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Effects of changes in leaf quality and diversity of the riparian vegetation on headwater streams ecosystem functioning

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PhD Dissertation

Effects of changes in leaf quality and diversity of the riparian vegetation on headwater streams ecosystem functioning

Efecto de los cambios en la calidad foliar y la diversidad de la vegetación de ribera sobre el funcionamiento de arroyos de cabecera

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Even if I knew that tomorrow the world would go to pieces,
I would still plant my apple tree.

Martin Luther King, Jr.

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ABSTRACT

In forested regions, stream primary production is low due to light limitation. In consequence, most streams' food webs appear to be mainly fueled by allochthonous organic matter inputs from their riparian vegetation. Under a global change scenario, the quality and quantity of such inputs are expected to change with potential implications for stream ecosystems. However, the effects of such modifications on stream ecosystem functioning are difficult to understand due to the existence of complex trade-offs within and among communities making use of leaf litter assemblages. This thesis aims to shed some light on how different changes in the properties of organic matter inputs to headwater streams may alter their ecosystem functioning. To address that objective, I developed different field and laboratory experiments and analyzed the leaf litter decomposition process as an integrative indicator of stream ecosystem status. In the first chapter, I assess how leaf traits may be affected by climate change focusing on persistent after-life traits that have been usually reported to affect leaf litter decomposition, and thus have the potential to impair pivotal effects on the functioning of stream ecosystems. The results from this chapter suggest a decrease of intraspecific leaf quality in riparian deciduous species with global warming in a relatively short term and point to significant implications for Mediterranean mountain streams currently under deciduous gallery forests. In chapters 2 and 3, I investigate how the loss of riparian plant functional diversity or the establishment of dense pine plantations on the slopes of the basins may alter key stream processes (litter decomposition, nutrient cycling, secondary production, fungal biomass), and the likely role of key plant species to alleviate such effects. Our results support a consistent slowing down of the decomposition process, and hence effects on stream ecosystem functioning, derived from plant biodiversity loss but not from pine plantations, as long as riparian vegetation strips along streams are present. Moreover, outcomes from both chapters, underscore the importance of key (N-fixing) species at different scales (instream and catchment) as drivers of plant diversity effects or as buffer of plantation-derived effects in the studied ecosystem processes. In chapter 4, I analyse how detritivores can cope with the invasion of riparian areas of the streams by alien plants. Results from this chapter suggest that big detritivores, with outstanding digestive capacity to process low-quality leaf litter from native or invasive species, may play a key role facilitating the access to nutrients of recalcitrant leaf litter to sympatric small detritivore species via coprophagy. Overall, the results presented in this thesis may help managers and policymakers in the design of ecologically sound conservation programs.

RESUMEN

En regiones boscosas, la producción primaria de los arroyos está limitada debido a la escasa disponibilidad de luz. En consecuencia, la mayoría de las redes tróficas de los arroyos parecen estar alimentadas principalmente por aportes de materia orgánica alóctona procedente de la vegetación de ribera. Bajo un escenario de cambio global, se espera que la calidad y cantidad de dichos insumos cambien con implicaciones potenciales para los ecosistemas fluviales. Sin embargo, los efectos de tales cambios en el funcionamiento de los ecosistemas fluviales son difíciles de entender debido a la existencia de complejas interacciones dentro y entre las comunidades que hacen uso de los acúmulos de hojarasca. Esta tesis pretende arrojar algo de luz sobre cómo diferentes cambios en las propiedades de los aportes de materia orgánica a los ríos de cabecera pueden alterar el funcionamiento de estos ecosistemas. Para abordar ese objetivo, desarrollé diferentes experimentos de campo y laboratorio y analicé el proceso de descomposición de hojarasca como un indicador integrador del estado del ecosistema de los arroyos. En el primer capítulo, evalúo cómo los rasgos de las hojas pueden verse afectados por el cambio climático centrándome en rasgos persistentes tras la senescencia que generalmente han sido señalados como controladores de la descomposición de la hojarasca y, por lo tanto, tienen el potencial de ejercer importantes efectos en el funcionamiento de los ecosistemas fluviales. Los resultados de este capítulo sugieren una disminución de la calidad foliar intraespecífica en especies de ribera caducifolias con el calentamiento global en un plazo relativamente corto y apuntan a implicaciones significativas para los arroyos de montaña mediterráneos que actualmente se encuentran bajo galerías de bosque caducifolio. En los capítulos 2 y 3, investigo cómo la pérdida de la diversidad funcional de la vegetación de ribera o el establecimiento de densas plantaciones de pinos en las laderas de las cuencas pueden alterar procesos fluviales clave (descomposición de hojarasca, reciclado de nutrientes, producción secundaria, biomasa fúngica) y el papel potencial de las especies vegetales clave para aliviar tales efectos. Nuestros resultados respaldan una ralentización constante del proceso de descomposición y, por lo tanto, efectos sobre el funcionamiento del ecosistema fluvial, derivados de la pérdida de biodiversidad vegetal pero no como consecuencia de las plantaciones de pinos, siempre que existan franjas de vegetación ribereña a lo largo de los arroyos. Además, los resultados de ambos capítulos subrayan la importancia de las especies clave (fijadoras de N) a diferentes escalas (dentro del río y en la cuenca) como impulsores de los efectos de la diversidad de plantas o como amortiguadores de los efectos derivados de las plantaciones en los procesos del ecosistema estudiados. En el capítulo 4, analizo cómo los detritívoros pueden hacer frente a la invasión de las áreas ribereñas de sus arroyos por parte de plantas exóticas. Los resultados de este capítulo sugieren que detritívoros grandes con una capacidad digestiva excepcional para procesar hojarasca de baja calidad de especies nativas o invasoras pueden desempeñar un papel clave al facilitar el acceso a los nutrientes de la hojarasca recalcitrante a las especies de detritívoros pequeños simpátricos a través de la coprofagia. En general, los resultados presentados en esta tesis pueden ayudar a los gestores y legisladores en el diseño de programas de conservación ecológicamente sensibles y con sólida base científica.

General Introduction

GENERAL INTRODUCTION

The importance of headwater streams….

Globally, rivers and streams have been estimated to cover approximately 0.6% of the Earth's non glaciated land surface or, in other words, 773000 ± 79000 km^2 (Allen & Pavelsky, 2018). Such a surface is spread following a hierarchical pattern, where small watercourses join as they flow downstream forming larger ones (Fig. 1.I.). This brief description of a river network reveals that small streams are, imperatively, much more numerous than large streams. Furthermore, besides their number, headwater streams have been estimated to represent more than 75% of total stream length in most catchments (Benda et al., 2004; Leopold et al., 1964). Considering this hierarchical pattern of river networks, it is logical to think that everything in a river or stream depends, one way or another, on what is happening upstream. Following that premise, everywhere on Earth, larger streams and rivers are somehow contingent on headwater streams (Lowe & Likens, 2005).

Figure 1.I. Top-down illustration of a dendritic drainage basin.

Despite the small cover of continental surface waters, they are key components of the global carbon (C) cycle. Headwater streams mineralize organic C entering from terrestrial ecosystems, reducing the terrestrial CO_2 released to the atmosphere (Butman & Raymond, 2011; Öquist et al., 2009). Likewise, C is also generated (primary production) and removed (respiration) through instream processes (Battin et al., 2008). The C stored in streams can be (i) transported downstream or (ii) released to the atmosphere as gas (Argerich et al., 2016). Through the latter, they undoubtedly contribute to natural CO_2 emissions to the atmosphere (Battin et al., 2009; Cole et al., 2007; Raymond et al., 2013). Some estimations (Allen & Pavelsky, 2018; Marx et al., 2017), suggest that headwaters could be contributing with *ca.* 7.2% of global carbon (C) emissions as CO_2 (~648 million tons year^{-1}), with most of these emissions deriving from the processing of terrestrial organic matter rather than from aquatic metabolism, which contribution to CO_2 emissions seem to increase with stream size (Hotchkiss et al., 2015).

Besides their importance in global C pathways, freshwater ecosystems have been estimated to host *ca.* 9.5% of animal global biodiversity (Balian et al., 2008) and *ca.* 6% of total biodiversity (Hawksworth, 1995). These estimates have recently increased to 11 and 8%, respectively (Román-Palacios et al., 2022). Although no assessment of the isolated contribution of headwater streams to global biodiversity has been carried out, their characteristics can provide habitat to many species that may not thrive in downstream reaches (Finn et al., 2011; Meyer et al., 2007). For instance, predator-free habitats, thermal refuge or other specific niche space (Richardson, 2019). Moreover, freshwaters have been recognized to play a key role in the maintenance of adjacent terrestrial food webs (Kautza & Sullivan, 2016; Martin-Creuzburg et al., 2017; Richardson et al., 2010). For example, emergence of aquatic insects as winged adults is an important energy source to predators in riparian zones, such as spiders, birds or bats (Burdon & Harding, 2008; Gratton & Zanden, 2009; Kato et al., 2004; Martin-Creuzburg et al., 2017).

Notwithstanding the above, fluvial ecosystems are among the most severely endangered all over the world (Reid et al., 2019). The combination of anthropic (land-use changes, hydrological alterations, pollution, global warming, increase of extreme climate events, biological invasions) and natural disturbances (drought, floods) is causing its biodiversity to decline at a much higher rate than that of their terrestrial or marine counterparts (Fig. 2.I.; Reid et al., 2019; WWF, 2020).

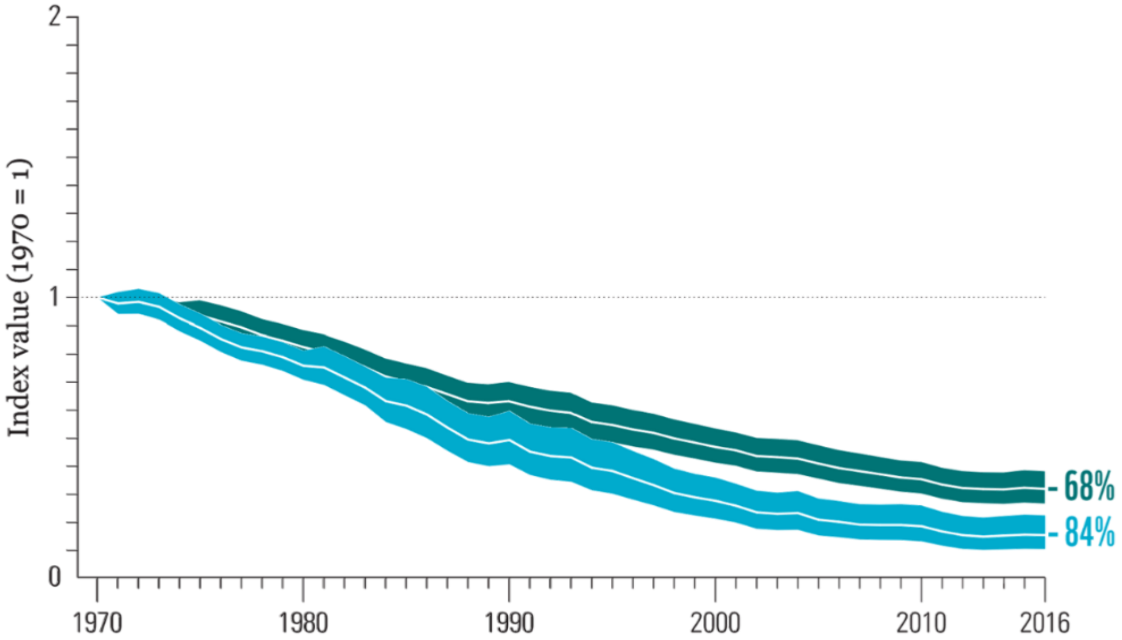

Figure 2.I. The Global (green) and Freshwater (blue) Living Planet Index: 1970 to 2016. Average decline was 68% (range: -73% to -62%) in global monitored populations and 84% (range 89% to -77%) in freshwater populations. The white line shows the index values, and the shaded areas represent the statistical certainty surrounding the trend. Modified from WWF (2020).

On top of that, the unequivocal warming of the earth system (Masson-Delmotte et al., 2021) may make non-perennial rivers the rule rather than the exception around the globe (Messager et al., 2021). This situation may be even more pronounced in the Mediterranean region where forecasts predict a significant decrease of precipitation (around 34%) as well (Harris et al., 2013). Under this context, headwater streams which are more naturally prone to flow intermittence (Datry et al., 2014), could suffer fundamental changes in their community composition and, consequently, in their ecosystem functioning (Bruder et al., 2011; Leberfinger et al., 2010).

… and their riparian vegetation

Headwater streams, with no tributaries, have higher levels of hydrological and ecological independence than larger streams. Nevertheless, they are characterized by close interactions with the surrounding terrestrial ecosystem making them vulnerable to both, aquatic and terrestrial biodiversity losses (Kominoski et al., 2013). In fact, headwater streams strongly rely on terrestrial inputs from their riparian areas (Vannote et al., 1980). Riparian areas are ecotones, or transitional zones, between adjacent terrestrial and aquatic realms, whose main component is riparian vegetation (Capon, 2020). Riparian forests usually reduce stream autotrophic production by shading and promote heterotrophy by supplying large amounts of allochthonous detritus, mainly in the form of leaf litter (Vannote et al., 1980; J. B. Wallace et al., 1997). Consequently, instream decomposition of leaf litter is a crucial ecosystem process involving the cycling of nutrients and fueling stream secondary production (Marks, 2019) which is carried out by microbial decomposers (bacteria and fungi) and detritivores (mainly shredders) (Fig. 3.I.; Gessner et al., 1999; Marks, 2019).

This strong dependence on terrestrial inputs makes headwater streams especially susceptible to natural or anthropogenic disturbances of riparian plant communities. For example, the rate at which leaf litter breaks down and is incorporated into food webs highly rely on its quality, which is fundamentally determined by after-life persistent traits (Graça et al., 2001; Graça & Cressa, 2010; Zhang et al., 2019). Consequently, headwater streams ecosystem functioning, and therefore global biogeochemical cycles (Battin et al., 2009; Raymond et al., 2013; Romeijn et al., 2019), may be altered if such leaf litter inputs experience physical or chemical changes (e.g. Alonso et al., 2022; Casas et al., 2013; López-Rojo et al., 2019) and/or if the amount of high-quality inputs decreases (Arias-Real et al., 2018). In a global change context, large alterations on the quality, quantity and seasonality of leaf litter inputs to streams can be derived, for example, from (i) variations of particular leaf traits in response to environmental changes (Graça & Poquet, 2014; Rubio-Ríos et al., 2022), (ii) the forecasted substitution of deciduous by evergreen species (Kominoski et al., 2013; Salinas et al., 2018); (iii) the decline of key plant species populations (J. Pérez et al., 2021); (iv) the establishment of monospecific plantations (Larrañaga et al., 2021; Martínez et al., 2013); and/or (v) the expansion of invasive plant species (Castro Díez & Alonso Fernández, 2017). These factors being non-mutually exclusive and potentially interactive (Fig. 4.I.).

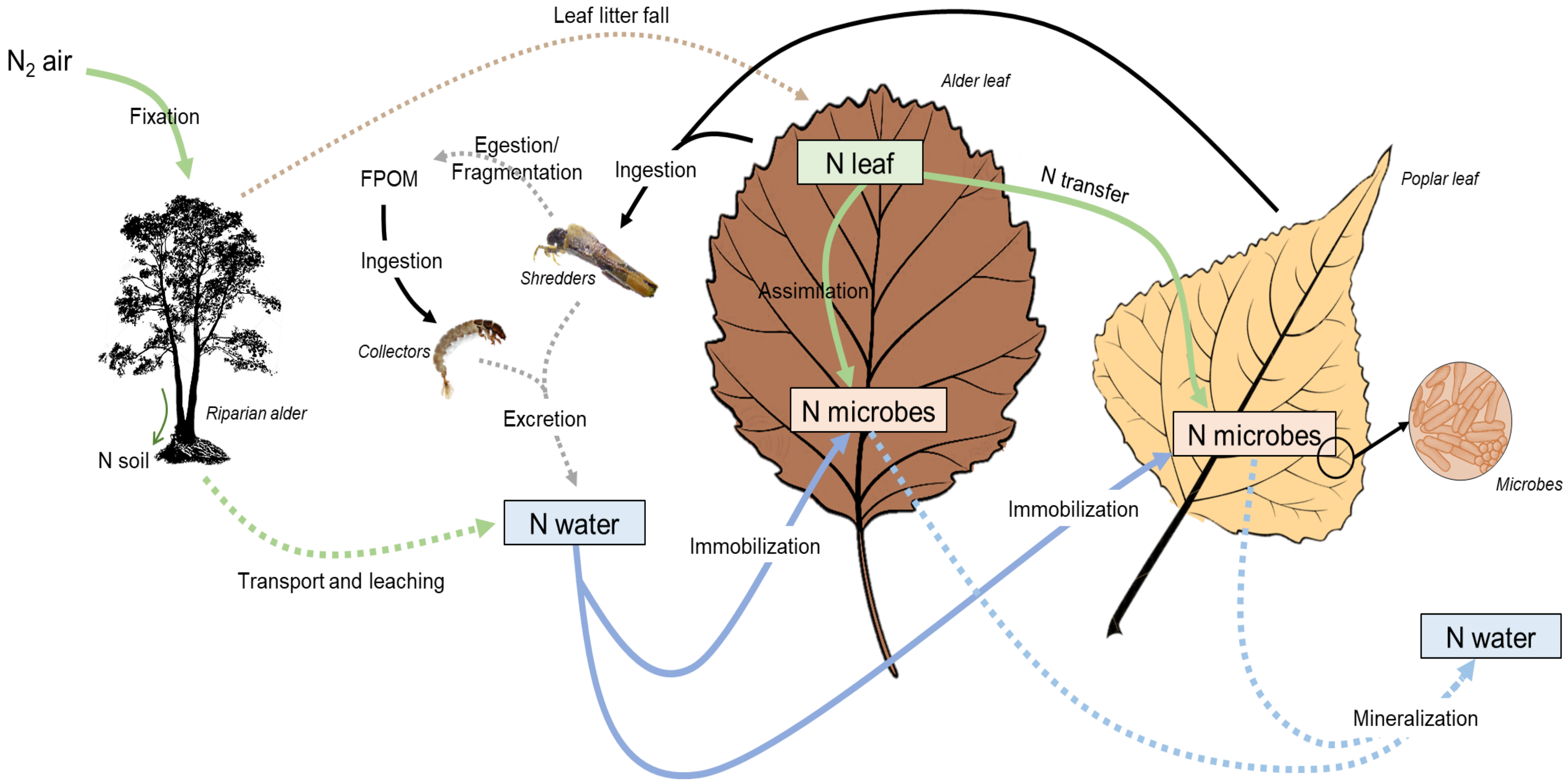

Figure 3.I. Conceptual diagram of nitrogen (N) cycling during instream leaf litter decomposition. Solid arrows represent feeding relationships, and dotted arrows represent the release of substances to the water column. Modified form Cheever et al. (2013) and Halvorson et al. (2015).

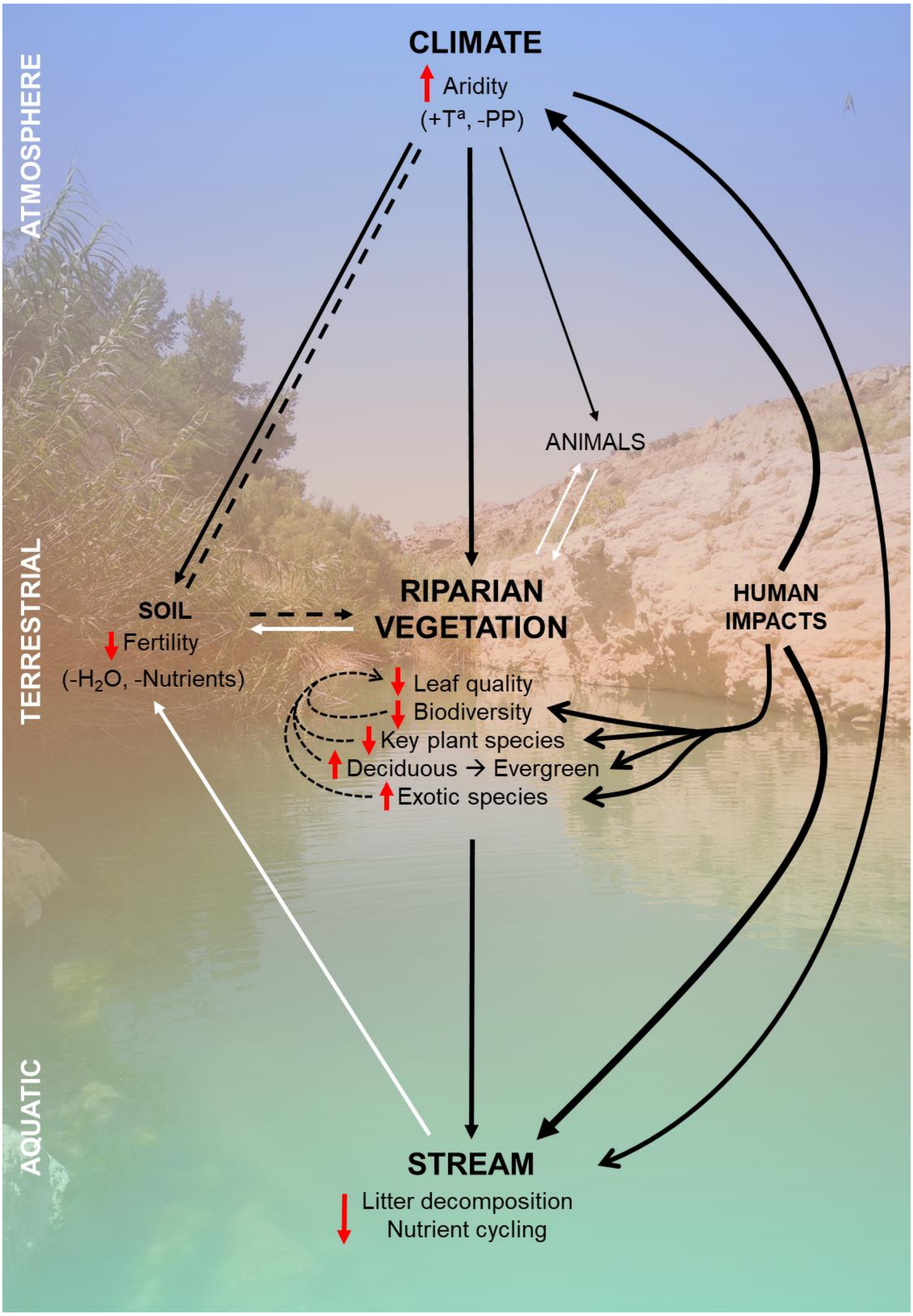

Figure 4.I. Conceptual diagram showing interactive factors driving changes on leaf litter inputs to streams. Solid arrows represent direct effects. Dashed arrows depict indirect effects. Black arrows denote deleterious effects. White arrows can stand for positive or negative effects. Modified from Chapin et al. (1995).

Forecasted changes in riparian vegetation

In general, litter decomposition rates are mainly promoted by high N and P concentrations (Fig. 5.I.; García-Palacios, McKie, et al., 2016; MacKenzie et al., 2013), although high concentrations of other nutrients as Ca or Mg (e.g. Jenkins & Suberkropp, 1995; Makkonen et al., 2012; National Research Council, 2005) can also have an important role on decomposition rates (Santonja et al., 2019). On the other hand, other leaf chemical (e.g., tannins, lignin, etc; Coq et al., 2010; Ferreira, Raposeiro, et al., 2016; Ramos et al., 2021; Schindler & Gessner, 2009) or physical (e.g., toughness; Fenoy et al., 2021; Li et al., 2009) traits tend to slow down litter breakdown. At the global scale, warming and reduced rainfall, i.e., increasing aridity, tend to promote the production of tougher (Wright et al., 2004) and poor-nutrient leaves (Reich & Oleksyn, 2004). Such changes usually entail a decrease of leaf litter quality (understood as decomposability and palatability to detritivores) of riparian vegetation (lower N, P and specific leaf area and higher N:P ratios with increasing Mean Annual Temperature; Boyero et al., 2017). However, this general pattern can differ among species (Graça & Poquet, 2014) and can be also strongly modulated by soil characteristics (Ordoñez et al., 2009).

Shifts in riparian plant communities involving the exchange of some plant functional groups by others, due to natural or anthropogenic reasons, may have important cascading effects on stream ecosystems (Kominoski et al., 2021). In a close future, increasing aridity may promote a retreat of deciduous species in favor of more drought-tolerant species (Gritti et al., 2006; Kominoski et al., 2013) what may imply a reduction of the diversity and palatability of leaf litter inputs to streams. Considering that soils from riparian areas of headwater streams use to contain constant water availability and high nutrients concentrations (due to constant stream sediment deposition; Naiman & Decamps, 1997) it could be expected that riparian vegetation of headwater streams differed from this general pattern being less susceptible to drought-derived effects. Nonetheless, recent research on riparian communities of Mediterranean headwater streams (Salinas et al., 2018) have pointed to a potential expansion of giant graminoids or evergreen species to the detriment of deciduous ones with increasing aridity or just rising warming, respectively.

Such changes could be boosted by the spread of emerging diseases. For example, in Europe, oomycetes of the genus *Phytophthora* and ascomycetes of the genus *Hymenoscyphus* have been recognized as an important threat to plant diversity, affecting directly to some dominant species of European forests as alder or oak (Bjelke et al., 2016; Brasier et al., 1993) and ash (George et al., 2022), respectively. In addition, these pathogens are expected to further expand favoured by climate change (Aguayo et al., 2014). Particularly, the dieback of key plant species as the N-fixing *Alnus glutinosa* could cause a notable impact on the functioning of headwater streams (Alonso et al., 2022; Alonso et al., 2021). This species provides high-palatable and nutrient-rich leaf litter that is rapidly colonized by microorganisms and consumed by detritivores (Graça et al., 2001; Graça & Cressa, 2010). Moreover, alder can promote the decomposition of other leaf litter species directly —acting as an attractant for detritivores (Ferreira et al.,

2012) or by direct nutrient transfer through fungal hyphae (Fig. 3.I.; Gessner et al., 2010; Tiunov, 2009)— or indirectly —by increasing the N concentration of stream water (Fig. 3.I.; Compton et al., 2003; Shaftel et al., 2012). This can mitigate the usual N limitation to which microbial communities are subjected in headwater streams (Ferreira et al., 2006).

The establishment of monospecific tree plantations in their surroundings can be another important impact on stream ecosystems. For instance, during the last century, aiming to recover part of the forest surface lost over centuries of human exploitation, the Mediterranean basin has been intensely afforested with pine and other fast-growing species plantations. Different studies assessing the effects of such plantations on instream functioning have reported contradictory results (see Larrañaga et al., 2021) with the exception of the unquestionable lower quality of conifer needles in comparison to deciduous broadleaves, and their, consequently, slower decomposition (Casas et al., 2013; Martínez et al., 2013). Following this statement, it may be expected a general decrease in leaf litter decomposition and nutrient cycling rates in those streams flowing through pine plantations (e.g. Kominoski et al., 2011; Whiles & Wallace, 1997).

One potential factor involved in those contradictory results may be the presence of other tree species in the riparian corridors of headwater streams flowing through plantations. The presence of strips of native riparian vegetation along streams could act as a buffer, cushioning plantation-derived negative effects on their ecosystem functioning (e.g. Casotti et al., 2015; Chellaiah & Yule, 2018; Ferreira, Koricheva, et al., 2016), especially if they contain key plant species.

Another threat to the conservation of native riparian vegetation are alien plant species. In a climate change context, plant invasions have been reported to likely modify the vegetation in some regions of the Mediterranean basin (Gritti et al., 2006). This may add to the high vulnerability of riparian areas to invasions. Riparian areas provide a corridor for the dispersal of biota and are exposed to frequent disturbances (natural and/or anthropic) involving clearance of riparian vegetation and thus release space and resources

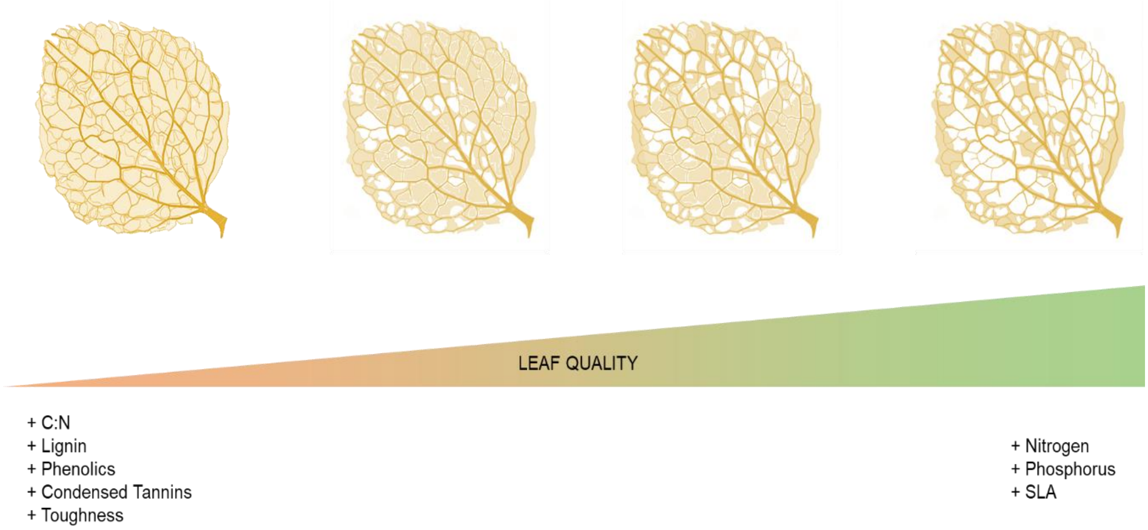

Figure 5.I. Leaf litter decomposition in a gradient of leaf quality based on specific leaf traits. Litter of higher quality tends to decompose faster. Modified from Canhoto et al. (2021).

that can be used by new species (Lake & Leishman, 2004; Naiman & Decamps, 1997). Moreover, their favorable conditions for plant life promote the entrance, establishment, and dispersal of non-native species (Castro Díez & Alonso Fernández, 2017), which may be capable of displacing native species if they are competitive enough (Belcher & Wilson, 1989; Trabaud, 1987). Leaf litter of invasive species can decompose faster or slower than native litter, but in both cases, it may alter stream communities and related ecosystem processes (Marks, 2019; and references therein).

Thesis outline

The south of the Iberian Peninsula (Andalusia) has numerous characteristics that makes it especially suitable to explore all the above-mentioned potential causes in shifts on leaf litter inputs and, consequently, on headwater streams functioning. First, this region encompasses a wide range of bioclimatic types (from semiarid to humid) embracing the projected aridification forecasts for the Mediterranean region at the end of the century (Fig. 1.C1; Seager et al., 2014). Such climatic variability makes possible approaches for the assessment of future climate-induced changes in leaf litter quality and riparian communities (e.g. Salinas et al., 2018), for example, using ‘space-for-time’ substitution designs (SFT; Blois et al., 2013; Pickett, 1989). Second, as in most of the Mediterranean region, many Andalusian mountainous areas have been subjected to intense afforestation programs whose main aim was the recovery of forest surface after the abandonment of agricultural lands (Allue Andrade & Garnica, 1970; Pausas et al., 2004). Further, the presence of key plant species (e.g., alder) in some riparian corridors allows for the evaluation of their role driving instream litter decomposition and associated processes. Last, but not least, although many headwater streams in this region are currently not threatened by plant invasions due to their location at high altitudes, there are some lowland headwater streams whose native riparian vegetation has endured for centuries the invasion of alien species such as the giant reed (*Arundo donax*). Therefore, the existence of these invaded reaches enables the assessment of how the detritivore guild face riparian plant invasions.

The effects of such changes on stream ecosystem functioning are difficult to understand due to the existence of complex trade-offs within and among decomposers and detritivores using leaf litter assemblages (Gessner et al., 2010). Taking advantage of such regional scenario, the main objective of this thesis is to expand our knowledge about how different processes altering the properties of organic matter inputs to headwater streams may modify the functioning of these ecosystems, with the purpose of helping managers and policymakers during their decision-making process for the establishment of ecologically sound and scientifically based conservation programs. To address that objective, I carried out different field and laboratory experiments. The work is structured in four chapters with the following aims:

- **Chapter 1** investigates how climate change might affect leaf quality, focusing on persistent after-life traits affecting leaf decomposition. Using a SFT substitution approach this chapter evaluates the leaf traits plasticity of four common riparian plant species in the Mediterranean basin (Fig. 6.I.-H1).

- **Chapter 2** assesses, by means of a microcosms assay, how the loss of plant functional diversity may alter key stream processes (litter decomposition, nutrient cycling, secondary production, fungal biomass) and the potential role of key plant species alleviating such effects (Fig. 6.I.-H2).
- **Chapter 3** explores, with a field experiment, the impact of pine plantations on ecosystem functioning (litter decomposition and nutrient cycling) and how the presence of key plant species at two scales (in the riparian area and/or within the stream in the form of leaf litter) may buffer the putative negative plantations-derived effects (Fig. 6.I.-H3).
- **Chapter 4** analyses the response of two detritivore species to riparian plant invasions. Using a feeding trial, this chapter tests to what extent detritivores can use conspecific and cross-species coprophagy, as a successful feeding strategy to deal with low-quality litter of a non-native species (Fig. 6.I.-H4).

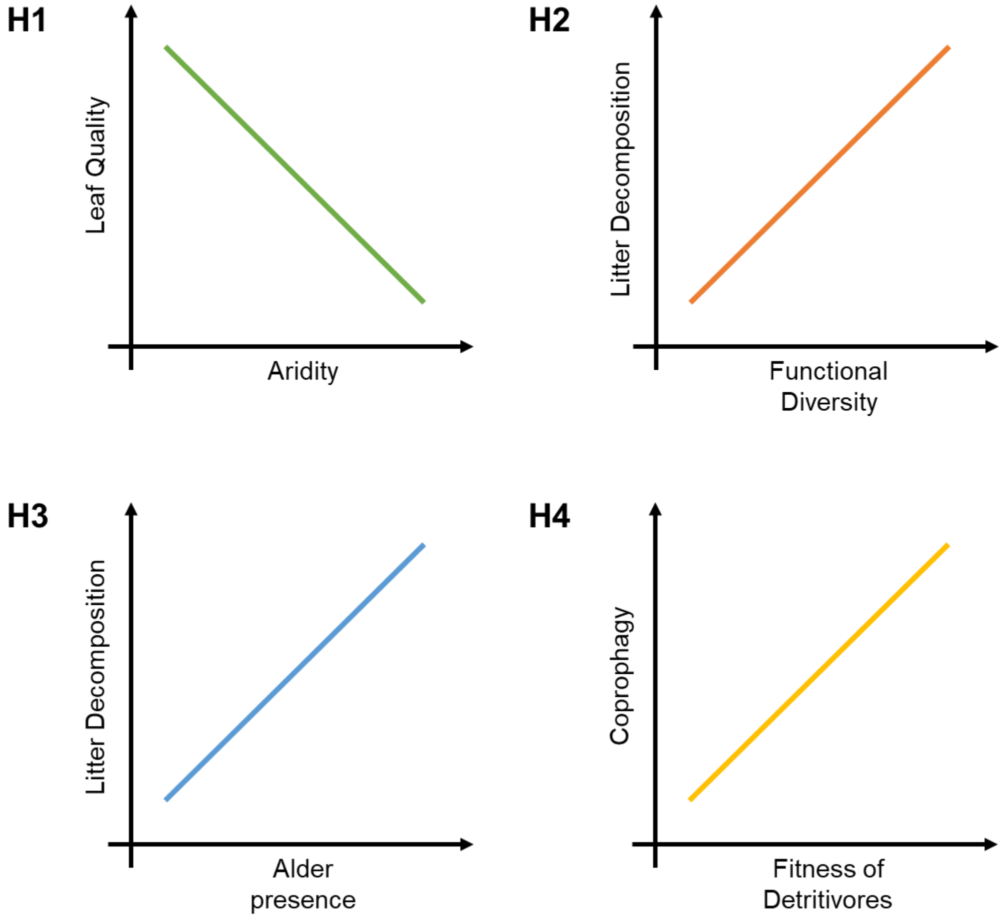

Figure 6.I. Main hypothesis of each chapter.

Chapter 1

Climate-induced plasticity in leaf traits of riparian plants

Rubio-Ríos J, Pérez J, Salinas MJ, Fenoy E, Boyero L & J.J. Casas

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ABSTRACT

Leaf litter inputs from riparian vegetation and its decomposition play a key role in energy and nutrient transfer in many stream ecosystems. Instream leaf litter decomposition is driven by both leaf traits and environmental conditions. Therefore, understanding and predicting leaf trait variation under current environmental changes and their putative interactive effects on stream food webs is a critical challenge. Most studies have focused on the assumed higher interspecific leaf trait variability, with little research addressing an intraspecific perspective. We assessed the relative effects of climate and soil conditions on the plasticity of leaf traits of four common woody riparian species in permanent low-order Mediterranean streams across a wide aridity gradient. We used a space-for-time substitution approach to predict leaf trait changes and consequences for stream food webs in a future climate change scenario. Overall, we found that aridity had a major influence on leaf trait plasticity but with opposite patterns depending on plant functional type, although soil was the strongest predictor in some cases. Results indicated that leaf quality—linked to palatability and decomposability—of *Alnus glutinosa*, *Salix atrocinerea* and *Rubus ulmifolius* (deciduous/semideciduous) will decrease with forecasted aridification, whereas the palatability of the evergreen *Nerium oleander* will increase. We observed higher trait plasticity than interspecific variation for leaf P, Ca, and Mg concentrations and C:P ratio. Our findings suggest a decrease of intraspecific leaf quality in riparian deciduous species with global warming in a relatively short term. In a longer term, this may merge with the forecasted dieback of deciduous species in riparian corridors of temperate climate zones. These changes have the potential to significantly impair ecosystem functioning of Mediterranean mountain streams currently under deciduous gallery forests.

Keywords: Aridification, deciduous, evergreen, instream decomposition, litter quality, soil, space-for-time substitution

INTRODUCTION

The warming of the Earth system is unequivocal (IPCC, 2021). Globally, precipitation is also predicted to increase in the long-term (Hewitson et al., 2015). However, forecasts in the Mediterranean basin point to a precipitation decrease of around 34%, along with a temperature increase of *ca*. 5 ºC for the period 2000-2099 (Harris et al., 2013). As a result, this region will face a climate much drier and hotter than at present, especially during warm seasons (Giorgi & Lionello, 2008), with direct effects on hydrologic regimes (Nohara et al., 2006; Vicente-Serrano et al., 2014) and soil moisture (Manabe et al., 2004). These changes may alter the functioning and structure of plant communities (e.g. Carnicer et al., 2011; Trivedi et al., 2008; Vicente-Serrano et al., 2012).

Small streams flowing through forested areas can be especially susceptible to climate-change induced alterations in plant communities, owing to their high dependence on organic matter inputs from the riparian vegetation, i.e. leaf litter (Wallace et al., 2015). Instream decomposition of leaf litter is a crucial ecosystem process, involving the cycling of nutrients and fuelling stream secondary production (Marks, 2019). The rate at which leaf litter decomposes and is incorporated into food webs highly depends on its quality, which fundamentally depends on after-life persistent traits (Graça & Cressa, 2010; Graça et al., 2001; Zhang et al., 2019). Thus, ecosystem functioning can be significantly altered if leaf litter inputs to streams experience physical and chemical changes (e.g. Casas et al., 2013; del Campo et al., 2021; López-Rojo et al., 2019). These changes can be interspecific, e.g. resulting from the forecasted substitution of deciduous by evergreen species (Kominoski et al., 2013; Salinas et al., 2018) and/or the decline of key plant species populations (e.g. alder; Alonso et al., 2021; Rubio-Ríos et al., 2021). Moreover, given that leaf traits are highly responsive to environmental changes (Heilmeier, 2019; Soudzilovskaia et al., 2013), intraspecific changes may also occur, e.g. due to genetic variability (Crutsinger et al., 2014; LeRoy et al., 2012) or phenotypic plasticity (Graça & Poquet, 2014; Henn et al., 2018; Jung et al., 2014).

Such relationship between leaf traits and the environment has been a recurrent theme of study (e.g. Ordoñez et al., 2009; Read et al., 2014; Reich & Oleksyn, 2004). However, although recent results indicate that intraspecific variation may represent up to *ca.* 30% of total functional trait variability in plant communities (Albert et al., 2010; Siefert et al., 2015), most studies have focused on the often assumed higher interspecific variability of many leaf traits (e.g. Hulshof & Swenson, 2010; Wright et al., 2004).

High rates of plasticity in leaf traits are expected in species distributed across ample environmental gradients (Cordell et al., 1998; Fajardo & Piper, 2011; Umaña & Swenson, 2019), as increases in niche breadth allow plants to respond to variation in climatic and other environmental conditions (Henn et al., 2018), whereas nearby individuals may share biotic and abiotic pressures and have close genetic relationships. Warming and reduced rainfall, i.e. increasing aridity, are usually reported to promote the production of thicker and smaller leaves (Wright et al., 2004)—in order to improve their water use efficiency and to increase their leaf lifespan—with low nutrient concentrations

(Reich & Oleksyn, 2004). Such plasticity in important traits can, in turn, affect the palatability and decomposability of leaves, i.e. their acceptability and easiness to be consumed, along environmental gradients (Boyero et al., 2017; Graça & Poquet, 2014; Lecerf & Chauvet, 2008; LeRoy et al., 2007). Understanding how individual species traits, or their syndromes, are modulated by climatic, or other environmental characteristics, could allow us to refine predictions of potential effects on stream ecosystem functioning, both in green (based on primary production) and brown (based on detritus) food webs, in the face of climate change (Kominoski et al., 2021).

Here, using a ‘space-for-time’ (SFT) substitution approach (Blois et al., 2013; Pickett, 1989), we investigated how climate change might affect leaf quality, focusing on after-life traits affecting leaf decomposition. The SFT substitution approach is a useful tool to anticipate changes taking advantage of natural gradients (Fukami & Wardle, 2005); in the present study, a natural aridity gradient represents the forecasted aridification of the Mediterranean basin (Seager et al., 2014). We assessed plasticity in leaf traits of four common riparian species, with contrasting functional traits, in permanent low-order streams [*Alnus glutinosa* (L.) Gaertn., *Salix atrocinerea* Brot., *Rubus ulmifolius* Schott and *Nerium oleander* L.], extrapolating their possible variation in the forecasted climatic scenarios from that observed across a wide environmental gradient studied within a relatively small region. Using the same species along many areas differing in environmental conditions allowed us to control for species-specific traits, but not to assess the amount of trait variability due to genetic variability.

Given the high responsiveness of leaves to climate changes (Heilmeier, 2019; Soudzilovskaia et al., 2013) and the high water and nutrient availability in riparian soils of permanent streams (Naiman & Decamps, 1997), we hypothesize that (1) climate will exert a higher influence on leaf trait plasticity of the studied species compared to soil variables. We (2) expect a general trend of decreasing leaf quality—i.e. lower nutrient concentration, higher toughness—with the forecasted aridification (increasing temperature and decreasing precipitation) (Reich & Oleksyn, 2004). However, we also expect that the strength of the effects will vary among different species, as they belong to different functional groups (i.e. C allocation and/or N-fixing) and therefore have low similarity in their leaf traits (Salinas et al., 2018). Thus, we also hypothesize that (3) trait plasticity will be relatively low compared to interspecific variation.

MATERIAL AND METHODS

Area of study and selected plant species

Our study was conducted during summer 2013 in the riverbanks of 34 headwater streams with permanent flows distributed across nine natural protected areas (considered as pristine) located in Andalusia (south of the Iberian Peninsula), covering *ca*. 88000 km^2. These locations represent a wide climatic gradient within the context of a Mediterranean-type climate, and possess a considerable lithological and topographical heterogeneity (Fig. 1.C1.). The present (mean annual temperature range 10.8 – 17.4 ºC; mean annual precipitation range 261 – 845 mm; Table S1.C1.) and the projected climatic gradient studied (by the end of the 21st century) cover from arid to humid conditions according to the Emberger's bioclimatic coefficient (Table 1.C1., Fig. 2.C1.). This embraces the forecasted aridification, i.e. warming (mean temperature rise of 2 – 4 ºC) and reduction of precipitation (mean precipitation decrease of 10 – 40%), for the Mediterranean region (Seager et al., 2014), as a consequence of climate change towards the year 2100 (reviewed by Giorgi & Lionello, 2008).

We selected four abundant riparian plant species which represent different functional groups featuring different characteristics, including two deciduous riparian trees: black alder ─*Alnus glutinosa* (an N-fixer), and grey willow, *Salix atrocinerea*; one semi-deciduous shrub: blackberry, *Rubus ulmifolius*; and one evergreen shrub: oleander, *Nerium oleander*, also known as laurel rose. Leaves of these species were collected (June-July 2013) from each sampling site where present (Table 1.C1.) from robust, well grown and totally unshaded plants distanced from the stream by a maximum of 6 m. Those leaves directly exposed to sun light and without herbivory or pathogen symptoms were selected (Cornelissen et al., 2003). In each stream and for each species we collected 102 leaves from 6 individuals (17 leaves per individual) randomly distributed on both stream sides along a 100 m stream reach. Leaves were air-dried at room temperature (20 – 23 ºC) for 1 week and stored in darkness in paper bags until processed. At each stream, the cover of each species was estimated using the Domin-Krajina scale of cover and abundance (Kent & Coker, 1992) in six plots (36 m^2 each) randomly distributed in both stream sides — three plots per side arranged from the edge of the wetted channel — along a 100 m stream reach (Salinas et al. (2018).

Environmental variables

Thirty-two environmental variables (altitude, 20 climatic and 11 edaphic; Tables 1.C1. & S1.C1.) were selected as potential predictors of leaf trait plasticity. Altitude was obtained *in situ* using a portable GPS. Historical (monthly average for the years 1970-2000) values of bioclimatic variables (spatial resolution of 30 seconds, i.e. ~1×1 km) recorded along the last period with available climatic data were obtained from the WorldClim database (Table S1.C1.; version 2.1; www.worldclim.org, Fick & Hijmans, 2017) using site location information (latitude and longitude). Future monthly values were estimated from the NCAR Community Model version 3 (2×CO_2 climate change scenario, CCM3) for the year 2100 (Govindasamy et al., 2003) and subsequently downscaled and matched to the WorldClim estimates of current climate at a resolution of 2.5 minutes (i.e. ~4.5× 4.5 km). From these variables the Emberger's bioclimatic coefficient (Q2) for each site was calculated following Condés and García-Robredo (2012) as $100P/((M^2 - m^2))$, where P is the annual rainfall in mm, M the average maximum of the warmest month, and m the average minimum of the coldest month. To

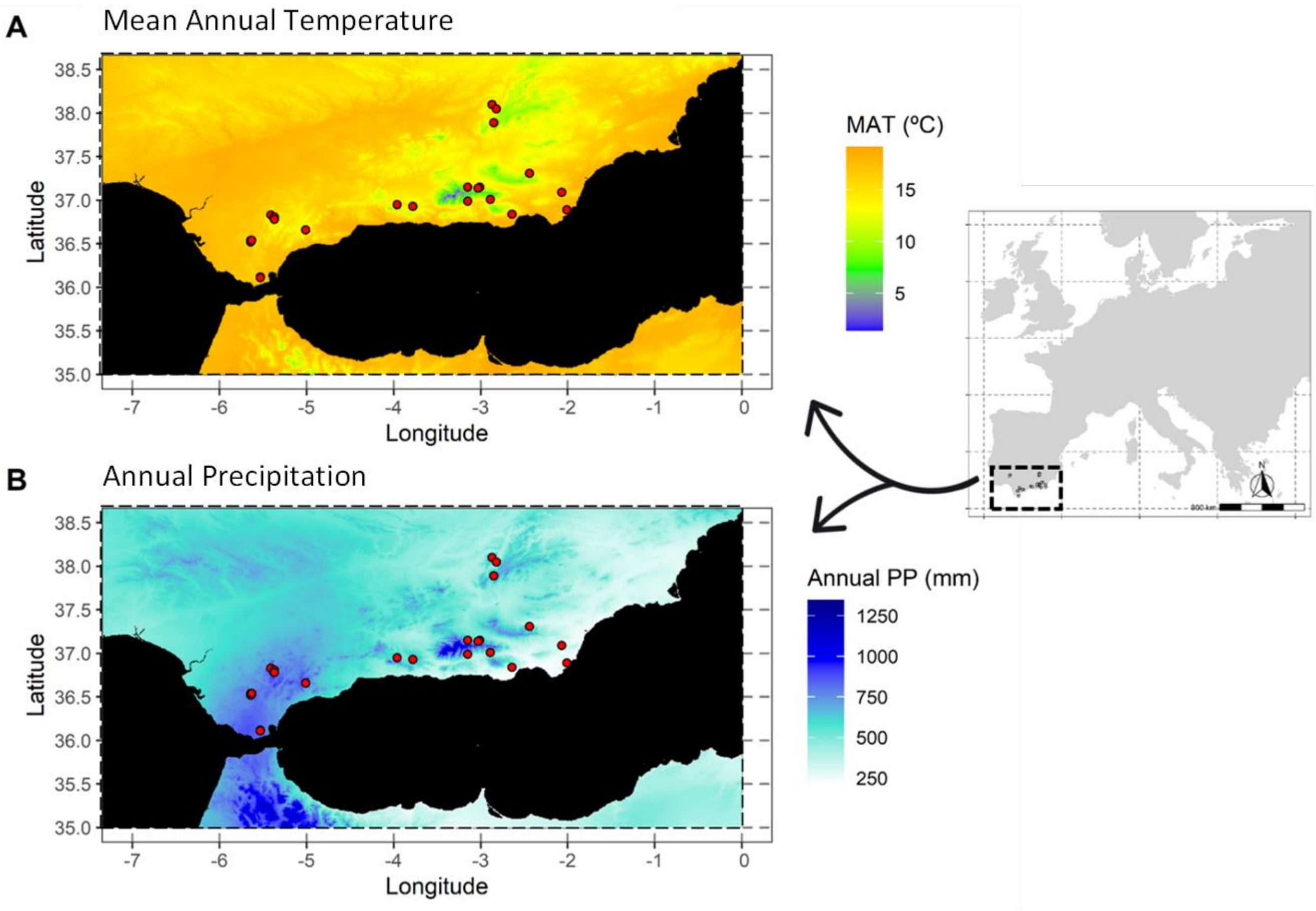

Figure 1.C1. Map of the 34 sites of study located within Europe and over the mean annual temperature (A) and the annual precipitation (B) gradients.

measure soil variables, we collected a sample consisting of six core samples of the top 20 cm of the riverbank soil profile, obtained by a randomly stratified method from each stream side at a distance of approximately 3 m from the active channel. Samples from each site were mixed, air dried, sieved (2 mm) and stored in sealed polyethylene bags until analysed. Soil physical and chemical variables (Table S1.C1.) were measured as in Gil et al. (2004).

Leaf traits

We measured nine leaf traits that often correlate with leaf litter decomposition rate (see Graça et al., 2015; Tonin et al., 2021) for each species: N, P, Ca, Mg, condensed tannins and lignin concentrations, C:N and C:P molar ratios, and toughness. Before measurements, leaves were rehydrated by spraying with distilled water and stored for 12 h at 5 ºC. Leaf toughness, expressed in units of mass (g), was measured by performing distal and proximal punctures per individual leaf using a Texture Analyzer TA.XTPlus (Stable Micro Systems) equipped with a needle of 0.38 mm^2 tip surface. Thereafter, leaves were oven dried (60ºC, 72h) and ground to fine powder (Mixer Mill RETSCH MM 200). Concentrations of C and N (% dry mass, DM) of leaves were determined using a mass spectrometer (EA-Thermo DELTA V Advantage, Fisher Scientific®) following standard

Table 1.C1. Characteristics of sampling sites, and cover (%) of each plant species studied. Land type was defined by altitude (m a.s.l.): Low <500, Mid 500-1000, High >1000. Sites ranked in order of decreasing aridity according to the Emberger's bioclimatic coefficient Q2.

Basin name	**Latitude**	**Longitude**	**Altitude (m a.s.l.)**	**Lithology**	**Land type**	**Emberger's coefficient (Q2)**	**Climate category according to Q2**	*Alnus glutinosa*	*Salix atrocinerea*	*Nerium oleander*	*Rubus ulmifolius*
Rambla de las Negras	36.89	-2.01	47	Calcareous	Lowland	31.5	Semiarid			13.8	
Río Aguas	37.09	-2.07	270	Calcareous	Lowland	33.8	Semiarid			2.0	1.5
Barranco del Cura	36.84	-2.64	291	Calcareous	Lowland	40.4	Semiarid			22.3	1.0
Arroyo de Aguamulas	38.05	-2.82	689	Calcareous	Midland	42.6	Semiarid				8.8
Río Bacares	37.31	-2.44	943	Calcareous	Midland	51.9	Subhumid		22.8		25.7
Río Vacal	36.92	-3.81	956	Calcareous	Midland	54.5	Subhumid				6.8
Arroyo Los Marcos	37.30	-2.58	1019	Calcareous	Highland	55.1	Subhumid		20.5		10.3
Río Mecina	36.99	-3.15	1136	Siliceous	Highland	56.5	Subhumid	11.0	24.8		9.2
Río Turrillas	36.93	-3.78	991	Calcareous	Midland	59.0	Subhumid				19.3
Río Andarax	37.01	-2.89	1013	Siliceous	Highland	60.4	Subhumid	43.8	12.8		
Río Alhama	36.95	-3.96	959	Calcareous	Midland	61.2	Subhumid				7.3
Ribera de Santa Ana	37.87	-6.70	546	Siliceous	Midland	62.5	Subhumid	66.3			9.3
Arroyo Corterrangel	37.94	-6.60	462	Siliceous	Lowland	62.6	Subhumid	15.8			12.7
Río Nacimiento	37.15	-2.91	1149	Siliceous	Highland	63.6	Subhumid		17.9		2.4
Barranco del Dun Dun	37.94	-6.64	554	Siliceous	Midland	66.3	Subhumid	52.5		1.5	3.0
Río Guadalentin	37.89	-2.85	1273	Calcareous	Highland	66.3	Subhumid		22.9		5.5
Arroyo de Aguascebas	38.10	-2.87	1063	Calcareous	Highland	66.9	Subhumid		3.0		4.0
Arroyo de la Garganta	37.90	-2.89	1356	Calcareous	Highland	67.1	Subhumid				11.6
Río Chico Ohanes	37.05	-2.76	1038	Siliceous	Highland	72.1	Subhumid		34.8	1.5	20.1
Barranco del Pueblo	37.15	-3.15	1394	Siliceous	Highland	74.8	Subhumid	29.7	6.0		3.5
Arroyo Hondo	37.14	-3.03	1438	Siliceous	Highland	75.3	Subhumid	51.4	3.5		15.0
Arroyo de los Castaños	37.15	-3.01	1321	Siliceous	Highland	75.3	Subhumid	62.0	16.3		2.8
Arroyo de los Caballos	36.68	-4.91	350	Calcareous	Lowland	77.5	Subhumid			17.7	7.1
Arroyo de los Molinos	36.81	-5.37	379	Calcareous	Lowland	85.0	Subhumid			11.3	8.2
Arroyo de Bocaleones	36.83	-5.41	316	Calcareous	Lowland	88.6	Subhumid			11.3	9.5
Arroyo Gaidovar	36.78	-5.37	695	Calcareous	Midland	92.3	Humid				36.8
Río Verde	36.66	-5.01	662	Calcareous	Midland	94.5	Humid			32.4	10.0
Arroyo de la Cruz	36.64	-5.03	897	Calcareous	Midland	94.5	Humid				1.2
Garganta del Caballo	36.54	-5.64	401	Siliceous	Lowland	102.2	Humid			11.9	35.9
Garganta del Aljibe	36.54	-5.63	432	Siliceous	Lowland	102.2	Humid	35.9			1.3
Garganta del Medio	36.54	-5.64	423	Siliceous	Lowland	102.2	Humid	17.3			2.3
Garganta de la Cierva	36.52	-5.64	550	Siliceous	Midland	102.2	Humid	42.9		6.7	8.5
Garganta de la Garza	36.12	-5.53	429	Siliceous	Lowland	109.5	Humid	30.5			6.3
Garganta del Tesorillo	36.11	-5.53	532	Siliceous	Midland	109.5	Humid	28.0			6.3

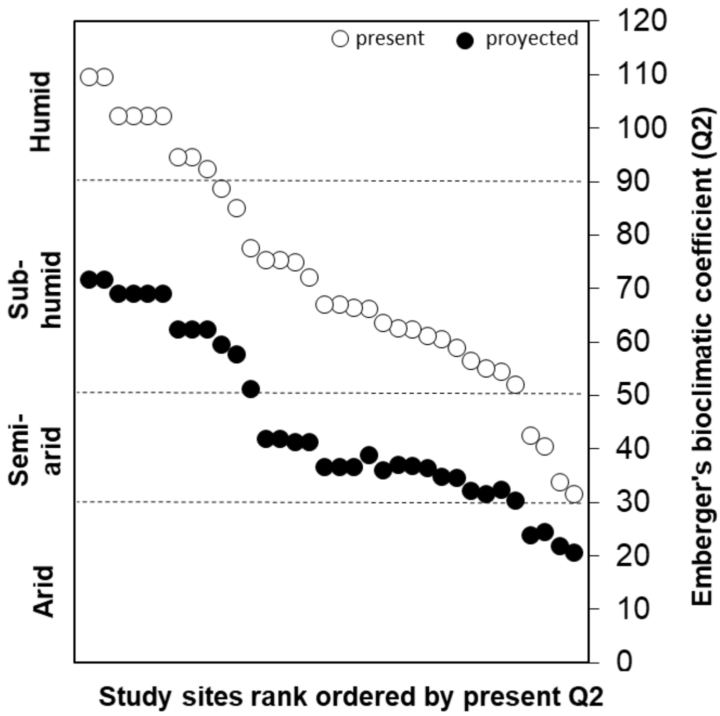

Figure 2.C1. Present (open, 2000) and projected (closed, 2100) Emberger's bioclimatic coefficient values (Q2), estimated from the NCAR Community Model version 3 (CCM3) for the year 2100 (Govindasamy et al., 2003), for each of the 34 streams studied. Note that higher Q2 values denote lower aridity.

procedures (Flindt et al., 2020). The concentration of P (% DM) was measured spectrophotometrically after autoclave-assisted extraction (APHA, 1998; Flindt et al., 2020). Concentrations of Ca and Mg (% DM) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II). Condensed tannins (mg Catechin Hydrate Equivalent per g of DM) were measured by the acid butanol assay (Gessner & Steiner, 2020). Concentration of lignin (% DM) was estimated gravimetrically using the acid-detergent method of Goering and Van Soest (1970).

Data analysis

To elucidate the relationships between species cover and environmental variables, we ran a Canonical Correspondence Analysis (CCA; cca function of the 'vegan' package, Oksanen et al., 2019), after a forward selection (*ordistep* function of the 'vegan' package with 9999 permutations) of the most parsimonious subset of explanatory variables (PPSeasonality, PWettestM, MaxT, MinT and soil pH). Significance of all testable fractions was assessed using permutation tests. Environmental variables were transformed to improve the structure of the residuals using *log* or *arcsin* transformations for decimal and percentage values, respectively.

Differences in individual traits among species were assessed using one-way ANOVA and post-hoc Tukey tests (*anova* and *TukeyHSD* functions of the 'stats' package). We performed Principal Component Analyses (PCAs; *prcomp* function in the 'stats' package) to examine patterns in leaf trait variability: one pooling the four species to examine interspecific variation *vs.* trait plasticity, and one for each species to extract the main gradients (2 first PCs) of trait plasticity (i.e. leaf quality). Previously, using Spearman rank correlations, leaf traits with high (> 0.85) collinearity were removed (Fig. S1.C1.). Seven traits were finally included in the PCA: N, P, Ca, Mg, condensed tannins, lignin and toughness. *Log* or *arcsin* transformations of variables were used when required in ANOVA and PCA analyses. The relative magnitude of interspecific variation *vs*. species plasticity for the overall pool of traits for each species was estimated as the proportion that each species covered in each of the dimensions of the general PCA. Besides, to quantify the relative magnitude of interspecific variation *vs*. species plasticity for each leaf trait, we performed variance partitioning analyses (varcomp function of the 'ape' package, Paradis & Schliep, 2019).

We carried out partial least squares regressions (PLS; plsr function in the 'pls' package, Mevik et al., 2020) to evaluate the relative importance of climate and soil as predictors of leaf trait plasticity (first two PCA axes). Preliminary PLS regressions for each environmental matrix and plant species (Table S2.C1.) were used to reduce the number of variables by selecting those with the highest variable importance in projection (VIP; VIP function in the 'plsVarSel' package, Mehmood et al., 2012). Those variables with VIP $\gtrsim$ 1 were considered relevant (Andersen & Bro, 2010). Spearman rank correlation analyses were used to equalize the size of the two matrices of environmental variables removing those variables with high collinearity within those with higher VIP values (Fig. S2.C1., Table S3.C1., S4.C1.). A second PLS regression was performed for each species using the selected variables, and the influence of each group of environmental variables (climate and soil) and their combination (climate + soil) on leaf plasticity was assessed using the goodness of prediction (Q^2) and the goodness of fit ($R^2(Y)$) of models. A model was considered significant when $Q^2 > 0.097$ (Friden et al., 1994).

In PLS regressions all explanatory variables were scaled to unit variance (*scale* function) to give all variables the same relative importance. Regressions were carried out separately for each species and the number of extracted components (latent variables) and the robustness of the resulting models were determined by leave-one-out cross-validation (LOO). For each model, we determined the number of dimensions with the lowest cross-validation error. PLS regressions built with climatic variables, when statistically significant ($Q^2 > 0.097$; Table 2.C1.), were used to estimate the projected change of leaf quality under the forecasted climate change scenarios for 2100, using the *predict* function of the 'stats' package. Current and projected values of leaf quality (i.e. mean position over PC 1 or PC 2 of separate PCAs for each species) were compared using t-tests for paired samples. Hedge's *g* effect size was estimated using the *cohen.d* function of the 'effsize' package (Torchiano, 2020). See Supporting Methods in Supplementary Material for further details of data analyses.

RESULTS

Environmental variables and species distribution

Overall, the four species covered a large gradient of climatic conditions from semiarid to humid bioclimatic types according to the Emberger's coefficient (Q2) (Table 1.C1., Fig.

2.C1.). The scenario for 2100 developed by the NCAR Community Model version 3 (CCM3) (Govindasamy et al., 2003) forecasts a significant aridification in the studied region, greater in presently humid sites (Fig. 2.C1.).

Distribution of each species was rather clearly separated by the environmental gradients established by the first two dimensions of the CCA ($P < 0.001$; Fig. 3.C1.-A), which explained 91% of fitted constrained variation (53% of total variation). Soil pH was the most important variable explaining species distribution, given its significant and positive load (0.72) on CCA 1 (also loading - 0.60 on CCA 2). Moreover, minimum annual temperature was positively correlated (0.43) with CCA 1. The above variables essentially determined the separation of the acidophilic *A. glutinosa* (hereafter *Alnus*) from other species, particularly from *N. oleander* (hereafter *Nerium*), which can tolerate high pH soils and prefers lowland sites with mild winters (Fig. 3.C1.-A, B, D; Table S5.C1.). Precipitation seasonality and precipitation of the wettest month loaded significantly and positively (0.70 and 0.53, respectively) on CCA 2. This dimension basically segregated *S. atrocinerea* (hereafter *Salix*), abundant at low-precipitation and neutral to basic soil sites, from other species (Fig. 3.C1.-C; Table S5.C1.). *Rubus ulmifolius* (hereafter *Rubus*) showed its highest cover at sites with basic soils and/or mild winters, where deciduous tree species developing dense canopy cover (alder, willow or other) were absent or scarce (Fig. 3.C1.-E; Table S5.C1.). The studied species varied in the range of environmental conditions they occupied. *Rubus* was the most widely distributed species, occupying 100% and 92% of CCA1 and CCA2 gradients, respectively, followed by *Nerium* (80.1% of CCA1 and 84.2% of CCA2), *Alnus* (45.5% of CCA1 and 78.5% of CCA2), and *Salix* with the most constricted distribution (44.6% of CCA1 and 71.7% of CCA2) (Table 1.C1., S1.C1. & S2.C1.; Fig. 3.C1.).

Table 2.C1. Summary of univariate dependent variable PLS models fitted to the first two principal components of PCA (PC1 & PC2), summarizing leaf trait plasticity for each species, using three matrices (C, S and C+S) of selected (in preliminary PLS regressions) environmental variables as predictors. The number of PLS dimensions with lowest cross validation error (N), goodness of prediction (Q^2) and coefficient of determination of dependent variable (R^2) are shown for each model. Significant models ($Q^2 > 0.097$) are in bold.

Functional type	Plant species	Set of environmental predictors or combination	Dependent variable					
			PC1			PC2		
			N	Q^2	R^2(Y)	N	Q^2	R^2(Y)
Deciduous N-fixer	*Alnus glutinosa*	Climate (C)	1	**0.63**	0.76	0	-	-
		Soil (S)	4	**0.26**	0.74	1	0.09	0.43
		C+S	1	**0.64**	0.78	1	-0.02	0.37
Deciduous	*Salix atrocinerea*	Climate (C)	2	**0.51**	0.74	1	**0.10**	0.42
		Soil (S)	1	**0.23**	0.47	2	**0.71**	0.91
		C+S	1	**0.36**	0.59	4	**0.58**	0.92
Evergreen	*Nerium oleander*	Climate (C)	3	**0.30**	0.71	1	-0.02	0.46
		Soil (S)	1	-0.01	0.42	**2**	**0.15**	0.60
		C+S	6	**0.70**	0.98	**1**	**0.13**	0.54
Semideciduous	*Rubus ulmifolius*	Climate (C)	1	**0.32**	0.41	1	-0.05	0.09
		Soil (S)	1	0.03	0.19	1	-0.02	0.11
		C+S	1	**0.26**	0.40	2	0.06	0.32

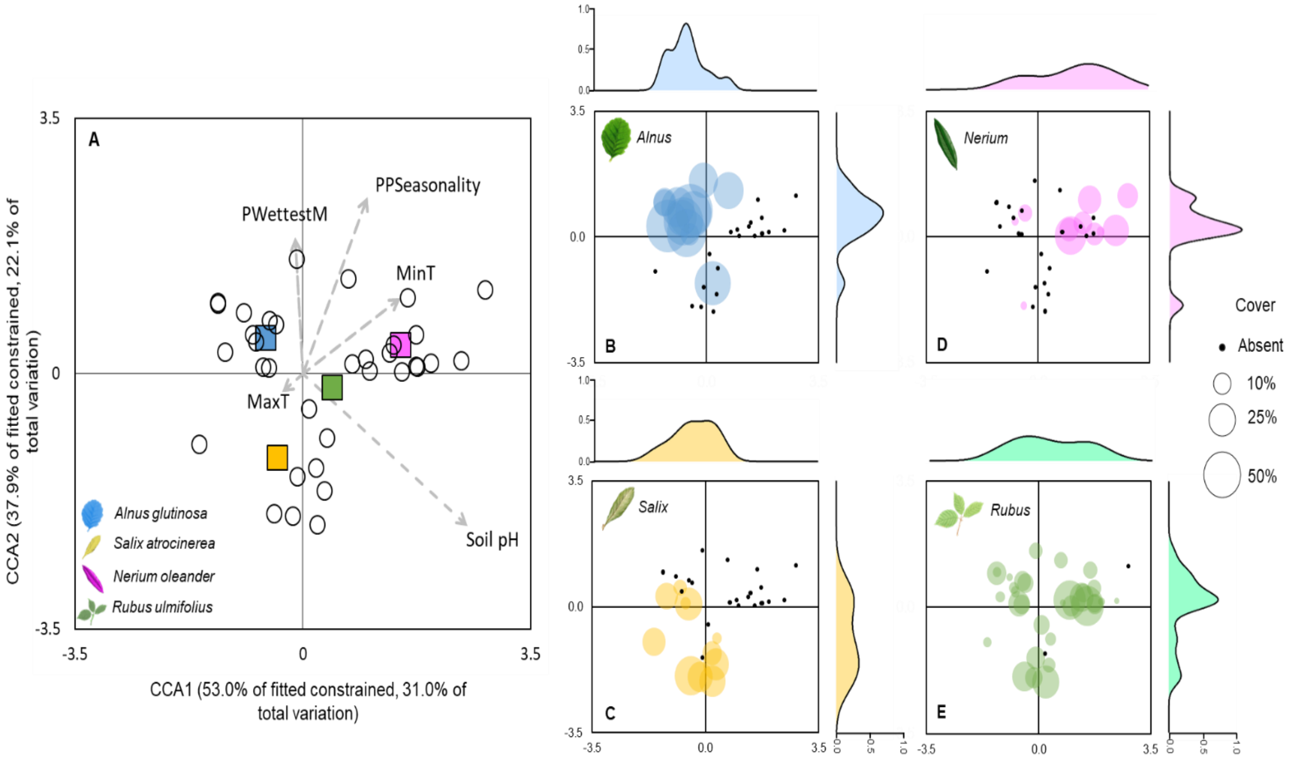

Figure 3.C1. Ordination of sampling sites for the first two CCA axes (90.87% of fitted constrained variance explained) based on the environmental variables. In A, vectors represent the weight of each environmental variable; open circles denote sampling sites; and squares show the mean weighted position of each plant species over the environmental gradient. In B-E, colored circles and their sizes represent the sampling site where each plant species was present and the percentage of canopy cover in each site, respectively; black circles represent sampling sites where each corresponding species did not appear. Marginal density plots show distribution of data for parsimonious CCA values of each plant species.

Interspecific variation and species plasticity of leaf traits

Species differed significantly in all leaf traits measured (one-way ANOVAs, all $P <$ 0.0001) (Fig. 4.C1., Table S6.C1.). *Alnus* showed the lowest toughness and the highest N concentration, and consequently the lowest C:N ratio, being for these traits antithetical to *Nerium,* which in turn showed the highest Ca concentration and C:P ratio. *Salix* exhibited the highest P, tannins, and lignin concentrations, and *Rubus* the highest Mg concentrations. The first two components of the PCA on leaf traits for the four pooled species explained 62.5% of the variation (Fig. 5.C1.-A): PC 1 represented a gradient of increasing nutrients (N and P) parallel to decreasing leaf toughness, segregating the deciduous (*Alnus* and *Salix*) and semi-deciduous (*Rubus*) species, from the evergreen *Nerium* with the highest toughness and lowest nutrient concentrations. Tannins and lignin heavily loaded (0.67 and 0.80, respectively) on positive PC 2, where *Salix* samples were clustered.

Overall, interspecific variation was higher than trait plasticity (Fig. 5.C1.-A). *Rubus*, the most widely distributed species, showed higher trait plasticity on PC 1, occupying 54% of this leaf quality gradient while other species ranged between 23-38%. However, the two species with more restricted distribution, *Alnus* and *Salix*, showed the highest trait plasticity on PC 2, occupying 66% and 51% of this leaf quality gradient,

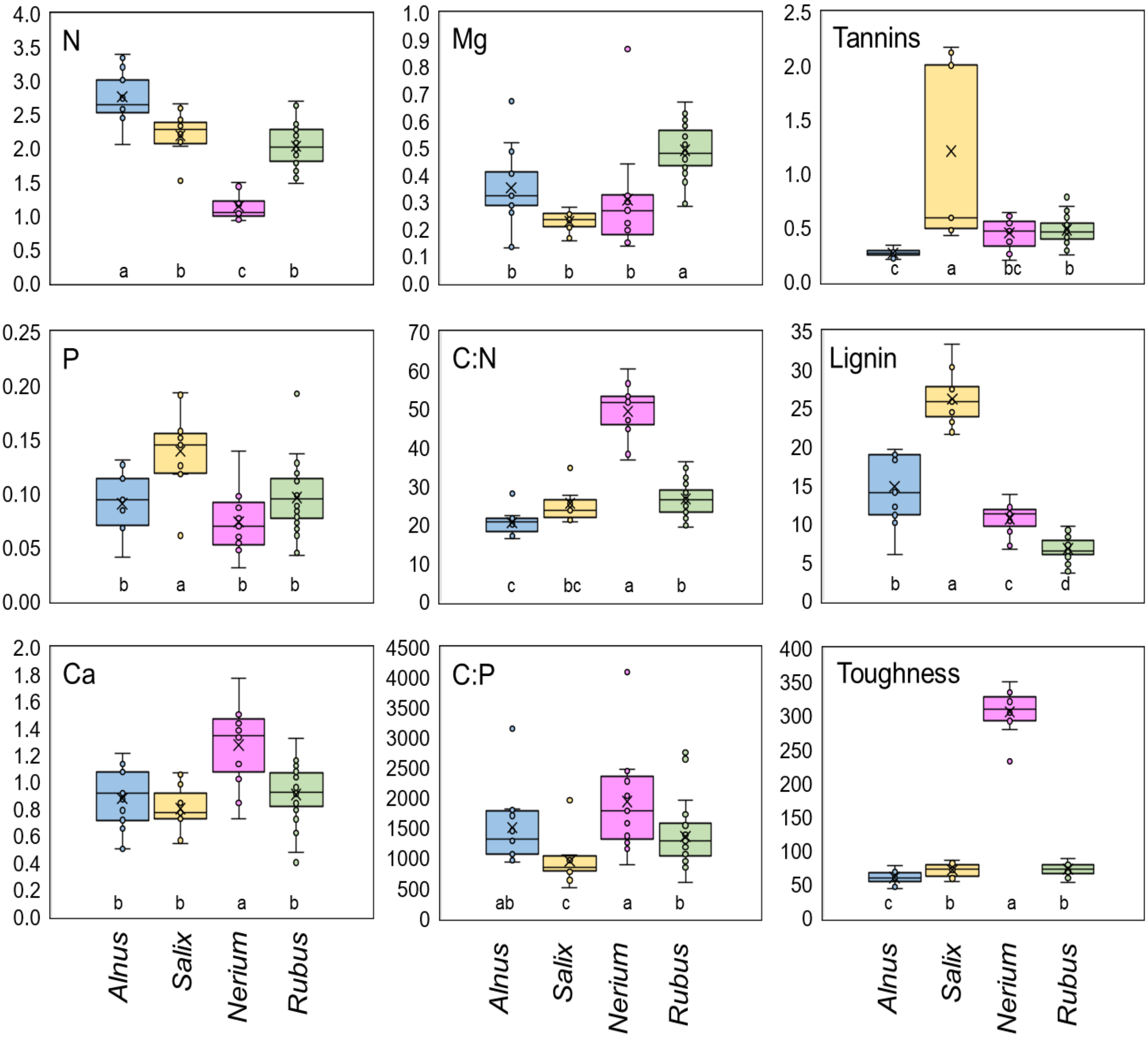

Figure 4.C1. Box-and-whisker plots for selected leaf trait variables of the four plant species studied: nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) concentrations (% DM), molar elemental ratios (C:N and C:P), lignin concentrations (% DM), condensed tannins concentrations (mg Catechin Hydrate Equivalent g DM^{-1}) and toughness (g) of each plant species. Box represents median and 25th and 75th percentile levels, crosses are the mean, whiskers are the range, and dots are replicates. Different letters indicate significant differences ($p < 0.05$) among plant species, on the basis of linear models followed by pairwise multiple comparisons (Tukey test).

respectively, compared to the more widely distributed, *Rubus* and *Nerium* (both 40%) (Fig. 5.C1.-A).

Regarding individual traits, variance partitioning analyses indicated, overall, higher interspecific variation than species plasticity in leaf traits (Fig. S3.C1.). The highest interspecific variation (> 80%) occurred in traits considered major determinants of litter decomposability-palatability—toughness, lignin, N and C:N—as expected dealing with species across different plant functional types. However, trait plasticity was higher than interspecific variation for P, Ca, Mg and C:P (ranging between 55-71%), and noticeably high for tannins (Fig. S3.C1.).

Trait plasticity was described by the first two principal components of the PCA performed for each species (Fig. 5.C1.-B-E), which explained a considerable proportion of trait plasticity: ranging between 54% in *Nerium* and 73% in *Alnus*. The first principal component (PC 1) represented for all species a gradient of increasing leaf quality (Fig. 5.C1.-B-E; Table S7.C1.) positively related with decomposability and digestibility, owing to its high positive correlation with leaf N (0.57 – 0.87) or P (0.56 – 0.93) concentrations, but negative with tannins (-0.90 – 0.29), lignin (-0.73 – 0.08) or toughness (-0.82 – -0.32). However, dimension PC 2 did not exhibit a common trend across species (Fig. 5.C1.-B-E; Table S7.C1.). For *Alnus* and *Salix*, PC 2 was positively correlated with N, but negatively with tannins and Mg, respectively; this component covaried positively with Ca but negatively with tannins in *Nerium*, and negatively with Ca, Mg, lignin, and toughness in *Rubus.*

Relative importance of climate and soil factors, and best climatic predictors of leaf trait plasticity

Univariate dependent variable PLS models indicated that leaf trait plasticity (PC 1) of the four species responded significantly and predominantly to climatic variables (Table 2.C1.). Adding soil factors to climate increased noticeably the goodness of prediction in

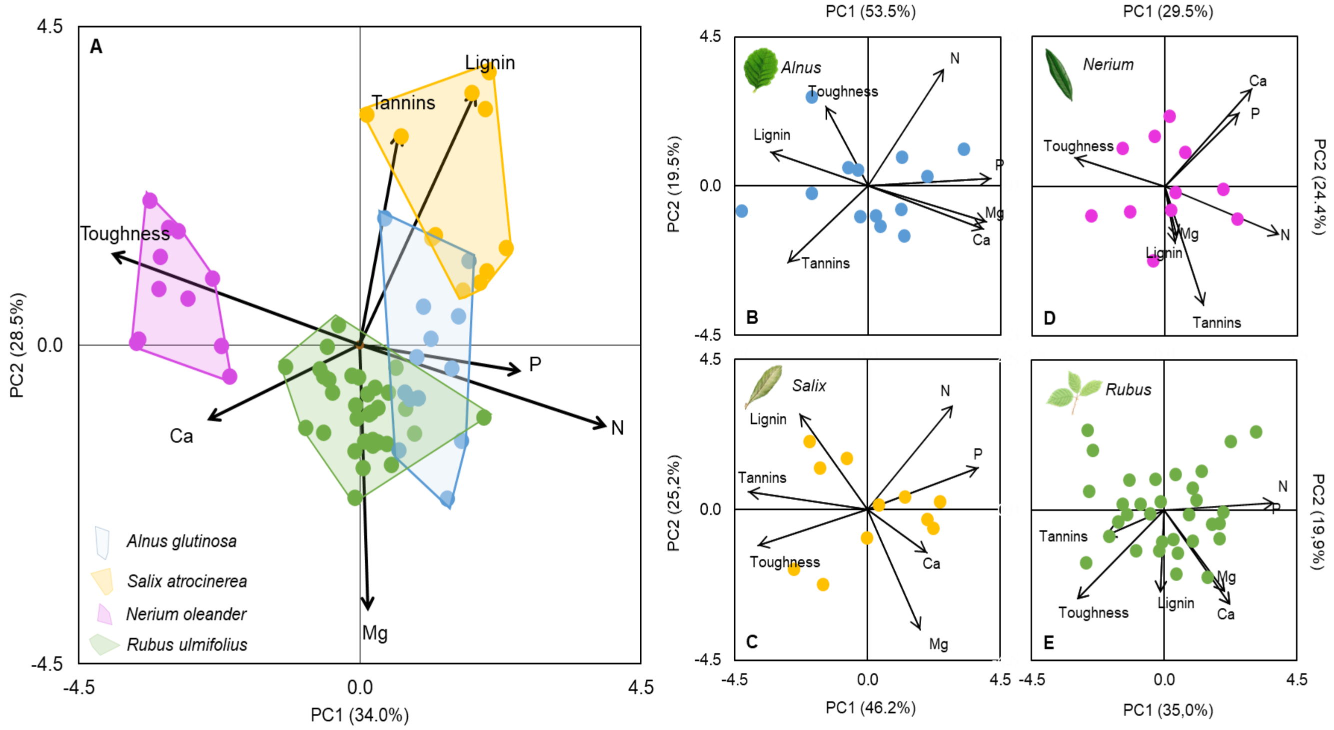

Figure 5.C1. Projection of the first two principal components of PCAs showing ordination of leaf samples of four species (circles) as a function of selected leaf traits (vectors). (A) interspecific variation (polygons of different colors) *versus* trait plasticity (circles in each polygon); (B-E) trait plasticity of each plant species separately: (B) *Alnus glutinosa*, (C) *Salix atrocinerea*, (D) *Nerium oleander*, (E) *Rubus ulmifolius*.

