



Global Change and stream ecosystem functioning: repercussions on the leaf litter decomposition

Silvia Monroy Zarzuelo

PhD Thesis

July 2017

supervised by Jesús Pozo Martínez

Acknowledgements

I want to give thanks to my supervisor Professor Jesús Pozo Martínez and my laboratory colleagues for their efforts and help to finish this dissertation.

I acknowledge the Spanish Ministry of Economy and Competitiveness for financial support in terms of a predoctoral grant (BES-2012-060743). This work was financially supported by European Union (FPT-ENV-2013-603629, GLOBAQUA), Basque Government (IT302-10; IT577-13; IT-951-16), Spanish Ministry of Science and Innovation and FEDER (CGL2011-23984-C04-01), MICINN-BFU2010-16349/BFI co-funded by ERDF; K-EGOKITZEN IE10-277; Training and Research Units from the University of the Basque Country (UFI11/24). I thank the technicians of SGIker's General Analysis Service (UPV/EHU) for nutrient measurements and the University of the Basque Country (UPV/EHU), Confederación Hidrográfica del Ebro (CHE) and Diputación Foral de Bizkaia.

CONTENTS

Summary	
Chapter 1: General introduction.....	1
Chapter 2: Elevated atmospheric CO ₂ interacts with drought and competition to produce complex responses in plant quality and subsequent microbial aquatic decomposition	15
Chapter 3: Temperature sensitivity of leaf litter decomposition in headwater forested streams: potential role of litter quality and stream environmental characteristics.....	33
Chapter 4: Structural and functional recovery of macroinvertebrate communities and leaf litter decomposition after a marked drought: does vegetation type matter?.....	65
Chapter 5: Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain).....	93
Chapter 6: Plant functional diversity and leaf-litter decomposition in Mediterranean streams along a drought gradient.....	119
Chapter 7: General discussion.....	145
Chapter 8: Conclusions.....	155

Summary

This dissertation explores the effects of various components of Global Change on stream ecosystem functioning through the study of leaf-litter decomposition and combining field and laboratory experiments. We first examine the effects of elevated atmospheric CO₂ and water availability on plant material quality from two species differing in biological traits and their subsequent microbial decomposition in a laboratory experiment. Water shortage reduced plant quality and its effects surpassed those of elevated CO₂ which were species-specific and did not affect equally nutrient content. The subsequent microbial decomposition depended on species. Therefore, the decomposition rates of plant materials grown under future scenarios of elevated CO₂ could be difficult to predict due to species-specific responses and interaction with other factors.

In the second chapter, we examine, in a field and laboratory experiment, the temperature sensitivity of microbial-mediated decomposition and associated functional variables (i.e. microbial respiration, fungal growth, leaf-nutrient changes) in leaf litter of high- and low-quality species and explore whether fungal communities adapted to different thermal regime influence the response. The decomposition rate increased with temperature and both types of species showed a similar temperature sensitivity (i.e. activation energy), suggesting that species contrasting in litter quality could be similarly affected by temperature rises. The patterns of respiration, fungal growth and litter nutrient change were erratic and less predictable to temperature rise, suggesting that litter decomposition could be a better indicator because it integrates all processes occurring during the processing time. The response of decomposition rate and microbial activities to temperature from streams with different thermal regimes followed similar patterns. The responses in the field were less clear and predictable, underlining the difficulty of direct extrapolation of the results obtained under controlled laboratory conditions.

Finally, we focus on potential effects of drought. First, we examine structural and functional responses after a drought-period in perennial temperate streams flowing through native forests and catchments already affected by exotic plantations. The recovery dynamic of benthic macroinvertebrate communities considering taxonomic and functional-traits composition depended on catchment vegetation type. Leaf decomposition rate after drought was little affected by exotic tree plantations. This result suggests that drought period would have more severe effects on functional and taxonomic recovery of benthic macroinvertebrates in perennial temperate streams already affected by exotic plantations but, in general, the stream functioning could not be such severely affected. Second, we examine leaf-litter decomposition across a regional gradient of aridity in Mediterranean calcareous streams and checked how microbial or

detritivore activities are affected, and whether the effects are consistent for leaves with contrasting quality. Decomposition rates differed among sites, being lowest in the 2 most water-stressed sites, but showed no general correlation with the gradient. In general, the results point to a complex pattern at the regional scale, as drought affect decomposition directly by emersion of litter and, indirectly, by affecting the functional composition and density of detritivores. This response was more evident in high-quality leaf litter species, suggesting higher sensitivity to drought than that of low-quality litter. In the last chapter, we wanted to explore the effect of leaf-litter functional diversity on the total decomposition rate in streams along a climate drought gradient by using plant species with contrasting carbon allocation strategies to disentangle its role under this environmental stressor. Although the results suggested that the effect of species diversity could be more prevalent under dry conditions, the results point to stronger effects of the environmental context than leaf functional diversity on the decomposition process in Mediterranean calcareous streams, overriding any potential effect of the later one.

The overall results provide evidences of relevant effects of climate change and vegetation change on structural and functional attributes of stream ecosystem. In addition, the results point to the need to evaluate their interaction for improving realistic estimation of stream ecosystem responses to future environmental changes. It also highlights the utility of leaf-litter decomposition as sensitive indicator of ecosystem status.

Resumen

Esta tesis explora los efectos de varios componentes del Cambio Global en el funcionamiento del ecosistema fluvial a través del estudio de la descomposición de hojarasca y mediante de la combinación de experimentos en campo y en laboratorio. En primer lugar, nosotros examinamos los efectos de una elevada concentración de CO₂ atmosférico y la disponibilidad de agua sobre la calidad del material vegetal de dos especies de plantas que difieren en sus rasgos biológicos y su posterior descomposición por microorganismos en un experimento de laboratorio. La escasez de agua redujo la calidad de la planta y sus efectos fueron mayores a los de un elevado CO₂, cuyos efectos fueron específicos para cada especie y no afectaron por igual al contenido de los diferentes nutrientes. La descomposición microbiana de estos materiales dependió de las especies. Por lo tanto, las tasas de descomposición de los materiales crecidos bajo futuros escenarios de elevado CO₂ serán difíciles de predecir debido a las respuestas específicas de las especies y a la interacción con otros factores.

En el segundo capítulo se examina la sensibilidad a la temperatura de la descomposición microbiana y las variables asociadas a este proceso de descomposición (respiración microbiana, crecimiento fúngico, cambio en nutrientes de las hojas) en hojarasca de distinta calidad. Además, se estudia si comunidades fúngicas adaptadas a diferente régimen térmico influenciaría la respuesta. La tasa de descomposición incrementó con la temperatura y ambos tipos de especies mostraron similar sensibilidad a la temperatura (energía de activación), sugiriendo que hojarascas de especies de distinta calidad podrían ser afectadas en un modo similar por los aumentos en la temperatura. Los patrones de respiración, crecimiento fúngico y cambio de nutrientes en las hojas fueron variables y menos predecibles con el aumento de temperatura, sugiriendo que la descomposición podría ser un mejor indicador del status del río ya que integra todos procesos que ocurren durante el tiempo de descomposición. La respuesta de la tasa de descomposición y las actividades microbianas a la temperatura procedente de ríos con diferente régimen térmico siguió patrones similares. La respuesta en el campo fue menos clara y predecible, poniendo de manifiesto la dificultad de una directa extrapolación de los resultados obtenidos bajo condiciones controladas en laboratorio.

Por último, nos centramos en los efectos potenciales de la sequía. Primero, examinamos las respuestas estructurales y funcionales tras un periodo de sequía en ríos perennes de clima templado que fluyen a través de bosques nativos y a través de cuencas afectadas por plantaciones de especies exóticas. La dinámica de recuperación de las comunidades de macroinvertebrados bentónicos, considerando su composición taxonómica y sus características funcionales, dependió del tipo de vegetación de la cuenca. La tasa de descomposición después de este periodo de sequía fue poco afectada por las plantaciones. Este resultado sugiere que

periodos de sequía tendrían efectos más severos sobre la recuperación de los macroinvertebrados bentónicos en ríos perennes ya afectados por plantaciones de especies exóticas, pero que el funcionamiento del río podría no verse afectado tan severamente. En segundo lugar, examinamos la descomposición de la hojarasca a través de un gradiente regional de aridez en ríos mediterráneos calcáreos, comprobamos cómo las actividades microbianas y detritívoras se ven afectadas, y si los efectos son consistentes para hojarasca de distinta calidad. Las tasas de descomposición difirieron entre sitios y fue más baja en los 2 ríos sometidos a más estrés hídrico, pero no mostraron una correlación general con el gradiente. En general, los resultados señalan un patrón complejo a escala regional ya que la sequía afectó a la descomposición directamente por la emersión de la hojarasca e indirectamente, a través de sus efectos sobre composición de los detritívoros. Esta respuesta fue más evidente en la hojarasca de mayor calidad lo que sugiere mayor sensibilidad a la sequía que la hojarasca de menor calidad. En el último capítulo, exploramos el efecto de la diversidad funcional de la hojarasca sobre la tasa de descomposición en ríos a lo largo de un gradiente climático de sequía para conocer su rol bajo este estresor ambiental. Para ello empleamos especies de plantas con estrategias de asignación de carbono diferentes. Aunque los resultados sugirieron que el efecto de la diversidad de especies podría ser más manifiesto en condiciones de más estrés, los resultados apuntan a efectos más fuertes del contexto ambiental en el proceso de descomposición in ríos mediterráneos calcáreos que la diversidad funcional.

En general, los resultados aportan evidencias de relevantes efectos del cambio en el clima y en la vegetación en la estructura y funcionamiento del ecosistema fluvial. Además, señalan la necesidad de evaluar su interacción para obtener una estima más realista de las respuestas de los ecosistemas fluviales a futuros escenarios de cambio ambiental. También destaca la utilidad de la descomposición de la hojarasca como un sensible indicador del estado del ecosistema.

CHAPTER 1

General introduction

General Introduction

The growing human population and the increase in the consumption of resources in recent decades are resulting in environmental changes at an unprecedented rate and whose repercussions extend to global scales affecting whole functioning of the Earth. The particular characteristic of this global change led to propose the term “Anthropocene” to designate a new geological age in which human population has emerged as a new force capable of controlling the fundamental processes of the biosphere (Crutzen 2002). Global Change is an amalgam of environmental changes that go beyond those of Climate Change (IPCC, 2013; Vitousek, 1994). Increases in nutrient availability, pollution as well as changes in land use are key drivers of ongoing global environmental change faced by ecosystems worldwide. The multiple consequences of such changes include, among others, the loss of biodiversity (Pereira et al., 2010) and widespread degradation of ecosystems that, in turn, ultimately affect the good and services that they provide to society (Millennium Ecosystem Assessment, 2005, 2003).

Freshwater ecosystems are essential contributors to biodiversity, ecological productivity and human well-being (Dudgeon et al., 2006). However, they are particularly vulnerable to multiple global change disturbances (Malmqvist *et al.* 2008; Woodward, Perkins & Brown 2010) and are among the most heavily threatened ecosystems (Dudgeon et al., 2006; Vörösmarty et al., 2010). Of special importance are headwater streams which could account for nearly 90 percent of river drainage channel length and for up to one third of the river networks (Downing et al., 2012; Leopold et al., 1964). They play a major role in global nutrient dynamic of entire stream system (Benstead and Leigh, 2012) and significantly contribute to whole-stream biodiversity due to the great biodiversity supported by their high levels of habitat diversity (Finn et al., 2011; Meyer et al., 2007). Consequently, disturbances in the headwaters will have negative consequences for the diversity and ecological integrity downstream with which they are intimately linked. Headwater streams are extremely vulnerable to global change disturbances because they are relatively isolated and fragmented within terrestrial landscapes (Perkins et al., 2010) and because of their small size and link to adjacent terrestrial ecosystem. Therefore, it is necessary to understand the key processes governing the structure and functioning of streams and how these are affected by multiple anthropogenic stress for more effective management of freshwater ecosystems.

In headwater forested streams, primary production is limited by shading from riparian vegetation (Vannote et al., 1980) and organic matter from the terrestrial ecosystem is the primary source of matter and energy for these systems (Vannote et al., 1980; Wallace et al., 1997). Most of the allochthonous organic matter inputs consist of leaf litter (Jesús Pozo et al., 1997), and its decomposition constitutes a pivotal ecosystem-level process, which encompasses

the recycling of organic and inorganic matter and the transfer of energy across food webs (Gessner, 1999; Perkins et al., 2010; Tank et al., 2010). Plant litter decomposition is a complex process that involves leaching of soluble compounds, physical fragmentation and degradation by microbial decomposers and invertebrate detritivores (Abelho, 2001; Tank et al., 2010). Microbial decomposers, particularly fungi, are primary actors in the leaf decomposition (Pascoal and Cássio, 2004) and crucial link between the riparian vegetation and consumer communities. They assimilate and convert organic matter into new fungal biomass and inorganic compounds (Gessner and Chauvet, 1994), enhancing litter nutritional value and palatability of detritus for detritivores. Both fungi and invertebrate detritivore are the basis of detritus food webs (Wallace *et al.* 1997). These chemical, physical and biotic processes are governed by an array of both intrinsic (leaf litter quality) and extrinsic (e.g. temperature, dissolved nutrients) factors (Tank et al., 2010) that ultimately determine the rate of conversion of plant litter to either CO₂ and other mineral substances (mineralization) or to other forms of organic matter. Consequently, it is critical understand how litter decomposition is affected by multiple global environmental changes since this process is also essential in controlling nutrient cycling and global carbon cycle (Battin et al., 2009).

Climate Change itself represents a complex set of stressors including alteration of atmospheric conditions and concomitant changes in temperature and precipitation patterns (IPCC, 2014; Milly et al., 2005). Future scenarios of global environmental change forecast that atmospheric carbon dioxide concentrations (CO₂) could be nearly double ca. 740 ppm by the end of the century as a consequence of human activity (IPCC, 2013). Elevated atmospheric CO₂ level might trigger profound changes in terrestrial vegetation by stimulating plant productivity (Norby and Zak, 2011; Norby et al., 2001), altering phenology (Taylor et al., 2008) and plant tissue composition which would represent a reduction of litter quality (Cotrufo et al., 1998; Norby et al., 2001; Tuchman et al., 2002). Changes in atmospheric CO₂ concentration are likely to be indirectly manifested in freshwater ecosystem through altering basal resources inputs (Kominoski and Rosemond, 2012) since the stream food webs depend heavily on terrestrial subsidies (Woodward et al., 2010) and substrate quality exerts a strong influence on organic matter processing (García-Palacios et al., 2016; Hladyz et al., 2009). Despite the current scarcity of studies, experiments with leaf plants grown in CO₂ enriched media point to weak effects of reduced litter quality on microbial- and invertebrate -mediated decomposition (Dray et al., 2014; Ferreira et al., 2010; Tuchman et al., 2002), but this conclusion is based on a limited number of leaf species and environmental scenarios.

Increase in air temperature and alterations of spatial patterns in precipitation are also forecast in climate scenarios (IPCC, 2013). Temperature and precipitation regimes modulate hydrogeological processes (Huang et al., 2016; Papadaki et al., 2016) and biotic communities

(Bhowmik and Schäfer, 2015; Heino et al., 2013). Mean global **temperatures** are predicted to increase between 1.5 and 4.6 °C by the end of the 21st century (IPCC, 2014), and a parallel response in water temperature of streams is expected due to its correlation with air temperature (Kaushal et al., 2010; Molinero et al., 2016). Temperature is a primary factor influencing chemical reactions and metabolic rates of organisms (Brown et al., 2004; Davidson and Janssens, 2006). Increasing temperature has the potential to influence the biological activities by altering physiological rates (Ferreira and Chauvet, 2011; Perkins et al., 2012; Yvon-Durocher et al., 2012), change the community structure (Dang et al., 2009; Ferreira and Chauvet, 2011; Martínez et al., 2014), species distribution and interspecific relationships (Duarte et al., 2013), and probably affecting the quantity and timing of terrestrial detrital inputs to streams (Menzel et al., 2006). Therefore, temperature increases can strongly compromise ecosystem processes in freshwater systems. Although previous studies addressing the temperature effects on litter decomposition reported a potential increase in its rate with temperature, the response to temperature is not well understood yet because other key drivers on metabolic rate could influence the temperature sensitivity (Ferreira and Chauvet, 2011; Follstad Shah et al., 2017; Martínez et al., 2014). It is thus critical to understand how other factors interact with temperature to predict the consequences of its changes on plant litter decomposition under global-change scenarios.

Future climate scenarios also predict changes in **precipitation** patterns with decreases in rainfall and increases in the occurrence of extreme events (e.g. droughts and floods), but such changes will not be uniform over space and time (Milly et al., 2005). In addition to climate-related variations in water flow, the increasing abstraction by human demands could exacerbate these change in stream runoff causing intensification of drought effect on rivers (Cooper et al., 2013; Vörösmarty et al., 2010). Drought is an important stressor for freshwater ecosystems (Lake, 2011; Sabater and Tockner, 2010; Sabater, 2008). It produces strong variation in the river discharge and implies a sequential decline in the flow that results in the decrease of water velocity and depth, reduction of hydrological connectivity (habitat contraction and fragmentation), alteration water physicochemical conditions (Dewson et al., 2007; Rolls et al., 2012) subsequent shifts in the biotic system (Datry et al., 2014; Filipe et al., 2013; Ledger et al., 2013). The potential consequences for aquatic communities and the processes they support are likely to be complicated to predict by different abilities of biota to cope with environmental changes (Chessman, 2015; Leigh et al., 2016).

In addition to climate-related disturbances, freshwater ecosystems are subjected to other global stressors like the **land use change (catchment vegetation change)**. Anthropogenic activities such as agricultural practises, urbanization, deforestation or replacement of native vegetation alter land cover (Allan and Castillo, 2007; Kominoski and Rosemond, 2012). The replacement

of native vegetation with exotic monospecific plantations is one of the most widespread worldwide practises (Kominoski et al., 2013). Changes in the diversity and composition of terrestrial vegetation can affect aquatic ecosystem through alteration in inputs of organic matter to streams and rivers (Martínez et al., 2016; J. Pozo et al., 1997). Despite the effects of exotic plantations has been widely assessed, the outcomes tend to provide contrasting results (Ferreira et al., 2016; Graça et al., 2002). Furthermore, it is not well known the potential consequences for stream ecosystems when different stressors act simultaneously, particularly on the taxonomic and functional diversity of biotic communities, since biological and ecological traits could be directly or indirectly related to ecological functions (Martínez et al., 2016).

The consequences of global environmental changes include a decline in global **biodiversity** (Pereira et al., 2010) that in freshwater ecosystems even exceed those in terrestrial ecosystems (Sala et al., 2000). There are serious concerns about the implications of the current high rate of biodiversity loss, as biodiversity has been shown to be related to ecosystem processes (Cardinale et al., 2000; Gessner et al., 2010; Lecerf and Richardson, 2010). Despite research efforts, the role of biodiversity in the functioning of stream ecosystems remains unclear and previous studies provided mixed results (Gessner et al., 2010). Species richness is the most commonly used measure of biodiversity; however, functional diversity (e.g. diversity of traits) represents an emerging approach to address the biodiversity and ecosystem functioning relationships (Reiss et al., 2009) that could help to reveal the potential effect of biodiversity on ecosystem, including pivotal processes such as litter decomposition (Handa et al., 2014). Moreover, to date, relatively few studies have addressed biodiversity-ecosystem functioning (B-EF) relationships under different situations of global environmental change in spite of environmental change might be important to modulate the role of litter diversity on decomposition rates in streams (Kominoski et al., 2010; Tonin et al., 2017).

This dissertation is focused on how drivers of global environmental change such as climate and catchment vegetation change affect the functioning of freshwater ecosystems with the aim of improving the understanding of their role under different global-change scenarios and predict their potential consequences. In particular, this work examines the effects of elevated CO₂, increasing temperature, drought and catchment vegetation change, and some of their interactions on plant litter processing in streams by combining field and laboratory experiments. This thesis has been structured in five chapters and the specific objectives are the following:

1. Examine the effects of changes in atmospheric CO₂ concentration and water availability on quality of plant materials from species differing in biological traits under two competitive situations, as well as their subsequent microbial decomposition. (Laboratory experiment).

2. Assess the temperature sensitivity of microbial-mediated decomposition and associated functional variables (i.e. microbial respiration, fungal growth, leaf-nutrient changes) in leaf litter contrasting in quality and to explore whether fungal communities adapted to different thermal regime influence the response to temperature increases. We explore these issues from field and laboratory approaches to disentangle temperature effect from potentially confounding environmental factors. (Field and laboratory experiment).

3. Examine structural and functional responses of drought-event in perennial temperate streams flowing through catchment already affected by exotic plantations and if responses to droughts are similar to those of native forested streams. We explore the recovery dynamic of macroinvertebrate communities taking into account taxonomic and functional-traits composition, and analyse its repercussion on leaf litter decomposition. (Field experiment).

4. Examine the effect of drought on leaf litter decomposition across a regional gradient of aridity in Mediterranean streams and to check whether microbial or detritivore activities are affected, and whether the effects are consistent for leaves with contrasting quality. (Field experiment).

5. Explore the effect of leaf-litter functional trait diversity on the decomposition rate in Mediterranean calcareous streams along a climate drought gradient by using plant species with contrasting carbon allocation strategies to disentangle its role under this environmental stressor. (Field experiment).

References

- Abelho, M., 2001. From Litterfall to Breakdown in Streams: A Review. *Sci. World J.* 1, 656–680. doi:10.1100/tsw.2001.103
- Allan, J.D., Castillo, M.M., 2007. *Stream Ecology: Structure and Function of Running Waters*. Springer Netherlands.
- Battin, T.J., Luysaert, S., Kaplan, L. a., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., Biology, G.C., Sciences, A., Box, P.O., Davidson, E.A., Janssens, I.A., Quality, L., Sensitivity, T.H.E.T., Decomposition, O.F., Resource, N., Collins, F., Science, E., Review, E., 2009. The boundless carbon cycle. *Nat. Geosci.* 2, 598–600. doi:10.1038/ngeo618
- Benstead, J.P., Leigh, D.S., 2012. An expanded role for river networks. *Nat. Geosci.* 5, 678–679. doi:10.1038/ngeo1593
- Bhowmik, A.K., Schäfer, R.B., 2015. Large Scale Relationship between Aquatic Insect Traits and Climate. *PLoS One* 10, e0130025.

- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. *Ecology* 85, 1771–1789.
- Cardinale, B.J., Nelson, K., Palmer, M.A., 2000. Linking species diversity to the functioning of ecosystems : on the importance of environmental context. *Oikos* 1, 175–183.
- Chessman, B.C., 2015. Relationships between lotic macroinvertebrate traits and responses to extreme drought. *Freshw. Biol.* 60, 50–63. doi:10.1111/fwb.12466
- Cooper, S.D., Lake, P.S., Sabater, S., Melack, J.M., Sabo, J.L., 2013. The effects of land use changes on streams and rivers in mediterranean climates. *Hydrobiologia* 719, 383–425.
- Cotrufo, M.F., Ineson, P., Scott, A., 1998. Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Glob. Chang. Biol.* 4, 43–54. doi:10.1046/j.1365-2486.1998.00101.x
- Dang, C.K., Schindler, M., Chauvet, E., Gessner, M.O., 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology* 90, 122–31.
- Datry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I., Santos, A.N., 2014. Broad-scale patterns of invertebrate richness and community composition in temporary rivers: effects of flow intermittence. *Ecography (Cop.)*. 37, 94–104.
- Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165–173.
- Dewson, Z.S., James, A.B.W., Death, R.G., 2007. Stream Ecosystem Functioning under Reduced Flow Conditions. *Ecol. Appl.* 17, 1797–1808. doi:1
- Downing, J.A., Cole, J.J., Duarte, C.M., Middelburg, J.J., Melack, J.M., Prairie, Y.T., Kortelainen, P., Striegl, R.G., McDowell, W.H., Tranvik, L.J., 2012. Global abundance and size distribution of streams and rivers. *Inl. Waters* 2, 229–236. doi:10.5268/IW-2.4.502
- Dray, M.W., Crowther, T.W., Thomas, S.M., A’Bear, a. D., Godbold, D.L., Ormerod, S.J., Hartley, S.E., Jones, T.H., 2014. Effects of elevated CO₂ on litter chemistry and subsequent invertebrate detritivore feeding responses. *PLoS One* 9, 1–7. doi:10.1371/journal.pone.0086246
- Duarte, S., Fernandes, I., Nogueira, M.J., Cássio, F., Pascoal, C., 2013. Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecol.* 6, 187–191. doi:https://doi.org/10.1016/j.funeco.2013.02.001

- Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev. Camb. Philos. Soc.* 81, 163–82. doi:10.1017/S1464793105006950
- Ferreira, V., Chauvet, E., 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Glob. Chang. Biol.* 17, 551–564. doi:10.1111/j.1365-2486.2010.02185.x
- Ferreira, V., Gonçalves, A.L., Godbold, D.L., Canhoto, C., 2010. Effect of increased atmospheric CO₂ on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Glob. Chang. Biol.* 16, 3284–3296. doi:10.1111/j.1365-2486.2009.02153.x
- Ferreira, V., Koricheva, J., Pozo, J., Graça, M.A.S., 2016. A meta-analysis on the effects of changes in the composition of native forests on litter decomposition in streams. *For. Ecol. Manage.* 364, 27–38. doi:10.1016/j.foreco.2016.01.002
- Filipe, A.F., Lawrence, J.E., Bonada, N., 2013. Vulnerability of stream biota to climate change in mediterranean climate regions: A synthesis of ecological responses and conservation challenges. *Hydrobiologia* 719, 331–351. doi:10.1007/s10750-012-1244-4
- Finn, D.S., Bonada, N., Múrria, C., Hughes, J.M., 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. *J. North Am. Benthol. Soc.* 30, 963–980. doi:10.1899/11-012.1
- Follstad Shah, J.J., Kominoski, J.S., Ardón, M., Dodds, W.K., Gessner, M.O., Griffiths, N.A., Hawkins, C.P., Johnson, S.L., Lecerf, A., Leroy, C.J., Manning, D.W.P., Rosemond, A.D., Sinsabaugh, R.L., Swan, C.M., Webster, J.R., Zeglin, L.H., 2017. Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. *Glob. Chang. Biol.* doi:10.1111/gcb.13609
- García-Palacios, P., McKie, B.G., Handa, I.T., Frainer, A., Hättenschwiler, S., 2016. The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. *Funct. Ecol.* 30, 819–829. doi:10.1111/1365-2435.12589
- Gessner, M.O., 1999. A perspective on leaf litter breakdown in streams. *OIKOS* 85, 377–384.
- Gessner, M.O., Chauvet, E., 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* 75, 1807–1817.

- Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. *Trends Ecol. Evol.* 25, 372–380.
- Graça, M.A.S., Pozo, J., Canhoto, C., Eloşegi, A., 2002. Effects of Eucalyptus plantations on detritus, decomposers, and detritivores in streams. *Sci. World J.* 2, 1173–1185.
- Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., van Ruijven, J., Vos, V.C. a, Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. *Nature* 509, 218–21. doi:10.1038/nature13247
- Heino, J., Schmera, D., Erős, T., 2013. A macroecological perspective of trait patterns in stream communities. *Freshw. Biol.* 58, 1539–1555. doi:10.1111/fwb.12164
- Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J., Woodward, G., 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshw. Biol.* 54, 957–970. doi:10.1111/j.1365-2427.2008.02138.x
- Huang, S., Huang, Q., Chang, J., Leng, G., 2016. Linkages between hydrological drought, climate indices and human activities: a case study in the Columbia River basin. *Int. J. Climatol.* 36, 280–290.
- IPCC, 2014. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK, and New York, NY, USA.
- IPCC, 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge and New York.
- Kaushal, S.S., Likens, G.E., Jaworski, N.A., Pace, M.L., Sides, A.M., Seekell, D., Belt, K.T., Secor, D.H., Wingate, R.L., 2010. Rising stream and river temperatures in the United States. *Front. