	(19.83, 44.19)
Detritivore biomass	Low	19.46	(10.55, 38.91)
	High	17.02	(11.41, 29.33)

Table S6. Results of linear models examining the variability of litter decomposition (litter mass loss, LML; litter nitrogen loss, LNL; and litter phosphorus loss, LPL) and diversity effects on decomposition (net, complementarity and selection effects), based on weighted mean litter trait values ("mean") and their variability ("var"; measured with RaoQ), in litter assemblages within fine-mesh and coarse-mesh bags; + and – indicate positive and negative relationships, respectively. Traits: see Fig. 1. AIC: Akaike information criteria. Retained variance: proportion of the variance explained by each factor.

Response variable	AIC	Selected factors	Retained variance	p-value
Fine mesh				
LML	-112.242	Mean (P) +	0.553	<0.001
		Var (Al) –	0.051	0.084
LNL	-53.004	Var (Pb) +	0.405	<0.001
		Var (P) +	0.243	<0.001
		Mean (Na) –	0.034	0.121
LPL	-62.878	Mean (Tan) –	0.637	<0.001
		Mean (N) –	0.060	0.035
Net (LML)	-110.451	Mean (C) +	0.409	<0.001
		Var (Hemicel) +	0.203	<0.001
		Var (P) –	0.076	0.023
Comp. (LML)	-107.539	Mean (C) +	0.401	<0.001
		Var (Hemicel) +	0.159	0.005
		Mean (Na) +	0.036	0.151
Sel. (LML)	-156.305	Mean (Tan) +	0.320	0.001
		Var (Hemicel) +	0.085	0.069
Net (LNL)	-55.455	Mean (Tan) +	0.132	0.094
		Var (Pb) +	0.093	0.049
Net (LPL)	-62.086	Var (P) –	0.404	<0.001
		Mean (Tan) –	0.206	0.001
		Var (Al) –	0.035	0.135
Coarse mesh				
LML	-54.625	Mean (P) +	0.654	<0.001
		Mean (N) +	0.159	<0.001
		Var (Mn) –	0.065	0.003
LNL	-30.328	Mean (N) +	0.708	<0.001
		Mean (Na)	0.096	0.001
		Var (Ti) +	0.039	0.022
		Var (N) +	0.036	0.027
LPL	-44.588	Var (P) –	0.715	<0.001
		Mean (N) +	0.088	0.002
		Mean (P) +	0.046	0.022
Net (LML)	-56.092	Mean (N) +	0.487	<0.001
		Var (Pb) –	0.075	0.064
		Mean (C) +	0.045	0.142
Compl. (LML)	-56.164	Mean (N) +	0.483	<0.001
		Var (Pb) –	0.149	0.004
		Mean (P) –	0.082	0.026
Sel. (LML)	-126.437	Var (C) +	0.294	<0.001
		Var (N) +	0.273	<0.001
		Mean (Tan) +	0.203	<0.001
Net (LNL)	-32.450	Var (C) –	0.360	<0.001
		Var (Pb) +	0.170	0.008
		Var (P) +	0.076	0.063
Net (LPL)	-47.539	Var (C) –	0.207	0.025

Table S7. Results of linear models examining the variability of community descriptors (richness, abundance and biomass for all invertebrates and detritivores) and community structure based on weighted mean litter trait values ("mean") and their variability ("var"; measured with RaoQ); + and – indicate positive and negative relationships, respectively. Traits: see Fig. 1. AIC: Akaike information criteria. Retained variance: proportion of the variance explained by each factor.

Response variable	AIC	Selected factors	Retained variance	p-value
All invertebrates				
Richness	125.565	Var (N) +	0.116	0.102
Abundance	201.376	Var (N) +	0.099	0.122
		Mean (C) -	0.091	0.137
Biomass	222.167	Mean (N) +	0.134	0.068
		Var (Pb) -	0.099	0.113
Detritivores				
Richness	90.734	Mean (P) +	0.210	0.017
		Var (N) +	0.099	0.088
		Var (Ti) +	0.071	0.143
Abundance	157.964	Var (N) +	0.229	0.013
		Var (B) +	0.085	0.112
		Mean (P) +	0.068	0.153
Biomass	205.238	Mean (N) +	0.358	0.002
Community structure		Mean (C)	0.064	0.036
		Mean (N)	0.045	0.237
		Var (N)	0.043	0.287
		Mean (P)	0.036	0.482
		Mean (Co)	0.027	0.780
		Var (C)	0.022	0.903

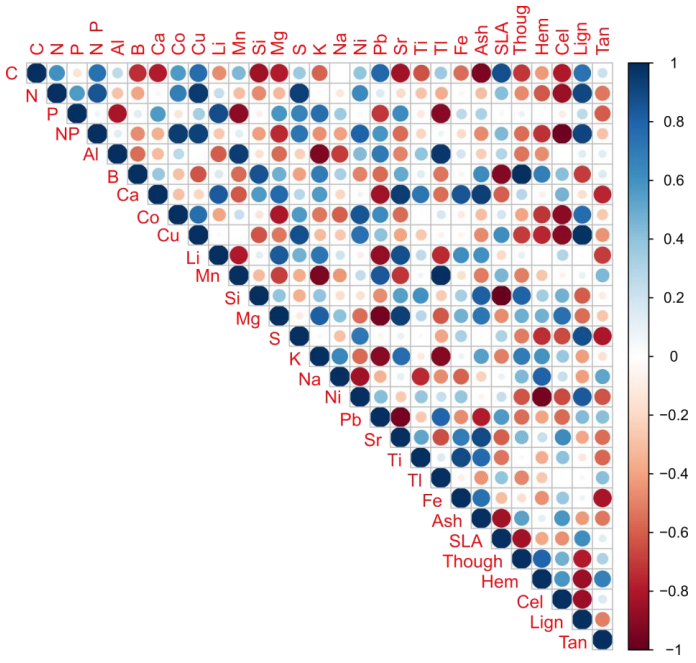

Fig. S1. Pairwise correlations between mean litter trait values in litter assemblages. Traits: see Fig. 1.

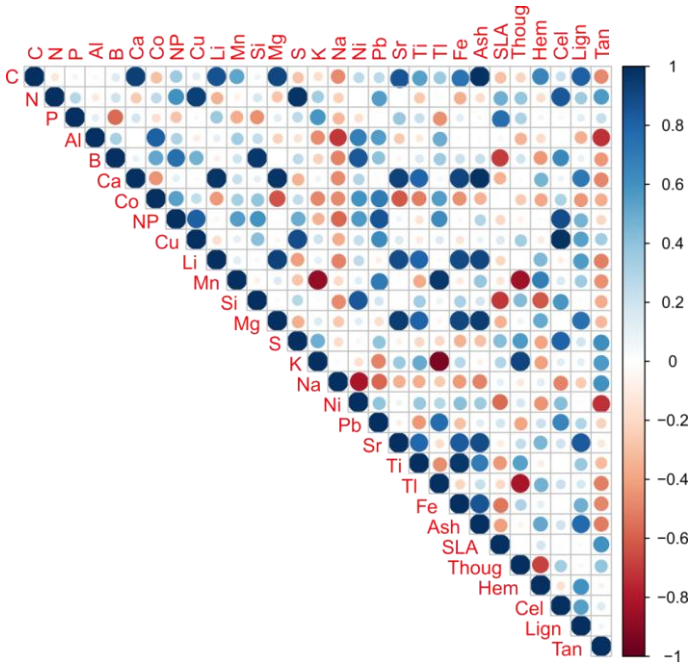

Fig. S2. Pairwise correlations between litter trait variabilities (measured with RaoQ) in litter assemblages. Traits: see Fig. 1.

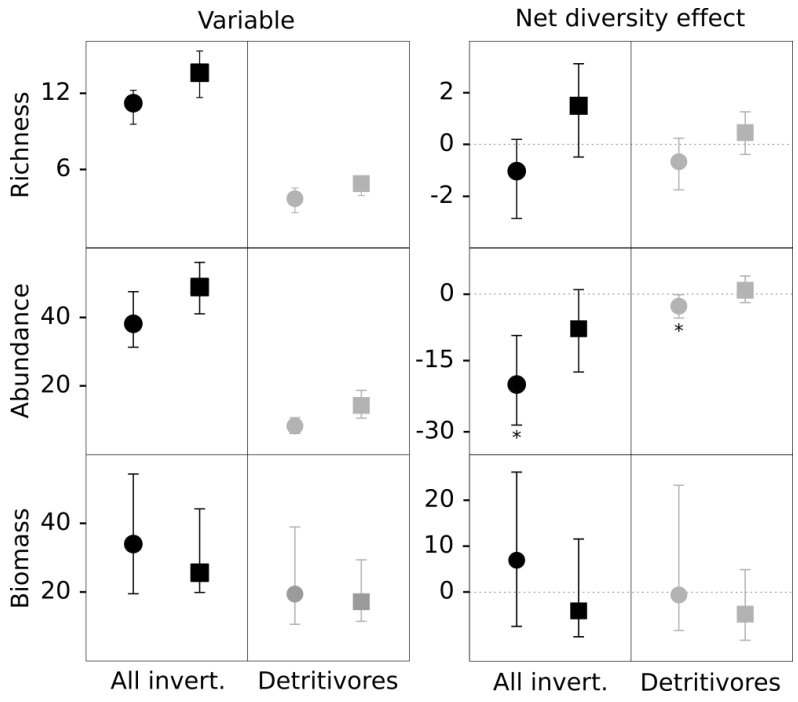

Fig S3. Invertebrate (black symbols) and detritivore (grey symbols) taxon richness (no. taxa per bag), abundance (individuals per bag) and biomass (mg per bag) (left panel), and net diversity effects (proportion) on these variables (right panel), for low-PD (circles) and high-PD (squares) assemblages. Symbols are means, and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Significant net diversity effects (i.e., intervals that do not contain the value of zero) are marked with asterisks.

CHAPTER 5

Effects of two measures of riparian plant biodiversity on litter decomposition and associated processes in stream microcosms

This chapter is published with the following reference

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ABSTRACT

Plant litter decomposition is a key ecosystem process that can be altered by global changes such as biodiversity loss. These effects can be particularly important in detritus-based ecosystems, such as headwater streams, which are mainly fuelled by allochthonous plant litter inputs. However, experiments examining effects of plant diversity on litter decomposition in streams have not reached consensus about which measures of biodiversity are more relevant. We explored the influence of two of these measures, plant species richness (SR; monocultures vs. 3-species mixtures) and phylogenetic distance (PD; species belonging to the same family vs. different families), on leaf litter decomposition and associated processes (nutrient dynamics and fungal and detritivore biomass production), in a stream microcosm experiment using litter from 9 tree species belonging to 3 families. We found a negative effect of SR on decomposition (which contradicted the results of previous experiments) but a positive effect on fungal biomass production. While PD did not affect decomposition, both SR and PD altered nutrient dynamics: there was greater litter and detritivore N loss in low-PD mixtures, and greater litter P loss and detritivore P gain in monocultures. This suggested that the number of species in mixtures and the similarity of their traits both modulated nutrient availability and utilization by detritivores. Moreover, the greater fungal biomass production with higher SR could imply positive effects on detritivores in the longer term. Our results provide new insights of the functional repercussions of biodiversity loss by going beyond the often-explored relationship between SR and decomposition, and reveal an influence of plant species phylogenetic relatedness on nutrient cycling that merits further investigation.

KEY WORDS: phylogenetic distance, species richness, litter traits, nitrogen, phosphorus, detritivores, fungi

INTRODUCTION

Current rates of biodiversity loss are far greater than those before human dominance of Earth (Lawton et al. 1995, Loh and Wackernagel 2004, Barnosky et al. 2011), as a result of multiple environmental changes of anthropogenic origin such as land transformation, climate change and species invasions (Vitousek et al. 1997, Mack et al. 2000, Leroy and Marks 2006, Amici et al. 2015). Biodiversity loss, in turn, can alter ecosystem processes such as plant litter decomposition, which is key for the functioning of ecosystems (Hooper et al. 2012). Headwater streams are detritus-based ecosystems that are fuelled by allochthonous plant litter detritus inputs from the surrounding terrestrial catchment (Vannote et al. 1980, Wallace et al. 1997, Suurkuukka et al. 2014). Once in the stream, plant litter is decomposed by

microorganisms (mainly fungi) and invertebrates (litter-consuming detritivores), which involves the cycling of major nutrients such as nitrogen (N) and phosphorus (P), and the production of microbial and invertebrate biomass (Marks 2019). All these stream processes can be altered by multiple global environmental drivers (e.g., climate warming, eutrophication) and by terrestrial plant diversity loss, which is caused by widespread forestry practices such as monospecific plantations (Kominoski et al. 2013).

There is evidence that plant diversity loss affects litter decomposition (Swan and Palmer 2004), nutrient cycling (López-Rojo et al. 2019) and biomass production (Stout III et al. 1993), with effects mediated by complementary resource by detritivores (i.e., complementarity effects) or by the presence of particular litter types that decompose faster or slower than others (i.e., selection effects) (Loreau and Hector 2001). However, inconsistencies between field and laboratory studies and across experiments (Gessner et al. 2010) suggest that there are still important gaps within this research field. A key question is whether species richness (SR; which has been used in most relevant studies) is the most appropriate measure of biodiversity, compared to other measures that consider the diversity of species traits (Hillebrand and Matthiessen 2009, Krause et al. 2014). Trait-related biodiversity measures could be expected to have greater influence on ecosystem processes than SR, because traits have direct functional repercussions (Petchey and Gaston 2006). For example, phylogenetic distance (PD) is often a good predictor of species trait variation (Cavender-Bares et al. 2009, Burns and Strauss 2011, Mouquet et al. 2012, López-Rojo et al. 2020b), and it has shown relationships with ecosystem processes such as primary production (Cadotte et al. 2008) and litter decomposition (Boyero et al. 2016).

We experimentally explored how both plant SR and PD within litter assemblages influenced litter decomposition and associated processes (nutrient cycling and the production of fungal and detritivore biomass) in stream microcosms. We examined the net diversity effect (i.e., the deviation between observed decomposition values in litter assemblages and the values expected from the corresponding monocultures) and, when possible, partitioned this effect into complementarity and selection effects (Loreau and Hector 2001). We used leaf litter from 9 tree species belonging to 3 families (Betulaceae, Salicaceae and Fagaceae), which were introduced in microcosms (with and without detritivores) as monocultures (SR = 1) or mixtures (SR = 3) with either low PD (3 species from the same family) or high PD (3 species from 3 different families). The above processes were quantified after 6 weeks, and the following hypotheses were examined: (1) plant SR enhances all studied processes (i.e., they have greater values in mixtures than in monocultures) (Fernandes et al. 2015, López-Rojo et al. 2019), mostly due to complementarity effects (Handa et al. 2014); (2) the difference between monocultures and mixtures is greater for high-PD than for low-PD mixtures; and (3) all the above patterns are more marked

in the presence of detritivores, which often are key drivers of biodiversity-ecosystem process relationships (Vos et al. 2011, Tonin et al. 2017, López-Rojo et al. 2018).

MATERIALS AND METHODS

Litter and detritivores

The plants used in the experiment were 3 species from the family Betulaceae (*Alnus glutinosa* (L.) Gaertner*, Corylus avellana* L. and *Betula celtiberica* Rothm. & Vasc.*),* 3 from the family Salicaceae (*Populus nigra* L.*, Salix alba* L. and *Salix atrocinerea* Brot.) and 3 from the family Fagaceae (*Castanea sativa* Mill., *Fagus sylvatica* L. and *Quercus robur* L.). These 9 species represented common litter inputs to headwater streams in our study area. Leaves were collected from the forest floor immediately after natural abscission in the autumn of 2017 from different locations in northern Spain: *A. glutinosa, C. avellana, C. sativa* and *Q. robur* at the Agüera stream catchment (43.20 °N, 3.26 °W); *B. celtiberica* and *F. sylvatica* at Urkiola natural park (43.32 °N, 2.97 °W); *S. alba* at Mungia (43.33 °N, 2.80 °W); *S.atrocinera* at the Biscay campus of the University of the Basque Country (43.32 °N; 2.97 °W); and *P. nigra* at Barakaldo (43.29 °N; 2.99 °W). Leaves were cut in fragments of about 4 cm^2 avoiding the basal midrib, air dried, and weighed to the nearest 0.01 mg using a precision balance.

Detritivores were larvae of the cased caddisfly *Sericostoma pyrenaicum*, a common invertebrate in the study area that has been often used in microcosm experiments assessing litter decomposition (Correa-Araneda et al. 2017, López-Rojo et al. 2018). Detritivores were collected manually from the benthos of Perea stream (43.291 °N, 3.243 °W) in March 2018. The initial dry mass (DM) of experimental larvae (mean ± SE: 13.87 ± 0.56 mg) was estimated from their case length (CL, measured under a binocular microscope with an accuracy of 0.5 mm; mean ± SE: 12.51 ± 0.22 mm) and the relationship $DM = 0.1398e^{CL*0.2818}$ (r^2 = 0.899). This relationship was calculated using 35 additional larvae that were collected simultaneously and with a similar case length range to experimental larvae (mean ± SE: 11.65 ± 0.52 mm), measured as above, uncased, freeze-dried and weighed. Experimental larvae were starved for 48 h just before the start of the experiment; the additional larvae were also starved for 48h before being measured and weighed.

Experimental setup

Litter treatments consisted of the 9 monocultures and six 3-species mixtures, either of low PD (species from the same family) or high PD (each species randomly assigned from each of the 3 families; Table 1). We explored whether high-PD mixtures had greater trait variability than low-PD mixtures using Rao's quadratic diversity (RaoQ; dbFD function in the 'FD' package), which is the sum of pairwise functional distances of

measured traits between species in a mixture weighted by their relative abundances (Rao 1982, Roscher et al. 2012). RaoQ was higher in two high-PD mixtures than in low-PD ones, with the exception of the mixture composed by the 3 species of family Betulaceae, which had a higher value that one of the high-PD mixtures (Table 1).

Table 1. Species comprising low-PD and high-PD litter mixtures (i.e., 3 plant species from the same family, or 3 species each from a different family, respectively), and trait variability (measured through RaoQ; value for each mixture and mean ± SE for each mixture type); PD: phylogenetic distance.

Litter mixtures	**RaoQ**
Low-PD	1.84 ± 0.46
Alnus glutinosa + Betula celtiberica + Corylus avellana	2.74
Populus nigra + Salix alba + Salix atrocinerea	1.55
Castanea sativa + Fagus sylvatica + Quercus robur	1.23
High-PD	3.24 ± 0.89
A. glutinosa + S. alba + C. sativa	4.79
C. avellana + S. atrocinerea + F. sylvatica	1.83
B. celtiberica + P. nigra + Q. robur	3.11

The experiment was carried out in March-April 2018 in 150 microcosms placed within a temperature-controlled room at 10 ºC (which mimicked natural conditions and minimized evaporation), with constant aeration and a light:dark regime of 12:12 h. The microcosms consisted of 580-mL glass jars (8 cm diameter, 11 cm height) containing 400 mL of stream water (Perea stream; soluble reactive phosphorous (P): 4.32 ± 1.25 µg P L^{-1}; dissolved inorganic N: 369.55 ± 37.59 µg N L^{-1}; n = 8) filtered through a 100-µm mesh (which allowed the entrance of microorganisms); and 30 cm^3 of sediment, composed of equal parts of fine sand (200 µm-1 mm) and small gravel (0.5 cm-1.5 cm), collected from the river bed and sterilized by incineration (550 °C, 4h). Each microcosm received 1.5 g of air-dried litter fragments (an amount that avoided resource limitation during the experiment) belonging to 1 plant species (monocultures) or to 3 species (0.5 g per species), with 10 microcosms per litter treatment. Litter fragments of the same species were kept together using safety pins to facilitate species identification at the end of the experiment; the same was done in monocultures to avoid any possible confounding effect. Litter was incubated for 72 h (with water replacement after the first 48 h) to allow the leaching of soluble compounds and initial microbial conditioning. Water was replaced with filtered (100 µm) stream water, and 7 microcosms per treatment received detritivores (2 larvae per

microcosm), while 3 microcosms per treatment remained without detritivores (in order to quantify microbial processes). We used higher replication in microcosms with detritivores because these have shown greater variability than microcosms without detritivores in previous experiments (Tonin et al. 2017, López-Rojo et al. 2019).

During the experiment, water was replaced weekly (days 7, 14, 21, 28 and 35), and the experiment finished on day 42; on each replacement, water was filtered through a 100-µm mesh in order to avoid loss of litter fragments and detritivores. At the end of the experiment (day 42) litter was collected, sorted by species in mixtures, oven-dried, weighed to determine final DM, and then divided in two subsamples. One was incinerated and re-weighed to determine final ash free dry mass (AFDM); the other was used to determine final nitrogen (N) content (using a Perkin Elmer series II CHNS/O elemental analyzer) and P content (measured spectrophotometrically after autoclave-assisted extraction (APHA 1998). From the 3 microcosms without detritivores and 3 out of the 7 microcosms with detritivores in each treatment, and before oven-drying the litter, we cut 12-mm diameter discs (5 per species) using a cork borer; discs were freeze-dried, weighed and processed in order to measure lipid ergosterol, with procedures slightly modified from Newell et al. (1988) and Suberkropp and Weyers (1996) (Supplementary Methods). Detritivores remained 48 h in starvation within the microcosms, so they were in the same conditions as at the start of the experiment; on day 44 they were uncased, freeze-dried, weighed individually to calculate their final DM, and their final N and P contents were determined as above.

Twenty-seven extra microcosms (3 per species, each containing 1.5 g of air-dried litter fragments) were used to estimate the initial (post-leaching) AFDM and several litter traits. Litter fragments were collected after 72 h, and leaf toughness was measured as the pressure required to pierce the leaf tissue using a steel rod (kPa). Then litter was oven-dried (70 °C, 72 h), weighed and divided in two subsamples. One was used to determine initial N and P contents (as above) and SLA [ratio of disc area (mm^2) to DM (mg)]. The other was incinerated (550 °C, 4 h) and re-weighed to determine the ash content and the relationships between air-dried and oven-dried DM, and between post-leaching DM and AFDM.

Data analyses

Survival of detritivores was 100% during the experiment but 2 larvae pupated, so those microcosms were excluded for the analyses. We calculated RaoQ for each litter mixture (see above) and for each litter trait (i.e., the variability of each particular trait in a mixture). Litter decomposition was quantified through proportional litter mass loss (LML), calculated as the difference between initial and final AFDM divided by initial AFDM. In microcosms with detritivores, we standardized LML using mean detritivore initial DM, in order to remove any possible effects due to differences in detritivore size

across microcosms. Detritivore biomass production was measured through proportional growth, calculated as the difference between final and initial DM divided by the initial DM. We quantified nutrient dynamics through the proportional change in litter and detritivore N and P contents (i.e., the difference between final and initial N or P content divided by initial N or P content). Initial data exploration using Cleveland dot- and boxplots revealed some potential outliers (2 data points for LML, including 1 in microcosms with detritivores and 1 in microcosms without detritivores, and 4 for detritivore growth; <5% of the data), which were removed for subsequent analyses (Ieno and Zuur 2015).

We explored the effect of plant SR on LML and ergosterol through the net diversity effect, which is the difference between the observed value of the response variable in a mixture and the expected value based on the values of the corresponding monocultures ($net_{LML} = LML_O - LML_E$) (Loreau and Hector 2001). Moreover, in order to explore the mechanisms driving any net diversity effect, we partitioned this net diversity effect into complementarity effects and selection effects. The complementarity effect was calculated as the average deviation from expected LML of species in a mixture multiplied by the mean LML of species in monoculture and the number of species (n) in the mixture (mean ΔLML × mean LML × n), and the selection effect was calculated as the covariance between deviation from expected LML of species in a mixture and in monoculture, multiplied by the number of species [cov(ΔLML,LML) × n](Loreau and Hector 2001, Handa et al. 2014, López-Rojo et al. 2018). For nutrient dynamics and detritivore growth, the existence of both positive and negative values precluded the interpretation of net diversity effects, so we directly examined differences among monocultures, low-PD and high-PD mixtures.

We ran linear mixed-effects models (lme function, 'nlme' package) testing for the effect of PD and detritivore presence (fixed factor fitted as an interaction) on all measured variables. Litter mixture was a random factor, and differences in variance between treatments with and without detritivores were considered using the VarIdent structure. As the interaction between PD and detritivore presence was not significant for any variable, we used ordinary nonparametric bootstrapped 95% confidence intervals (BCa method using the boot function on 'boot' R package, based on 999 bootstrap replicates (Davison and Hinkley 1997, Canty and Ripley 2016)), which are not subject to requirements of parametric analysis, to represent: (i) differences in net diversity, complementarity and selection effects on LML and ergosterol between low-PD and high-PD mixtures and between microcosms with and without detritivores; (ii) differences in litter N and P change between monocultures and low-PD and high-PD mixtures and between microcosms with and without detritivores; and (iii) differences in detritivore growth and detritivore N and P change between monocultures and low-PD and high-PD mixtures in microcosms with detritivores. In all cases, we determined whether the confidence intervals contained the value of zero (i.e., the null expectation

of no effect or no change) and whether the confidence intervals of different treatments overlapped (i.e., the null expectation of no differences between treatments). All statistical analyses were performed in R statistics software (R Core Team 2020).

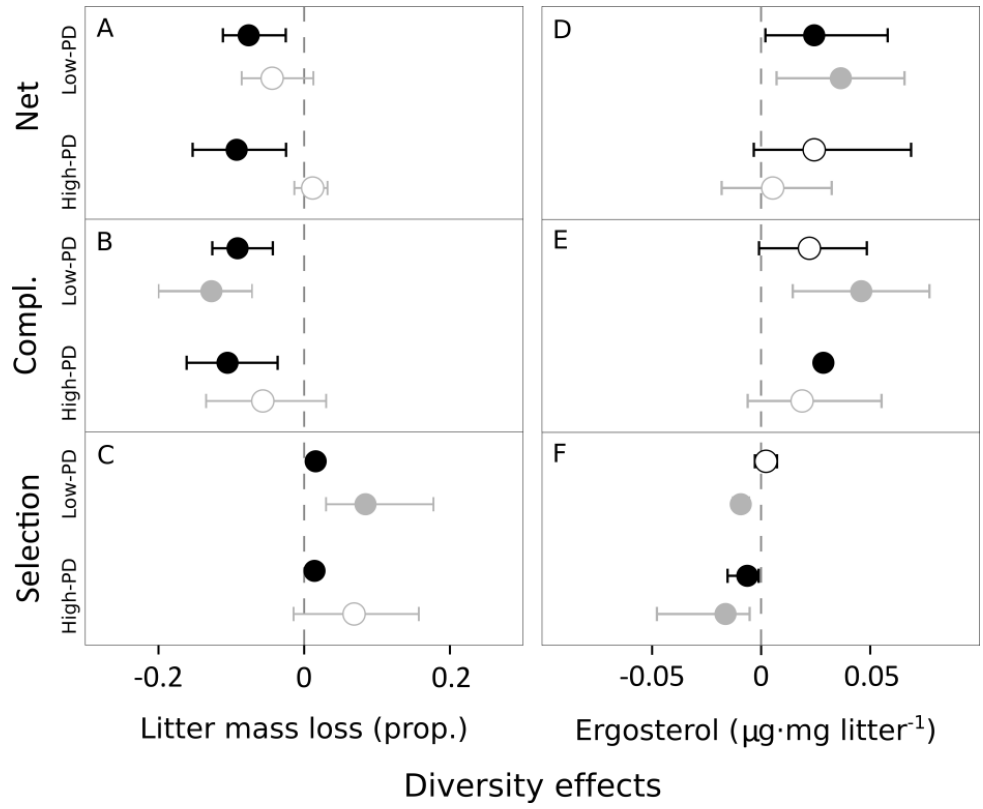

Fig. 1. Net diversity, complementarity and selection effects for litter mass loss (proportion) and ergosterol content (µg · mg litter $^{-1}$) for low-PD and high-PD treatments, with (black) and without (grey) detritivores. Circles are means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis (i.e., do not contain the value of zero) and open circles represent intervals that do not reject the null hypothesis.

RESULTS

The net diversity effect on decomposition (quantified through LML) was mostly negative (i.e., LML was higher in monocultures than in mixtures), and significant only in the presence of detritivores (Fig. 1A). In contrast, the net diversity effect on fungal biomass production [quantified through lipid ergosterol (Gessner and Chauvet 1993)] was positive (i.e., there was more ergosterol in mixtures than in monocultures), and the effect was significant only for low-PD mixtures (Fig. 1D). When the net diversity effect was partitioned into complementarity and selection effects, results again differed for LML and ergosterol: for LML, complementarity was negative (Fig. 1B) and

selection was positive in low-PD (with and without detritivores) and high-PD mixtures (with detritivores: Fig. 1C); for ergosterol, there was positive complementarity (significant in low-PD mixtures without detritivores and high-PD mixtures with detritivores; Fig. 1E) and negative selection (except for low-PD mixtures with detritivores; Fig. 1F). Diversity effects were thus almost entirely driven by complementarity effects in the presence of detritivores, with important contribution of selection effects in their absence (Table S4).

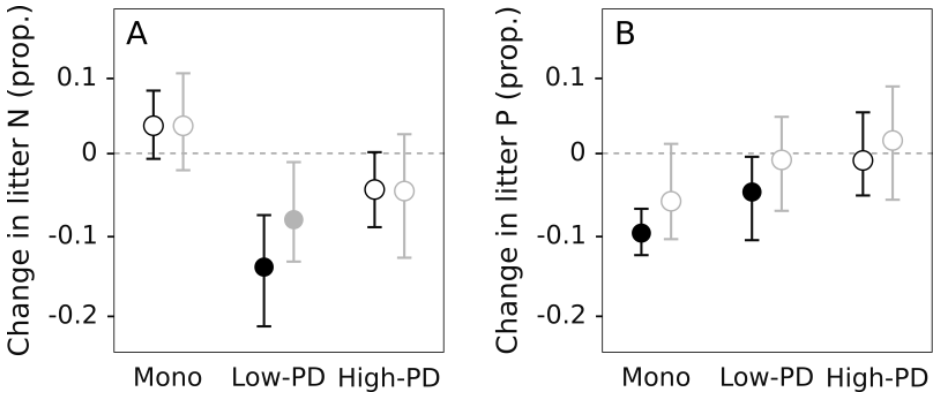

Fig. 2. Proportional change in litter nitrogen and phosphorus content (mg · g litter^{-1}) for monocultures (Mono), low-PD and high-PD litter mixtures, with (black) and without (grey) detritivores. Circles represent means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis (i.e., do not contain the value of zero) and open circles represent intervals that do not reject the null hypothesis.

Nutrient dynamics in litter showed differences between monocultures and mixtures. Litter N concentration tended to increase in monocultures and decrease in mixtures, although the difference was only significant for low-PD mixtures in the presence of detritivores (Fig. 2A). Litter P concentration decreased in the presence of detritivores in monocultures and low-PD mixtures, with no change in high-PD mixtures; there was an increasing trend from monocultures to high-PD mixtures both in the presence and absence of detritivores, but it was not significant (Fig. 2B). Detritivore biomass production (quantified through growth) was highly variable and showed no differences between treatments (Fig. 3A). Detritivores decreased their N proportional content, and the decrease was higher in mixtures than in monocultures (Fig. 3B). In contrast, detritivores increased their P proportional content in monocultures and low-PD mixtures and showed a similar but nonsignificant trend in high-PD mixtures; the pattern shown was opposite to that in P litter concentration (Fig. 3C).

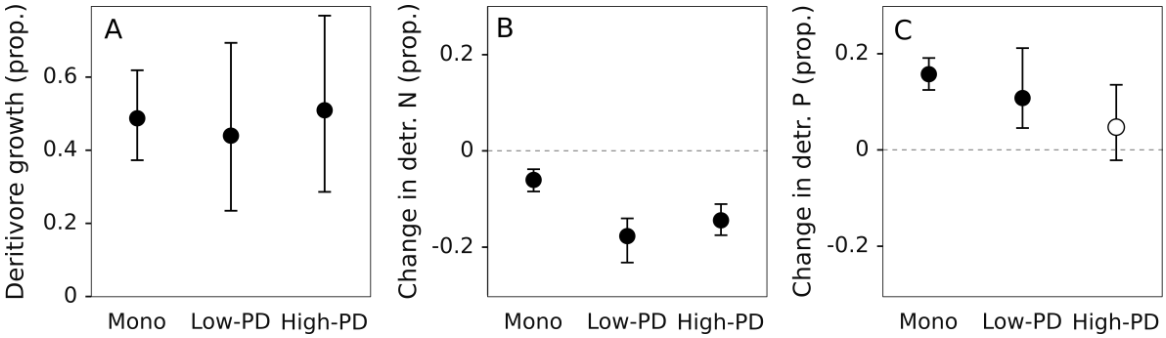

Fig. 3. Detritivore biomass production and change in nitrogen and phosphorus (proportion) for monocultures (Mono), low-PD and high-PD litter mixtures. Circles represent means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis (i.e., do not contain the value of zero) and open circles represent intervals that do not reject the null hypothesis.

DISCUSSION

Litter decomposition was lower in mixtures than in monocultures due to negative complementarity

Our experiment revealed a negative effect of plant species richness on litter decomposition: monocultures decomposed, on average, faster than litter mixtures. This result was unexpected when compared with several other microcosm experiments, which have found faster decomposition of litter mixtures than monocultures (Fernandes et al. 2015, Tonin et al. 2017, López-Rojo et al. 2018, López-Rojo et al. 2019). In most of the above-mentioned microcosm experiments, diversity effects occurred only in the presence of detritivores, suggesting that they were the key drivers of such effects, and the main underlying mechanism was a positive complementarity effect. Similarly, in our study, the diversity effect was significant in the presence of detritivores; in their absence, complementarity and selection effects presented similar but opposite values that counterbalanced each other (see below).

Positive complementarity can occur when different litter types offer complementary resources to consumers, or when the presence of one litter type enhances the consumption of another (i.e., facilitation), and is often greater than the positive selection effect (i.e., when a given litter type is decomposed faster than others). For example, an experiment found that complementarity accounted for 66% of the diversity effect on decomposition on average (and up to 99%) in several litter mixtures (López-Rojo et al. 2018). In our study, we also found that complementarity was the dominant mechanism behind diversity effects on decomposition in the presence of detritivores (selection effects were significant and positive, but only accounted for 13% of the net diversity effect on average) but, in this case, it was negative complementarity.

Negative complementarity was also found in a field study with a similar design to ours (López-Rojo et al. 2020b), and could indicate some kind of physical or chemical interference between litter types. For example, toxic compounds present in one species could inhibit the consumption of another that would otherwise be consumed faster (McArthur et al. 1994, Graça et al. 2001). In our study, in the absence of detritivores, negative complementarity and positive selection were similar in magnitude (53% and 47% on average, respectively), resulting in a non-significant net diversity effect. This suggests that selection effects were more relevant for microbial than for detritivore-mediated decomposition, and indicates that the lack of net diversity effects on microbial decomposition found here and in other studies (López-Rojo et al. 2018, López-Rojo et al. 2019) could be due to different mechanisms operating in opposite ways, rather than to the absence of interactions between litter diversity and microbial decomposers.

Fungal biomass production was higher in litter mixtures, mostly in those with low phylogenetic distance

Despite the negative effect of plant species richness on decomposition, the effect on fungal biomass production was opposite, that is, litter mixtures produced more fungal biomass than expected from monocultures. This may result in greater litter conditioning (Gessner et al. 1999) and thus enhance detritivore-mediated decomposition in the longer term. However, this was significant only for low-PD mixtures (with and without detritivores), and driven by positive complementarity (which accounted for 87% of the net diversity effect on average), suggesting the existence of resource partitioning or facilitation among fungal species. This can occur if different species within the fungal assemblage differ in their enzymatic complements or activity patterns (Gessner et al. 2010), or benefit from the presence of litter types differing in physical structure [e.g., contrasting toughness or specific leaf area (SLA)], which increase habitat complexity and stability. However, we cannot confirm this as we did not characterise fungal assemblages. Moreover, in our case, such effects did not translate into differences in microbial decomposition, such as those shown in terrestrial ecosystems (Hättenschwiler and Gasser 2005), possibly due to functional redundancy of fungal species (Gessner et al. 2010).

Although we did not quantify fungal species richness, other studies have found that it is positively related to plant (litter) species richness, in relation to a higher functional trait diversity (Rajashekhar and Kaveriappa 2003, Laitung and Chauvet 2005). In our study, high-PD mixtures tended to have higher trait diversity than lower-PD mixtures. Thus, it is possible that fungal assemblages growing on our high-PD mixtures were more diverse than those growing on low-PD mixtures, and more diverse fungal assemblages generally show slower production due to increased interspecific competitive interactions (Gessner et al. 2010). In high-PD assemblages, positive

complementarity was the dominant mechanism (82% of the net diversity effect) only in the presence of detritivores, which most likely mediated this complementarity effect. In the absence of detritivores, positive complementarity and negative selection were similar in magnitude (53% and 47%, respectively), as occurred for decomposition, resulting in a very low and non-significant net diversity effect.

Nutrient dynamics was influenced by plant species richness, with a lower influence of phylogenetic distance

Plant species richness affected the dynamics of N and P in litter and detritivores, but had no effect on detritivore biomass production, which was 42% on average (i.e., 1.14% per day); this is within the range reported elsewhere for *Sericostoma* spp. (0.75-2.99%) (Friberg and Jacobsen 1999, López-Rojo et al. 2019). While litter monocultures tended to present higher N concentration (although the trend was not significant), it tended to be lower in mixtures (being the reduction significant only for low-PD mixtures, and significantly different from that of monocultures only in the presence of detritivores). This suggests that more N was used from litter in mixtures, which is in accordance with their higher fungal biomass production, and with the key role of microorganisms in N dynamics shown elsewhere (Tonin et al. 2017, López-Rojo et al. 2019). We note, however, that N litter content cannot be separated from N content of colonising fungi. In contrast, detritivores reduced their N proportional content in all cases, but less so when exposed to monocultures, suggesting that detritivores were able to use more N from litter when fungal activity was lower. The general reduction in detritivore N content could be due to the fact no litter type fulfilled their N demands (even if *A. glutinosa* had high N concentration; Table S1); these demands are usually high for caddisflies because they use it for the production of silk and N-rich chitin for the exoskeleton (Frainer et al. 2016).

The dynamics of P showed a different pattern, which was opposite in litter and detritivores: litter decreased its P proportional content in monocultures and low-PD mixtures in the presence of detritivores, and detritivores increased their P proportional content when exposed to monocultures and low-PD mixtures; the trend was similar for high-PD mixtures in both cases, albeit not significant. This suggests that P dynamics were highly dependent on detritivores, which used P from monocultures and low-PD mixtures more efficiently than from high-PD mixtures, that is, from litter with lower diversity of functional traits in general or P in particular (P variability was 0.17% ± 0.09 SE in low-PD mixtures and 0.84% ± 0.39 in high-PD mixtures; Supplementary Table 2). This agrees with studies suggesting that detritivores can benefit from the concentration of resources (Friberg and Jacobsen 1994, Boyero et al. 2016) and with the fact that detritivore biomass production was not constrained by P supply, as shown elsewhere (Frainer et al. 2016).

Biodiversity effects on ecosystem functioning may depend on experimental conditions and on the biodiversity measure used

Our study supports previous evidence that plant biodiversity loss can affect litter decomposition and associated processes in stream ecosystems. However, it suggests that effects can be variable depending on the available litter, fungal assemblages and detritivore numbers used, and hence the biological interactions allowed. Experimental conditions thus seem to be main determinants of outcomes, which have been variable among different field and microcosm experiments (Lecerf and Richardson 2010). This is particularly true for field studies, which have often found positive, negative and/or no effects at different sites (Handa et al. 2014) or for different litter mixtures (Lecerf et al. 2007, Taylor et al. 2007). Many microcosm experiments have found positive diversity effects (Vos et al. 2013, Fernandes et al. 2015, Tonin et al. 2017, López-Rojo et al. 2019), but these sometimes depended on which species were lost (Boyero et al. 2014), and here we found negative diversity effects. Contrasting results could be related to differences in experimental conditions, mainly regarding two aspects.

Firstly, studies or sites with more diverse detritivore assemblages have more potential for complementary resource use (Handa et al. 2014). However, at the same time, the balance between different positive and negative interspecific and intraspecific interactions mediating diversity effects is more variable (McKie et al. 2009, Tonin et al. 2018), which may obscure the results (as discussed for microbial decomposition above). This, however, may not apply to many microcosm experiments, which use a single detritivore species, although intraspecific interactions could also play a role (Boyero and Pearson 2006), for example between individuals with different body size (Reiss et al. 2011), and due to density-dependent effects (McKie et al. 2008). In our experiment, each microcosm contained 2 individuals, which differed from other experiments using more individuals per microcosm [e.g., 3 in López-Rojo et al. (2019); 6 in Boyero et al. (2014)], hence with more potential for intraspecific interactions (and positive diversity effects) in the latter.

Secondly, the amount and types of litter provided could influence the results of microcosm experiments (but not so much in field studies, where litter other than that provided within litter bags is generally available in the stream). This may also help explain the outcome of our study (a negative diversity effect on decomposition) compared to other microcosm experiments. In particular, we provided litter in large excess, and > 60% of the litter preferred by detritivores (*A. glutinosa*) remained at the end of the experiment in mixtures; in contrast, others have provided more limited amounts (Tonin et al. 2017). The presence of a limiting amount of the preferred litter in mixtures may enhance the consumption of other litter types, and thus enhance overall decomposition compared to monocultures. However, this may not happen if

the preferred litter is highly abundant in mixtures, because detritivores would feed mostly on it and there would be no differences with monocultures.

Another relevant question raised here is how to measure biodiversity in these studies. We found that phylogenetic distance had no effect on decomposition, but it influenced nutrient dynamics, which would have been only partially assessed by exploring species richness only. This is despite the fact that phylogenetic distance and trait variability was not strongly related, at least in relation to the traits that we measured; the inclusion of other traits such as tannins (which are generally high in the Fagaceae) would most likely have increased this relationship. Nevertheless, we provide evidence of a key role of trait-based biodiversity measures such as phylogenetic distance on nutrient dynamics (which are little explored compared to decomposition) highlights the relevance of compositional changes in vegetation for stream ecosystem functioning, regardless of changes in species richness. Still, our results should be taken with caution because we did not include all possible low-PD and high-PD mixtures resulting from different combinations of the plant species used. Given that other studies have found either significant (Boyero et al. 2016, LeRoy et al. 2019) or non-significant effects (López-Rojo et al. 2020b) of phylogenetic distance on litter decomposition in streams, and that its effects on associated processes (such as those examined here) are mostly unknown, we suggest that this issue merits further investigation.

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SUPPORTING MATERIAL

Supplementary Methodology

Ergosterol was extracted from the frozen-dried litter discs (≈50 mg) in 10 mL screw-cap test tubes by 30 m of refluxing in 2 mL of KOH-methanol at 80 °C using a dry-bath system. Once cooled at room temperature, the mixture was treated with 1 mL of a saturated NaCl solution (≈ 0.36 g mL^{-1}) to saturate the aqueous-phase, and sterols were extracted from the alcoholic base by partitioning with the addition of 1 mL of n-hexane (HPLC grade). Samples were then stirred in a vortex mixer for 30 s and centrifuged for 4 m at 1165RCF. The supernatant (n-hexane phase containing sterols) was collected, transferred to 1.5 mL HPLC vials, and evaporated to dryness under a stream of N_2. A second extraction was carried out adding another 1 mL of n-hexane to the sample test tube, and repeating the above process in the same HPLC vial. The dry residue was dissolved in 1 mL of methanol (HPLC grade) and immediately injected into a high-pressure liquid chromatography system (HPLC).

Table S1. Litter traits (mean ± SE) measured for each plant species: nitrogen (N), phosphorus (P) and ash contents (%), specific leaf area (SLA; $mm^2 \cdot mg^{-1}$) and leaf toughness (kPa).

Plant species	%N	%P	%Ash	SLA	Toughness
A. glutinosa	3.05 ± 0.11	0.08 ± 2e-3	13.81 ± 3.33	17.47 ± 0.29	1672 ± 174
C. avellana	1.38 ± 0.05	0.07 ± 4e-3	18.60 ± 3.13	19.54 ± 2.74	1563 ± 116
B. celtibérica	1.61 ± 0.13	0.05 ± 1e-3	9.38 ± 1.26	16.41 ± 0.49	2271 ± 161
P. nigra	1.53 ± 0.09	0.13 ± 2e-3	15.57 ± 0.64	11.90 ± 0.75	3859 ± 715
S. alba	1.80 ± 0.10	0.12 ± 2e-3	23.41 ± 1.10	12.97 ± 0.51	3036 ± 116
S. atrocinerea	1.79 ± 0.06	0.09 ± 5e-3	21.36 ± 6.27	13.86 ± 0.56	2371 ± 137
C. sativa	1.40 ± 0.07	0.05 ± 3e-3	12.63 ± 2.06	19.64 ± 0.99	1640 ± 107
F. sylvatica	0.91 ± 0.12	0.05 ± 3e-3	14.70 ± 4.26	17.87 ± 2.25	2142 ± 187
Q. robur	1.41 ± 0.13	0.05 ± 6e-3	9.57 ± 1.14	14.71 ± 0.86	2752 ± 117

Table S2. Mean value and variability (RaoQ) of litter traits for each litter mixture: nitrogen (N), phosphorus (P) and ash contents (%), specific leaf area (SLA; $mm^2 \cdot mg^{-1}$) and leaf toughness (kPa).

	%N		%P		%Ash		SLA		Toughness	
Litter mixture	**mean**	**RaoQ**	**mean**	**RaoQ**	**mean**	**RaoQ**	**mean**	**RaoQ**	**mean**	**RaoQ**
Low-PD										
A. glutinosa + B. celtiberica + C. avellana	2.00	1.56	0.07	0.19	13.90	0.60	17.80	0.21	1840	0.17
P. nigra + S. alba + S. atrocinerea	1.71	0.04	0.12	0.32	20.14	0.47	12.90	0.08	3095	0.64
C. sativa + F. sylvatica + Q. robur	1.24	0.16	0.05	0.00	12.28	0.19	17.34	0.52	2192	0.36
High-PD										
A. glutinosa + S. alba + C. sativa	2.06	1.42	0.08	0.67	16.60	0.98	16.72	0.98	2115	0.75
C. avellana + S. atrocinerea + F. sylvatica	1.34	0.37	0.07	0.27	18.05	0.32	17.18	0.68	2023	0.20
B. celtiberica + P. nigra + Q. robur	1.51	0.02	0.08	1.60	11.38	0.33	14.39	0.41	2939	0.74

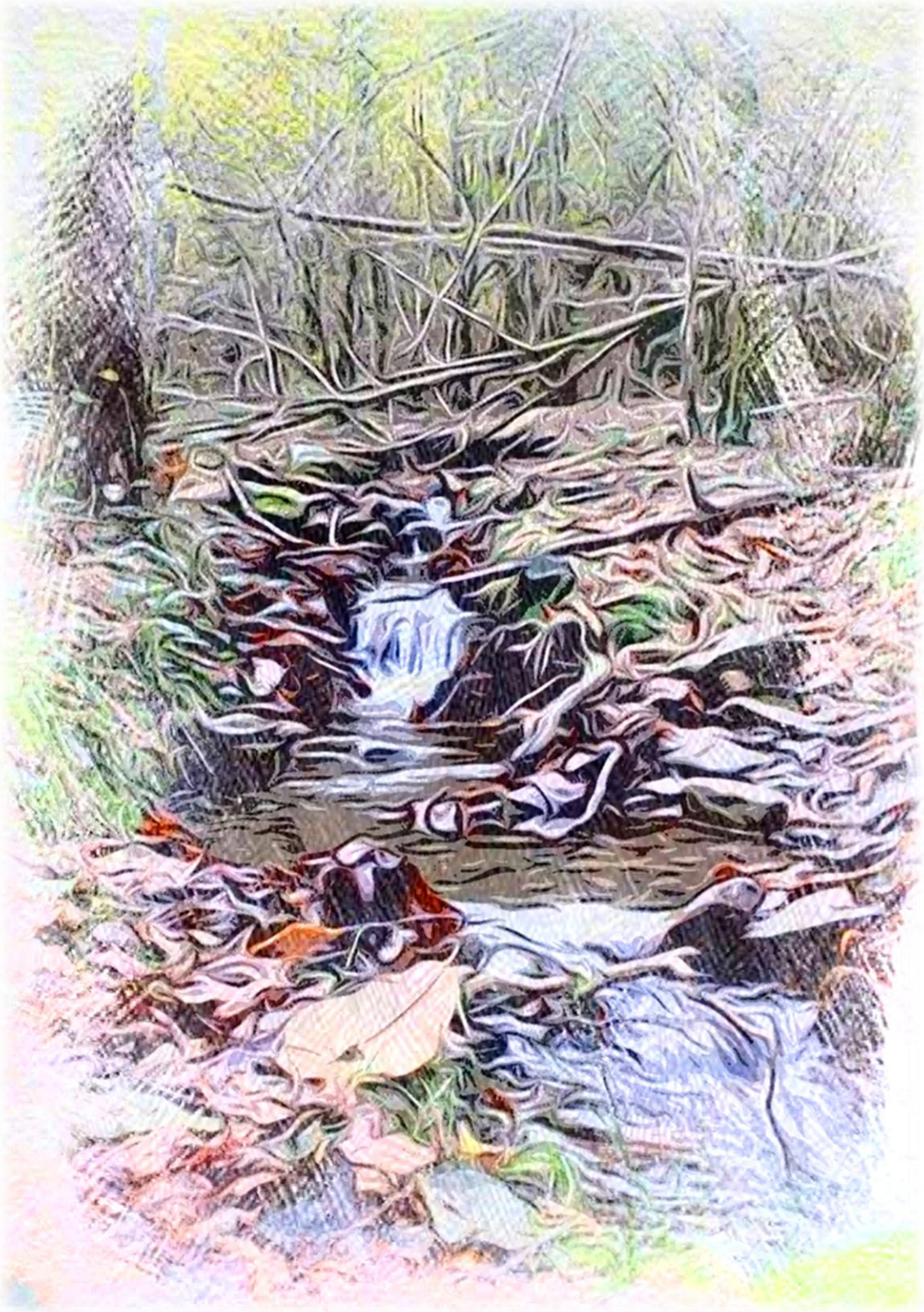
Table S3. Results of linear mixed-effects models testing for the effect of diversity, detritivore presence and their interaction on the response variables. Diversity levels were low-PD and high-PD treatments for net, complementarity and selection effects on decomposition (measured through leaf mass loss, LML) and fungal biomass production (measured through ergosterol); and monocultures vs. low-PD vs. high-PD treatments for the change in litter and detritivore nitrogen (N) and phosphorus (P) and detritivore growth. df: numerator and denominator degrees of freedom; F: F-statistic value; p: p-value.

Variable	Effect	df	F	p
Net LML	Diversity	1,4	0.25	0.639
	Detritivore presence	1,49	3.34	0.073
	Diversity: Detr. presence	1,49	0.08	0.769
Complementarity LML	Diversity	1,4	0.04	0.854
	Detritivore presence	1,49	3.11	0.084
	Diversity: Detr. presence	1,49	0.08	0.777
Selection LML	Diversity	1,4	0.36	0.578
	Detritivore presence	1,49	0.04	0.837
	Diversity: Detr. presence	1,49	1.11	0.297
Net Ergosterol	Diversity	1,4	0.49	0.519
	Detritivore presence	1,28	0.02	0.884
	Diversity: Detr. presence	1,28	1.08	0.306
Complementarity Ergosterol	Diversity	1,4	0.86	0.406
	Detritivore presence	1,28	0.09	0.759
	Diversity: Detr. presence	1,28	0.14	0.706
Selection Ergosterol	Diversity	1,4	1.10	0.353
	Detritivore presence	1,28	5.30	0.029
	Diversity: Detr. presence	1,28	0.01	0.900
Change in litter N	Diversity	2,12	1.98	0.179
	Detritivore presence	1,13	0.66	0.418
	Diversity: Detr. presence	2,13	0.75	0.474
Change in litter P	Diversity	2,12	1.353	0.295
	Detritivore presence	1,13	4.955	0.027
	Diversity: Detr. presence	2,13	0.068	0.933
Detritivore growth	Diversity	2,12	0.049	0.951
Change in detritivore N	Diversity	2,12	14.538	<0.001
Change in detritivore P	Diversity	2,12	1.429	0.279

Table S4. Contribution (%) of complementarity and selection effects to the net diversity effect (sum of absolute value complementarity and selection effects) of litter mass loss (LML) and ergosterol content for each treatment (low- or high-PD) with and without detritivores.

	With detritivores		Without detritivores	
Variable	**Complementarity**	**Selection**	**Complementarity**	**Selection**
LML				
Low PD	85.66	14.34	60.38	39.62
High PD	88.47	11.53	45.40	54.60
Ergosterol				
Low PD	91.04	8.96	83.01	16.99
High PD	81.68	18.32	53.35	46.65

GENERAL DISCUSSION



GENERAL DISCUSSION

This thesis aimed to shed light on several important gaps of BEF research, by addressing key questions regarding the effects of plant litter diversity loss on stream ecosystem functioning. Despite the large number of experimental studies that have addressed BEF relationships in the last two decades (Tilman and Downing 1994, Cardinale et al. 2011, Mora et al. 2011), there still is much uncertainty concerning the key processes operating in detrital food webs, particularly in stream ecosystems (Mori et al. 2020). In this synthesis I try to provide some answers to these issues that derive from the different chapters of the thesis, and raise new questions that may stimulate future research.

The first fundamental issue addressed here was the lack of information on how biodiversity could simultaneously impact multiple processes, all of which are fundamental to the functioning of stream ecosystems. Many stream food webs are to a large extent detrital, with most energy deriving from allochthonous leaf litter (Tank et al. 2010), and so most stream BEF studies have focused on the process of litter decomposition, often quantified as the rate of litter mass loss (Hector et al. 2000). However, I predicted that examining this process in isolation would not be sufficient to understand how the ecosystem is affected by changes in plant litter diversity. A number of other processes occur and are intimately linked to decomposition (Gessner et al. 2010), so I expected that their joint examination would provide a more comprehensive picture of how stream ecosystems may be altered by changes in plant litter diversity (**Chapters 1 and 2**). Moreover, I explored the concept of multifunctionality (**Chapter 2**), which intends to encapsulate ecosystem functioning in a single measurement (Byrnes et al. 2014) and has received considerable attention in recent literature, but at the same time has been controversial (Gamfeldt and Roger 2017).

My results provided novel evidence of simultaneous effects on an array of relevant processes (i.e., decomposition, nutrient cycling and biomass production, among others) and demonstrated that (1) different processes provide complementary information that cannot be ignored when assessing effects at the ecosystem level; and (2) despite the advantages of using multifunctionality indices in some instances, the separate analysis of different processes is essential to understand how organisms, food webs and, ultimately, ecosystems, will be altered by changes in plant litter diversity. It was particularly interesting to see that processes related to nutrient cycling (i.e., nutrient loss from litter and increases in the water and in detritivores) were more often affected by changes in plant litter diversity than decomposition. Given that the latter process is by far the most examined process in relevant studies, many effects of diversity loss on ecosystem functioning may have been overlooked by ignoring nutrient

cycling. A thorough assessment of BEF relationships thus requires an understanding of which processes are fundamental to the functioning of ecosystems, and the examination of these individual processes, which may also be examined using multifunctionality metrics for further verification of overall effects.

The second question explored here was the potential interaction between detrital and autotrophic stream food webs (**Chapter 3**), which often coexist, mostly downstream the headwaters that have been explored in other chapters (Halvorson et al. 2020). Part of the terrestrial litter entering these low-order streams is not processed in situ, being transported downstream and processed in mid-order reaches, where the contribution of the autotrophic pathway gains importance (Vannote et al. 1980). Here, I explored BEF relationships between microbial decomposers and primary producers, a topic that has been understudied. Although the results did not support the hypothesis of bidirectional effects of biodiversity on ecosystem functioning between both food webs, I found that the green food web was in fact affected by the presence and identity of leaf litter, which mainly determined the structure of periphytic algal assemblages) and by several litter traits, which influenced net primary production.

The third issue examined in this thesis was whether different types of biodiversity were more or less relevant within the context of BEF research. Thus, I went beyond the assessment of species richness effects and considered also functional aspects of biodiversity based on biological traits and species phylogenetic relatedness. To explore this question I used two approaches, a field experiment (**Chapter 4**) and a microcosm experiment (**Chapter 5**), which offered different perspectives. Thus, while the field experiment allowed the examination of processes and effects mediated by whole stream assemblages and under natural environmental conditions, microcosms offered the opportunity to study processes that are difficult to explore in the field, mostly those related to C and nutrient cycling. In both cases I looked at the consequences of changing plant species richness, functional diversity (measured through a set of relevant biological traits) and phylogenetic distance on decomposition and other processes. My results revealed important differences among these biodiversity measures, and highlighted the central role of several litter traits (mostly the concentrations of major nutrients, N and P) as drivers of changes in decomposition and associated processes. Plant species richness (the most used metric in BEF research) influenced decomposition and other processes, but effects were not entirely consistent across experiments, which suggests that this metric should not be used solely without considering more functional aspects such as the mean concentrations and diversity of traits. Lastly, effects of plant phylogenetic distance were in most cases weaker than those of the other metrics, so it should be used with caution. Given that some important traits might not be phylogenetically conserved (Moles et al. 2013) or may show convergent evolution (Ackerly and Reich 1999) or phenotypic plasticity

(Valladares et al. 2007), more detailed information about these issues may be required when phylogenetic distance is used.

Finally, I addressed the scarce knowledge about the biological mechanisms underlying BEF relationships (**Chapters 1, 4 and 5**). In order to improve our understanding of such mechanisms I used the diversity partitioning approach (Loreau and Hector 2001), which separates net diversity effects into complementarity (which evidences a key role of interspecific interactions) and selection (which indicates that effects are mainly driven by a particular species), both of which can either be positive or negative. I observed that diversity effects on ecosystem functioning were in most cases driven by complementarity (Fig. 1), which was greater than selection, both in laboratory and field experiments. This occurred even in the presence of especially nutrient-rich species, which may be expected to drive large selection effects. Moreover, all these patterns were particularly evident in the presence of detritivores, with differences in magnitude between different mechanisms being less marked in the absence of detritivores.

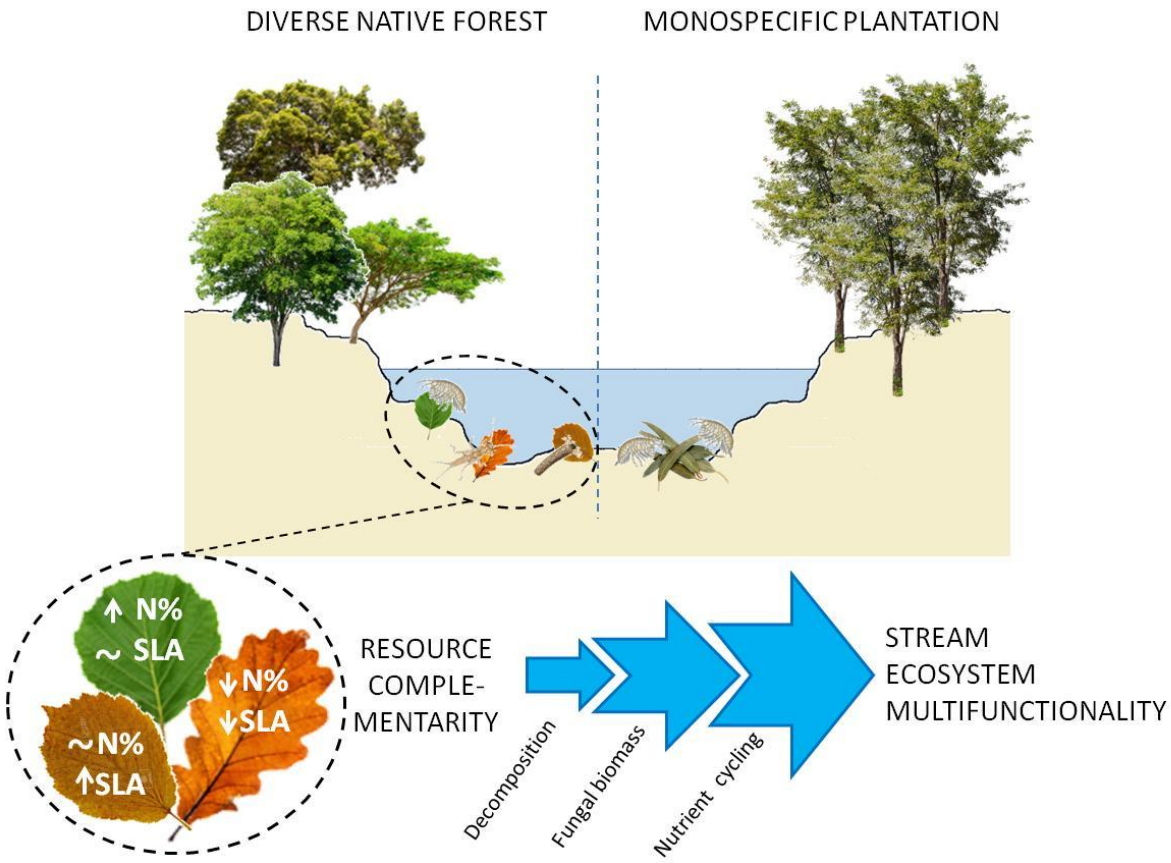


Fig 1. Graphical illustration of main processes and mechanisms identified to drive BEF relationships in streams

Below I discuss the main findings of each of the above topics, their potential implications within and beyond streams, and some insights and future research directions that arise from my results.

MULTIFUNCTIONALITY: LITTER DIVERSITY EFFECTS GO BEYOND THE DECOMPOSITION PROCESS

I found that, in the presence of detritivores, several ecosystem processes were negatively affected by the loss of plant litter diversity, quantified as species richness. When I explored diversity effects on multiple processes simultaneously through the use of multifunctionality indices (with two different approaches: averaging and multiple threshold) I found an overall, strong alteration of ecosystem functioning. In contrast, when detritivores were absent, only two of the measured processes (P loss from litter and N release to the water) showed a significant (and negative) relationship with diversity, which were translated in an overall effect on multifunctionality. The negative relationship between diversity and N release to the water indicated greater N uptake from the water column (**Chapter 2**); this, together with the significant diversity effect on ergosterol content (an indicator of fungal biomass, Gessner and Chauvet 1993) found in **Chapter 4,** suggested a positive effect of plant litter diversity on fungal growth. During the experiment, however, enhanced fungal growth did not imply higher microbial decomposition, although it is possible that effects may arise in the longer term, as microbial leaf litter processing generally occurs at a slower pace than detritivore feeding (Hieber and Gessner 2002). The lack of diversity effects on microbially-mediated decomposition, commonly found in BEF studies, does not necessarily mean that microbial decomposers are not affected by plant litter diversity, as reported elsewhere (Swan and Palmer 2004, Sanpera-Calbet et al. 2009). Conversely, the strong effects of plant litter diversity on nutrient dynamics and fungal biomass found here suggest that more attention should be paid to the microbial compartment, particularly to predict changes in ecosystem functioning in the long term.

In the presence of detritivores, the greatest diversity effect was observed for P release to the water, with the most diverse polyculture outperforming the best monoculture. This phenomenon, often called transgressive overyielding, has been seldom reported and demonstrates a strong diversity effect (Cardinale et al. 2007). The alteration of nutrient transfer across different compartments of the ecosystem (litter, water, and detritivores) is an issue rarely addressed in BEF research, but here I show its relevance when predicting biodiversity effects on ecosystem functioning. It is worth noting, however, that changes in nutrient cycling may be overestimated in microcosm experiments because nutrients are so readily available in streams, due to the action of

flow. Nevertheless, this applies to the transfer of nutrients from and to the water at small scales, but not to transfer through fungal hyphae (Handa et al. 2014). For example, in **Chapter 3**, I found positive diversity effects on litter P loss in the field, which contrasted with the negative effects on decomposition.

Terrestrial litter decomposition and the concomitant recycling of nutrients and production of detritivore biomass are central processes maintaining the functioning of headwater stream ecosystems. My results highlight the advantage of considering these processes together rather than focusing on decomposition only as proxy for stream ecosystem functioning (Chauvet et al. 2016) when exploring effects of biodiversity loss. Moreover, plant litter diversity effects on decomposition were more variable across experiments than effects on nutrient cycling, and seemed to be more influenced by experimental methodology (e.g., the amount and types of litter provided). In contrast, diversity effects on nutrient cycling were more consistent and are thus likely to be highly relevant for future BEF studies.

NO EVIDENCE OF BEF RELATIONSHIPS ACROSS DIFFERENT FOOD WEB PATHWAYS

Most BEF studies have examined biodiversity loss effects on ecosystem processes that occur within a particular pathway of the stream food web (e.g., the detrital or brown pathway in **Chapters 1, 2, 4 and 5**). Such interactions are to be expected in low-order streams where the detrital pathway is dominant, as mentioned above. However, interactions between brown and green pathways occur in streams (Danger et al. 2007, Halvorson et al. 2020), and hence BEF relationships between both pathways may be expected and were examined in **Chapter 3**. However, I was unable to find evidence of such reciprocal biodiversity effects. Interestingly, I observed that the presence and identity of plant litter had an influence on algal assemblage structure, and on the relationship between algal diversity and carrying capacity. This influence may be important because part of the litter entering headwater streams is transported downstream to mid and low reaches, where the green pathway is dominant (Vannote et al. 1980). I suggest that future studies should further explore BEF relationships across food web compartments, including interactions across multiple trophic levels, which are also underexplored (but see Srivastava et al. 2009).

THE DIVERSITY OF KEY TRAITS IS HIGHLY RELEVANT FOR ECOSYSTEM FUNCTIONING

Biodiversity measures other than species richness have been rarely used when addressing plant diversity effects on decomposition, but inconsistencies across studies suggest that species richness might not be the most important feature of biodiversity within this context. However, I found no clear effects of plant phylogenetic distance on

litter decomposition, either in field or laboratory approaches (**Chapters 4 and 5**). This result contrasts with several terrestrial studies showing that plant phylogenetic distance is a good predictor of plant biomass accumulation (Cadotte et al. 2008, Flynn et al. 2011), a contrast that might be explained by differences in the relevant biological traits. The assumption that plant phylogenetic distance can influence an ecosystem process is based on the existence of a strong phylogenetic signal in the traits driving the process, meaning that traits present more similar values in close relatives than in distant ones (Flynn et al. 2011). For example, it is known that the main traits determining grassland primary production exhibit strong phylogenetic signals (Srivastava et al. 2012). In contrast, riparian leaf litter traits relevant for decomposition have shown weak phylogenetic signal (Boyero et al. 2017). These differences between 'live' leaves and 'dead' leaf litter may be related to the nutrient resorption that occurs prior to leaf senescence (Yuan and Chen 2009) and merit further attention.

In contrast to the lack of effect of phylogenetic distance on stream ecosystem functioning, I found strong effects of several litter traits and their diversity. The most relevant ones were the concentrations of essential nutrients (i.e., N and P) and secondary compounds (i.e., condensed tannins), which together largely contribute to litter quality. Previous experiments at large spatial scales and systematic reviews have demonstrated that litter quality, together with environmental conditions, is a major driver of litter decomposition in both aquatic and terrestrial ecosystems (Cornwell et al. 2008, García-Palacios et al. 2016). Here, I demonstrated that not only the mean value of these traits is relevant, but also their diversity or variability within litter mixtures is important. Interestingly, I observed that traits related to litter chemical composition were relevant mostly for detritivores, while traits related to its physical structure (e.g., hemicellulose and specific leaf area) were mostly relevant for microorganisms (**Chapter 3**). This finding seems counterintuitive, given that detritivores mostly shred on litter and thus would be expected to be affected by their toughness; microorganisms, in contrast, secrete enzymes that decompose litter and subsequently use the released C and nutrients, so they should be mostly affected by the chemical composition of litter (Marks 2019). This is thus an interesting avenue that merits further exploration in future studies.

The importance of litter trait variability for decomposition and nutrient cycling had been previously shown for terrestrial ecosystems. For example, García-Palacios et al. (2017) found that both mean trait values and their variability had a large influence on litter C and N loss, even when compared with the influence of environmental factors. Here, I found that effects of trait variability were higher for nutrient cycling than for decomposition, as occurred with species richness. Another important finding was the fundamental role of the variability of N concentration in litter mixtures (**Chapters 4, 5**). In particular, the high N concentration of alder litter probably enhanced the use of other litter types that are poorer in this nutrient (see discussion

about complementarity effects below). It is worth noting that trait variability can be measured using different indices, which might lead to different results, although large differences are unlikely.

COMPLEMENTARITY IS A MAJOR MECHANISM DRIVING BEF RELATIONSHIPS

I found ample evidence that complementarity effects were a main driver of BEF relationships in stream ecosystems, as they were always greater in magnitude than selection effects. This is consistent with results of previous field and microcosm studies (e.g., Handa et al. 2014, Tonin et al. 2017), and indicates that detritivores (and, to a lesser extent, microorganisms) can benefit from the use of complementary resources provided by different litter types (i.e., species). For example, the presence of litter with different nutrient concentrations can help organisms meeting with their stoichiometric requirements (Cross et al. 2005, Manzoni et al. 2010). Detritivores often feed preferentially on certain litter types (Graça et al. 2001), and fungal mycelia can colonize different litter types at the same time and selectively take different nutrients from them. The latter phenomenon has been described as nutrient transfer between litter types (Handa et al. 2014) and has also been observed in terrestrial studies, not only for major nutrients such as N but also for labile C (Schimel and Hättenschwiler 2007) and micronutrients such as potassium, calcium and magnesium (Briones and Ineson 1996). Interestingly, these studies pointed to a greater complementarity effect on nutrient dynamics than on decomposition, which agrees with my results discussed above.

Litter mixtures can be more or less diverse not only based on their nutrient concentrations, but also due to their different structural traits (e.g., leaf size, toughness, surface structure or specific leaf area). Thus, leaves of different toughness may promote complementary resource use by different detritivore species, with large caddisflies with strong mouthparts being able to deal with tougher leaves, and small stoneflies preferentially feeding on those that are softer (Tonin et al. 2018). Tougher leaves, however, may provide a better habitat of longer duration than softer ones, so different litter types should be seen not only as food resources, but also as microhabitats that could drive complementarity effects. For example, in terrestrial ecosystems, Wardle et al. (2003) found higher decomposition and N loss in mixtures containing slow-decomposing moss species that had particularly high water holding capacity. It must be highlighted that complementary resource use by different detritivore species could not occur in my microcosm experiments, as I only used one species, but the field approach allowed litter colonization by natural assemblages and I found a positive relationship between litter diversity and invertebrate diversity. Future

studies should also consider the temporal dimension, which can also influence the role on resource complementarity (Van Groenigen et al. 2015).

KEY FINDINGS OF THIS THESIS AND SUGGESTIONS FOR FUTURE RESEARCH

In this thesis, I have shown that the loss of riparian tree biodiversity can cause significant alterations in multiple ecosystem processes that are central to the functioning of many headwater streams. Importantly, I have demonstrated strong effects on the cycling of major nutrients, which had been overlooked in many studies. Thus, when native forests are replaced by monoespecific plantations (Fig. 3), we may expect large shifts in nutrient balances regardless of the magnitude of changes in litter decomposition rates. I have also shown significant effects on the secondary production of detritivores, and my results suggest potential effects on other detritivorous groups through altered production of fine particulate organic matter. All these effects add to other consequences of forestry practices on stream ecosystems that have been explored elsewhere, such as inputs of pesticides that can ultimately end in the stream water and alter ecosystem functioning (Cornejo et al. 2020). I also found some evidence of interactions across different components of the stream food web; changes in biodiversity in one food web compartment did not seem to affect processes occurring in another compartment, but further exploration of these interactions, as well as effects of changes in biodiversity simultaneously in multiple trophic levels, are worthwhile.

Biodiversity can be measured in different ways, and here I explored the relevance of different measures of taxonomic and functional diversity, all of which provided complementary information on how ecosystem functioning is impacted by biodiversity loss. Importantly, I found that good knowledge of the studied processes and the organisms involved is fundamental to determine the biological traits that mediate BEF relationships. Understanding which mechanisms underlie these relationships is also important, and the partitioning method proposed two decades ago (Loreau and Hector 2001) still holds effective in disentangling mechanisms related to biological interactions from purely random effects.

Although not addressed in this thesis, changes in the diversity and composition of riparian vegetation can interact with other stressors of anthropogenic origin (Fig. 2) and produce complex effects (Piggott et al. 2015). This multiple-stressor approach is rarely found in BEF literature, and it may be a promising research avenue. The magnitude of biodiversity effects on ecosystem functioning is comparable to that of major environmental drivers (Hooper et al. 2012, Mori et al. 2020), so assessing potential synergisms between them seems highly relevant. This is particularly true for

stressors which effects are still poorly, known, such as emerging pollutants (López-Rojo et al. 2020a) or extreme climatic events (Correa-Araneda et al. 2015). However, exploring these interactions in the field can be complicated, due to the lack of control of many factors and because sufficient replication is sometimes unattainable. These drawbacks can be avoided in microcosm experiments, where multi-factorial designs with high numbers of experimental units can be used (Fox 2004), although extrapolation to natural systems should always be taken with caution. I thus advocate future BEF studies that include a multiple-stressor approach, together with realistic scenarios of loss of species and traits and emphasis on nutrient cycling, ideally at different spatial and temporal scales.

Fig. 4. Examples of multiple stressors that can affect the functioning of stream ecosystems.

"Look closely at nature. Every species is a masterpiece, exquisitely adapted to the particular environment in which it has survived. Who are we to destroy or even diminish biodiversity?

E. O. Wilson

GENERAL CONCLUSIONS

1. Plant litter diversity altered stream multifunctionality. Effects differed depending on whether detritivores were present or absent but, in both cases, they were mostly driven by changes in nutrient cycling.

2. The use of multifunctionality metrics precluded the observation of effects on different processes. The examination of processes individually was necessary to fully understand how the ecosystem could be impaired by plant diversity loss.

3. There was a weak connection between the brown and green pathways of the stream food web, with no BEF relationships across pathways. This suggested that effects of biodiversity loss on ecosystem functioning are less complex than could be expected.

4. Different measures of biodiversity (i.e., species richness, phylogenetic distance and trait variability) revealed effects on ecosystem functioning. However, the strongest and most consistent effects were those of litter trait variability, together with mean values of certain traits (mostly nitrogen, phosphorus and tannin concentrations). Effects of species richness and phylogenetic distance varied depending on the species/traits involved and were also influenced by the experimental setting.

5. The complementarity effect consistently was the major biological mechanism underlying plant diversity effects on stream ecosystem processes, mostly in the presence of detritivores. The selection effect gained importance when processes were mediated by microbial decomposers.

6. Riparian plant biodiversity loss and compositional changes can cause significant alterations in multiple processes that are central to the functioning of many stream ecosystems. The overriding repercussions of such changes on the cycling of major nutrients indicated potential consequences for global biogeochemical cycles.

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