Nerium, but produced a highly complex model with six latent variables. Models predicting leaf trait plasticity associated to PC 2 were only significant for *Nerium* and *Salix*, but especially for the latter, in which the set of soil variables significantly predicted a high proportion of variance of leaf trait plasticity, but the model including just the set of climate variables was still significant (Table 2.C1.).

Overall, climatic predictors with the highest influence (VIP close or >1) on leaf trait plasticity associated to PC 1 (Table 3.C1.) varied among species, although most notable differences arose between broad functional groups. Mean temperature of the wettest quarter (late winter-early spring) was an important predictor with negative effects on leaf quality for deciduous/semideciduous species. Conversely, maximum annual temperature was the main predictor with high positive effect on leaf quality for the evergreen *Nerium*. Temperature annual range was an important predictor of leaf quality (PC 1) for *Nerium* and *Alnus*, although with contrasting sign (negative and positive, respectively), highlighting the opposite response that species belonging to different plant functional types may have to the same climatic variable. Moreover, precipitation variables (Table 3.C1.) did not have substantial effects on the evergreen *Nerium*, but were important predictors of leaf quality (PC 1) for deciduous/semideciduous species, with notable positive effects on *Salix* and *Rubus*, but slightly negative on *Alnus*. Leaf quality of *Salix* associated to PC 2 was primarily predicted by temperature annual range (positive effect) and winter temperature (negative effect), with precipitation variables (Table 3.C1.) being other important predictors with positive effects on leaf quality. Over this dimension, soil EC and P (with negative effects) and soil $CaCO_3$ (with positive effects) were important predictors on leaf quality of *Salix*.

Forecasted intraspecific changes in leaf quality induced by climate change

Our modelling projections showed that the four plant species would respond differently to the forecasted scenario of aridification by the year 2100 (2×CO_2 climate change scenario) in the studied region, although with remarkable congruence within broad functional groups in terms of response direction (Fig. 6.C1.; Fig. S4.C1.). For *Alnus* and *Salix* (PC 1), we observed weak evidence of overall variation in leaf quality (t = 1.523, p = 0.154; t = -2.071, p = 0.065, respectively; *Hedge's g* = 0.232 and -0.295, respectively; Fig. 6.C1.). *Salix* (PC 2; Fig. S4.C1.) and *Rubus* (PC 1; Fig. 6.C1.) showed large (62% and 57% decrease in mean position, respectively) and significant depletion of their leaf quality (t = 2.423, p = 0.036; t = -8.277, p < 0.0001; respectively), with large effect size (*Hedge's g* = 1.242 and 1.064, respectively). Conversely, leaf quality of *Nerium* is projected to increase consistently and significantly (87% increase in PC 1; t = -8.277, p < 0.0001, *Hedge's g* = -1.437) in the scenario of rising aridity used for our predictions.

DISCUSSION

Functional trait-based approaches are potentially useful to understand how species respond to environmental changes (Soudzilovskaia et al., 2013; Zhang et al., 2020) and,

Table 3.C1. Variable importance in the projection (VIP) and standardized coefficients of the environmental variables used in the PLS models developed for the first dimension of the PCA (PC1) of each species and also for the second dimension of the PCA (PC 2) of *Salix* and *Nerium* as response variables. Isothermality (%); TSeasonility, Temperature Seasonality (%); MaxT, Maximum Temperature of Warmest Month (°C), TAnnualRange, Temperature Annual Range (°C); TColdestQ, Mean Temperature of Coldest Quarter (°C); TWettestQ, Mean Temperature of Wettest Quarter (°C); TDriestQ, Mean Temperature of Driest Quarter (°C); PDriestM, Precipitation of Driest Month (mm); PPSeasonality, Precipitation Seasonality (%); PWettestQ, Precipitation of Wettest Quarter (mm); PDriestQ, Precipitation of Driest Quarter (mm); PWarmestQ, Precipitation of Warmest Quarter (mm); EC, Soil Electric conductivity (µS cm^{-1}); pH, Soil pH; $CaCO_3$, Soil $CaCO_3$ (%); Organic C, Soil Organic Carbon (%); CEC, Soil Cation exchange capacity ($cmol^{+}kg^{-1}$); BasSat, Soil Base saturation (%), ESP, Soil Exchangeable Sodium Percentage (%); P, Soils Phosphorus (%).

	Alnus glutinosa		***Salix atrocinerea***				***Nerium oleander***				***Rubus ulmifolius***	
	PC1		**PC1**		**PC2**		**PC1**		**PC2**		**PC1**	
Climatic predictor	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**
Isothermality			0.83	0.03								
TSeasonility	**1.05**	**0.06**										
MaxT							**1.13**	**0.54**				
TAnnualRange	**1.03**	**0.06**			**1.11**	**0.05**	**1.04**	**-0.36**				
TColdestQ					**1.03**	**-0.04**						
TWettestQ	0.95	-0.05	**1.33**	**-0.21**							**1.00**	**-0.10**
TDriestQ							0.79	-0.53				
PDriestM					0.99	0.04						
PPSeasonality			**1.14**	**0.14**								
PWettestQ	0.97	-0.06			0.92	0.04						
PDriestQ			0.47	0.04							**1.00**	**0.10**
PWarmestQ					0.95	0.04						
Soil predictor												
EC					**1.25**	**-0.21**						
pH			**1.15**	**-0.08**					0.60	0.03		
$CaCO_3$			0.93	-0.07	**1.16**	**0.12**						
Organic C									0.71	-0.01		
CEC	**1.00**	**-0.15**							**1.28**	**-0.15**		
BasSat	0.80	-0.05	0.99	-0.07	0.7	-0.09						
ESP	0.64	-0.02			0.59	-0.1						
P	**1.40**	**0.27**	0.90	0.06	**1.11**	**-0.1**			**1.23**	**0.16**		

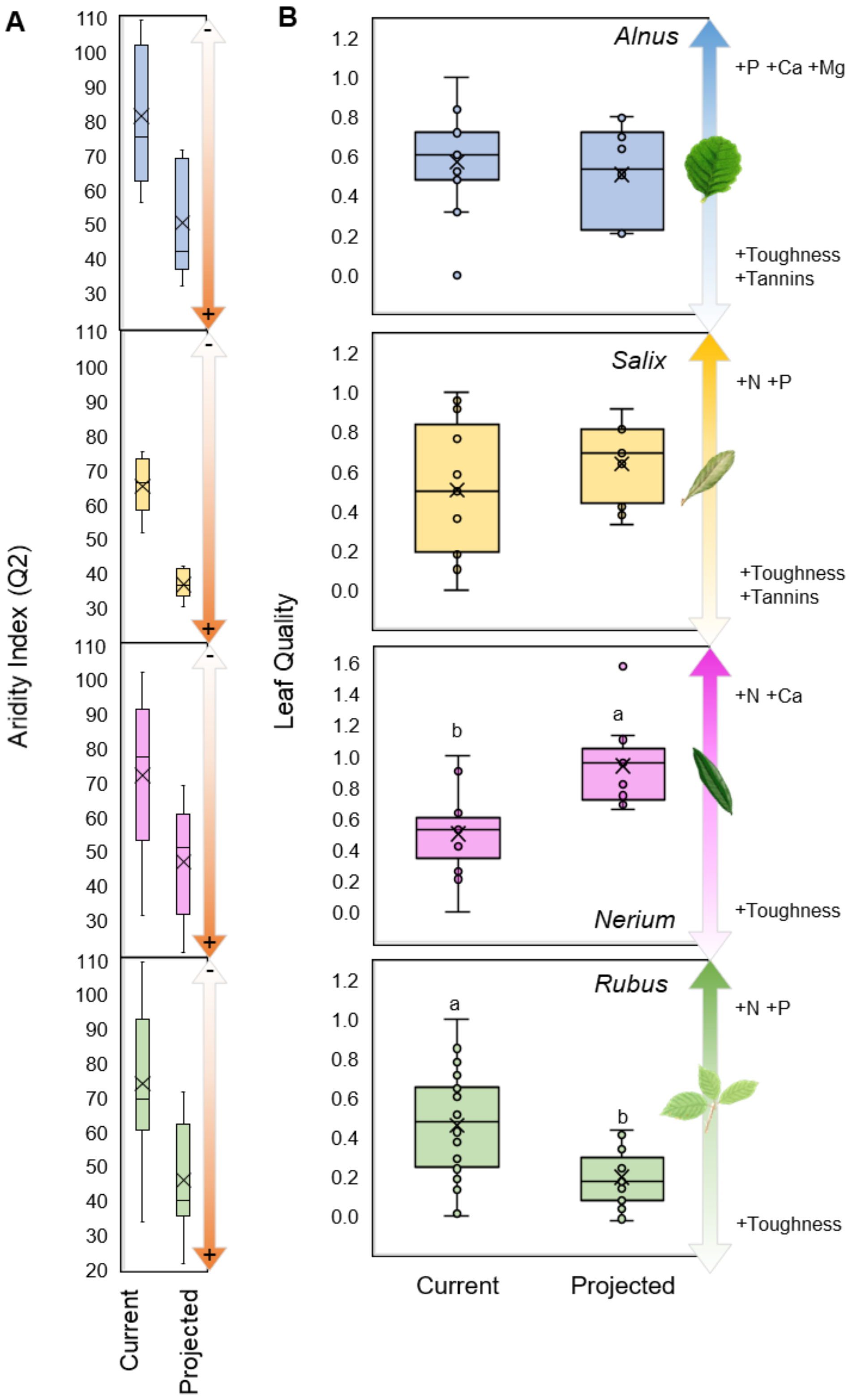

Figure 6.C1. Boxplots showing (A) the aridity range covered by the distribution of each species according to the Emberger's bioclimatic coefficient (Q2); and (B) the leaf quality (PC1) of each plant species at present (Current) and in future climate change scenarios according to the NCAR Community Model version 3 (CCM3) for the year 2100 (Projected). Note that leaf quality ranges (PCA dimensions) are scaled to unit for simplicity. Different letters indicate significant different based on t-test analyses.

therefore, are important for an ecologically sensitive management of ecosystems. Here, we assessed how climate change might affect leaf quality of different riparian woody species from an intraspecific perspective, which has been much disregarded based on the general assumption that intraspecific variation accounts only for an irrelevant portion of total trait variability (Garnier et al., 2001). Overall, in support of our first hypothesis, but contrary to previous studies (Graça & Poquet, 2014; Ordoñez et al., 2009), climate showed larger influence than soil explaining most leaf trait plasticity. Our second hypothesis of decreasing intraspecific leaf quality—linked to determinant traits of palatability and decomposability—with increasing aridity was partially supported, given that increasing temperature had negative effects on leaf quality of deciduous and semi-deciduous species, but not on the evergreen *Nerium*, which displayed the opposite response. These results suggest potential effects on stream ecosystem functioning (Fenoy et al., 2021; A. Martínez et al., 2013), but with inverse sign depending on the identity of dominant species in the riparian vegetation. Moreover, in support of our third hypothesis, we generally observed higher variation among species than plasticity within species, except for a few traits (e.g. P, Ca, and Mg concentrations and C:P ratio) that exhibited remarkable leaf trait plasticity (Albert et al., 2010; Fajardo & Piper, 2011). Nonetheless, ranges of trait plasticity found here for some traits (e.g. %N, %P and %lignin) are similar, or higher, than those reported before for other species (e.g. Lecerf & Chauvet, 2008; LeRoy et al., 2007; Oliveira et al., 2021).

Relative influence of climate and soil factors

Over the environmental gradient studied, climate exhibited an overall higher influence than soil on most species' leaf trait plasticity, although soil was the strongest predictor in some cases (e.g. *Salix* and *Nerium* PC 2). We presumed higher responsiveness of leaf traits to climate than soil in species with distributions highly constrained by soil conditions. This appears to be the case for the acidophilic *Alnus* (Miles, 1985), the species with the highest control of climate on its leaf trait plasticity. The fact that *Alnus* is an N-fixer may have further contributed to make this species less sensitive to soil nutrients. However, other species with less restricted soil-related distributions, such as *Rubus*—spread out across almost the entire study area—or *Nerium*, also showed a prominent role of climate influence on leaf trait plasticity. Similar patterns have been observed when assessing the abundance of plant functional types in the same region and across environmental gradients (Salinas et al., 2018).This lower predictive role of soil variables may stem from the high dynamics of alluvial soils and their permanent water availability, which would tend to homogenize conditions—i.e. nutrient availability—among sites (Naiman & Decamps, 1997). Yet our results are counter to other findings recorded at much larger spatial scales that observed substantial importance of soil predictors explaining intraspecific changes in leaf traits (Graça & Poquet, 2014; Ordoñez et al., 2009). This suggests that other factors not considered here, such as the great topographic variability present in our spatial gradient, or genotype differences, might be overriding soil effects.

Main climatic predictors of leaf trait plasticity

Among climatic factors temperature exhibited much clearer patterns than precipitation on the main dimension of leaf trait plasticity (PC 1). This is to be expected in riparian belts of permanent streams where soil moisture tends to be relatively high and constant in the absence of extreme drought events (Moore et al., 2016), preventing major water stress in plants and its consequences on leaf characteristics (e.g. García-Palacios, Prieto, et al., 2016; LeRoy et al., 2014). However, climate-driven changes in streamflow may worsen the effects of aridification on such ecosystems (Perry et al., 2012).

Despite clear differentiation in distribution extent among species, we detected a common negative relationship between temperature and leaf quality in the deciduous and semi-deciduous species. On the contrary, this relationship was positive for the evergreen *Nerium*. Overall, nutrient concentrations (N, P, Ca, Mg) decreased but tannin and/or lignin concentrations, and/or toughness increased with increasing temperature for deciduous/semi-deciduous species, whereas *Nerium* roughly exhibited the opposite pattern. Thus, within the frame of the leaf economic spectrum (Reich et al., 1997; Wright et al., 2004), the above seems to reveal antithetical syndromes of leaf traits between functional groups in response to temperature, in which the intraspecific inter-correlated leaf traits along our quality gradient represent physiological and structural trade-offs (Boyero et al., 2017; Onoda et al., 2017).

Decreasing leaf N and/or P concentrations with increasing temperature has been reported before in woody deciduous species (Chen et al., 2011; Kudo et al., 2001; Sun et al., 2015). This may be explained by an increase of the catalytic capacity of photosynthetic enzymes at higher temperatures, requiring lower enzyme amounts (e.g. lower N concentration) to maintain photosynthetic rates (i.e., the photosynthetic rate is achieved with lower amounts of such enzyme; Scafaro et al., 2017). Alternatively, or additionally, higher temperature is often associated with increasing length of the growing season in deciduous species, which in turn promotes long leaf lifespan. Long-lived leaves often invest more in structure/protection at the expense of reducing photosynthetic efficiency (Kudo et al., 2001; Onoda et al., 2017). Similarly, other authors have reported that deciduous plants growing under relatively elevated temperatures develop tougher leaves (Wright et al., 2017), or leaves with higher tannin (Top et al., 2017) and lignin (Graça & Poquet, 2014) concentrations.

Reduction of nutrients and strengthening of leaf traits to confer resistance (e.g., increasing toughness) have been reported in evergreen *Quercus* species in response to decreasing winter temperatures. This is interpreted as a higher cost for evergreens at cooler sites compared to deciduous trees (González-Zurdo et al., 2016). However, this finding is not totally consistent with our results for *Nerium* as winter temperatures did not exhibit any effect on its leaf quality. We observed the strongest positive effect on leaf quality of *Nerium* from maximum temperature, but a more negative effect from annual temperature range. This suggests that *Nerium* develops more nutrient rich and softer leaves in its optimum distributional range (areas with mild winters and maritime influence), with negligible effects from harsh low-winter temperatures, which are infrequent in its area of distribution. Nevertheless, we cannot rule out the possibility that our results are species-specific, and projection of such results to the entire functional group need to be confirmed with the study of further evergreen species.

A substantial amount of leaf trait plasticity (25%) in *Salix* (PC 2)—positively related to leaf N and lignin, and negatively to Mg concentrations—was significantly explained by climatic conditions, but much more by soil variables. The strong positive association of N and lignin on PC 2 suggest that this N fraction is structural, possibly lignin-bound N, therefore not readily available to decomposers and detritivores (Berendse et al., 1987). Thus, PC 2 represents a structural reinforcement of *Salix* leaves positively related with temperature annual range and negatively with winter temperature, but also, and mostly, negatively with soil P. A structural reinforcement of leaves (increasing leaf mass per area and lignin concentration) with decreasing soil fertility has been documented elsewhere (e.g. Diehl et al., 2008).

The trait plasticity observed in this study can arise from responses to environmental conditions, but also from genetic variability. Genotypes, although largely influenced and selected by local environments, represent an important source of trait variability unaccounted for here. Genetic variability has been exhibited to strongly influence litter quality and, consequently, associated ecosystem processes (e.g. litter decomposition) and communities (Crutsinger et al., 2014; LeRoy et al., 2006; LeRoy et al., 2007; LeRoy et al., 2012). Given that leaf traits differ in their heritability, for example tannins appear to be highly heritable whereas C:N ratios are environmentally controlled (Crutsinger et al., 2014), further research assessing how environment × genotype interaction affect leaf traits is important for improving predictions of potential effects on ecosystem functioning, particularly, in the face of climate change.

Projecting climate-change driven variation of species leaf quality: implications for stream ecosystems

Litter trait variation across species constitutes the main driver of instream litter decomposition worldwide (Boyero et al., 2017; García-Palacios, McKie, et al., 2016; Zhang et al., 2019), indicating an essential role of plant phylogenetic history on controlling such process (LeRoy et al., 2019). Although less studied, some evidence indicates that the control exerted by trait plasticity on litter decomposition, nutrient cycling and trophic dynamics could be almost as important as interspecific changes (Jackrel & Morton, 2018; Jackrel et al., 2016; Lecerf & Chauvet, 2008; LeRoy et al., 2007; Oliveira et al., 2021). Here, we assessed the plasticity of selected traits of green leaves of riparian plants aimed at forecasting potential consequences of climate change on stream ecosystems highly dependent on these resources (i.e., forest streams; Wallace et al., 2015). Although inputs of leaves to streams are mainly in the form of leaf litter, it has been reported that some traits of green leaves tend to persist after senescence and control rates of litter decomposition (Cornelissen et al., 1999; Cornwell et al., 2008). Therefore, if nutrient resorption efficiency remains fundamentally invariable across climatic conditions (Aerts et al., 2007; Norby et al., 2000; but see Z. Yuan & H. Y. H. Chen, 2009), understanding how green leaves respond to climate change may allow us to anticipate effects of leaf quality changes on stream ecosystem functioning. In support of this idea, a recent study suggests that traits of green leaves can be used to accurately predict decomposition rates (Rosenfield et al., 2020). However, as others have pointed out that traits of litter can differ from those of fresh leaves (Hättenschwiler et al., 2008; Hättenschwiler & Vitousek, 2000; Horner et al., 1987; Z. Yuan & H. Y. Chen, 2009), the potential effects on headwater stream functioning exposed here should be interpreted with caution.

Litter decomposition is often reported to be enhanced by its high N and P concentrations (García-Palacios, McKie, et al., 2016; MacKenzie et al., 2013). Elevated litter concentrations of Ca and Mg—reported to be important for fungal decomposers (Jenkins and Suberkropp, 1995) and macroinvertebrates (Makkonen et al., 2012; National Research Council, 2005)—can also accelerate decomposition (Santonja et al., 2019). Moreover, tannins (Coq et al., 2010; Irons et al., 1988), lignin (Ferreira, Raposeiro, et al., 2016; Ramos et al., 2021; Schindler & Gessner, 2009) and toughness (Fenoy et al., 2021; Li et al., 2009) primarily tend to reduce litter consumption by detritivores. Our results point to a general decrease in leaf quality as a response to aridification in the three deciduous/semi-deciduous species. This decrease was generally related to a reduction in leaf N and P, but also Ca and Mg, versus an increase in tannins or lignin, and leaf toughness.

In particular, changes in leaf quality of the deciduous N-fixer *Alnus* could have major consequences given the key role of this species on stream ecosystem processes (Alonso et al., 2021; J. Pérez et al., 2021; Rubio-Ríos et al., 2021). We reported here for *Alnus* ranges of %N, %P and %lignin variation similar to those reported at the European continental scale (Lecerf & Chauvet, 2008), and 53% of its species leaf trait plasticity was remarkably explained by climatic variables, yet our forecasted decrease in leaf quality was relatively low (11%) and not statistically significant, compared to other species. Nonetheless, apparent subtle changes in litter traits, might result in major effects in consumer fitness (Javier Pérez et al., 2021). Furthermore, this projected minor decrease in leaf quality, adds to the decline of populations of this key species through Europe due to a disease caused by the pathogen *Phytophthora alni* (Bjelke et al., 2016), which also has been recently reported to alters nutritional quality of leaf litter (Ferreira et al., 2021). Both factors are likely to trigger significant alterations to the functioning of forested streams (Alonso et al., 2021). Moreover, if a general decrease in leaf quality occurs in other deciduous species, as those forecasted here for *Salix* and *Rubus*, the negative influences on stream food webs will increase.

Thus, our results indicated that decreases of leaf quality of individual deciduous species may occur in a relatively short term (via phenotypic plasticity; Nicotra et al. 2010; but see Valladares et al. 2007) , which in the long term will add to the forecasted dieback of deciduous woody species in riparian corridors of temperate climate zones (Kominoski et al., 2013; Salinas et al., 2018). Both riparian changes have the potential to significantly impair instream ecosystem processes, particularly in mountain streams presently dominated by deciduous vegetation (Fenoy et al., 2021), more than in lowland streams where deciduous species actually represent a minor component of the riparian belt.

Chapter 2

Key plant species and detritivores drive diversity effects on instream leaf litter decomposition more than functional diversity: A microcosm study

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ABSTRACT

Anthropogenic impacts on freshwater ecosystems cause critical losses of biodiversity that can in turn impair key processes such as decomposition and nutrient cycling. Forest streams are mainly subsidized by terrestrial organic detritus, so their functioning and conservation status can be altered by changes in forest biodiversity and composition, particularly if these changes involve the replacement of functional groups or the loss of key species. We examined this issue using a microcosm experiment where we manipulated plant functional diversity (FD) (monocultures and low-FD and high-FD mixtures, resulting from different combinations of deciduous and evergreen *Quercus* species) and the presence of a key species (*Alnus glutinosa*), all in presence and absence of detritivores, and assessed effects on litter decomposition, nutrient cycling, and fungal and detritivore biomass. We found (i) positive diversity effects on detritivore-mediated decomposition, litter nutrient losses and detritivore biomass exclusively when *A. glutinosa* was present; and (ii) negative effects on the same processes when microbially mediated and on fungal biomass. Most positive trends could be explained by the higher litter palatability and litter trait variability obtained with the inclusion of alder leaves in the mixture. Our results support the hypothesis of a consistent slowing down of the decomposition process as a result of plant biodiversity loss, and hence effects on stream ecosystem functioning, especially when a key (N-fixing) species is lost; and underscore the importance of detritivores as drivers of plant diversity effects in the studied ecosystem processes.

Keywords: *Alnus glutinosa*, biodiversity-ecosystem functioning, complementarity and selection effects, microbial, net diversity effect, leaf litter mixtures

INTRODUCTION

The current human-induced biodiversity decline (Ceballos & Ehrlich, 2018) and its consequences for ecosystem functioning (hereafter B-EF) have attracted much attention since the seminal paper by Naeem et al. (1994)). Headwater stream ecosystems in forested regions may be especially sensitive to plant biodiversity loss, owing to their dependence on allochthonous organic matter, mostly in the form of leaf litter (Kominoski et al., 2011; J. B. Wallace et al., 1997). Instream litter decomposition is a crucial process involving the cycling of nutrients and the production of microbial and invertebrate biomass (Marks, 2019). Therefore, ongoing changes in riparian vegetation affecting litter inputs (e.g. dominance of evergreen versus deciduous plants; Kominoski et al., 2013; Salinas et al., 2018) may alter the stream food web structure (Bärlocher & Graça, 2002; Martínez et al., 2013; Seena et al., 2017) as well as biogeochemical cycles at multiple scales (Battin et al., 2009; Casas et al., 2013).

Plant biodiversity loss effects on instream decomposition have been addressed by numerous studies. However, their outcomes have been idiosyncratic (Gessner et al., 2010), showing positive (e.g., Gartner & Cardon, 2004; Leroy & Marks, 2006) to negative diversity effects (e.g., Swan & Palmer, 2006). These contrasting results may arise from differences in experimental designs (field vs. laboratory experiments), the selected plant species or the use of different diversity measures. An alternative approach to species richness is to focus on functional diversity (FD), i.e. the diversity of functional traits, which has been reported to have a pivotal role on ecosystem processes (Petchey & Gaston, 2006) by favoring different organisms (niche or resource partitioning; Finke & Snyder, 2008). However, there is no consensus about which is the most suitable FD measure (Flynn et al., 2011; Petchey et al., 2009), or which traits are the most relevant for a particular process.

Another controversial question relates to the primary role of single species, in particular key species (also called keystone process species sensu Folke et al., 1996), which might drive critical processes and thus determine the overall ecosystem functioning (Ellison et al., 2005). Litter from these key species could promote diversity effects through a variety of underlying mechanisms, including an increase in the mean value of the nutrient pool in litter mixtures (mass ratio hypothesis; Grime, 1998), or via nutrient transfer between litter types (Gessner et al., 2010; Tiunov, 2009).

Our study addresses two main questions. First, by mixing tree species belonging to the same or different functional types in terms of their leaf habits (deciduous, semi-deciduous or evergreen), we explored plant FD effects on several stream ecosystem processes (litter decomposition, nutrient cycling and changes in biomass of fungi and detritivores) and the relative role of microbial decomposers and detritivores as drivers of these effects. Second, we examined the role of black alder, *Alnus gutinosa* (L.) Gaertn., a deciduous nitrogen (N)-fixing plant with soft, nutrient-rich and fast decomposing litter (Casas et al., 2013; Hladyz et al., 2009), as key species with direct repercussions on the above processes (e.g., Graça et al., 2015; J. Pérez et al., 2021; Piccolo & Wipfli,

2002) and B-EF relationships (Alonso et al., 2021; López-Rojo et al., 2018). In Europe, populations of *A. glutinosa* are declining since 1990 (Bjelke et al., 2016; Gibbs et al., 1999) due to a fungal disease caused by *Phytophthora alni "*sensu lato" (Husson et al., 2015). We used microcosms, with and without a stream detritivore to conduct an experiment using monocultures, low-FD and high-FD mixtures (resulting from different combinations of deciduous and evergreen *Quercus* species) and the key species *A. glutinosa*. We tested the following hypotheses: (1) FD increases trait variability of litter mixtures (Flynn et al., 2011; Hillebrand & Matthiessen, 2009); (2) litter FD enhances decomposition and nutrient cycling through resource partitioning (Handa et al., 2014; López-Rojo et al., 2019) and (3) leads to a higher fungal (Kominoski et al., 2009) and detritivore biomass through a balanced diet effect (DeMott, 1998; Duffy et al., 2007); (4) the above diversity effects are greater when a key plant species is present due to overall increased nutrient content and/or increased trait variability (Larrañaga et al., 2020; López-Rojo et al., 2018); and (5) such effects are mostly mediated by detritivores (Tonin et al., 2017).

MATERIAL AND METHODS

Selection of litter and detritivore species

We selected 7 oak species belonging to 3 different plant functional groups in terms of their leaf habits, and consequently with regard to leaf traits and carbon (C) gain strategies (see Escudero et al., 2017; Gil-Pelegrín et al., 2017 for more details) to examine effects of plant FD on stream processes: 3 deciduous (*Quercus robur* L., *Q. canariensis* Willd. and *Q. pyrenaica* Willd.), 1 semi-deciduous (*Q. faginea* Lam.) and 3 evergreen species (*Q. coccifera* L., *Q. suber* L. *and Q. rotundifolia* Lam.). Deciduous *Quercus* species have leaves that show functional traits values such as greater leaf area, and lower Leaf Mass per Area (LMA) and leaf thickness (considered proxies for mass allocation and related processes, Onoda et al., 2011), and shorter leaf lifespan than leaves of evergreen *Quercus* species (Escudero et al., 2017). *Quercus faginea* is a semi-deciduous tree (Sanz-Pérez et al., 2009), i.e. its old and withered leaves remain on the plant for the most of the winter until the emergence of new leaves the following spring (see Veselá et al., 2018). Additionally, we chose the deciduous N-fixing *A. glutinosa* as key species (J. Pérez et al., 2021). The 8 species covered a broad litter quality spectrum (Table 1.C2., Table S1.C2.). Leaves were collected in autumn 2017 from forests of different catchments in Southern Spain, except for *Q. robur*, which was collected in Northern Spain as it is absent at lower latitudes (Table S2.C2.). Deciduous leaves were gathered from the forest floor just after senescence and evergreen leaves were collected from dry, broken branches, with no symptoms of diseases. Leaves were air dried at room temperature (20-23 ºC) for 1 week and stored in darkness until used for experimentation.

We selected larvae of the caddisfly *Allogamus mortoni* Navás (hereafter *Allogamus* or detritivores), an endemic leaf-shredding species common in streams of the Iberian

Table 1.C2. Experimental treatments of varying functional diversity, *Quercus* and *Alnus* species used in the microcosm experiment. Leaf litter quality index (LQI) of each species is showed between parenthesis and LQI and Rao´s quadratic diversity (Rao's Q) of litter mixtures traits is presented in the corresponding columns.

Treat ID	Functional diversity	Functional group	Sp1	Sp2	Sp3	LQI	Rao's Q
Low FD-D	Low	Deciduous	*Q. robur* (0.36)	*Q. canariensis* (0.34)	*Q. pyrenaica* (0.35)	0.35	4.78
Low FD-E	Low	Evergreen	*Q. coccifera* (0.23)	*Q. suber* (0.35)	*Q. rotundifolia* (0.36)	0.31	4.86
High FD-O	High	Deciduous, semi-deciduous and evergreen	*Q. robur* (0.36)	*Q. faginea* (0.37)	*Q. rotundifolia* (0.36)	0.36	9.37
High FD- K	High	Deciduous and N-fixing, semi-deciduous and evergreen	*A. glutinosa* (0.58)	*Q. faginea* (0.37)	*Q. rotundifolia* (0.36)	0.43	10.59

Peninsula (Ruiz-García et al., 2004). This species is widespread within the Iberian Peninsula and likely coexist with all the plant species selected. Detritivores were collected from Río del Pueblo, a second-order stream located at 1405 m a.s.l in the Sierra Nevada Natural Park (37.15°N, 3.15°W; Southeast Spain). Riparian vegetation consists of mixed deciduous tree species dominated by alder groves (*Alnus glutinosa*) and plantations of *Populus nigra* L., followed out to the slopes by natural forests of the evergreen holm-oak (*Quercus rotundifolia*) and plantations of the conifer *Pinus pinaster* Aiton. Other frequent plant species encountered in the stream bank include *Salix atrocinerea* Brot. *Castanea sativa* Mill, *Populus alba* L., or *Rubus ulmifolius* Schott. Detritivores were acclimated to experimental conditions (see below) for 1 week and starved for 48 h just prior to starting the experiment. Initial detritivore dry mass (DM) was estimated from a case aperture diameter (CD) (mm) / DM (mg) relationship, using additional *Allogamus* larvae (DM = $0.097\ e^{1.362\ CD}$, $R^2 = 0.87$, $p < 0.001$, root mean square error = 1.64, $n = 55$). CD was measured under a binocular microscope (0.1 mm), and DM was weighed (0.1 mg) in freeze-dried uncased individuals that were later used to analyse their initial body N and phosphorus (P) (see Supplementary Methods in Supplementary Material).

Experimental setup

A total of 120 microcosms were placed in a temperature-controlled room set at 10 ºC (the mean of hourly records obtained in the stream during the experiment season (April-May) in previous years) and a light:dark 12:12 h photoperiod (based on the natural cycle at that time of the year), and provided with continuous air supply (Fig. S1.C2.). Each microcosm consisted of a 500-mL glass jar containing 30 cm^3 of stream sediment, previously furnaced (500 ºC, 5 h) and washed with distilled water, and 400 mL of filtered (100 µm) stream water, which was oligotrophic (soluble reactive P: 7 µg P L^{-1}; nitrate-N: 24 µg N L^{-1}) and soft circumneutral (pH: 7.10; electrical conductivity: 60 µS cm^{-1}; alkalinity: 0.32 meq L^{-1}). Sediment and water were collected from the same stream as detritivores (Río del Pueblo). Just before the experiment, litter was cut (≈ 2x2 cm, avoiding the basal midrib) and weighed to the nearest 0.1 mg. Each microcosm received 1.516 ± 0.001 g (mean ± SE) of air-dried litter pieces belonging to 1 (monocultures) or 3 plant species (litter mixtures containing 0.5 g per species), combined as shown in Table 1.C2. to create the highest gradient of FD possible among all the potential combinations (Table S3.C2.; 12 treatments in total: 8 monocultures and 4 mixtures). Mixtures consisted of 2 low FD (deciduous: Low FD-D, or evergreen: Low FD-E, oak species) and 2 high FD treatments (one including all the leaf habits ─deciduous, semi-deciduous and evergreen─ within oak species: High FD-O, and the other including also the key species: High FD-K).

Litter fragments of the same species were color-coded and kept together using 3 safety pins per microcosm; the same was done in monocultures to mirror the conditions of mixtures. In each litter treatment, detritivores were added to 7 microcosms (2 larvae of *Allogamus* per microcosm); the other 3 remained without detritivores in order to separate their contribution from that of microorganisms (Fig. S1.C2.). Detritivore initial biomass per microcosm was on average 12.94 ± 0.98 mg (mean ± SE), without significant differences across litter treatments (One-way ANOVA, $p = 0.97$). The experiment lasted 42 days (April-May 2018). Larval mortality was recorded daily, and dead larvae (52 out of 244) were replaced by a new one only when both individuals died in a given jar (4 out of 84 microcosms with detritivores).

Experimental procedure

Before adding the detritivores to microcosms, litter fragments were incubated for 5 days in microcosms with aeration (with stream water renewal on the third day) to allow the leaching of soluble compounds and initial microbial colonization. Twenty-four extra microcosms without detritivores (3 per plant species in monoculture), containing ca. 1.5 g of litter, were used to measure initial (post-leaching; Fig. S2.C2.) ash-free dry mass (AFDM) and litter traits (Supplementary Methods). Thereafter, water was renewed weekly with filtered (100 µm) stream water collected the same day. On the last day, litter material was separated by species, and 5 discs (12 mm Ø) of each species (3 replicates with and 3 without detritivores per treatment) were cut out with a cork borer, freeze-dried, weighed and used to estimate fungal biomass, which was measured through ergosterol concentration following Suberkropp and Weyers (1996) with some modifications (Supplementary Methods). The remaining litter material was oven-dried (70 ºC, 72 h), weighed to determine final DM, and ground to leaf powder (Mixer Mill RETSCH MM 200). An aliquot of each sample was furnaced (550ºC, 5 h) and weighed to estimate final AFDM, and another aliquot was used for elemental analyses (C, N and P; Supplementary Methods). At the end of the experiment, detritivores from each microcosm were starved for 48 h. Then, the diameter of their cases was measured as above and individuals were uncased, freeze-dried and stored until N and P analyses (Supplementary Methods).

Data analyses

We examined trait variability among litter treatments using principal component analysis (PCA; *prcomp* function in the ‘stats’ package of R statistical software (R Core Team, 2020). Previously, a Pearson correlation analysis was performed to remove litter traits with high (>0.85) collinearity (Fig. S3.C2.). Twelve litter traits were included in the PCA: toughness, N, P, N:P molar ratio, hemicellulose, lignin, total phenolics, Ca, K, Mg, Si and non-structural carbohydrates (NSC). To test our 1st hypothesis we assessed trait variability in litter mixtures using the Rao’s quadratic diversity coefficient (Rao, 1982), widely applied in functional ecology analyses [Rao’s Q; *dbFD* function in the ‘FD’ R package (Laliberté et al., 2014)]. Statistical differences among species or mixture traits were assessed using one-way ANOVA and Tukey multiple comparisons tests with the *anova* and *TukeyHSD* functions of ‘stats’ R package (R Core Team, 2020). *Log* or *arcsin* transformations of variables were used when required.

Litter palatability was estimated using the litter quality index (LQI) equation proposed by Solagaistua et al. (2019): LQI: $1 - [(T_1/(n \times T_{1(\max)})) + ... + (T_n/(n \times T_{n(\max)}))]$; where T is the average of the measured value for the trait and n the number of measured traits. In order to ease interpretation, we selected a limited number of traits (5 out of 20: toughness, C:N and C:P molar ratios, phenols and NSC) that are relevant for detritivore- (e.g., Hladyz et al., 2009) and microbially-mediated decomposition (Fernandes et al., 2012; López-Rojo et al., 2020), being the latter usually favored by a high nutrient and structural carbohydrates content.

Litter decomposition was quantified as the proportion of litter mass loss [LML = (initial – final AFDM)/initial AFDM)], after correcting initial litter mass for humidity, ash content and leaching losses. LML in microcosms without detritivores (i.e., microbially-mediated LML) was used to correct initial AFDM in microcosms with detritivores and thus isolate detritivore-mediated LML, allowing us to partially test our 5th hypothesis (i.e. FD effects are mostly mediated by detritivores). Litter N loss (LNL) and litter P loss (LPL) were calculated as: (initial – final)/initial N or P content. Positive values represented nutrient mineralization, and negative ones nutrient immobilization. Detritivore relative growth (DMG), detritivore N gain (DNG) and detritivore P gain (DPG) were quantified as the proportional change in detritivore DM, N and P content, respectively [(final – initial)/initial DM, N or P content]; and were estimated only in those microcosms with no animal replacements. Finally, proportional changes in nutrient concentration of litter and detritivores were determined as: [(final – initial) / initial N or P %)].

We examined differences in all response variables (LML, LNL, LPL, ergosterol, DMG, DNG and DPG) among monocultures and among litter mixtures using one-way ANOVAs and Tukey multiple comparisons tests (see above function and package). To avoid very complex models with many interactions, we examined detritivore- and microbially-mediated variables separately. We applied the Kaplan-Meier test to compare *Allogamus* survivorship among treatments using the *survfit* function of ‘survival’ R package (Therneau, 2015). Any possible effect of mortality on other detritivore related variables was prevented standardizing by the number of days that each animal survived relative to the experimental time.

We explored our 2nd and 3rd hypotheses through the difference between observed (Obs.) and expected (Exp.) values (i.e. the net diversity effect, NDE = Obs. – Exp.), with expected values estimated based on monocultures. NDE was estimated in microcosms with and without detritivores enabling us to better assess our fifth hypothesis. To assess the magnitude of these effects we calculated the relationship between the NDE and expected values [NDE magnitude (%): (Obs. – Exp.) / Exp. × 100]. We explored differences among treatments with nonparametric bootstrapped 95% confidence intervals using the BCa method in the *boot.ci* function of `boot' R package (Canty & Ripley, 2019), which allowed us to test if FD effects were greater when the key plant species is present (our 4th hypothesis). For LML and ergosterol, the NDE was partitioned into complementarity and selection effects using the additive partitioning method (Loreau & Hector, 2001). In the case of LML, we were able to split diversity effects for total, detritivore- and microbially-mediated decomposition, to further assess the role of detritivores on BE-F relationships (5th hypothesis). Complementarity effects are explained by synergistic (positive) or antagonistic (negative) interactions; and selection effects represent positive (or negative) effects due to the presence of a dominant species in the mixture, which enhances (or inhibits) overall decomposition of the mixture (Handa et al., 2014). In addition, we carried out intraspecific comparisons, i.e. we compared LML for each species in monocultures and in each mixture where present. We finally explored the relationships between litter palatability (LQI) or litter traits variability (Rao’s Q) with the measured variables and diversity effects in mixtures using linear models (*lm* function of ‘stats’ R package) in order to better explain the observed patterns and separate the FD effects from that caused by the presence of the key species.

RESULTS

Litter trait variation across species and mixtures

Trait variability within a mixture (represented by polygon size, Fig. 1.C2.) was the highest in High FD-K followed by High FD-O, Low FD-E and Low FD-D, matching the FD assessment by means of Rao’s Q (Table 1.C2.). The first 2 dimensions of the PCA explained 68.7% of trait variation (Fig. 1.C2., Table S1.C2.) and showed a clear separation of evergreen and deciduous species and their mixtures; however, low-FD and high-FD mixtures (which shared species) overlapped. PC1 mostly represented a gradient of structural and secondary metabolites, and PC2 represented a gradient in elemental composition. *A. glutinosa* was clearly separated from oak species due to its particularly high concentration of lignin and N. LQI (Table 1.C2.) was lowest for *Q. coccifera* and the evergreen mixture (Low FD-E), and highest for *A. glutinosa* and the High FD-K mixture. The third dimension of PCA explained 15.9% of traits variation and helped to further separate *A. glutinosa* from the rest of species.

Litter decomposition

In monocultures, the highest decomposition was found for *A. glutinosa* in presence of detritivores, and also for the semi-deciduous *Q. faginea* in their absence; decomposition was lowest for the deciduous *Q. robur* and *Q. canariensis* with and without detritivores

(Fig. S4.C2.-A, B). Decomposition of monocultures was explained by LQI only when detritivores where present (R=0.60, p<0.001); microbially-mediated decomposition showed a similar but not significant trend (R=0.37, p=0.076). The mixture with *A. glutinosa* (High FD-K) showed the highest decomposition and the deciduous mixture (Low FD-D) the lowest, regardless of detritivore presence (Fig. S4.C2.-A, B), but only in their presence the difference was significant (One-way ANOVA; $F_{3,24} = 11.474$, $p <$ 0.001). Decomposition of mixtures was positively related with Rao's Q, but LQI only explained decomposition in microcosms with detritivores (Table S4.C2.).

Net diversity effects (NDE) on decomposition were only significant in the High FD-K mixture, being positive for total (detritivores + microorganisms; Fig. 2.C2.-A) and detritivore-mediated decomposition (Fig. 2.C2.-B), but negative for microbially-mediated decomposition (Fig. 2.C2.-C). The magnitude of these effects was 23.8%, 50.5% and 11.4% for total, detritivore-mediated and for microbial decomposition, respectively. When partitioning NDEs (Fig. 2.C2.-D-I), we found negative complementarity effects only in microbially-mediated decomposition of the High FD-K mixture. This mixture always showed positive selection effects. The NDE on detritivore-mediated decomposition was correlated with LQI, while selection effects on total and detritivore-mediated decomposition were correlated with both, LQI and Rao's Q (Table S4.C2.).

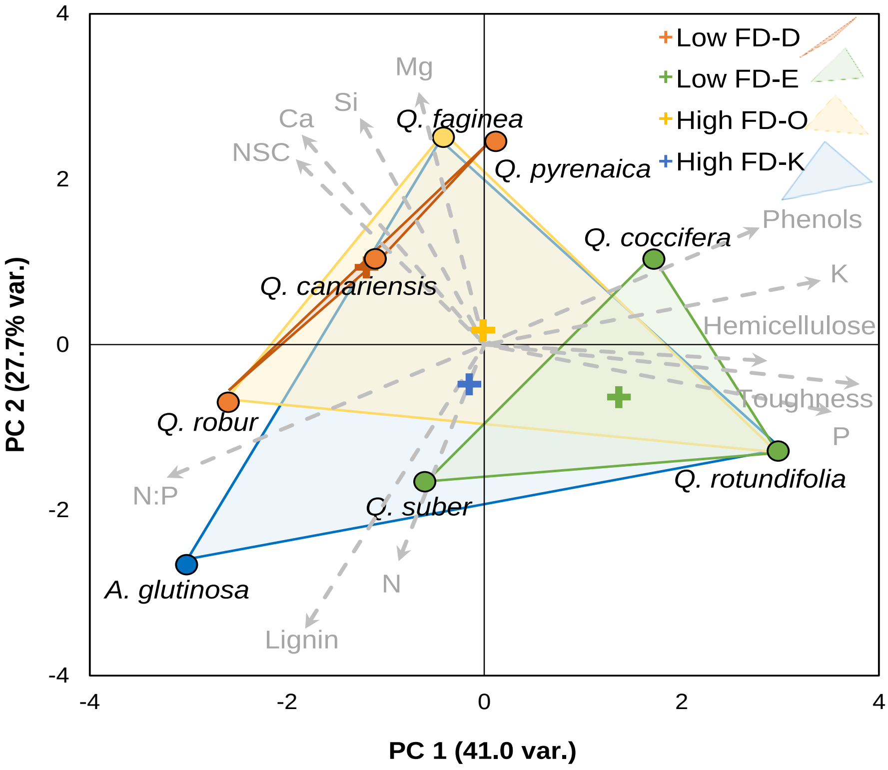

Figure 1.C2. Projection of the 2 first principal component axis showing differences among litter traits of the 8 individual species and 4 litter mixtures (Explained variance = 68.7%). Vectors represent each leaf trait weight and circles each plant litter position: deciduous in brown, semi-deciduous in yellow, evergreen in green and key species in blue. Triangular shapes represent the mixtures and crosses the weighted means of trait values calculated for each mixture.

Furthermore, intraspecific comparisons of litter decomposition (i.e. comparison of LML of a particular species in monocultures and mixtures) highlighted significant mixing effects for some litter species (Fig. 3.C2.), being noteworthy the consistent pattern found for *A. glutinosa*: faster decomposition in the mixture (High FD-K) than in monocultures with detritivores, but lower without them.

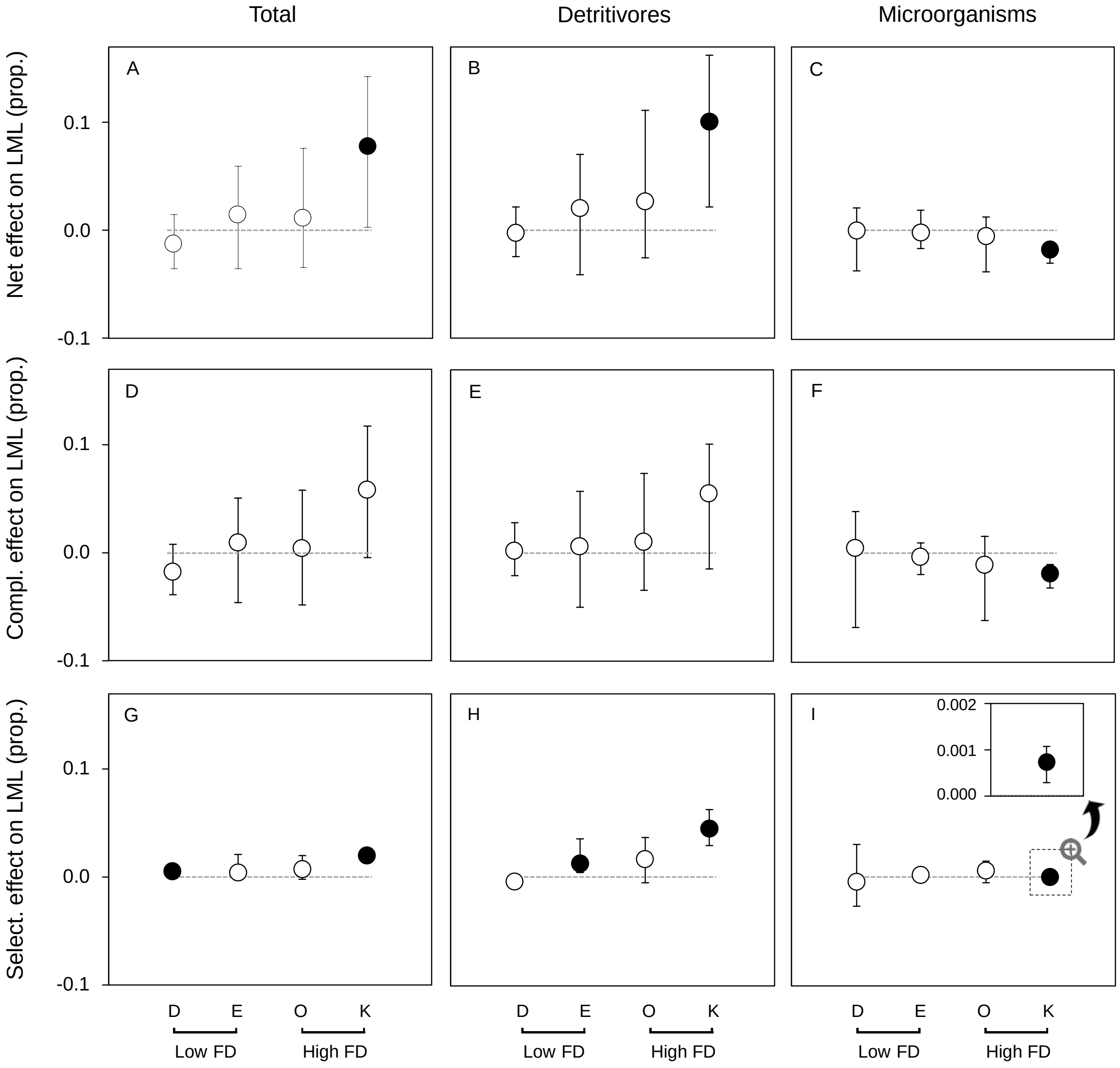

Figure 2.C2. Net diversity, complementarity and selection effects on total (A, D and G), detritivore-mediated (B, E and H) and microbially-mediated (C, F and I) litter mass loss (LML; proportion). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect, i.e. the null expectation that mixtures do not differ from expected ones, estimated from monocultures. Closed circles represent intervals that reject the null hypothesis (i.e., confidence interval do not contain the 0-value) and open circles represent intervals that accept the null hypothesis.

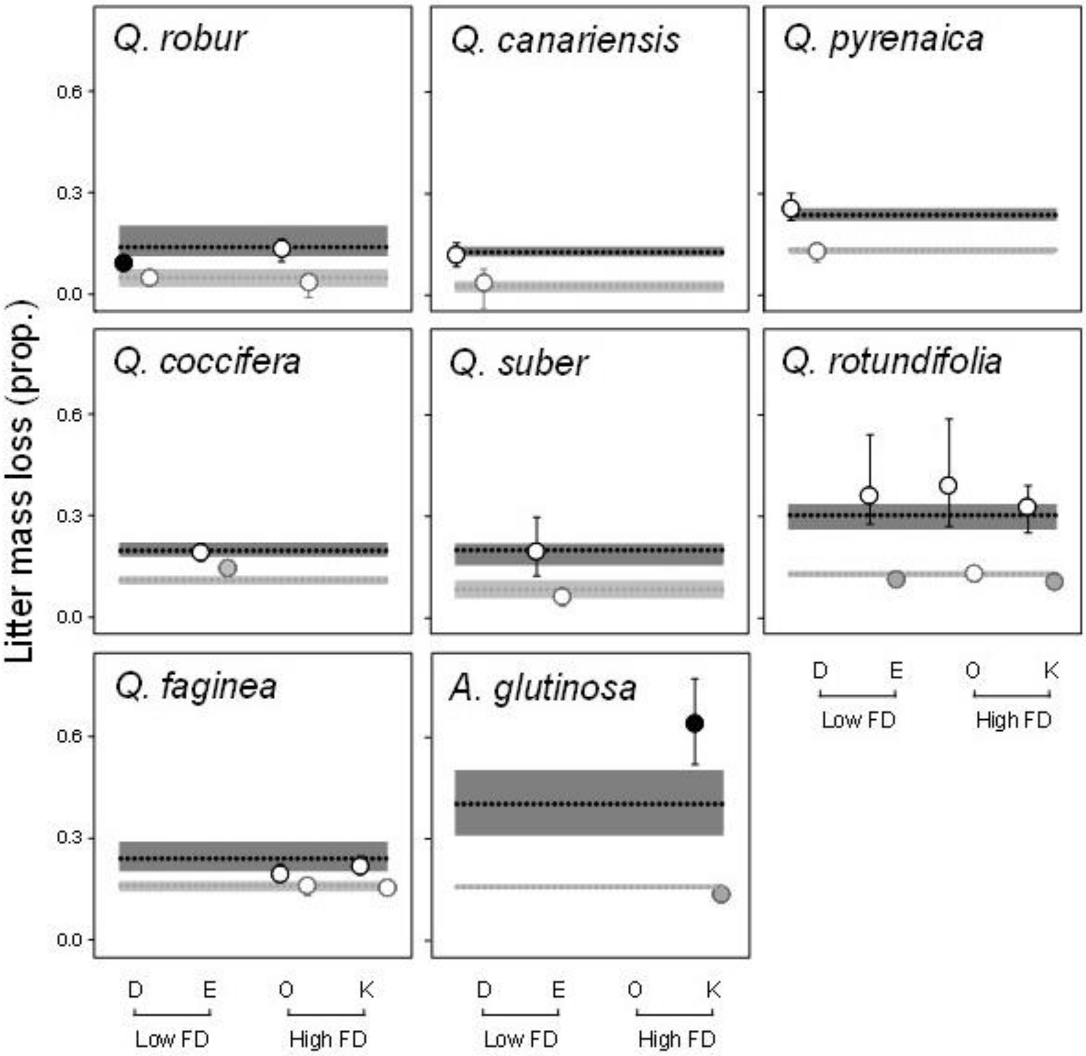

Figure 3.C2. Comparison of litter mass loss (LML; proportion) of each litter species among treatments where present. Circles are mean values in mixtures (black and grey represent treatments with and without detritivores, respectively). Dotted lines represent monoculture values, following the same color scale. Whiskers and shadows (dark grey for treatments with detritivores) denote upper and lower limits of 95% nonparametric bootstrapped confidence intervals for mixtures and monoculture values, respectively. Closed symbols represent intervals that are significantly different of monoculture values.

Litter nutrient dynamics

When detritivores were present, LNL was mostly positive in monocultures and mixtures (Fig. S4.C2.-C), accompanied, in general, by an increase of litter N concentration (N%; range of increase: 1.4-23.8%). LNL increased with LQI in monocultures (R=0.54, *p*=0.007) and also with Rao's Q in mixtures (Table S4.C2.). Mean NDEs on LNL were positive for all mixtures, but only significant (24% increase) in the High FD-K mixture (Fig. 4.C2.-A).

Without detritivores we observed net N immobilization (i.e., negative LNL) in low-diversity mixtures (Low FD-D and Low FD-E; Fig. S4.C2.-D). In contrast, most monocultures and high-diversity mixtures (High FD-O and High FD-K; Fig. S4.C2.-D) showed positive LNL. Litter N concentration (N%) generally increased (3.9-17.7%). LNL in mixtures was positively related with LQI and Rao’s Q (Table S4.C2.). NDEs on microbially-mediated LNL were negative in all mixtures (Fig. 4.C2.-B).

In presence of detritivores, LPL was positive in mixtures and most monocultures (Fig. S4.C2.-E), increasing with mixture’s Rao’s Q (Table S4.C2.). In these microcosms, litter P concentration (P%) generally increased in deciduous oaks (1.6-36.2%) but decreased in the other species. No diversity effects on LPL were detected in presence of detritivores (Fig. 4.C2.-C).

Without detritivores, litter P content tended to increase (negative LPL values) in low-diversity mixtures (Low FD-D and Low FD-E), and to decrease (positive LPL values) in high-diversity mixtures (High FD-O and High FD-K; Fig. S4.C2.-F), being positively related to Rao’s Q (Table S4.C2.). Litter P concentration (P%) increased in all litter species (2.8-56.3%) except *Q. faginea* (11.3-14.4% of reduction). NDE was negative for all mixtures without detritivores, excepting High FD-O (Fig. 4.C2.-D).

Fungal biomass

In monocultures, ergosterol concentration varied among species (with detritivores: $F_{7,16}$ = 6.28, *p* = 0.001; without detritivores: $F_{7,16}$ = 3.01, *p* = 0.032), being on average higher

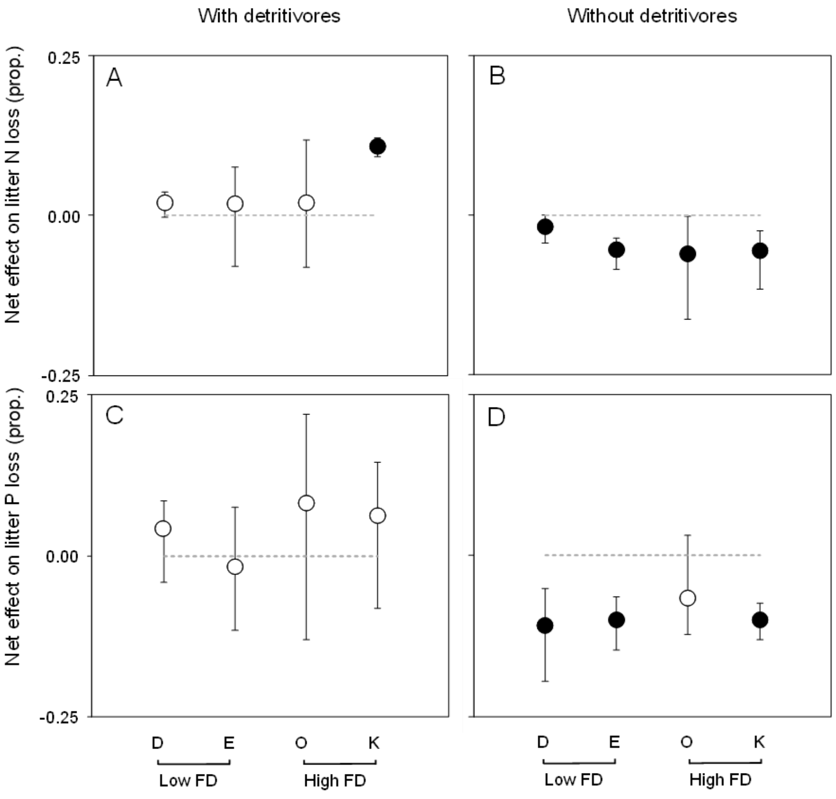

Figure 4.C2. Net diversity effect on litter nutrient loss (LNL and LPL; proportion). Litter N loss (A and B) and litter P loss (C and D); in microcosmos with detritivores (A and C) and without them (B and D). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

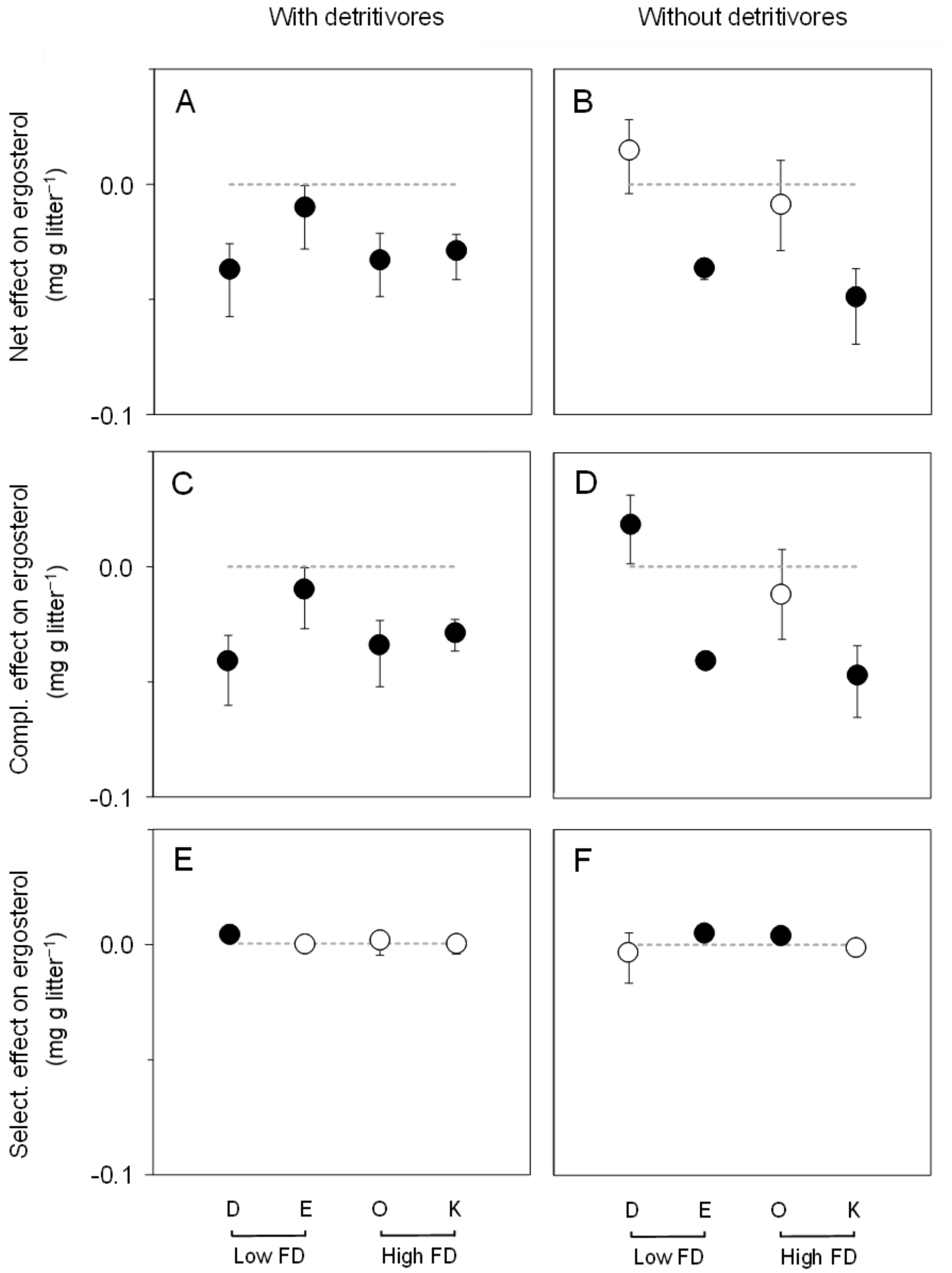

Figure 5.C2. Net diversity, complementarity and selection effects on fungal biomass (Ergosterol content; mg g litter^{-1}): with detritivores (A, C and E) and without detritivores (B, D and F). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

in deciduous than evergreen species (Fig. S4.C2.-G, H). Similarly, comparisons across litter mixtures showed significant differences in ergosterol concentration within microcosms with ($F_{3,8}$=10.79, *p*=0.003) and without ($F_{3,8}$=12.68, *p*=0.002) detritivores, being the lowest in the evergreen mixture (Fig. S4.C2.-G, H). NDE on fungal biomass was always negative in microcosms with detritivores (effects from -13.5% to -21.4%), and more variable without them (Fig. 5.C2.-A, B), being highest (ca. -35%) in Low FD-E and High FD-K mixtures (Fig. S4.C2.-H). Complementarity effects followed the net diversity effects' pattern (Fig. 5.C2.-C, D). Selection effects, always of lower magnitude than complementarity, arose only in few combinations (Fig. 5.C2.-E, F). No relationships between ergosterol accrual and LQI or Rao's Q were found (Table S4.C2.).

Detritivore performance

In monocultures, the highest survival of detritivores was found in *Q. suber* and *A. glutinosa* microcosms, whereas the lowest was observed in 2 deciduous oaks (*Q. robur* and *Q. pyrenaica;* Fig. S5.C2.-A). Within mixtures, no statistically significant differences were found (Fig. S5.C2.-B).

In monocultures, DMG was the highest when fed *Q. rotundifolia* and *A. glutinosa*, and the lowest when fed deciduous oak litter (Fig. S6.C2.-A), exhibiting a linear relationship with LQI (R=0.45, *p*<0.010). DNG and DPG followed a similar pattern to that of DMG (Fig S6B, C). DNG in monocultures was also related to LQI (R=0.37, *p*=0.027). However, *Allogamus* larvae decreased their N and P body concentration regardless of the treatment (ca. 8.6% and 14.4% of reductions of N and P, respectively). NDE on DMG was positive in the high FD-K mixture (Fig. 6.C2.-A; effect size: 49%), whereas no NDE was found for DNG (Fig. 6.C2.-B), and DPG exhibited a positive NDE on individuals fed Low FD-D litter (Fig. 6.C2.-C).

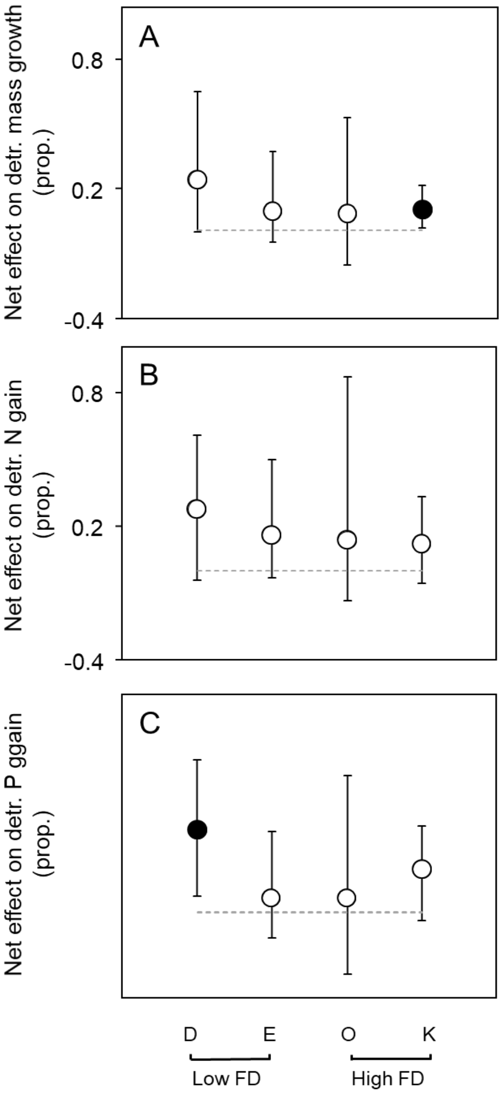

Figure 6.C2. Net diversity effect on detritivore growth (mass: DMG; proportion) and elemental gain (N: DNG, and P: DPG; proportion). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

DISCUSSION

Global change forecast scenarios consider the possibility of a replacement of functional groups or the loss of key plant species in riparian areas (Kominoski et al., 2013; Salinas et al., 2018). However, the effects on stream ecosystem functioning appear to be difficult to understand due to the complex trade-offs within and among decomposers exploiting mosaics of litter species (Gessner et al., 2010; Santonja et al., 2019). This study assesses this topic from a functional approach. Our results exhibit different diversity effects on the performance of detritivores and microbial decomposers, being such effects more related with the presence of the key plant species *A. glutinosa* rather than with the FD derived from the variation in leaf habits. This highlights the crucial role of N-fixing species, and point to litter nutrient concentration as driver of B-EF relationships (Flynn et al., 2011; Santonja et al., 2019).

Functional diversity and key plant species increase trait variability of litter mixtures

The lack of consensus about which is the most suitable measure of FD in leaf litter (Lecerf et al., 2011; Petchey et al., 2009) has prevented a comprehensive assessment of its effects on stream ecosystem processes. Here, by mixing species from different functional groups (based on their leaf habits and N-fixing strategies) we confirmed the increasing trait variability along with FD, especially when *A. glutinosa* was present. That is, although FD related to leaf habits increased trait variability, it was the inclusion of the key species what made the difference. Although previous studies have attributed the observed mixing effects to an increase in functional dissimilarity (e.g., Santschi et al., 2018; Vos et al., 2013), others (e.g., Santonja et al., 2019) pointed to species-specific effects through increasing mean nutrient concentrations of litter mixtures (mass ratio hypothesis; Grime, 1998). Since species phylogenetically closer are expected to share more traits, some authors (Boyero et al., 2016; López-Rojo et al., 2020) have used phylogenetic distance as a proxy of trait diversity. Yet, our results concerning oak species show that even congeneric taxa can greatly differ in relevant functional traits. Nonetheless, whatever the approach, functional diversity or phylogenetic distance, the pivotal role of both litter quality and trait variability explaining biodiversity-litter decomposition interactions, often emerges (Handa et al., 2014; Lecerf et al., 2011; López-Rojo et al., 2020).

Detritivore- and microbially-mediated decomposition respond differently to litter functional diversity

In agreement with previous studies, our results reveal a trend of increasing litter total decomposition with FD (Lecerf et al., 2011; Tonin et al., 2017). However, whereas Tonin et al. (2017)) reported positive net diversity effects on both detritivore- and microbially-mediated decomposition, although of lower magnitude on the second; here we detected positive net diversity effects on detritivore- and negative on microbially-mediated decomposition, but only in the mixture with the highest diversity of traits and containing alder leaf litter (i.e. High FD-K). This, and similar findings (Larrañaga et al., 2020; López-Rojo et al., 2018), point to the existence of a threshold of litter quality dissimilarity above which the effects of diversity arise. The net diversity effect on litter decomposition was mainly explained by complementarity effects, in agreement with previous studies (Handa et al., 2014; López-Rojo et al., 2018): positive (although not significant) in presence of detritivores, and negative for microbially-mediated decomposition. Often, positive complementarity effects lead to positive diversity effects on detritivore-mediated decomposition (e.g., Larrañaga et al., 2020). Here, positive diversity effects emerged from concomitant positive complementarity and selection effects in the mixture with *A. glutinosa*, the latter effect exhibiting its highest value when detritivore-mediated decomposition was isolated. *Allogamus* fed preferentially on the more palatable litter (selection), without altering consumption of other resources, as previously observed in field (Sanpera-Calbet et al., 2009) and microcosm experiments (Tonin et al., 2017). This contrasts with the general assumption that high-quality litter enhances decomposition of concurrent low-quality one (Gartner & Cardon, 2004), but supports the ‘clutching at straws’ effect (sensu Landeira-Dabarca et al., 2019), i.e. the presence of relatively recalcitrant litter, may enhance overall mixture consumption despite the reduction of average mixture quality. This emphasizes detritivore capability to feed simultaneously on resources of contrasting quality in order to balance their diet (Carvalho & Graça, 2007; Leroy & Marks, 2006).

The positive selection effect found on microbial decomposition suggests substrate preference (Gulis, 2001), however, contrary to our expectations, this selection effect was not translated into a greater decay of alder litter than that of oaks as usually reported (e.g., Cornejo et al., 2020; Monroy et al., 2016). Yet, the observed negative diversity effect on microbial decomposition was driven by negative complementarity. Three main factors could have conditioned this negative effect in our microcosms: low microbial colonization of litter as a consequence of the short incubation period, nutrient limitation (V. Ferreira et al., 2015) caused by the low amount of dissolved nutrients incorporated with water renewal, and/or the accumulation of leached secondary compounds, common in oak litter (McArthur et al., 1994), with putative toxic effects on microbes (Canhoto et al., 2013). However, the latter seems more likely, since the initial colonization and the nutrient limitation appeared to be the same in all microcosms, whereas the toxic effects of accumulated soluble secondary metabolites could have differed between monoculture and mixtures treatments. These effects are more likely to be detected in microcosms with lower water renewal than in field conditions where leaf leachates are constantly washed out, and can be potentiated with increasing mixture diversity, as more different compounds coming from diverse litters may create a toxic cocktail, as defined in allelopathy studies (Reigosa et al., 1999).

Functional diversity differently affects litter nutrient recycling by detritivores and microbial decomposers

We found a general trend of accelerated nutrient dynamics with detritivores as FD raised, as reported before in large-scale field (Handa et al., 2014) and microcosm (López-Rojo et al., 2019) studies. Here, significant positive diversity effects occurred only for N cycling in the mixture with *A. glutinosa*; this exhibited, although not significantly, higher litter-N loss rates than the best performing species, supporting the premise of the limiting role of N for litter consumer detritivores in headwater streams (Balseiro & Albariño, 2006; Fenoy et al., 2020; McManamay et al., 2011). Thus, litter N variability seems to drive N loss (García-Palacios et al., 2017) and, subsequently, diversity effects (López-Rojo et al., 2020) on leaf litter decomposition.

Microbially-mediated nutrient dynamics were also determined by FD and showed mostly significant negative diversity effects, i.e. mixtures lost less nutrients than expected from monocultures. However, N and P net losses (mineralization) occurred in the two mixtures with higher FD, and nutrient sequestration (immobilization) appeared in low FD mixtures, in which microbes might have used nutrients from the water (López-Rojo et al., 2019; Suberkropp & Chauvet, 1995; Tonin et al., 2017). The latter process is expected to be higher in microcosms with detritivores, due to increased nutrient release to water (López-Rojo et al., 2019; Tonin et al., 2017), but might be masked by the high detritivore-mediated decomposition. Thus, similar negative diversity effects resulted from opposed underlying mechanisms: nutrient sequestration in low FD mixtures and nutrient mobilization in high FD ones. Most likely, the negative diversity effect (negative complementarity) also observed for microbially-mediated decomposition (i.e. microbial inhibition due to the leaching of secondary compounds; see section 4.2.) must underlie the corresponding effects on nutrients, but the specific mechanisms need further study.

Fungal biomass and detritivore performance in response to functional diversity

Contrary to our expectations (e.g., Kominoski et al., 2009), fungal biomass was, in general, negatively affected by FD. A plausible explanation for such negative diversity effect is the leaching of toxic compounds from oaks, as discussed in section 4.2. Furthermore, high FD mixtures might promote fungal species richness (e.g., Fernandes et al., 2013) and, therefore, competitive interactions (Gessner et al., 2010; Gulis & Stephanovich, 1999), resulting in negative complementarity effects. Nevertheless, the potential limitations that our microcosms may have imposed to fungal performance (i.e., nutrient limitation and poor fungal colonization due to the short pre-incubation period of litter and the relatively lack of water renewal) could have distorted diversity effects on fungal biomass. Nonetheless, a comparison of ergosterol concentrations in alder leaf litter between our experiment (monocultures) and a field experiment carried out in streams of Sierra Nevada (Casas et al., 2011) revealed very similar values. Whatever the cause, our results contrast with other studies which found that litter mixtures may improve nutrient availability, and thus microbial performance (Handa et al., 2014; Schimel & Hättenschwiler, 2007), failing to support a positive effect of plant diversity on microbial decomposers.

Survival of detritivores was variable and appeared to depend on intraspecific interactions (Boyero & Pearson, 2006) coupled with litter quality, more than litter diversity. We observed cannibalism, likely promoted in those mixtures lacking a litter type that fulfils the nutrient requirements of detritivores (e.g., Bastian et al., 2008).

Detritivore mass growth followed a similar pattern to that of detritivore-mediated decomposition, which often increased with litter quality and diversity (Fernandes et al., 2015). However, similarly to other studies (e.g., Tonin et al., 2017), detritivore growth did not increase with increasing litter diversity, although a slight trend of higher mass and nutrient gain with increasing litter FD was discerned, giving some support to the balanced diet hypothesis. Diverse mixtures provide more varied nutritional resources, which effects translate into higher consumer biomass (DeMott, 1998), what could subsequently shift from a bottom-up into a top-down control of the process (Duffy et al., 2007). This trend was, nonetheless, not entirely clear since significant positive diversity effects on growth (ca. 50%) were only detected in the highest FD treatment (High FD-K). The combination of two nutrient-rich litter species (*A. glutinosa* and *Q. rotundifolia*) in this mixture clearly favored litter consumption (Santonja et al., 2019; Vos et al., 2013) and detritivore growth (Danger et al., 2013). Cannibalism may have altered N and P gains to some extent. However, *Allogamus* larvae were only able to significantly gain N and P when fed two monocultures where no signals of cannibalism were observed and containing the most nutrient-rich litters (i.e. *A. glutinosa*-N and *Q. rotundifolia*-P), possibly because no other litter type fulfilled their, usually high, nutrients demands. N is used by caddisflies to produce somatic proteins, silk, and N-rich chitin for case building (Frainer et al., 2016), while P is required in ribosomal RNA (Sterner & Elser, 2002). Consumption of litter assemblages lacking a nutrient-rich litter may lead to a decrease in detritivore growth, which can cause a reduction of adult size (Carvalho & Graça, 2007; Flores et al., 2014), consequently hindering their reproductive success and survivorship and pointing to bottom-up effects (Martínez et al., 2013; Swan & Palmer, 2006). Despite all the aforementioned, it is important to consider that our results may not be applicable to other detritivore species, e.g., if they were more sensitive to leached secondary compounds or unable to face tough and recalcitrant leaves such as those from oaks; nor to the entire detritivore community present in the streams due to interspecific interactions.

Detritivores and A. glutinosa: key players of diversity effects on decomposition

Direct assessment of mixing effects on a particular plant species in different mixtures, allowed us to delve into mechanisms driving net diversity effects beyond complementarity and selection effects (Kominoski et al., 2009). This approach further supports the determinant role of *A. glutinosa* mediating diversity effects. In the presence of detritivores, the species accompanying *A. glutinosa* in the High FD-K treatment (*Q. rotundifolia* and *Q. faginea*) decomposed at a similar rate than in their monocultures. Therefore, suggesting that the observed significant net diversity effect found in this mixture relied mainly on an increased consumption of *A. glutinosa* within the mixture compared to its monoculture. The scarcity of the preferred litter within an assemblage may enhance the consumption of the co-existing resources (Bastian et al., 2008). Nonetheless, we provided *A. glutinosa* litter in excess (>50% of *A. glutinosa* litter remaining at the end of the experiment), what further supports the diversity effect found in our experiment. In contrast, microbially-mediated decomposition reveals the opposite pattern: nutrient-rich litters (*A. glutinosa* and *Q. rotundifolia*) exhibited lower decomposition rates in the High FD-K mixture than in monocultures, driving the observed negative diversity effect. This is possibly due to short initial litter colonization period carried out, the leaching of secondary compounds (as discussed in section 4.2), or because positive diversity effects on microbially-mediated decomposition require longer incubation times (e.g., Fernandes et al., 2013; Santschi et al., 2018).

CONCLUSIONS

Our outcomes suggest combining the use of functional diversity and leaf quality when assessing B-EF relationships. This study underlines that litter nutrient content had greater relevance for diversity effects compared to diversity of other litter traits. N-fixing plant species play a prominent key role, entailing a leap of quality and trait variability of litter assemblages, which drives diversity effects on ecosystem functioning. Moreover, our results underscore the combined role of detritivores and plant key species supporting positive diversity effects on instream decomposition in headwaters. However, these outcomes must be interpreted cautiously given the difficulty to conciliate the required time to decompose of microorganisms and shredders, and the usual constraints of microcosms studies; for example, the use of only one stream detritivore species, and the fact that the microbial community was reduced to that present in the weekly water renewal, added to the possible existence of a nutrient limitation and an accumulation of litter leachates. Considering the forecasted loss of deciduous in favor of evergreen plant species (Kominoski et al., 2013; Salinas et al., 2018) and, in particular, the black alder dieback due to an emergent fungal disease (Bjelke et al., 2016; Gibbs et al., 1999), further studies should be carried out to improve our knowledge on effects of such changes and to advice managers about which species should receive special attention in conservation programs, in order to mitigate instream effects of functional changes in riparian plant communities.

Chapter 3

Alder groves promote N-cycling but not leaf litter decomposition in streams flowing through pine plantations

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ABSTRACT

During the last century, the abandonment of traditional dryland farming and pastures in the Mediterranean basin promoted the development of ambitious afforestation programs causing a drastic transformation of the landscape. Afforestation programs were usually accomplished without considering the potential ecological impacts on the recipient ecosystems. Forest streams highly rely on terrestrial organic detritus, so their functioning and conservation status can be altered by changes in forest biodiversity and composition. However, conifer plantations-derived effects on stream functioning are still unclear, possibly because the presence of other species intermingled within the plantations or along the riparian areas of streams may act as a buffer of such effects. Here, by means of a field experiment we assessed whether the presence of black alder in the riparian area and/or as leaf litter within the stream may mitigate the putative impacts exerted by pine plantations on stream ecosystem functioning. We found (i) that streams were functionally similar but differed in water N concentrations; (ii) no differences on litter decomposition rates between riparian types, but higher total decomposition of those assemblages containing alder leaf litter; and (iii) higher N losses (or lower N gains) in all the litter types at streams without riparian alder. These results demonstrate that microbial decomposers can use either stream water or litter N when required, and the ability of detritivores to feed simultaneously on resources of contrasting quality to balance their diet. Our study underscores the importance of riparian vegetation strips along streams to alleviate pine plantation-derived effects on leaf litter decomposition and suggest that even low riparian covers of alder can further expand its buffer action promoting microbial nutrient cycling through by increasing water N concentration.

Keywords: afforestation, *Alnus glutinosa*, net diversity effect, N-fixing species, poplar, riparian buffer

INTRODUCTION

Current Mediterranean landscapes are the result of the joint development of natural systems and human societies through the centuries (Blondel, 2006). At present, millions of hectares of the Mediterranean Basin are covered by forest plantations, which are predominantly composed of pines and other fast-growing species. Plantations were widely used during the last century as a tool for recovery of the forest surface lost over centuries of human exploitation (Pausas et al., 2004). Afforestation programs were usually accomplished without ecologically sound design and management, which could have avoided potential impacts on the recipient ecosystems (e.g. Andrés & Ojeda, 2002; Martín-Peinado et al., 2016; Molina et al., 2021).

Forest streams highly rely on leaf litter from riparian and basin vegetation (J. Wallace et al., 1997) and, therefore, their structure and functioning may be altered by the establishment of plantations in their surroundings (Kominoski et al., 2021). In conifer plantations, although litter inputs to streams are steady throughout the year (Inoue et al., 2012), conifer needles are not easily processed by stream biota (Collen et al., 2004; Márquez et al., 2017; Principe et al., 2015). Their low nutrient concentration and palatability, compared to these from many broadleaf deciduous species (Casas et al., 2013; Graça & Cressa, 2010; Martínez et al., 2013; Martínez et al., 2016), can hinder the performance of fluvial communities and, therefore, the rates of some key stream ecosystem processes (e.g. Ferreira et al., 2017; Kominoski et al., 2011; Wipfli & Musslewhite, 2004). Thus, it may be expected a general decrease in leaf litter decomposition and nutrient cycling rates in those streams flowing through conifer plantations (e.g. Kominoski et al., 2011; Whiles & Wallace, 1997). Nevertheless, the effects of conifer plantations on stream communities and litter decomposition are still unclear, with field studies reporting contrasting results (see Larrañaga et al., 2021), which only concurred that needles decompose more slowly than deciduous broadleaf litter (e.g. Albariño & Balseiro, 2002; Casas et al., 2013; Collen et al., 2004).

These discrepancies can derive from the presence or absence of other plant species in the surroundings of streams flowing through plantations. Plant diversity promotes functional diversity of instream litter assemblages, usually boosting community productivity (e.g. Fernandes et al., 2013). Thus, the presence of different species intermingled within the plantation or in riparian areas may buffer impacts of plantations on stream functioning (Casotti et al., 2015; Ferreira, Koricheva, et al., 2016; Larrañaga et al., 2021). For instance, alder species are usually considered key species (J. Pérez et al., 2021), since they can stimulate breakdown of litter assemblages in two different ways: (i) directly, supplying streams with nitrogen-rich and tender leaves (e.g. Rubio-Ríos et al., 2021; Rubio-Ríos et al., 2022) which may attract detritivores (Ferreira et al., 2012) and/or promote nutrient transfer between litter types (Handa et al., 2014; Tiunov, 2009); or (ii) indirectly, through an increase in stream water N concentration (Compton et al., 2003; Shaftel et al., 2012), therefore mitigating the usual N limitation to which microbial communities are subjected in headwater streams (Ferreira et al., 2006). Such mechanisms point to a key role of alder on stream ecosystem functioning at two different scales: instream (e.g. Alonso et al., 2022; Alonso et al., 2021) and riparian area or catchment (e.g. Shaftel et al., 2012). However, to our knowledge, the relative importance of both mechanisms has not been tested simultaneously in field experiments.

Here, we aim to assess whether the presence of black alder, *Alnus glutinosa* (L.) Gaertn, in the riparian area and/or within the stream (in the form of leaf litter) can mitigate the negative effects exerted by pine plantations on stream ecosystem functioning. We carried out a field experiment by means of the litter bag technique using monocultures (single species) and 5 different mixtures resulting from the combinations of 3 species: 2 species widely used in afforestation programs during the 1950s in southeastern Spain, the evergreen needle conifer *Pinus pinaster* Aiton and the deciduous broadleaf *Populus nigra* L.; and the key species *A. glutinosa*. The experiment was developed in 6 headwater streams flowing through pine plantations differing in the presence of alder in their riparian areas. We tested the following hypotheses: (1) water N concentration will be higher in streams with riparian alder groves (Compton et al., 2003; Shaftel et al., 2012); (2) the presence of alder in the riparian areas will enhance litter decomposition, both microbial and total (Kominoski et al., 2011), and microbial nutrient cycling via stream nutrient enrichment (Ferreira et al., 2006); (3) litter diversity effects on decomposition will be greater when litter from the key plant species is present in the assemblage (Larrañaga et al., 2020; Rubio-Ríos et al., 2021); and (4) these diversity effects will be mostly mediated by detritivores (Rubio-Ríos et al., 2021; Tonin et al., 2017).

MATERIAL AND METHODS

Study region and streams

We conducted a field experiment in 6 low-order streams located in the Sierra Nevada mountains (southeastern Spain), at elevations ranging between *ca*. 1500-1700 m a.s.l. and located relatively close to each other (max. straight line distance 11 km) **(**Fig. 1.C3.; Table S1.C3.). These streams have their sources at altitudes of ~2500 m a.s.l., show steep topographic gradients, and pluvio-nival regimes. The stream bottom is composed of heterogeneous sediments of siliceous nature dominated by sand and gravel along with large boulders. All the streams were located within the protected area of Sierra Nevada National Park. The climate is Mediterranean, with cold, wet winters and warm, dry summers (Esteban-Parra et al., 2022).

The vegetation of the Mediterranean region has changed over the last three millennia due to climate changes and human pressures. In Sierra Nevada, some of the most profound modifications have occurred during the last hundred years. The abandonment of traditional dryland farming and pastures was followed, since the 1950s, by the development of ambitious afforestation programs. This caused a drastic transformation of the landscape, with pine forests now dominating at higher zones (below

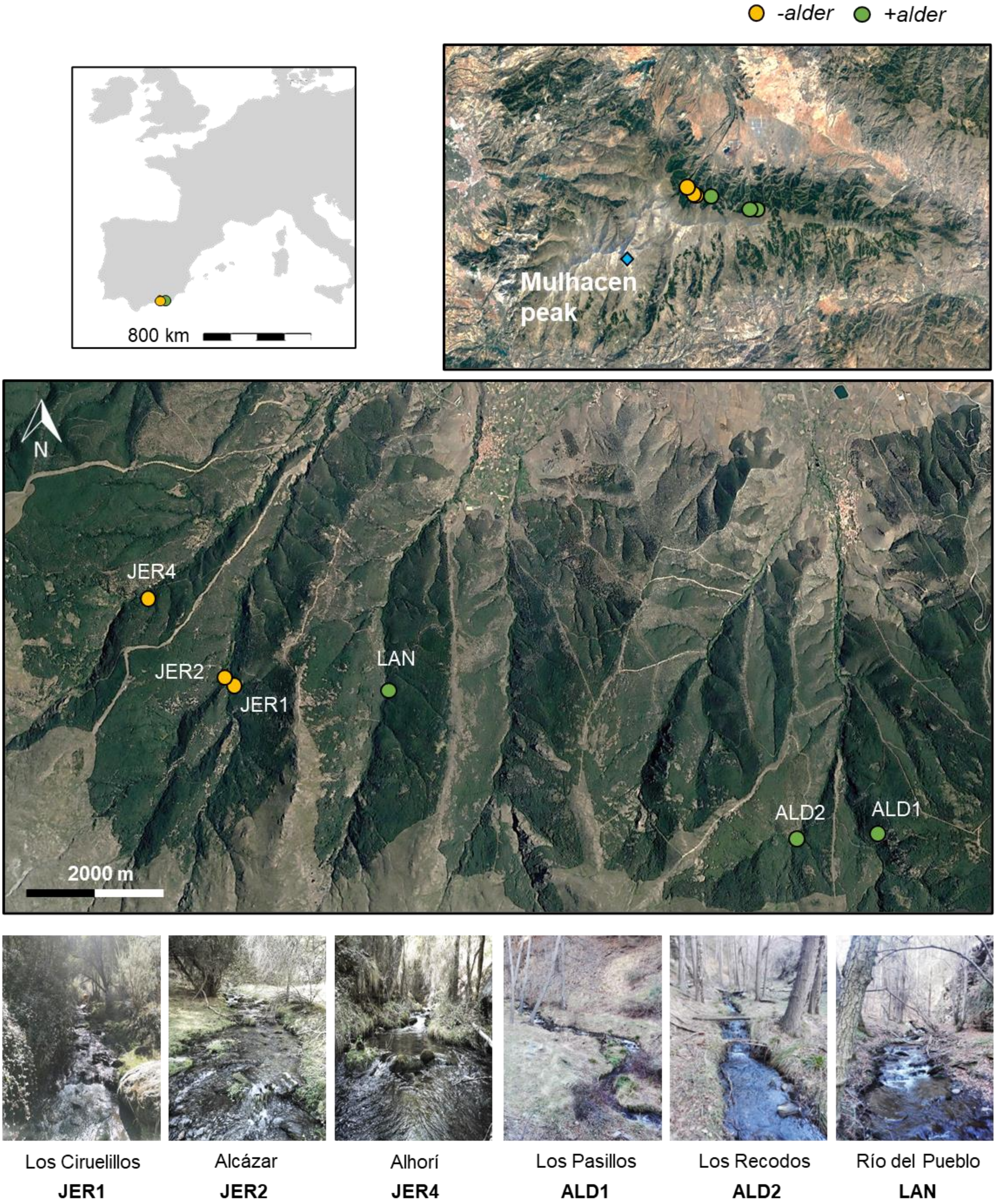

Figure 1.C3. Location of the six streams in Sierra Nevada, southeastern Spain.

2100 m a.s.l) and old abandoned farmlands (Olivencia et al., 2015). A favorable climate and the lack of appropriate management resulted in the current dense, nearly monospecific, stands of pine afforestations, which account for ~79% of the forested area of the national park. Only the remaining ~20% is covered by native forests of the evergreen holm oak *Quercus rotundifolia* Lam. or the deciduous oak *Quercus pyrenaica* Willd. (Pérez-Raya et al., 2001). The main species used in afforestation programs were maritime pine (*Pinus pinaster* Aiton), Aleppo pine (*Pinus halepensis* Mill.), black pine (*Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco) and Scotch pine (*Pinus sylvestris* L.), which were planted depending on altitudinal preferences of each species (Arias Abellán, 1981; Gómez-Aparicio et al., 2009; Mesa Garrido, 2019).

At present, afforestations located at the altitudinal range studied here are mainly composed of maritime pine, mixed with a few regenerated holm oaks, and black poplars (*Populus nigra* L.) in riparian areas (Arias Abellán, 1981; Padilla et al., 2010). Thus, the tree riparian vegetation in the 6 streams we selected (Table 1.C3.) consisted mostly of naturally grown willows (*Salix atrocinerea* Brot.) and plantations of black poplars, closely flanked on the slopes by maritime pines. Furthermore, three out the six selected streams also presented black alder groves in their riparian belts. Other riparian tree species in the selected streams were much less abundant (Table 1.C3.).

Stream water characterization

During the full period of the litter decomposition experiment (January–March 2020) we monitored water temperature in each stream with HOBO Pendant® loggers (Onset Computer Corporation). At the beginning, middle and end of the experiment, we measured in each stream water pH, electric conductivity, dissolved oxygen concentration and % saturation with a multiparametric probe (HACH® model HQ-30d, Loveland, CO, USA). Additionally, samples of filtered (47-mm diameter, 1.2-µm pore size, APFC, Merk Millipore) and non-filtered stream water were collected at the beginning and middle of the experiment into acid washed plastic bottles and transported to the laboratory. There, total alkalinity was immediately measured in non-filtered samples, by acid titration to a pH endpoint of 4.25 (Wetzel and Likens, 1991). Filtered samples were stored frozen (-20 ºC) until used for nutrient analyses. Total dissolved nitrogen (TDN) and phosphorus (TDP), nitrates (NO_3–N), soluble reactive phosphorus (SRP) and ammonium (NH_4-N) were measured. For TDN and TDP an aliquot of 100 mL of filtered water was wet mineralized for 30 min at 120°C in an autoclave. After cooling to room temperature, an aliquot (50 mL) was acidified with concentrated sulphuric acid to determine TDN (absorbance at 220 nm), whereas TDP, mineralized to phosphate, was determined in the remaining 50 mL (Wetzel & Likens, 1991). Nitrates were determined by the sodium salicylate method (APHA, 2005), SRP by the ascorbic acid method (Wetzel & Likens, 1991) and ammonium by the salicylate method (APHA, 2005). Streamflow was estimated at the beginning and end of the experiment by measuring current velocity (SEBA Mini Current Meter M1, SEBA Hydrometrie GmbH & Co, Kaufbeuren, Germany) along a known cross-section of the stream.

Sampling of stream benthos and estimation of ecosystem attributes

In each stream we collected 10 sampling units of benthos using a Hess sampler (area 0.09 m^2, mesh size 0.5 mm), by means of a stratified random design, differentiating two strata: riffles and pools. Each sampling unit was divided in two fractions, coarse (> 1 cm: leaves, twigs, cones…) and fine (> 0.45 mm – 1 cm: macroinvertebrates, inorganic and organic particles of sediment), and transported to the laboratory in a cool box. There, leaves were sorted by plant species, rinsed with distilled water to remove sediment particles, oven-dried (70 ºC, 72 h) and weighed to estimate the litter benthic composition and abundance in each stream.

Macroinvertebrates were sorted and identified to genus, or species level when possible, using identification keys (Tachet et al., 2010). Then, they were counted and assigned to functional feeding groups (FFG), oven dried (24h, 60°C) and weighed to estimate biomass of each FFG. Biomass of FFGs was used to estimate FFG ratios as indicators of stream ecosystem attributes (Merritt et al., 2017). Ratios estimated were the Autotrophy to Heterotrophy Index (Auto/Hetero), as *Scrapers to Shredders + Total Collectors* ratio; the Coarse Particulate Organic Matter to Fine Particulate Organic Matter Index (CPOM/FPOM), as *Shredders to Total Collectors* ratio; and the Top-Down Predator Control Index, as *Predators to All other groups* ratio.

Experimental design

We designed a field experiment to test the effects of black alder leaf litter on litter decomposition and nutrient dynamics of maritime pine, employed in extensive catchment plantations, and black poplar, used in

Table 1.C3. Location, geographic characteristics and dominant tree species density (ind./dam^2) in the riparian community of the selected.

Riparian type	With alder				Without alder			
Stream ID	ALD1	ALD2	LAN		JER1	JER2	JER4	
Stream	Barranco de los Pasillos	Barranco de los Recodos	Río del Pueblo		Barranco de los Ciruelillos	Barranco de Alcázar	Barranco de Alhorí	
Municipality	Aldeire	Aldeire	Lanteira		Jérez del Marquesado	Jérez del Marquesado	Jérez del Marquesado	
Basin	Guadalquivir	Guadalquivir	Guadalquivir		Guadalquivir	Guadalquivir	Guadalquivir	
Altitude (m a.s.l.)	1682	1643	1543		1519	1522	1507	
Geographic coordinates	37.12 N 3.07 W	37.12 N 3.08 W	37.14 N 3.16 W		37.14 N 3.18 W	37.14 N 3.19 W	37.15 N 3.19 W	
				Mean				Mean
Alnus glutinosa **(ind./dam^2)**	11.76	2.56	30.19	14.84	0.00	0.00	0.00	0.00
Populus nigra **(ind./dam^2)**	5.88	33.33	9.43	16.22	2.08	6.06	25.93	11.36
Pinus pinaster **(ind./dam^2)**	1.96	5.13	1.89	2.99	0.00	3.03	9.26	4.10
Betula pendula (ind./dam^2)	15.69	0.00	0.00	5.23	0.00	0.00	0.00	0.00
Quercus rotundifolia (ind./dam^2)	1.96	5.13	0.00	2.36	0.00	0.00	0.00	0.00
Salix atrocinerea (ind./dam^2)	1.96	0.00	5.66	2.54	29.17	15.15	12.96	19.09

riparian plantations (hereinafter alder, pine and poplar, respectively). We considered two experimental scales: stream and leaf-pack. We collected leaves of these three species just after abscission and dried them at room temperature (1 week) prior to initial weighing. We enclosed 5 g of litter (weighed to the nearest 0.1 mg) in each litter bag. Litter bags included single species (monocultures) and mixtures of all possible two species combinations (Fig. S1.C3.). Proportions of species in mixtures (¼ alnus, ¾ poplar or pine; 1⁄4 pine, ¾ poplar) were based on the natural average accumulated in the bottom of streams with alder in their riparian areas: ~25% out of the total for alder and ~3 times more poplar than pine (~39% and ~12% out of the total, respectively; Fig. S2.C3.; see above). Additionally, we prepared two extra mixture treatments using 3× more alder than poplar or pine (Fig. S1.C3.). We constructed litter bags of two different mesh sizes, fine (1-mm) and coarse (5-mm), to isolate microbial from total decomposition. Fine-mesh bags only allow the access and activity of fungi and bacteria, whereas coarse-mesh bags allow the access to decomposers and detritivores. All litter mixtures were placed in the different mesh sizes and replicated 3 or 5 times for fine and coarse mesh, respectively (total of 384 bags) (Fig. S1.C3.). Sets of bags containing one replicate per treatment were incubated in separate riffle areas (distant ~10 m) in a reach of ~50-m long at each stream. Ninety extra litter bags of coarse mesh (5 per species and stream, each containing 1.66 g of leaves belonging to each plant species) were used to estimate initial (post-leaching, 48h instream incubation) ash free dry mass (AFDM) of leaves. These leaves were used for initial leaf trait analyses (see below).

The experiment lasted 61 days (from 8 January to 9 March 2020). Thereafter, litter bags were collected, stored individually in zip-lock bags and transported to the laboratory in a cool box. There, remaining leaves were meticulously washed with stream water to remove fine particulate matter, sorted by species, oven-dried to constant mass (72 h at 60 °C), weighed to determine final dry mass (DM) and ground to leaf powder (Mixer Mill RETSCH MM 200). An aliquot of each sample was combusted (550 ºC, 5 h), and re-weighed to determine ash concentration (% DM) and estimate final AFDM. Another aliquot of each sample from those leaves incubated in fine mesh bags was stored for nutrient (CNP) analyses.

Litter trait characterization

Initial physical and chemical trait characterization (Table S2.C3.) was performed on post-leached litter (n=6). Discs of each species were cut to measure specific leaf area (SLA) and leaf toughness. The remaining litter fragments were oven-dried (70 ºC, 72 h), and ground for subsequent litter composition analyses. Specific leaf area (SLA; cm^2 g^{-1}) was estimated as the coefficient between leaf disc area and disc DM. Leaf toughness (g) was measured using a Texture Analyzer TA.XTPlus equipped with a 0.7 mm Ø steel rod. Percentages of lignin were calculated as in Fenoy et al. (2016), using an ANKOM 200/220 fibre analyser (ANKOM Technologies, Macedon, NY, USA). Concentration of Ca and Mg (% DM) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II). Silicon (Si) concentration was measured using inductively coupled plasma atomic emission spectroscopy (Thermo ICAP 6500 duo, Thermo Fisher Scientific, Cambridge, UK), after microwave sample digestion in nitric acid (65%) and hydrogen peroxide (30%). Condensed tannins were estimated by the acid butanol assay (Gessner & Steiner, 2005). Total phenols were determined following the Folin & Ciocalteu procedure as described in Bärlocher and Graça (2005). Concentrations of C and N (% DM) were estimated using a Perkin Elmer series II CHNS/O elemental analyser. P concentration (% DM) was measured spectrophotometrically after autoclave-assisted extraction (APHA, 2005). Concentrations of C, N and P were measured at the beginning (post-leached) and on litter in fine-mesh bags at the end of the experiment (n=3). Molar ratios C:N, C:P and N:P were also calculated.

Data analysis

Stream water characteristics were compared between riparian areas (-alder *vs.* +alder) and among sampling dates by two-way ANOVAs using the *aov* function of the ‘stats’ R package (R Core Team, 2020). Differences between stream-riparian types in ecosystem attributes, deduced from FFG characteristics, were assessed using T-tests (*t.test* function of ‘stats’ package). Trait variability among species or mixture traits were assessed using one-way ANOVA and post-hoc Tukey tests with the *anova* and *TukeyHSD* functions of ‘stats’ package. *Log* or *arcsin* transformations of variables were used when required.

Litter decomposition was quantified as the proportion of litter mass loss [LML = (initial – final AFDM)/initial AFDM); prop.], after correcting initial litter mass for humidity, ash content and leaching losses. To account for temperature differences among streams, litter decomposition rates were calculated in degree-days, dividing the mass loss by the sum of the accumulated mean daily temperature during the experiment. Changes in litter N (N change; prop.) and P (P change; prop.) were calculated as: (final – initial)/initial N or P content. Positive values represented nutrient immobilization, and negative ones nutrient mineralization. Changes in litter nutrients were only estimated from fine-mesh bags. Net diversity effects (NDE) of leaf litter mixtures on decomposition and nutrient changes were explored through the difference between observed (Obs.) and expected (Exp.) values (i.e., NDE = Obs. – Exp.), being expected values estimated based on monocultures (species incubated alone) (Loreau & Hector, 2001).

We used linear mixed effects regression (LMER) analyses at the leaf-pack level to assess the significance of the effects of riparian type (-alder *vs.* +alder), litter species combinations and their interaction (riparian area × litter mixture) on litter mass loss (LML), nutrient (N and P) changes and net diversity effects. Stream was included as a random factor nested within riparian type. Regressions were performed for each mesh size independently to avoid very complex models with many interactions (see Tonin et al., 2017). Linear models were fitted using the *lmer* function using restricted maximum likelihood (REML) estimation in the ‘lme4’ R package (Bates et al., 2015). When necessary to meet ANOVA assumptions, LML, nutrient changes and NDE were transformed using the *orderNorm* function transformation which was chosen by the *bestNormalize* function of the ‘bestNormalize’ R package (Peterson & Cavanaugh, 2020).

As random factors did not show significance (Table S3.C3.), the following comparisons for LML and nutrient changes were assessed using two-way ANOVAs, followed by Tukey's HSD tests when significant differences were detected. In these analyses, each litter bag within each stream and riparian area was considered a replicate (9 and 15 for fine and coarse mesh, respectively) for each litter mixture (Fig. S1.C3.).

We explored differences of NDEs among treatments with nonparametric bootstrapped 95% confidence intervals using the BCa method in the *boot.ci* function of `boot' R package (Canty & Ripley, 2019). For LML, the NDE was partitioned into complementarity and selection effects using the additive partitioning method (Loreau & Hector, 2001). Complementarity effects may derive from synergistic (positive) or antagonistic (negative) interactions; while selection effects represent positive (or negative) effects due to the presence of a dominant species in the mixture, which enhances (or inhibits) overall decomposition of the mixture (Handa et al., 2014). In addition, we compared LML for each species in monocultures and in each mixture where present. Outliers were detected using boxplots and removed from the analyses.

RESULTS

Water physicochemical characteristics

Water temperature of all streams ranged between 0.2 and 6.4°C (mean: 3.5 °C ± 0.06 SE) in January–March 2020, with a slightly, but significantly, higher mean temperature in streams without black alder (Table S1.C3.). Stream water of both riparian types was soft circumneutral (pH: 7.45 (7.10-7.95), electrical conductivity: 35.5 (25.5-48.3) µS cm^{-1}; alkalinity: 0.3 (0.1-0.4) meq L^{-1}), and oligotrophic (nitrate-N: 134 (45-318) µg N L^{-1}; soluble reactive P: 6 (2-12) µg P L^{-1}; NH_4-N: 20 (0 – 69) µg N L^{-1}; total dissolved-N: 0.72 (0-2.29) mg N L^{-1}; total dissolved-P: 11 (0-39) µg P L^{-1}; Table S1.C3.). Remarkably, streams with alder had significantly higher dissolved N, with on average double nitrate-N and 1.4 times more total dissolved-N concentrations than their counterparts without alder (Table S1.C3.).

Stream ecosystem attributes based on macroinvertebrates

The Autotrophy to Heterotrophy Index (Auto/Hetero) indicated that all streams were heterotrophic, dependent on allochthonous organic matter inputs (Table S4.C3.). The CPOM/FPOM Index demonstrated that all streams were winter shredder streams, with macroinvertebrates mainly dependent on leaf litter. Streams with riparian alder presented higher values of CPOM/FPOM, but no significant differences were found compared to the other riparian type (T-test, p = 0.1563; Table S4.C3.). Most streams also showed a normal predator to prey balance based on the Top-Down Predator Control Index (Table S4.C3.).

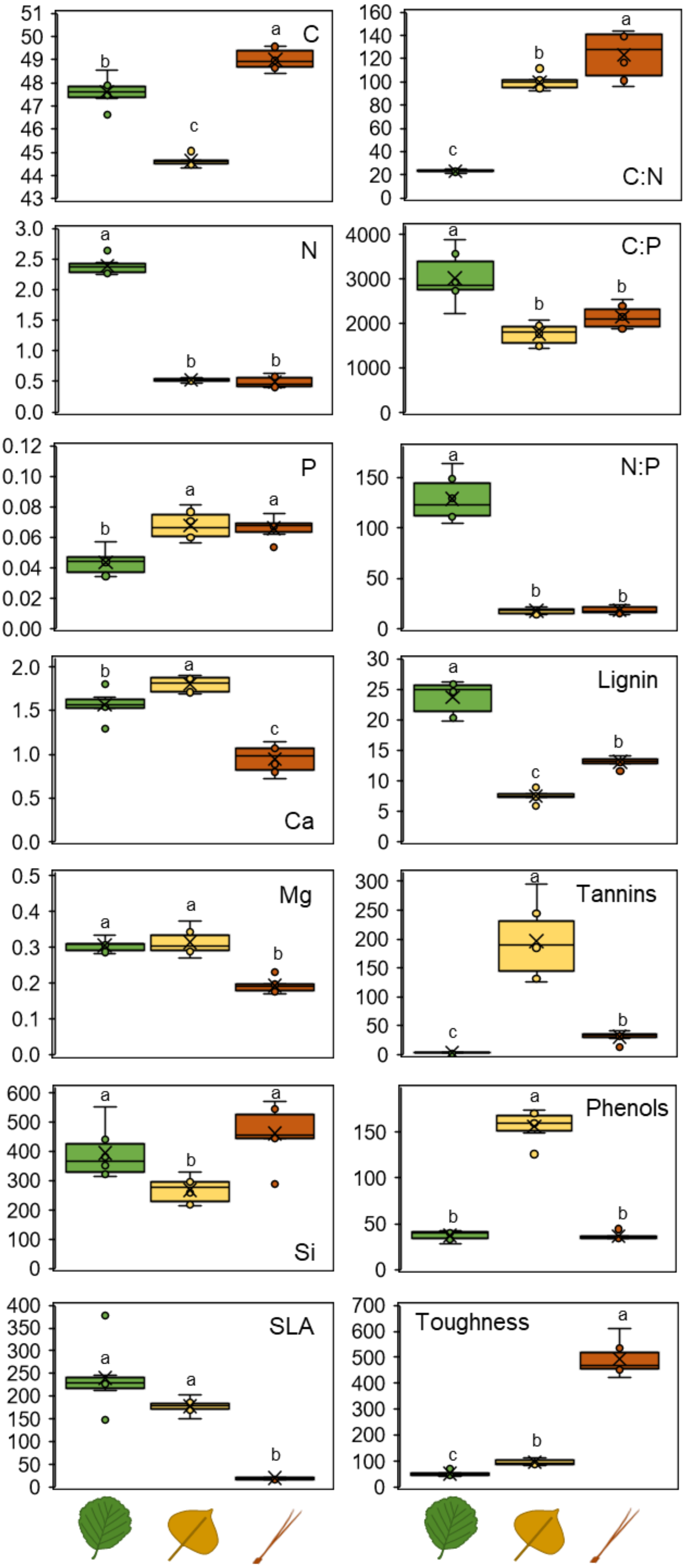

Figure 2.C3. Box-and-whisker plots for selected leaf trait variables of the three plant species studied (green, black alder; yellow, black poplar; brown, maritime pine): carbon (C), nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg) and silicon (Si) concentrations (% DM), molar elemental ratios (C:N, C:P and N:P), lignin concentrations (% DM), condensed tannins and phenols concentrations (mg g litter-1), specific leaf area (SLA; cm2 g-1) and toughness (g) of each plant species. Box represents median and 25th and 75th percentile levels, crosses are the mean, whiskers are the range, and dots are replicates. Different letters indicate significant differences ($p < 0.05$) among plant species, on the basis of linear models followed by pairwise multiple comparisons (Tukey test).

Initial litter traits across species and mixtures

Leaf litter of the 3 species selected differed in leaf traits (Fig. 2.C3.; Table S2.C3.). Alder litter exhibited the highest N and lignin concentrations and, C:P and N:P ratios, but the lowest P and tannin concentrations, C:N ratio and toughness (Fig. 2.C3.; Table S2.C3.). Poplar litter had the highest Ca, tannin and phenolic but the lowest Si and lignin concentrations (Fig. 2.C3.; Table S2.C3.). Pine litter had the highest toughness, C:N ratio and C concentration but the lowest SLA, Mg and Ca concentrations (Fig. 2.C3.; Table S2.C3.). Mixtures followed the patterns of the individual species making them up (Table S2.C3.).

Litter decomposition

Leaf mass loss (LML) of litter mixtures, either microbial or total LML, was only significantly affected by the type of mixture (Table 2.C3.). Microbial LML of monocultures was the lowest for pine (~35% lower in average), while higher, and very similar, for alder and poplar, independently of the riparian type (Fig. 3.C3.). Total LML of alder was 1.9 and 5.4 times higher than poplar and pine, respectively (Fig. 3.C3.).

Generally, microbial LML of mixtures containing alder was slightly faster. However, no significant differences were detected, except in the case of the mixture with high proportion of pine (PP+AG), which LML was significantly lower than most others (Fig. 3.C3.). Total LML of mixtures followed the pattern of monocultures, i.e., assemblages containing a high proportion of alder (AG+PN and AG+PP) decomposed faster than assemblages without alder (PN+PP), or with a high proportion of pine (PN+AG), which decomposed the slowest (Fig. 3.C3.).

Net diversity effects (NDE) on microbial LML were significantly affected by the interaction riparian type × litter-assemblage (Table 2.C3.). In riparian areas with alder, the AG+PN mixture (i.e., 75% alder + 25% poplar) exhibited negative NDE, as a result of negative complementarity effects (Fig. 4.C3.). In streams without riparian alder, AG+PN, PP+AG and AG+PP exhibited a positive NDE (resulting from positive complementarity), whereas PN+PP presented a negative NDE due to negative selection effects (Fig 4.C3.). NDE in total LML were only affected by litter assemblage (Table 2.C3.). These presented overall positive effects at both riparian types due to positive complementarity effects, except for PP+AG at both riparian types and for PN+PP at those without alder, for which the positive NDE appears to be derived from positive selection effects (Fig. 4.C3.).

Intraspecific comparisons of litter decomposition (i.e., comparison of LML of a particular species in monocultures and mixtures) highlighted significant mixing effects for some litter species (Fig. S3.C3.). It was noteworthy the consistent pattern found for alder, which tended to decompose faster in mixtures than in monocultures when incubated in streams without riparian alder (Fig. S3.C3.-A, B). Total decomposition of poplar was

Table 2.C3. ANOVA results testing the effects of riparian type and litter assemblage on leaf mass loss (LML), N and P changes, and net diversity effects (NDE) of mixture treatments. Each variable was tested against three fixed factors (Riparian type, Litter assemblage and their interaction). Analyses were carried out separately for each mesh type.

	Microbial Decomposition							Total Decomposition						
Factor	df	Sum Sq	Mean Sq	*F-value*	*p-value*	Multiple R^2	Adjusted R^2	df	Sum Sq	Mean Sq	*F-value*	*p-value*	Multiple R^2	Adjusted R^2
LML						0.61	0.56						0.68	0.65
n type	1	$5.80\cdot10^{-8}$	$5.80\cdot10^{-8}$	1.37	0.2462			1	0.290	0.290	0.84	0.3609		
blage	4	$4.38\cdot10^{-6}$	$1.10\cdot10^{-6}$	25.79	**<0.0001**			4	93.257	23.324	67.66	**<0.0001**		
n type × Assemblage	4	$2.76\cdot10^{-7}$	$6.89\cdot10^{-8}$	1.62	0.1774			4	0.384	0.096	0.28	0.8914		
als	72	$3.06\cdot10^{-6}$	$4.24\cdot10^{-8}$					130	44.79	0.345				
NDE on LML						0.32	0.24						0.15	0.09
Riparian type	1	$5.75\cdot10^{-7}$	$5.76\cdot10^{-7}$	12.19	**0.0008**			1	3.451	3.451	3.80	0.0536		
Assemblage	4	$4.16\cdot10^{-7}$	$1.04\cdot10^{-7}$	2.20	0.0769			4	13.782	3.446	3.79	**0.0060**		
Riparian type × Assemblage	4	$6.37\cdot10^{-7}$	$1.59\cdot10^{-7}$	3.38	**0.0137**			4	3.097	0.774	0.85	0.4952		
Residuals	73	$3.45\cdot10^{-6}$	$4.72\cdot10^{-8}$					128	116.395	0.909				
N change														
Riparian type	1	0.353	0.353	49.28	**<0.0001**	0.77	0.74							
Assemblage	4	1.469	0.367	51.23	**<0.0001**									
Riparian type × Assemblage	4	0.039	0.010	1.35	0.2608									
Residuals	78	0.559	0.002											
NDE on N change														
Riparian type	1	0.054	0.054	7.37	**0.0082**	0.64	0.60							
Assemblage	4	0.676	0.169	23.10	**<0.0001**									
Riparian type × Assemblage	4	0.279	0.070	9.53	**<0.0001**									
Residuals	77	0.564	0.007											
P change														
Riparian type	1	0.032	0.032	0.85	0.3585	0.44	0.38							
Assemblage	4	2.292	0.573	15.11	**<0.0001**									
Riparian type × Assemblage	4	0.038	0.010	0.25	0.9082									
Residuals	79	2.996	0.038											
NDE on P change														
Riparian type	1	1.078	1.078	28.19	**<0.0001**	0.55	0.50							
Assemblage	4	2.168	0.512	14.17	**<0.0001**									
Riparian type × Assemblage	4	0.514	0.128	3.36	**0.0136**									
Residuals	79	3.022	0.038											

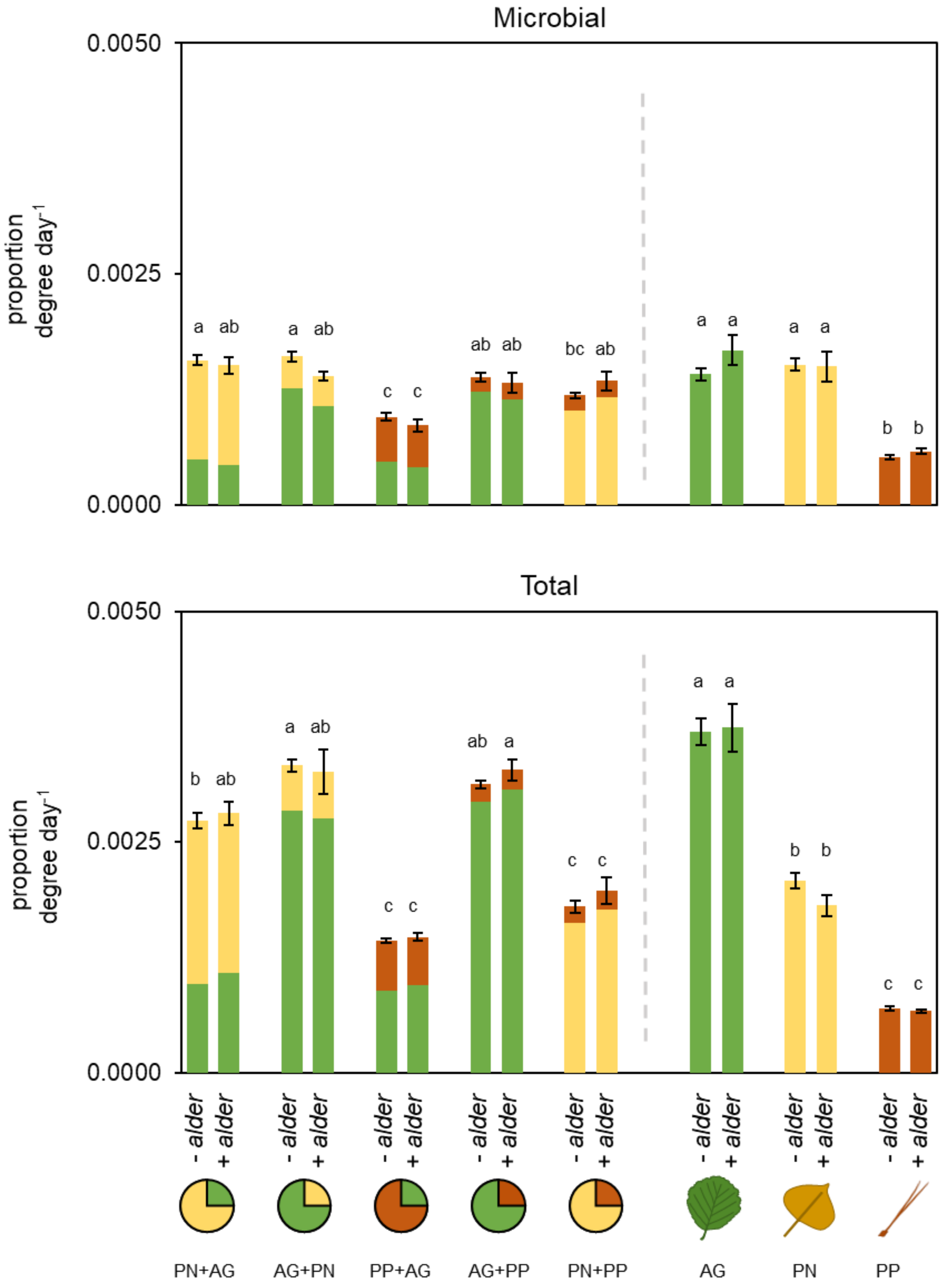

Figure 3.C3. Mean (±SE) litter mass loss (LML; proportion, prop.) of mixtures and monocultures. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 2-spps litter mixtures independently on the basis of linear models followed by pairwise multiple comparisons. Streams with or without riparian alder are stated using +alder or +alder, respectively.

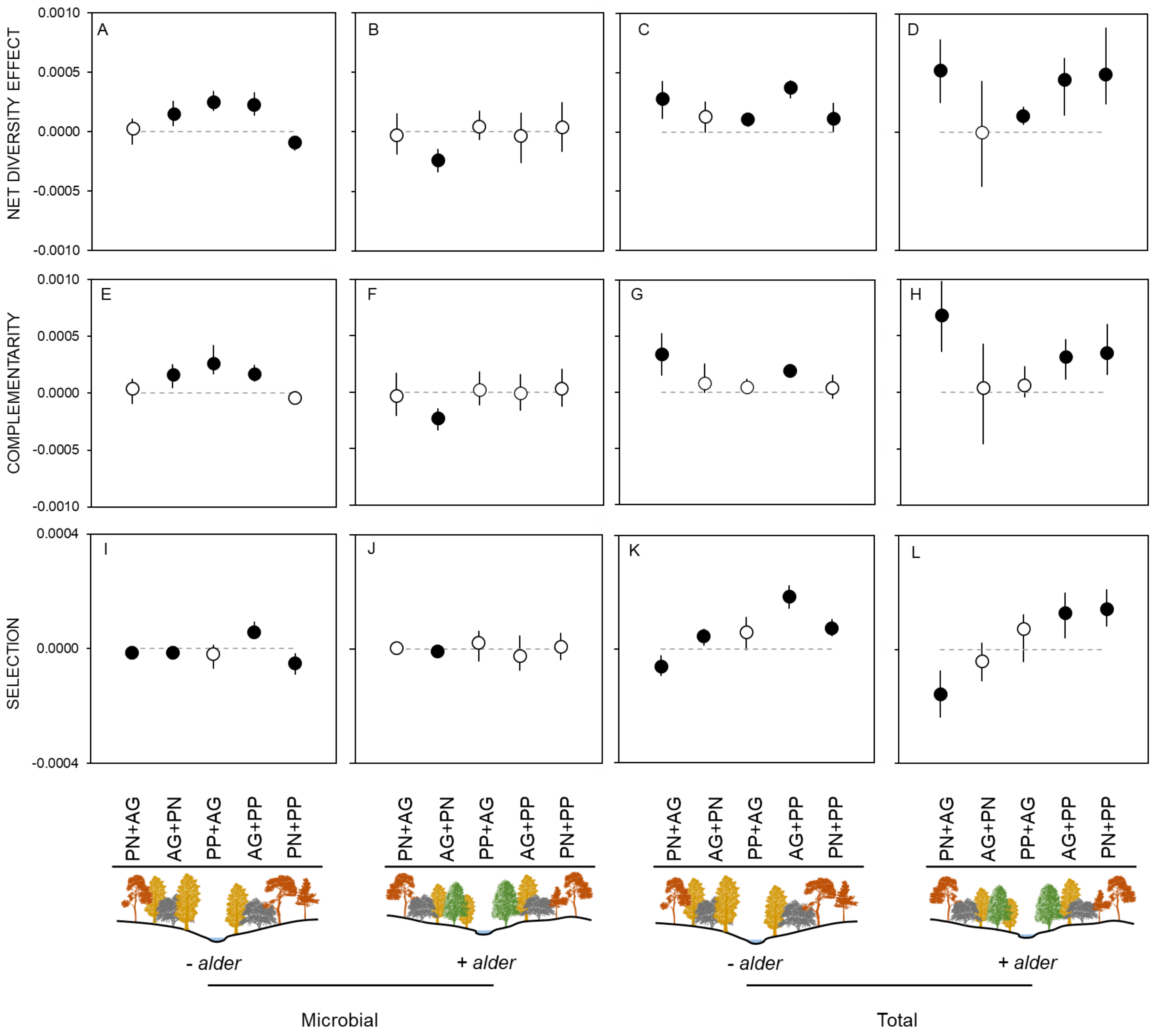

Figure 4.C3. Net diversity, complementarity and selection effects on microbially-mediated (A, B, E, F, I and J) and total (C, D, G, H, K and L) litter mass loss (LML; proportion degree day-1) after a 61 days incubation in streams with (+ alder) and without (-alder) alder in their riverbanks. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect, i.e., the null expectation that mixtures do not differ from expected ones, estimated from monocultures. Closed circles represent intervals that reject the null hypothesis (i.e., confidence interval do not contain the 0-value) and open circles represent intervals that accept the null hypothesis.

higher at riparian areas with alder when paired with pine (PN+PP; Fig S3.C3.-D), whereas at those without alder, poplar decomposed similarly to the monoculture whatever the assemblage in which it was present (Fig S3.C3.-C). Pine exhibited higher microbial LML in the PP+AG mixture than in monocultures at riparian areas without alder (Fig. S3.C3.-E).

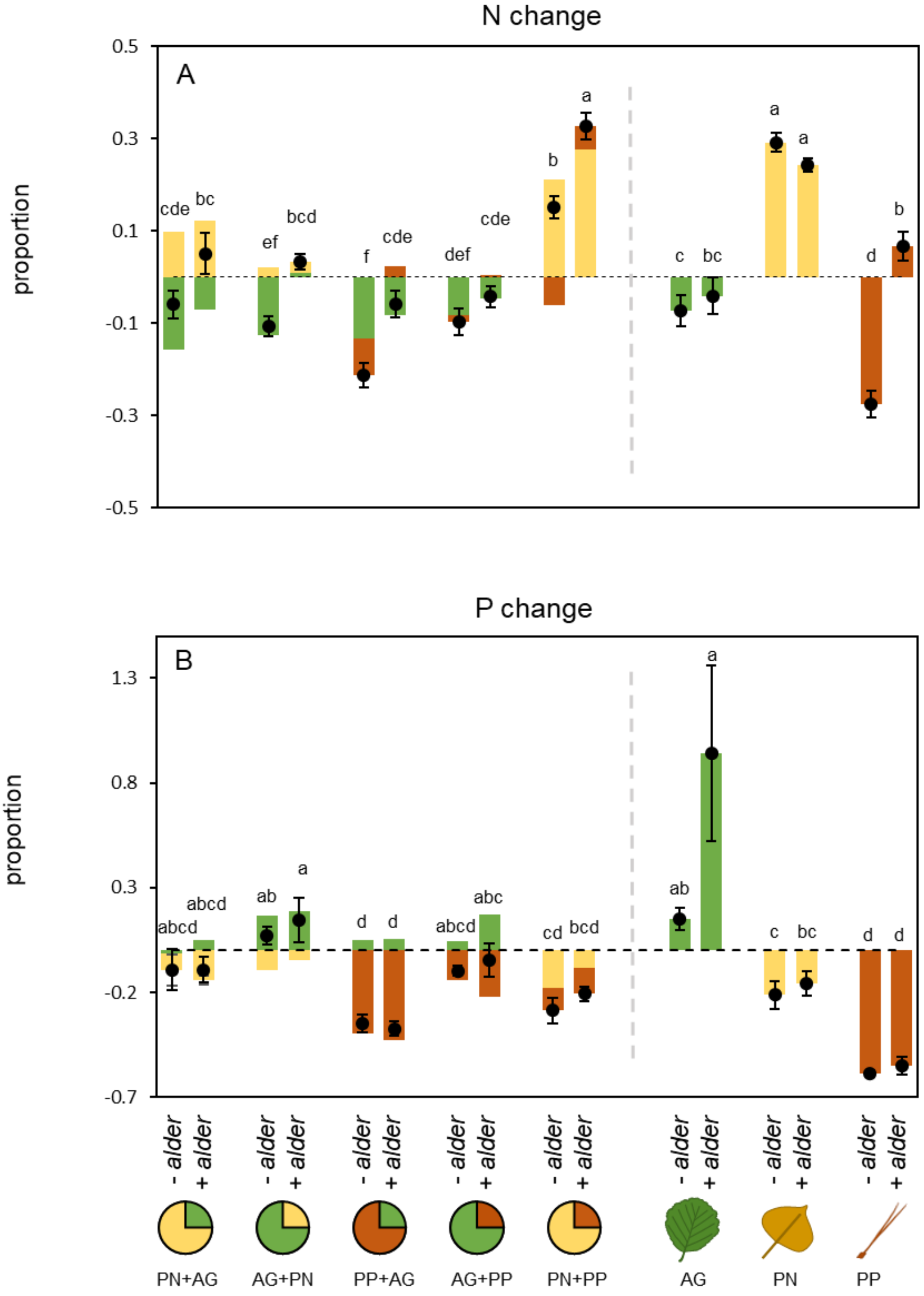

Figure 5.C3. Mean (±SE) N and P change (proportion, prop.) of mixtures and monocultures after a 61 days incubation in fine mesh bags in streams with (+ *alder*) and without (- *alder*) alder in their riverbanks. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 2-spps litter mixtures independently on the basis of linear models followed by pairwise multiple comparisons. Note that positive and negative values represent microbial immobilization and mineralization, respectively (i.e., increase or decrease of N or P content), respectively.

Litter nutrient dynamics

Nutrient changes were only assessed on fine-mesh bags. N concentrations increased in all the treatments independently of riparian type, except for the pine monoculture and the PN+AG mixture whose N concentration decreased ~3% and ~19%, respectively, at riparian areas without alder (Table S5.C3.). P concentrations increased in all treatments except for pine monoculture (decrease of ~50%) and the PN+AG mixture (decrease of ~20%) independently of the riparian type and for the PN+PP mixture (decrease of ~4%) at streams without riparian alder (Table S5.C3.).

N change was affected by riparian type and litter assemblage but not by their interaction (Table 2.C3.). Overall, N mineralization was observed in mixtures containing alder, exhibiting higher rates at streams without riparian alder (Fig. 5.C3.-A). Noteworthy, each litter species acted differently: alder and poplar litters always lost (mineralization) or immobilized N, respectively; whereas pine needles mineralized or immobilized N depending on the riparian type where they were incubated (Fig. 5.C3.-A). The NDE on N change was affected by the interaction between riparian types and litter assemblages (Table 2.C3.). Mean NDE on N change was overall negative when alder was present in the mixture independently of the riparian type, but positive when absent in streams with riparian alder (Fig. 6.C3.-A).

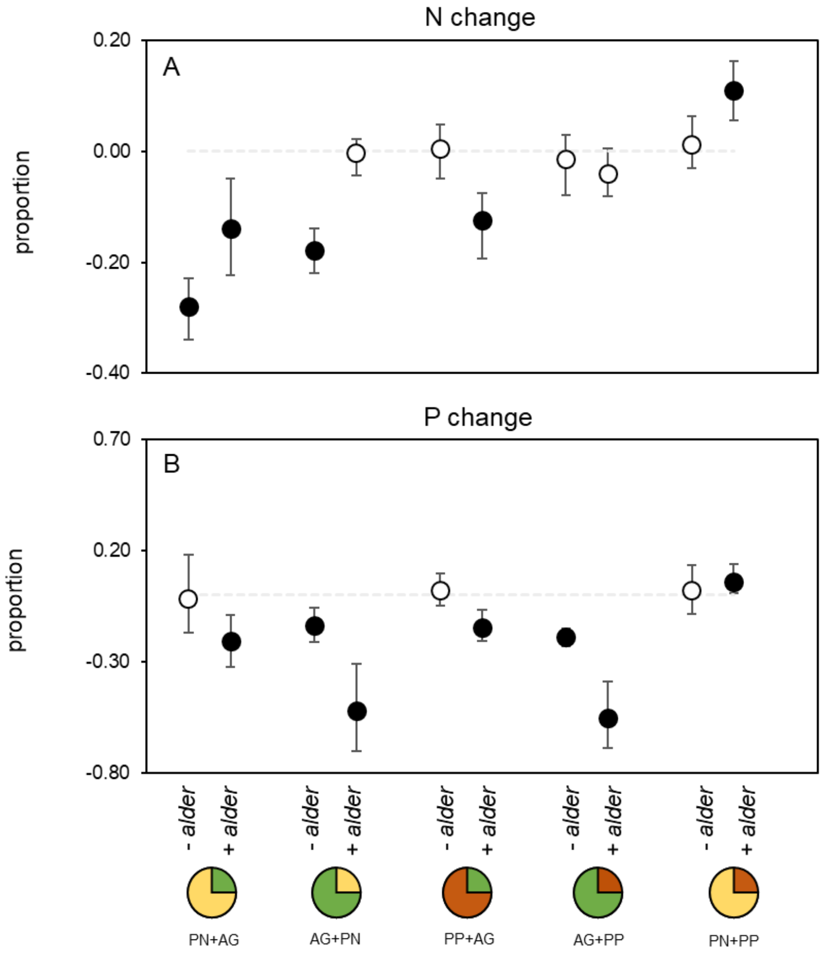

Figure 6.C3. Net diversity effects on microbially-mediated N (A) and P change (B) (NDE; proportion) after a 61 days incubation in streams with (+ alder) and without (- alder) alder in their riverbanks. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect, i.e., the null expectation that mixtures do not differ from expected ones, estimated from monocultures. Closed circles represent intervals that reject the null hypothesis (i.e., confidence interval does not contain the 0-value) and open circles represent intervals that accept the null hypothesis.

Litter assemblage significantly affected P change (Table 2.C3.). Most mixtures exhibited P mineralization independently of the riparian type, except AG+PN (Fig. 5.C3.-B). P followed the opposite pattern than N, with alder litter immobilizing P, but poplar and pine litter mineralizing it (Fig. 5.C3.-B). NDE on P change was affected by the interaction between riparian types and litter assemblages (Table 2.C3.) and followed a similar pattern of that for N change, with overall negative effects, except for the PN+PP mixtures in streams with riparian alder (Fig. 6B.C3.).

DISCUSSION

By the middle of the last century, the Mediterranean basin underwent the development of intensive afforestation campaigns which bet it all to the use of rapid-growing pine species (Allue Andrade & Garnica, 1970; Olivencia et al., 2015; Pausas et al., 2004), without considering the consequences on the recipient ecosystems (Larrañaga et al., 2021). In forested stream ecosystems, the complex interactions among decomposers and their resources hinder the understanding of plantation effects on the functioning of these ecosystems (Gessner et al., 2010; Santonja et al., 2019). Thus, studies have often reported contradictory results (see Larrañaga et al., 2021). Here we assess, by means of a field experiment, whether the presence of an N-fixing tree (*A. glutinosa*) in the riparian area of streams flowing through coniferous plantations may be key to buffer conifer plantation effects on instream leaf litter decomposition and nutrient cycling. Our results exhibit no obvious effect of this species on decomposition rates, but a crucial role on litter N cycling.

Streams ecosystems flowing through plantations with and without alder are functionally similar but differ in water nitrogen concentrations

Water physicochemical characteristics were very similar across the six streams studied. Their proximity, shared lithology and pluvio-nival origin of the water may explain this similarity. Mean water temperature was slightly, but significantly, higher in streams without alder in their riparian areas. However, the difference between the mean temperatures of riparian types was less than 0.2 ºC, which appears to be insufficient to significantly affect the community of decomposers and detritivores or stream ecosystem functioning (e.g. Ferreira & Canhoto, 2015; Verónica Ferreira et al., 2015). Noteworthy, the main difference observed between the two types of riparian areas was related to water N concentrations. Streams with alder in their riversides presented higher concentrations in water of nitrate-N and total dissolved N (TDN). This agrees with previous studies suggesting that riparian alder could be an important source of N to stream water (Compton et al., 2003; Shaftel et al., 2012). Moreover, our results also agree with studies assessing the effects of the N-fixing tree *Acacia melanoxylon* R. Br., which observed higher nitrate and dissolved inorganic N concentrations in streams with this invasive species in their riparian areas (Pereira & Ferreira, 2021). Differences in N concentrations between our riparian types could have been also strengthened by the season of the study (end of winter). Streams surrounded by alder trees may receive higher N inputs when snow starts to melt and sweeps along the N mineralized under the snowpack during winter (Devotta et al., 2021).

No differences in stream water P concentrations were observed between riparian types. However, since alder presence influence stream water N concentration, so does its N to P ratio. Thus, in some situations, alder N-derived inputs to streams may result in a P limitation in these ecosystems (Devotta et al., 2021). The TDN:TDP ratios of our streams were well above the Redfield ratio (16:1) at both riparian types (~74 and ~47 for streams with and without alder, respectively) indicating potential P limitation independently of alder presence (Güsewell & Gessner, 2009; Sterner & Elser, 2002).

Macroinvertebrate FFG ratios, used as surrogates of ecosystem attributes (Merritt et al., 2017), indicated high similarity among streams. Accordingly, all streams were heterotrophic, with “normal” shredder associations linked to Fall-Winter functioning riparian systems and normal predator to prey balance. Although non-significant, those streams flowing through riparian areas with alder groves maintained slightly higher shredder relevance (high CPOM/FPOM Index values) than those without riparian alder, which agrees with studies reporting lower shredder biomass in streams flowing through conifer plantations (Riipinen et al., 2010; Whiles & Wallace, 1997). This may be related with the accumulation of high-quality organic matter stocks from alder. However, such CPOM stocks appear to be insufficient to determine statistically significant differences between riparian types.

Litter decomposition did not differ between riparian types, but it did among litter assemblages

In this study, neither rates of microbial nor those of total decomposition significantly differed between riparian types, despite the significantly higher water N concentration in the streams with riparian alder. This contrasts with previous studies (Kominoski et al., 2011; Pereira & Ferreira, 2021) but agrees with others reporting similar decomposition rates in streams flowing through conifer plantations and broadleaf forests (Ferreira et al., 2017; Riipinen et al., 2010). Higher N concentrations are expected to promote decomposition rates (Ferreira et al., 2015; Pereira & Ferreira, 2021; Rosemond et al., 2015), due to stimulation of microbial decomposer activity (Ferreira et al., 2006; Gulis & Suberkropp, 2003; Pereira & Ferreira, 2021; Pereira et al., 2021) and a reduction of nutrient imbalance for shredders (Cross et al., 2005; Lauridsen et al., 2012). Following the classification of stream trophic state suggested by Dodds et al. (1998), our streams with riparian alder are in the mesotrophic range, whereas those without alder are in the oligotrophic range. Despite that, both stream types are close to the oligotrophic-mesotrophic boundary (~700 mg TN L^{-1}), which may have prevented evident effects of higher N concentrations on litter decomposition.

Litter decomposition differed among monocultures and among litter assemblages as expected from litter differences in physical and chemical traits (Casas et al., 2013; Zhang et al., 2019). In monocultures, alder decomposed the fastest and pine the slowest, although poplar equaled breakdown rates of alder in fine mesh bags, despite its high tannins and phenols concentrations, which might be leached during the first weeks after litter incubation (Gessner, 1991; Schofield et al., 1998). This result are similar to previous studies reporting a significant relationship between leaf litter decomposition and litter quality only for total and detritivore-mediated decomposition (Rubio-Ríos et al., 2021). As our streams appear to be P limited (see above), higher P concentrations in poplar leaves may have favored their use by microbial decomposers to overcome potential stoichiometric imbalances (Gessner et al., 2010; Tonin et al., 2017)—communing with the Growth Rate Hypothesis (Elser et al., 2000)—. Nonetheless, pine needles, with similar P concentrations than poplar leaves, exhibited a much slower microbial decomposition. Possibly, the higher concentration of other nutrients in poplar litter, as Ca, can mean the difference. Ca concentration in leaf litter is known to promote aquatic hyphomycete performance (García-Palacios, McKie, et al., 2016; Jenkins & Suberkropp, 1995) and has been reported as an important driver of litter diversity effects on decomposition (Santonja et al., 2019).

The above-mentioned mechanisms seem to be also operating on microbial decomposition of litter mixtures, which apparently followed the patterns observed for monocultures. Except for the mixture containing a high proportion of pine needles (PP+AG), our results are in line with others reporting similar rates of microbial decomposition independently of the alder proportion within the litter assemblage (ranging from 0 to 50%; Alonso et al., 2022). On the other hand, total decomposition of litter mixtures did not totally follow the trend of monocultures. Noteworthy, the PN+AG mixture decomposed at a rate similar to AG+PN and AG+PP, although containing three times less alder. This result contrasts with a recent study, which reported differences on decomposition rates between litter assemblages containing 0-15 % and those containing 50% of alder litter (Alonso et al., 2022). In accordance with that study**,** we found significant differences between the decomposition rate of PP+AG or PN+PP and the other three mixtures. Nonetheless, such differences can be explained by the consistently reported slower decomposition of pine needles when compared to deciduous leaves (e.g. Albariño & Balseiro, 2002; Casas et al., 2013; Hisabae et al., 2011; Martínez et al., 2013). Thus, the high proportion of pine needles within the PP+AG assemblage and the replacement of alder by pine in the PN+PP (compared to the PN+AG) appear to be the reason for the significant differences observed here. These results may indicate that, whereas microbial decomposition rates appear to be unrelated to the presence of alder in the stream riparian areas, small proportions of alder litter (~25%) can exert a similar effect on total decomposition of the whole assemblage than very high proportions (~75%), when mixed with other deciduous species, but not when mixed with coniferous needles. Presumably, continuous water flow may provide microbial decomposers with the dissolved N supply necessary to cope with nutrient imbalances in the litter (Cheever et al., 2012; Suberkropp, 1998), whereas detritivores' feeding strategies rely on the food resources present in the stream.

Riparian alder modulates diversity effects on microbial litter decomposition

The presence of alder in riparian areas did not appear to affect the decomposition rates but seems to influence litter diversity effects on microbial decomposition. Noteworthy, we observed an overall positive NDE at streams without riparian alder, when this species was present in the litter mixture, but none or negative effects at streams with alder. These results are fundamentally opposed to those reported by a microcosm study (Tonin et al., 2017), observing positive NDE on microbial decomposition when water NO_3-N was increased 5 times over natural concentration (~10-fold higher than our streams with riparian alder) but no effects at natural concentrations (2-fold higher). Possibly, weekly water renewal was insufficient to prevent nutrient limitation for microorganisms in microcosms receiving water with natural nutrient concentrations, whereas in the field, continuous flow of low nutrient concentrations, often precludes such strong limiting conditions (Cheever et al., 2012; Suberkropp, 1998). Previous results (Larrañaga et al., 2020; Rubio-Ríos et al., 2021) point to a threshold of litter quality dissimilarity above which diversity effects would be detectable. Here, we have probably exceeded that threshold in those treatments containing alder litter. However, higher water N concentrations at streams with riparian alder may have reduced the interspecific variability among plant litters, thus altering the way litter species interacted. This may have suppressed the positive complementarity effects (and thus NDE) found at streams without riparian alder (Rosemond et al., 2010). Conversely, in the mixture without alder litter, higher N concentrations in stream water appeared to compensate for negative NDE through a reduction of negative selection effects, which were probably derived from the low microbial decomposition rate of pine needles, due to their antifungal compounds (Bärlocher & Oertli, 1978).

The overall positive NDE found for total decomposition agrees with many other studies (e.g. López-Rojo et al., 2018; Tonin et al., 2017), but contrast with the results of a field study assessing effects of alder loss on litter decomposition (Alonso et al., 2021). The length of their study (42 days) may have prevented the observation of positive NDE in mixtures with alder, as they reported a significant increase of complementarity effects with incubation time. Furthermore, a microcosm study assessing a similar topic observed a positive NDE on total decomposition after 42 days (Rubio-Ríos et al., 2021). Presumably, such effects are more likely to be detected in shorter times in microcosm experiments where detritivores have no other food available. These results manifest the ability of detritivores to feed simultaneously on resources of contrasting quality to balance their diet (Carvalho & Graça, 2007; Leroy & Marks, 2006), independently of water N availability.

Riparian alder can influence stream N budgets

Higher nitrate-N and TDN water concentrations at streams with riparian alder point to the importance of alder as a source of N to streams (Compton et al., 2003; Shaftel et al., 2012), which can have considerable implications for nutrient cycling in such ecosystems. Our results indicating similar decomposition rates among litter assemblages differing in

N concentrations at different riparian types demonstrated that microbial decomposers were able to use either stream water or leaf litter N when required (Cheever et al., 2013; Vitousek & Hobbie, 2000). This was evidenced by the N losses underwent by alder litter in all cases, but of lower magnitude at streams with riparian alder (Fig. 5.C3.). It seems that microorganisms at streams without riparian alder might increase N assimilation from litter to compensate for the lower N concentration in stream water. Poplar presented an interesting pattern, gaining more N the less the amount of alder accompanying it within the mixture. Probably, fungal ability to transfer N between litter species (Tiunov, 2009) was likely promoted by poplar Ca content (Jenkins & Suberkropp, 1995), becoming more active the scarcer the N was within the mixture. Further, such gain was overall higher at streams with riparian alder, supporting the alternation of immobilization of exogenous N and assimilation of endogenous N as a potential strategy of microorganisms to avoid N limitation (Cheever et al., 2013). This inference is further supported by the N gain and loss undergone by pine litter at streams with and without riparian alder, respectively. Thus, these results suggest that microbial nutrient usage of two litters with similar N and P content can be significantly different. Here, microbial decomposers may have used litter P from both, poplar and pine, but only poplar was able to promote fungal N assimilation from other litter species likely due to its higher Ca concentrations (see above), whereas N was only immobilized on pine needles at streams with riparian alder. Previous studies (Handa et al., 2014) suggest that N transfer is stoichiometrically controlled and thus depends on the demand for N relative to the availability of C. Therefore, besides its higher Ca content, poplar litter with higher C quality (e.g., lower lignin content) than pine needles may have favored rapid microbial growth and, consequently, a higher N demand.

Diversity effects on litter N change were affected by the interaction between riparian types and litter assemblages. In general, we observed none or negative NDE on N change in mixtures containing alder, i.e., mixtures gained similar or less N (or lost more N) than expected based on monocultures. Other field studies supporting our results found none or positive diversity effects on litter N loss rates (Handa et al., 2014; López-Rojo et al., 2020). Further, Handa et al. (2014) observed that litter from N-fixing species tended to have higher N loss rates in mixtures than in monocultures, similarly to what we found. On the other hand, the positive NDE on N change observed for the PN+PP mixture at streams with riparian alder manifests again the ability of microorganisms to immobilize exogenous N when an N-rich litter is lacking.

Higher negative NDE on P change at streams with riparian alder, indicate that mixtures lost more (or gained less) P than expected from monocultures (López-Rojo et al., 2020). The combination of an isolated N-rich litter with high-water N concentration likely promoted higher microbial immobilization of P from water on alder monocultures at these riparian areas, whereas within mixtures the presence of other P-rich litter reduced P immobilization rates. At streams without riparian alder, only those mixtures containing high proportions of alder presented negative NDE on P change. Presumably, high proportions of the N-rich alder litter promoted P assimilation from other species. These results manifest that the presence of riparian alder may influence, not only N, but also P instream cycling.

Riparian vegetation: a belt that can buffer pine plantation effects on stream functioning

Riparian areas constitute boundaries where terrestrial and aquatic ecosystems meet and mingle. Maintaining strips of riparian vegetation along streams to mitigate plantation-derived impacts on fluvial ecosystems have been suggested and implemented (Eivers, 2006; Martínez et al., 2013; Pozo et al., 1997), and their potential role in reducing the arrival of diffuse pollution to aquatic systems has been reported (e.g. Naiman & Décamps, 1990).

Our study indicates that the presence of riparian vegetation strips along streams can mitigate pine plantation-derived effects on leaf litter decomposition. In addition, even relatively low cover of a key plant species, such as *A. glutinosa*, can further enlarge the action of this buffer by promoting microbial nutrient recycling through an increase of water N concentration. Previous studies have pointed to a threshold in alder riparian cover of *ca*. 20-30 % above which the profile of stream N can be altered (Compton et al., 2003; Devotta et al., 2021; Shaftel et al., 2012). Our streams hold riparian alder covers between 5 and 60 % when only considering the dominant tree community (Table 1.C3.). However, the amount of alder litter present within the litter stocks in our stream was 24 – 37% (Figure S2.C3.). Thus, lower alder covers may also be able to influence fluvial N dynamics if they are close enough to provide the streams with substantial amounts of litter. These results underscore the key role of alder in driving ecosystem N dynamics in headwaters stream.

Chapter 4

Cross-species coprophagy in small stream detritivores counteracts low-quality litter: native vs. invasive plant litter

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ABSTRACT

Understanding how stream detritivores can cope with riparian plant invasions is relevant to predict future impacts on detritivore assemblages and the functioning of small stream ecosystems, where litter decomposition mainly fuels food webs. In a microcosm feeding trial, we examined survival, consumption, growth, and energetic status of two detritivores coexisting in nature: the amphipod *Echinogammarus obtusidens,* and the snail *Melanopsis praemorsa*. Detritivores were fed two types of leaf litter—the native tree *Populus alba,* and the invasive reed *Arundo donax*—and the corresponding con- and heterospecifics faeces produced from the ingestion of the litter from these species. We aimed to assess whether lowland stream detritivores can use coprophagy as a suitable feeding strategy alternatively to *Arundo* litter; and how coprophagy affects their fitness compared to litter from native and non-native plants. *Echinogammarus* presented the highest performance when fed *Melanopsis*' faeces but the lowest when fed leaf litter, especially the invasive one, exhibiting a high mortality rate. *Melanopsis* exhibited good and similar performance when fed on both leaf litter species, as well as on conspecific faeces. *Echinogammarus* performed best as cross-species coprophagous, enabling the amphipods to thrive on the low-quality litter species offered when *Melanopsis* was present. These results suggest that *Echinogammarus obtusidens* may act as a coprophagous when no high-quality litter is available. Thus, the snail seems to play a key role facilitating the access to nutrients of recalcitrant leaf litter to sympatric detritivore species via coprophagy.

Keywords: *Arundo donax, Echinogammarus obtusidens,* facilitation, faeces, plant invasion, *Melanopsis praemorsa*

INTRODUCTION

The structure and function of first-order streams greatly rely on allochthonous inputs of organic matter (OM), mostly riparian leaf litter (e.g. Abelho, 2001; J. B. Wallace et al., 1997) which is processed by decomposers and detritivores. Therefore, changes affecting the quality and/or magnitude of litter inputs to streams may alter their community structure (Bärlocher & Graca, 2002; A. Martínez et al., 2013) and ecosystem functioning (Casas et al., 2013; Hladyz et al., 2011; Larrañaga et al., 2021). This reliance on leaf litter from riparian vegetation makes small forested streams especially sensitive to plant invasions (e.g. Pereira & Ferreira, 2021). The favourable conditions for plant life in riparian zones and the reduction of the biotic resistance of native vegetation to invasions caused by natural and/or unceasing anthropic disturbances, promote the proliferation of non-native species in these ecosystems (Castro Díez & Alonso Fernández, 2017).

Giant reed, *Arundo donax* L. (Poaceae; hereafter *Arundo*), is among the 100 world's worst invasive alien species (Lowe et al., 2000), particularly in sub-tropical and temperate wetlands (Canavan et al., 2017). This species is considered one of the greatest threats for the conservation of lowland riparian ecosystems in Mediterranean-type climates (Aguiar & Ferreira, 2013), since it creates mono-specific stands by displacing native vegetation, causing an impoverishment of the native riverine biodiversity (Maceda-Veiga et al., 2016). Regarding the impacts caused by *Arundo*, most studies have focused on the riparian corridor (e.g. Herrera & Dudley, 2003; Maceda-Veiga et al., 2016), while its effects on fluvial food webs remain almost unexplored (but see Going & Dudley, 2008). Leaf litter of non-native invasive plants can decompose more rapidly or slowly than native species, but, in both cases, it may alter stream ecosystem processes (Marks, 2019; and references therein). Leaf litter of *Arundo* begin decomposing with poor nutritional value for aquatic detritivores due to its high toughness and silicon concentration, and low nitrogen content, even compared with low-quality native species such as *Populus* spp. (Going & Dudley, 2008; Salinas et al., 2018).

The generation of fine particulate organic matter (FPOM) is closely linked to detritivores litter consumption (Halvorson, Hall, et al., 2017; Santonja et al., 2018). Small detritivores benefit from this FPOM produced by the feeding activity of larger detritivore shredders (Tonin et al., 2018), particularly by ingesting the abundant pool of FPOM in the form of faecal aggregates (Cummins et al., 1989) which, in some cases, can even equal the amount of detritus directly derived from leaf litter inputs (Malmqvist et al., 2001). Overall, coprophagy may provide nutritional benefits to detritivores (Weiss, 2006), due to the often increased nutritional value of shredder's faecal pellets (Halvorson, Sperfeld, et al., 2017; Hood et al., 2014) which is further promoted by microbial action at warm temperatures (> 10ºC; Joyce & Wotton, 2008). However, this mechanism is under-studied in lotic ecosystems (Wotton, 2007; Wotton & Malmqvist, 2001), probably because egested material have been often considered to lack labile C (high lignin content; Yoshimura et al., 2008) and to possess higher C:N and C:P ratios than original resources (Callisto & Graça, 2013) enabling it to remain unaltered for months (Joyce et al., 2007).

Here, we intend to assess responses of two major detritivore species to the invasion of riparian zones by *Arundo* in lowland Mediterranean low-order streams: the small shredding amphipod, *Echinogammarus obtusidens* (Pinkster & Stock, 1972; hereafter *Echinogammarus*) and the large grazing snail, *Melanopsis praemorsa* (Linnaeus, 1758; hereafter *Melanopsis*). The snail is an abundant generalist feeder in this stream-type, which behaves as an important consumer of leaf litter (Casas et al., 2011), with outstanding capacity to digest low-quality litter (Fenoy et al., 2021). We aimed to: (i) estimate the nutritional value of leaf litter from the invasive *Arundo* compared to that from the native *Populus alba* L. (Salicaceae; hereafter *Populus*) and faeces; (ii) test to what extend these detritivores can use coprophagy, as a successful feeding strategy to deal with low-quality litter from the non-native species; and (iii) compare the performance (survivorship, litter consumption, growth, and energetic reserves) of both detritivores when offered the different diets. To this end, we designed laboratory feeding trials in which both detritivore species were fed with leaf litter, con- or hetero-specifics faeces (i.e., faeces from the same or from other species, respectively). After 21 days of microcosm incubation the following hypotheses were examined: (i) both detritivore species will exhibit a superior performance when fed litter of higher nutritional quality (*Populus*) over litter of lower quality (*Arundo*); (ii) faeces represent a more nutritive food than the original leaf litter; (iii) both detritivores can use coprophagy as a suitable feeding strategy; and (iv) performance of *Echinogammarus* will improve when fed on faeces from *Melanopsis*.

MATERIALS AND METHODS

Field procedures used with leaf-litter and detritivores

For feeding tests we selected leaf-litter of the invasive perennial grass giant reed (*Arundo donax)* and the native semi-deciduous tree white poplar (*Populus alba*). Both are species common in warm temperate and Mediterranean zones, particularly in lowland streams. Senescent leaves were collected from the riparian vegetation before the experiment, air-dried at room temperature (≈ 23ºC) for 1 week and stored in the dark until needed.

Portions (≈ 5 g each) of each leaf litter species were introduced within mesh bags (0.5 mm mesh size and 30 cm^2 each bag) and submerged during two weeks, to allow leaching of soluble compounds and microbial conditioning, along a 50 m stream-reach in the Barranco del Cura stream (291 m *a.s.l*, 36.84ºN 2.64ºW; mean summer water temperature 21.5 ± 0.1ºC, mean ± SD) approx. 3 weeks before the experiment. This is a non-polluted low-order stream notably invaded by giant reed (*ca.* 75% cover), where there are still a few individual trees or clumps of white poplar. Additional environmental information of the basin and stream can be found in Table S1.C4. After retrieval, litter was transported to the laboratory, and 12 mm Ø leaf discs were cut with a cork borer. Leaf discs were air-dried at room temperature (≈ 23ºC) for 1 week, weighted to the nearest 0.1 mg and frozen (-20ºC) until needed in the feeding experiment. An extra set of leaf discs was used to measure initial dry mass (DM; 70ºC, 72 h) of the discs offered to the animals and litter traits (toughness, C, N, P, Si, Ca, K and Mg concentrations, and lignin, total phenols and condensed tannins contents; Supplementary methods).

Detritivores were collected from the same stream, placed in plastic containers filled with stream water and transported to laboratory in a portable refrigerator. There, animals were acclimatized to experimental conditions (see below) for one week fed litter from the stream. Animals were starved for 24 h prior the start of the experiment to allow evacuation of their gut contents.

Experimental set-up in the laboratory

Microcosms were placed in a temperature-controlled chamber at 20ºC, in order to mimic summer temperature in Barranco del Cura stream, under a light:dark regime of 12:12 h and with constant aeration of water. Each microcosm consisted of a 300-mL glass jar filled with 250 mL of stream water: total dissolved P 7 µg P L^{-1}; N-NO_3 723 µg N L^{-1}; pH 8.13; electric conductivity 896 µS cm^{-1}; alkalinity 250 mg $CaCO_3$ L^{-1}. Water was previously filtered through 1.2 µm pore size glass fibre filters (Merk Millipore Ltd.). Microcosms were horizontally divided into two compartments using a septum of 1 mm mesh size to allow the passage of faecal pellets from the upper to the lower compartment (Fig. S1.C4.). *Echinogammarus* were supplied with a pebble, previously incinerated, to serve as a shelter. In the upper compartment, detritivores were fed microbially conditioned leaf litter discs of *Arundo* or *Populus*, while in the lower compartment were fed faeces falling from the upper compartment. Finally, only six out of the eight possible treatments were carried out: both litters (*Arundo* and *Populus*) and faeces of *Echinogammarus* (E-Ad or E-Pa) or *Melanopsis* (M-Ad or M-Pa) fed on them. (Fig. S1.C4.). The two treatments of *Melanopsis* fed on faeces from *Echinogammarus* were not conducted assuming low nutritional significance of faeces from *Echinogammarus* for *Melanopsis*, given the notable differences in body mass between species (~10 times higher in *Melanopsis*). Fifteen replicates of each treatment were performed, totalling 90 microcosms and 180 animals (two individuals per microcosms, one in each compartment). Two leaf litter discs were offered to each individual in the upper compartments, being replaced by new discs every 4 days to prevent food deprivation whereas availability of fecal pellets was visually verified by daily observations of the lower part of the microcosms. Removed leaf discs were oven dried (70ºC, 72 h), and weighed to the nearest 0.1 mg to determine DM loss during the time of exposure. Five control discs per plant species were randomly incubated in upper compartments inside 0.5 mm mesh size bags to isolate detritivores consumption from microbial decomposition. Simultaneously, additional pools of individuals of each detritivore species were placed in extra microcosms and fed litter of *Arundo* and *Populus*, and their faeces were collected daily using a Pasteur pipette, frozen and used to estimate nutrient content analyses (Supplementary methods). Water was renewed every 5 days with fresh filtered stream water to prevent excessive microbial proliferation and to compensate for water losses. The experiment lasted 21 days. Mortality was recorded daily and dead animals were measured as explained in the next section and rapidly replaced by a new one. The last day, survivors from each microcosm were starved (24 h), measured and frozen (-20ºC) until needed for total lipids and glycogen analyses.

Analytical procedures with detritivores

Just before and after the experiment, each experimental individual was photographed under a binocular microscope, and their total length—TL = dorsal length of the ten thoracic segments in *Echinogammarus* and shell length in *Melanopsis*—was measured using the *SigmaScan Pro v 5.0* image analyser. Detritivores dry mass (DM) was estimated from TL (mm)-DM (mg) relationships, established using additional animals of each species collected simultaneously to the experimental individuals: $DM_{Echinogammarus}$ (mg) = $0.5743 \times TL - 1.3934$; $R^2 = 0.71$; $DM_{Melanopsis}$ (mg, without shell) = $8.8483 \times TL - 61.862$; $R^2 = 0.87$. We used a linear equation as it fitted better than the exponential one. Detritivores initial biomass per microcosm was on average 7.10 ± 0.44 mg for *Echinogammarus*, and 70.23 ± 1.67 mg (mean ± SE) for *Melanopsis* (Fig. S2.C4.).

Energetic reserves of detritivores, lipids and glycogen, were measured using the sulfo-phospho-vanillin and the anthrone reactions respectively, following the methods described in (Charron et al., 2014) with minor modifications (see Fenoy et al., 2021). After removal of shells if necessary, each animal was homogenized in 1 mL methanol using 5 mm Ø stainless balls and a vortex mixer for 2 minutes. The homogenized mix was then divided in two identical aliquots and frozen until used in analyses. Optical density was measured at 525 nm for lipids and at 630 nm for glycogen. Calibration solutions were prepared, for lipids using a commercial olive oil solution (5 g L^{-1}) solubilized in chloroform, and for glycogen (2.5 g L^{-1}) solubilized in distilled water.

Data analysis

We carried out comparisons of litter traits among plant species (Table S2.C4.) and of nutrient concentrations among litter and faeces (Table 1.C4.) using t-test and one-way ANOVAs followed by Tukey's Honestly Significant Difference (HSD) analyses (*anova* and *TukeyHSD* functions of 'stats' R package), respectively. *Log* or *logit* transformations of variables were used when required for decimal and proportion values, respectively.

Additionally, we characterised nutrient (C, N and P) variability among litter and faeces using a principal component analysis (PCA) with the *prcomp* function in the 'stats' package of R statistical software (R Core Team, 2020; Fig. 1.C4.). A second PCA was used to assess litter traits variability (Fig. S3.C4.). Previously, we calculated Spearman paired correlations (*cor* function of 'stats' R package) between 14 leaf traits and, within each highly correlated pair ($r > 0.80$; Fig. S4.C4.), we selected only one. Therefore, the eight less correlated litter traits were included in the PCA: N, P, N:P, total phenols, condensed tannins, Si and Mg.

Table 1.C4. Mean (± SE) of carbon (C), nitrogen (N) and phosphorus (P) concentrations (% DM) and molar elemental ratios (C:N, C:P and N:P), of each type of leaf litter and faeces used in this study. Different letters indicate significant differences ($p < 0.05$) on the basis of linear models followed by pairwise multiple comparisons.

	Arundo	*Populus*	E-AD	E-PA	M-AD	M-PA	*p value*
C	43.7 ± 0.19^{a}	46.6 ± 0.24^{a}	22.1 ± 0.9^{c}	26.9 ± 2.7^{bc}	31.6 ± 1.3^{b}	40.9 ± 1.5^{a}	< 0.0001
N	0.6 ± 0.018^{d}	2.2 ± 0.081^{a}	0.9 ± 0.05^{c}	1.2 ± 0.1^{b}	0.9 ± 0.05^{c}	2.1 ± 0.1^{a}	< 0.0001
P	0.02 ± 0.001^{b}	0.06 ± 0.003^{a}	0.07 ± 0.016^{a}	0.07 ± 0.006^{a}	0.07 ± 0.003^{a}	0.06 ± 0.002^{a}	< 0.0001
C:N	92.1 ± 3.01^{a}	25.1 ± 1.07^{cd}	30.4 ± 2.4^{c}	26.8 ± 1.3^{cd}	42.7 ± 1.7^{b}	22.5 ± 1.0^{d}	< 0.0001
C:P	7143 ± 449^{a}	2098 ± 126.9^{b}	982 ± 298^{d}	1033 ± 93.2^{cd}	1233 ± 61.2^{c}	1858 ± 93.8^{b}	< 0.0001
N:P	91.4 ± 5.7^{a}	98.8 ± 6.0^{a}	38.7 ± 11.7^{b}	45.5 ± 4.1^{b}	34.3 ± 1.7^{b}	97.6 ± 4.9^{a}	< 0.0001

We used the differences in stoichiometry between food and faeces to assess whether faeces become an enriched resource in N and/or P for consumers. In addition, we calculated the elemental imbalance (EI; see Frainer et al., 2016) between leaf litter and faeces to quantify such differences as the quotient between leaf litter and faeces C:N, C:P or N:P molar ratios (Supplementary methods).

Kaplan-Meier test was used to compare survivorship among treatments using the *survfit* function of `survival' R package (Therneau, 2015). The median time to death was calculated using the Kaplan-Meier product-limit method (Ferreira et al., 2010). Significant differences in survivorship among treatments were compared using log-rank test (Ferreira et al., 2010) which is based on the difference between observed and expected death data.

Litter consumption was quantified through litter mass loss as the difference between initial and final DM. To isolate shredders litter consumption, initial discs DM was corrected by microbial decomposition based on the litter mass loss observed in control discs. Consumption rate was then calculated as: $(DM_i - DM_f) / (DM_{detritivore} \times d)$; where DM_i and DM_f are the leaf disc DM (mg) at the beginning and at the end of the experiment, respectively; $DM_{detritivore}$ is the mean dry mass (g) of the detritivore during the experiment and d is the number of days the experiment lasted. The experiment design did not allow us to estimate faeces consumption rates. To assess differences on leaf litter consumption rates we used two-sample t-test or Welch two-sample t-test analyses if homoscedasticity was not achieved.

We measured individual growth of each detritivore species when fed each of the treatments. Detritivores growth rate was calculated as the difference between final and initial mass against initial mass and the number of days exposed. Differences in growth and, total lipids and glycogen content were assessed using one-way ANOVA followed by Tukey's HSD test (see function and package above) or Welch's ANOVA (*oneway.test* function of the 'stats' R package) followed by Games Howell post-hoc test (*games_howell_test* function of the 'rstatix' R package) if homoscedasticity assumptions were not fulfilled. Total lipids and glycogen contents were transformed when required using the *orderNorm* function transformation which was chosen by the *bestNormalize* function of the 'bestNormalize' R package (Peterson & Cavanaugh, 2019) to meet the assumptions of normality and equal variance. However, as homoscedasticity was not achieved for the glycogen content of *Echinogammarus*, Welch's ANOVA was used.

For these raw variables (litter consumption, growth, lipids and glycogen content) associated effect sizes (Cohen's d) and 95% confidence intervals (CI) were calculated applying bootstrapping procedures (1000 repetitions) using the *cohens_d* function of "rstatix" R package (Kassambara, 2020). We estimated effect sizes for all the possible two sample comparisons. We report mean treatment effect sizes (d) and 95% confidence intervals (CI), where effect sizes of 0.20–0.49, 0.50–0.79 and ≥0.80 were considered small, medium and large, respectively (Cohen, 1988). All statistical analyses were performed using R software version 4.0.5 (R Core Team, 2020).

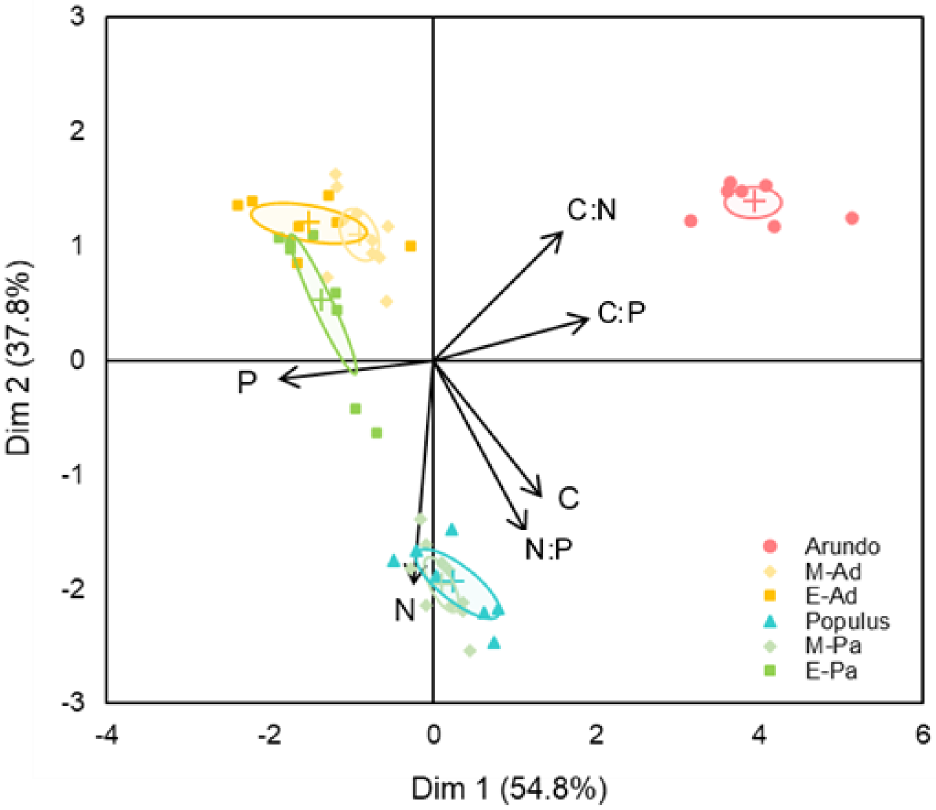


Fig. 1.C4. Projection of the 2 first principal component axis showing differences among nutrient concentrations (C, N and P) and nutrient ratios (C:N, C:P and N:P) between the 2 plant species (*Arundo* [Ad] and *Populus* [Pa]) and the different types of faeces produced by amphipod *Echinogammarus* (E-Ad, E-Pa) and snail *Melanopsis* (M-Ad, M-Pa) (Explained variance = 92.6%). Vectors represent each trait weight and symbols each litter or faeces position. Ellipses represent 95% confidence and centroids (crosses) the average position of each diet over the PCA axes (n = 7−10).

RESULTS

Differences between the diets offered to detritivores

There were significant differences on leaf traits across the selected plant species (Table S2.C4., Fig. S3.C4.). Overall, *Arundo* presented a much more recalcitrant litter than *Populus*, with higher toughness and Si concentration, but lower concentrations of nutrients (N, P, Ca, K and Mg). The first two dimensions of the PCA explained 84.9% of the variation. Dimension 1 (66.6% var. expl.) represented a gradient of decreasing nutrients (N, P and Mg) versus increasing structural components (Si and condensed tannins), which clearly segregated both litter species. Dimension 2 (18.3% var. expl.) was positively correlated with total phenols and negatively with N:P ratio, reflecting within-species variability.

We observed significant differences between nutrient ratios (C:N, C:P, N:P) of leaf litter and faeces from both detritivores (Fig 1.C4., Table 1.C4.), suggesting a general relative nutrient enrichment of faeces, especially when fed on *Arundo* litter (Fig. 2.C4., Fig. S5.C4.). Faeces of both detritivores exhibited lower C:N and C:P ratios than the ingested litter of *Arundo* (Fig. 2.C4.-A-D; Fig. S5.C4.) due to increasing N and P, but decreasing C concentrations (Table 1.C4.). Likely, faeces had lower N:P ratios than *Arundo* litter (Fig. 2.C4.-E, F; Fig. S5.C4.). When fed *Populus, Echinogammarus'* faeces exhibited a relative enrichment in P (lower C:P and N:P ratios) due to decreasing C and N concentrations (Fig. 2.C4.-C, E; Table 1.C4.), whereas those of *Melanopsis* exhibited slightly lower C:N and C:P ratios (Fig. 2.C4.-B, D), as a consequence of a small decrease in C concentrations (Table 1.C4.).

Detritivores performance

Survival probability of *Echinogammarus* significantly differed among treatments (Log-rank test, $\chi^2 = 13.5$, $P = 0.02$; Fig. S6.C4.; Table S3.C4.), being the highest for individuals fed M-Pa faeces (88.2%), followed by those fed *Populus* (83.3%), E-Pa (75.0%), M-Ad (71.4%) and E-Ad faeces (55.6%), being the lowest when fed *Arundo* (43.8%). Mortality started before the third day for all the diets except *Populus* for which deaths did not occur until day 6. Median time to death was 18 days in *Arundo* treatment. All individuals of *Melanopsis* survived regardless of the food offered.

Overall, the consumption rates of both detritivores were higher when fed *Populus* over *Arundo* leaf litter (T-test, $P < 0.0001$; Fig. 3.C4.), with large effect sizes (Table S3.C4.). *Echinogammarus* was incapable to grow significantly when fed any diet except *Melanopsis* faeces (Fig. 4.C4.-A); the highest growth was found when fed M-Pa (3.20 ± 1.14 mg g^{-1} day^{-1}, mean ± SE), whereas animals fed *Arundo* and *Populus* leaf litter underwent weight losses (Welch's ANOVA, $F_{5,37.9} = 3.95$, $P = 0.005$; Fig. 4.C4.-A). Accordingly, associated effect sizes (Table S3.C4.) were large when *Echinogammarus* fed *Melanopsis* faeces (M-Ad and M-Pa) compared with leaf litter of *Arundo* and *Populus*. Unexpectedly, *Melanopsis* exhibited the greatest growth rate (0.39 ± 0.13 mg g^{-1} day^{-1}, mean ± SE) when fed *Arundo*. However, they were able to grow regardless of the

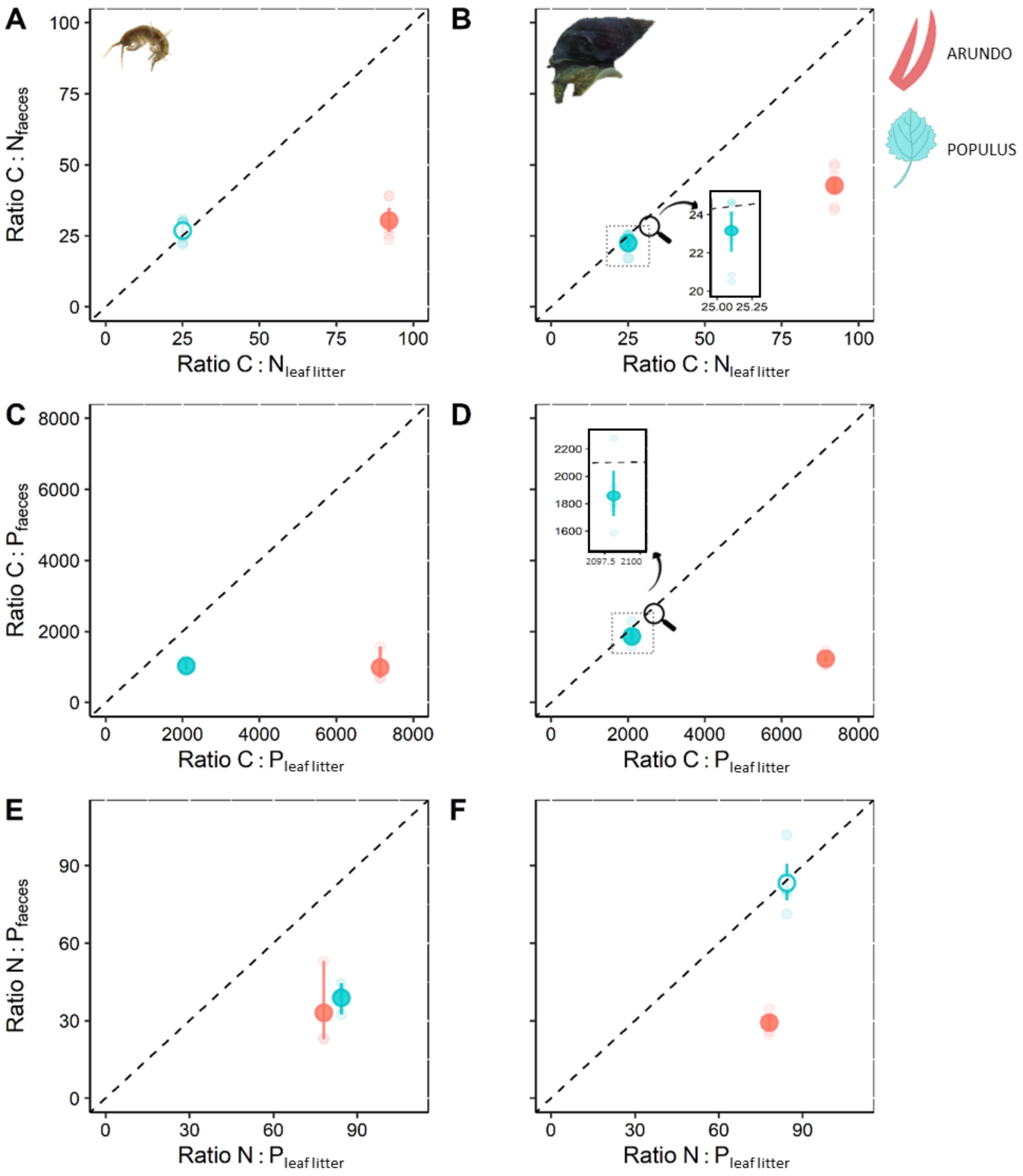


Fig. 2.C4. Relationship of molar C:N, C:P, and N:P ratios between faeces (Y-axes) and the two leaf-litter species offered (X-axes) to *Echinogammarus obtusidens* (A,C,E) and *Melanopsis praemorsa* (B, D, F). Dotted lines represent the ratio 1:1. Circles are means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis of no differences between litter and faeces nutrient ratios (i.e., do not overlap the 1:1 line) and open circles represent intervals that do not reject the null hypothesis. Points under the reference line indicate an enrichment in faeces of the limiting nutrient (N or P, denominator in the ratio). Zoom insets are included in B and D to show that confidence intervals do not overlap the 1:1 line.

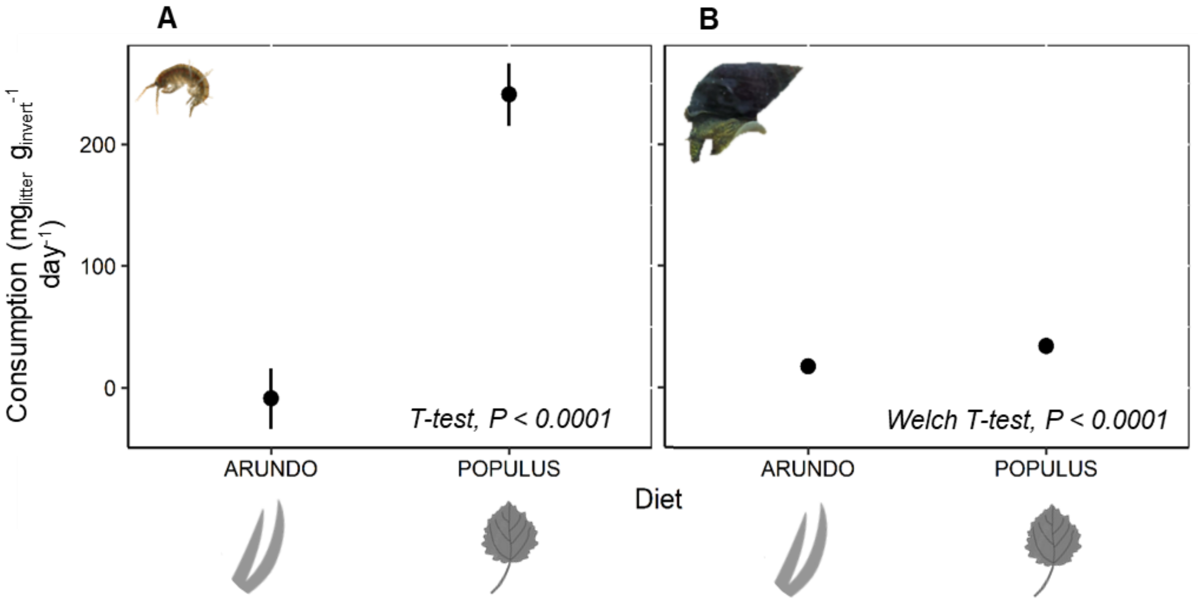


Fig. 3.C4. Effects of treatments (*Arundo donax* and *Populus alba* litter) on *Echinogammarus obtusidens* (A) and *Melanopsis praemorsa* (B) consumption rates during 21 days experiments. Different superscript letters indicate significant differences ($P < 0.05$) across treatments on the basis of linear models followed by pairwise multiple comparisons. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented.

diet offered (Fig. 4.C4.-B) and no differences were observed among diets (One-way ANOVA, $F_{3,85} = 0.77$, $P = 0.515$; Table S3.C4.).

The energetic status of *Echinogammarus* fed on *Melanopsis* faeces was higher compared to other treatments. *Echinogammarus* fed on M-Pa (89.7 ± 14.1 mg g_{animal}^{-1}) and M-Ad (72.33 ± 12.11 mg g_{animal}^{-1}, mean ± SE) exhibited the highest lipid concentrations (One-way ANOVA, $F_{5,41} = 7.44$, $P < 0.0001$; Fig. 4.C4.-C), with most of the associated effect sizes being large when comparing litter and faeces (Table S3.C4.). Glycogen showed a similar trend, and *Echinogammarus* fed M-Ad (51.98 ± 3.83 mg g_{animal}^{-1}) had the highest glycogen accumulation (Welch's Anova, $F_{5,16.9} = 6.60$, $P = 0.0014$; Fig. 4.C4.-E). Associated effect sizes were mostly large when comparing M-Ad or M-Pa with other treatments (Table S3.C4.).

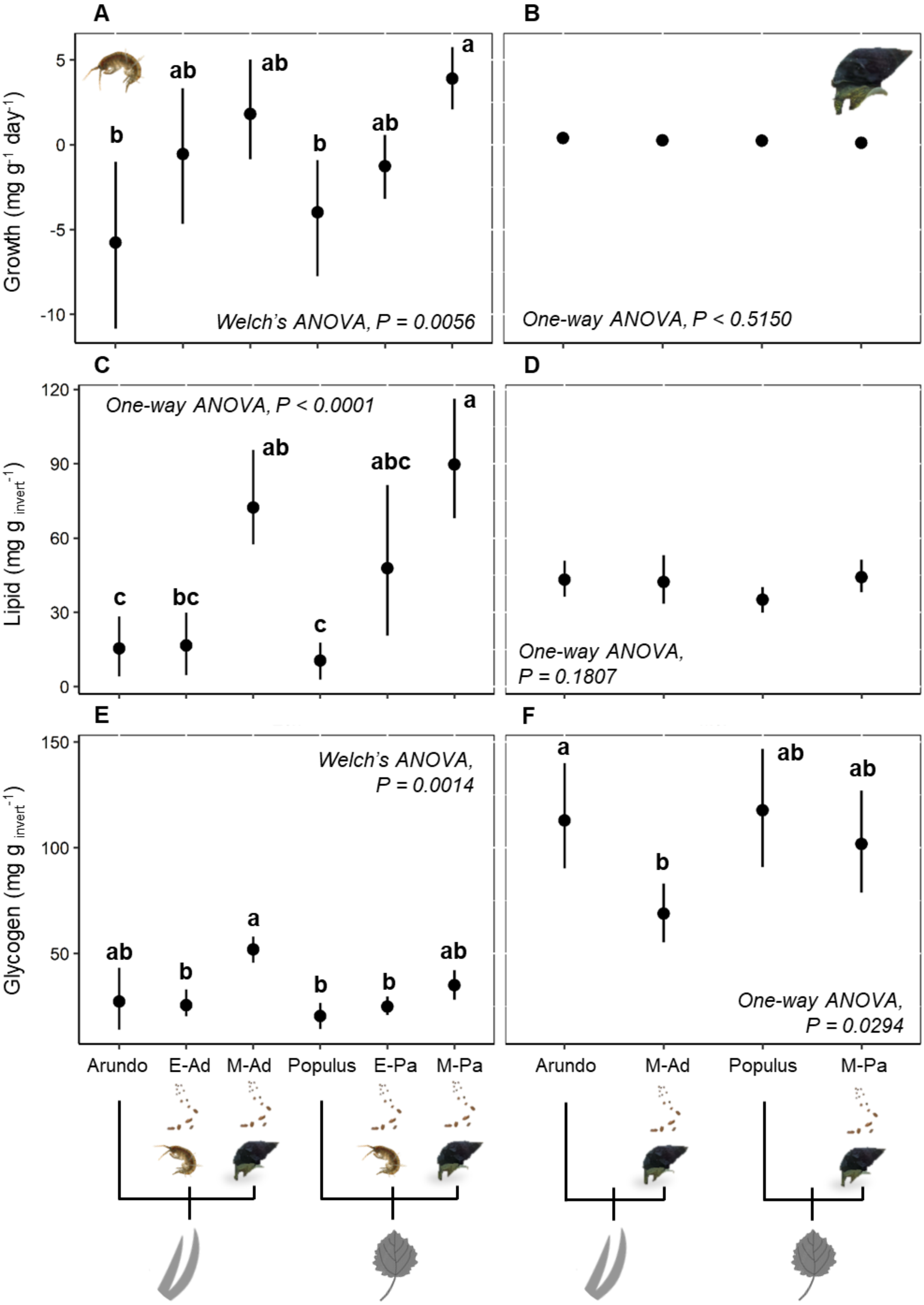
Melanopsis lipids content (range = 35.11 – 44.18 mg g_{animal}^{-1}, mean ± SE) did not exhibited significant differences among treatments (One-way ANOVA, $F_{3,56} = 1.68$, $P = 0.181$; Fig. 4.C4.-D). However, a large effect size was found between the lipids content of snails fed M-Pa and of those fed *Populus* litter (effect size = 0.82; Table S3.C4.). Glycogen content was in general higher when fed on litter than on faeces (One-way ANOVA, $F_{3,56} = 3.22$, $P = 0.029$; Fig. 4.C4.-F), with large associated effect sizes when comparing snails fed faeces M-Ad with those fed either litter species (Table S3.C4.) .

DISCUSSION

Our study strongly suggests that cross-species coprophagy can be a useful feeding strategy for small stream detritivores facing shrinking leaf-litter quality, e.g. due to riparian invasions by giant reed but also due to climate-induced changes in the riparian community composition or leaves traits (Kominoski et al., 2021; Rubio-Ríos et al., 2022; Salinas et al., 2018). Previous studies (Tonin et al., 2018) find that small detritivores are facilitated by larger ones which produce fine particulate organic matter, whereas when two large detritivore species interact, niche partitioning seems to be the subjacent mechanism. Here, we show that one small and one large detritivore species are able to survive feeding exclusively conspecific faeces (intraspecific facilitation), and that the small species develops its best performance when fed on heterospecific faeces (interspecific facilitation). These results may help explain how some detritivores can maintain viable populations in streams mostly receiving highly recalcitrant leaf-litter inputs, as in those invaded by giant reed, and highlight the prime role of a large detritivore, *Melanopsis praemorsa,* facilitating sympatric detritivore species via faecal production.

Some studies show detritivores’ preference for non-native leaf litter even when is a resource of poorer quality than the native one (Lewis et al., 2017; Moretti et al., 2020). Here, however, detritivores consumed more of the native than the invasive litter species. This can be satisfactorily explained by differences in litter traits related with its nutritional value and palatability, i.e. higher nutrient concentrations and lower C:N and C:P molar ratios (Graça et al., 2001; Santonja et al., 2019), and lower tannins and toughness (Ardon et al., 2009; Li et al., 2009) in *Populus*. Moreover, the extremely high Si concentration of *Arundo* may also have dissuaded detritivores from feeding due to mandible wear caused by phytoliths (Massey & Hartley, 2009). Even though snails seems to avoid phytoliths when grazing on leaf litter (Schaller, 2013), *Melanopsis* still consumed significantly more *Populus* than *Arundo*. Higher feeding rates of this snail on *Populus alba vs.* litter of higher dietary quality—*Alnus glutinosa*—have been recently reported (Fenoy et al., 2021), suggesting that the preference for the native species could depend on detritivore idiosyncrasies interacting with certain preferred litter traits (Frainer et al., 2016) more than simply on litter nutrient content. Nevertheless, overall, the two litter species used in our study may be of low-quality for detritivores —in particular for *Echinogammarus* species— compared to those from other species as alder or ash tree (e.g. Fenoy et al., 2021; Solagaistua et al., 2019), especially due to their high Si concentrations.

Contrary to our first hypothesis, and despite greater dietary quality and consumption rates in *Populus* than in *Arundo*, neither of the two detritivores showed superior performance when fed the native species (excepting survival of *Echinogammarus*). *Melanopsis* had similar survival (100% survivors in all treatments), growth rates and accumulation of reserves in both litter species, as well as when fed faeces. This is not surprising given the extraordinary physiological flexibility reported for freshwater snails (Fink & Von Elert, 2006; Rollo & Hawryluk, 1988), including *Melanopsis praemorsa* (Fenoy et al., 2021), as an adaptation to thrive feeding on high-C



diets—e.g. *Arundo* litter—by allocating the excess of C acquired to respiration or accumulation of reserves. On the contrary, *Echinogammarus* was not able to grow when

Fig. 4.C4. Effects of treatments (*Arundo donax* and *Populus alba* litter, and E-Ad, M-Ad, E-Pa, M-Pa faeces) on *Echinogammarus obtusidens* (A, C, E) and *Melanopsis praemorsa* (B, D, F) growth rates and energy reserves during 21 days experiments. Different superscript letters indicate significant differences (*P*

< 0.05) across treatments on the basis of linear models followed by pairwise multiple comparisons. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented.

fed leaf litter, not even *Populus* despite consuming it abundantly compared to *Arundo*, which was virtually unconsumed. Thus, while some freshwater crustacean detritivores show enzymatic adaptation to digest leaf litter (Zimmer & Bartholmé, 2003), our results suggest that *E. obtusidens* may need more than just litter to grow (Tonin et al., 2018). However, given the high survival rate of the amphipod when fed on the native compared to the exotic litter (Going & Dudley, 2008), it possibly would be able to perform much better if a higher quality litter, especially in terms of Si concentration, was offered (see above).

According to the assimilation hypothesis (Hessen, 1997), animals under high-C diets tend, in order to balance their stoichiometry (~5.8 and ~5.5 for *Echinogammarus* and *Melanopsis* C:N ratios, respectively; Unpublished data) retain the limiting nutrients (N or P) more efficiently than C. Thus, C:nutrients ratios in faeces should be higher than in the ingested litter. However, in support of our second hypothesis, we reported much lower C:N and C:P molar ratios of faeces, from both detritivores, relative to the ingested *Arundo* litter—the highest-C diet—but not *Populus*, as a consequence of a simultaneous increase of N and P and decrease of C concentrations. Consequently, the passage of *Arundo*-litter through the gut of detritivores determined a relative nutrient enrichment of its egesta, which roughly equaled it with that of *Populus*. This suggests limited control of nutrient assimilation by our detritivores when fed on the recalcitrant non-native litter. Likewise, N assimilation appeared to be higher than that of P when both detritivores fed *Arundo* litter, as inferred from the higher N:P molar ratios in faeces than in litter. Probably, our detritivores are maintaining their elemental homeostasis by regulating excretion rather than assimilation (excretion hypothesis; Balseiro & Albariño, 2006; Sterner & Elser, 2002) although other mechanisms, as selective feeding of nutrient-rich fractions of leaf litter (Hood et al., 2014), may be also operating.

Furthermore, nutrient enrichment of faeces can be boosted by microorganisms that thrive within the gut of aquatic invertebrates (e.g. in crustaceans and molluscs) contributing with exudates, living and dead cells to the egesta (Wotton & Malmqvist, 2001), and by microbial colonization during its storage in the riverbed (up to months; Joyce & Wotton, 2008), therefore making faeces—especially those from recalcitrant litters as *Arundo*—a presumably more palatable and nutrient-rich food resource for detritivores (Joyce et al., 2007; Shepard & Minshall, 1981).

In accordance with the above, and at least partly with our third hypothesis, both detritivores showed high survival when fed faeces. Particularly, it was the consumption of faeces from the snail that led the greatest performance to *Echinogammarus*: reaching the highest accumulation of reserves and being the only food that promoted its growth. Previous studies have reported some genera of Gammaridae as generalist feeders, having a flexible omnivory including the consumption of faeces (Agnew & Moore, 1986), but without documenting the nutritional value of faeces for consumers. Some other studies, however, suggest that *Echigammarus* species—i.e. *E. berilloni*—might be unable to ingest faeces due to unsuitable morphological adaptation to sieve particles (Mas-Marti et al., 2015; Mayer et al., 2012). Here, we clearly evinced that *E. obtusidens* was able to grow up consuming faeces, possibly due to an intrinsic ability of some amphipods to adjust their feeding mode depending on food availability (Kulesza & Holomuzki, 2006).

Crustaceans usually metabolize lipids slowly and use them in long-term process as growth, body maintenance or reproduction (Sánchez-Paz et al., 2006). Thus, their lipid body concentration may reflect better than glycogen shifts in energy demand or food availability (Becker et al., 2013). Accordingly, lipids content of *Echinogammarus* had a slight but significant correlation with growth rate (r = 0.639; R^2 = 0.409, P < 0.001; Fig. S7.C4.-A), and, overall, was lower when fed on litter than when fed on faeces, primarily *Melanopsis* faeces, supporting our fourth hypothesis. For example, average lipids content was 4 and 8 times higher when fed M-Ad and M-Pa, respectively, than when fed the original leaf litter. Previous studies have reported a decrease of the triglyceride content in a *Gammarus* species when fed on leaf litter, independently of consumption rate (Foucreau et al., 2013). Since in our experiment we did not analyse the initial energy reserves of the animals, we cannot determine if the experimental individuals increased or decreased their lipids content during the experiment. Even so, we unquestionably show that *Echinogammarus* hold the highest lipids content when fed on *Melanopsis* faeces compared to other food items offered.

In our research we only assessed the role of resource quality, but not resource quantity (see Halvorson, Sperfeld, et al., 2017), on the performance of the two detritivore species. The amount of resource present in each microcosm could have played an important role on the response of our detritivores as have been reported by other studies (e.g. Arias-Real et al., 2018; Marcarelli et al., 2011). Therefore, although the availability of fecal material was verified by daily observations of the lower part of the microcosms (even in those fed E-AD), responses of detritivores when feed on faecal material exposed here should be interpreted with caution. Likewise, despite the FPOM produced by *Melanopsis* and *Echinogammarus* is primarily composed by faeces—≥95%, personal observation under microscope—(as seen for other species; Patrick, 2013) we cannot totally rule out the possibility that detritivores took advantage of small litter particles reaching the lower part of our microcosms.

Invasive species are among the main threats to biodiversity conservation (Bellard et al., 2016). Long-term human impacts in riparian ecosystems have favoured the proliferation of such species (Castro Díez & Alonso Fernández, 2017). Particularly, *Arundo* has been reported to significantly impair riparian habitats (Jiménez-Ruiz et al., 2021), altering vegetation structure and displacing native vegetation (Maceda-Veiga et al., 2016) with subsequent negative effects on arthropod abundance and diversity and on wildlife which diet rely on them (Herrera & Dudley, 2003). Our study assesses how two aquatic detritivores species may face riparian invasion by the giant reed and shows that cross-species coprophagy may be a successful feeding strategy for *Echinogammarus* to overcome the impacts of such invasion, but also to prosper in systems where low-quality litter inputs (native or not) are abundant. Furthermore, the role of the snail as a key species facilitating the access to nutrients to other detritivore species in lowland streams was highlighted. Managers should pay attention to the conservation of such relevant detritivore species for the functioning of lowland stream ecosystems, supervising activities that could threaten their populations (e.g. aquifer exploitation or water contamination; Bartolini et al., 2017), especially in streams with reduced detritivore diversity (Boyero et al., 2021) as these located in Mediterranean lowlands.

General Discussion

GENERAL DISCUSSION

Globally, less than 10% of the terrestrial plant biomass produced is consumed by herbivores. That means that over 90 gigatons of leaves, wood and roots turn out being part of the detrital pool in both, terrestrial and aquatic ecosystems (Cebrian, 1999; Gessner et al., 2010). The processing of this organic matter by detritivores and decomposers will then determine the rates at which carbon and other nutrients are sequestered, respired off as CO_2 through microbial respiration, or transferred to higher trophic levels of the food webs. Thus, any alteration of these rates and pathways may in turn impair biodiversity conservation and other stream ecosystem services, and likely global biogeochemical cycles.

Although primary production is also taking place, in forested regions most streams' food webs appear to be mainly fueled by allochthonous organic matter inputs from their riparian vegetation (Marcarelli et al., 2011). In consequence, streams are highly dependent on—and influenceable by—their surroundings. This situation underlines the susceptibility of streams to changes in their riparian vegetation. For instance, physical or chemical changes in litter traits (e.g. Alonso et al., 2022; Casas et al., 2013; López-Rojo et al., 2019) and/or decreasing availability of high-quality inputs (Arias-Real et al., 2018) may derive in important impacts on streams functioning. Despite the large number of studies addressing this topic, the complex —and sometimes subtle— biotic and abiotic interactions existing within the communities exploiting leaf litter assemblages (Gessner et al., 2010), hinder our understanding of how changes in litter quality or quantity may alter the functioning of these ecosystems.

This dissertation, by means of the works presented in the previous chapters, aims to shed some light on the topic, expanding our knowledge about the potential consequences of altering the properties of organic matter inputs to headwater streams on their functioning. Making use of a space-for-time substitution approach and, laboratory and field experiments, we assessed the effects of some of the main direct drivers of such changes —climate change, land-use changes, biodiversity loss, invasive species—on the functioning of headwater streams (Perkins et al., 2010), through the analysis of an integrative indicator of stream ecosystem status as the leaf litter decomposition process (Hieber & Gessner, 2002; Tank et al., 2010). This discussion aims to briefly place the main results of the different chapters in a common but wider context beyond this thesis.

Leaf quality in a changing climate

The rate at which leaf litter decomposes depends on a series of biotic and abiotic factors: litter quality and quantity, water temperature, water chemistry, water flow, streambed substrate and biogeography and phylogeny of the community of decomposers and detritivores (Tonin et al., 2021). Among them, litter quality has been marked as one of the main drivers of litter decomposition rates worldwide (e.g. García-Palacios, McKie, et al., 2016; Zhang et al., 2019) and as such, it has been assessed in many works.

The quality of the leaf litter reaching the streams is determined by the species composition of the riparian vegetation and their physiological and morphological traits. Usually, litter quality is defined by its content in nutrients (especially N and P) and recalcitrant (e.g., lignin and silicon) or toxic (e.g., phenols and tannins) compounds. These traits can be influenced by climate, landscape, soil, biogeography, phylogeny, and species interactions (Boyero et al., 2017; Siefert et al., 2015), therefore similar plant communities from different regions may differ in their litter quality. Assuming the high responsiveness of leaf traits to climate and making use of a wide environmental gradient, in **Chapter 1** we attempt to forecast intraspecific changes on leaf traits of four different riparian plant species under a climate change scenario.

Based on the species used in our study, overall, results from **Chapter 1** agree with other at wider geographical scales reporting the production of tougher (Wright et al., 2017), poorer in nutrient (Graça & Poquet, 2014) and tannin richer (Top et al., 2017) leaves with increasing aridity. This pattern predicts a decrease of the intraspecific leaf quality — i.e., a reduction of N and P concentrations but an increase of toughness and tannins content— of riparian deciduous species in a relatively short term as a consequence of warming and decreasing precipitation (Fig. 1.D.). Such decrease in leaf quality is expected to have potential effects on litter mass loss and nutrient cycling in the recipient ecosystems by affecting both microbial and detritivore-mediated decomposition (e.g. Lecerf & Chauvet, 2008), and, consequently, the transference of nutrients to higher trophic levels. This is partially supported by the outcomes found in **Chapters 2-4**, where detritivore-mediated or total litter decomposition were always higher for species with higher litter quality. However, in **Chapters 2 and 3**, microbial decomposition was not

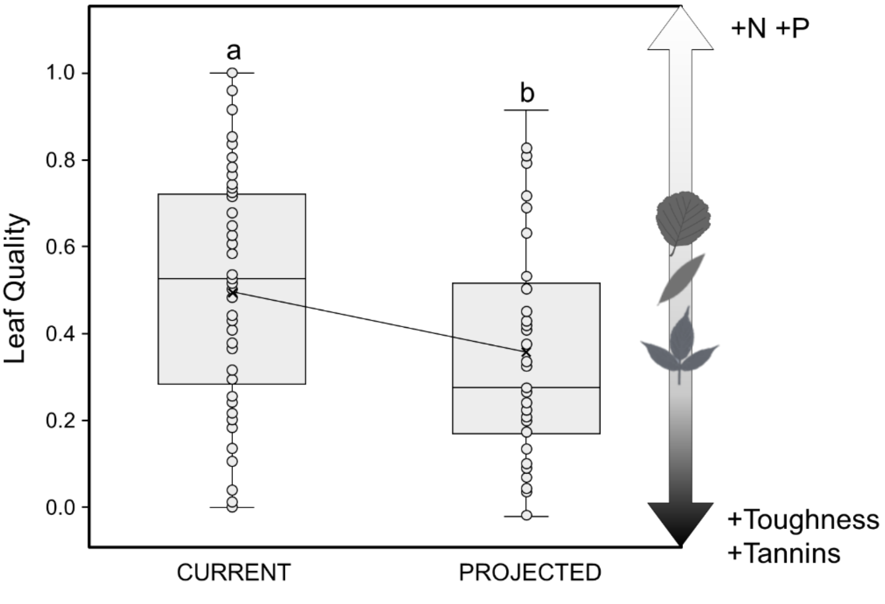


Figure 1.D. Boxplot showing the leaf quality (PC1) of the overall group of deciduous species assessed in **Chapter 1** at present (Current) and in a future climate change scenario according to the NCAR Community Model version 3 (CCM3) for the year 2100 (Projected). Leaf quality range (PCA dimension) is scaled to unit for simplicity. Different letters indicate significant differences based on paired t-test analyses ($p <$ 0.0001).

unambiguously faster for the, a priori, higher quality litter (i.e., black alder, *Alnus glutinosa*), since those litter with high Ca and Mg concentrations (i.e., Portuguese oak, *Quercus faginea*, and black poplar, *Populus nigra*, respectively) exhibited similar decay rates than the former despite their lower N concentrations but higher toughness and, tannins and phenolics contents. Together, these results suggest that caution has to be taken when associating decomposition rates with litter quality (see Marks, 2019) since the latter concept usually does not consider the organisms making use of leaf litter (e.g. bacteria, fungi, invertebrates) or the pathways and fates for which leaf litter is used (e.g. transference to higher trophic levels, microbial biomass, dissolved organic matter, released as CO_2). For example, some studies have demonstrated that some litter species decomposing at slow rates may promote higher C and N assimilation by macroinvertebrates than these decomposing at a faster rate, the latter supporting more microbial productivity (Compson et al., 2018; Fuller et al., 2015; Siders et al., 2018). Therefore, ours and similar results must be considered within the context investigated and not been assumed as a general statement.

Mass-ratio vs. Niche complementarity

As explained above, litter quality has been reported as one of the main drivers of litter decomposition. However, most of those studies are based on the assessment of monocultures (i.e., single species). Litter monocultures do not represent the plant diversity usually found in riparian areas; therefore, the use of litter mixtures allows a more realistic assessment of the litter decomposition process (Gessner et al., 2010). After some decades of research, there is still controversy about what is more important for litter mixtures decomposition, whether trait mean values (mass-ratio) or trait dissimilarity (niche complementarity) (e.g. García-Palacios et al., 2017; Fig. 2.D.). Understanding how these two drivers of litter decomposition work is important to assess the consequences of current and future variation of physiological or morphological leaf traits on the functioning of headwater streams facing the worldwide decline in diversity of riparian vegetation.

Based on the niche complementarity hypothesis, higher trait variability may promote litter decomposition through the complementarity of different litter species in terms of resource availability for decomposers and detritivores (e.g. Vos et al., 2013; Fig. 2.D.). Supporting this statement, results from **Chapter 2** exhibited a strong relationship of litter decomposition, but also N and P loss, with trait dissimilarity (Rao's Q) of litter mixtures independently of the executor of the process (microorganisms, detritivores, or both). However, litter quality (estimated using a multi-trait index as a proxy of the mass-ratio mechanism) was also significantly related to litter decomposition and N loss when detritivores were present. Moreover, as found by others (Santonja et al., 2019), increasing nutrient content (or litter quality in our case) of mixtures appeared to be the main driver of the positive net diversity effects (NDE) found in **Chapter 2** for detritivore-mediated decomposition, and may be the cause of the positive NDE observed in **Chapter 3** for microbial and total decomposition. This point, totally matches with the results obtained

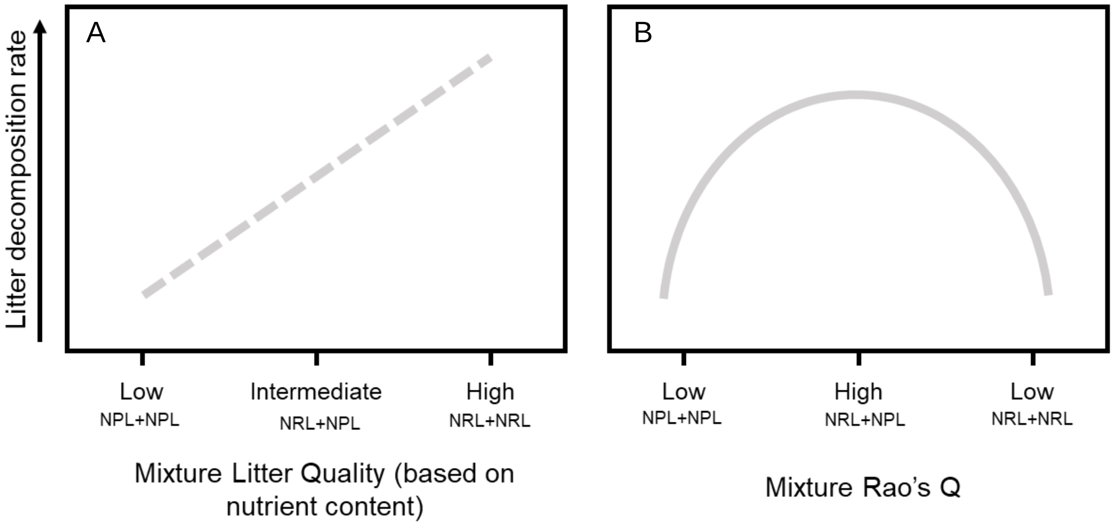


Figure 2.D. Hypothetical relationship between litter mixtures differing in their nutrient concentrations and their litter decomposition rate according to the mass-ratio hypothesis (**A)** and the niche complementarity hypothesis **(B)**. According to the mass-ratio hypothesis the decomposition rate will rise with the increased average nutrient concentration of the mixture. According to the niche complementarity hypothesis, the magnitude of litter diversity effects, and thus litter decomposition, will increase with the increase in functional diversity values in nutrient concentration of the mixture. NPL = nutrient poor litter; NRL = nutrient rich litter. Modified from Santonja et al. (2019).

in **Chapter 1,** predicting a direct impact on diversity effects as a consequence of the decrease in leaf quality forecasted in this chapter.

Climate and water characteristics can also exert a great control on litter decomposition rates (Boyero et al., 2011; Woodward et al., 2012), even altering the way mass-ratio or niche complementarity mechanisms operate. For example, in **Chapter 3**, higher water N concentrations at riverbanks with black alder appeared to suppress the diversity effects on microbially-mediated decomposition. Probably, it led to a reduction of the interspecific C:N variability among plant litters (Rosemond et al., 2010) through microbial N immobilization from the water column (Cheever et al., 2013; Suberkropp, 1998) and, consequently, limiting the role of N transfer among litter species (Handa et al., 2014; Schimel & Hättenschwiler, 2007). Nitrogen transfer is one of the main drivers of complementarity and, therefore, of diversity effects (Handa et al., 2014; López-Rojo et al., 2018). In **Chapters 2 and 3**, this mechanism seems to be operating since the N-rich litter of black alder always exhibited the highest N release within mixtures, especially when was accompanied by Ca-rich litter types (Jenkins & Suberkropp, 1995; Santonja et al., 2019); and, interestingly, N transfer appeared to become more active the scarcer the N within the mixture (**Chapter 3**).

Altogether, these results suggest that both mechanisms can operate simultaneously, playing an essential role in the decomposition of litter assemblages, but that the relative importance of each will depend on the specific process assessed and the particular characteristics of the system (García-Palacios et al., 2017).

Changes in riparian vegetation and stream food webs in the face of global change

Streams and their riparian areas are considered as hotspots of biodiversity; being among the most degraded ecosystems (e.g., channelization, dewatering, dams, deforestation, forest plantations, invasive species). The species composition of riparian vegetation is changing worldwide in favor of drought-tolerant, evergreen, and non-native species (Kominoski et al., 2021; Kominoski et al., 2013; Salinas et al., 2018). Understanding how litter is processed in streams is essential to forecast the potential consequences of global change on these ecosystems and may help managers and policymakers during the decision-making process for the conservation of biodiversity and ecosystem services.

The presence of leaves decomposing at different rates in streams can improve the availability of resources for detritivores during longer periods (Ferreira, Castela, et al., 2016; Siders et al., 2018). For instance, looking at the species used in our research **(Chapters 2 and 3)**, black alder litter (for the N) and holm oak (*Quercus rotundifolia*) (for the P) may represent a fast pulse of nutrients to invertebrates shortly after entering the stream, whereas black poplar or Portuguese oak litter may be more available later in the season after being fully conditioned by microorganisms, the main responsible of litter mass loss of these species observed in our experiments (Fig. 3.D.). Likewise, more diverse litter assemblages are usually reported to undergo faster decomposition (e.g. Handa et al., 2014; Chapters 2 and 3). This is in part promoted by the support of a diverse invertebrate community which accelerates decomposition through facilitation and niche partitioning (Jabiol et al., 2013; Tonin et al., 2018).

Compositional changes in riparian vegetation can exert important effects on stream food webs and functioning. For example, the substitution of deciduous by evergreen species (Salinas et al., 2018; Chapter 2), the dieback of key plant species (Alonso et al., 2022; Alonso et al., 2021; Chapters 2 and 3), the establishment of monospecific forest

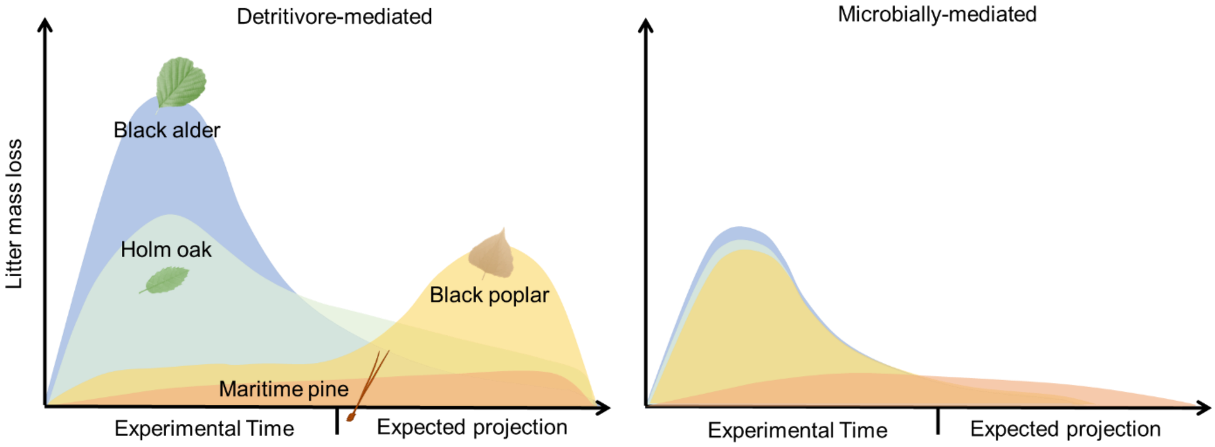


Figure 3.D. Diagram illustrating the observed relative decomposition of some litter species used in **Chapters 2 and 3** by detritivores and microbial decomposers. We show the hypothetical pathway litter decomposition has followed during the experimental time and the potential fate expected in longer periods of incubation. Modified from Marks (2019). Black alder, *Alnus glutinosa*. Black poplar, *Populus nigra*. Holm oak, *Quercus rotundifolia*. Maritime pine, *Pinus pinaster*.

plantations (Larrañaga et al., 2021; Chapter 3) or the invasion by alien plant species (Castro Díez & Alonso Fernández, 2017; Chapter 4), may promote a significant reduction of riparian plant diversity, weakening or suppressing diversity effects on litter decomposition (**Chapters 2 and 3**), thereby altering the way organic matter is processed. Further, contrary to what is usually expected, non-native plants can alter stream ecosystems independently of their decomposition rate (see Marks, 2019). For instance, fast decomposing tamarisk and low decomposing eucalyptus or pine have been reported to exert negative effects on macroinvertebrates communities (Larrañaga et al., 2021; Larrañaga et al., 2014; Martínez et al., 2013), although the former may promote detritivores growth in the short term (Going & Dudley, 2008; Moline & Poff, 2008).

In our studies, the decomposition rates of pine needles (**Chapter 3**) and giant reed (*Arundo donax*) leaves (**Chapter 4**) were slowed down by defensive and structural compounds, which significantly increased their toughness and reduced their palatability. As a case in point, pine can produce antifungal compounds (Bärlocher & Oertli, 1978) limiting microbial colonization of its needles (**Chapter 3**), while giant reed litter contain huge amounts of silicon which can produce excessive wear of detritivores' mandibles (Massey & Hartley, 2009) (**Chapter 4).** Both factors may greatly dissuade detritivores from feeding, reducing the amount of energy flowing through the trophic web. Nevertheless, as observed in **Chapter 4**, the presence of some invertebrate key species, with outstanding digestive capability (e.g., *Melanopsis praemorsa*), can ease the access to nutrients to other detritivore species of smaller size (in this case through cross-species coprophagy), thus facilitating the overcoming of the impacts exerted by invasive plant species or other drivers of changes in riparian vegetation.

With the decline of invertebrate diversity, an increase in the CO_2 release from streams is expected due to higher microbially-mediated decomposition of organic matter (Boyero et al., 2021; Boyero et al., 2011). Microbes' ability to alternate the acquisition of nutrients from stream water and leaf litter depending on environmental constraints (Cheever et al., 2013), may make them less vulnerable in the face of the projected shifts on riparian vegetation induced by global change. In **Chapter 3** this strategy is evidenced. Black alder litter underwent N losses in any case, but of lower magnitude at streams with higher N concentrations. Further, two litters with similar N and P concentrations, as black poplar and maritime pine (*Pinus pinaster*), exhibited two different patterns. Black poplar gained more N the less the amount of N-rich alder litter accompanying it within the mixture, whereas pine needles lost or gained N with low and high-N concentrations in water, respectively. Interestingly, and contrasting with the pattern observed for black poplar litter, the litter with highest Ca concentration (i.e., Portuguese oak) in **Chapter 2** exhibited the highest N loss. Possibly, weekly water renewal of microcosms was insufficient to avoid nutrient limitation and, therefore, microorganisms were forced to use N from litter, whereas in field (**Chapter 3)** flow of very low concentrations of nutrients precluded such limitation. These results also underscore how the presence of N-fixing species in riparian areas can promote different pathways of nutrient cycling through an increase of water N concentration. (**Chapter 3**).

What's next?

Multiple stressors

From the results obtained during this PhD thesis, some new questions emerged. The first question is related to how the interaction between the factors determining those qualitative and quantitative changes assessed here may alter the functioning of headwater streams. In a realistic scenario, such changes will not act isolated from each other but interacting and thus, potentially exerting considerably different effects on the invaded ecosystems.

Likewise, whether invertebrate populations already adapted to one stressor (e.g., thriving in forest plantations) may be able to overcome or cope with new stressors (e.g., invasive plant species and/or temperature) easier than non-adapted populations is worthy of study. Previous research on rotifers of the species *Brachionus calyciflorus*, have reported that temperature-adapted populations responded better to stress caused by copper than non-adapted populations. Another interesting question is whether removing a stressor is always beneficial. Previous research has reported that evolved populations do not necessarily increase fitness when the stressor is removed, and that different populations may evolve differently in response to the same stressor (Orr et al., 2022). In a global change context, these results suggest that the management and restoration of ecosystems affected by multiple stressors cannot be carried out without considering rapid adaptation. Further research using common garden experiments would be useful to improve our comprehension about potential micro-evolutionary adaptations of detritivores and their ecological implications.

Trying to shed some light on these questions, a group of three young limnologists and myself joined forces and created the **INVASORIAN** project (granted in the *First call of SIBECOL Projects for Early Career Researchers – 2020*) aiming to increase the current knowledge about the interactive effects of some global change drivers (e.g., invasive plant species × land-use changes or invasive plant species × global warming) on the functioning of headwater stream ecosystems.

Interaction between invasive species

Another open front derives from the increasing accumulation of invasive species in many ecosystems. Coexisting invasive species may strongly alter the invaded communities due to their potential interactive effects, particularly when they belong to different trophic levels (e.g., primary producers and consumers). Invasive species can transform food webs by altering available resources (bottom-up effects) when the invasive species is a primary producer, or through predation of native species (top-down effects) when the invader is a consumer (Figure 4.D.-A). When several invasive species coexist in the same ecosystem, the combined ecological effects of multiple invaders may be the sum of their individual effects (additive effect) or, because of their potential to interact, greater (synergistic effect) or less (antagonistic effect) than the sum of their individual effects (Fig. 4.D.-B). However, despite the growing interest in the study of the effects of multiple stressors on ecosystems, there is little information available about the interactive effects of exotic species on stream ecosystem functioning.

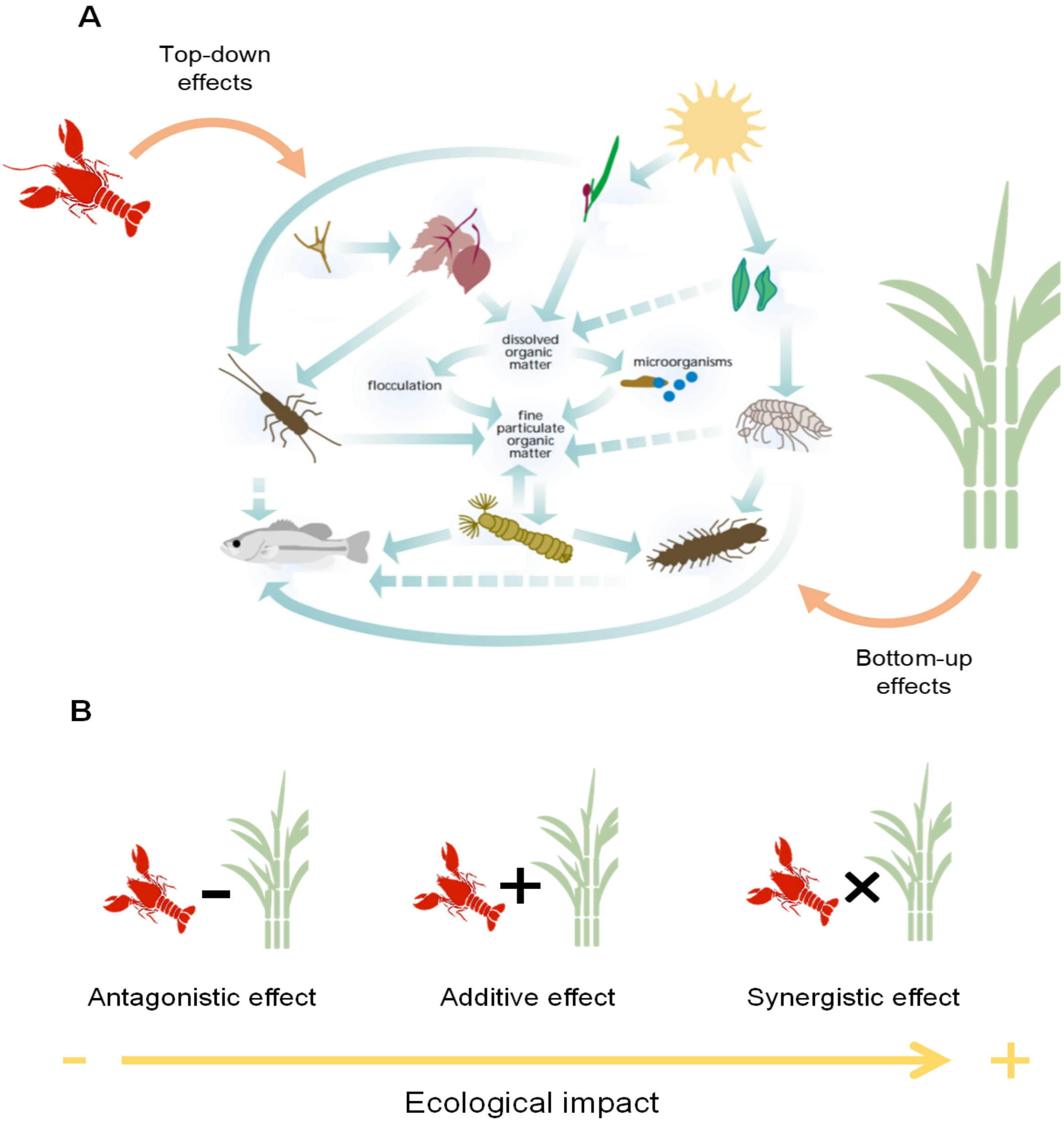


Figure 4.D. (A) Diagram illustrating the usual effects caused by an invasive consumer (e.g., *Procambarus clarkii*) and a primary producer (e.g., *Arundo donax*) on streams detrital food webs. (B) Potential ecological impact derived from the interactive effects of these two invasive species.

Conclusions

CONCLUSIONS

The main conclusions derived from this thesis are the following:

1. We forecasted a decrease of intraspecific leaf quality, mainly consisting in decreasing N and P concentrations, but increasing toughness, of riparian deciduous species with global warming in a relatively short term.

2. The dieback of key riparian plant species and species loss of invertebrate detritivores, which are the main drivers of positive diversity effects, can cause a significant alteration of essential processes of ecosystem functioning in forested streams. The major repercussions of such changes are on litter N losses and detritivore biomass, which point to important consequences for instream nutrient cycling and maintenance of aquatic and adjacent terrestrial food webs.

3. Positive diversity effects on detritivore-mediated processes were mostly derived from complementarity effects, underlying their outstanding ability to feed simultaneously on resources of contrasting quality to balance their diet. However, selection effects were also important, especially in mixtures containing litter species of high nutrient dissimilarity.

4. The presence of riparian vegetation strips can buffer the negative pine plantations-derived effect on leaf litter decomposition. Further, the presence of N-fixing black alder within these strips can promote microbial nutrient recycling through an increase of water nitrogen concentrations.

5. Cross-species coprophagy can be a successful strategy to overcome the impacts of invasive plant species by small aquatic detritivores, highlighting the role of detritivores of higher size as key species facilitating the access to nutrients to other sympatric detritivore species in systems dominated by low-quality litter inputs.

Supplementary material

Supplementary material **Chapter 1**

Climate-induced plasticity in leaf traits of riparian plants

SUPPORTING METHODS

Data analysis

To elucidate the relationships between species cover and environmental variables, we first determined whether species responses were linear or unimodal (i.e., length of the gradient below 3 standard deviation units for linear and above 4 for unimodal; Ter Braak & Smilauer, 2002), by running a Detrended Correspondence Analysis (DCA) using the function *decorana* of the 'vegan' package of R statistical software (R Core Team, 2020). The length of the first DCA axis of ordinated species cover data was 3.1, indicating that both linear and unimodal methods were suitable. Thus, we ran a Canonical Correspondence Analysis (CCA) using the *cca* function of the 'vegan' package (Oksanen et al., 2019), after a forward selection of the most parsimonious subsets of explanatory variables (PPSeasonality, PWettestM, MaxT, MinT and soil pH) using the *ordistep* function of the 'vegan' package with 9999 permutations. Significance of all testable fractions was assessed using permutation tests. Environmental variables were transformed to improve the structure of the residuals using *log* or *arcsin* transformations for decimal and percentage values, respectively.

Statistical differences in individual traits among species were assessed using one-way ANOVA and post-hoc Tukey tests with the *anova* and *TukeyHSD* functions of the 'stats' package. We performed Principal Component Analyses (PCAs) to examine patterns in leaf trait variability: one PCA pooling the four species to examine interspecific variation *vs.* trait plasticity, and one PCA per species to extract the main gradients (2 first PCs) of intraspecific trait plasticity. Previously, Spearman rank correlations were performed to remove leaf traits with high (> 0.85) collinearity (Fig. S1). Seven out of 9 traits were finally included in the PCA: N, P, Ca, Mg, condensed tannins, lignin and toughness. We used the *prcomp* function in the 'stats' package, retaining the first two PC axes explaining most of the variance of mean trait values as integrative proxies of leaf quality. *Log* or *arcsin* transformations of variables were used when required in ANOVA and PCA analyses. The relative magnitude of interspecific variation vs. plasticity for the overall pool of traits for each species was estimated as the proportion that each species covered in each of the dimensions of the general PCA. Besides, to quantify the relative magnitude of interspecific variation vs. plasticity for each leaf trait, we performed variance partitioning analyses using the *varcomp* function of the 'ape' package (Paradis & Schliep, 2019). For each level, the function first calculates the group mean. It then compares the variance around the group mean to the mean of the next level (e.g., variance of genus level is compared to the mean of family level).

We carried out partial least squares regressions (PLS), using the *plsr* function in the 'pls' package (Mevik et al., 2020), to evaluate the relative importance of independent climatic and soil variables as predictors of leaf traits plasticity (first two PCA axes). PLS regression allows handling multiple dependent variables simultaneously and works effectively in situations where the number of independent variables exceeds the number of observations (Tenenhaus, 1998; Wold, 1995; Wold et al., 2001). Preliminary PLS regressions for each environmental matrix and plant species (Table S2) were used to reduce the number of variables by selecting those with the highest variable importance in projection (VIP; Mehmood et al., 2020), using the *VIP* function in the ‘plsVarSel’ package (Mehmood et al., 2012). Those variables with VIP close or greater than 1 were considered the most relevant to explain variation in the response variable (Andersen & Bro, 2010). The strength and direction of the effect of each variable in PLS models are indicated by the standardized model coefficients (Guo et al., 2014; Luedeling et al., 2013), which represent the change in mean response (i.e., leaf quality) associated with a change in an independent variable while the other variables are held constant. Spearman rank correlation analyses were used in order to equalize the size of the two matrices of environmental variables removing those variables with high collinearity within those with higher VIP values (Fig. S2, Table S3, S4). A second PLS regression was performed for each species using the variables previously selected, and the influence of each group of environmental variables (climate and soil) and their combination (climate + soil) on leaf trait variability was assessed using the goodness of prediction (Q^2) and the goodness of fit ($R^2(Y)$) of models. A model was considered significant when $Q^2 > 0.097$ (Friden et al., 1994), and those values of $R^2(Y)$ above 0.67, around 0.33, or below 0.19 were considered as substantial, moderated or weak fit, respectively (Chin, 1998).

In PLS regressions all explanatory variables were scaled to unit variance, using the *scale* function, to give all variables the same relative importance. Regressions were carried out separately for each species and the number of extracted components (latent variables) and the robustness of the resulting models were determined by leave-one-out cross-validation (LOO). For each model, we determined the number of dimensions with the lowest cross-validation error. PLS regressions models built with climatic variables, those statistically significant for PC 1 and PC 2 of each species (Table 2), were used to estimate the projected change of leaf quality under the forecasted climate change scenarios for 2100, using the *predict* function of the ‘stats’ package. Current and projected values of leaf quality (i.e. mean position over PC 1 or PC 2 of separate PCAs for each species) were compared using t-tests for paired samples. Hedge’s *g* effect size was estimated using the *cohen.d* function of the ‘effsize’ package (Torchiano, 2020).

Glossary

Environmental variable	Bioclimatic variable	Code	Definition
Altitude (m a.s.l)	-	-	Altitude measured in meters above the sea level
Annual Mean Temperature (°C)	BIO1	MAT	The annual mean temperature
Mean Monthly Temperature Range (°C)	BIO2	TRangeM	Mean of monthly temperature variation over a given period.
Isothermality (%)	BIO3	-	Isothermality quantifies how large the day to-night temperatures oscillate relative to the summer to-winter (annual) oscillations
Temperature Seasonality (%)	BIO4	TSeason	The amount of temperature variation over a given period based on the ratio of the standard deviation of the monthly mean temperatures to the mean monthly temperature (also known as the coefficient of variation (CV)).
Max. Temperature of Warmest Month (°C)	BIO5	MaxT	The maximum monthly temperature occurrence over a given year (time-series) or averaged span of years (normal).
Min. Temperature of Coldest Month (°C)	BIO6	MinT	The minimum monthly temperature occurrence over a given year (time-series) or averaged span of years (normal).
Temperature Annual Range (°C)	BIO7	TRangeA	A measure of temperature variation over a given period.
Mean Temperature of Wettest Quarter (°C)	BIO8	TWettestQ	This quarterly index approximates mean temperatures that prevail during the wettest season
Mean Temperature of Driest Quarter (°C)	BIO9	TDriestQ	This quarterly index approximates mean temperatures that prevail during the driest quarter.
Mean Temperature of Warmest Quarter (°C)	BIO10	TWarmestQ	This quarterly index approximates mean temperatures that prevail during the warmest quarter
Mean Temperature of Coldest Quarter (°C)	BIO11	TColdestQ	This quarterly index approximates mean temperatures that prevail during the coldest quarter.
Annual Precipitation (mm)	BIO12	PP	This is the sum of all total monthly precipitation values.
Precipitation of Wettest Month (mm)	BIO13	PWettestM	This index identifies the total precipitation that prevails during the wettest month.
Precipitation of Driest Month (mm)	BIO14	PDriestM	This index identifies the total precipitation that prevails during the driest month.
Precipitation Seasonality (%)	BIO15	PSeason	This is a measure of the variation in monthly precipitation totals over the course of the year. This index is the ratio of the standard deviation of the monthly total precipitation to the mean monthly total precipitation (also known as the coefficient of variation) and is expressed as a percentage.
Precipitation of Wettest Quarter (mm)	BIO16	PWettestQ	This quarterly index approximates total precipitation that prevails during the wettest quarter.
Precipitation of Driest Quarter (mm)	BIO17	PDriestQ	This quarterly index approximates total precipitation that prevails during the driest quarter.
Precipitation of Warmest Quarter (mm)	BIO18	PWarmestQ	This quarterly index approximates total precipitation that prevails during the warmest quarter.
Precipitation of Coldest Quarter (mm)	BIO19	PColdestQ	This quarterly index approximates total precipitation that prevails during the coldest quarter.
Emberger's bioclimatic coefficient	-	Q2	One hundred times annual precipitations divided by the subtraction of the square maximum temperature of the warmest month minus the square minimum temperature of the coldest month.
Soil Electric conductivity (µS cm-1)	-	EC	Electric conductivity of riparian soil.
Soil pH	-	pH	pH of riparian soil.
Soil $CaCO_3$ (%)	-	$CaCO_3$	$CaCO_3$ content of the riparian soil.
Soil Organic Carbon (%)	-	OC	Organic carbon content of the riparian soil.
Soil Nitrogen (%)	-	N	N content of the riparian soil.
Soil CN	-	CN	CN molar ratio of the riparian soil.
Soil Cation exchange capacity ($cmol^{+}kg^{-1}$)	-	CEC	Cation exchange capacity of the riparian soil.
Soil Base saturation (%)	-	BasSat	Percent of base saturation in the riparian soil.
Soil Exchangeable Sodium Percentage (%)	-	ESP	Percent of exchangeable sodium present in the riparian soil.
Soil available water (%)	-	AW	Percentage of available water present in the soil
Soils Phosphorus (%)	-	P	P content of the riparian soil.

Table S1.C1. Environmental values of climatic and soil variables in each sampling site.

Basin name	Annual Mean Temperature (MAT; °C)	Mean Monthly Temperature Range (°C)	Isothermality (%)	Temperature Seasonality (%)	Max. Temperature of Warmest Month (°C)	Min. Temperature of Coldest Month (°C)	Temperature Annual Range (°C)	Mean Temperature of Wettest Quarter (°C)	Mean Temperature of Driest Quarter (°C)	Mean Temperature of Warmest Quarter (°C)
Rambla de las Negras	17.4	8.6	37.8	29.5	29.6	6.9	22.7	15.2	23.7	24.2
Río Aguas	16.6	9.4	38.2	33.0	30.0	5.5	24.5	14.0	23.5	23.8
Barranco del Cura	15.4	9.8	38.0	37.6	29.8	3.9	25.9	9.9	22.7	23.0
Arroyo de Aguamulas	14.5	11.8	38.0	47.1	32.5	1.5	31.0	12.5	23.3	23.3
Río Bacares	13.3	11.1	38.2	48.0	30.1	1.0	29.1	11.1	21.5	21.7
Río Vacal	13.9	11.7	38.6	46.4	31.5	1.2	30.3	7.5	22.3	22.3
Arrollo Los Marcos	12.9	11.3	38.0	50.4	30.1	0.4	29.7	8.2	21.3	21.5
Río Mecina	13.0	11.6	38.3	50.2	30.6	0.4	30.2	8.3	21.4	21.5
Río Turrillas	13.3	11.8	38.6	49.1	31.2	0.6	30.6	6.9	21.8	21.8
Río Andarax	12.3	11.5	38.1	52.9	30.0	-0.2	30.2	7.6	20.8	21.0
Río Alhama	13.4	11.7	38.6	48.0	31.0	0.8	30.2	7.1	21.8	21.8
Ribera de Santa Ana	15.5	10.8	38.6	39.7	31.8	3.7	28.1	9.3	23.2	23.4
Arroyo Corterrangel	15.3	11.1	38.6	41.1	32.1	3.3	28.8	8.9	23.2	23.4
Río Nacimiento	11.9	11.7	38.1	55.7	30.0	-0.7	30.7	7.1	20.6	20.7
Barranco del Dun Dun	15.0	11.0	38.4	41.9	31.7	3.0	28.7	8.6	22.8	23.0
Río Guadalentin	11.3	12.1	37.8	61.7	30.3	-1.7	32.0	9.1	20.4	20.5
Arroyo de Aguascebas	11.1	12.2	37.9	63.2	30.3	-1.9	32.2	8.9	20.3	20.3
Arroyo de la Garganta	11.2	12.1	37.9	62.2	30.3	-1.7	32.0	9.0	20.4	20.4
Río Chico Ohanes	11.1	11.7	38.3	59.5	29.1	-1.4	30.5	6.3	19.7	19.9
Barranco del Pueblo	10.9	12.0	38.1	62.2	29.6	-1.9	31.5	6.0	19.8	19.8
Arroyo Hondo	10.8	12.0	38.2	62.5	29.4	-1.9	31.3	5.9	19.7	19.7
Arroyo de los Castaños	10.8	12.0	38.2	62.5	29.4	-1.9	31.3	5.9	19.7	19.7
Arroyo de los Caballos	16.4	9.6	39.9	31.8	29.9	5.8	24.1	11.5	23.0	23.3
Arroyo de los Molinos	15.5	10.2	39.5	36.4	30.3	4.4	25.9	10.0	22.6	22.8
Arroyo de Bocaleones	15.0	10.4	39.6	37.9	30.0	3.8	26.2	9.5	22.2	22.4
Arroyo Gaidovar	14.6	10.5	39.6	39.2	29.7	3.3	26.4	9.1	21.8	22.1
Río Verde	13.8	10.7	39.9	41.6	29.1	2.3	26.8	8.3	21.1	21.3
Arroyo de la Cruz	13.8	10.7	39.9	41.6	29.1	2.3	26.8	8.3	21.1	21.3
Garganta del Caballo	15.5	9.2	38.6	33.7	28.7	5.0	23.7	10.5	22.0	22.3
Garganta del Aljibe	15.5	9.2	38.6	33.7	28.7	5.0	23.7	10.5	22.0	22.3
Garganta del Medio	15.5	9.2	38.6	33.7	28.7	5.0	23.7	10.5	22.0	22.3
Garganta de la Cierva	15.5	9.2	38.6	33.7	28.7	5.0	23.7	10.5	22.0	22.3
Garganta de la Garza	15.7	9.2	40.2	30.7	28.3	5.4	22.9	10.2	21.7	22.1
Garganta del Tesorillo	15.7	9.2	40.2	30.7	28.3	5.4	22.9	10.2	21.7	22.1

Table S1.C1. (cont.)

Basin name	Mean Temperature of Coldest Quarter (°C)	Annual Precipitation (mm)	Precipitation of Wettest Month (mm)	Precipitation of Driest Month (mm)	Precipitation Seasonality (%)	Precipitation of Wettest Quarter (mm)	Precipitation of Driest Quarter (mm)	Precipitation of Warmest Quarter (mm)	Precipitation of Coldest Quarter (mm)	Emberger's coefficient (Q2)
Rambla de las Negras	11.6	261.0	36.0	2.0	53.0	100.0	15.0	22.0	88.0	31.5
Río Aguas	10.4	294.0	39.0	3.0	48.2	107.0	21.0	28.0	94.0	33.8
Barranco del Cura	8.9	353.0	44.0	4.0	51.0	126.0	22.0	29.0	122.0	40.4
Arroyo de Aguamulas	6.7	449.0	57.0	7.0	44.6	156.0	39.0	44.0	135.0	42.6
Río Bacares	6.2	470.0	59.0	10.0	42.2	156.0	45.0	50.0	143.0	51.9
Río Vacal	6.6	540.0	72.0	6.0	57.1	212.0	28.0	28.0	205.0	54.5
Arrollo Los Marcos	5.7	499.0	63.0	11.0	42.7	168.0	47.0	53.0	155.0	55.1
Río Mecina	5.7	529.0	65.0	9.0	50.3	185.0	37.0	45.0	184.0	56.5
Río Turrillas	6.0	574.0	75.0	7.0	56.1	220.0	31.0	31.0	215.0	59.0
Río Andarax	5.1	544.0	68.0	11.0	46.6	188.0	45.0	52.0	181.0	60.4
Río Alhama	6.2	588.0	78.0	7.0	57.4	231.0	31.0	31.0	224.0	61.2
Ribera de Santa Ana	8.4	623.0	88.0	3.0	60.8	253.0	28.0	33.0	239.0	62.5
Arroyo Corterrangel	8.0	638.0	89.0	4.0	60.2	257.0	30.0	34.0	244.0	62.6
Río Nacimiento	4.6	572.0	72.0	12.0	45.4	199.0	50.0	56.0	186.0	63.6
Barranco del Dun Dun	7.7	660.0	91.0	4.0	59.8	265.0	32.0	36.0	253.0	66.3
Río Guadalentin	3.6	607.0	76.0	15.0	40.7	210.0	64.0	67.0	180.0	66.3
Arroyo de Aguascebas	3.3	612.0	76.0	15.0	39.5	212.0	68.0	69.0	179.0	66.9
Arroyo de la Garganta	3.5	614.0	77.0	15.0	41.2	213.0	64.0	67.0	184.0	67.1
Río Chico Ohanes	3.8	609.0	77.0	15.0	43.1	210.0	59.0	64.0	194.0	72.1
Barranco del Pueblo	3.4	653.0	80.0	14.0	45.8	229.0	57.0	64.0	216.0	74.8
Arroyo Hondo	3.4	648.0	81.0	15.0	44.9	227.0	59.0	65.0	211.0	75.3
Arroyo de los Castaños	3.4	648.0	81.0	15.0	44.9	227.0	59.0	65.0	211.0	75.3
Arroyo de los Caballos	10.5	667.0	107.0	2.0	72.7	312.0	18.0	25.0	293.0	77.5
Arroyo de los Molinos	9.1	764.0	119.0	2.0	70.9	343.0	21.0	29.0	327.0	85.0
Arroyo de Bocaleones	8.5	785.0	121.0	2.0	70.7	349.0	22.0	29.0	336.0	88.6
Arroyo Gaidovar	8.1	804.0	122.0	3.0	70.4	356.0	23.0	31.0	344.0	92.3
Río Verde	7.3	795.0	116.0	4.0	69.0	348.0	26.0	35.0	339.0	94.5
Arroyo de la Cruz	7.3	795.0	116.0	4.0	69.0	348.0	26.0	35.0	339.0	94.5
Garganta del Caballo	9.5	816.0	131.0	1.0	74.1	370.0	18.0	27.0	359.0	102.2
Garganta del Aljibe	9.5	816.0	131.0	1.0	74.1	370.0	18.0	27.0	359.0	102.2
Garganta del Medio	9.5	816.0	131.0	1.0	74.1	370.0	18.0	27.0	359.0	102.2
Garganta de la Cierva	9.5	816.0	131.0	1.0	74.1	370.0	18.0	27.0	359.0	102.2
Garganta de la Garza	10.2	845.0	140.0	1.0	79.5	399.0	14.0	25.0	399.0	109.5
Garganta del Tesorillo	10.2	845.0	140.0	1.0	79.5	399.0	14.0	25.0	399.0	109.5

Table S1.C1. (cont.)

Basin name	Soil EC (µS cm^{-1})	Soil pH	Soil CaCO$_3$ (%)	Soil Organic C (%)	Soil N (%)	Soil CN ratio	Soil Cation Exchange Capacity (cmol^{+} kg^{-1})	Soil Base saturation (%)	Soil Echangeable sodium (%)	Soil Avaialable water (%)	Soil P (%)
Rambla de las Negras	2969.4	8.7	8.2	0.5	0.03	13.0	23.3	100.0	32.4	6.1	0.04
Río Aguas	2898.7	8.6	15.1	0.1	0.02	4.4	4.0	100.0	5.2	6.5	0.03
Barranco del Cura	1197.9	8.8	25.6	0.1	0.02	6.5	2.7	100.0	6.3	5.0	0.02
Arroyo de Aguamulas	925.7	8.0	67.0	3.2	0.10	45.4	14.3	100.0	0.6	3.6	0.02
Río Bacares	1155.0	8.2	14.4	2.6	0.14	18.8	10.7	100.0	1.1	2.3	0.06
Río Vacal	640.0	8.4	56.2	1.2	0.06	21.4	4.3	100.0	1.9	3.2	0.01
Arrollo Los Marcos	1364.0	7.9	0.8	5.1	0.25	20.7	17.5	100.0	1.1	5.8	0.05
Río Mecina	729.0	6.9	0.6	2.0	0.13	14.9	10.4	84.8	0.7	3.8	0.05
Río Turrillas	776.0	8.2	23.5	1.1	0.04	24.6	16.9	100.0	0.5	9.3	0.02
Río Andarax	517.0	7.9	1.7	0.5	0.02	21.9	4.0	100.0	2.8	1.6	0.04
Río Alhama	578.0	8.5	41.0	0.4	0.05	9.3	9.5	100.0	0.8	1.5	0.03
Ribera de Santa Ana	559.0	6.8	0.0	1.1	0.03	35.9	5.9	100.0	1.8	3.7	0.02
Arroyo Corterrangel	573.0	6.2	0.1	4.6	0.13	34.8	22.0	98.4	0.7	11.1	0.05
Río Nacimiento	365.0	6.5	0.0	0.8	0.05	18.1	2.7	86.8	3.3	2.8	0.05
Barranco del Dun Dun	395.0	6.7	0.1	1.1	0.04	26.0	5.9	80.3	2.2	3.8	0.02
Río Guadalentin	755.0	8.2	85.9	2.1	0.06	34.7	8.8	100.0	0.7	1.3	0.02
Arroyo de Aguascebas	838.3	8.0	81.2	2.8	0.03	79.8	11.4	100.0	0.6	5.2	0.01
Arroyo de la Garganta	1183.7	7.9	34.0	4.6	0.21	21.8	22.7	100.0	0.5	4.3	0.03
Río Chico Ohanes	301.0	7.5	0.5	0.4	0.06	6.7	3.3	83.9	1.0	1.9	0.05
Barranco del Pueblo	796.9	6.4	0.4	5.4	0.21	27.4	21.6	61.8	0.9	5.4	0.07
Arroyo Hondo	806.0	6.7	0.6	5.3	0.25	21.1	19.8	77.9	1.0	4.6	0.07
Arroyo de los Castaños	787.8	6.2	0.1	5.4	0.16	33.8	23.3	45.7	0.7	6.2	0.08
Arroyo de los Caballos	763.6	7.6	0.4	1.3	0.07	19.2	20.4	100.0	0.7	3.2	0.01
Arroyo de los Molinos	1321.1	7.8	43.2	5.1	0.36	14.3	28.2	100.0	0.7	3.3	0.04
Arroyo de Bocaleones	1354.0	8.1	69.2	3.2	0.04	75.9	8.8	100.0	1.6	8.0	0.03
Arroyo Gaidovar	1612.0	7.9	33.9	2.4	0.11	21.0	47.8	78.5	0.4	3.9	0.04
Río Verde	829.2	7.4	20.0	2.1	0.15	14.6	25.6	19.7	0.1	6.6	0.03
Arroyo de la Cruz	583.8	7.2	0.5	2.9	0.13	21.7	24.0	84.3	0.3	6.6	0.01
Garganta del Caballo	3290.0	7.2	0.0	2.3	0.08	27.7	12.7	100.0	11.8	5.3	0.03
Garganta del Aljibe	699.0	5.6	0.0	5.4	0.21	25.4	6.9	100.0	2.9	6.1	0.03
Garganta del Medio	574.0	5.3	0.5	4.0	0.20	19.9	18.2	54.4	1.1	3.6	0.03
Garganta de la Cierva	1521.0	6.0	0.2	3.9	0.17	24.3	12.6	84.8	5.3	5.0	0.03
Garganta de la Garza	649.0	6.4	0.1	2.2	0.11	19.9	7.5	100.0	1.4	6.1	0.01
Garganta del Tesorillo	645.0	5.7	0.1	4.7	0.29	16.1	17.2	72.6	1.6	2.6	0.03

Table S2.C1. Summary of univariate dependent variable preliminary PLS models fitted to the first two axes of the PCA (PC 1 and PC 2) using all the environmental variables. The number of dimensions with lowest cross validation error (N), the proportion of the variance in the response variables that can be predicted by the model (Q^2), and the coefficient of determination (R^2) are shown for each model. Significant models ($Q^2 > 0.097$) are in bold.

Plant species	**Set of environmental variables or combinations**	**PC1**			**PC2**		
		N	**Q^2**	**R^2(Y)**	**N**	**Q^2**	**R^2(Y)**
Alnus glutinosa	Climate (C)	1	**0.65**	0.78	1	-0.48	0.14
	Soil (S)	1	**0.24**	0.63	1	-0.04	0.42
Salix atrocinerea	Climate (C)	2	**0.52**	0.73	1	0.01	0.39
	Soil (S)	1	0.04	0.49	3	**0.55**	0.89
Nerium oleander	Climate (C)	3	**0.39**	0.73	1	-0.23	0.36
	Soil (S)	1	-0.66	0.41	1	-1.48	0.54
Rubus ulmifolius	Climate (C)	1	**0.25**	0.36	1	-0.15	0.08
	Soil (S)	1	-0.54	0.24	1	-0.12	0.20

Table S3.C1. Variable importance in the projection (VIP), standardized coefficients and loadings of the predictor variables used in the preliminary PLS models (Table S4) developed for the PC1 of the PCA analyses of each species as response variables. Selected variables for equalizing matrices sizes are in bold. Meaning of predictor variable codes are in Supplementary material (Glossary).

		Alnus glutinosa			***Salix atrocinerea***			***Nerium oleander***			***Rubus ulmifolius***		
Independent variables	**Predictor variable**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**
Climate	MAT	1.00	-0.01	-0.23	0.82	-0.01	0.17	0.97	0.01		1.22	-0.01	-0.26
	TMonthlyRange	1.07	0.01	0.25	0.69	0.00	-0.23	1.21	0.06	0.14	1.27	0.01	0.26
	Isothermality	1.07	-0.01	-0.20	**1.06**	**0.05**	**0.38**	0.84	0.04	-0.24	0.78	-0.01	-0.20
	TSeasonility	**1.13**	**0.01**	**0.25**	0.82	-0.02	-0.33	1.01	0.05	0.24	1.25	0.01	0.27
	MaxT	0.51	0.01	0.11	0.73	-0.03		**2.19**	**0.12**	**0.25**	0.54	0.01	
	MinT	1.07	-0.01	-0.24	0.80	-0.01	0.21	0.89	-0.01	-0.11	1.25	-0.01	-0.28
	TAnnualRange	**1.11**	**0.01**	**0.25**	0.68	-0.01	-0.26	**1.09**	**0.05**	**0.20**	1.26	0.01	0.27
	TWettestQ	**1.02**	**-0.01**	**-0.24**	**1.40**	**-0.07**	**-0.14**	0.96	-0.01		**1.18**	**-0.01**	**-0.18**
	TDriestQ	0.62	-0.01	-0.15	0.88	-0.02		**1.19**	**0.04**		0.91	-0.01	-0.19
	TWarmestQ	0.70	-0.01	-0.17	0.88	-0.02	0.11	1.14	0.03		0.98	-0.01	-0.21
	TColdestQ	1.09	-0.01	-0.24	0.80	0.00	0.21	0.90	-0.01		1.28	-0.01	-0.27
	PP	0.95	-0.01	-0.22	0.90	0.02	-0.12	0.78	0.03	-0.30	0.29	0.00	-0.14
	PWettestM	**1.05**	**-0.01**	**-0.24**	0.88	0.02	-0.13	0.66	0.02	-0.34	0.63	-0.01	-0.19
	PDriestM	1.03	0.01	0.24	0.91	-0.02	-0.27	0.86	-0.02	0.21	1.17	0.01	0.27
	PPSeasonality	1.12	-0.01	-0.25	1.77	0.09	0.40	0.52	0.01	-0.36	0.97	-0.01	-0.26
	PWettestQ	1.05	-0.01	-0.24	0.94	0.03		0.69	0.02	-0.34	0.62	-0.01	-0.20
	PDriestQ	1.08	0.01	0.24	**1.11**	**-0.04**	**-0.35**	0.79	0.01	0.19	**1.19**	**0.01**	**0.27**
	PWarmestQ	1.05	0.01	0.24	0.96	-0.03	-0.31	0.83	0.00	0.15	1.11	0.01	0.26
	PColdestQ	1.08	-0.01	-0.24	**1.35**	**0.06**		0.68	0.02	-0.34	0.65	-0.01	-0.20
	Q2	0.90	-0.01	-0.20	0.93	0.03		0.61	0.00	-0.34	0.49	-0.01	-0.15
Soil	EC	0.32	-0.01		0.89	-0.03		**1.30**	**-0.04**	**-0.45**	**1.06**	**-0.04**	**-0.54**
	pH	1.00	0.04	0.13	**1.64**	**-0.06**	**-0.46**	0.87	-0.03	-0.34	0.50	0.02	
	CaCO3	0.85	0.04	0.14	**1.32**	**-0.05**	**-0.40**	0.68	0.02	0.16	0.26	0.01	
	OC	0.55	0.02	0.28	0.56	0.02	0.26	0.98	0.03	0.41	0.25	-0.01	0.13
	N	0.05	0.00	0.13	0.53	0.02	0.30	**1.17**	**0.04**	**0.39**	0.54	-0.02	
	CN	0.98	0.04	0.20	0.51	-0.02	-0.21	0.44	0.02	0.14	0.13	0.01	
	CEC	**1.00**	**0.04**	**0.49**	0.71	0.03	0.29	0.16	-0.01		0.34	-0.01	
	BasSat	**1.02**	**-0.04**	**-0.38**	**1.42**	**-0.06**	**-0.44**	0.47	-0.02	-0.21	0.66	-0.03	-0.39
	ESP	**1.02**	**-0.04**	**-0.43**	0.67	0.03		**2.13**	**-0.07**	**-0.52**	0.89	-0.04	-0.59
	AW	0.01	0.00	0.16	0.54	0.02	0.26	0.82	-0.03	-0.24	0.54	-0.02	-0.20
	P	**2.20**	**0.09**	**0.63**	**1.29**	**0.05**	**0.46**	0.28	0.01		**2.75**	**0.11**	**0.72**

Table S4.C1. Variable importance in the projection (VIP), standardized coefficients and loadings of the predictor variables used in the preliminary PLS models (Table S4) developed for the PC 2 of the PCA analyses of each species as response variables. Selected variables for equalizing matrices sizes are in bold. Note that only variables with VIP > 1 were selected, excepting in the PLS built for the PC 2 of *Nerium oleander*, where soil Ph and OC were selected to equalize the number of variables to those selected for climate since some authors point those variables with VIPs over 0.83 can be relevant. Meaning of predictor variable codes are in Supplementary material.

		Alnus glutinosa			***Salix atrocinerea***			***Nerium oleander***			***Rubus ulmifolius***		
Independent variables	**Predictor variable**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**	**VIP**	**Standardized coefficient**	**Loading**
Climate	MAT	0.18	0.00	0.22	1.11	-0.01	-0.26	1.21	-0.02	-0.30	0.01	0.00	0.17
	TMonthlyRange	**1.04**	**-0.01**	**-0.29**	1.31	0.01	0.24	0.54	0.01	0.26	0.89	-0.01	-0.28
	Isothermality	1.24	0.01	0.24	0.46	-0.01		**1.28**	**-0.02**	**-0.11**	1.68	0.01	0.30
	TSeasonility	1.22	-0.01	-0.30	1.27	0.01	0.23	1.03	0.01	0.30	1.10	-0.01	-0.31
	MaxT	**1.75**	**-0.01**	**-0.21**	0.17	0.00	-0.13	0.82	-0.01		**1.99**	**-0.01**	**-0.29**
	MinT	0.48	0.00	0.25	1.18	-0.01	-0.26	1.20	-0.02	-0.31	0.40	0.00	0.23
	TAnnualRange	1.09	-0.01	-0.29	**1.29**	**0.01**	**0.24**	0.84	0.01	0.29	**1.05**	**-0.01**	**-0.30**
	TWettestQ	0.62	0.00	0.24	0.33	0.00	-0.16	0.67	-0.01	-0.22	0.29	0.00	
	TDriestQ	0.68	0.00		0.93	-0.01	-0.24	**1.12**	**-0.02**	**-0.25**	0.94	-0.01	
	TWarmestQ	0.56	0.00	0.12	0.99	-0.01	-0.25	1.06	-0.01	-0.25	0.79	-0.01	
	TColdestQ	0.54	0.00	0.25	**1.19**	**-0.01**	**-0.26**	1.18	-0.02	-0.31	0.36	0.00	0.22
	PP	**1.21**	**0.01**	**0.29**	1.07	0.01	0.26	0.47	-0.01		1.17	0.01	0.33
	PWettestM	**1.17**	**0.01**	**0.30**	1.08	0.01	0.26	0.64	-0.01		1.20	0.01	0.36
	PDriestM	0.43	0.00	-0.24	**1.14**	**0.01**	**0.25**	1.33	0.02	0.33	0.06	0.00	-0.23
	PPSeasonality	**0.98**	**0.01**	**0.29**	0.39	0.00		**1.32**	**-0.02**	**-0.23**	0.79	0.00	0.33
	PWettestQ	1.16	0.01	0.30	**1.06**	**0.01**	**0.25**	0.72	-0.01		1.21	0.01	0.36
	PDriestQ	0.75	-0.01	-0.25	1.12	0.01	0.24	**1.23**	**0.02**	**0.33**	0.05	0.00	-0.22
	PWarmestQ	0.49	0.00	-0.24	**1.10**	**0.01**	**0.25**	1.30	0.02	0.33	0.29	0.00	-0.19
	PColdestQ	1.24	0.01	0.30	0.76	0.01	0.21	0.70	-0.01		**1.10**	**0.01**	**0.36**
	Q2	1.50	0.01	0.28	0.93	0.01	0.25	0.33	0.00		1.43	0.01	0.36
Soil	EC	0.26	0.01	0.18	**1.26**	**-0.15**	**-0.54**	0.83	0.04	0.23	0.93	-0.03	-0.30
	pH	**1.35**	**-0.04**	**-0.41**	0.70	-0.03	-0.30	**0.86**	**0.04**	**0.42**	0.12	0.00	0.20
	CaCO3	0.31	-0.01	-0.16	**1.11**	**0.09**	**-0.43**	0.10	0.01		0.38	-0.01	
	OC	**1.12**	**0.03**	**0.47**	0.87	-0.03	-0.32	**0.85**	**-0.04**	**-0.51**	**1.63**	**-0.05**	**-0.65**
	N	**1.76**	**0.05**	**0.54**	**1.12**	**-0.07**		0.51	-0.03	-0.44	**1.20**	**-0.03**	**-0.48**
	CN	**1.26**	**-0.04**	**-0.19**	0.86	0.03	-0.65	0.75	-0.04	-0.19	0.91	-0.03	-0.23
	CEC	0.85	0.03	0.42	0.87	0.00	-0.26	**2.00**	**-0.10**	**-0.55**	0.09	0.00	-0.28
	BasSat	**1.32**	**-0.04**	**-0.41**	**1.11**	**-0.09**	**-0.27**	0.61	0.03	0.36	0.43	-0.01	
	ESP	0.32	-0.01	-0.13	**1.12**	**-0.13**	**0.16**	0.11	-0.01	0.23	0.75	-0.02	
	AW	0.69	-0.02		0.80	-0.05	-0.40	0.26	-0.01		0.88	-0.02	-0.33
	P	0.22	0.01	0.19	**1.02**	**-0.05**	**0.30**	**1.88**	**0.10**	**0.36**	**1.87**	**-0.05**	**-0.50**

Table S5.C1. Mean, standard error of the mean (SE), minimum (Min.) and maximum (Max.) values of all the environmental variables measured for the sampling sites of each species**.**

		Alnus glutinosa				*Salix atrocinerea*				*Nerium oleander*				*Rubus ulmifolius*			
		MEAN	SE	Max	Min	MEAN	SE	Max	Min	MEAN	SE	Max	Min	MEAN	SE	Max	Min
	Altitude (m a.s.l.)	787	113	1438	423	1162	51	1438	943	442	78	1038	47	779	65	1438	270
Climate	Annual Mean Temperature (MAT; °C)	14.0	0.6	15.7	10.8	11.8	0.3	13.3	10.8	15.2	0.5	17.4	11.1	13.9	0.3	16.6	10.8
	Mean Monthly Temperature Range (°C)	10.6	0.3	12.0	9.2	11.7	0.1	12.2	11.1	10.0	0.3	11.7	8.6	10.8	0.2	12.2	9.2
	Isothermality (%)	38.7	0.2	40.2	38.1	38.1	0.0	38.3	37.8	38.8	0.2	39.9	37.8	38.7	0.1	40.2	37.8
	Temperature Seasonality (%)	44.3	3.4	62.5	30.7	57.2	1.8	63.2	48.0	37.9	2.5	59.5	29.5	45.2	1.9	63.2	30.7
	Max. Temperature of Warmest Month (°C)	29.8	0.4	32.1	28.3	29.9	0.1	30.6	29.1	29.7	0.3	31.7	28.7	30.0	0.2	32.5	28.3
	Min. Temperature of Coldest Month (°C)	2.3	0.8	5.4	-1.9	-0.9	0.3	1.0	-1.9	4.0	0.7	6.9	-1.4	2.0	0.5	5.8	-1.9
	Temperature Annual Range (°C)	27.5	1.0	31.5	22.9	30.8	0.3	32.2	29.1	25.7	0.7	30.5	22.7	28.0	0.6	32.2	22.9
	Mean Temperature of Wettest Quarter (°C)	8.6	0.5	10.5	5.9	7.7	0.5	11.1	5.9	10.4	0.8	15.2	6.3	9.0	0.3	14.0	5.9
	Mean Temperature of Driest Quarter (°C)	21.5	0.3	23.2	19.7	20.5	0.2	21.5	19.7	22.3	0.3	23.7	19.7	21.6	0.2	23.5	19.7
	Mean Temperature of Warmest Quarter (°C)	21.7	0.4	23.4	19.7	20.6	0.2	21.7	19.7	22.6	0.4	24.2	19.9	21.8	0.2	23.8	19.7
	Mean Temperature of Coldest Quarter (°C)	7.2	0.7	10.2	3.4	4.4	0.3	6.2	3.3	8.8	0.6	11.6	3.8	7.0	0.4	10.5	3.3
	Annual Precipitation (PP; mm)	699	32	845	529	581	19	653	470	620	65	816	261	648	25	845	294
	Precipitation of Wettest Month (mm)	101.2	7.9	140.0	65.0	72.5	2.3	81.0	59.0	92.0	11.2	131.0	36.0	92.0	5.1	140.0	39.0
	Precipitation of Driest Month (mm)	6.2	1.6	15.0	1.0	12.9	0.7	15.0	9.0	3.6	1.2	15.0	1.0	6.7	0.9	15.0	1.0
	Precipitation Seasonality (%)	61.1	3.8	79.5	44.9	44.2	0.9	50.3	39.5	62.4	3.5	74.1	43.1	57.9	2.4	79.5	39.5
	Precipitation of Wettest Quarter (mm)	288	23	399	185	201	7	229	156	264	33	370	100	262	15	399	107
	Precipitation of Driest Quarter (mm)	33.0	4.8	59.0	14.0	53.6	2.9	68.0	37.0	24.7	3.7	59.0	15.0	34.6	3.0	68.0	14.0
	Precipitation of Warmest Quarter (mm)	40.4	4.4	65.0	25.0	59.1	2.4	69.0	45.0	31.9	3.4	64.0	22.0	40.7	2.8	69.0	25.0
	Precipitation of Coldest Quarter (mm)	278.0	23.1	399.0	181.0	185.5	6.8	216.0	143.0	251.3	32.6	359.0	88.0	249.6	15.6	399.0	94.0
	Emberger's index (Q2)	81.5	5.7	109.5	56.5	65.3	2.6	75.3	51.9	72.2	7.9	102.2	31.5	74.0	3.7	109.5	33.8
Soil	EC (µS cm^{-1})	712	75	1521	395	765	93	1364	301.0	1531	318	3290	301	983	114	3290	301
	pH	6.4	0.2	7.9	5.3	7.3	0.2	8.2	6.2	7.7	0.3	8.8	6.0	7.3	0.2	8.8	5.3
	$CaCO_3$ (%)	0.4	0.1	1.7	0.0	16.9	10.0	85.9	0.0	16.6	6.7	69.2	0.0	19.2	4.8	85.9	0.0
	Organic C (%)	3.5	0.5	5.4	0.5	2.9	0.6	5.4	0.4	1.8	0.5	5.1	0.1	2.8	0.3	5.4	0.1
	N (%)	0.15	0.02	0.29	0.02	0.12	0.03	0.25	0.02	0.09	0.03	0.36	0.02	0.12	0.02	0.36	0.02
	CN ratio	24.7	1.9	35.9	14.9	27.1	5.8	79.8	6.7	21.2	6.0	75.9	4.4	25.2	2.9	79.8	4.4
	Cation Exchange Capacity (cmol$^+$ kg^{-1})	13.5	2.0	23.3	4.0	12.1	2.2	23.3	2.7	13.4	2.9	28.2	2.7	14.6	1.7	47.8	2.7
	Base saturation (%)	81.6	5.2	100.0	45.7	85.5	5.4	100.0	45.7	88.1	7.2	100.0	19.7	87.9	3.4	100.0	19.7
	Echangeable sodium (%)	1.8	0.4	5.3	0.7	1.3	0.3	3.3	0.6	6.1	2.8	32.4	0.1	1.9	0.4	11.8	0.1
	Avaialable water (%)	4.9	0.6	11.1	1.6	3.7	0.5	6.2	1.3	5.0	0.5	8.0	1.9	4.7	0.4	11.1	1.3
	P (%)	0.04	0.01	0.08	0.01	0.05	0.01	0.08	0.01	0.03	0.00	0.05	0.01	0.03	0.00	0.08	0.01

Table S6.C1. Mean (± SE), and maximum and minimum values of nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) concentrations (% DM), molar elemental ratios (CN and CP), condensed tannins content (mg Catechin Hydrate Equivalent g DM^{-1}), lignin content (% DM) and toughness (g) of the leaves each plant species. Different letters indicate significant differences ($p < 0.05$) obtained using linear models followed by pairwise multiple comparisons (Tukey test).

	Alnus glutinosa				*Salix atrocinerea*				*Nerium oleander*				*Rubus ulmifolius*			
	Mean	SE	Max	Min	Mean	SE	Max	Min	Mean	SE	Max	Min	Mean	SE	Max	Min
% N	2.77^{a}	0.11	3.40	2.06	2.19^{b}	0.11	2.67	1.52	1.14^{c}	0.06	1.50	0.94	2.03^{b}	0.05	2.70	1.48
% P	0.09^{b}	0.01	0.13	0.04	0.14^{a}	0.01	0.19	0.06	0.07^{b}	0.01	0.14	0.03	0.10^{b}	0.01	0.19	0.04
% Ca	0.88^{b}	0.07	1.21	0.50	0.80^{b}	0.05	1.07	0.54	1.28^{a}	0.09	1.77	0.73	0.91^{b}	0.04	1.33	0.40
% Mg	0.35^{b}	0.04	0.67	0.13	0.23^{b}	0.01	0.28	0.16	0.31^{b}	0.06	0.87	0.14	0.49^{a}	0.02	0.67	0.29
CN	20.51^{c}	0.8	28.2	16.4	25.55^{bc}	1.6	36.0	20.8	49.47^{a}	2.2	60.5	36.8	26.69^{b}	0.7	36.4	19.4
CP	1507.61^{ab}	164.5	3152.0	939.0	947.52^{c}	113.8	1960.9	514.7	1942.93^{a}	269.2	4092.8	892.4	1347.51^{b}	84.5	2760.6	603.6
Tannins	0.27^{c}	0.01	0.34	0.21	1.21^{a}	0.25	2.17	0.43	0.45^{bc}	0.05	0.65	0.20	0.48^{b}	0.02	0.80	0.25
% Lignin	14.82^{b}	1.24	19.71	5.98	26.20^{a}	1.08	33.26	21.56	10.64^{c}	0.66	13.77	6.75	6.78^{d}	0..29	9.69	3.60
Toughness	60.31^{c}	2.8	77.7	43.4	71.42^{b}	3.2	85.5	54.9	306.29^{a}	9.8	350.1	233.0	72.65^{b}	1.5	89.0	53.2

Table S7.C1. Weights of selected leaf traits on each of the first two principal components (PCs) of PCAs: the four species pooled and each species separately.

	All species		*Alnus glutinosa*		*Salix atrocinerea*		*Nerium oleander*		*Rubus ulmifolius*	
Leaf trait	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
N	0.88	-0.25	0.57	0.79	0.65	0.70	0.87	-0.32	0.83	0.05
P	0.57	-0.08	0.93	0.05	0.84	0.28	0.56	0.49	0.82	0.05
Ca	-0.54	-0.23	0.87	-0.29	0.45	-0.29	0.66	0.65	0.50	-0.63
Mg	0.03	-0.83	0.89	-0.24	0.40	-0.80	0.09	-0.34	0.46	-0.55
Tannins	0.14	0.67	-0.60	-0.51	-0.90	0.12	0.29	-0.80	-0.44	-0.17
Lignin	0.41	0.80	-0.73	0.23	-0.51	0.64	0.08	-0.38	-0.03	-0.55
Toughness	-0.89	0.29	-0.32	0.54	-0.82	-0.24	-0.68	0.19	-0.66	-0.60

Figure S1.C1. Visual Spearman correlation matrix between leaf traits. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

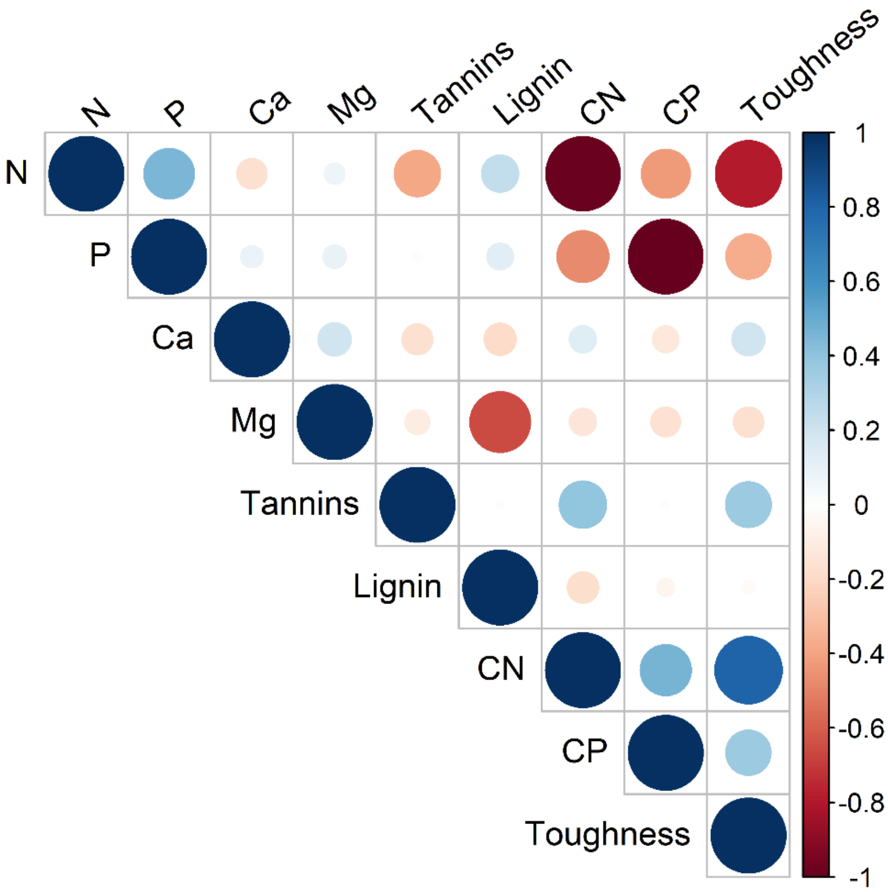


Figure S2.C1. Visual Spearman correlation matrix between environmental variables among the sites where each species is present: (A) *Alnus glutinosa*, (B) *Salix atrocinerea*, (C) *Nerium oleander*, (D) *Rubus ulmifolius*. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

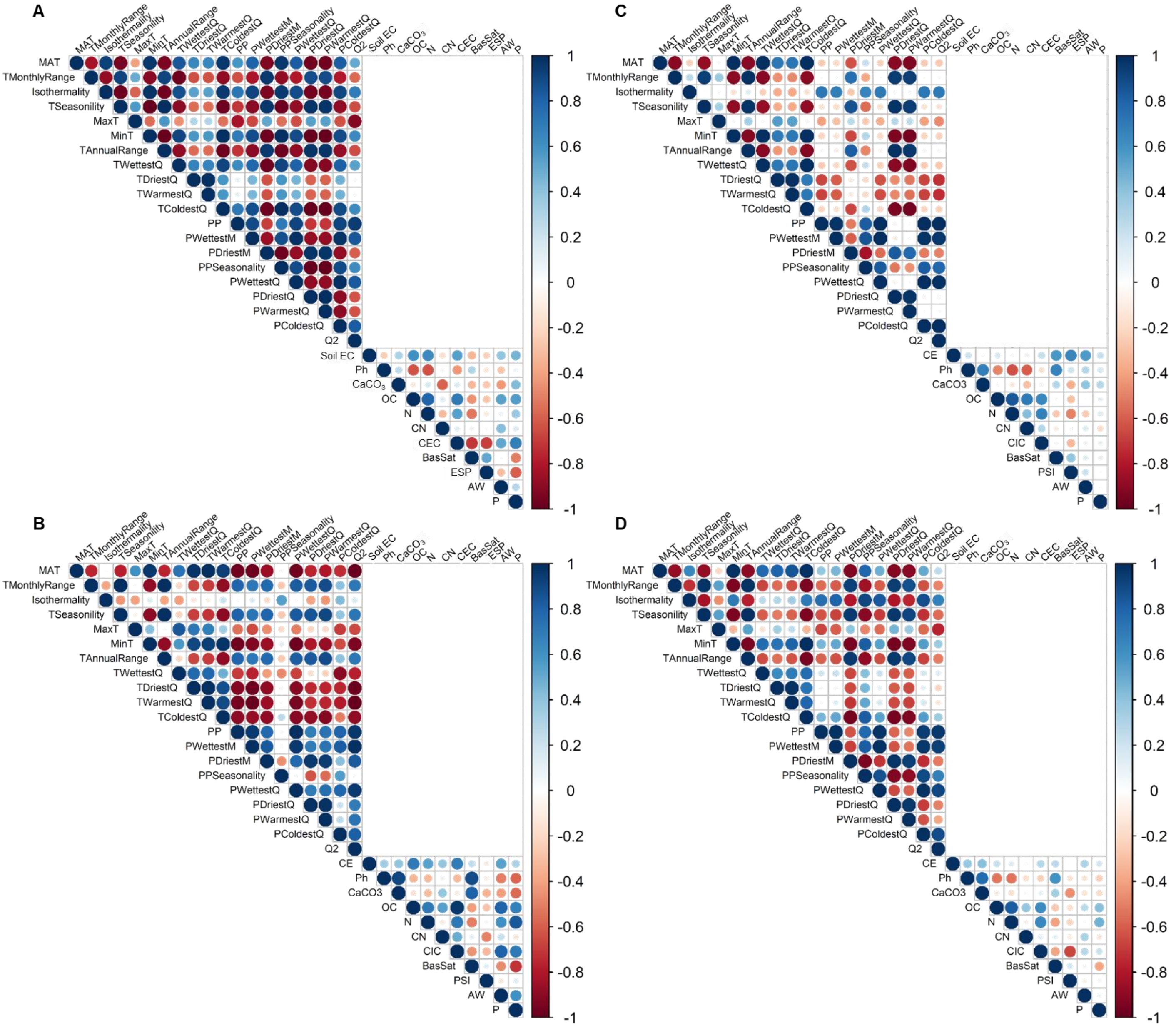


Figure S3.C1. Variance partitioning of leaf traits at different levels along the environmental gradient. %N, % nitrogen; %P, % phosphorus; %Ca, % calcium; %Mg, % magnesium; C:N, carbon:nitrogen molar ratio; C:P, carbon:phosphorus molar ratio; Tannins, condensed tannins content (mg Catechin Hydrate Equivalent g DM-1); % Lignin, % lignin; and Toughness, toughness of leaves (g).

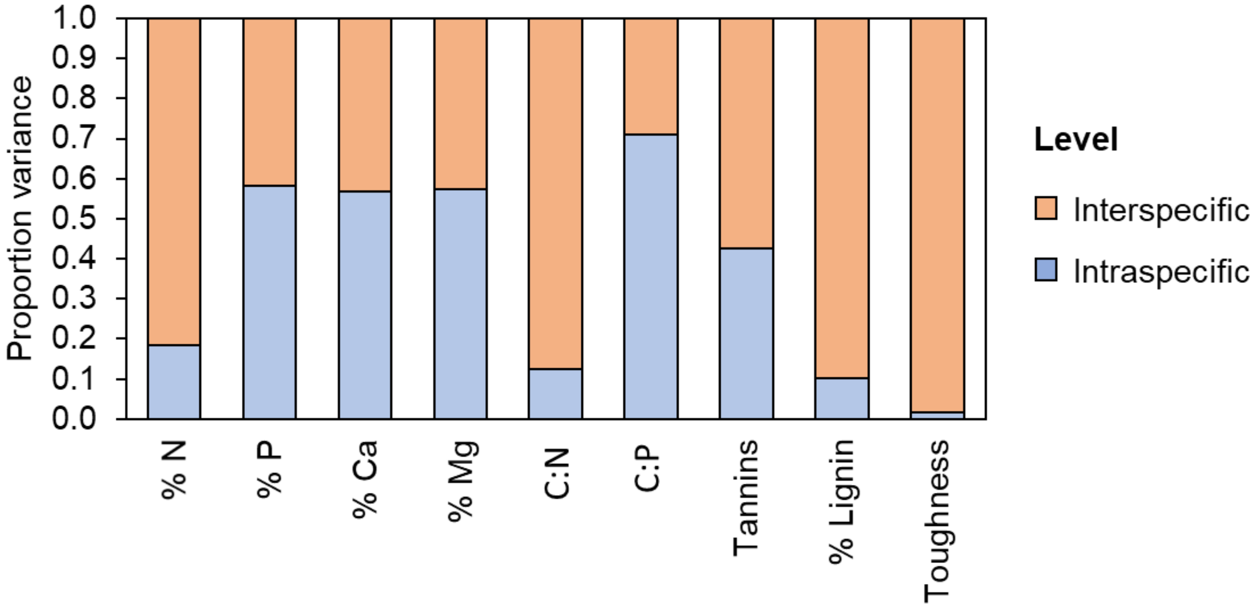
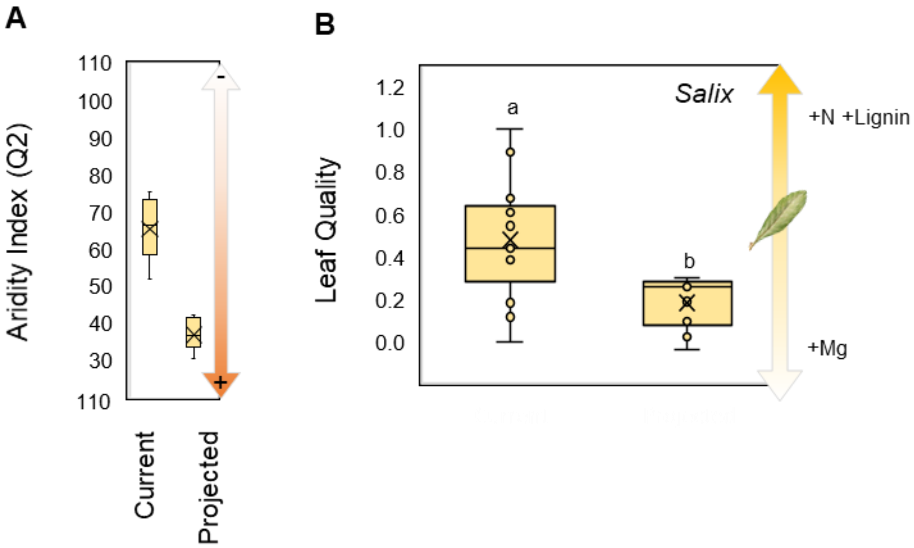


Figure S4.C1. Box-plots showing (A) the aridity range covered by the distribution of each species according to the Emberger's bioclimatic coefficient (Q2) ; and (B) the leaf quality (Dim 2) of *S. atrocinerea* at present (Current) and in future climate change scenarios according to the CCM3 (Projected). Note that leaf quality range (PCA dimension) is scaled to unit for simplicity. Different letters indicate significant difference based on t-test analyses.



Supplementary material ***Chapter 2***

Key plant species and detritivores drive diversity effects on instream leaf litter decomposition more than functional diversity: A microcosm study

SUPPLEMENTARY METHODS

Detritivore elemental composition analysis

The concentrations of C and N (%DM) on detritivores were estimated using a Perkin Elmer series II CHNS/O elemental analyser. P content (% DM) was measured spectrophotometrically after autoclave-assisted extraction (APHA, 1998). C, N and P concentrations of detritivores were measured at the beginning (pooling pairs of individuals from those used in the size-biomass curve, n=12) and at the end of the experiment (using only individuals which completed the experimental period; n=4-5 per treatment).

Litter traits characterization

Initial physical and chemical trait characterization (Table 1, Table S1) was performed on post-leached litter (n=3). Ten discs of each sample were cut out to measure specific leaf area (SLA) and leaf toughness. The remaining litter fragments were oven-dried (70 ºC, 72 h), and ground for subsequent litter composition analyses. Specific leaf area (SLA; cm^2 g^{-1}) was estimated as the coefficient between leaf disc area and disc DM. Leaf toughness (g) was measured using a Texture Analyzer TA.XTPlus equipped with a 0.7 mm Ø steel rod. Ash content (% DM) was estimated as explained in the main text. Concentrations of C, N and P were estimated following the same procedure as explained for detritivores and were measured at the beginning (post- leached) and at the end of the experiment (n=3). Molar ratios C:N, C:P and N:P were also calculated. Percentages of hemicellulose, cellulose and lignin were calculated as exposed in Fenoy et al. (2016), using an ANKOM 200/220 fibre analyser (ANKOM Technologies, Macedon, NY, USA). Total phenols were determined following the Folin & Ciocalteu procedure as described in Bärlocher and Graça (2005)). Condensed tannins were estimated by the acid butanol assay (Gessner & Steiner, 2005). Concentration of Ca, K, Mg and Na (% DM) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II). Silicon (Si) concentration was determined using inductively coupled plasma atomic emission spectroscopy (Thermo ICAP 6500 duo, Thermo Fisher Scientific, Cambridge, UK), after microwave sample digestion in nitric acid (65%) and hydrogen peroxide (30%). Non-structural carbohydrates of each species were estimated using the following formula: NSC (%) = [100 – (*Cp* + *Fc* + *ash)*], where *Cp* is the crude protein calculated as N (%) multiplied by 6.25 (Williams, 1984); *Fc* is the sum of the structural carbohydrates (hemicellulose, cellulose and lignin) percentages and *ash* (%) is the ash concentration.

Ergosterol analysis

Ergosterol was extracted from frozen-dried leaf discs (≈50 mg) in 10 mL screw-cap test tubes by 30 minutes of refluxing in 2 mL KOH-methanol (0.4 g in 50 ml) at 80ºC 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 seconds and centrifuged for 4 minutes at 1165 g. The supernatant, corresponding to the n-hexane phase containing sterols was collected, transferred to 1.5 mL HPLC vials and evaporated to dryness under a stream of N2. To apply a low-temperature heating (e.g. 40ºC) during evaporation will accelerate the process. A second extraction was carried out adding another 1 mLof n-hexane to the sample test tube and repeating the subsequently 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) equipped with a UV detector and a Luna C18 reversed-phase column (250 × 4.6 mm, 5 µm particle size; Phenomenex, Torrance, CA, USA) operating at a fixed temperature of 30°C. The mobile phase was methanol:acetonitrile (80:20, v/v), at a flow rate of 1 mL min^{-1}, and the injection volume was 20 µL. The detection was performed at 282 nm. Ergosterol eluted approximately after 6.5 min

Table S1.C2. Mean (± SE) of specific leaf area (SLA; mm^{2} mg^{-1}), toughness (g), ash, carbon (C), nitrogen (N) and phosphorus (P) concentrations (% DM), molar elemental ratios (C:N, C:P and N:P), hemicellulose, cellulose and lignin content (% DM), total phenolics (mg tannic acid equivalents g litter DM^{-1}) and condensed tannins (mg Quebracho tannins equivalents g litter DM^{-1}) content, concentrations of Ca, K, Mg, Na and Si (% DM) and non-structural carbohydrates (NSC, % DM) of each plant species and litter mixtures. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 3-Sps litter mixtures independently, on the basis of linear models followed by pairwise multiple comparisons (Tukey test).

Plant species	SLA	Toughness	Ash	C	N	P	C:N	C:P	N:P	Hemicellulose
Quercus robur	165.1±11.4^{a}	74.4±0.9^{de}	5.1±0.1^{bc}	49.97±0.80^{a}	1.24±0.05^{c}	0.030±0.000^{c}	47.1±1.3^{bc}	4293.0±101.6^{a}	91.3±3.8^{b}	12.6±1.7^{abc}
Q. canariensis	124.3±2.5^{b}	101.4±3.1^{cd}	8.9±1.2^{a}	48.93±1.06^{ab}	1.22±0.03^{c}	0.033±0.003^{c}	46.7±0.3^{bc}	3865.8±221.9^{a}	82.8±4.8^{b}	15.4±0.8^{abc}
Q. pyrenaica	121.1±6.2^{b}	88.1±5.9^{cd}	7.9±0.5^{a}	46.52±0.37^{b}	0.62±0.03^{d}	0.042±0.001^{b}	88.3±4.1^{a}	2832.2±12.3^{b}	32.2±1.6^{d}	16.7±0.6^{ab}
Q. coccifera	77.6±4.9^{cd}	150.7±0.4^{b}	5.1±0.1^{bc}	49.99±0.87^{a}	1.15±0.03^{c}	0.051±0.001^{b}	50.8±1.1^{b}	2549.1±51.6^{b}	50.29±1.8^{c}	13.0±0.5^{abc}
Q. suber	100.3±4.5^{bc}	135.7±8.2^{bc}	3.5±0.2^{c}	51.48±0.44^{a}	1.25±0.03^{c}	0.046±0.002^{b}	48.0±1.5^{bc}	2930.4±171.3^{b}	60.95±1.7^{c}	10.6±0.4c
Q. rotundifolia	59.5±1.0^{d}	221.2±13.7^{a}	4.4±0.3^{bc}	49.72±0.23^{ab}	1.43±0.02^{b}	0.070±0.002^{a}	40.6±0.2^{d}	1842.3±42.1^{c}	45.3±0.8^{cd}	18.3±0.5^{a}
Q. faginea	105.0±3.6^{bc}	103.6±4.3^{bc}	7.9±0.7^{a}	48.67±0.71^{ab}	1.29±0.04^{bc}	0.049±0.002^{b}	44.1±0.9^{cd}	2549.6±83.4^{b}	57.81±2.8^{c}	13.4±1.2^{abc}
Alnus glutinosa	176.0±7.0^{a}	52.2±5.1^{e}	6.3±0.5^{ab}	51.72±0.37^{a}	2.71±0.02^{a}	0.045±0.002^{b}	22.3±0.3^{e}	2950.7±110.4^{b}	132.45±6.1^{a}	12.7±1.6^{bc}
Litter mixtures										
Low FD-D	137.6±3.6^{a}	87.5±3.0^{c}	7.3±0.4^{a}	48.51±0.29^{b}	1.03±0.03^{c}	0.035±0.001^{c}	60.5±1.7^{a}	3677.7±100.3^{a}	69.2±2.3^{ab}	14.9±0.6^{a}
Low FD-E	79.8±2.7^{c}	167.8±6.5^{a}	4.3±0.1^{c}	50.43±0.39^{a}	1.27±0.02^{b}	0.055±0.000^{a}	46.6±0.8^{b}	2458.6±29.7^{c}	52.5±0.4^{c}	13.8±0.3^{a}
High FD-O	111.7±5.3^{b}	130.9±6.1^{b}	5.8±0.2^{b}	49.47±0.49^{ab}	1.31±0.03^{b}	0.049±0.000^{b}	44.1±0.7^{b}	2940.2±35.3^{b}	65.7±1.8^{b}	14.7±1.1^{a}
High FD-K	112.6±0.9^{b}	126.6±7.6^{b}	6.2±0.2^{ab}	50.01±0.12^{ab}	1.79±0.02^{a}	0.055±0.000^{a}	35.9±0.4^{c}	2441.3±49.8^{c}	77.7±2.7^{a}	14.6±0.7^{a}

Table S1.C2. (cont.)

Plant species	Cellulose	Lignin	Total phenolics	Condensed tannins	Ca	K	Mg	Na	Si	NSC
Quercus robur	19.7 ± 1.5^{a}	18.45 ± 1.1^{ab}	119.6 ± 3.4^{d}	47.6 ± 2.1^{b}	1.17 ± 0.03^{cd}	0.021 ± 0.001^{d}	0.149 ± 0.003^{cd}	0.010 ± 0.001^{ab}	0.167 ± 0.036^{bc}	36.37 ± 3.57^{ab}
Q. canariensis	21.3 ± 1.7^{a}	12.80 ± 0.9^{cd}	162.2 ± 3.8^{c}	54.6 ± 4.5^{ab}	1.69 ± 0.15^{b}	0.029 ± 0.003^{d}	0.155 ± 0.008^{cd}	0.012 ± 0.002^{ab}	0.347 ± 0.071^{ab}	33.95 ± 2.31^{ab}
Q. pyrenaica	25.9 ± 1.0^{a}	11.39 ± 0.6^{d}	96.3 ± 4.0^{e}	25.4 ± 8.9^{c}	1.43 ± 0.09^{bc}	0.045 ± 0.003^{bc}	0.224 ± 0.005^{b}	0.013 ± 0.001^{ab}	0.356 ± 0.011^{a}	34.22 ± 1.10^{ab}
Q. coccifera	20.7 ± 0.7^{a}	13.77 ± 0.7^{bcd}	285.3 ± 10.0^{a}	55.1 ± 2.2^{ab}	1.20 ± 0.05^{cd}	0.084 ± 0.011^{a}	0.174 ± 0.008^{cd}	0.009 ± 0.001^{ab}	0.105 ± 0.009^{c}	40.28 ± 0.78^{a}
Q. suber	22.2 ± 1.1^{a}	19.16 ± 1.3^{ab}	144.4 ± 5.6^{c}	50.3 ± 2.8^{ab}	0.86 ± 0.03^{d}	0.025 ± 0.002^{d}	0.138 ± 0.008^{d}	0.009 ± 0.001^{ab}	0.078 ± 0.003^{c}	37.62 ± 2.73^{ab}
Q. rotundifolia	24.7 ± 1.5^{a}	17.30 ± 0.9^{abc}	185.2 ± 3.4^{b}	68.9 ± 4.7^{a}	0.92 ± 0.08^{d}	0.059 ± 0.006^{ab}	0.142 ± 0.008^{cd}	0.008 ± 0.001^{b}	0.121 ± 0.039^{c}	26.41 ± 1.62^{b}
Q. faginea	19.6 ± 1.1^{a}	14.75 ± 1.4^{bcd}	201.7 ± 4.5^{b}	50.5 ± 1.0^{ab}	2.26 ± 0.00^{a}	0.044 ± 0.002^{bc}	0.338 ± 0.017^{a}	0.011 ± 0.001^{ab}	0.252 ± 0.004^{ab}	36.36 ± 3.47^{ab}
Alnus glutinosa	11.5 ± 0.9^{b}	24.17 ± 2.2^{a}	64.5 ± 2.4^{f}	11.6 ± 1.3^{c}	1.81 ± 0.09^{b}	0.030 ± 0.001^{cd}	0.185 ± 0.007^{bc}	0.014 ± 0.001^{a}	0.174 ± 0.020^{abc}	29.24 ± 4.23^{ab}
Litter mixtures										
Low FD-D	22.2 ± 1.2^{a}	14.33 ± 0.5^{b}	125.6 ± 1.8^{d}	42.6 ± 5.1^{b}	1.42 ± 0.02^{b}	0.031 ± 0.001^{c}	0.176 ± 0.002^{b}	0.012 ± 0.001^{a}	0.285 ± 0.026^{a}	34.89 ± 2.02^{a}
Low FD-E	22.5 ± 0.9^{a}	16.55 ± 0.4^{ab}	204.3 ± 5.4^{a}	57.8 ± 2.0^{a}	0.99 ± 0.03^{c}	0.056 ± 0.003^{a}	0.151 ± 0.003^{c}	0.009 ± 0.001^{a}	0.100 ± 0.009^{b}	34.94 ± 1.24^{a}
High FD-O	21.3 ± 1.3^{a}	16.88 ± 0.6^{ab}	167.4 ± 2.0^{b}	55.3 ± 1.2^{ab}	1.45 ± 0.01^{b}	0.041 ± 0.003^{bc}	0.209 ± 0.005^{a}	0.010 ± 0.001^{a}	0.179 ± 0.020^{b}	33.18 ± 2.72^{a}
High FD-K	18.8 ± 1.1^{a}	18.57 ± 1.2^{a}	151.8 ± 1.8^{c}	44.1 ± 1.6^{b}	1.66 ± 0.03^{a}	0.045 ± 0.002^{ab}	0.223 ± 0.006^{a}	0.011 ± 0.001^{a}	0.183 ± 0.018^{b}	30.71 ± 2.36^{a}

Table S2.C2. Origin of the different leaf litter species collected.

Leaf-litter species	Region	Mountain chain	Basin	Alt. (m a.s.l.)	Coordinates
Quercus robur	Cantabria	Ordunte	Agüera	320	43.2 N 3.26 W
Q. canariensis	Andalusia	Alcornocales	De la Miel	678	36.1 N 5.51 W
Q. pyrenaica	Andalusia	S. Nevada	Guadalfeo	1446	37.0 N 3.26 W
Q. coccifera	Andalusia	S. de Albuñuelas	Guadalfeo	733	36.9 N 3.61 W
Q. suber	Andalusia	Alcornocales	De la Miel	678	36.1 N 5.51 W
Q. rotundifolia	Andalusia	S. Nevada	Guadalquivir	1368	37.2 N 3.25 W
Q. faginea	Andalusia	S. de Albuñuelas	Guadalfeo	733	36.9 N 3.61 W
Alnus glutinosa	Andalusia	S. Nevada	Guadalquivir	1256	37.2 N 3.15 W

Table S3.C2. Leaf litter quality index (LQI) and Rao´s quadratic diversity (Rao’s Q) of experimental litter mixtures (in bold) and of all potential combinations of high-diversity mixtures containing one species from each functional group. *Q. robur (Q. rob), Q. canariensis (Q. can), Q. pyrenaica (Q. pyr), Q. coccifera (Q. coc), Q. suber (Q. sub), Q rotundifolia (Q. rot), Q. faginea (Q. fag), A. glutinosa (A. glu)*

Functional Diversity	Deciduous	Semi-deciduous	Evergreen	LQI	Rao's Q
Low FD-D	***Q. rob Q. can Q. pyr***			**0.35**	**4.78**
Low FD-E			***Q. coc Q. sub Q. rot***	**0.31**	**4.86**
High FD-O	*Q. rob*	*Q. fag*	*Q. coc*	0.32	7.22
High FD-O	*Q. rob*	*Q. fag*	*Q. sub*	0.36	6.49
High FD-O	***Q. rob***	***Q. fag***	***Q. rot***	**0.36**	**9.37**
High FD-O	*Q. can*	*Q. fag*	*Q. coc*	0.31	5.41
High FD-O	*Q. can*	*Q. fag*	*Q. sub*	0.35	6.81
High FD-O	*Q. can*	*Q. fag*	*Q. rot*	0.36	7.07
High FD-O	*Q. pyr*	*Q. fag*	*Q. coc*	0.31	5.46
High FD-O	*Q. pyr*	*Q. fag*	*Q. sub*	0.35	7.51
High FD-O	*Q. pyr*	*Q. fag*	*Q. rot*	0.36	7.01
High FD-K	*A. glu*	*Q. fag*	*Q. coc*	0.39	9.58
High FD-K	*A. glu*	*Q. fag*	*Q. sub*	0.42	9.13
High FD-K	***A.glu***	***Q. fag***	***Q. rot***	**0.43**	**10.59**

Table S4.C2. Results of linear models exploring effects of litter palatability (LQI) and trait diversity (Rao’s Q) on litter decomposition, associated processes and diversity effects in mixtures (df = degrees of freedom (n-2); R: Pearson correlation; F: F-statistic; *p*: p-value). Significant models are in bold.

	With detritivores										Without detritivores									
	LQI					Rao's Q					LQI					Rao's Q				
Variable	**df**	**F**	**R**	**R^2**	***p***	**df**	**F**	**R**	**R^2**	***p***	**df**	**F**	**R**	**R^2**	***p***	**df**	**F**	**R**	**R^2**	***p***
LML	**26**	**12.41**	**0.57**	**0.32**	**0.002**	**26**	**13.80**	**0.59**	**0.35**	**0.001**	10	2.61	0.45	0.21	0.138	**10**	**5.30**	**0.59**	**0.35**	**0.044**
Detritivore LML	**26**	**9.13**	**0.51**	**0.26**	**0.006**	**26**	**9.19**	**0.51**	**0.26**	**0.005**										
LNL	**10**	**16.35**	**0.79**	**0.62**	**0.002**	**10**	**21.25**	**0.82**	**0.68**	**0.001**	**10**	**20.82**	**0.82**	**0.68**	**0.001**	**10**	**31.21**	**0.87**	**0.76**	**0.001**
LPL	10	4.76	0.57	0.32	0.054	**10**	**12.11**	**0.74**	**0.55**	**0.006**	10	2.54	0.45	0.20	0.142	**10**	**12.25**	**0.74**	**0.55**	**0.006**
Ergosterol accrual	10	1.92	0.40	0.16	0.197	10	0.45	0.21	0.04	0.517	10	0.02	0.05	0.00	0.884	10	0.13	-0.11	0.01	0.728
Detritivore Growth	23	2.31	0.30	0.09	0.142	23	2.87	0.33	0.11	0.104										
Detritivore N growth	16	0.47	0.17	0.03	0.503	16	0.95	0.24	0.06	0.345										
Detritivore P growth	16	1.36	0.28	0.08	0.261	16	1.84	0.32	0.10	0.194										
NDE on total LML	26	3.35	0.34	0.11	0.079	26	3.00	0.32	0.10	0.095	10	0.94	-0.29	0.09	0.355	10	0.86	-0.28	0.08	0.375
NDE on detritivore LML	**26**	**4.48**	**0.38**	**0.15**	**0.044**	26	4.03	0.37	0.08	0.055										
NDE on LNL	10	1.86	0.40	0.16	0.202	10	1.07	0.31	0.10	0.326	10	0.04	-0.06	0.00	0.842	10	0.49	-0.22	0.05	0.499
NDE on LPL	10	0.42	0.20	0.04	0.533	10	0.50	0.22	0.05	0.495	10	0.00	0.00	0.00	0.998	10	0.21	0.14	0.02	0.658
NDE on ergosterol accrual	10	0.99	-0.30	0.09	0.344	10	0.39	-0.19	0.04	0.545	10	1.00	-0.30	0.09	0.341	10	1.71	-0.38	0.15	0.220
NDE on detritivore growth	23	0.00	0.01	0.00	0.945	23	0.01	-0.02	0.00	0.915										
NDE on N growth	16	0.06	-0.06	0.00	0.811	16	0.26	-0.13	0.02	0.617										
NDE on P growth	16	0.07	0.06	0.00	0.801	16	0.27	-0.13	0.02	0.611										
Compl. on total LML	26	2.44	0.29	0.09	0.131	26	2.36	0.29	0.08	0.137	10	0.35	-0.18	0.03	0.567	10	0.57	-0.23	0.05	0.469
Compl. on detritivore LML	26	2.18	0.28	0.08	0.152	26	1.63	0.24	0.06	0.213										
Compl. on ergosterol accrual	10	0.90	-0.29	0.08	0.365	10	0.24	-0.15	0.02	0.633	10	0.56	-0.23	0.05	0.471	10	1.52	-0.36	0.13	0.247
Sel. on total LML	**26**	**6.90**	**0.46**	**0.21**	**0.014**	**26**	**4.42**	**0.38**	**0.15**	**0.045**	10	0.00	0.01	0.00	0.984	10	0.19	0.14	0.02	0.674
Sel. on detritivore LML	**26**	**9.04**	**0.51**	**0.26**	**0.006**	**26**	**10.95**	**0.54**	**0.30**	**0.003**										
Sel. on ergosterol accrual	10	0.01	-0.03	0.00	0.920	10	0.26	-0.16	0.03	0.619	10	0.63	-0.24	0.06	0.446	10	0.00	0.01	0.00	0.974

Figure S1.C2. Experimental set up.

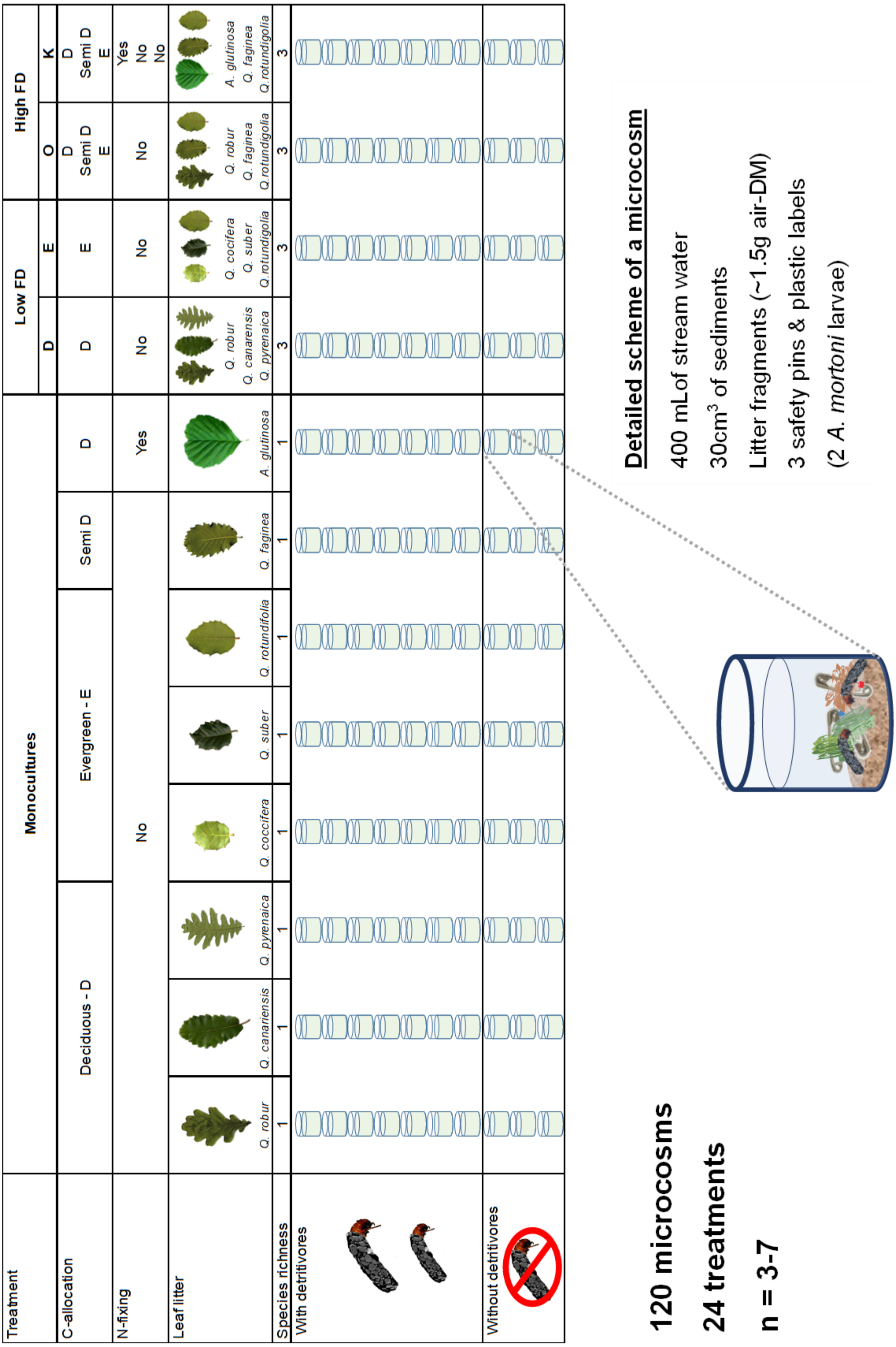


Figure S2.C2. Temporal dynamics of leachates of each leaf litter species during 12 days (corresponding to the initial incubation period of 5 days and the first experimental week of 7 days). Water replacement was carried out in days 3 and 5. Values (mean ±SE) are expressed as proportion of AFDM (n=3).

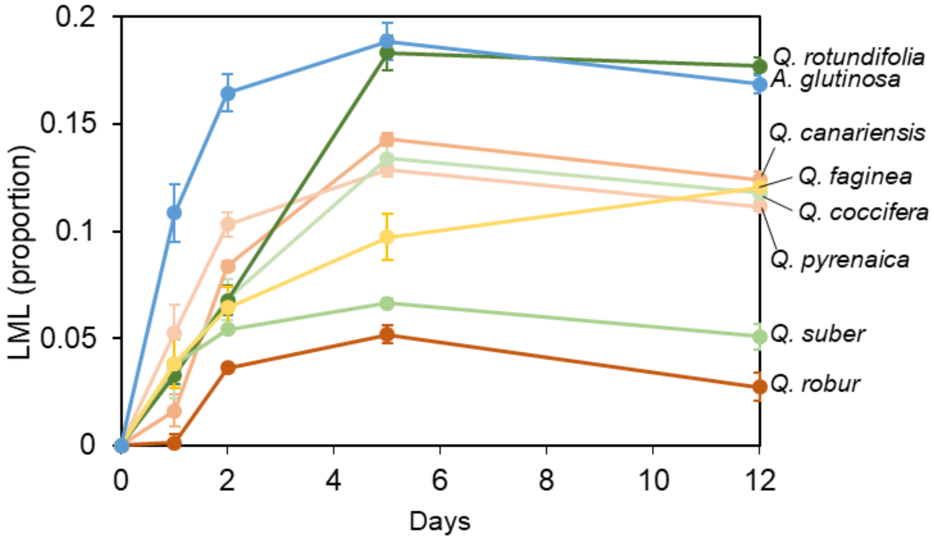


Figure S3.C2. Visual Pearson correlation matrix between leaf litter traits. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

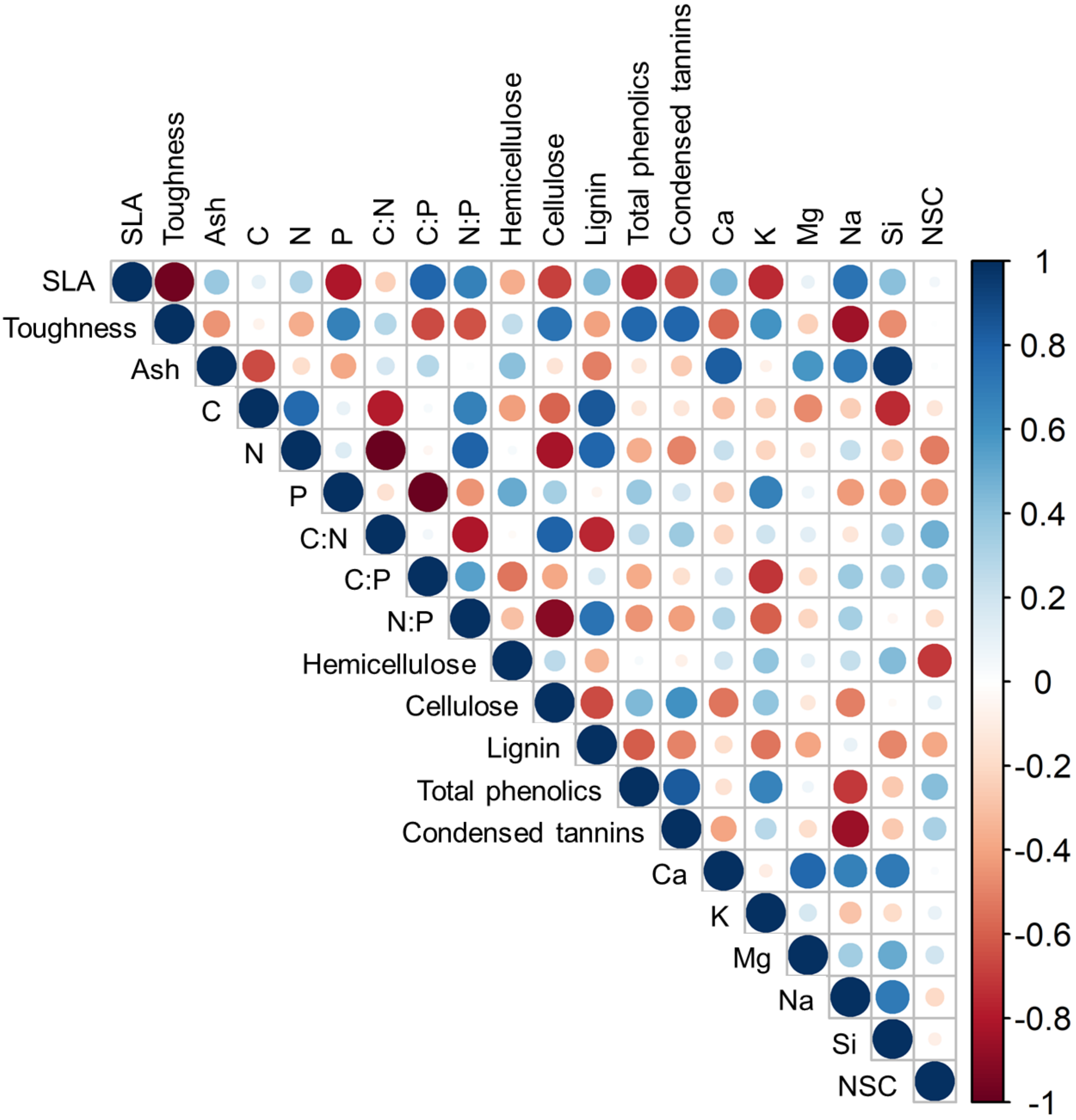


Figure S4.C2. Mean (±SE) litter mass loss (LML), litter N loss, litter P loss (proportion; prop.) and ergosterol concentration (mg g litter^{-1}) of monocultures and mixtures in microcosms with and without detritivores. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 3-spps litter mixtures independently, on the basis of linear models followed by pairwise multiple comparisons. Note that in C, D, E and F positive values indicate litter nutrient losses (decomposition) and negative one's nutrient gains (net immobilization). When necessary (coexistence of negative and positive values) a grey circle shows the mean value for the mixture.

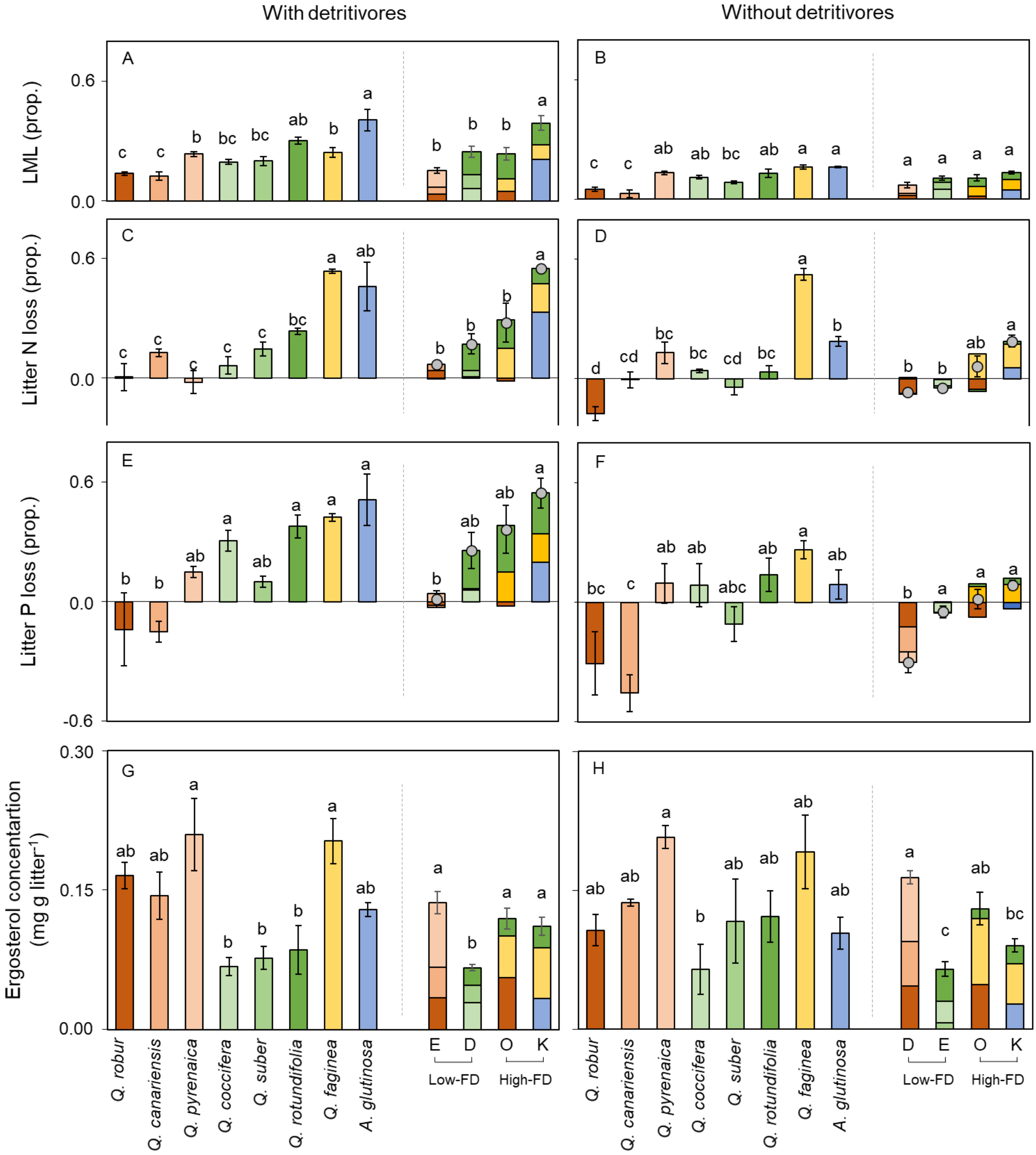


Figure S5.C2. Effects of treatments on *Allogamus mortoni* survival in monocultures (A) and mixtures (B) during the 42 days experiment. Values are expressed as % of survival estimated with Kaplan-Meier method. Different superscript letters indicate significant differences (Log-Rank test pairwise comparison, $p < 0.05$). In B no significant differences were found.

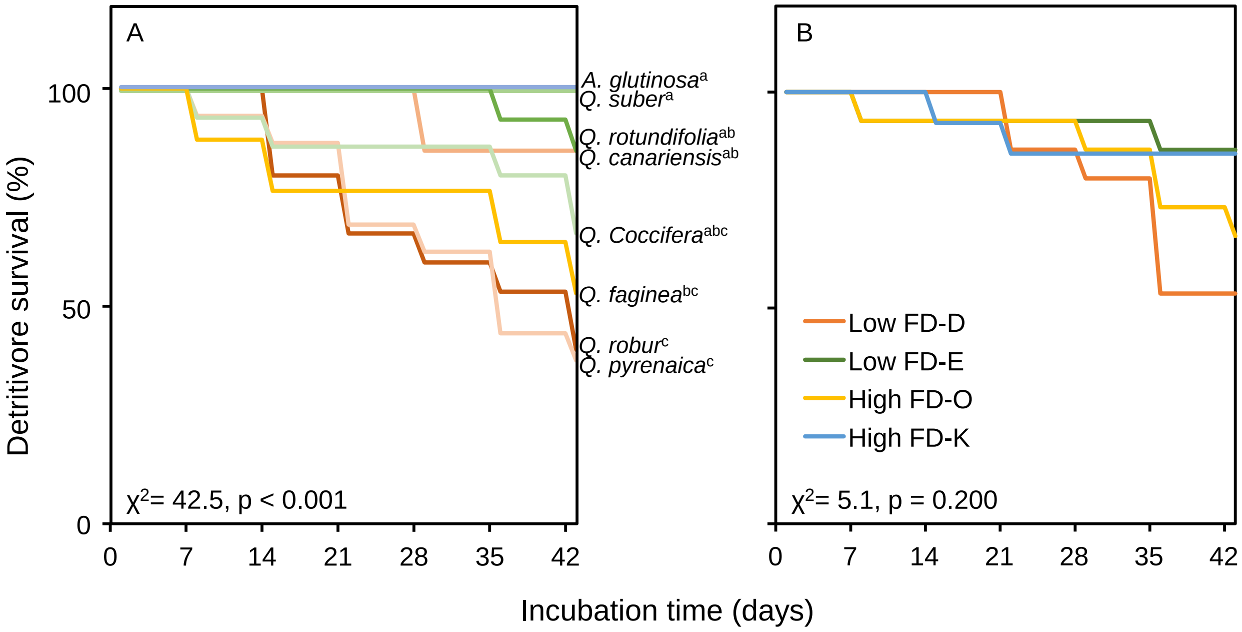
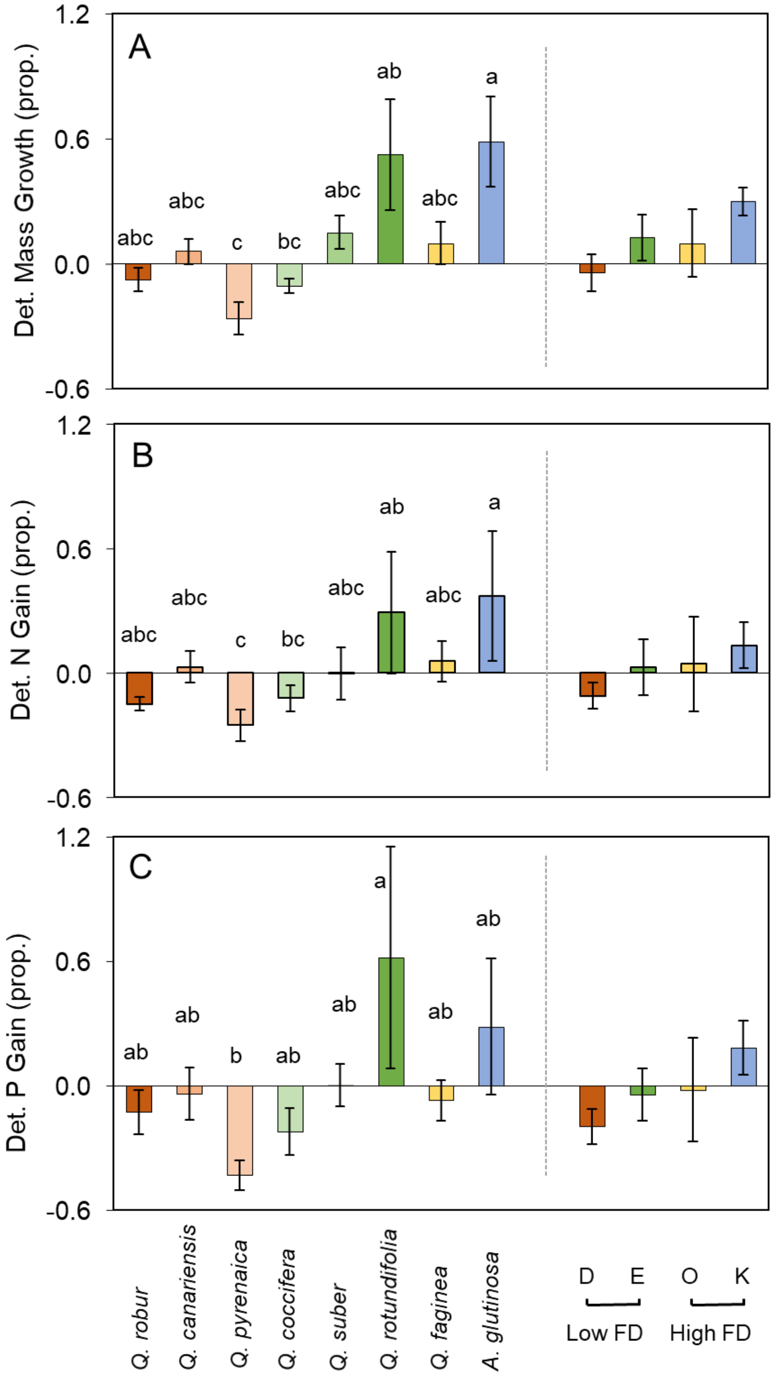


Figure S6.C2. Mean (±SEM) detritivore growth (DMG; proportion; prop.) and elemental gain (N: DNG, and P: DPG; proportion; prop.) in monocultures and mixtures. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 3-spps litter mixtures independently, on the basis of linear models followed by pairwise multiple comparisons (Tukey test). Note that positive values indicate mass, N or P gains and negative one's mass, N or P losses.



Supplementary material **Chapter 3**

Alder groves promote N-cycling but not leaf litter decomposition in streams flowing through pine plantations

Table S1.C3. Water physicochemical characteristics of the six streams (Mean ± SE) : Temperature (ºC, Temp.); pH, alkalinity (meq $CaCO_3$ L^{-1}, Alk.); conductivity (µS cm^{-1}, Cond.); dissolved oxygen (mg L^{-1}, DO); oxygen saturation (%, Oxygen sat.); nitrate-N (µg L^{-1}, N-NO_3); soluble reactive P (µg L^{-1}, SRP); ammonium-N (ppb, NH_4-N); total dissolved nitrogen (mg L^{-1}, TDN) and phosphorus (µg L^{-1}, TDP) and flow (L s^{-1}). Different superscript letters indicate significance differences ($p < 0.005$) between riparian types based on two-way ANOVAs. All values are mean values within stream or riparian type.

Stream ID	**Stream**	**Temp. (ºC)**	**pH**	**Alk. (meq $CaCO_3$ L^{-1})**	**Cond. (µS cm^{-1})**	**DO (mg L^{-1})**	**Oxygen sat. (%)**
	With alder	**3.41 ± 0.08^b**	**7.41 ± 0.09**	**0.30 ± 0.02**	**38.03 ± 3.24**	**10.38 ± 0.06**	**94.62 ± 0.33^b**
ALD1	Barranco de los Pasillos	3.26	7.26	0.26	31.90	10.49	95.23
ALD2	Barranco de los Recodos	3.37	7.40	0.30	34.05	10.31	94.13
LAN	Río del Pueblo	3.61	7.56	0.34	48.15	10.33	94.50
	Without alder	**3.60 ± 0.10^a**	**7.49 ± 0.10**	**0.28 ± 0.04**	**33.81 ± 2.19**	**10.40 ± 0.13**	**96.07 ± 0.14^a**
JER1	Barranco de los Ciruelillos	3.78	7.48	0.18	26.37	10.36	96.07
JER2	Barranco de Alcázar	3.19	7.51	0.38	41.13	10.33	96.07
JER4	Barranco de Alhorí	3.81	7.47	0.28	33.93	10.50	96.07

Stream ID	**Stream**	**NO_3-N (µg L^{-1})**	**SRP (µg L^{-1})**	**NH_4-N (µg L^{-1})**	**TDN (mg L^{-1})**	**TDP (µg L^{-1})**	**Flow (L s^{-1})**
	With alder	**180.85 ± 20.20^a**	**6.33 ± 0.60**	**21.25 ± 6.84**	**0.83 ± 0.17^a**	**11.20 ± 1.33**	**40.31 ± 12.15**
ALD1	Barranco de los Pasillos	236.15	7.40	17.91	0.94	11.78	53.93
ALD2	Barranco de los Recodos	172.11	5.79	15.91	0.81	10.09	16.06
LAN	Río del Pueblo	134.29	5.79	29.93	0.73	11.63	50.94
	Without alder	**84.64 ± 7.63^b**	**6.34 ± 0.84**	**19.02 ± 5.62**	**0.61 ± 0.18^b**	**13.13 ± 2.41**	**82.52 ± 20.12**
JER1	Barranco de los Ciruelillos	98.01	7.71	12.57	0.47	19.12	83.98
JER2	Barranco de Alcázar	75.04	4.21	13.23	0.82	10.30	46.97
JER4	Barranco de Alhorí	80.87	7.10	31.27	0.55	9.96	116.62

Values are means ± SE (n = 12-18, except for temperature where n = 183 per riparian type).

Table S2.C3. Mean (± SE) of carbon (C), nitrogen (N) and phosphorus (P) concentrations (% DM), molar elemental ratios (C:N, C:P and N:P), concentrations of Ca, Mg and Si (% DM), lignin content (% DM), condensed tannins (mg Quebracho tannins equivalents g litter DM^{-1}) and total phenolics (mg tannic acid equivalents g litter DM^{-1}) content, specific leaf area (SLA; mm^2 mg^{-1}) and toughness (g) of each plant species and litter mixtures. Different superscript letters indicate significant differences ($p < 0.05$) across single species and 3-Sps litter mixtures independently, on the basis of linear models followed by pairwise multiple comparisons (Tukey test). PN+AG, ¾ poplar + ¼ alder; AG+PN, ¾ alder + ¼ poplar; PP+AG, ¾ pine + ¼ alder; AG+PP, ¾ alder + ¼ pine; PN+PP, ¾ poplar + ¼ pine.

Plant species	**C**	**N**	**P**	**C:N**	**C:P**	**N:P**	**Ca**
A. glutinosa (AG)	47.6 ± 0.3b	2.39 ± 0.06a	0.044 ± 0.004b	23.3 ± 0.5c	3020.1 ± 244.6a	129.1 ± 9.4a	1.57 ± 0.07b
P. nigra (PN)	44.6 ± 0.1c	0.52 ± 0.01b	0.068 ± 0.004a	99.9 ± 2.7b	1768.4 ± 103.6b	17.7 ± 1.1b	1.80 ± 0.04a
P. pinaster (PP)	49.0 ± 0.2a	0.49 ± 0.04b	0.066 ± 0.003a	123.0 ± 8.7a	2147.1 ± 111.9b	18.6 ± 1.6b	0.95 ± 0.07c
Litter mixtures							
PN+AG	45.4 ± 0.1c	0.99 ± 0.01b	0.062 ± 0.003a	80.9 ± 2.0b	2078.9 ± 103.4b	45.4 ± 2.9b	1.74 ± 0.04a
AG+PN	46.9 ± 0.2b	1.92 ± 0.04a	0.050 ± 0.003b	42.5 ± 0.5c	2707.6 ± 187.9a	101.3 ± 7.2a	1.63 ± 0.06a
PP+AG	48.7 ± 0.2a	0.89 ± 0.04b	0.061 ± 0.003ab	101.7 ± 6.9a	2333.4 ± 121.0ab	42.1 ± 2.0b	1.08 ± 0.07c
AG+PP	48.0 ± 0.2a	1.84 ± 0.05a	0.050 ± 0.003b	52.4 ± 2.8c	2767.4 ± 190.5a	96.9 ± 6.5a	1.39 ± 0.07b
PN+PP	45.9 ± 0.1c	0.51 ± 0.01c	0.067 ± 0.002a	106.6 ± 1.6a	1877.4 ± 76.7b	18.0 ± 0.7c	1.55 ± 0.03ab

Plant species	**Mg**	**Si**	**Lignin**	**Tannins**	**Phenols**	**Toughness**	**SLA**
A. glutinosa (AG)	0.31 ± 0.01a	394.3 ± 37.0a	23.7 ± 1.2a	4.3 ± 0.8c	37.2 ± 2.3b	51.8 ± 4.5c	240.3 ± 30.6a
P. nigra (PN)	0.31 ± 0.02a	268.9 ± 19.0b	7.4 ± 0.4c	195.8 ± 26.6a	156.6 ± 7.0a	94.8 ± 5.0b	177.7 ± 7.0a
P. pinaster (PP)	0.19 ± 0.01b	461.0 ± 40.5a	13.0 ± 0.4b	30.9 ± 3.8b	36.6 ± 1.6b	492.6 ± 28.0a	20.1 ± 1.9b
Litter mixtures							
PN+AG	0.31 ± 0.01a	300.0 ± 20.7b	11.5 ± 0.3c	148.3 ± 20.0a	127.0 ± 5.5a	84.2 ± 4.4c	193.2 ± 8.2a
AG+PN	0.31 ± 0.01ab	363.0 ± 30.6ab	19.6 ± 0.8a	52.1 ± 6.5b	67.0 ± 2.9b	62.5 ± 4.2d	224.7 ± 22.6a
PP+AG	0.22 ± 0.01c	446.8 ± 37.0a	15.3 ± 0.4b	25.2 ± 3.0c	36.8 ± 1.6c	398.6 ± 21.8a	67.1 ± 6.4c
AG+PP	0.27 ± 0.01b	414.1 ± 34.5ab	20.6 ± 0.8a	12.1 ± 1.4d	37.1 ± 1.9c	180.5 ± 7.8b	176.2 ± 21.6ab
PN+PP	0.28 ± 0.01ab	324.2 ± 15.3b	9.1 ± 0.3c	148.4 ± 19.3a	122.1 ± 5.0a	209.3 ± 9.8b	132.3 ± 5.1b

Table S3.C3. Analysis of variance results for the effects of riparian type (-alder *vs.* +alder) and litter assemblage on leaf mass loss (LML), N and P changes, and net diversity effects (NDE) of mixture treatments. Each variable was tested against three fixed factors (Riparian type, Litter assemblage and their interaction) and a random factor (Stream nested within Riparian type). Analyses were carried out separately for each mesh type.

		Microbial Decomposition					Total Decomposition				
Factor	Fixed/ Random	χ^2	df	*p-value*	R^2 marginal	R^2 conditional	χ^2	df	*p-value*	R^2 marginal	R^2 conditional
LML					0.54	0.80				0.62	0.81
(Intercept)		1040.28	1	**<0.0001**			55.64	1	**<0.0001**		
Riparian type	F	2.39	1	0.1223			0.03	1	0.8651		
Litter assemblage	F	111.70	4	**<0.0001**			203.23	4	**<0.0001**		
Riparian type × Litter assemblage	F	12.45	4	**0.0143**			0.87	4	0.9287		
Stream (Riparian type)	R	-	2	0.1148			-	2	0.0517		
NDE on LML					0.27	0.68				0.11	0.56
(Intercept)		8.05	1	**0.0045**			2.97	1	0.0851		
Riparian type	F	6.59	1	**0.0103**			0.00	1	0.9597		
Litter assemblage	F	25.08	4	**<0.0001**			11.31	4	**0.0233**		
Riparian type × Litter assemblage	F	28.88	4	**<0.0001**			6.86	4	0.1433		
Stream (Riparian type)	R	-	2	0.1145			-	2	0.0667		
N change					0.74	0.81					
(Intercept)		2.49	1	0.1144							
Riparian type	F	4.55	1	**0.0330**							
Litter assemblage	F	114.68	4	**<0.0001**							
Riparian type × Litter assemblage	F	6.50	4	0.1650							
Stream (Riparian type)	R	-	2	0.9847							
NDE on N change					0.61	0.72					
(Intercept)		53.90	1	**<0.0001**							
Riparian type	F	7.32	1	**0.0068**							
Litter assemblage	F	108.50	4	**<0.0001**							
Riparian type × Litter assemblage	F	49.07	4	**<0.0001**							
Stream (Riparian type)	R	-	2	0.9834							
P change					0.39	0.61					
(Intercept)		1.70	1	0.1917							
Riparian type	F	0.00	1	0.9921							
Litter assemblage	F	38.17	4	**<0.0001**							
Riparian type × Litter assemblage	F	1.78	4	0.7770							
Stream (Riparian type)	R	-	2	0.6843							
NDE on P change					0.51	0.69					
(Intercept)		0.06	1	0.8020							
Riparian type	F	2.16	1	0.1416							
Litter assemblage	F	12.69	4	**0.0129**							
Riparian type × Litter assemblage	F	20.71	4	**0.0004**							
Stream (Riparian type)	R	-	2	0.6523							

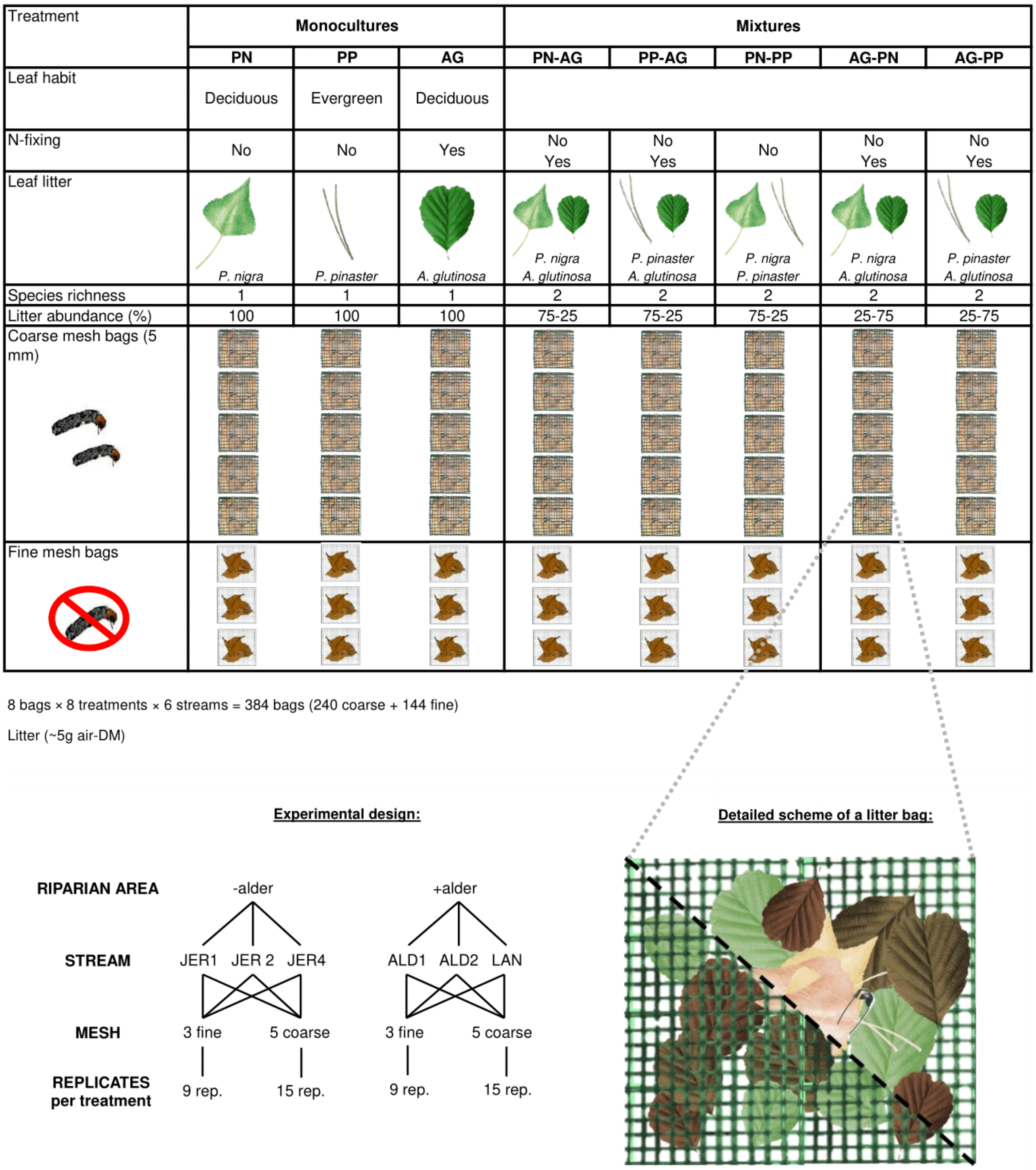
Table S4.C3. Indicators of stream ecosystem attributes based on Functional Feeding Group (FFG) ratios for each of the studied streams before the development of the experiment. No significant differences were found between riparian types (T-test, $p > 0.05$). Auto/Hetero: Autotrophy to Heterotrophy Index, estimated as Scrapers to Shredders + Total Collectors ratio; CPOM/FPOM: the Coarse Particulate Organic Matter to Fine Particulate Organic Matter Index, estimated as Shredders to Total Collectors ratio; and Top-Down Control: Top-Down Predator Control Index, estimated as Predators to total All other groups ratio.

Stream ID	**Stream**	**Auto/ Hetero**	**CPOM/ FPOM**	**Top-Down Control**
With alder		**0.57**	**11.77**	**0.09**
ALD1	Barranco de los Pasillos	0.41	12.41	0.11
ALD2	Barranco de los Recodos	0.71	20.14	0.08
LAN	Río del Pueblo	0.58	2.76	0.09
Without alder		**0.39**	**2.74**	**0.09**
JER1	Barranco de los Ciruelillos	0.44	5.47	0.10
JER2	Barranco de Alcázar	0.36	1.57	0.13
JER4	Barranco de Alhorí	0.38	1.17	0.04

Table S5.C3. Mean nutrient concentrations at the beginning and the end of the experiment and relative nutrient concentration change of each of the assemblages used in the experiment at each of the riparian types.

Riparian type	Assemblage	Initial N (%)	Final N (%)	Change (%)	Initial P (%)	Final P (%)	Change (%)
Without alder	AG	2.49	3.38	35.97	0.05	0.09	80.77
	PN	0.50	0.99	96.36	0.07	0.08	16.33
	PP	0.57	0.46	-19.01	0.07	0.03	-51.31
	PN+AG	0.98	1.41	43.60	0.07	0.09	37.06
	AG+PN	1.98	2.49	25.95	0.05	0.08	51.78
	PP+AG	0.98	0.95	-3.36	0.06	0.05	-20.03
	AG+PP	1.94	2.34	20.31	0.05	0.07	23.92
	PN+PP	0.52	0.82	57.71	0.07	0.07	-4.18
With alder	AG	2.29	3.21	40.16	0.04	0.10	163.69
	PN	0.54	1.01	85.70	0.06	0.08	23.78
	PP	0.41	0.51	24.25	0.07	0.03	-48.67
	PN+AG	0.97	1.47	51.54	0.06	0.08	32.59
	AG+PN	1.85	2.56	38.05	0.05	0.07	51.94
	PP+AG	0.82	0.93	13.42	0.06	0.05	-23.73
	AG+PP	1.75	2.23	27.46	0.05	0.06	28.08
	PN+PP	0.50	0.90	79.10	0.06	0.07	11.14

Figure S1.C3. Experimental set up.



Treatment	Monocultures			Mixtures				
	PN	PP	AG	PN-AG	PP-AG	PN-PP	AG-PN	AG-PP
Leaf habit	Deciduous	Evergreen	Deciduous					
N-fixing	No	No	Yes	No Yes	No Yes	No	No Yes	No Yes
Leaf litter	*P. nigra*	*P. pinaster*	*A. glutinosa*	*P. nigra* *A. glutinosa*	*P. pinaster* *A. glutinosa*	*P. nigra* *P. pinaster*	*P. nigra* *A. glutinosa*	*P. pinaster* *A. glutinosa*
Species richness	1	1	1	2	2	2	2	2
Litter abundance (%)	100	100	100	75-25	75-25	75-25	25-75	25-75
Coarse mesh bags (5 mm)								
Fine mesh bags								

8 bags × 8 treatments × 6 streams = 384 bags (240 coarse + 144 fine)

Litter (~5g air-DM)

Figure S2.C3. Mean (proportion) FPOM (i.e. leaf litter) available in the studied streams before the development of the experiment.

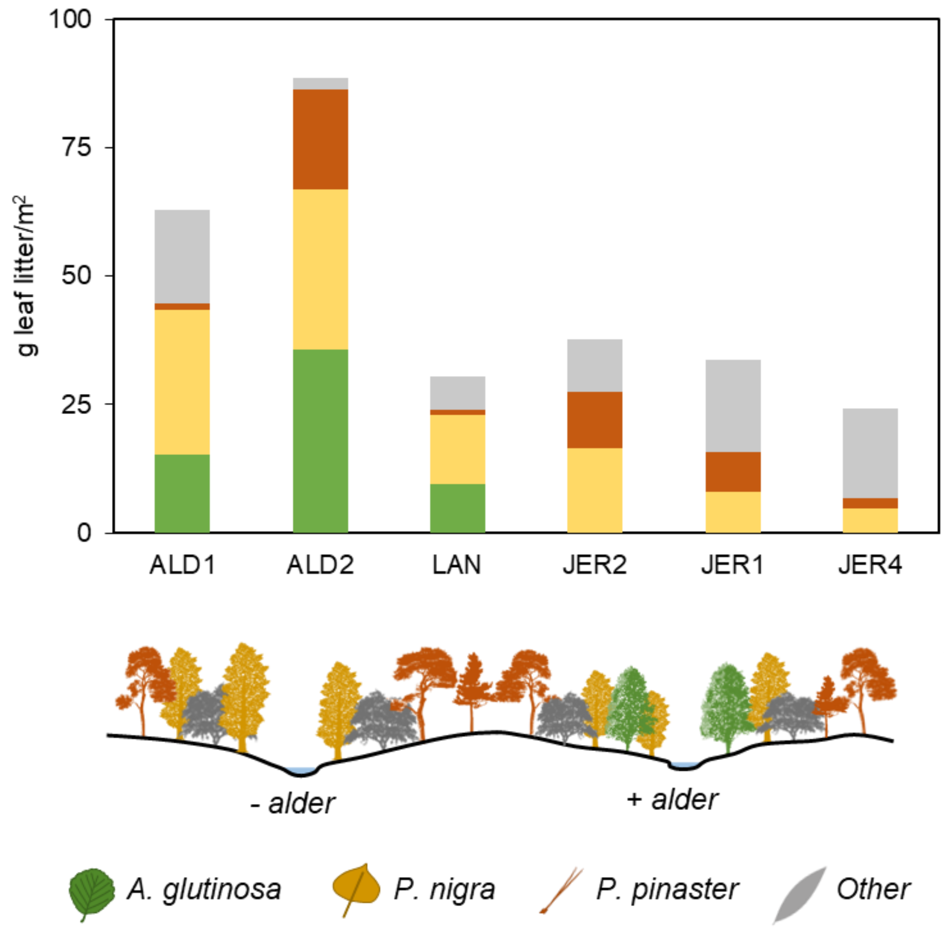
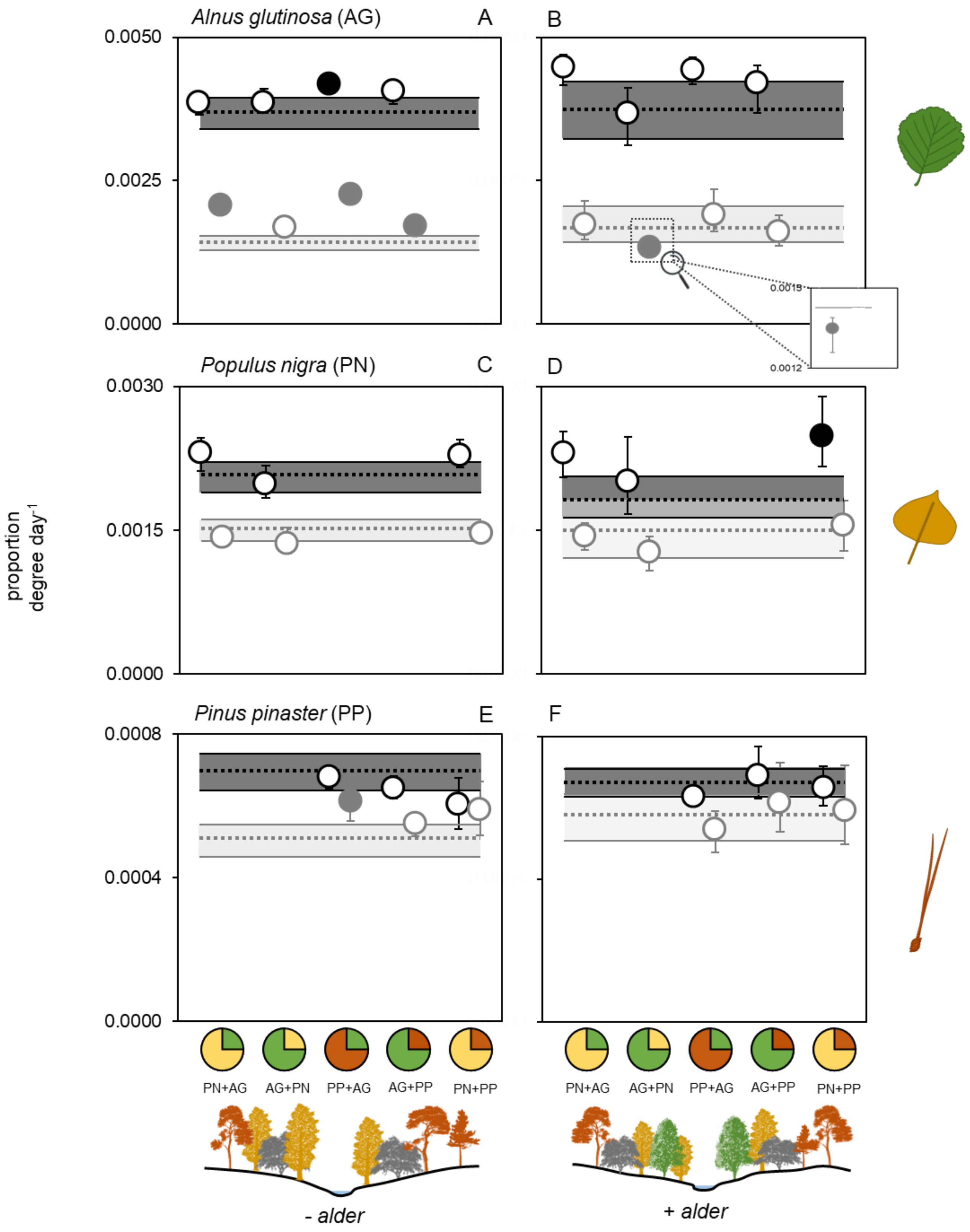


Figure S3.C3. Comparison of litter mass loss (LML; proportion degree day^{-1}) of each litter species among treatments where present. Circles are mean values in mixtures (black and grey represent total and microbial decomposition, respectively). Dotted lines represent monoculture values, following the same color scale. Whiskers and shadows (dark grey for total decomposition) denote upper and lower limits of 95% nonparametric bootstrapped confidence intervals for mixtures and monoculture values, respectively. Closed symbols represent intervals that are significantly different of monoculture values.



Supplementary material **Chapter 4**

Cross-species coprophagy in small stream detritivores counteracts low-quality litter: native vs. invasive plant litter

SUPPLEMENTARY METHODS

Analyses of leaf-litter traits and nutrients in faeces

Litter trait characterization was performed on post-leached discs (n = 7). Toughness was measured on wet litter discs as the force needed to pierce a leaf disc using a Texture Analyzer (TA.XTPlus) equipped with a steel rod (0.7 mm diameter). For composition analyses, leaf discs were desiccated (70 ºC, 72 h) and ground (Mixer Mill RETSCH MM 200) to fine powder (< 1 mm particle size). C and N concentration were determined using a Perkin Elmer series II CHNS/O elemental analyser. P concentration was measured spectrophotometrically after autoclave-assisted extraction (APHA, 1998). Percentage lignin was determined using an ANKOM 200/220 fibre analyser (ANKOM Technologies, Macedon, NY, USA). Total phenolics were determined following the Folin & Ciocalteu procedure (Bärlocher & Graça, 2005). Condensed tannins were estimated by the acid butanol assay (Gessner & Steiner, 2020). The concentration of Si was determined using inductively coupled plasma atomic emission spectroscopy (Thermo ICAP 6500 duo, Thermo Fisher Scientific, Cambridge, UK), after microwave sample digestion in nitric acid (65%) and hydrogen peroxide (30%). Finally, Ca, K and Mg concentration (% DM) were determined by an inductively coupled plasma mass spectrometry (ICP-MS,Perkin Elmer DRC II). Faeces C, N and P concentrations were determined as for leaf litter.

Elemental imbalance between litter and faeces

We calculated the elemental imbalance (EI; see Frainer et al., 2016) between leaf litter and faeces to quantify such differences as the quotient between leaf litter and faeces C:N, C:P or N:P molar ratios as follows:

$$EI_{X:Y^{lf}} = \frac{X:Y^{l}}{X:Y^{f}}$$

where X:Y is the molar ratio (C:N, C:P or N:P) of litter *l* and faeces *f*. Values higher than 1 indicate an enrichment of nutrient Y in faeces; whereas values lower than 1 indicate an impoverishment of nutrient Y in faeces (i.e. nutrient Y is being assimilated by the animals). We estimated upper and lower bounds of 95% nonparametric bootstrapped confidence intervals with *boot.ci* function of `boot' R package (Canty & Ripley, 2019), to explore those intervals that do not reject the null hypothesis (i.e., do not contain the value of one). To explore differences among treatments we used two-sample t-test or Welch two-sample t-test analyses if homoscedasticity was not achieved.

Table S1.C4. Environmental characteristics (mean ± SD) of the stream of origin of detritivores. Climatic data was obtained from the Environmental Information Network of Andalusia (REDIAM, http://www.juntadeandalucia.es/medioambiente/site/web/rediam). Stream water data was obtained in-situ (discharge, pH, CE, alkalinity and DO) or in laboratory from samples collected at the same time than detritivores (P-PO_4, total dissolved P and N-NO_3).

Stream & locality	Barranco del Cura (Almería, Spain)
Protected Natural Area	Sierra de Gádor
Longitude/Latitude	36.84 N / 2.64 W
Altitude (m a.s.l.)	291
Climate	
Mean Temp. (ºC)	16.27 ± 0.37
Max Temp. (ºC)	21.34 ± 0.40
Min Temp. (ºC)	11.20 ± 0.33
Annual PP (mm)	387.80 ± 17.25
Days with PP	31.56 ± 0.09
Stream water	
Discharge (L s-1)	9
pH	8.13
CE (µS cm^{-1})	896
Alkalinity (mg $CaCO_3$ L^{-1})	250
DO (mg O2 L^{-1})	8.12
DO (% SAT)	88.3
P-PO_4 (µg P L^{-1})	1.4
Total dissolved P (µg P L^{-1})	7
N-NO_3 (µg L^{-1})	723

Table S2.C4. Mean ± SE of toughness (g), carbon (C), nitrogen (N) and phosphorus (P) concentrations (% DM), molar elemental ratios (C:N, C:P and N:P), lignin content (% DM), total phenols (mg tannic acid equivalents /g litter DM) and condensed tannins (mg Quebracho tannins equivalents/g litter DM), and concentration of Si, Ca, K and Mg (% DM) of each plant species (*Arundo* and *Populus*) determined on instream conditioned litter. Differences ($p < 0.05$) are shown on the basis of T-test analyses (n = 7).

	Arundo	*Populus*	*p*-value
Toughness	393.64 ± 50.90	82.03 ± 5.91	0.0005
C	43.68 ± 0.19	46.59 ± 0.20	<0.0001
N	0.56 ± 0.02	2.19 ± 0.07	<0.0001
P	0.016 ± 0.001	0.059 ± 0.004	<0.0001
C:N	92.13 ± 3.09	25.07 ± 0.90	<0.0001
C:P	7143.54 ± 449.13	2098.13 ± 126.98	<0.0001
N:P	78.05 ± 4.91	84.34 ± 5.10	0.3804
Lignin	2.40 ± 0.09	14.49 ± 3.68	0.0029
Total phenols	0.04 ± 0.02	0.04 ± 0.02	0.8197
Condensed tannins	0.75 ± 0.07	0.53 ± 0.08	0.1086
Si	1.22 ± 0.14	0.58 ± 0.03	0.0103
Ca	0.39 ± 0.02	2.73 ± 0.05	<0.0001
K	0.036 ± 0.004	0.119 ± 0.009	0.0008
Mg	0.11 ± 0.01	0.25 ± 0.01	0.0009

Table S3.C4. Effect sizes for all the possible two sample comparisons. We report mean treatment effect sizes (effsize) and 95% confidence intervals (conf.) where effect sizes of 0.20–0.49, 0.50–0.79 and ≥0.80 were considered small, medium and large, respectively (Cohen, 1988). Large effect sizes are in bold.

Variable	group1	group2	effsize	n1	n2	conf. low	conf. high	magnitude	effsize	n1	n2	conf. low	conf. high	magnitude
Litter consumption	*Arundo*	*Populus*	**-4.72**	**15**	**15**	**-6.16**	**-3.11**	**large**	**-3.87**	**30**	**30**	**-4.62**	**-2.99**	**large**
Growth	*Arundo*	E-AD	-0.57	15	14	-1.32	0.24	moderate						
	Arundo	M-AD	**-0.91**	**15**	**15**	**-1.57**	**-0.09**	**large**	0.2	30	15	-0.41	0.76	small
	Arundo	*Populus*	-0.21	15	15	-0.93	0.69	small	0.23	30	30	-0.27	0.82	small
	Arundo	E-PA	-0.6	15	15	-1.3	0.27	moderate						
	Arundo	M-PA	**-1.17**	**15**	**15**	**-1.97**	**-0.35**	**large**	0.45	30	15	-0.18	1.06	small
	E-AD	M-AD	-0.32	14	15	-1.06	0.45	small						
	E-AD	*Populus*	0.46	14	15	-0.36	1.17	small						
	E-AD	E-PA	0.11	14	15	-0.81	0.89	negligible						
	E-AD	M-PA	-0.58	14	15	-1.35	0.27	moderate						
	M-AD	*Populus*	**0.89**	**15**	**15**	**0.2**	**1.49**	**large**	0.04	15	30	-0.53	0.71	negligible
	M-AD	E-PA	0.59	15	15	-0.14	1.23	moderate						
	M-AD	M-PA	-0.26	15	15	-1.1	0.53	small	0.33	15	15	-0.37	1.15	small
	Populus	E-PA	-0.5	15	15	-1.19	0.37	small						
	Populus	M-PA	**-1.27**	**15**	**15**	**-1.92**	**-0.49**	**large**	0.22	30	15	-0.39	0.75	small
	E-PA	M-PA	**-1.1**	**15**	**15**	**-1.98**	**-0.26**	**large**						
Lipids content	*Arundo*	E-AD	-0.12	11	9	-1.16	1.08	negligible						
	Arundo	E-PA	**-1.04**	**11**	**7**	**-2.14**	**0.23**	**large**						
	Arundo	M-AD	**-2.07**	**11**	**4**	**-3.86**	**-0.56**	**large**	0.16	15	15	-0.63	0.9	negligible
	Arundo	M-PA	**-2.39**	**11**	**7**	**-3.53**	**-1.24**	**large**	-0.22	15	15	-1.04	0.6	small
	Arundo	*Populus*	0.16	11	9	-0.85	1.23	negligible	0.58	15	15	-0.22	1.28	moderate
	E-AD	E-PA	**-0.83**	**9**	**7**	**-1.87**	**0.35**	**large**						
	E-AD	M-AD	**-1.65**	**9**	**4**	**-2.79**	**-0.23**	**large**						
	E-AD	M-PA	**-1.99**	**9**	**7**	**-2.87**	**-0.86**	**large**						
	E-AD	*Populus*	0.26	9	9	-1.02	1.21	small						
	E-PA	M-AD	-0.67	7	4	-2.01	0.74	moderate						
	E-PA	M-PA	**-1.02**	**7**	**7**	**-2.15**	**0.29**	**large**						
	E-PA	*Populus*	**1.21**	**7**	**9**	**-0.38**	**2.49**	**large**						
	M-AD	M-PA	-0.5	4	7	-1.47	2.03	small	-0.38	15	15	-1.15	0.41	small
	M-AD	*Populus*	**2.49**	**4**	**9**	**-0.81**	**3.64**	**large**	0.39	15	15	-0.38	1.1	small
	M-PA	*Populus*	**2.72**	**7**	**9**	**1.91**	**3.56**	**large**	**0.82**	**15**	**15**	**0.04**	**1.5**	**large**
Glycogen content	*Arundo*	E-AD	-0.08	11	9	-1.05	0.81	negligible						
	Arundo	E-PA	-0.1	11	7	-1.27	0.92	negligible						
	Arundo	M-AD	**-1.39**	**11**	**4**	**-2.75**	**-0.16**	**large**	**1.21**	**15**	**15**	**0.46**	**2.02**	**large**
	Arundo	M-PA	-0.6	11	7	-1.8	0.41	moderate	0.34	15	15	-0.42	1.09	small
	Arundo	*Populus*	0.33	11	9	-0.6	1.37	small	0.04	15	15	-0.83	0.73	negligible
	E-AD	E-PA	-0.04	9	7	-1.08	0.99	negligible						
	E-AD	M-AD	**-2.47**	**9**	**4**	**-4.47**	**-0.22**	**large**						
	E-AD	M-PA	**-0.96**	**9**	**7**	**-2.1**	**0.28**	**large**						
	E-AD	*Populus*	0.61	9	9	-0.5	1.57	moderate						
	E-PA	M-AD	**-2.96**	**7**	**4**	**-4.88**	**0.0027**	**large**						
	E-PA	M-PA	**-1.11**	**7**	**7**	**-2.51**	**0.19**	**large**						
	E-PA	*Populus*	0.7	7	9	-0.38	1.82	moderate						
	M-AD	M-PA	**1.83**	**4**	**7**	**0.8**	**3.54**	**large**	-0.67	15	15	-1.44	0.2	moderate
	M-AD	*Populus*	**2.68**	**4**	**9**	**-0.72**	**4.07**	**large**	**-0.96**	**15**	**15**	**-1.72**	**-0.1**	**large**
	M-PA	*Populus*	**1.45**	**7**	**9**	**0.35**	**2.53**	**large**	-0.26	15	15	-1	0.56	small
Survival	*Arundo*	E-AD	-0.234	32	27	-0.81	0.29	small						
	Arundo	E-PA	-0.648	32	20	-1.31	-0.05	moderate						
	Arundo	M-AD	-0.567	32	21	-1.14	0.04	moderate						
	Arundo	*M-PA*	**-0.982**	**32**	**17**	**-1.63**	**-0.34**	**large**						
	Arundo	*Populus*	**-0.851**	**32**	**18**	**-1.47**	**-0.21**	**large**						
	E-AD	E-PA	-0.404	27	20	-1.03	0.27	small						
	E-AD	M-AD	-0.325	27	21	-0.87	0.28	small						
	E-AD	*M-PA*	-0.729	27	17	-1.28	-0.05	moderate						
	E-AD	*Populus*	-0.602	27	18	-1.26	0.03	moderate						
	E-PA	M-AD	0.0787	20	21	-0.56	0.74	negligible						
	E-PA	*M-PA*	-0.333	20	17	-0.93	0.43	small						
	E-PA	*Populus*	-0.2	20	18	-0.86	0.54	small						
	M-AD	M-PA	-0.41	21	17	-0.97	0.28	small						
	M-AD	*Populus*	-0.28	21	18	-0.88	0.43	small						
	M-PA	*Populus*	0.136	17	18	-0.61	0.79	negligible						

Figure S1.C4. Experimental set up. Each glass microcosm has a continuous air supply and is divided in two parts with a mesh net. In the upper part one individual is fed leaf-litter discs. In the lower part, one individual is fed faeces produced by the individual present in the upper part.

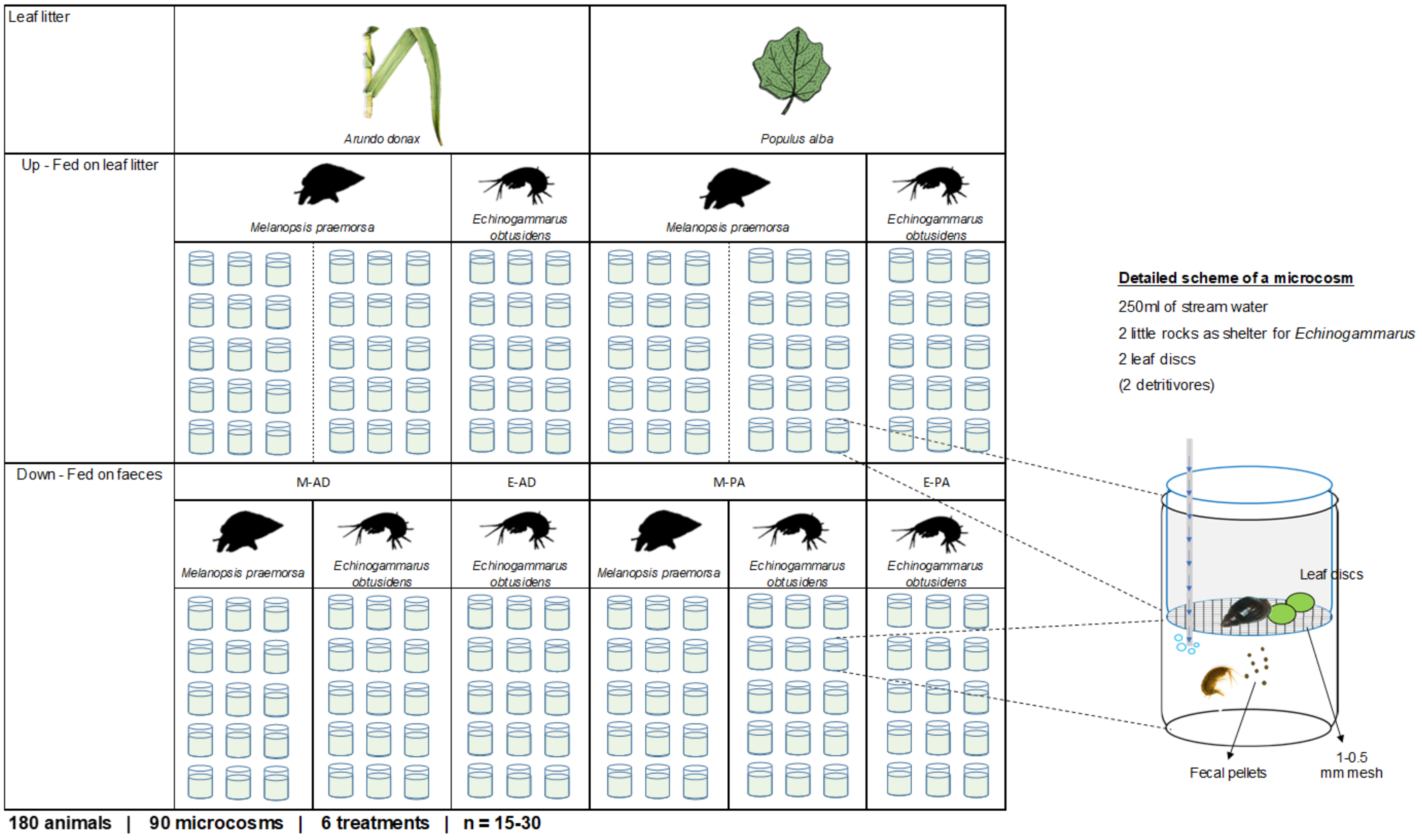


Figure S2.C4. Relationships between total body length and body dry mass (DM) for *Echinogammarus obtusidens* (A) and between total shell length and dry mass (DM) for *Melanopsis praemorsa* (B), used to estimate initial detritivore biomass in experimental microcosms.

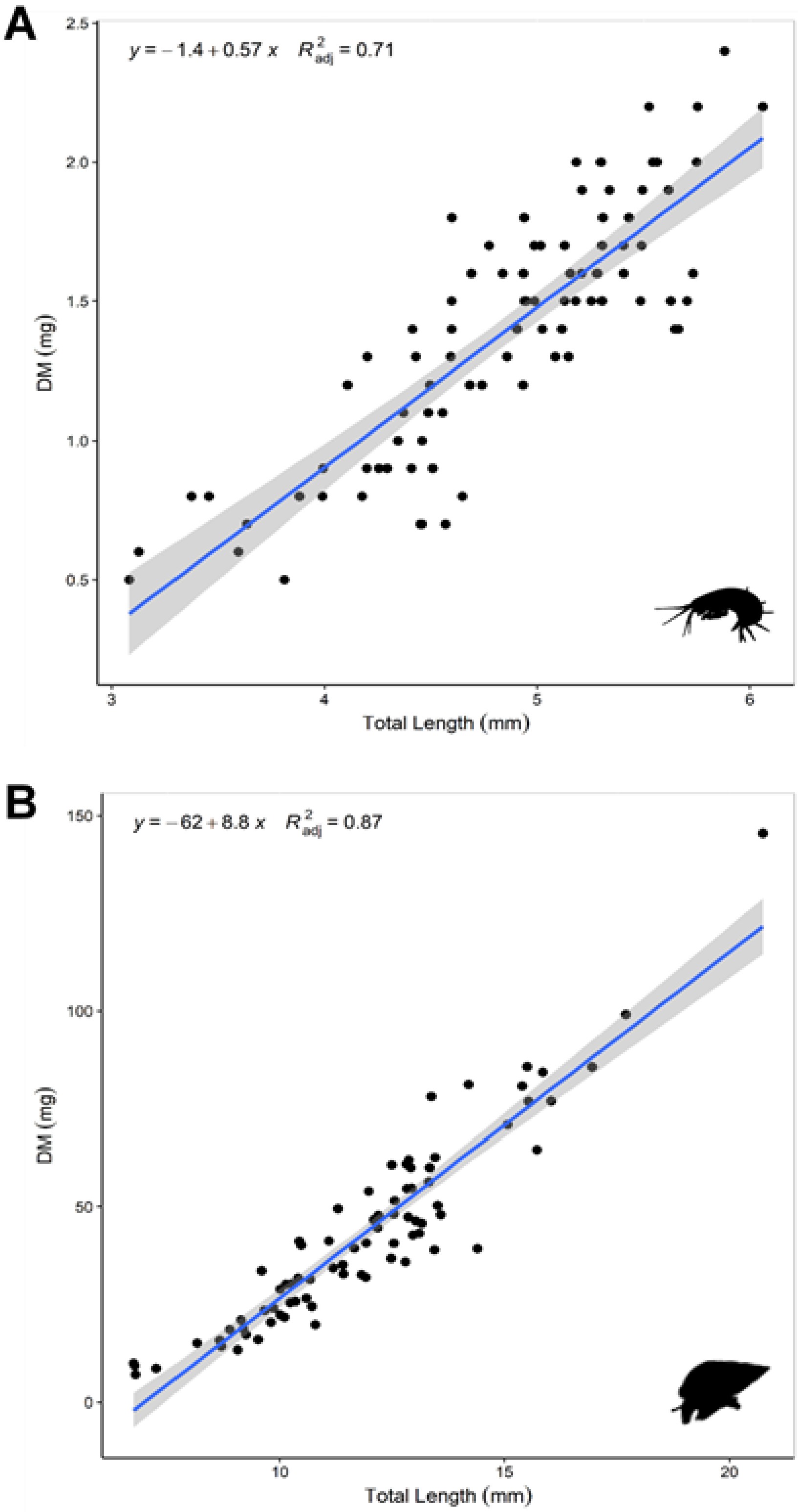


Figure S3.C4. Projection of the 2 first principal component axis showing differences among litter traits of the 2 plant species, after incubation for 2 weeks in the stream (Explained variance = 84.9%). Vectors represent each leaf trait weight and symbols each plant litter position: red circles and blue triangles represent trait values calculated for *Arundo donax* and *Populus alba*, respectively. Ellipses represent 95% confidence and centroids the average position of each species over the PCA axes (n = 7).

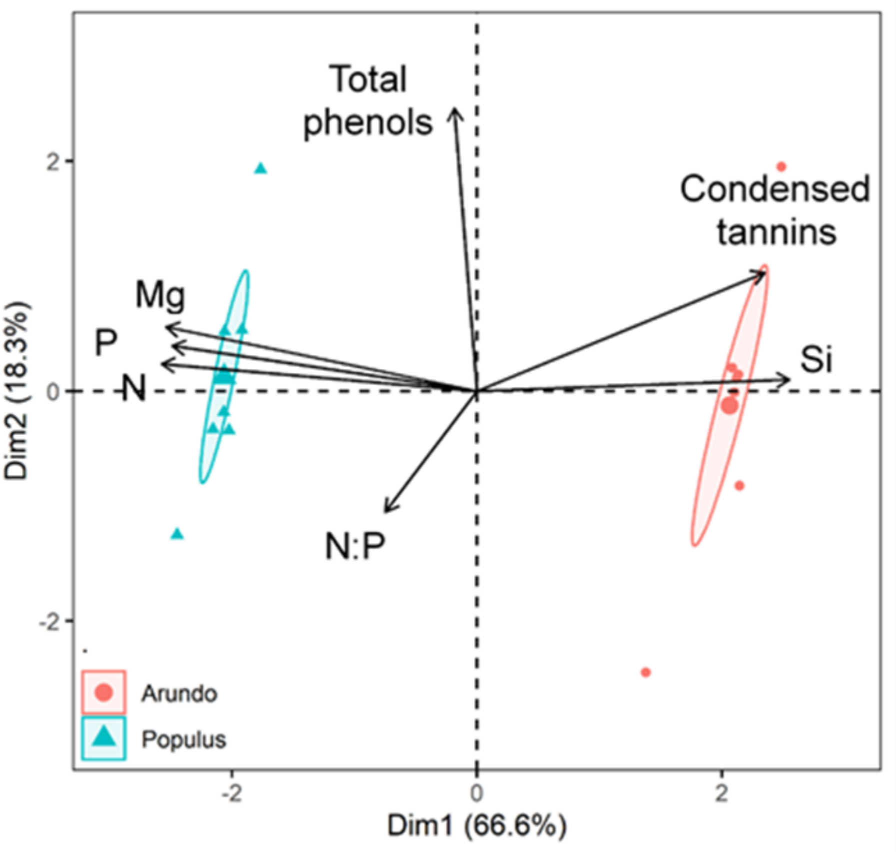
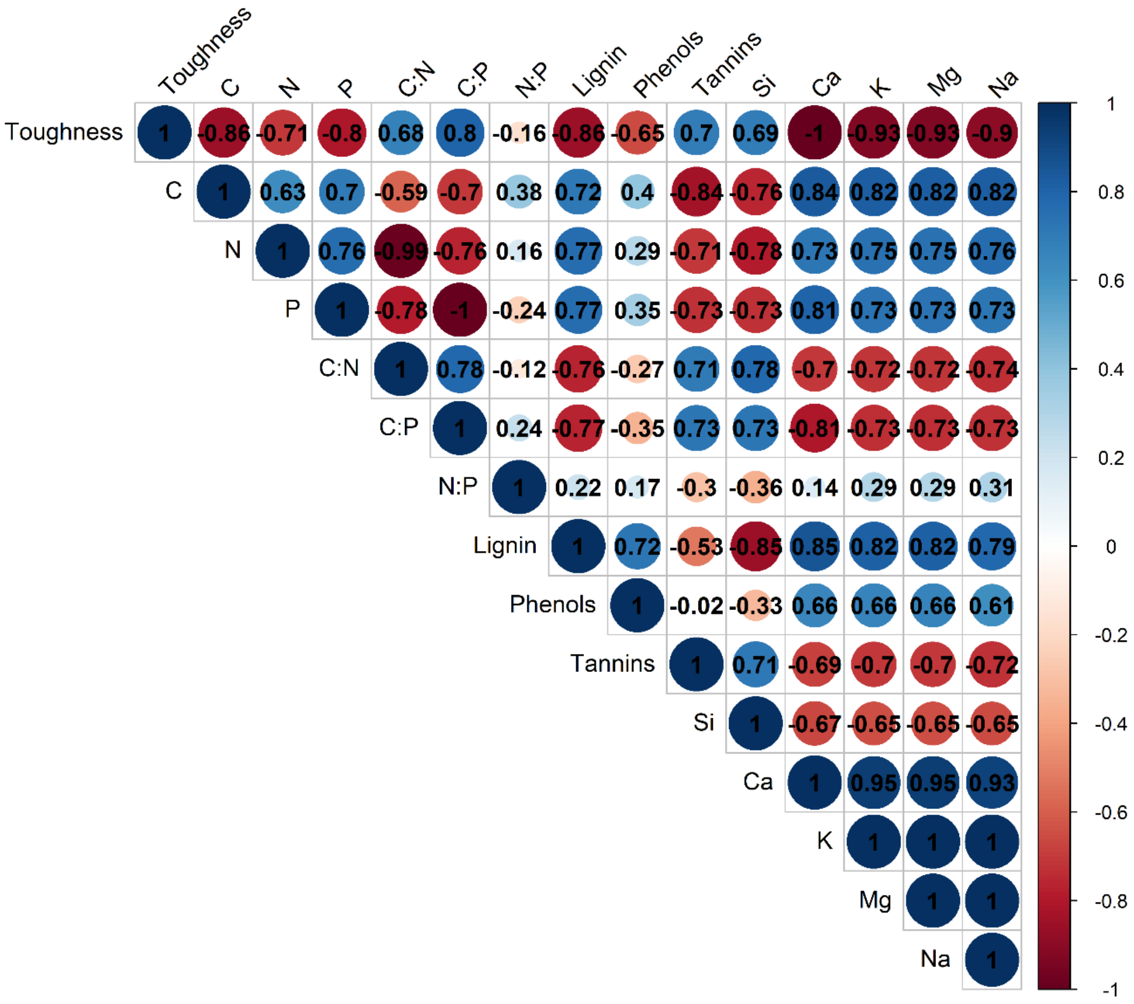


Figure S4.C4. Visual Spearman correlation matrix between leaf litter traits. Positive correlations are displayed in blue and negative correlations in red colour. Values represent the correlation coefficients. Colour intensity and the size of the circle are proportional to the correlation coefficients.



	Toughness	C	N	P	C:N	C:P	N:P	Lignin	Phenols	Tannins	Si	Ca	K	Mg	Na
Toughness	1	-0.86	-0.71	-0.8	0.68	0.8	-0.16	-0.86	-0.65	0.7	0.69	-1	-0.93	-0.93	-0.9
C		1	0.63	0.7	-0.59	-0.7	0.38	0.72	0.4	-0.84	-0.76	0.84	0.82	0.82	0.82
N			1	0.76	-0.99	-0.76	0.16	0.77	0.29	-0.71	-0.78	0.73	0.75	0.75	0.76
P				1	-0.78	-1	-0.24	0.77	0.35	-0.73	-0.73	0.81	0.73	0.73	0.73
C:N					1	0.78	-0.12	-0.76	-0.27	0.71	0.78	-0.7	-0.72	-0.72	-0.74
C:P						1	0.24	-0.77	-0.35	0.73	0.73	-0.81	-0.73	-0.73	-0.73
N:P							1	0.22	0.17	-0.3	-0.36	0.14	0.29	0.29	0.31
Lignin								1	0.72	-0.53	-0.85	0.85	0.82	0.82	0.79
Phenols									1	-0.02	-0.33	0.66	0.66	0.66	0.61
Tannins										1	0.71	-0.69	-0.7	-0.7	-0.72
Si											1	-0.67	-0.65	-0.65	-0.65
Ca												1	0.95	0.95	0.93
K													1	1	1
Mg														1	1
Na															1

Figure S5.C4. Elemental imbalance between C:N (A), C:P (B) and N:P (C) ratios in leaves of *Arundo donax* (1) and *Populus alba* (2) and in faeces of *Echinogammarus obtusidens* and *Melanopsis praemorsa* fed on those leaves during 21 days experiments. Values are expressed as the quotient between leaf litter and faeces nutrient molar ratios (X:Ylitter/X:Yfaeces). Circles are means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that do not reject the null hypothesis (i.e., do not contain the value of one) and open circles represent intervals that do reject the null hypothesis. Dash lines show 1:1 relationships. Values > 1 indicate an enrichment of nutrient Y in faeces; values <1 indicate an impoverishment of nutrient Y in faeces.

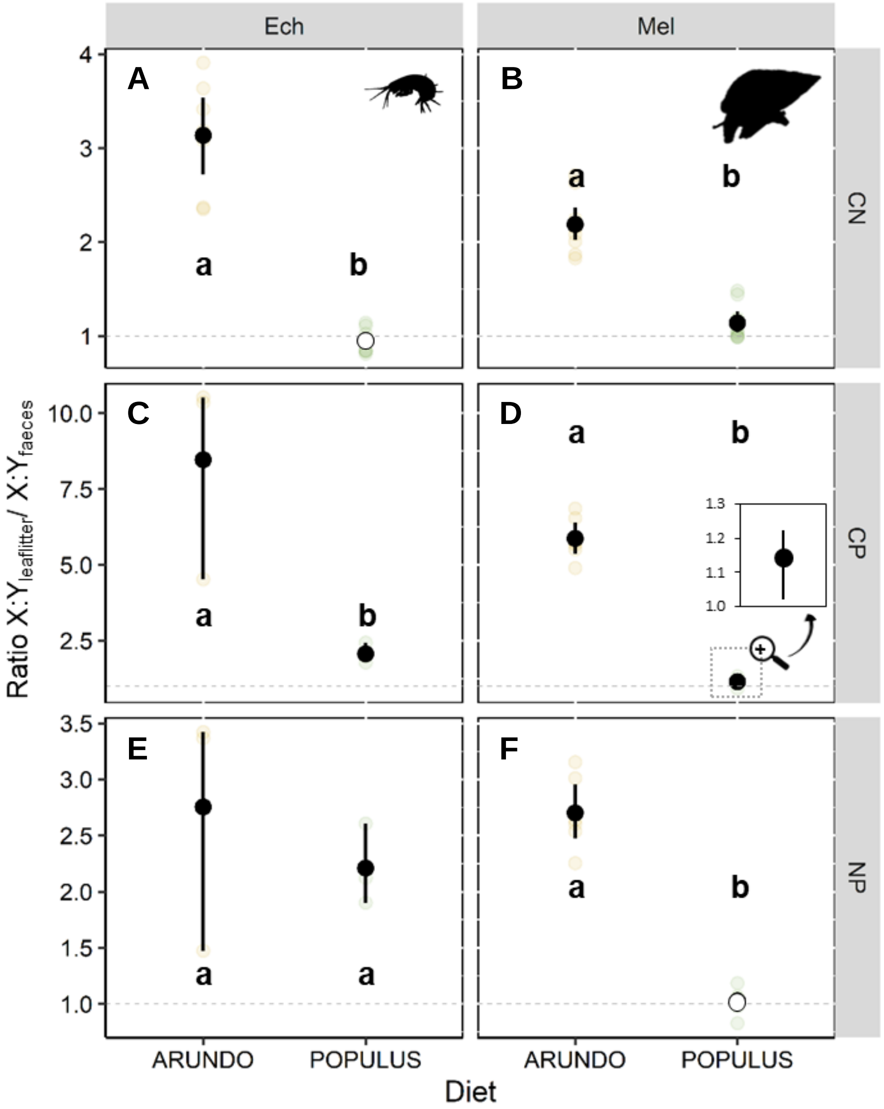


Figure S6.C4. Effects of treatments on *Echinogammarus obtusidens* survival probability during 21 days experiments. Values are expressed as % of survival. Different letters indicate significant differences (Log rank test, p<0.05).

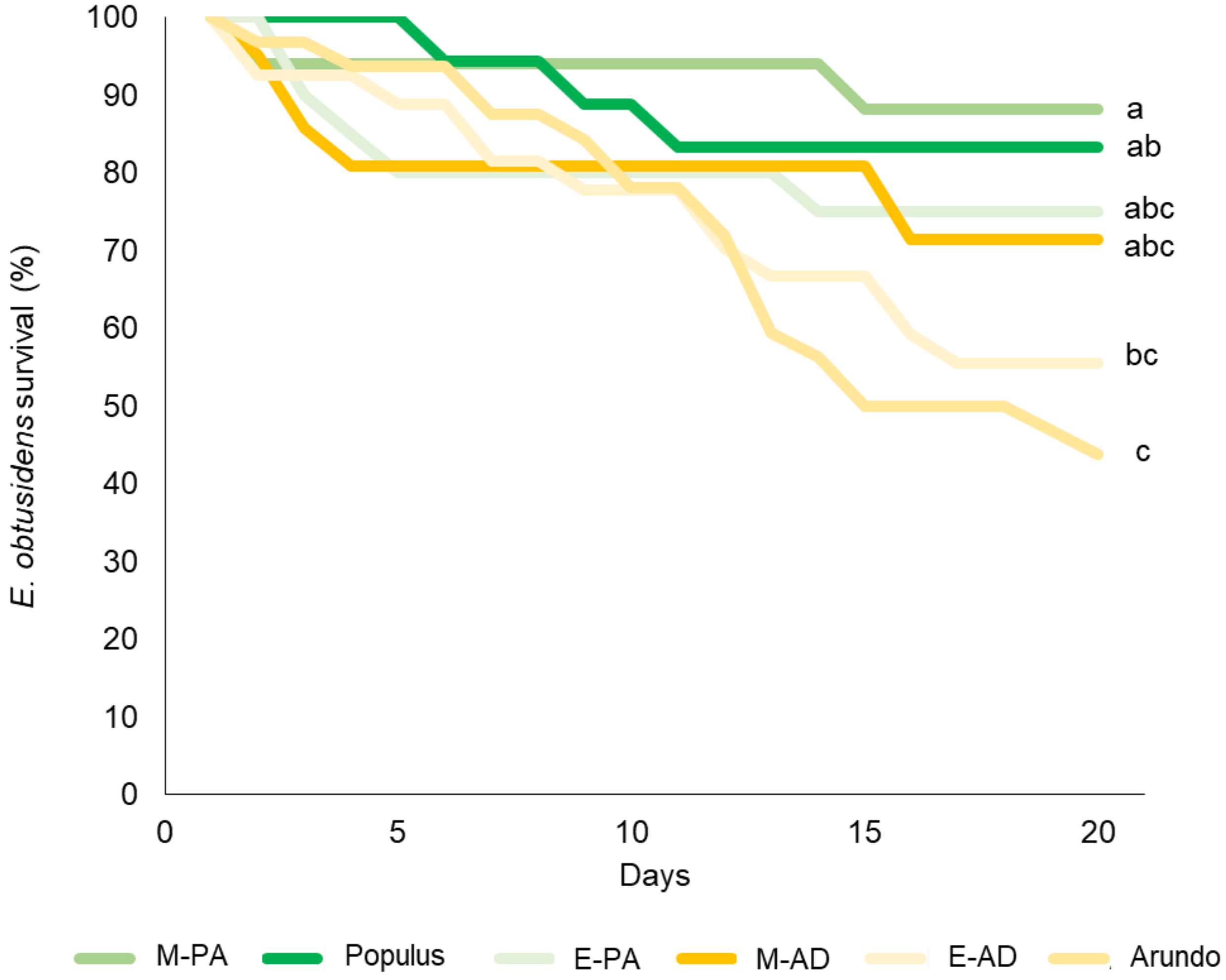
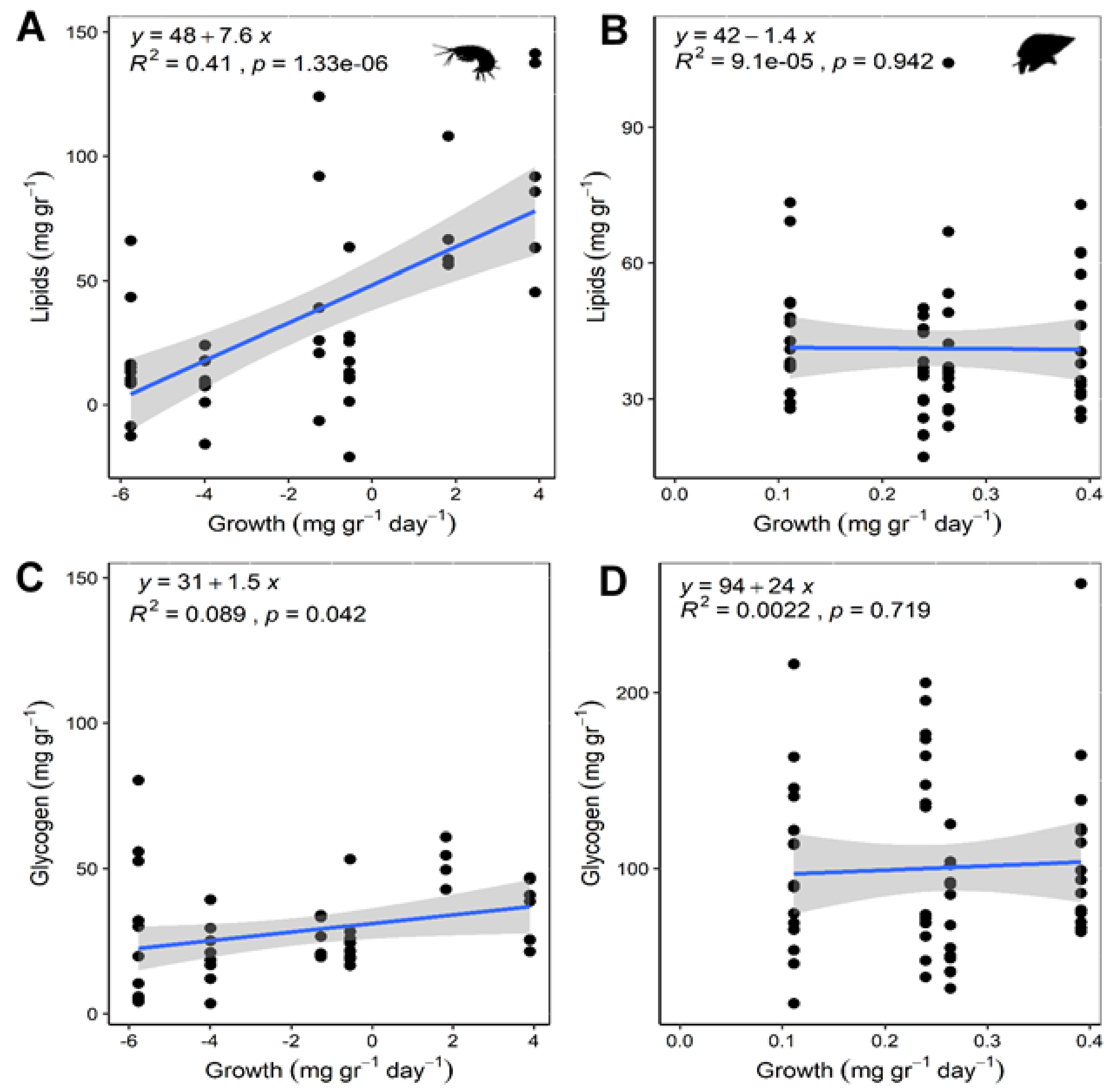


Figure S7.C4. Relationships between lipids and glycogen content and growth rates of *Echinogammarus obtusidens* and *Melanopsis praemorsa*.



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RESEARCH ARTICLE

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Climate-induced plasticity in leaf traits of riparian plants

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Abstract

Aim: Leaf litter inputs from riparian vegetation and its decomposition play a key role in energy and nutrient transfer in many stream ecosystems. Instream leaf litter decomposition is driven by both leaf traits and environmental conditions. Therefore, understanding and predicting leaf trait variation under current environmental changes and their putative interactive effects on stream food webs is a critical challenge. Most studies have focussed on the assumed higher interspecific leaf trait variability, with little research addressing an intraspecific perspective.

Location: Andalusia, Spain.

Methods: We assessed the relative effects of climate and soil conditions on the plasticity of leaf traits of four common woody riparian species in permanent low-order Mediterranean streams across a wide aridity gradient. We used a space-for-time substitution approach to predict leaf trait changes and consequences for stream food webs in a future climate change scenario.

Results: Overall, we found that aridity had a major influence on leaf trait plasticity but with opposite patterns depending on plant functional type, although soil was the strongest predictor in some cases. Results indicated that leaf quality—linked to palatability and decomposability—of *Alnus glutinosa*, *Salix atrocinerea* and *Rubus ulmifolius* (deciduous/semi-deciduous) will decrease with forecasted aridification, whereas the palatability of the evergreen *Nerium oleander* will increase. We observed higher trait plasticity than interspecific variation for leaf P, Ca and Mg concentrations and C:P ratio.

Main conclusions: Our findings suggest a decrease of intraspecific leaf quality in riparian deciduous species with global warming in a relatively short term. In a longer term, this may merge with the forecasted dieback of deciduous species in riparian corridors of temperate climate zones. These changes have the potential to significantly impair ecosystem functioning of Mediterranean mountain streams currently under deciduous gallery forests.

KEYWORDS
Aridification, deciduous, evergreen, instream decomposition, litter quality, soil, space-for-time substitution

1 | INTRODUCTION

The warming of the Earth system is unequivocal (IPCC, 2021). Globally, precipitation is also predicted to increase in the long term (Hewitson et al., 2015). However, forecasts in the Mediterranean basin point to a precipitation decrease of around 34%, along with a temperature increase of *ca.* 5°C for the period 2000–2099 (Harris et al., 2013). As a result, this region will face a climate much drier and hotter than at present, especially during warm seasons (Giorgi & Lionello, 2008), with direct effects on hydrologic regimes (Nohara et al., 2006; Vicente-Serrano et al., 2014) and soil moisture (Manabe et al., 2004). These changes may alter the functioning and structure of plant communities (e.g. Carnicer et al., 2011; Trivedi et al., 2008; Vicente-Serrano et al., 2012).

Small streams flowing through forested areas can be especially susceptible to climate change-induced alterations in plant communities, owing to their high dependence on organic matter inputs from the riparian vegetation, i.e. leaf litter (Wallace et al., 2015). Instream decomposition of leaf litter is a crucial ecosystem process, involving the cycling of nutrients and fuelling stream secondary production (Marks, 2019). The rate at which leaf litter decomposes and is incorporated into food webs highly depends on its quality, which fundamentally depends on after-life persistent traits (Graça & Cressa, 2010; Graça et al., 2001; Zhang et al., 2019). Thus, ecosystem functioning can be significantly altered if leaf litter inputs to streams experience physical and chemical changes (e.g. Casas et al., 2013; del Campo et al., 2021; López-Rojo et al., 2019). These changes can be interspecific, e.g. resulting from the forecasted substitution of deciduous by evergreen species (Kominoski et al., 2013; Salinas et al., 2018) and/or the decline of key plant species populations (e.g. alder; Alonso et al., 2021; Rubio-Ríos et al., 2021). Moreover, given that leaf traits are highly responsive to environmental changes (Heilmeier, 2019; Soudzilovskaia et al., 2013), intraspecific changes may also occur, e.g. due to genetic variability (Crutsinger et al., 2014; LeRoy et al., 2012) or phenotypic plasticity (Graça & Poquet, 2014; Henn et al., 2018; Jung et al., 2014).

Such relationship between leaf traits and the environment has been a recurrent theme of the study (e.g. Ordoñez et al., 2009; Read et al., 2014; Reich & Oleksyn, 2004). However, although recent results indicate that intraspecific variation may represent up to *ca.* 30% of total functional trait variability in plant communities (Albert et al., 2010; Siefert et al., 2015), most studies have focussed on the often assumed higher interspecific variability of many leaf traits (e.g. Hulshof & Swenson, 2010; Wright et al., 2004).

High rates of plasticity in leaf traits are expected in species distributed across ample environmental gradients (Cordell et al., 1998; Fajardo & Piper, 2011; Umaña & Swenson, 2019), as increases in niche breadth allow plants to respond to variation in climatic and other environmental conditions (Henn et al., 2018), whereas nearby individuals may share biotic and abiotic pressures and have close genetic relationships. Warming and reduced rainfall, i.e. increasing aridity, are usually reported to promote the production of thicker and smaller leaves (Wright et al., 2004)—in order to improve their water use efficiency and to increase their leaf life span—with low nutrient concentrations (Reich & Oleksyn, 2004). Such plasticity in important traits can, in turn, affect the palatability and decomposability of leaves, i.e. their acceptability and easiness to be consumed, along environmental gradients (Boyero et al., 2017; Graça & Poquet, 2014; Lecerf & Chauvet, 2008; LeRoy et al., 2007). Understanding how individual species traits, or their syndromes, are modulated by climatic or other environmental characteristics could allow us to refine predictions of potential effects on stream ecosystem functioning, both in green (based on primary production) and brown (based on detritus) food webs, in the face of climate change (Kominoski et al., 2021).

Here, using a 'space-for-time' (SFT) substitution approach (Blois et al., 2013; Pickett, 1989), we investigated how climate change might affect leaf quality, focusing on after-life traits affecting leaf decomposition. The SFT substitution approach is a useful tool to anticipate changes taking advantage of natural gradients (Fukami & Wardle, 2005); in the present study, a natural aridity gradient represents the forecasted aridification of the Mediterranean basin (Seager et al., 2014). We assessed plasticity in leaf traits of four common riparian species, with contrasting functional traits, in permanent low-order streams [*Alnus glutinosa* (L.) Gaertn., *Salix atrocinerea* Brot., *Rubus ulmifolius* Schott and *Nerium oleander* L.], extrapolating their possible variation in the forecasted climatic scenarios from that observed across a wide environmental gradient studied within a relatively small region. Using the same species along many areas differing in environmental conditions allowed us to control for species-specific traits, but not to assess the amount of trait variability due to genetic variability.

Given the high responsiveness of leaves to climate changes (Heilmeier, 2019; Soudzilovskaia et al., 2013) and the high water and nutrient availability in riparian soils of permanent streams (Naiman & Decamps, 1997), we hypothesize that (1) climate will exert a higher influence on leaf trait plasticity of the studied species compared to soil variables. We (2) expect a general trend of decreasing leaf quality—i.e. lower nutrient concentration, higher toughness—with the forecasted aridification (increasing temperature and decreasing precipitation) (Reich & Oleksyn, 2004). However, we also expect that the strength of the effects will vary among different species, as they belong to different functional groups (i.e. C allocation and/or N-fixing) and therefore have low similarity in their leaf traits (Salinas et al., 2018). Thus, we also hypothesize that (3) trait plasticity will be relatively low compared to interspecific variation.

2 | MATERIALS AND METHODS

2.1 | Area of study and selected plant species

Our study was conducted during summer 2013 in the riverbanks of 34 headwater streams with permanent flows distributed across nine natural protected areas (considered as pristine) located in Andalusia (south of the Iberian Peninsula), covering *ca.* 88,000 km^2. These locations represent a wide climatic gradient within the context of a Mediterranean-type climate and possess a considerable lithological and topographical heterogeneity (Figure 1). The present (mean annual temperature range 10.8–17.4°C; mean annual precipitation range 261–845 mm; Table S1) and the projected climatic gradient studied (by the end of the 21st century) covers from arid to humid conditions according to the Emberger's bioclimatic coefficient (Table 1, Figure 2). This embraces the forecasted aridification, i.e. warming (mean temperature rise of 2–4°C) and reduction of precipitation (mean precipitation decrease of 10–40%), for the Mediterranean region (Seager et al., 2014), as a consequence of climate change towards the year 2100 (reviewed by Giorgi & Lionello, 2008).

We selected four abundant riparian plant species which represent different functional groups featuring different characteristics, including two deciduous riparian trees: black alder—*Alnus glutinosa* (an N-fixer), and grey willow, *Salix atrocinerea*; one semi-deciduous shrub: blackberry, *Rubus ulmifolius*; and one evergreen shrub: oleander, *Nerium oleander*, also known as laurel rose. Leaves of these species collected (June-July 2013) from each sampling sites were present (Table 1) from robust, well-grown and totally unshaded plants distanced from the stream by a maximum of 6 m. Those leaves directly exposed to sun light and without herbivory or pathogen symptoms were selected (Cornelissen et al., 2003). In each stream and for each species, we collected 102 leaves from six individuals (17 leaves per individual) randomly distributed on both stream sides along a 100 m stream reach. Leaves were air-dried at room temperature (20–23°C) for one week and stored in darkness in paper bags until processed. At each stream, the cover of each species was estimated using the Domin–Krajina scale of cover and abundance (Kent & Coker, 1992) in six plots (36 m^2 each) randomly distributed in both stream sides—three plots per side arranged from the edge of the wetted channel—along a 100 m stream reach (Salinas et al., 2018).

2.2 | Environmental variables

Thirty-two environmental variables (altitude, 20 climatic and 11 edaphic; Table 1 and S1) were selected as potential predictors of leaf trait plasticity. Altitude was obtained *in situ* using a portable GPS. Historical (monthly average for the years 1970–2000) values of bioclimatic variables (spatial resolution of 30 seconds, i.e. ~1 × 1 km) recorded along the last period with available climatic data were obtained from the WorldClim database (Table S1; version 2.1; www.worldclim.org, Fick & Hijmans, 2017) using site location information (latitude and longitude). Future monthly values were estimated from the NCAR Community Model version 3 (2 × CO_2 climate change

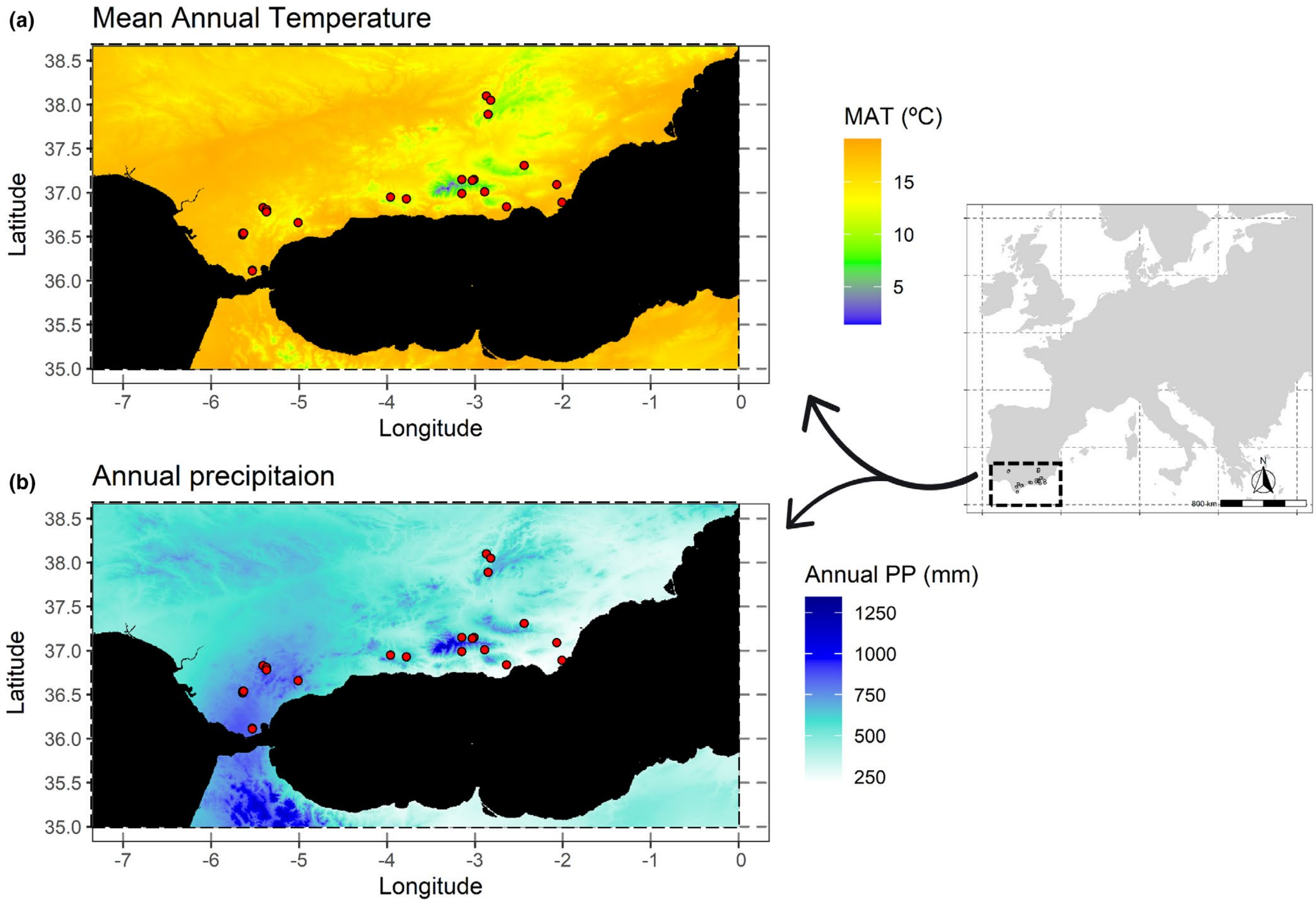

FIGURE 1 Map of the 34 sites of study located within Europe and over the mean annual temperature (a) and the annual precipitation (b) gradients

TABLE 1 Characteristics of sampling sites and cover (%) of each plant species studied

Basin name	Latitude	Longitude	Altitude (m a.s.l.)	Lithology	Land type	Emberger's coefficient (Q2)	Climate category according to Q2	*Alnus glutinosa*	*Salix atrocinerea*	*Nerium oleander*	*Rubus ulmifolius*
Rambla de las Negras	36.89	−2.01	47	Calcareous	Lowland	31.5	Semi-arid			13.8	
Río Aguas	37.09	−2.07	270	Calcareous	Lowland	33.8	Semi-arid			2.0	1.5
Barranco del Cura	36.84	−2.64	291	Calcareous	Lowland	40.4	Semi-arid			22.3	1.0
Arroyo de Aguamulas	38.05	−2.82	689	Calcareous	Midland	42.6	Semi-arid				8.8
Río Bacares	37.31	−2.44	943	Calcareous	Midland	51.9	Sub-humid		22.8		25.7
Río Vacal	36.92	−3.81	956	Calcareous	Midland	54.5	Sub-humid				6.8
Arroyo Los Marcos	37.30	−2.58	1019	Calcareous	Highland	55.1	Sub-humid		20.5		10.3
Río Mecina	36.99	−3.15	1136	Siliceous	Highland	56.5	Sub-humid	11.0	24.8		9.2
Río Turrillas	36.93	−3.78	991	Calcareous	Midland	59.0	Sub-humid				19.3
Río Andarax	37.01	−2.89	1013	Siliceous	Highland	60.4	Sub-humid	43.8	12.8		
Río Alhama	36.95	−3.96	959	Calcareous	Midland	61.2	Sub-humid				7.3
Ribera de Santa Ana	37.87	−6.70	546	Siliceous	Midland	62.5	Sub-humid	66.3			9.3
Arroyo Corterrangel	37.94	−6.60	462	Siliceous	Lowland	62.6	Sub-humid	15.8			12.7
Río Nacimiento	37.15	−2.91	1149	Siliceous	Highland	63.6	Sub-humid		17.9		2.4
Barranco del Dun Dun	37.94	−6.64	554	Siliceous	Midland	66.3	Sub-humid	52.5		1.5	3.0
Río Guadalentin	37.89	−2.85	1273	Calcareous	Highland	66.3	Sub-humid		22.9		5.5
Arroyo de Aguascebas	38.10	−2.87	1063	Calcareous	Highland	66.9	Sub-humid		3.0		4.0
Arroyo de la Garganta	37.90	−2.89	1356	Calcareous	Highland	67.1	Sub-humid				11.6
Río Chico Ohanes	37.05	−2.76	1038	Siliceous	Highland	72.1	Sub-humid		34.8	1.5	20.1
Barranco del Pueblo	37.15	−3.15	1394	Siliceous	Highland	74.8	Sub-humid	29.7	6.0		3.5
Arroyo Hondo	37.14	−3.03	1438	Siliceous	Highland	75.3	Sub-humid	51.4	3.5		15.0
Arroyo de los Castaños	37.15	−3.01	1321	Siliceous	Highland	75.3	Sub-humid	62.0	16.3		2.8
Arroyo de los Caballos	36.68	−4.91	350	Calcareous	Lowland	77.5	Sub-humid			17.7	7.1
Arroyo de los Molinos	36.81	−5.37	379	Calcareous	Lowland	85.0	Sub-humid			11.3	8.2
Arroyo de Bocaleones	36.83	−5.41	316	Calcareous	Lowland	88.6	Sub-humid			11.3	9.5
Arroyo Gaidovar	36.78	−5.37	695	Calcareous	Midland	92.3	Humid				36.8
Río Verde	36.66	−5.01	662	Calcareous	Midland	94.5	Humid			32.4	10.0
Arroyo de la Cruz	36.64	−5.03	897	Calcareous	Midland	94.5	Humid				1.2
Garganta del Caballo	36.54	−5.64	401	Siliceous	Lowland	102.2	Humid			11.9	35.9
Garganta del Aljibe	36.54	−5.63	432	Siliceous	Lowland	102.2	Humid	35.9			1.3
Garganta del Medio	36.54	−5.64	423	Siliceous	Lowland	102.2	Humid	17.3			2.3
Garganta de la Cierva	36.52	−5.64	550	Siliceous	Midland	102.2	Humid	42.9		6.7	8.5

TABLE 1 (Continued)

Basin name	Latitude	Longitude	Altitude (m a.s.l.)	Lithology	Land type	Emberger's coefficient (Q2)	Climate category according to Q2	*Alnus glutinosa*	*Salix atrocinerea*	*Nerium oleander*	*Rubus ulmifolius*
Garganta de la Garza	36.12	−5.53	429	Siliceous	Lowland	109.5	Humid	30.5			6.3
Garganta del Tesorillo	36.11	−5.53	532	Siliceous	Midland	109.5	Humid	28.0			6.3

Notes: Land type was defined by altitude (m a.s.l.): Low <500, Mid 500–1000, High >1000. Sites ranked in order of decreasing aridity according to the Emberger's bioclimatic coefficient Q2.

scenario, CCM3) for the year 2100 (Govindasamy et al., 2003) and subsequently downscaled and matched to the WorldClim estimates of current climate at a resolution of 2.5 minutes (i.e. ~4.5 × 4.5 km). From these variables, the Emberger's bioclimatic coefficient (Q2) for each site was calculated following Condés and García-Robredo (2012) as $100P/((M^2–m^2))$, where P is the annual rainfall in mm, M the average maximum of the warmest month, and m the average minimum of the coldest month. To measure soil variables, we collected a sample consisting of six core samples of the top 20 cm of the riverbank soil profile, obtained by a randomly stratified method from each stream side at a distance of approximately 3 m from the active channel. Samples from each site were mixed, air dried, sieved (2 mm) and stored in sealed polyethylene bags until analysed. Soil physical and chemical variables (Table S1) were measured as in Gil et al. (2004).

2.3 | Leaf traits

We measured nine leaf traits that often correlate with leaf litter decomposition rate (see Graça et al., 2015; Tonin et al., 2021) for each species: N, P, Ca, Mg, condensed tannins and lignin concentrations, C:N and C:P molar ratios and toughness. Before measurements, leaves were rehydrated by spraying with distilled water and stored for 12 h at 5°C. Leaf toughness, expressed in units of mass (g), was measured by performing distal and proximal punctures per individual leaf using a Texture Analyzer TA.XTPlus (Stable Micro Systems) equipped with a needle of 0.38 mm^2 tip surface. Thereafter, leaves were oven dried (60°C, 72 h) and ground to fine powder (Mixer Mill RETSCH MM 200). Concentrations of C and N (% dry mass, DM) of leaves were determined using a mass spectrometer (EA-Thermo DELTA V Advantage, Fisher Scientific®) following standard procedures (Flindt et al., 2020). The concentration of P (% DM) was measured spectrophotometrically after autoclave-assisted extraction (APHA 1998, Flindt et al., 2020). Concentrations of Ca and Mg (% DM) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer DRC II). Condensed tannins (mg Catechin Hydrate Equivalent per g of DM) were measured by the acid butanol assay (Gessner & Steiner, 2020). Concentration of lignin (% DM) was estimated gravimetrically using the acid detergent method of Goering and Van Soest (1970).

2.4 | Data analysis

To elucidate the relationships between species cover and environmental variables, we ran a Canonical Correspondence Analysis (CCA; *cca* function of the 'vegan' package, Oksanen et al., 2019), after a forward selection (*ordistep* function of the 'vegan' package with 9999 permutations) of the most parsimonious subset of explanatory variables (PPSeasonality, PWettestM, MaxT, MinT and soil pH). Significance of all testable fractions was assessed using permutation tests. Environmental variables were transformed to improve the structure of the residuals using *log* or *arcsin* transformations for decimal and percentage values, respectively.

Differences in individual traits among species were assessed using one-way ANOVA and post hoc Tukey tests (*anova* and *TukeyHSD* functions of the 'stats' package). We performed Principal Component Analyses (PCAs; *prcomp* function in the 'stats' package) to examine patterns in leaf trait variability: one pooling the four species to examine interspecific variation *vs.* trait plasticity, and one for each species to extract the main gradients (2 first PCs) of trait plasticity (i.e. leaf quality). Previously, using Spearman rank correlations, leaf traits with high (>0.85) collinearity were removed (Figure S1). Seven traits were finally included in the PCA: N, P, Ca, Mg, condensed tannins, lignin and toughness. *Log* or *arcsin* transformations of variables were used when required in ANOVA and PCA analyses. The relative magnitude of interspecific variation *vs.* species plasticity for the overall pool of traits for each species was estimated as the proportion that each species covered in each of the dimensions of the general PCA. Besides, to quantify the relative magnitude of interspecific variation *vs.* species plasticity for each leaf trait, we performed variance partitioning analyses (*varcomp* function of the 'ape' package, Paradis & Schliep, 2019).

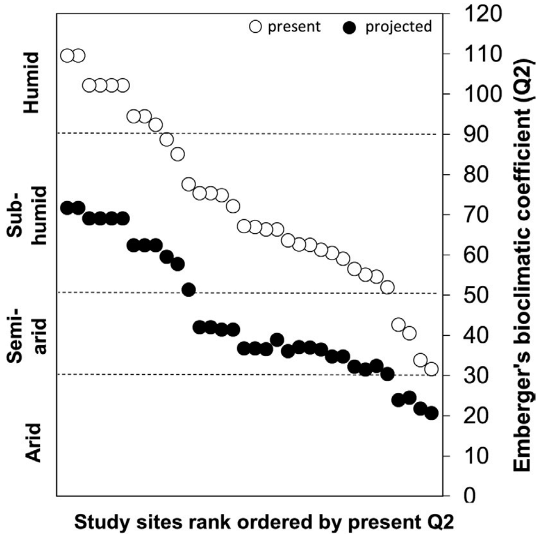

FIGURE 2 Present (open, 2000) and projected (closed, 2100) Emberger's bioclimatic coefficient values (Q2), estimated from the NCAR Community Model version 3 (CCM3) for the year 2100 (Govindasamy et al., 2003), for each of the 34 streams studied. Note that higher Q2 values denote lower aridity

We carried out partial least squares regressions (PLS; *plsr* function in the 'pls' package, Mevik et al., 2020) to evaluate the relative importance of climate and soil as predictors of leaf trait plasticity (first two PCA axes). Preliminary PLS regressions for each environmental matrix and plant species (Table S2) were used to reduce the number of variables by selecting those with the highest variable importance in projection (VIP; *VIP* function in the 'plsVarSel' package, Mehmood et al., 2012). Those variables with VIP ≥1 were considered relevant (Andersen & Bro, 2010). Spearman rank correlation analyses were used to equalize the size of the two matrices of environmental variables removing those variables with high collinearity within those with higher VIP values (Figure S2, Tables S3 and S4). A second PLS regression was performed for each species using the selected variables, and the influence of each group of environmental variables (climate and soil) and their combination (climate + soil) on leaf plasticity was assessed using the goodness of prediction (Q^2) and the goodness of fit (R^2(Y)) of models. A model was considered significant when $Q^2 > 0.097$ (Friden et al., 1994).

TABLE 2 Summary of univariate dependent variable PLS models fitted to the first two principal components of PCA (PC1 and PC2), summarizing leaf trait plasticity for each species, using three matrices (C, S and C+S) of selected (in preliminary PLS regressions) environmental variables as predictors

			Dependent variable					
			PC1			PC2		
Functional type	Plant species	Set of environmental predictors or combination	N	Q^2	R^2(Y)	N	Q^2	R^2(Y)
Deciduous N-fixer	*Alnus glutinosa*	Climate (C)	1	**0.63**	0.76	0	–	–
		Soil (S)	4	**0.26**	0.74	1	0.09	0.43
		C+S	1	**0.64**	0.78	1	−0.02	0.37
Deciduous	*Salix atrocinerea*	Climate (C)	2	**0.51**	0.74	1	**0.10**	0.42
		Soil (S)	1	**0.23**	0.47	2	**0.71**	0.91
		C+S	1	**0.36**	0.59	4	**0.58**	0.92
Evergreen	*Nerium oleander*	Climate (C)	3	**0.30**	0.71	1	−0.02	0.46
		Soil (S)	1	−0.01	0.42	**2**	**0.15**	0.60
		C+S	6	**0.70**	0.98	**1**	**0.13**	0.54
Semi-deciduous	*Rubus ulmifolius*	Climate (C)	1	**0.32**	0.41	1	−0.05	0.09
		Soil (S)	1	0.03	0.19	1	−0.02	0.11
		C+S	1	**0.26**	0.40	2	0.06	0.32

Notes: The number of PLS dimensions with lowest cross validation error (*N*), goodness of prediction (Q^2) and coefficient of determination of dependent variable (R^2) are shown for each model. Significant models ($Q^2 > 0.097$) are in bold.

In PLS regressions, all explanatory variables were scaled to unit variance (*scale* function) to give all variables the same relative importance. Regressions were carried out separately for each species and the number of extracted components (latent variables) and the robustness of the resulting models were determined by leave-one-out cross-validation (LOO). For each model, we determined the number of dimensions with the lowest cross-validation error. PLS regressions built with climatic variables, when statistically significant ($Q^2 > 0.097$; Table 2), were used to estimate the projected change of leaf quality under the forecasted climate change scenarios for 2100, using the *predict* function of the 'stats' package. Current and projected values of leaf quality (i.e. mean position over PC 1 or PC 2 of separate PCAs for each species) were compared using *t*-tests for paired samples. Hedge's *g* effect size was estimated using the *cohen.d* function of the 'effsize' package (Torchiano, 2020). See Supporting Methods in Supplementary Material for further details of data analyses.

3 | RESULTS

3.1 | Environmental variables and species distribution

Overall, the four species covered a large gradient of climatic conditions from semi-arid to humid bioclimatic types according to the Emberger's coefficient (Q2) (Table 1, Figure 2). The scenario for 2100 developed by the NCAR Community Model version 3 (CCM3) (Govindasamy et al., 2003) forecasts a significant aridification in the studied region, greater in presently humid sites (Figure 2).

Distribution of each species was rather clearly separated by the environmental gradients established by the first two dimensions of the CCA ($p < .001$; Figure 3a), which explained 91% of fitted constrained variation (53% of total variation). Soil pH was the most important variable explaining species distribution, given its significant and positive load (0.72) on CCA 1 (also loading - 0.60 on CCA 2). Moreover, minimum annual temperature was positively correlated (0.43) with CCA 1. The above variables essentially determined the separation of the acidophilic *A. glutinosa* (hereafter *Alnus*) from other species, particularly from *N. oleander* (hereafter *Nerium*), which can tolerate high pH soils and prefers lowland sites with mild winters (Figure 3a, b, d; Table S5). Precipitation seasonality and precipitation of the wettest month loaded significantly and positively (0.70 and 0.53, respectively) on CCA 2. This dimension basically segregated *S. atrocinerea* (hereafter *Salix*), abundant at low-precipitation and neutral to basic soil sites, from other species (Figure 3c; Table S5). *Rubus ulmifolius* (hereafter *Rubus*) showed its highest cover at sites with basic soils and/or mild winters, where deciduous tree species developing dense canopy cover (alder, willow or other) were absent or scarce (Figure 3e; Table S5). The studied species varied in the range of environmental conditions they occupied. *Rubus* was the most widely distributed species, occupying 100% and 92% of CCA1 and CCA2 gradients, respectively, followed by *Nerium* (80.1% of CCA1 and 84.2% of CCA2), *Alnus* (45.5% of CCA1 and 78.5% of CCA2) and *Salix* with the most constricted distribution (44.6% of CCA1 and 71.7% of CCA2) (Table 1, Tables S1 and S2; Figure 3).

3.2 | Interspecific variation and species plasticity of leaf traits

Species differed significantly in all leaf traits measured (one-way ANOVAs, all $p < .0001$) (Figure 4, Table S6). *Alnus* showed the lowest toughness and the highest N concentration, and consequently the lowest C:N ratio, being for these traits antithetical to *Nerium*, which in turn showed the highest Ca concentration and C:P ratio. *Salix* exhibited the highest P, tannins and lignin concentrations, and *Rubus* the highest Mg concentrations. The first two components of the PCA on leaf traits for the four pooled species explained 62.5% of the variation (Figure 5a): PC 1 represented a gradient of increasing nutrients (N and P) parallel to decreasing leaf toughness, segregating the deciduous (*Alnus* and *Salix*) and semi-deciduous (*Rubus*) species, from the evergreen *Nerium* with the highest toughness and lowest nutrient concentrations. Tannins and lignin heavily loaded (0.67 and 0.80, respectively) on positive PC 2, where *Salix* samples were clustered.

Overall, interspecific variation was higher than trait plasticity (Figure 5a). *Rubus*, the most widely distributed species, showed higher trait plasticity on PC 1, occupying 54% of this leaf quality gradient while other species ranged between 23% and 38%. However, the two species with more restricted distribution, *Alnus* and *Salix*, showed the highest trait plasticity on PC 2, occupying 66% and 51% of this leaf quality gradient, respectively, compared to the more widely distributed *Rubus* and *Nerium* (both 40%) (Figure 5a).

Regarding individual traits, variance partitioning analyses indicated, overall, higher interspecific variation than species plasticity in leaf traits (Figure S3). The highest interspecific variation (>80%) occurred in traits considered major determinants of litter decomposability-palatability—toughness, lignin, N and C:N—as expected dealing with species across different plant functional types. However, trait plasticity was higher than interspecific variation for P, Ca, Mg and C:P (ranging between 55% and 71%) and noticeably high for tannins (Figure S3).

Trait plasticity was described by the first two principal components of the PCA performed for each species (Figure 5b–e), which explained a considerable proportion of trait plasticity: ranging between 54% in *Nerium* and 73% in *Alnus*. The first principal component (PC 1) represented for all species a gradient of increasing leaf quality (Figure 5b–e; Table S7) positively related with decomposability and digestibility, owing to its high positive correlation with leaf N (0.57–0.87) or P (0.56–0.93) concentrations, but negative with tannins (−0.90–0.29), lignin (−0.73–0.08) or toughness (−0.82 to −0.32). However, dimension PC 2 did not exhibit a common trend across species (Figure 5b–e; Table S7). For *Alnus* and *Salix*, PC 2 was positively correlated with N, but negatively with tannins and Mg, respectively; this component covaried positively with Ca but

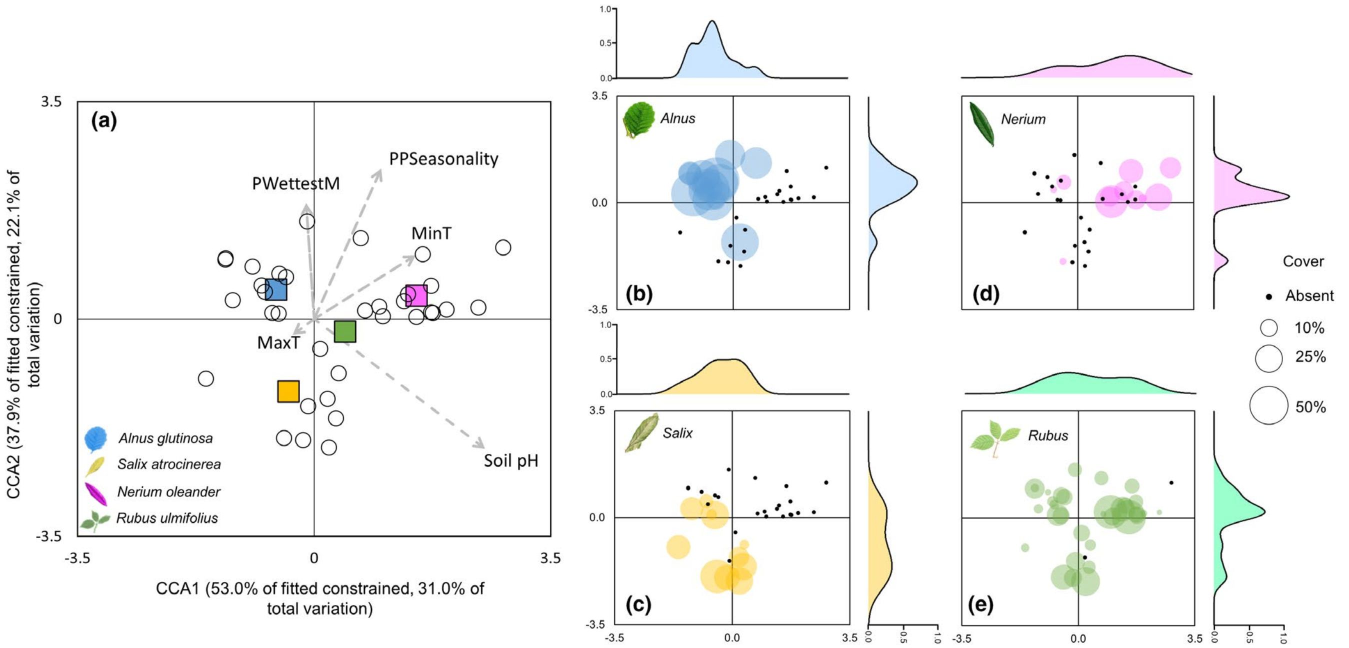

FIGURE 3 Ordination of sampling sites for the first two CCA axes (90.87% of fitted constrained variance explained) based on the environmental variables. In a, vectors represent the weight of each environmental variable; open circles denote sampling sites; and squares show the mean weighted position of each plant species over the environmental gradient. In b–e, coloured circles and their sizes represent the sampling site where each plant species was present and the percentage of canopy cover in each site, respectively; black circles represent sampling sites where each corresponding species did not appear. Marginal density plots show distribution of data for parsimonious CCA values of each plant species

negatively with tannins in *Nerium*, and negatively with Ca, Mg, lignin and toughness in *Rubus*.

3.3 | Relative importance of climate and soil factors, and best climatic predictors of leaf trait plasticity

Univariate dependent variable PLS models indicated that leaf trait plasticity (PC 1) of the four species responded significantly and predominantly to climatic variables (Table 2). Adding soil factors to climate increased noticeably the goodness of prediction in *Nerium*, but produced a highly complex model with six latent variables. Models predicting leaf trait plasticity associated to PC 2 were only significant for *Nerium* and *Salix*, but especially for the latter, in which the set of soil variables significantly predicted a high proportion of variance of leaf trait plasticity, but the model including just the set of climate variables was still significant (Table 2).

Overall, climatic predictors with the highest influence (VIP close or >1) on leaf trait plasticity associated to PC 1 (Table 3) varied among species, although most notable differences arose between broad functional groups. Mean temperature of the wettest quarter (late winter-early spring) was an important predictor with negative effects on leaf quality for deciduous/semi-deciduous species. Conversely, maximum annual temperature was the main predictor with high positive effect on leaf quality for the evergreen *Nerium*. Temperature annual range was an important predictor of leaf quality (PC 1) for *Nerium* and *Alnus*, although with contrasting sign (negative and positive, respectively), highlighting the opposite response that species belonging to different plant functional types may have the same climatic variable. Moreover, precipitation variables (Table 3) did not have substantial effects on the evergreen *Nerium*, but were important predictors of leaf quality (PC 1) for deciduous/semi-deciduous species, with notable positive effects on *Salix* and *Rubus*, but slightly negative on *Alnus*. Leaf quality of *Salix* associated to PC 2 was primarily predicted by temperature annual range (positive effect) and winter temperature (negative effect), with precipitation variables (Table 3) being other important predictors with positive effects on leaf quality. Over this dimension, soil EC and P (with negative effects) and soil $CaCO_3$ (with positive effects) were important predictors on leaf quality of *Salix*.

3.4 | Forecasted intraspecific changes in leaf quality induced by climate change

Our modelling projections showed that the four plant species would respond differently to the forecasted scenario of aridification by the year 2100 (2 × CO_2 climate change scenario) in the studied region, although with remarkable congruence within broad functional groups in terms of response direction (Figure 6; Figure S4). For *Alnus* and *Salix* (PC 1), we observed weak evidence of overall variation in leaf quality ($t = 1.523$, $p = .154$; $t = -2.071$, $p = .065$, respectively; *Hedge's* $g = 0.232$ and -0.295, respectively; Figure 6). *Salix* (PC 2;

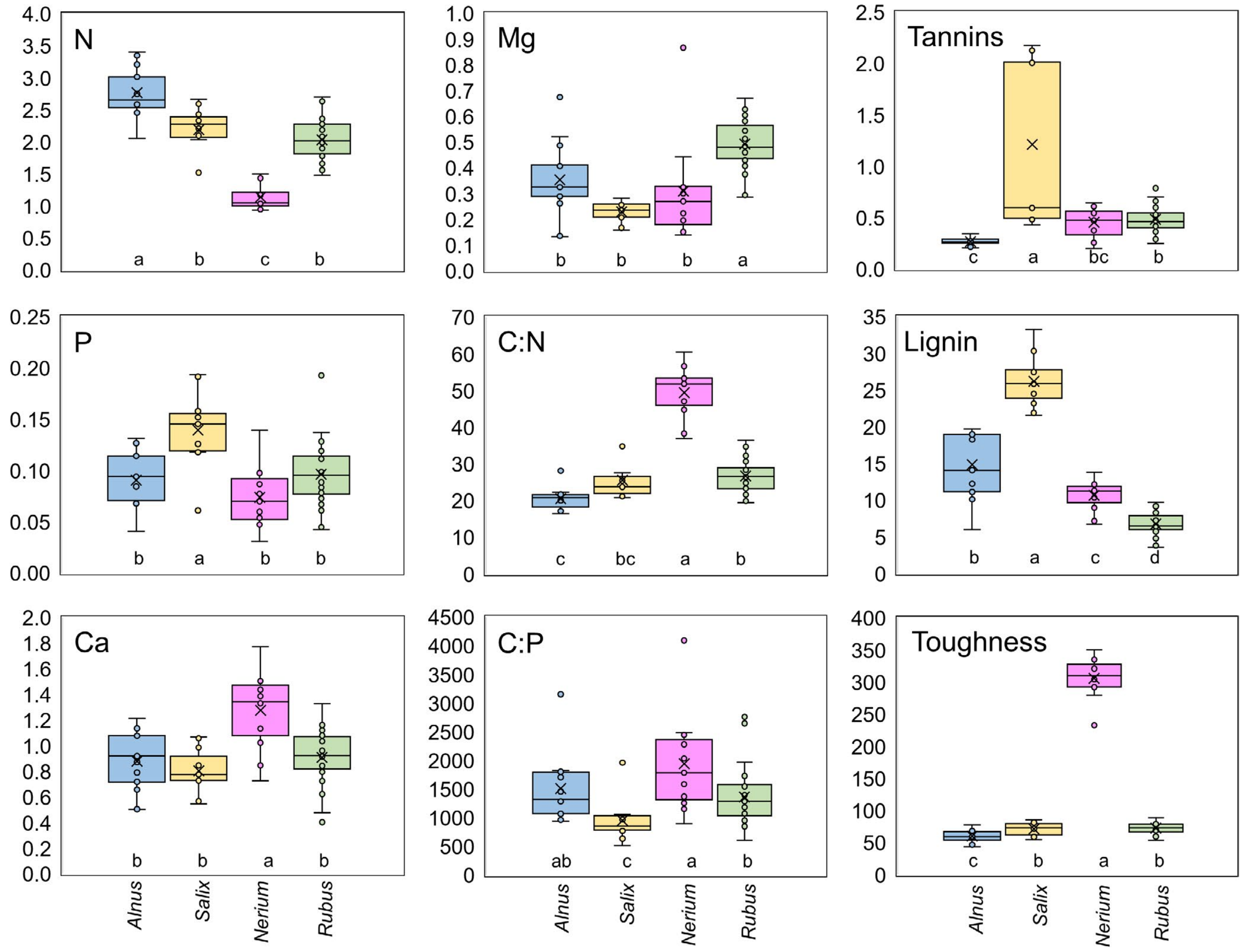

FIGURE 4 Box-and-whisker plots for selected leaf trait variables of the four plant species studied: nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) concentrations (% DM), molar elemental ratios (C:N and C:P), lignin concentrations (% DM), condensed tannins concentrations (mg Catechin Hydrate Equivalent g DM^{-1}) and toughness (g) of each plant species. Box represents median and 25th and 75th percentile levels, crosses are the mean, whiskers are the range, and dots are replicates. Different letters indicate significant differences ($p < .05$) among plant species, on the basis of linear models followed by pairwise multiple comparisons (Tukey test)

Figure S4) and *Rubus* (PC 1; Figure 6) showed large (62% and 57% decrease in mean position, respectively) and significant depletion of their leaf quality ($t = 2.423$, $p = .036$; $t = -8.277$, $p < .0001$; respectively), with large effect size (*Hedge's* $g = 1.242$ and 1.064, respectively). Conversely, leaf quality of *Nerium* is projected to increase consistently and significantly (87% increase in PC 1; $t = -8.277$, $p < .0001$, *Hedge's* $g = -1.437$) in the scenario of rising aridity used for our predictions.

4 | DISCUSSION

Functional trait-based approaches are potentially useful to understand how species respond to environmental changes (Soudzilovskaia et al., 2013; Zhang et al., 2020) and, therefore, are important for an ecologically sensitive management of ecosystems. Here, we assessed how climate change might affect leaf quality of different riparian woody species from an intraspecific perspective, which has been much disregarded based on the general assumption that intraspecific variation accounts only for an irrelevant portion of total trait variability (Garnier et al., 2001). Overall, in support of our first hypothesis, but contrary to previous studies (Graça & Poquet, 2014; Ordoñez et al., 2009), climate showed larger influence than soil explaining most leaf trait plasticity. Our second hypothesis of decreasing intraspecific leaf quality—linked to determinant traits of palatability and decomposability—with increasing aridity was partially supported, given that increasing temperature had negative effects on leaf quality of deciduous and semi-deciduous species, but not on the evergreen *Nerium*, which displayed the opposite response. These results suggest potential effects on stream ecosystem functioning (Fenoy et al., 2021; Martínez et al., 2013), but with inverse sign depending on the identity of dominant species in the riparian vegetation. Moreover, in support of our third hypothesis, we generally observed higher variation among species than plasticity within

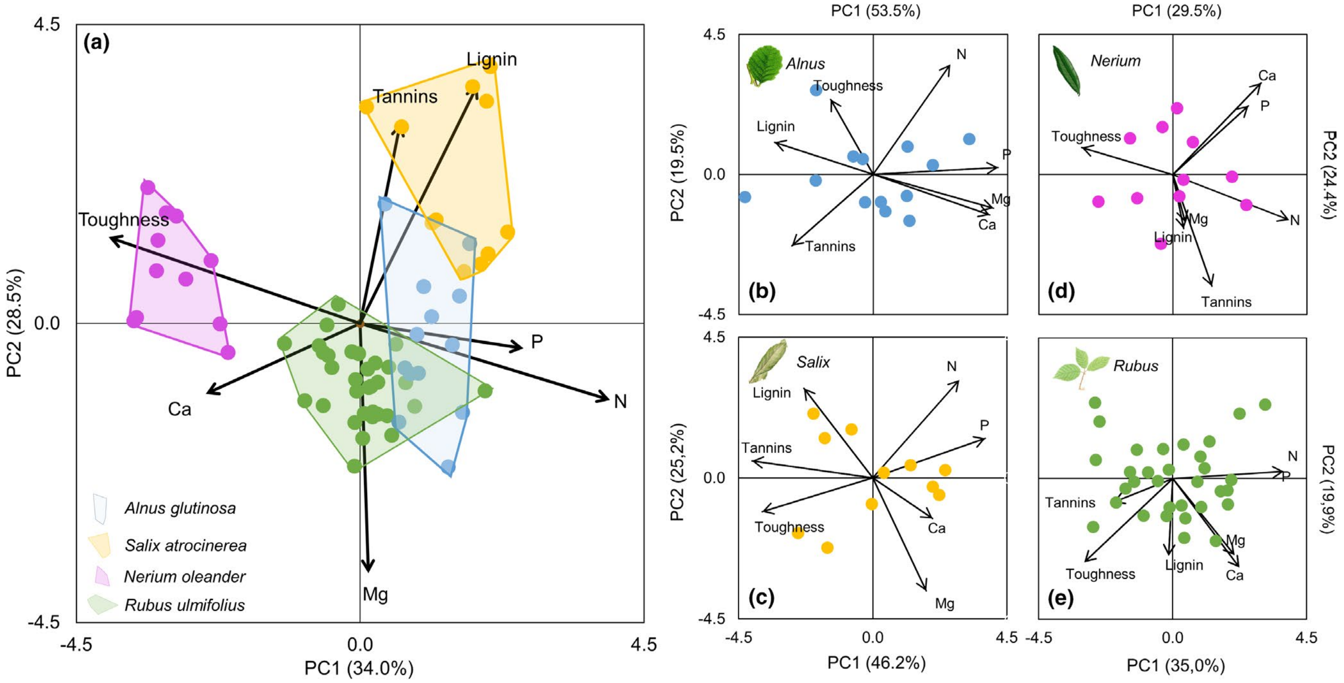

FIGURE 5 Projection of the first two principal components of PCAs showing ordination of leaf samples of four species (circles) as a function of selected leaf traits (vectors). (a) interspecific variation (polygons of different colours) *versus* trait plasticity (circles in each polygon); (b–e) trait plasticity of each plant species separately: (b) *Alnus glutinosa*, (c) *Salix atrocinerea*, (d) *Nerium oleander*, (e) *Rubus ulmifolius*

species, except for a few traits (e.g. P, Ca and Mg concentrations and C:P ratio) that exhibited remarkable leaf trait plasticity (Albert et al., 2010; Fajardo & Piper, 2011). Nonetheless, ranges of trait plasticity found here for some traits (e.g. %N, %P and %lignin) are similar, or higher, than those reported before for other species (e.g. Lecerf & Chauvet, 2008; LeRoy et al., 2007; Oliveira et al., 2021).

4.1 | Relative influence of climate and soil factors

Over the environmental gradient studied, climate exhibited an overall higher influence than soil on most species' leaf trait plasticity, although soil was the strongest predictor in some cases (e.g. *Salix* and *Nerium* PC 2). We presumed higher responsiveness of leaf traits to climate than soil in species with distributions highly constrained by soil conditions. This appears to be the case for the acidophilic *Alnus* (Miles, 1985), the species with the highest control of climate on its leaf trait plasticity. The fact that *Alnus* is an N-fixer may have further contributed to make this species less sensitive to soil nutrients. However, other species with less restricted soil-related distributions, such as *Rubus*—spread out across almost the entire study area—or *Nerium*, also showed a prominent role of climate influence on leaf trait plasticity. Similar patterns have been observed when assessing the abundance of plant functional types in the same region and across environmental gradients (Salinas et al., 2018). This lower predictive role of soil variables may stem from the high dynamics of alluvial soils and their permanent water availability, which would tend to homogenize conditions—i.e. nutrient availability—among sites (Naiman & Decamps, 1997). Yet our results are counter to other findings recorded at much larger spatial scales that observed substantial importance of soil predictors explaining intraspecific changes in leaf traits (Graça & Poquet, 2014; Ordoñez et al., 2009). This suggests that other factors not considered here, such as the great topographic variability present in our spatial gradient, or genotype differences, might be overriding soil effects.

4.2 | Main climatic predictors of leaf trait plasticity

Among climatic the factors, temperature exhibited much clearer patterns than precipitation on the main dimension of leaf trait plasticity (PC 1). This is to be expected in riparian belts of permanent streams where soil moisture tends to be relatively high and constant in the absence of extreme drought events (Moore et al., 2016), preventing major water stress in plants and its consequences on leaf characteristics (e.g. García-Palacios et al., 2016; LeRoy et al., 2014). However, climate-driven changes in streamflow may worsen the effects of aridification on such ecosystems (Perry et al., 2012).

Despite clear differentiation in distribution extent among species, we detected a common negative relationship between temperature and leaf quality in the deciduous and semi-deciduous species. On the contrary, this relationship was positive for the evergreen *Nerium*. Overall, nutrient concentrations (N, P, Ca and Mg) decreased, but tannin and/or lignin concentrations, and/or toughness increased with increasing temperature for deciduous/semi-deciduous species, whereas *Nerium* roughly exhibited the opposite pattern. Thus, within the frame of the leaf economic spectrum (Reich et al., 1997; Wright et al., 2004), the above seems to reveal antithetical syndromes of

TABLE 3 Variable importance in the projection (VIP) and standardized coefficients of the environmental variables used in the PLS models developed for the first dimension of the PCA (PC1) of each species and also for the second dimension of the PCA (PC 2) of *Salix* and *Nerium* as response variables

	Alnus glutinosa		*Salix atrocinerea*				*Nerium oleander*				*Rubus ulmifolius*	
	PC1		PC1		PC2		PC1		PC2		PC1	
Climatic predictor	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**	**VIP**	**Standardized coefficient**
Isothermality			0.83	0.03								
TSeasonility	**1.05**	**0.06**										
MaxT							**1.13**	**0.54**				
TAnnualRange	**1.03**	**0.06**			**1.11**	**0.05**	**1.04**	**−0.36**				
TColdestQ					**1.03**	**−0.04**						
TWettestQ	0.95	−0.05	**1.33**	**−0.21**							**1.00**	**−0.10**
TDriestQ							0.79	−0.53				
PDriestM					0.99	0.04						
PPSeasonality			**1.14**	**0.14**								
PWettestQ	0.97	−0.06			0.92	0.04						
PDriestQ			0.47	0.04							**1.00**	**0.10**
PWarmestQ					0.95	0.04						
Soil predictor												
EC					**1.25**	**−0.21**						
pH			**1.15**	**−0.08**					0.60	0.03		
$CaCO_3$			0.93	−0.07	**1.16**	**0.12**						
Organic C									0.71	−0.01		
CEC	**1.00**	**−0.15**							**1.28**	**−0.15**		
BasSat	0.80	−0.05	0.99	−0.07	0.7	−0.09						
ESP	0.64	−0.02			0.59	−0.1						
P	**1.40**	**0.27**	0.90	0.06	**1.11**	**−0.1**			**1.23**	**0.16**		

Notes: Isothermality (%); TSeasonility, Temperature Seasonality (%); MaxT, Maximum Temperature of Warmest Month (°C), TAnnualRange, Temperature Annual Range (°C); TColdestQ, Mean Temperature of Coldest Quarter (°C); TWettestQ, Mean Temperature of Wettest Quarter (°C); TDriestQ, Mean Temperature of Driest Quarter (°C); PDriestM, Precipitation of Driest Month (mm); PPSeasonality, Precipitation Seasonality (%); PWettestQ, Precipitation of Wettest Quarter (mm); PDriestQ, Precipitation of Driest Quarter (mm); PWarmestQ, Precipitation of Warmest Quarter (mm); EC, Soil Electric conductivity (µS cm^{-1}); pH, Soil pH; $CaCO_3$, Soil $CaCO_3$ (%); Organic C, Soil Organic Carbon (%); CEC, Soil Cation exchange capacity ($cmol^{+}$ kg^{-1}); BasSat, Soil Base saturation (%), ESP, Soil Exchangeable Sodium Percentage (%); P, Soils Phosphorus (%).

Relevant variables (VIP > 1) are in bold.

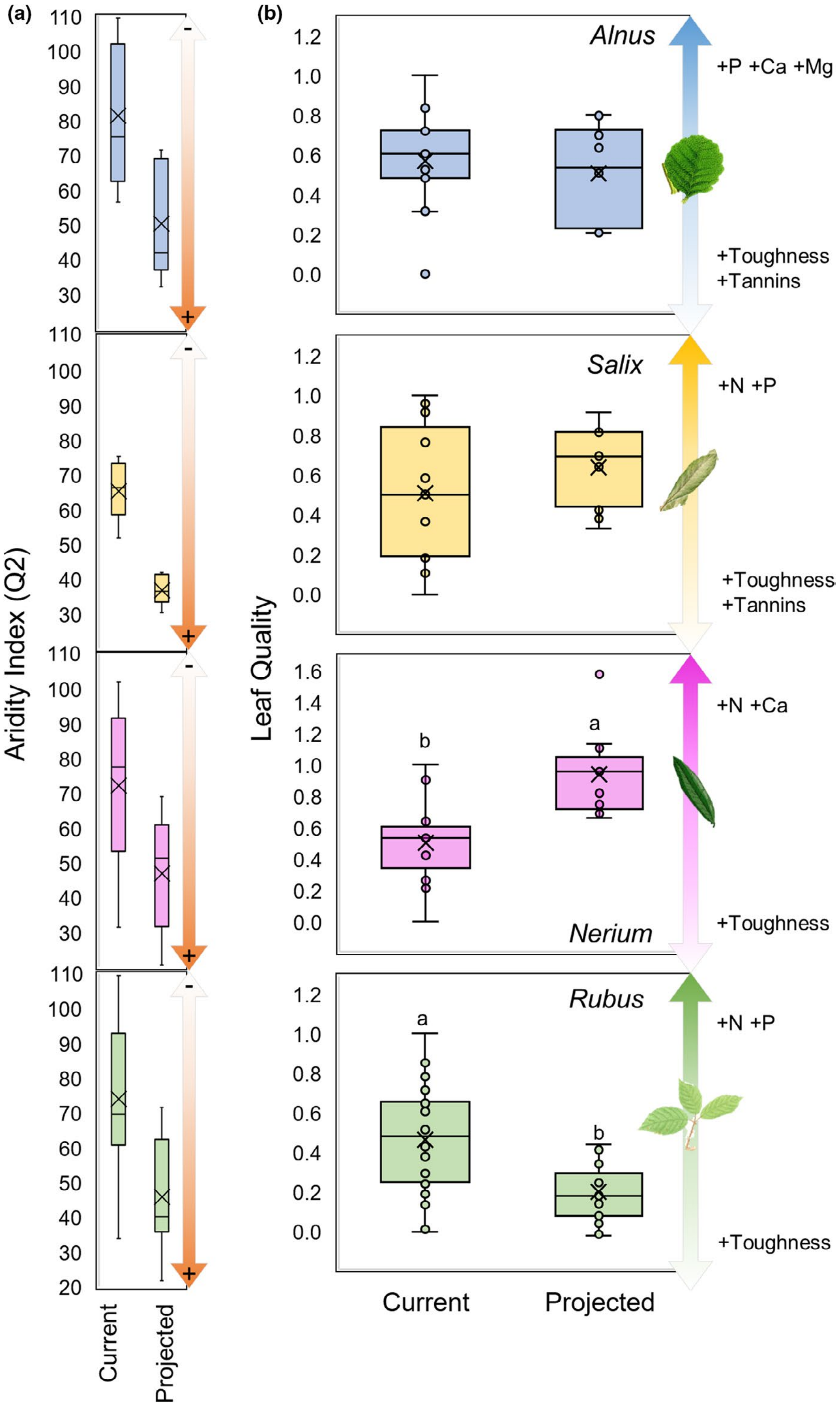

FIGURE 6 Boxplots showing (a) the aridity range covered by the distribution of each species according to the Emberger's bioclimatic coefficient (Q2); and (b) the leaf quality (PC1) of each plant species at present (Current) and in future climate change scenarios according to the NCAR Community Model version 3 (CCM3) for the year 2100 (Projected). Note that leaf quality ranges (PCA dimensions) are scaled to unit for simplicity. Different letters indicate significant different based on *t*-test analyses

leaf traits between functional groups in response to temperature, in which the intraspecific intercorrelated leaf traits along our quality gradient represent physiological and structural trade-offs (Boyero et al., 2017; Onoda et al., 2017).

Decreasing leaf N and/or P concentrations with increasing temperature has been reported before in woody deciduous species (Chen et al., 2011; Kudo et al., 2001; Sun et al., 2015). This may be explained by an increase of the catalytic capacity of photosynthetic enzymes at higher temperatures, requiring lower enzyme amounts (e.g. lower N concentration) to maintain photosynthetic rates (i.e. the photosynthetic rate is achieved with lower amounts of such enzyme; Scafaro et al., 2017). Alternatively, or additionally, higher temperature is often associated with increasing length of the growing season in deciduous species, which in turn promotes long leaf life span. Long-lived leaves often invest more in structure/protection at the expense of reducing photosynthetic efficiency (Kudo et al., 2001; Onoda et al., 2017). Similarly, other authors have reported that deciduous plants growing under relatively elevated temperatures develop tougher leaves (Wright et al., 2017) or leaves with higher tannin (Top et al., 2017) and lignin (Graça & Poquet, 2014) concentrations.

Reduction of nutrients and strengthening of leaf traits to confer resistance (e.g. increasing toughness) have been reported in evergreen *Quercus* species in response to decreasing winter temperatures. This is interpreted as a higher cost for evergreens at cooler sites compared with deciduous trees (González-Zurdo et al., 2016). However, this finding is not totally consistent with our results for *Nerium* as winter temperatures did not exhibit any effect on its leaf quality. We observed the strongest positive effect on leaf quality of *Nerium* from maximum temperature, but a more negative effect from annual temperature range. This suggests that *Nerium* develops more nutrient-rich and softer leaves in its optimum distributional range (areas with mild winters and maritime influence), with negligible effects from harsh low-winter temperatures, which are infrequent in its area of distribution. Nevertheless, we cannot rule out the possibility that our results are species-specific, and projection of such results to the entire functional group needs to be confirmed with the study of further evergreen species.

A substantial amount of leaf trait plasticity (25%) in *Salix* (PC 2)—positively related to leaf N and lignin, and negatively to Mg concentrations—was significantly explained by climatic conditions, but much more by soil variables. The strong positive association of N and lignin on PC 2 suggest that this N fraction is structural, possibly lignin-bound N, therefore not readily available to decomposers and detritivores (Berendse et al., 1987). Thus, PC 2 represents a structural reinforcement of *Salix* leaves positively related with temperature annual range and negatively with winter temperature, but also, and mostly, negatively with soil P. A structural reinforcement of leaves (increasing leaf mass per area and lignin concentration) with decreasing soil fertility has been documented elsewhere (e.g. Diehl et al., 2008).

The trait plasticity observed in this study can arise from responses to environmental conditions, but also from genetic variability. Genotypes, although largely influenced and selected by local environments, represent an important source of trait variability unaccounted for here. Genetic variability has been exhibited to strongly influence litter quality and, consequently, associated ecosystem processes (e.g. litter decomposition) and communities (Crutsinger et al., 2014; LeRoy et al., 2006, 2007, 2012). Given that leaf traits differ in their heritability, for example, tannins appear to be highly heritable whereas C:N ratios are environmentally controlled (Crutsinger et al., 2014), further research assessing how environment × genotype interaction affects leaf traits is important for improving predictions of potential effects on ecosystem functioning, particularly, in the face of climate change.

4.3 | Projecting climate change-driven variation of species leaf quality: implications for stream ecosystems

Litter trait variation across species constitutes the main driver of instream litter decomposition worldwide (Boyero et al., 2017; García-Palacios et al., 2016; Zhang et al., 2019), indicating an essential role of plant phylogenetic history on controlling such process (LeRoy et al., 2019). Although less studied, some evidence indicates that the control exerted by trait plasticity on litter decomposition, nutrient cycling and trophic dynamics could be almost as important as interspecific changes (Jackrel & Morton, 2018; Jackrel et al., 2016; Lecerf & Chauvet, 2008; LeRoy et al., 2007; Oliveira et al., 2021). Here, we assessed the plasticity of selected traits of green leaves of riparian plants aimed at forecasting potential consequences of climate change on stream ecosystems highly dependent on these resources (i.e. forest streams; Wallace et al., 2015). Although inputs of leaves to streams are mainly in the form of leaf litter, it has been reported that some traits of green leaves tend to persist after senescence and control rates of litter decomposition (Cornelissen et al., 1999; Cornwell et al., 2008). Therefore, if nutrient resorption efficiency remains fundamentally invariable across climatic conditions (Norby et al., 2000, Aerts et al., 2007, but see Yuan & Chen, 2009b), understanding how green leaves respond to climate change may allow us to anticipate effects of leaf quality changes on stream ecosystem functioning. In support of this idea, a recent study suggests that traits of green leaves can be used to accurately predict decomposition rates (Rosenfield et al., 2020). However, as others have pointed out that traits of litter can differ from those of fresh leaves (Hättenschwiler et al., 2008; Hättenschwiler & Vitousek, 2000; Horner et al., 1987; Yuan & Chen, 2009a), the potential effects on headwater stream functioning exposed here should be interpreted with caution.

Litter decomposition is often reported to be enhanced by its high N and P concentrations (García-Palacios, McKie, et al., 2016; MacKenzie et al., 2013). Elevated litter concentrations of Ca and Mg—reported to be important for fungal decomposers (Jenkins & Suberkropp, 1995) and macroinvertebrates (Makkonen et al., 2012; National Research Council, 2005)—can also accelerate decomposition (Santonja et al., 2019). Moreover, tannins (Coq et al., 2010; Irons et al., 1988), lignin (Ferreira et al., 2016; Ramos et al., 2021; Schindler & Gessner, 2009) and toughness (Fenoy et al., 2021; Li et al., 2009) primarily tend to reduce litter consumption by detritivores. Our results point to a general decrease in leaf quality as a response to aridification in the three deciduous/semi-deciduous species. This decrease was generally related to a reduction in leaf N and P, but also Ca and Mg, versus an increase in tannins or lignin, and leaf toughness.

In particular, changes in leaf quality of the deciduous N-fixer *Alnus* could have major consequences given the key role of this species on stream ecosystem processes (Alonso et al., 2021; Pérez, Basaguren, et al., 2021; Rubio-Ríos et al., 2021). We reported here for *Alnus* ranges of %N, %P and %lignin variation similar to those reported at the European continental scale (Lecerf & Chauvet, 2008), and 53% of its species leaf trait plasticity was remarkably explained by climatic variables, yet our forecasted decrease in leaf quality was relatively low (11%) and not statistically significant, compared to other species. Nonetheless, apparent subtle changes in litter traits might result in major effects in consumer fitness (Pérez et al., 2021). Furthermore, this projected minor decrease in leaf quality adds to the decline of populations of this key species through Europe due to a disease caused by the pathogen *Phytophthora alni* (Bjelke et al., 2016), which also has been recently reported to alter the nutritional quality of leaf litter (Ferreira et al., 2021). Both factors are likely to trigger significant alterations to the functioning of forested streams (Alonso et al., 2021). Moreover, if a general decrease in leaf quality occurs in other deciduous species, as those forecasted here for *Salix* and *Rubus*, the negative influences on stream food webs will increase.

Thus, our results indicated that decreases of leaf quality of individual deciduous species may occur in a relatively short term (via phenotypic plasticity; Nicotra et al., 2010; but see Valladares et al., 2007), which in the long term will add to the forecasted dieback of deciduous woody species in riparian corridors of temperate climate zones (Kominoski et al., 2013; Salinas et al., 2018). Both riparian changes have the potential to significantly impair instream ecosystem processes, particularly in mountain streams presently dominated by deciduous vegetation (Fenoy et al., 2021), more than in lowland streams where deciduous species actually represent a minor component of the riparian belt.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data openly available in a public repository https://doi.org/10.5061/dryad.bzkh1899h.

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BIOSKETCH

The research team is made up of ecologists and botanists whose work focuses on the basic and applied aspects of the ecology of streams and riparian zones. We aim to increase our knowledge about the relationships between riparian plant communities and stream ecosystem functioning.

Author contributions: JJC and MJS connceptualized the study. JRR, MJS and JJC contributed to methodology. JRR performed analyses with help of JP and EF. JRR wrote the original draft with help of JP and JJC. All authors reviewed and edited the manuscript. JJC provided resources and performed funding acquisition.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Key plant species and detritivores drive diversity effects on instream leaf litter decomposition more than functional diversity: A microcosm study

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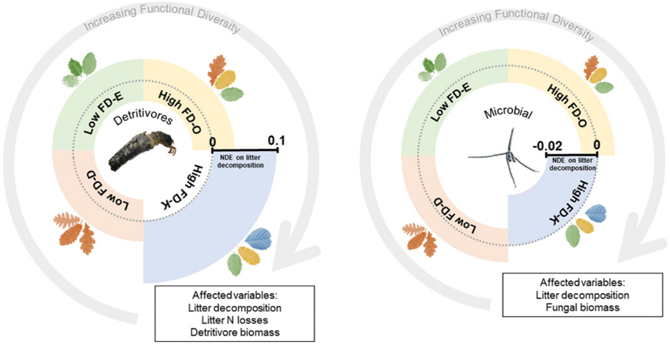
HIGHLIGHTS

- Effects of functional diversity loss on instream litter decomposition were assessed.
- Diversity effects were mostly controlled by the N-fixing *Alnus glutinosa*.
- Overall positive diversity effects were detected for detritivore-mediated processes.
- Overall negative diversity effects were detected for microbially-mediated processes.
- Key plant species and detritivores, more than functional diversity, were the essential drivers of diversity effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Anthropogenic impacts on freshwater ecosystems cause critical losses of biodiversity that can in turn impair key processes such as decomposition and nutrient cycling. Forest streams are mainly subsidized by terrestrial organic detritus, so their functioning and conservation status can be altered by changes in forest biodiversity and composition, particularly if these changes involve the replacement of functional groups or the loss of key species. We examined this issue using a microcosm experiment where we manipulated plant functional diversity (FD) (monocultures and low-FD and high-FD mixtures, resulting from different combinations of deciduous and evergreen *Quercus* species) and the presence of a key species (*Alnus glutinosa*), all in presence and absence of detritivores, and assessed effects on litter decomposition, nutrient cycling, and fungal and detritivore biomass. We found (i) positive diversity effects on detritivore-mediated decomposition, litter nutrient losses and detritivore biomass exclusively when *A. glutinosa* was present; and (ii) negative effects on the same processes when microbially mediated and on fungal biomass. Most positive trends could be explained by the higher litter palatability and litter trait variability obtained with the inclusion of alder leaves in the mixture. Our results support the hypothesis of a consistent slowing down of the decomposition process as a result of plant biodiversity loss, and hence effects on stream ecosystem functioning, especially when a key (N-fixing) species is lost; and underscore the importance of detritivores as drivers of plant diversity effects in the studied ecosystem processes.

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1. Introduction

The current human-induced biodiversity decline (Ceballos and Ehrlich, 2018) and its consequences for ecosystem functioning (hereafter B-EF) have attracted much attention since the seminal paper by Naeem et al. (1994). Headwater stream ecosystems in forested regions may be especially sensitive to plant biodiversity loss, owing to their dependence on allochthonous organic matter, mostly in the form of leaf litter (Kominoski et al., 2011; Wallace et al., 1997). Instream litter decomposition is a crucial process involving the cycling of nutrients and the production of microbial and invertebrate biomass (Marks, 2019). Therefore, ongoing changes in riparian vegetation affecting litter inputs (e.g. dominance of evergreen versus deciduous plants; Kominoski et al., 2013; Salinas et al., 2018) may alter the stream food web structure (Bärlocher and Graça, 2002; Martínez et al., 2013; Seena et al., 2017) as well as biogeochemical cycles at multiple scales (Battin et al., 2009; Casas et al., 2013).

Plant biodiversity loss effects on instream decomposition have been addressed by numerous studies. However, their outcomes have been idiosyncratic (Gessner et al., 2010), showing positive (e.g., Gartner and Cardon, 2004; Leroy and Marks, 2006) to negative diversity effects (e.g., Swan and Palmer, 2006). These contrasting results may arise from differences in experimental designs (field vs. laboratory experiments), the selected plant species or the use of different diversity measures. An alternative approach to species richness is to focus on functional diversity (FD), i.e. the diversity of functional traits, which has been reported to have a pivotal role on ecosystem processes (Petchey and Gaston, 2006) by favoring different organisms (niche or resource partitioning; Finke and Snyder, 2008). However, there is no consensus about which is the most suitable FD measure (Flynn et al., 2011; Petchey et al., 2009), or which traits are the most relevant for a particular process.

Another controversial question relates to the primary role of single species, in particular key species (also called keystone process species sensu Folke et al., 1996), which might drive critical processes and thus determine the overall ecosystem functioning (Ellison et al., 2005). Litter from these key species could promote diversity effects through a variety of underlying mechanisms, including an increase in the mean value of the nutrient pool in litter mixtures (mass ratio hypothesis; Grime, 1998), or via nutrient transfer between litter types (Gessner et al., 2010; Tiunov, 2009).

Our study addresses two main questions. First, by mixing tree species belonging to the same or different functional types in terms of their leaf habits (deciduous, semi-deciduous or evergreen), we explored plant FD effects on several stream ecosystem processes (litter decomposition, nutrient cycling and changes in biomass of fungi and detritivores) and the relative role of microbial decomposers and detritivores as drivers of these effects. Second, we examined the role of black alder, *Alnus glutinosa* (L.) Gaertn., a deciduous nitrogen (N)-fixing plant with soft, nutrient-rich and fast decomposing litter (Casas et al., 2013; Hladyz et al., 2009), as key species with direct repercussions on the above processes (e.g., Graça et al., 2015; Pérez et al., 2021; Piccolo and Wipfli, 2002) and B-EF relationships (Alonso et al., 2021; López-Rojo et al., 2018). In Europe, populations of *A. glutinosa* are declining since 1990 (Bjelke et al., 2016; Gibbs et al., 1999) due to a fungal disease caused by *Phytophthora alni* "sensu lato" (Husson et al., 2015). We used microcosms, with and without a stream detritivore to conduct an experiment using monocultures, low-FD and high-FD mixtures (resulting from different combinations of deciduous and evergreen *Quercus* species) and the key species *A. glutinosa*. We tested the following hypotheses: (1) FD increases trait variability of litter mixtures (Flynn et al., 2011; Hillebrand and Matthiessen, 2009); (2) litter FD enhances decomposition and nutrient cycling through resource partitioning (Handa et al., 2014; López-Rojo et al., 2019) and (3) leads to a higher fungal (Kominoski et al., 2009) and detritivore biomass through a balanced diet effect (DeMott, 1998; Duffy et al., 2007); (4) the above diversity effects are greater when a key plant species is present due to overall increased nutrient content and/or increased trait variability (Larrañaga et al., 2020; López-Rojo et al., 2018); and (5) such effects are mostly mediated by detritivores (Tonin et al., 2017).

2. Material and methods

2.1. Selection of litter and detritivore species

We selected 7 oak species belonging to 3 different plant functional groups in terms of their leaf habits, and consequently with regard to leaf traits and carbon (C) gain strategies (see Escudero et al., 2017; Gil-Pelegrín et al., 2017 for more details) to examine effects of plant FD on stream processes: 3 deciduous (*Quercus robur* L., *Q. canariensis* Willd. and *Q. pyrenaica* Willd.), 1 semi-deciduous (*Q. faginea* Lam.) and 3 evergreen species (*Q. coccifera* L., *Q. suber* L. *and Q. rotundifolia* Lam.). Deciduous *Quercus* species have leaves that show functional traits values such as greater leaf area, and lower Leaf Mass per Area (LMA) and leaf thickness (considered proxies for mass allocation and related processes, Onoda et al., 2011), and shorter leaf lifespan than leaves of evergreen *Quercus* species (Escudero et al., 2017). *Quercus faginea* is a semi-deciduous tree (Sanz-Pérez et al., 2009), i.e. its old and withered leaves remain on the plant for the most of the winter until the emergence of new leaves the following spring (see Veselá et al., 2018). Additionally, we chose the deciduous N-fixing *A. glutinosa* as key species (Pérez et al., 2021). The 8 species covered a broad litter quality spectrum (Tables 1, S1). Leaves were collected in autumn 2017 from forests of different catchments in Southern Spain, except for *Q. robur*, which was collected in Northern Spain as it is absent at lower latitudes (Table S2). Deciduous leaves were gathered from the forest floor just after senescence and evergreen leaves were collected from dry, broken branches, with no symptoms of diseases. Leaves were air dried at room temperature (20–23 °C) for 1 week and stored in darkness until used for experimentation.

We selected larvae of the caddisfly *Allogamus mortoni* Navás (hereafter *Allogamus* or detritivores), an endemic leaf-shredding species common in streams of the Iberian Peninsula (Ruiz-García et al., 2004). This species is widespread within the Iberian Peninsula and likely coexist with all the plant species selected. Detritivores were collected from Río del Pueblo, a second-order stream located at 1405 m a.s.l in the Sierra Nevada Natural Park (37.15°N, 3.15°W; Southeast Spain). Riparian vegetation consists of mixed deciduous tree species dominated by alder groves (*Alnus glutinosa*) and plantations of *Populus nigra* L., followed out to the slopes by natural forests of the evergreen holm-oak (*Quercus rotundifolia*) and plantations of the conifer *Pinus pinaster* Aiton. Other frequent plant species encountered in the stream bank include *Salix atrocinerea* Brot., *Castanea sativa* Mill, *Populus alba* L., or *Rubus ulmifolius* Schott. Detritivores were acclimated to experimental conditions (see below) for 1 week and starved for 48 h just prior to starting the experiment. Initial detritivore dry mass (DM) was estimated from a case aperture diameter (CD) (mm) / DM (mg) relationship, using additional *Allogamus* larvae ($DM = 0.097\ e^{1.362\ CD}$, $R^2 = 0.87$, $p < 0.001$, root mean square error $= 1.64$, $n = 55$). CD was measured under a binocular microscope (0.1 mm), and DM was weighed (0.1 mg) in freeze-dried uncased individuals that were later used to analyse their initial body N and phosphorus (P) (see Supplementary Methods in Supplementary Data).

2.2. Experimental setup

A total of 120 microcosms were placed in a temperature-controlled room set at 10 °C (the mean of hourly records obtained in the stream during the experiment season (April–May) in previous years) and a light:dark 12:12 h photoperiod (based on the natural cycle at that time of the year), and provided with continuous air supply (Fig. S1). Each microcosm consisted of a 500-mL glass jar containing 30 cm^3 of

Table 1
Experimental treatments of varying functional diversity, *Quercus* and *Alnus* species used in the microcosm experiment. Leaf litter quality index (LQI) of each species is showed between parenthesis and LQI and Rao's quadratic diversity (Rao's Q) of litter mixtures traits is presented in the corresponding columns.

Treat ID	Functional diversity	Functional group	Sp1	Sp2	Sp3	LQI	Rao's Q
Low FD-D	Low	Deciduous	*Q. robur* (0.36)	*Q. canariensis* (0.34)	*Q. pyrenaica* (0.35)	0.35	4.78
Low FD-E	Low	Evergreen	*Q. coccifera* (0.23)	*Q. suber* (0.35)	*Q. rotundifolia* (0.36)	0.31	4.86
High FD-O	High	Deciduous, semi-deciduous and evergreen	*Q. robur* (0.36)	*Q. faginea* (0.37)	*Q. rotundifolia* (0.36)	0.36	9.37
High FD-K	High	Deciduous and N-fixing, semi-deciduous and evergreen	*A. glutinosa* (0.58)	*Q. faginea* (0.37)	*Q. rotundifolia* (0.36)	0.43	10.59

stream sediment, previously furnaced (500 °C, 5 h) and washed with distilled water, and 400 mL of filtered (100 μm) stream water, which was oligotrophic (soluble reactive P: 7 μg P L^{-1}; nitrate-N: 24 μg N L^{-1}) and soft circumneutral (pH: 7.10; electrical conductivity: 60 μS cm^{-1}; alkalinity: 0.32 meq L^{-1}). Sediment and water were collected from the same stream as detritivores (Río del Pueblo). Just before the experiment, litter was cut (≈2 × 2 cm, avoiding the basal midrib) and weighed to the nearest 0.1 mg. Each microcosm received 1.516 ± 0.001 g (mean ± SE) of air-dried litter pieces belonging to 1 (monocultures) or 3 plant species (litter mixtures containing 0.5 g per species), combined as shown in Table 1 to create the highest gradient of FD possible among all the potential combinations (Table S3) (12 treatments in total: 8 monocultures and 4 mixtures). Mixtures consisted of 2 low FD (deciduous: Low FD-D, or evergreen: Low FD-E, oak species) and 2 high FD treatments (one including all the leaf habits —deciduous, semi-deciduous and evergreen— within oak species: High FD-O, and the other including also the key species: High FD-K).

Litter fragments of the same species were color-coded and kept together using 3 safety pins per microcosm; the same was done in monocultures to mirror the conditions of mixtures. In each litter treatment, detritivores were added to 7 microcosms (2 larvae of *Allogamus* per microcosm); the other 3 remained without detritivores in order to separate their contribution from that of microorganisms (Fig. S1). Detritivore initial biomass per microcosm was on average 12.94 ± 0.98 mg (mean ± SE), without significant differences across litter treatments (One-way ANOVA, $p = 0.97$). The experiment lasted 42 days (April–May 2018). Larval mortality was recorded daily, and dead larvae (52 out of 244) were replaced by a new one only when both individuals died in a given jar (4 out of 84 microcosms with detritivores).

2.3. Experimental procedure

Before adding the detritivores to microcosms, litter fragments were incubated for 5 days in microcosms with aeration (with stream water renewal on the third day) to allow the leaching of soluble compounds and initial microbial colonization. Twenty-four extra microcosms without detritivores (3 per plant species in monoculture), containing ca. 1.5 g of litter, were used to measure initial (post-leaching; Fig. S2) ash-free dry mass (AFDM) and litter traits (Supplementary Methods). Thereafter, water was renewed weekly with filtered (100 μm) stream water collected the same day. On the last day, litter material was separated by species, and 5 discs (12 mm ø) of each species (3 replicates with and 3 without detritivores per treatment) were cut out with a cork borer, freeze-dried, weighed and used to estimate fungal biomass, which was measured through ergosterol concentration following Suberkropp and Weyers (1996) with some modifications (Supplementary Methods). The remaining litter material was oven-dried (70 °C, 72 h), weighed to determine final DM, and ground to leaf powder (Mixer Mill RETSCH MM 200). An aliquot of each sample was furnaced (550 °C, 5 h) and weighed to estimate final AFDM, and another aliquot was used for elemental analyses (C, N and P; Supplementary Methods). At the end of the experiment, detritivores from each microcosm were starved for 48 h. Then, the diameter of their cases was measured as above and individuals were uncased, freeze-dried and stored until N and P analyses (Supplementary Methods).

2.4. Data analyses

We examined trait variability among litter treatments using principal component analysis (PCA; *prcomp* function in the 'stats' package of R statistical software (R Core Team, 2020)). Previously, a Pearson correlation analysis was performed to remove litter traits with high (>0.85) collinearity (Fig. S3). Twelve litter traits were included in the PCA: toughness, N, P, N:P molar ratio, hemicellulose, lignin, total phenolics, Ca, K, Mg, Si and non-structural carbohydrates (NSC). To test our 1st hypothesis we assessed trait variability in litter mixtures using the Rao's quadratic diversity coefficient (Rao, 1982), widely applied in functional ecology analyses [Rao's Q; *dbFD* function in the 'FD' R package (Laliberté et al., 2014)]. Statistical differences among species or mixture traits were assessed using one-way ANOVA and Tukey multiple comparisons tests with the *anova* and *TukeyHSD* functions of 'stats' R package (R Core Team, 2020). *Log* or arcsin transformations of variables were used when required.

Litter palatability was estimated using the litter quality index (LQI) equation proposed by Solagaistua et al. (2019): LQI: $1 - [(T_1 / (n \times T_{1(\max)})) + \ldots + (T_n / (n \times T_{n(\max)}))]$; where T is the average of the measured value for the trait and n the number of measured traits. In order to ease interpretation, we selected a limited number of traits (5 out of 20: toughness, C:N and C:P molar ratios, phenols and NSC) that are relevant for detritivore- (e.g., Hladyz et al., 2009) and microbially-mediated decomposition (Fernandes et al., 2012; López-Rojo et al., 2020), being the latter usually favored by a high nutrient and structural carbohydrates content.

Litter decomposition was quantified as the proportion of litter mass loss [LML = (initial − final AFDM) / (initial AFDM)], after correcting initial litter mass for humidity, ash content and leaching losses. LML in microcosms without detritivores (i.e., microbially-mediated LML) was used to correct initial AFDM in microcosms with detritivores and thus isolate detritivore-mediated LML, allowing us to partially test our 5th hypothesis (i.e. FD effects are mostly mediated by detritivores). Litter N loss (LNL) and litter P loss (LPL) were calculated as: (initial − final) / initial N or P content. Positive values represented nutrient mineralization, and negative ones nutrient immobilization. Detritivore relative growth (DMG), detritivore N gain (DNG) and detritivore P gain (DPG) were quantified as the proportional change in detritivore DM, N and P content, respectively [(final − initial) / initial DM, N or P content]; and were estimated only in those microcosms with no animal replacements. Finally, proportional changes in nutrient concentration of litter and detritivores were determined as: [(final − initial) / (initial N or P %)].

We examined differences in all response variables (LML, LNL, LPL, ergosterol, DMG, DNG and DPG) among monocultures and among litter mixtures using one-way ANOVAs and Tukey multiple comparisons tests (see above function and package). To avoid very complex models with many interactions, we examined detritivore- and microbially-mediated variables separately. We applied the Kaplan-Meier test to compare *Allogamus* survivorship among treatments using the *survfit* function of 'survival' R package (Therneau, 2015). Any possible effect of mortality on other detritivore related variables was prevented standardizing by the number of days that each animal survived relative to the experimental time.

We explored our 2nd and 3rd hypotheses through the difference between observed (Obs.) and expected (Exp.) values (i.e. the net diversity effect, NDE = Obs. − Exp.), with expected values estimated based on monocultures. NDE was estimated in microcosms with and without detritivores enabling us to better assess our fifth hypothesis. To assess the magnitude of these effects we calculated the relationship between the NDE and expected values [NDE magnitude (%): (Obs. − Exp.) / Exp. × 100]. We explored differences among treatments with nonparametric bootstrapped 95% confidence intervals using the BCa method in the *boot.ci* function of 'boot' R package (Canty and Ripley, 2019), which allowed us to test if FD effects were greater when the key plant species is present (our 4th hypothesis). For LML and ergosterol, the NDE was partitioned into complementarity and selection effects using the additive partitioning method (Loreau and Hector, 2001). In the case of LML, we were able to split diversity effects for total, detritivore- and microbially-mediated decomposition, to further assess the role of detritivores on BE-F relationships (5th hypothesis). Complementarity effects are explained by synergistic (positive) or antagonistic (negative) interactions; and selection effects represent positive (or negative) effects due to the presence of a dominant species in the mixture, which enhances (or inhibits) overall decomposition of the mixture (Handa et al., 2014). In addition, we carried out intraspecific comparisons, i.e. we compared LML for each species in monocultures and in each mixture where present. We finally explored the relationships between litter palatability (LQI) or litter traits variability (Rao's Q) with the measured variables and diversity effects in mixtures using linear models (*lm* function of 'stats' R package) in order to better explain the observed patterns and separate the FD effects from that caused by the presence of the key species.

3. Results

3.1. Litter trait variation across species and mixtures

Trait variability within a mixture (represented by polygon size, Fig. 1) was the highest in High FD-K followed by High FD-O, Low FD-E and Low FD-D, matching the FD assessment by means of Rao's Q (Table 1). The first 2 dimensions of the PCA explained 68.7% of trait variation (Fig. 1, Table S1) and showed a clear separation of evergreen and deciduous species and their mixtures; however, low-FD and high-FD mixtures (which shared species) overlapped. PC1 mostly represented a gradient of structural and secondary metabolites, and PC2 represented a gradient in elemental composition. *A. glutinosa* was clearly separated from oak species due to its particularly high concentration of lignin and N. LQI (Table 1) was lowest for *Q. coccifera* and the evergreen mixture (Low FD-E), and highest for *A. glutinosa* and the High FD-K mixture. The third dimension of PCA explained 15.9% of traits variation and helped to further separate *A. glutinosa* from the rest of species.

3.2. Litter decomposition

In monocultures, the highest decomposition was found for *A. glutinosa* in presence of detritivores, and also for the semi-deciduous *Q. faginea* in their absence; decomposition was lowest for the deciduous *Q. robur* and *Q. canariensis* with and without detritivores (Fig. S4A, B). Decomposition of monocultures was explained by LQI only when detritivores where present ($R = 0.60$, $p < 0.001$); microbially-mediated decomposition showed a similar but not significant trend ($R = 0.37$, $p = 0.076$). The mixture with *A. glutinosa* (High FD-K) showed the highest decomposition and the deciduous mixture (Low FD-D) the lowest, regardless of detritivore presence (Fig. S4A, B), but only in their presence the difference was significant (One-way ANOVA; $F_{3,24} = 11.474$, $p < 0.001$). Decomposition of mixtures was positively related with Rao's Q, but LQI only explained decomposition in microcosms with detritivores (Table S4).

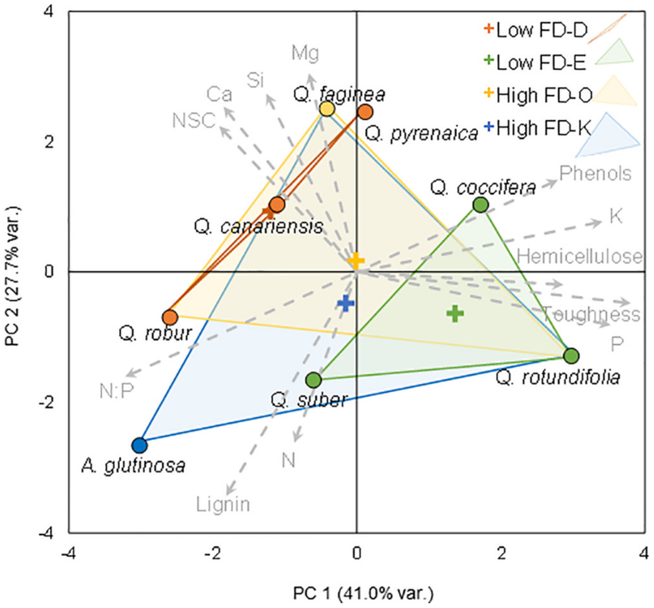

Fig. 1. Projection of the 2 first principal component axis showing differences among litter traits of the 8 individual species and 4 litter mixtures (Explained variance = 68.7%). Vectors represent each leaf trait weight and circles each plant litter position: deciduous in brown, semi-deciduous in yellow, evergreen in green and key species in blue. Triangular shapes represent the mixtures and crosses the weighted means of trait values calculated for each mixture. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Net diversity effects (NDE) on decomposition were only significant in the High FD-K mixture, being positive for total (detritivores + microorganisms; Fig. 2A) and detritivore-mediated decomposition (Fig. 2B), but negative for microbially-mediated decomposition (Fig. 2C). The magnitude of these effects was 23.8%, 50.5% and 11.4% for total, detritivore-mediated and for microbial decomposition, respectively. When partitioning NDE (Fig. 2D–I), we found negative complementarity effects only in microbially-mediated decomposition of the High FD-K mixture. This mixture always showed positive selection effects. The NDE on detritivore-mediated decomposition was correlated with LQI, while selection effects on total and detritivore-mediated decomposition were correlated with both, LQI and Rao's Q (Table S4).

Furthermore, intraspecific comparisons of litter decomposition (i.e. comparison of LML of a particular species in monocultures and mixtures) highlighted significant mixing effects for some litter species (Fig. 3), being noteworthy the consistent pattern found for *A. glutinosa*: faster decomposition in the mixture (High FD-K) than in monocultures with detritivores, but lower without them.

3.3. Litter nutrient dynamics

When detritivores were present, LNL was mostly positive in monocultures and mixtures (Fig. S4C), accompanied, in general, by an increase of litter N concentration (N%; range of increase: 1.4–23.8%). LNL increased with LQI in monocultures ($R = 0.54$, $p = 0.007$) and also with Rao's Q in mixtures (Table S4). Mean NDE on LNL was positive for all mixtures, but only significant (24% increase) in the High FD-K mixture (Fig. 4A).

Without detritivores we observed net N immobilization (i.e., negative LNL) in low-diversity mixtures (Low FD-D and Low FD-

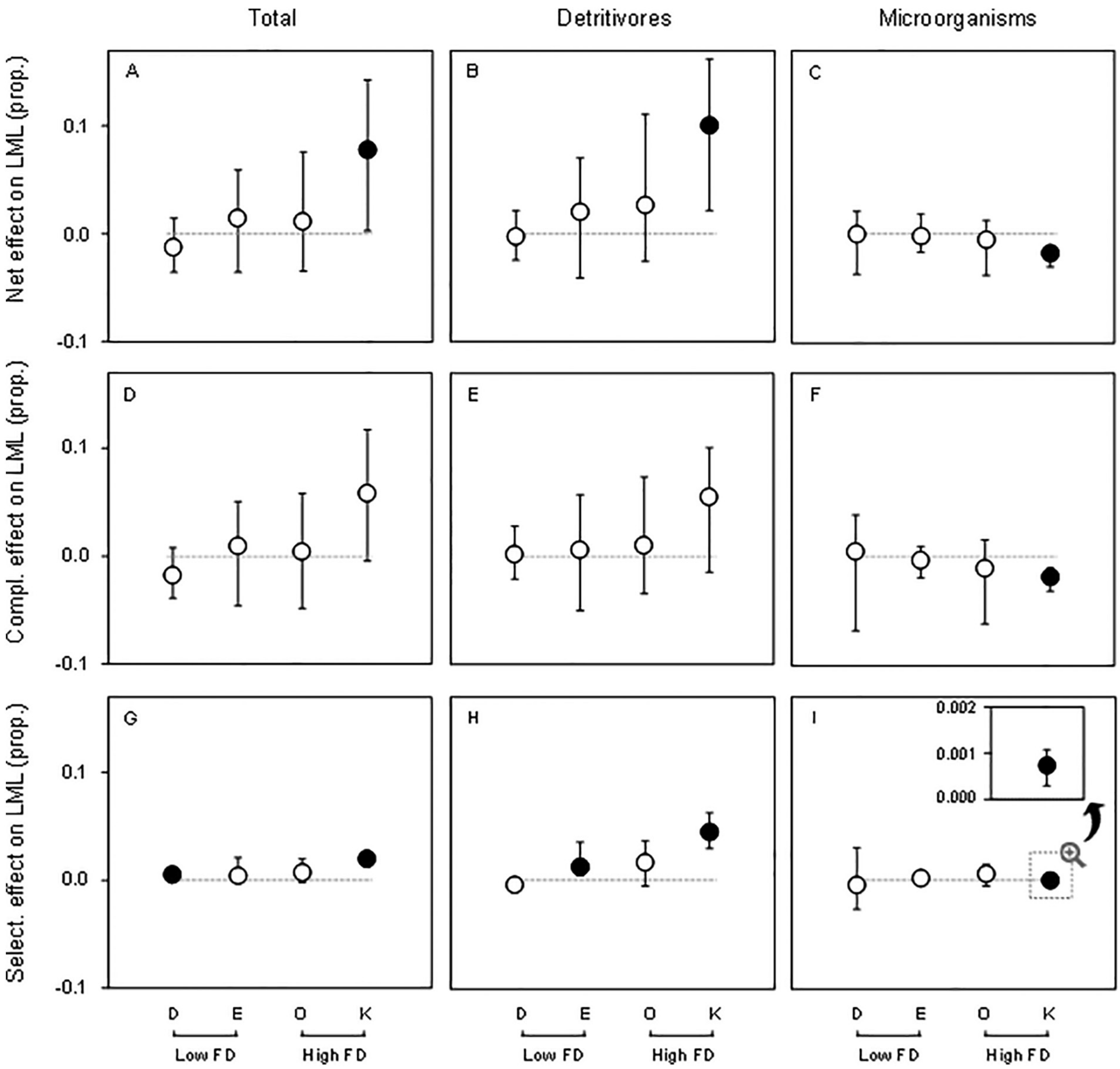

Fig. 2. Net diversity, complementarity and selection effects on total (A, D and G), detritivore-mediated (B, E and H) and microbially-mediated (C, F and I) litter mass loss (LML; proportion). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect, i.e. the null expectation that mixtures do not differ from expected ones, estimated from monocultures. Closed circles represent intervals that reject the null hypothesis (i.e., confidence interval do not contain the 0-value) and open circles represent intervals that accept the null hypothesis.

E; Fig. S4D). In contrast, most monocultures and high-diversity mixtures (High FD-O and High FD-K; Fig. S4D) showed positive LNL. Litter N concentration (N%) generally increased (3.9–17.7%). LNL in mixtures was positively related with LQI and Rao's Q (Table S4). NDE on microbially-mediated LNL was negative in all mixtures (Fig. 4B).

In presence of detritivores, LPL was positive in mixtures and most monocultures (Fig. S4E), increasing with mixture's Rao's Q (Table S4). In these microcosms, litter P concentration (P%) generally increased in deciduous oaks (1.6–36.2%) but decreased in the other species. No diversity effects on LPL were detected in presence of detritivores (Fig. 4C).

Without detritivores, litter P content tended to increase (negative LPL values) in low-diversity mixtures (Low FD-D and Low FD-E), and to decrease (positive LPL values) in high-diversity mixtures (High FD-O and High FD-K; Fig. S4F), being positively related to Rao's Q (Table S4). Litter P concentration (P%) increased in all litter species (2.8–56.3%) except *Q. faginea* (11.3–14.4% of reduction). NDE was negative for all mixtures without detritivores, excepting High FD-O (Fig. 4D).

3.4. Fungal biomass

In monocultures, ergosterol concentration varied among species (with detritivores: $F_{7,\,16} = 6.28$, $p = 0.001$; without detritivores: $F_{7,\,16} = 3.01$, $p = 0.032$), being on average higher in deciduous than evergreen species (Fig. S4G, H). Similarly, comparisons across litter mixtures showed significant differences in ergosterol concentration within microcosms with ($F_{3,8} = 10.79$, $p = 0.003$) and without ($F_{3,8} = 12.68$, $p = 0.002$) detritivores, being the lowest in the evergreen mixture (Fig. S4G, H). NDE on fungal biomass was always negative in microcosms with detritivores (effects from −13.5% to −21.4%), and more variable without them (Fig. 5A, B), being highest (ca. −35%) in Low FD-E and High FD-K mixtures (Fig. S4H). Complementarity effects followed the net diversity effects' pattern (Fig. 5C, D). Selection effects, always of lower magnitude than complementarity, arose only in few combinations (Fig. 5E, F). No relationships between ergosterol accrual and LQI or Rao's Q were found (Table S4).

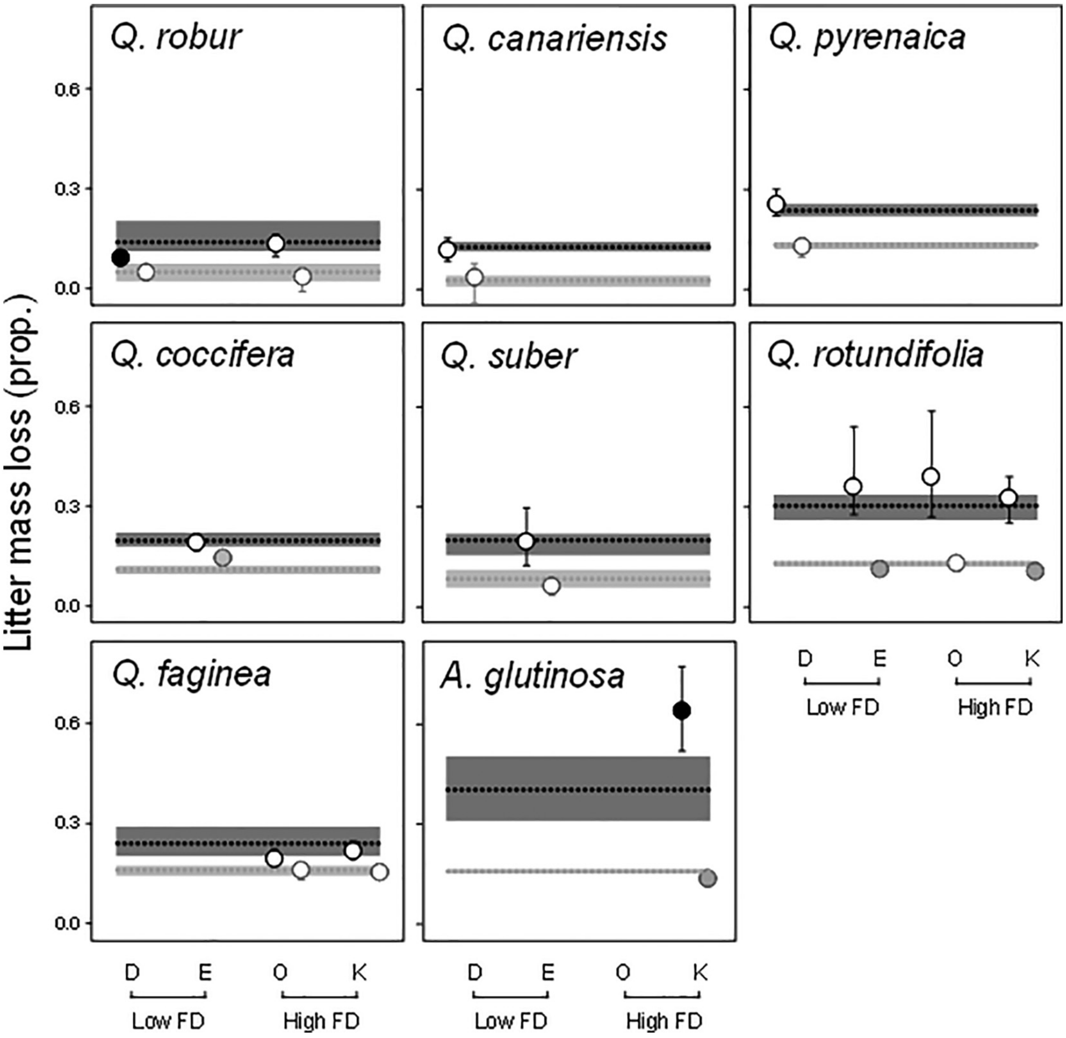

Fig. 3. Comparison of litter mass loss (LML; proportion) of each litter species among treatments where present. Circles are mean values in mixtures (black and grey represent treatments with and without detritivores, respectively). Dotted lines represent monoculture values, following the same color scale. Whiskers and shadows (dark grey for treatments with detritivores) denote upper and lower limits of 95% nonparametric bootstrapped confidence intervals for mixtures and monoculture values, respectively. Closed symbols represent intervals that are significantly different of monoculture values.

3.5. Detritivore performance

In monocultures, the highest survival of detritivores was found in *Q. suber* and *A. glutinosa* microcosms, whereas the lowest was observed in 2 deciduous oaks (*Q. robur* and *Q. pyrenaica;* Fig. S5A). Within mixtures, no statistically significant differences were found (Fig. S5B).

In monocultures, DMG was the highest when fed *Q. rotundifolia* and *A. glutinosa*, and the lowest when fed deciduous oak litter (Fig. S6A), exhibiting a linear relationship with LQI ($R = 0.45$, $p < 0.010$). DNG and DPG followed a similar pattern to that of DMG (Fig. S6B, C). DNG in monocultures was also related to LQI ($R = 0.37$, $p = 0.027$). However, *Allogamus* larvae decreased their N and P body concentration regardless of the treatment (ca. 8.6% and 14.4% of reductions of N and P, respectively). NDE on DMG was positive in the high FD-K mixture (Fig. 6A; effect size: 49%), whereas no NDE was found for DNG (Fig. 6B), and DPG exhibited a positive NDE on individuals fed Low FD-D litter (Fig. 6C).

4. Discussion

Global change forecast scenarios consider the possibility of a replacement of functional groups or the loss of key plant species in riparian areas (Kominoski et al., 2013; Salinas et al., 2018). However, the effects on stream ecosystem functioning appear to be difficult to understand due to the complex trade-offs within and among decomposers exploiting mosaics of litter species (Gessner et al., 2010; Santonja et al., 2019). This study assesses this topic from a functional approach. Our results exhibit different diversity effects on the performance of detritivores and microbial decomposers, being such effects more related with the presence of the key plant species *A. glutinosa* rather than with the FD derived from the variation in leaf habits. This highlights the crucial role of N-fixing species, and point to litter nutrient concentration as driver of B-EF relationships (Flynn et al., 2011; Santonja et al., 2019).

4.1. Functional diversity and key plant species increase trait variability of litter mixtures

The lack of consensus about which is the most suitable measure of FD in leaf litter (Lecerf et al., 2011; Petchey et al., 2009) has prevented a comprehensive assessment of its effects on stream ecosystem processes. Here, by mixing species from different functional groups (based on their leaf habits and N-fixing strategies) we confirmed the increasing trait variability along with FD, especially when *A. glutinosa* was present. That is, although FD related to leaf habits increased trait variability, it was the inclusion of the key species what made the difference. Although previous studies have attributed the observed mixing effects

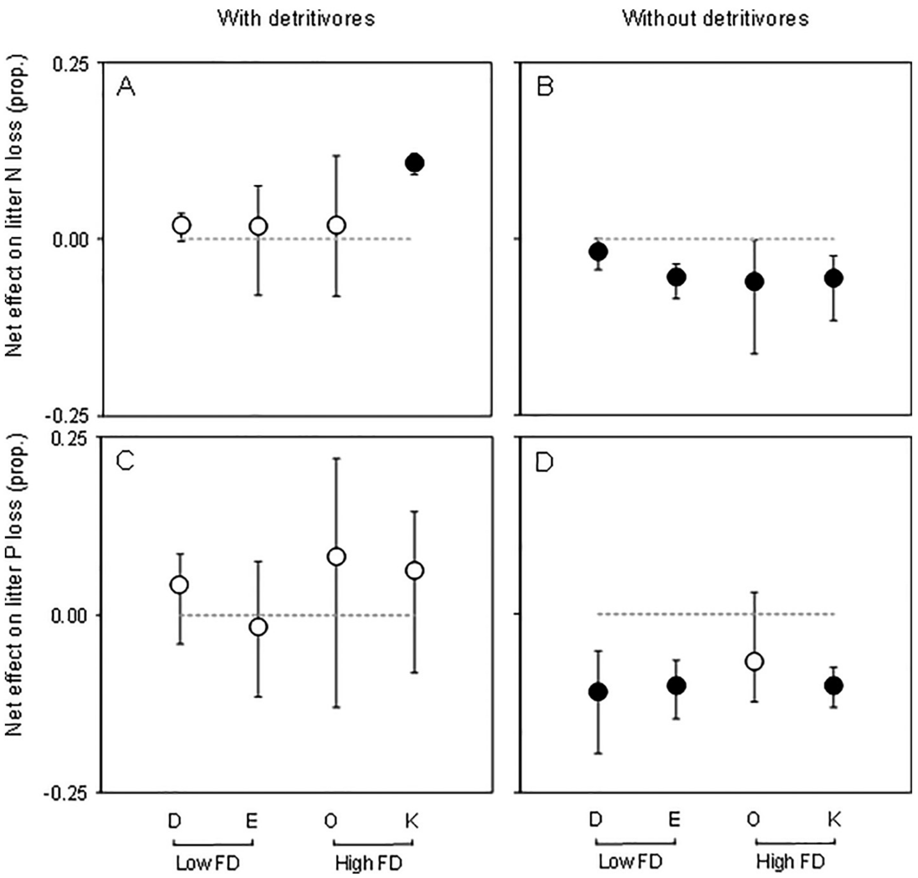

Fig. 4. Net diversity effect on litter nutrient loss (LNL and LPL; proportion). Litter N loss (A and B) and litter P loss (C and D); in microcosmos with detritivores (A and C) and without them (B and D). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

to an increase in functional dissimilarity (e.g., Santschi et al., 2018; Vos et al., 2013), others (e.g., Santonja et al., 2019) pointed to species-specific effects through increasing mean nutrient concentrations of litter mixtures (mass ratio hypothesis; Grime, 1998). Since species phylogenetically closer are expected to share more traits, some authors (Boyero et al., 2016; López-Rojo et al., 2020) have used phylogenetic distance as a proxy of trait diversity. Yet, our results concerning oak species show that even congeneric taxa can greatly differ in relevant functional traits. Nonetheless, whatever the approach, functional diversity or phylogenetic distance, the pivotal role of both litter quality and trait variability explaining biodiversity-litter decomposition interactions, often emerges (Handa et al., 2014; Lecerf et al., 2011; López-Rojo et al., 2020).

4.2. Detritivore- and microbially-mediated decomposition respond differently to litter functional diversity

In agreement with previous studies, our results reveal a trend of increasing litter total decomposition with FD (Lecerf et al., 2011; Tonin et al., 2017). However, whereas Tonin et al. (2017) reported positive net diversity effects on both detritivore- and microbially-mediated decomposition, although of lower magnitude on the second; here we detected positive net diversity effects on detritivore- and negative on microbially-mediated decomposition, but only in the mixture with the highest diversity of traits and containing alder leaf litter (i.e. High FD-K). This, and similar findings (Larrañaga et al., 2020; López-Rojo et al., 2018), point to the existence of a threshold of litter quality dissimilarity above which the effects of diversity arise. The net diversity effect on litter decomposition was mainly explained by complementarity effects, in agreement with previous studies (Handa et al., 2014; López-Rojo et al., 2018): positive (although not significant) in presence of detritivores, and negative for microbially-mediated decomposition. Often, positive complementarity effects lead to positive diversity effects on detritivore-mediated decomposition (e.g., Larrañaga et al., 2020). Here, positive diversity effects emerged from concomitant positive complementarity and selection effects in the mixture with *A. glutinosa*, the latter effect exhibiting its highest value when detritivore-mediated decomposition was isolated. *Allogamus* fed preferentially on the more palatable litter (selection), without altering consumption of other resources, as previously observed in field (Sanpera-Calbet et al., 2009) and microcosm experiments (Tonin et al., 2017). This contrasts with the general assumption that high-quality litter enhances decomposition of concurrent low-quality one (Gartner and Cardon, 2004), but supports the 'clutching at straws' effect (sensu Landeira-Dabarca et al., 2019), i.e. the presence of relatively recalcitrant litter, may enhance overall mixture consumption despite the reduction of average mixture quality. This emphasizes detritivore capability to feed simultaneously on resources of contrasting quality in order to balance their diet (Carvalho and Graça, 2007; Leroy and Marks, 2006).

The positive selection effect found on microbial decomposition suggests substrate preference (Gulis, 2001), however, contrary to our expectations, this selection effect was not translated into a greater decay of alder litter than that of oaks as usually reported (e.g., Cornejo et al., 2020; Monroy et al., 2016). Yet, the observed negative diversity effect on microbial decomposition was driven by negative complementarity. Three main factors could have conditioned this negative effect in our microcosms: low microbial colonization of litter as a consequence of the short incubation period, nutrient limitation (Ferreira et al., 2015)

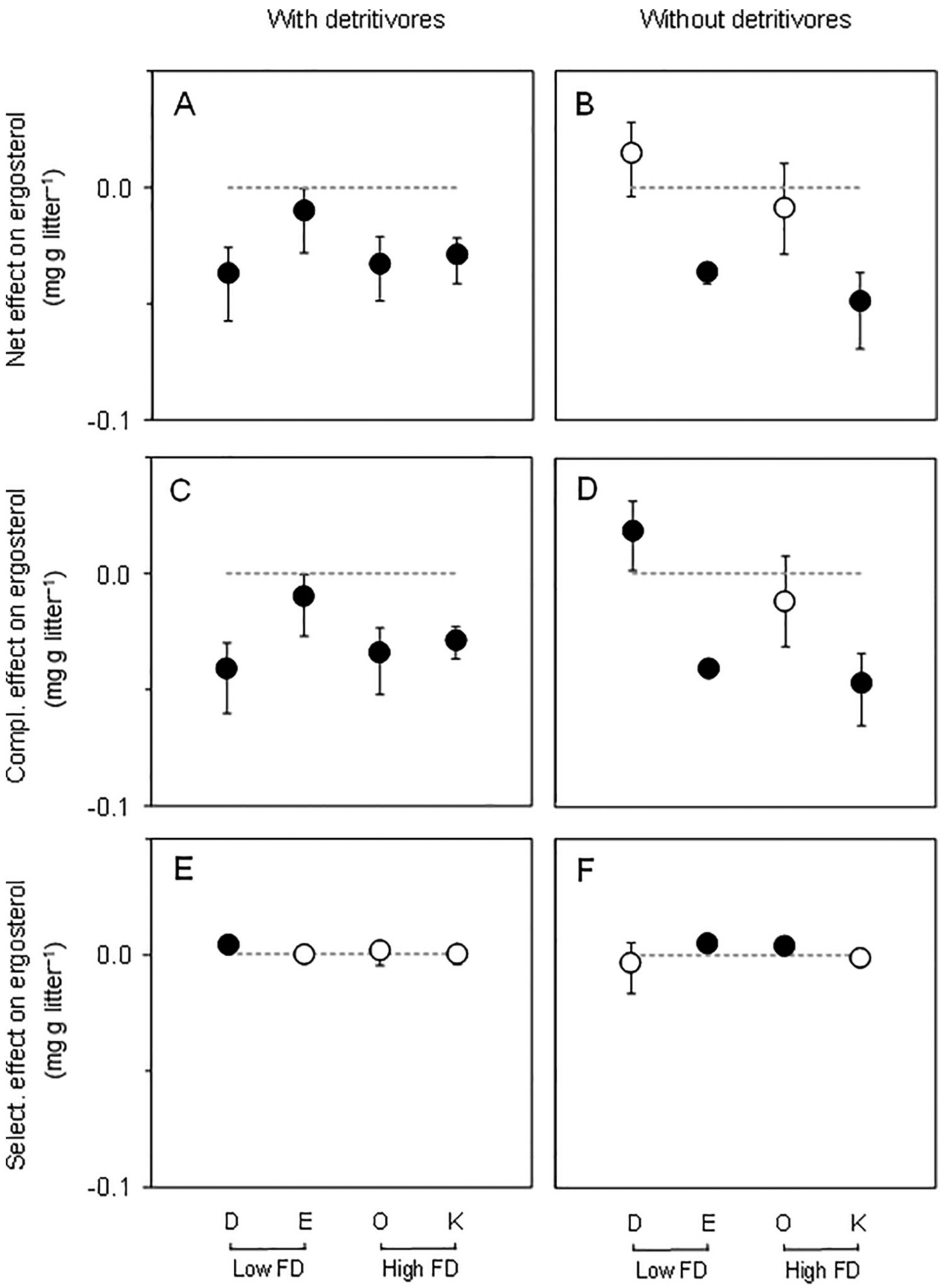

Fig. 5. Net diversity, complementarity and selection effects on fungal biomass (Ergosterol content; mg g litter^{-1}): with detritivores (A, C and E) and without detritivores (B, D and F). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

caused by the low amount of dissolved nutrients incorporated with water renewal, and/or the accumulation of leached secondary compounds, common in oak litter (McArthur et al., 1994), with putative toxic effects on microbes (Canhoto et al., 2013). However, the latter seems more likely, since the initial colonization and the nutrient limitation appeared to be the same in all microcosms, whereas the toxic effects of accumulated soluble secondary metabolites could have differed between monoculture and mixtures treatments. These effects are more likely to be detected in microcosms with lower water renewal than in field conditions where leaf leachates are constantly washed out, and can be potentiated with increasing mixture diversity, as more different compounds coming from diverse litters may create a toxic cocktail, as defined in allelopathy studies (Reigosa et al., 1999).

4.3. Functional diversity differently affects litter nutrient recycling by detritivores and microbial decomposers

We found a general trend of accelerated nutrient dynamics with detritivores as FD raised, as reported before in large-scale field (Handa et al., 2014) and microcosm (López-Rojo et al., 2019) studies. Here, significant positive diversity effects occurred only for N cycling in the mixture with *A. glutinosa*; this exhibited, although not significantly, higher litter-N loss rates than the best performing species, supporting the premise of the limiting role of N for litter consumer detritivores in headwater streams (Balseiro and Albariño, 2006; Fenoy et al., 2020; McManamay et al., 2011). Thus, litter N variability seems to drive N loss (García-Palacios et al., 2017) and, subsequently, diversity effects (López-Rojo et al., 2020) on leaf litter decomposition.

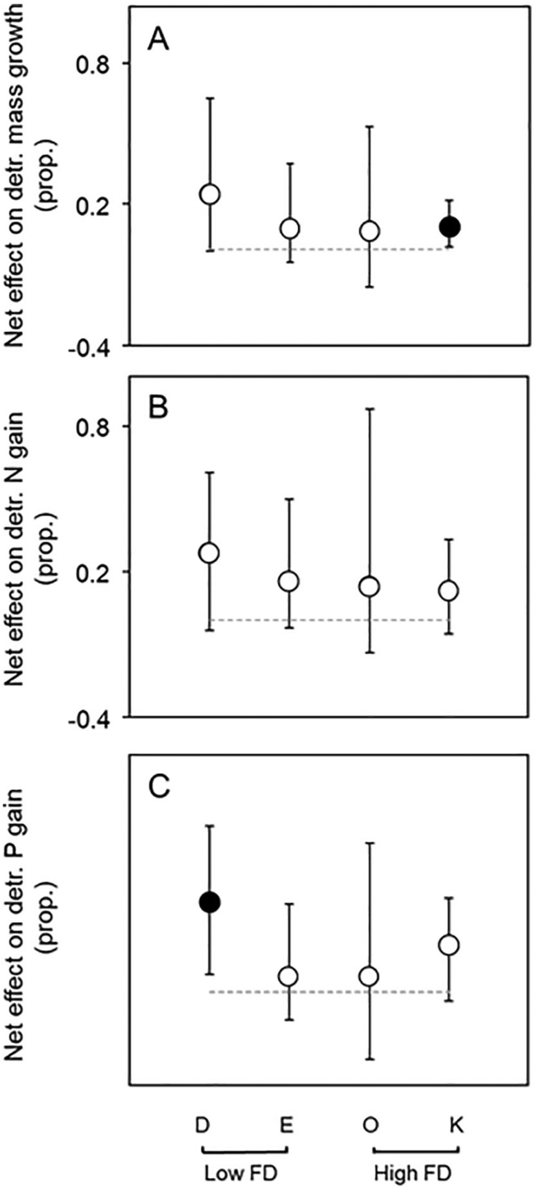

Fig. 6. Net diversity effect on detritivore growth (mass: DMG; proportion) and elemental gain (N: DNG, and P: DPG; proportion). Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented. Dashed lines denote no-effect. Closed and open circles represent intervals that reject or accept the null hypothesis, respectively.

Microbially-mediated nutrient dynamics were also determined by FD and showed mostly significant negative diversity effects, i.e. mixtures lost less nutrients than expected from monocultures. However, N and P net losses (mineralization) occurred in the two mixtures with higher FD, and nutrient sequestration (immobilization) appeared in low FD mixtures, in which microbes might have used nutrients from the water (López-Rojo et al., 2019; Suberkropp and Chauvet, 1995; Tonin et al., 2017). The latter process is expected to be higher in microcosms with detritivores, due to increased nutrient release to water (López-Rojo et al., 2019; Tonin et al., 2017), but might be masked by the high detritivore-mediated decomposition. Thus, similar negative diversity effects resulted from opposed underlying mechanisms: nutrient sequestration in low FD mixtures and nutrient mobilization in high FD ones. Most likely, the negative diversity effect (negative complementarity) also observed for microbially-mediated decomposition (i.e. microbial inhibition due to the leaching of secondary compounds; see Section 4.2) must underlie the corresponding effects on nutrients, but the specific mechanisms need further study.

4.4. Fungal biomass and detritivore performance in response to functional diversity

Contrary to our expectations (e.g., Kominoski et al., 2009), fungal biomass was, in general, negatively affected by FD. A plausible explanation for such negative diversity effect is the leaching of toxic compounds from oaks, as discussed in Section 4.2. Furthermore, high FD mixtures might promote fungal species richness (e.g., Fernandes et al., 2013) and, therefore, competitive interactions (Gessner et al., 2010; Gulis and Stephanovich, 1999), resulting in negative complementarity effects. Nevertheless, the potential limitations that our microcosms may have imposed to fungal performance (i.e., nutrient limitation and poor fungal colonization due to the short pre-incubation period of litter and the relatively lack of water renewal) could have distorted diversity effects on fungal biomass. Nonetheless, a comparison of ergosterol concentrations in alder leaf litter between our experiment (monocultures) and a field experiment carried out in streams of Sierra Nevada (Casas et al., 2011) revealed very similar values. Whatever the cause, our results contrast with other studies which found that litter mixtures may improve nutrient availability, and thus microbial performance (Handa et al., 2014; Schimel and Hättenschwiler, 2007), failing to support a positive effect of plant diversity on microbial decomposers.

Survival of detritivores was variable and appeared to depend on intraspecific interactions (Boyero and Pearson, 2006) coupled with litter quality, more than litter diversity. We observed cannibalism, likely promoted in those mixtures lacking a litter type that fulfils the nutrient requirements of detritivores (e.g., Bastian et al., 2008). Detritivore mass growth followed a similar pattern to that of detritivore-mediated decomposition, which often increased with litter quality and diversity (Fernandes et al., 2015). However, similarly to other studies (e.g., Tonin et al., 2017), detritivore growth did not increase with increasing litter diversity, although a slight trend of higher mass and nutrient gain with increasing litter FD was discerned, giving some support to the balanced diet hypothesis. Diverse mixtures provide more varied nutritional resources, which effects translate into higher consumer biomass (DeMott, 1998), what could subsequently shift from a bottom-up into a top-down control of the process (Duffy et al., 2007). This trend was, nonetheless, not entirely clear since significant positive diversity effects on growth (ca. 50%) were only detected in the highest FD treatment (High FD-K). The combination of two nutrient-rich litter species (*A. glutinosa* and *Q. rotundifolia*) in this mixture clearly favored litter consumption (Santonja et al., 2019; Vos et al., 2013) and detritivore growth (Danger et al., 2013). Cannibalism may have altered N and P gains to some extent. However, *Allogamus* larvae were only able to significantly gain N and P when fed two monocultures where no signals of cannibalism were observed and containing the most nutrient-rich litters (i.e. *A. glutinosa*-N and *Q. rotundifolia*-P), possibly because no other litter type fulfilled their, usually high, nutrients demands. N is used by caddisflies to produce somatic proteins, silk, and N-rich chitin for case building (Frainer et al., 2016), while P is required in ribosomal RNA (Sterner and Elser, 2002). Consumption of litter assemblages lacking a nutrient-rich litter may lead to a decrease in detritivore growth, which can cause a reduction of adult size (Carvalho and Graça, 2007; Flores et al., 2014), consequently hindering their reproductive success and survivorship and pointing to bottom-up effects (Martínez et al., 2013; Swan and Palmer, 2006). Despite all the aforementioned, it is important to consider that our results may not be applicable to other detritivore species, e.g., if they were more sensitive to leached secondary compounds or unable to face tough and recalcitrant leaves such as those from oaks; nor to the entire detritivore community present in the streams due to interspecific interactions.

4.5. Detritivores and A. glutinosa: key players of diversity effects on decomposition

Direct assessment of mixing effects on a particular plant species in different mixtures, allowed us to delve into mechanisms driving net diversity effects beyond complementarity and selection effects (Kominoski et al., 2009). This approach further supports the determinant role of *A. glutinosa* mediating diversity effects. In the presence of detritivores, the species accompanying *A. glutinosa* in the High FD-K treatment (*Q. rotundifolia* and *Q. faginea*) decomposed at a similar rate than in their monocultures. Therefore, suggesting that the observed significant net diversity effect found in this mixture relied mainly on an increased consumption of *A. glutinosa* within the mixture compared to its monoculture. The scarcity of the preferred litter within an assemblage may enhance the consumption of the co-existing resources (Bastian et al., 2008). Nonetheless, we provided *A. glutinosa* litter in excess (>50% of *A. glutinosa* litter remaining at the end of the experiment), what further supports the diversity effect found in our experiment. In contrast, microbially-mediated decomposition reveals the opposite pattern: nutrient-rich litters (*A. glutinosa* and *Q. rotundifolia*) exhibited lower decomposition rates in the High FD-K mixture than in monocultures, driving the observed negative diversity effect. This is possibly due to short initial litter colonization period carried out, the leaching of secondary compounds (as discussed in Section 4.2), or because positive diversity effects on microbially-mediated decomposition require longer incubation times (e.g., Fernandes et al., 2013; Santschi et al., 2018).

5. Conclusions

Our outcomes suggest combining the use of functional diversity and leaf quality when assessing B-EF relationships. This study underlines that litter nutrient content had greater relevance for diversity effects compared to diversity of other litter traits. N-fixing plant species play a prominent key role, entailing a leap of quality and trait variability of litter assemblages, which drives diversity effects on ecosystem functioning. Moreover, our results underscore the combined role of detritivores and plant key species supporting positive diversity effects on instream decomposition in headwaters. However, these outcomes must be interpreted cautiously given the difficulty to conciliate the required time to decompose of microorganisms and shredders, and the usual constraints of microcosms studies; for example, the use of only one stream detritivore species, and the fact that the microbial community was reduced to that present in the weekly water renewal, added to the possible existence of a nutrient limitation and an accumulation of litter leachates. Considering the forecasted loss of deciduous in favor of evergreen plant species (Kominoski et al., 2013; Salinas et al., 2018) and, in particular, the black alder dieback due to an emergent fungal disease (Bjelke et al., 2016; Gibbs et al., 1999), further studies should be carried out to improve our knowledge on effects of such changes and to advice managers about which species should receive special attention in conservation programs, in order to mitigate instream effects of functional changes in riparian plant communities.

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CRediT authorship contribution statement

J. Rubio-Ríos: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Visualization. **J. Pérez:** Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **M.J. Salinas:** Investigation, Writing – review & editing. **E. Fenoy:** Investigation, Visualization, Writing – review & editing. **N. López-Rojo:** Formal analysis, Investigation, Writing – review & editing. **L. Boyero:** Conceptualization, Funding acquisition, Writing – review & editing. **J.J. Casas:** Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149266.

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RESEARCH ARTICLE

Cross-species coprophagy in small stream detritivores counteracts low-quality litter: native versus invasive plant litter

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Abstract

Understanding how stream detritivores can cope with riparian plant invasions is relevant to predict future impacts on detritivore assemblages and the functioning of small stream ecosystems, where litter decomposition mainly fuels food webs. In a microcosm feeding trial, we examined survival, consumption, growth, and energetic status of two detritivores coexisting in nature: the amphipod *Echinogammarus obtusidens,* and the snail *Melanopsis praemorsa*. Detritivores were fed two types of leaf litter—the native tree *Populus alba*, and the invasive reed *Arundo donax*—and the corresponding con- and hetero-specifics faeces produced from the ingestion of the litter from these species. We aimed to assess whether lowland stream detritivores can use coprophagy as a suitable feeding strategy alternatively to *Arundo* litter; and how coprophagy affects their fitness compared to litter from native and non-native plants. *Echinogammarus* presented the highest performance when fed *Melanopsis*' faeces but the lowest when fed leaf litter, especially the invasive one, exhibiting a high mortality rate. *Melanopsis* exhibited good and similar performance when fed on both leaf litter species, as well as on conspecific faeces. *Echinogammarus* performed best as cross-species coprophagous, enabling the amphipods to thrive on the low-quality litter species offered when *Melanopsis* was present. These results suggest that *Echinogammarus obtusidens* may act as a coprophagous when no high-quality litter is available. Thus, the snail seems to play a key role facilitating the access to nutrients of recalcitrant leaf litter to sympatric detritivore species via coprophagy.

Keywords *Arundo donax* · *Echinogammarus obtusidens* · Facilitation · Faeces · Plant invasion · *Melanopsis praemorsa*

Introduction

The structure and function of first-order streams greatly rely on allochthonous inputs of organic matter (OM), mostly riparian leaf litter (e.g. Abelho 2001; Wallace et al. 1997) which is processed by decomposers and detritivores. Therefore, changes affecting the quality and/or magnitude of litter inputs to streams may alter their community structure (Bärlocher and Graca 2002; Martínez et al. 2013) and ecosystem functioning (Casas et al. 2013; Hladyz et al. 2011; Larrañaga et al. 2021). This reliance on leaf litter from riparian vegetation makes small forested streams especially sensitive to plant invasions (e.g. Pereira and Ferreira 2021). The favourable conditions for plant life in riparian zones and the reduction of the biotic resistance of native vegetation to invasions caused by natural and/or unceasing anthropic disturbances, promote the proliferation of non-native species in these ecosystems (Castro Díez and Alonso Fernández 2017).

Giant reed, *Arundo donax* L. (Poaceae; hereafter *Arundo*), is among the 100 world's worst invasive alien species (Lowe et al. 2000), particularly in sub-tropical and temperate wetlands (Canavan et al. 2017). This species is considered one of the greatest threats for the conservation of lowland riparian ecosystems in Mediterranean-type climates (Aguiar and Ferreira 2013), since it creates mono-specific stands by displacing native vegetation, causing an impoverishment of the native riverine biodiversity (Maceda-Veiga et al. 2016). Regarding the impacts caused by *Arundo*, most studies have focused on the riparian corridor (e.g. Herrera and Dudley 2003; Maceda-Veiga et al. 2016), while its effects on fluvial food webs remain almost unexplored (but see Going

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and Dudley 2008). Leaf litter of non-native invasive plants can decompose more rapidly or slowly than native species, but, in both cases, it may alter stream ecosystem processes (Marks 2019; and references therein). Leaf litter of *Arundo* begin decomposing with poor nutritional value for aquatic detritivores due to its high toughness and silicon concentration, and low nitrogen content, even compared with low-quality native species such as *Populus* spp. (Going and Dudley 2008; Salinas et al. 2018).

The generation of fine particulate organic matter (FPOM) is closely linked to detritivores litter consumption (Halvorson et al. 2017a, b; Santonja et al. 2018). Small detritivores benefit from this FPOM produced by the feeding activity of larger detritivore shredders (Tonin et al. 2018), particularly by ingesting the abundant pool of FPOM in the form of faecal aggregates (Cummins et al. 1989) which, in some cases, can even equal the amount of detritus directly derived from leaf litter inputs (Malmqvist et al. 2001). Overall, coprophagy may provide nutritional benefits to detritivores (Weiss 2006), due to the often increased nutritional value of shredder's faecal pellets (Halvorson et al. 2017b; Hood et al. 2014) which is further promoted by microbial action at warm temperatures (> 10 °C; Joyce and Wotton 2008). However, this mechanism is under-studied in lotic ecosystems (Wotton 2007; Wotton and Malmqvist 2001), probably because egested material have been often considered to lack labile C (high lignin content; Yoshimura et al. 2008) and to possess higher C:N and C:P ratios than original resources (Callisto and Graça 2013) enabling it to remain unaltered for months (Joyce et al. 2007).

Here, we intend to assess responses of two major detritivore species to the invasion of riparian zones by *Arundo* in lowland Mediterranean low-order streams: the small shredding amphipod, *Echinogammarus obtusidens* (Pinkster and Stock, 1972; hereafter *Echinogammarus*) and the large grazing snail, *Melanopsis praemorsa* (Linnaeus, 1758; hereafter *Melanopsis*). The snail is an abundant generalist feeder in this stream-type, which behaves as an important consumer of leaf litter (Casas et al. 2011), with outstanding capacity to digest low-quality litter (Fenoy et al. 2021). We aimed to: (i) estimate the nutritional value of leaf litter from the invasive *Arundo* compared to that from the native *Populus alba* L. (Salicaceae; hereafter *Populus*) and faeces; (ii) test to what extend these detritivores can use coprophagy, as a successful feeding strategy to deal with low-quality litter from the non-native species; and (iii) compare the performance (survivorship, litter consumption, growth, and energetic reserves) of both detritivores when offered the different diets. To this end, we designed laboratory feeding trials in which both detritivore species were fed with leaf litter, con- or heterospecifics faeces (i.e., faeces from the same or from other species, respectively). After 21 days of microcosm incubation, the following hypotheses were examined: (i) both detritivore species will exhibit a superior performance when fed litter of higher nutritional quality (*Populus*) over litter of lower quality (*Arundo*); (ii) faeces represent a more nutritive food than the original leaf litter; (iii) both detritivores can use coprophagy as a suitable feeding strategy; and (iv) performance of *Echinogammarus* will improve when fed on faeces from *Melanopsis*.

Materials and methods

Field procedures used with leaf litter and detritivores

For feeding tests, we selected leaf litter of the invasive perennial grass giant reed (*Arundo donax)* and the native semi-deciduous tree white poplar (*Populus alba*). Both are species common in warm temperate and Mediterranean zones, particularly in lowland streams. Senescent leaves were collected from the riparian vegetation before the experiment, air-dried at room temperature (≈ 23 °C) for 1 week and stored in the dark until needed.

Portions (≈ 5 g each) of each leaf litter species were introduced within mesh bags (0.5 mm mesh size and 30 cm^2 each bag) and submerged during 2 weeks, to allow leaching of soluble compounds and microbial conditioning, along a 50 m stream-reach in the Barranco del Cura stream (291 m *a.s.l*, 36.84°N 2.64°W; mean summer water temperature 21.5 ± 0.1 °C, mean ± SD) approx. 3 weeks before the experiment. This is a non-polluted low-order stream notably invaded by giant reed (*ca.* 75% cover), where there are still a few individual trees or clumps of white poplar. Additional environmental information of the basin and stream can be found in Table S1. After retrieval, litter was transported to the laboratory, and 12 mm Ø leaf discs were cut with a cork borer. Leaf discs were air-dried at room temperature (≈ 23 °C) for 1 week, weighted to the nearest 0.1 mg and frozen (− 20 °C) until needed in the feeding experiment. An extra set of leaf discs was used to measure initial dry mass (DM; 70 °C, 72 h) of the discs offered to the animals and litter traits (toughness, C, N, P, Si, Ca, K and Mg concentrations, and lignin, total phenols and condensed tannins contents; Supplementary methods).

Detritivores were collected from the same stream, placed in plastic containers filled with stream water and transported to laboratory in a portable refrigerator. There, animals were acclimatized to experimental conditions (see below) for 1 week fed litter from the stream. Animals were starved for 24 h prior the start of the experiment to allow evacuation of their gut contents.

Experimental set-up in the laboratory

Microcosms were placed in a temperature-controlled chamber at 20 °C, to mimic summer temperature in Barranco del Cura stream, under a light:dark regime of 12:12 h and with constant aeration of water. Each microcosm consisted of a 300-mL glass jar filled with 250 mL of stream water: total dissolved P 7 µg P L^{-1}; N-NO_3 723 µg N L^{-1}; pH 8.13; electric conductivity 896 µS cm^{-1}; alkalinity 250 mg $CaCO_3$ L^{-1}. Water was previously filtered through 1.2 µm pore size glass fibre filters (Merk Millipore Ltd.). Microcosms were horizontally divided into two compartments using a septum of 1 mm mesh size to allow the passage of faecal pellets from the upper to the lower compartment (Fig. S1). *Echinogammarus* were supplied with a pebble, previously incinerated, to serve as a shelter. In the upper compartment, detritivores were fed microbially conditioned leaf litter discs of *Arundo* or *Populus*, while in the lower compartment were fed faeces falling from the upper compartment. Finally, only six out of the eight possible treatments were carried out: both litters (*Arundo* and *Populus*) and faeces of *Echinogammarus* (E-Ad or E-Pa) or *Melanopsis* (M-Ad or M-Pa) fed on them. (Fig. S1). The two treatments of *Melanopsis* fed on faeces from *Echinogammarus* were not conducted assuming low nutritional significance of faeces from *Echinogammarus* for *Melanopsis*, given the notable differences in body mass between species (~ 10 times higher in *Melanopsis*). Fifteen replicates of each treatment were performed, totalling 90 microcosms and 180 animals (two individuals per microcosms, one in each compartment). Two leaf litter discs were offered to each individual in the upper compartments, being replaced by new discs every 4 days to prevent food deprivation, whereas availability of fecal pellets was visually verified by daily observations of the lower part of the microcosms. Removed leaf discs were oven dried (70 °C, 72 h), and weighed to the nearest 0.1 mg to determine DM loss during the time of exposure. Five control discs per plant species were randomly incubated in upper compartments inside 0.5 mm mesh size bags to isolate detritivores consumption from microbial decomposition. Simultaneously, additional pools of individuals of each detritivore species were placed in extra microcosms and fed litter of *Arundo* and *Populus*, and their faeces were collected daily using a Pasteur pipette, frozen and used to estimate nutrient content analyses (Supplementary methods). Water was renewed every 5 days with fresh filtered stream water to prevent excessive microbial proliferation and to compensate for water losses. The experiment lasted 21 days. Mortality was recorded daily and dead animals were measured as explained in the next section and rapidly replaced by a new one. The last day, survivors from each microcosm were starved (24 h), measured and frozen (− 20 °C) until needed for total lipids and glycogen analyses.

Analytical procedures with detritivores

Just before and after the experiment, each experimental individual was photographed under a binocular microscope, and their total length—TL = dorsal length of the ten thoracic segments in *Echinogammarus* and shell length in *Melanopsis*—was measured using the *SigmaScan Pro v 5.0* image analyser. Detritivores dry mass (DM) was estimated from TL (mm)-DM (mg) relationships, established using additional animals of each species collected simultaneously to the experimental individuals: $DM_{Echinogammarus}$ (mg) = 0.5743 × TL − 1.3934; $R^2 = 0.71$; $DM_{Melanopsis}$ (mg, without shell) = 8.8483 × TL − 61.862; $R^2 = 0.87$. We used a linear equation as it fitted better than the exponential one. Detritivores initial biomass per microcosm was on average 7.10 ± 0.44 mg for *Echinogammarus*, and 70.23 ± 1.67 mg (mean ± SE) for *Melanopsis* (Fig. S2).

Energetic reserves of detritivores, lipids and glycogen, were measured using the sulfo-phospho-vanillin and the anthrone reactions respectively, following the methods described in (Charron et al. 2014) with minor modifications (see Fenoy et al. 2021). After removal of shells if necessary, each animal was homogenized in 1 mL methanol using 5 mm Ø stainless balls and a vortex mixer for 2 min. The homogenized mix was then divided in two identical aliquots and frozen until used in analyses. Optical density was measured at 525 nm for lipids and at 630 nm for glycogen. Calibration solutions were prepared, for lipids using a commercial olive oil solution (5 g L^{-1}) solubilized in chloroform, and for glycogen (2.5 g L^{-1}) solubilized in distilled water.

Data analysis

We carried out comparisons of litter traits among plant species (Table S2) and of nutrient concentrations among litter and faeces (Table 1) using t-test and one-way ANOVAs followed by Tukey's Honestly Significant Difference (HSD) analyses (*anova* and *TukeyHSD* functions of 'stats' R package), respectively. *Log* or *logit* transformations of variables were used when required for decimal and proportion values, respectively.

Additionally, we characterised nutrient (C, N and P) variability among litter and faeces using a principal component analysis (PCA) with the *prcomp* function in the 'stats' package of R statistical software (R Core Team 2020; Fig. 1). A second PCA was used to assess litter traits variability (Fig. S3). Previously, we calculated Spearman paired correlations (*cor* function of 'stats' R package) between 14 leaf traits and, within each highly correlated pair ($r > 0.80$; Fig. S4), we selected only one. Therefore, the eight less correlated litter traits were included in the PCA: N, P, N:P, total phenols, condensed tannins, Si and Mg.

Table 1 Mean (±SE) of carbon (C), nitrogen (N) and phosphorus (P) concentrations (% DM) and molar elemental ratios (C:N, C:P and N:P), of each type of leaf litter and faeces used in this study

	Arundo	*Populus*	E-Ad	E-Pa	M-Ad	M-Pa	*P* value
C	43.7 ± 0.19^{a}	46.6 ± 0.24^{a}	22.1 ± 0.9^{c}	26.9 ± 2.7^{bc}	31.6 ± 1.3^{b}	40.9 ± 1.5^{a}	< 0.0001
N	0.6 ± 0.018^{d}	2.2 ± 0.081^{a}	0.9 ± 0.05^{c}	1.2 ± 0.1^{b}	0.9 ± 0.05^{c}	2.1 ± 0.1^{a}	< 0.0001
P	0.02 ± 0.001^{b}	0.06 ± 0.003^{a}	0.07 ± 0.016^{a}	0.07 ± 0.006^{a}	0.07 ± 0.003^{a}	0.06 ± 0.002^{a}	< 0.0001
C:N	92.1 ± 3.01^{a}	25.1 ± 1.07^{cd}	30.4 ± 2.4^{c}	26.8 ± 1.3^{cd}	42.7 ± 1.7^{b}	22.5 ± 1.0^{d}	< 0.0001
C:P	7143 ± 449^{a}	2098 ± 126.9^{b}	982 ± 298^{d}	1033 ± 93.2^{cd}	1233 ± 61.2^{c}	1858 ± 93.8^{b}	< 0.0001
N:P	91.4 ± 5.7^{a}	98.8 ± 6.0^{a}	38.7 ± 11.7^{b}	45.5 ± 4.1^{b}	34.3 ± 1.7^{b}	97.6 ± 4.9^{a}	< 0.0001

Different letters indicate significant differences ($P < 0.05$) on the basis of linear models followed by pairwise multiple comparisons

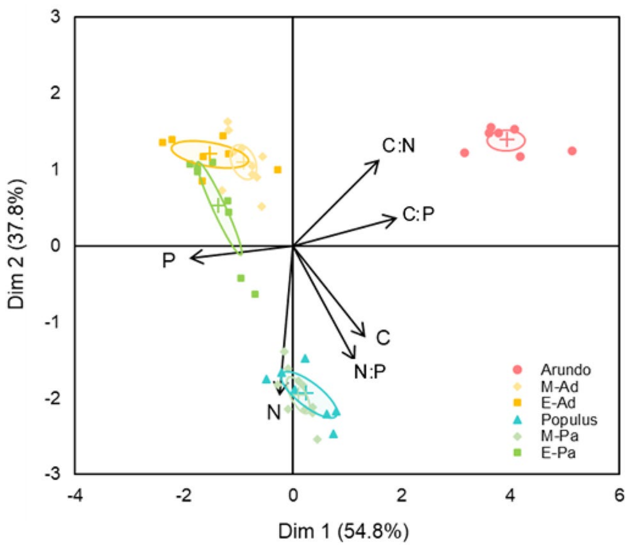

Fig. 1 Projection of the 2 first principal component axis showing differences among nutrient concentrations (C, N and P) and nutrient ratios (C:N, C:P and N:P) between the 2 plant species (*Arundo* [Ad] and *Populus* [Pa]) and the different types of faeces produced by amphipod *Echinogammarus* (E-Ad, E-Pa) and snail *Melanopsis* (M-Ad, M-Pa) (Explained variance = 92.6%). Vectors represent each trait weight and symbols each litter or faeces position. Ellipses represent 95% confidence and centroids (crosses) the average position of each diet over the PCA axes ($n = 7$–10)

We used the differences in stoichiometry between food and faeces to assess whether faeces become an enriched resource in N and/or P for consumers. In addition, we calculated the elemental imbalance (EI; see Frainer et al. 2016) between leaf litter and faeces to quantify such differences as the quotient between leaf litter and faeces C:N, C:P or N:P molar ratios (Supplementary methods).

Kaplan–Meier test was used to compare survivorship among treatments using the *survfit* function of 'survival' R package (Therneau 2015). The median time to death was calculated using the Kaplan–Meier product-limit method (Ferreira et al. 2010). Significant differences in survivorship among treatments were compared using log-rank test (Ferreira et al. 2010) which is based on the difference between observed and expected death data.

Litter consumption was quantified through litter mass loss as the difference between initial and final DM. To isolate shredders litter consumption, initial discs DM was corrected by microbial decomposition based on the litter mass loss observed in control discs. Consumption rate was then calculated as: $(DM_i - DM_f)/(DM_{detritivore} \times d)$; where DM_i and DM_f are the leaf disc DM (mg) at the beginning and at the end of the experiment, respectively; $DM_{detritivore}$ is the mean dry mass (g) of the detritivore during the experiment and d is the number of days the experiment lasted. The experiment design did not allow us to estimate faeces consumption rates. To assess differences on leaf litter consumption rates, we used two-sample t-test or Welch two-sample t-test analyses if homoscedasticity was not achieved.

We measured individual growth of each detritivore species when fed each of the treatments. Detritivores growth rate was calculated as the difference between final and initial mass against initial mass and the number of days exposed. Differences in growth and, total lipids and glycogen content were assessed using one-way ANOVA followed by Tukey's HSD test (see function and package above) or Welch's ANOVA (*oneway.test* function of the 'stats' R package) followed by Games Howell post hoc test (*games_howell_test* function of the 'rstatix' R package) if homoscedasticity assumptions were not fulfilled. Total lipids and glycogen contents were transformed when required using the *orderNorm* function transformation which was chosen by the *bestNormalize* function of the 'bestNormalize' R package (Peterson and Cavanaugh 2019) to meet the assumptions of normality and equal variance. However, as homoscedasticity was not achieved for the glycogen content of *Echinogammarus*, Welch's ANOVA was used.

For these raw variables (litter consumption, growth, lipids and glycogen content, and survival), associated effect sizes (Cohen's d) and 95% confidence intervals (CI) were calculated applying bootstrapping procedures (1000 repetitions) using the *cohens_d* function of "rstatix" R package (Kassambara 2020). We estimated effect sizes for all the possible two-sample comparisons. We report mean treatment effect sizes (*d*) and 95% confidence intervals (CI), where effect sizes of 0.20–0.49, 0.50–0.79 and ≥ 0.80 were considered small, medium and large, respectively (Cohen 1988). All statistical analyses were performed using R software version 4.0.5 (R Core Team 2020).

Results

Differences between the diets offered to detritivores

There were significant differences on leaf traits across the selected plant species (Table S2, Fig. S3). Overall, *Arundo* presented a much more recalcitrant litter than *Populus*, with higher toughness and Si concentration, but lower concentrations of nutrients (N, P, Ca, K and Mg). The first two dimensions of the PCA explained 84.9% of the variation. Dimension 1 (66.6% var. expl.) represented a gradient of decreasing nutrients (N, P and Mg) versus increasing structural components (Si and condensed tannins), which clearly segregated both litter species. Dimension 2 (18.3% var. expl.) was positively correlated with total phenols and negatively with N:P ratio, reflecting within-species variability.

We observed significant differences between nutrient ratios (C:N, C:P, N:P) of leaf litter and faeces from both detritivores (Fig. 1, Table 1), suggesting a general relative nutrient enrichment of faeces, especially when fed on *Arundo* litter (Figs. 2, S5). Faeces of both detritivores exhibited lower C:N and C:P ratios than the ingested litter of *Arundo* (Fig. 2a–d; Fig. S5) due to increasing N and P, but decreasing C concentrations (Table 1). Likely, faeces had lower N:P ratios than *Arundo* litter (Fig. 2e, f; Fig. S5). When fed *Populus*, *Echinogammarus'* faeces exhibited a relative enrichment in P (lower C:P and N:P ratios) due to decreasing C and N concentrations (Fig. 2c, e; Table 1), whereas those of *Melanopsis* exhibited slightly lower C:N

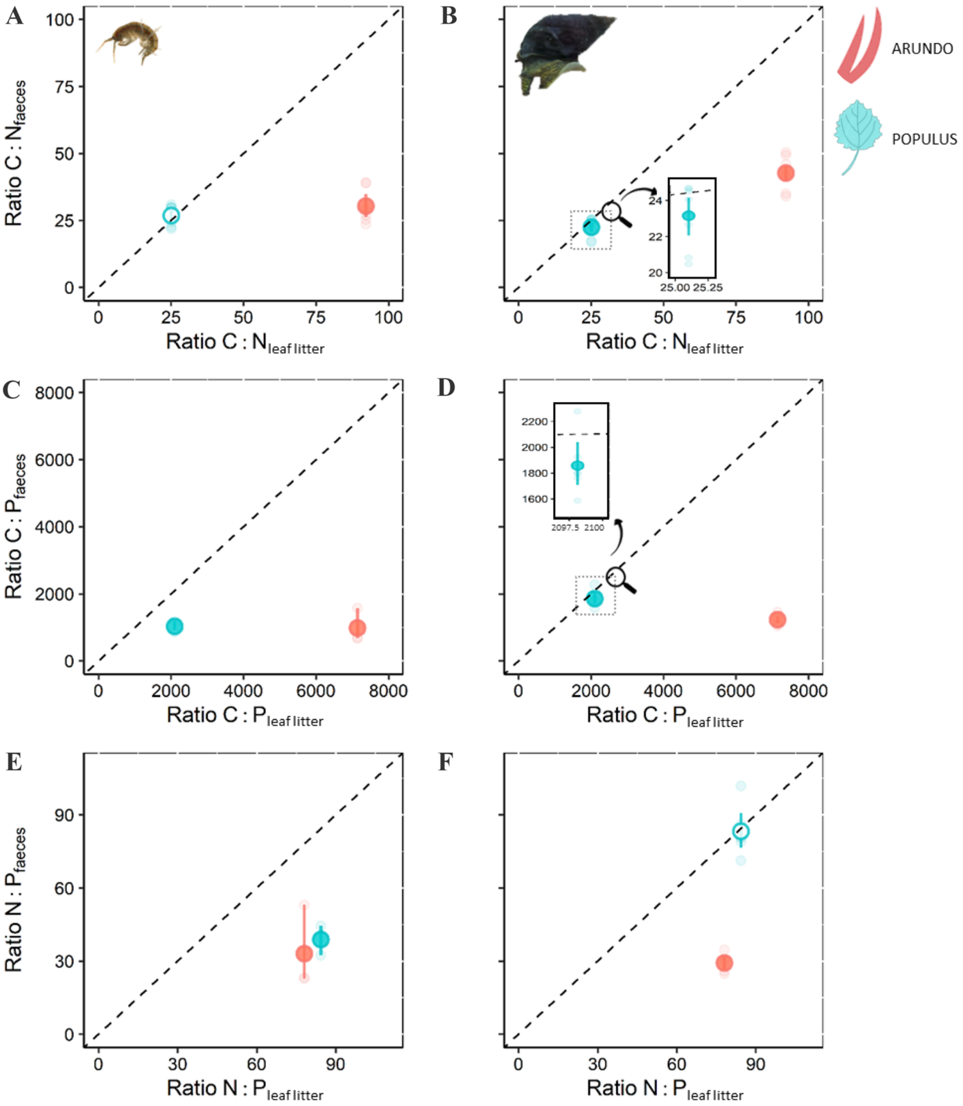

Fig. 2 Relationship of molar C:N, C:P, and N:P ratios between faeces (*Y*-axes) and the two leaf litter species offered (*X*-axes) to *Echinogammarus obtusidens* (**A**, **C**, **E**) and *Melanopsis praemorsa* (**B**, **D**, **F**). Dotted lines represent the ratio 1:1. Circles are means and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Closed circles represent intervals that reject the null hypothesis of no differences between litter and faeces nutrient ratios (i.e., do not overlap the 1:1 line) and open circles represent intervals that do not reject the null hypothesis. Points under the reference line indicate an enrichment in faeces of the limiting nutrient (N or P, denominator in the ratio). Zoom insets are included in **B** and **D** to show that confidence intervals do not overlap the 1:1 line

and C:P ratios (Fig. 2b, d), as a consequence of a small decrease in C concentrations (Table 1).

Detritivores performance

Survival probability of *Echinogammarus* significantly differed among treatments (Log-rank test, $\chi^2 = 13.5$, $P = 0.02$; Fig. S6; Table S3), being the highest for individuals fed M-Pa faeces (88.2%), followed by those fed *Populus* (83.3%), E-Pa (75.0%), M-Ad (71.4%) and E-Ad faeces (55.6%), being the lowest when fed *Arundo* (43.8%). Mortality started before the third day for all the diets except *Populus* for which deaths did not occur until day 6. Median time to death was 18 days in *Arundo* treatment. All individuals of *Melanopsis* survived regardless of the food offered.

Overall, the consumption rates of both detritivores were higher when fed *Populus* over *Arundo* leaf litter (T-test, $P < 0.0001$; Fig. 3), with large effect sizes (Table S3). *Echinogammarus* was incapable to grow significantly when fed any diet except *Melanopsis* faeces (Fig. 4a); the highest growth was found when fed M-Pa (3.20 ± 1.14 mg g^{-1} day^{-1}, mean ± SE), whereas animals fed *Arundo* and *Populus* leaf litter underwent weight losses (Welch's ANOVA, $F_{5,37.9} = 3.95$, $P = 0.005$; Fig. 4a). Accordingly, associated effect sizes (Table S3) were large when *Echinogammarus* fed *Melanopsis* faeces (M-Ad and M-Pa) compared with leaf litter of *Arundo* and *Populus*. Unexpectedly, *Melanopsis* exhibited the greatest growth rate (0.39 ± 0.13 mg g^{-1} day^{-1}, mean ± SE) when fed *Arundo*. However, they were able to grow regardless of the diet offered (Fig. 4b) and no differences were observed among diets (One-way ANOVA, $F_{3,85} = 0.77$, $P = 0.515$; Table S3).

The energetic status of *Echinogammarus* fed on *Melanopsis* faeces was higher compared to other treatments. *Echinogammarus* fed on M-Pa (89.7 ± 14.1 mg $\mathrm{g_{animal}}^{-1}$) and M-Ad (72.33 ± 12.11 mg $\mathrm{g_{animal}}^{-1}$, mean ± SE) exhibited the highest lipid concentrations (One-way ANOVA, $F_{5,41} = 7.44$, $P < 0.0001$; Fig. 4c), with most of the associated effect sizes being large when comparing litter and faeces (Table S3). Glycogen showed a similar trend, and *Echinogammarus* fed M-Ad (51.98 ± 3.83 mg $\mathrm{g_{animal}}^{-1}$) had the highest glycogen accumulation (Welch's Anova, $F_{5,16.9} = 6.60$, $P = 0.0014$; Fig. 4e). Associated effect sizes were mostly large when comparing M-Ad or M-Pa with other treatments (Table S3).

Melanopsis lipids content (range 35.11–44.18 mg $\mathrm{g_{animal}}^{-1}$, mean ± SE) did not exhibited significant differences among treatments (One-way ANOVA, $F_{3,56} = 1.68$, $P = 0.181$; Fig. 4d). However, a large effect size was found between the lipids content of snails fed M-Pa and of those fed *Populus* litter (effect size = 0.82; Table S3). Glycogen content was in general higher when fed on litter than on faeces (One-way ANOVA, $F_{3,56} = 3.22$, $P = 0.029$; Fig. 4f), with large associated effect sizes when comparing snails fed M-Ad with those fed either litter species (Table S3).

Discussion

Our study strongly suggests that cross-species coprophagy can be a useful feeding strategy for small stream detritivores facing shrinking leaf litter quality, e.g. due to riparian invasions by giant reed but also due to climate-induced changes in the riparian community composition or leaves traits (Kominoski et al. 2021; Rubio-Ríos et al. 2022; Salinas et al. 2018). Previous studies (Tonin et al. 2018) find that small detritivores are facilitated by larger ones which produce fine particulate organic matter, whereas when two large detritivore species interact, niche partitioning seems to be the subjacent mechanism. Here, we show that one small and one large detritivore species are able to survive feeding exclusively conspecific faeces (intraspecific facilitation), and that the small species develops its best performance when fed on heterospecific faeces (interspecific facilitation). These results may help explain how some detritivores can maintain

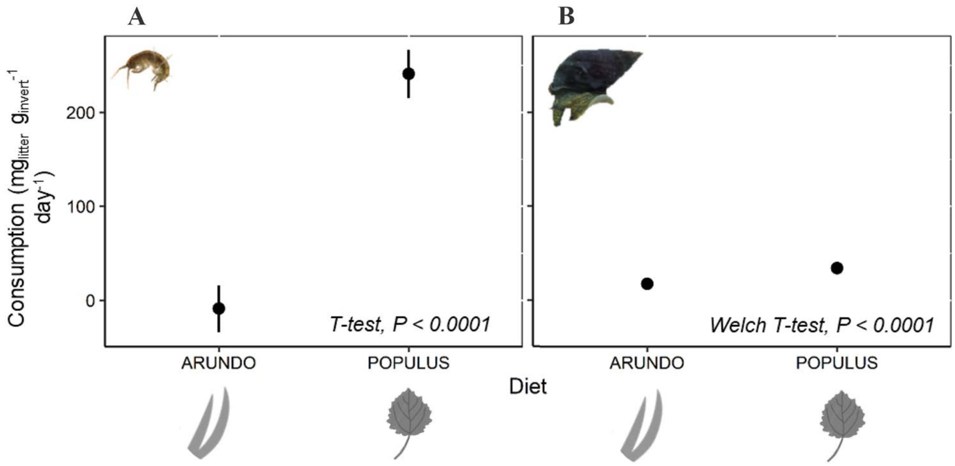

Fig. 3 Effects of treatments (*Arundo donax* and *Populus alba* litter) on *Echinogammarus obtusidens* (**A**) and *Melanopsis praemorsa* (**B**) consumption rates during 21 days experiments. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented

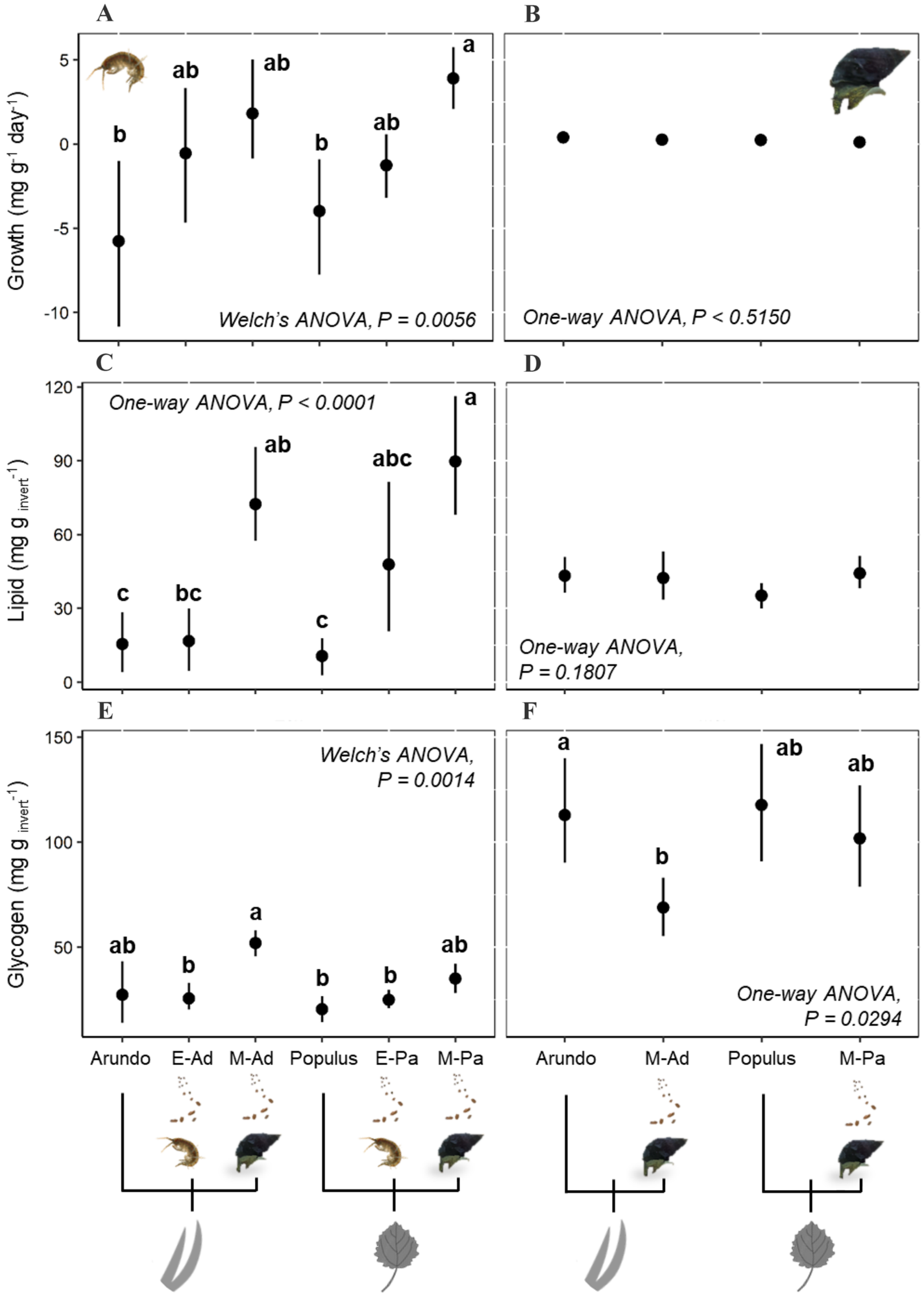

Fig. 4 Effects of treatments (*Arundo donax* and *Populus alba* litter, and E-Ad, M-Ad, E-Pa, M-Pa faeces) on *Echinogammarus obtusidens* (**A**, **C**, **E**) and *Melanopsis praemorsa* (**B**, **D**, **F**) growth rates and energy reserves during 21 days experiments. Different superscript letters indicate significant differences ($P < 0.05$) across treatments on the basis of linear models followed by pairwise multiple comparisons. Mean values (circles) and upper and lower limits of 95% nonparametric bootstrapped confidence intervals (whiskers) are presented

viable populations in streams mostly receiving highly recalcitrant leaf litter inputs, as in those invaded by giant reed, and highlight the prime role of a large detritivore, *Melanopsis praemorsa,* facilitating sympatric detritivore species via faecal production.

Some studies show detritivores' preference for non-native leaf litter even when is a resource of poorer quality than the native one (Lewis et al. 2017; Moretti et al. 2020). Here, however, detritivores consumed more of the native than the invasive litter species. This can be satisfactorily explained by differences in litter traits related with its nutritional value and palatability, i.e. higher nutrient concentrations and lower C:N and C:P molar ratios (Graça et al. 2001; Santonja et al. 2019), and lower tannins and toughness (Ardon et al. 2009; Li et al. 2009) in *Populus*. Moreover, the extremely high Si concentration of *Arundo* may also have dissuaded detritivores from feeding due to mandible wear caused by phytoliths (Massey and Hartley 2009). Even though snails seems to avoid phytoliths when grazing on leaf litter (Schaller 2013), *Melanopsis* still consumed significantly more *Populus* than *Arundo*. Higher feeding rates of this snail on *Populus alba vs.* litter of higher dietary quality—*Alnus glutinosa*—have been recently reported (Fenoy et al. 2021), suggesting that the preference for the native species could depend on detritivore idiosyncrasies interacting with certain preferred litter traits (Frainer et al. 2016) more than simply on litter nutrient content. Nevertheless, overall, the two litter species used in our study may be of low-quality for detritivores—in particular for *Echinogammarus* species—compared to those from other species as alder or ash tree (e.g. Fenoy et al. 2021; Solagaistua et al. 2019), especially due to their high Si concentrations.

Contrary to our first hypothesis, and despite greater dietary quality and consumption rates in *Populus* than in *Arundo*, neither of the two detritivores showed superior performance when fed the native species (excepting survival of *Echinogammarus*). *Melanopsis* had similar survival (100% survivors in all treatments), growth rates and accumulation of reserves in both litter species, as well as when fed faeces. This is not surprising given the extraordinary physiological flexibility reported for freshwater snails (Fink and Von Elert 2006; Rollo and Hawryluk 1988), including *Melanopsis praemorsa* (Fenoy et al. 2021), as an adaptation to thrive feeding on high-C diets—e.g. *Arundo* litter—by allocating the excess of C acquired to respiration or accumulation of reserves. On the contrary, *Echinogammarus* was not able to grow when fed leaf litter, not even *Populus* despite consuming it abundantly compared to *Arundo*, which was virtually unconsumed. Thus, while some freshwater crustacean detritivores show enzymatic adaptation to digest leaf litter (Zimmer and Bartholmé 2003), our results suggest that *E. obtusidens* may need more than just litter to grow (Tonin et al. 2018). However, given the high survival rate of the amphipod when fed on the native compared to the exotic litter (Going and Dudley 2008), it possibly would be able to perform much better if a higher quality litter, especially in terms of Si concentration, was offered (see above).

According to the assimilation hypothesis (Hessen 1997), animals under high-C diets tend, to balance their stoichiometry (~5.8 and ~5.5 for *Echinogammarus* and *Melanopsis* C:N ratios, respectively; Unpublished data) retain the limiting nutrients (N or P) more efficiently than C. Thus, C:nutrients ratios in faeces should be higher than in the ingested litter. However, in support of our second hypothesis, we reported much lower C:N and C:P molar ratios of faeces, from both detritivores, relative to the ingested *Arundo* litter—the highest-C diet—but not *Populus*, as a consequence of a simultaneous increase of N and P and decrease of C concentrations. Consequently, the passage of *Arundo* litter through the gut of detritivores determined a relative nutrient enrichment of its egesta, which roughly equaled it with that of *Populus*. This suggests limited control of nutrient assimilation by our detritivores when fed on the recalcitrant non-native litter. Likewise, N assimilation appeared to be higher than that of P when both detritivores fed *Arundo* litter, as inferred from the higher N:P molar ratios in faeces than in litter. Probably, our detritivores are maintaining their elemental homeostasis by regulating excretion rather than assimilation (excretion hypothesis; Balseiro and Albariño 2006; Sterner and Elser 2002), although other mechanisms, as selective feeding of nutrient-rich fractions of leaf litter (Hood et al. 2014), may be also operating.

Furthermore, nutrient enrichment of faeces can be boosted by microorganisms that thrive within the gut of aquatic invertebrates (e.g. in crustaceans and molluscs) contributing with exudates, living and dead cells to the egesta (Wotton and Malmqvist 2001), and by microbial colonization during its storage in the riverbed (up to months; Joyce and Wotton 2008), therefore making faeces—especially those from recalcitrant litters as *Arundo*—a presumably more palatable and nutrient-rich food resource for detritivores (Joyce et al. 2007; Shepard and Minshall 1981).

In accordance with the above, and at least partly with our third hypothesis, both detritivores showed high survival when fed faeces. Particularly, it was the consumption of faeces from the snail that led the greatest performance to *Echinogammarus*: reaching the highest accumulation of reserves and being the only food that promoted its growth. Previous studies have reported some genera of Gammaridae as generalist feeders, having a flexible omnivory including the consumption of faeces (Agnew and Moore 1986), but without documenting the nutritional value of faeces for consumers. Some other studies, however, suggest that *Echigammarus* species—i.e. *E. berilloni*—might be unable to ingest faeces due to unsuitable morphological adaptation to sieve particles (Mas-Marti et al. 2015; Mayer et al. 2012). Here, we clearly evinced that *E. obtusidens* was able to grow up consuming faeces, possibly due to an intrinsic ability of some amphipods to adjust their feeding mode depending on food availability (Kulesza and Holomuzki 2006).

Crustaceans usually metabolize lipids slowly and use them in long-term process as growth, body maintenance or reproduction (Sánchez-Paz et al. 2006). Thus, their lipid body concentration may reflect better than glycogen shifts in energy demand or food availability (Becker et al. 2013). Accordingly, lipids content of *Echinogammarus* had a slight but significant correlation with growth rate ($r = 0.639$; $R^2 = 0.409$, $P < 0.001$; Fig. S7A), and, overall, was lower when fed on litter than when fed on faeces, primarily *Melanopsis* faeces, supporting our fourth hypothesis. For example, average lipids content was 4 and 8 times higher when fed M-Ad and M-Pa, respectively, than when fed the original leaf litter. Previous studies have reported a decrease of the triglyceride content in a *Gammarus* species when fed on leaf litter, independently of consumption rate (Foucreau et al. 2013). Since in our experiment, we did not analyse the initial energy reserves of the animals, we cannot determine if the experimental individuals increased or decreased their lipids content during the experiment. Even so, we unquestionably show that *Echinogammarus* hold the highest lipids content when fed on *Melanopsis* faeces compared to other food items offered.

In our research, we only assessed the role of resource quality, but not resource quantity (see Halvorson et al. 2017b), on the performance of the two detritivore species. The amount of resource present in each microcosm could have played an important role on the response of our detritivores as have been reported by other studies (e.g. Arias-Real et al. 2018; Marcarelli et al. 2011). Therefore, although the availability of fecal material was verified by daily observations of the lower part of the microcosms (even in those fed E-Ad), responses of detritivores when feed on faecal material exposed here should be interpreted with caution. Likewise, despite the FPOM produced by *Melanopsis* and *Echinogammarus* is primarily composed by faeces—$\geq 95\%$, personal observation under microscope—(as seen for other species; Patrick 2013), we cannot totally rule out the possibility that detritivores took advantage of small litter particles reaching the lower part of our microcosms.

Invasive species are among the main threats to biodiversity conservation (Bellard et al. 2016). Long-term human impacts in riparian ecosystems have favoured the proliferation of such species (Castro Díez and Alonso Fernández 2017). Particularly, *Arundo* has been reported to significantly impair riparian habitats (Jiménez-Ruiz et al. 2021), altering vegetation structure and displacing native vegetation (Maceda-Veiga et al. 2016) with subsequent negative effects on arthropod abundance and diversity and on wildlife which diet rely on them (Herrera and Dudley 2003). Our study assesses how two aquatic detritivores species may face riparian invasion by the giant reed and shows that cross-species coprophagy may be a successful feeding strategy for *Echinogammarus* to overcome the impacts of such invasion, but also to prosper in systems where low-quality litter inputs (native or not) are abundant. Furthermore, the role of the snail as a key species facilitating the access to nutrients to other detritivore species in lowland streams was highlighted. Managers should pay attention to the conservation of such relevant detritivore species for the functioning of lowland stream ecosystems, supervising activities that could threaten their populations (e.g. aquifer exploitation or water contamination; Bartolini et al. 2017), especially in streams with reduced detritivore diversity (Boyero et al. 2021) as these located in Mediterranean lowlands.

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Author contributions JRR and JJC conceived the ideas and designed the methodology; JRR, MJS and EF collected the data; JRR and JP analysed the data with help from MJS; JRR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability All data used to conduct the analyses are available from the corresponding author, upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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No man ever steps in the same river twice, for it is not the same river and he is not the same man.

Heraclitus

Hope you are not the same after reading this thesis.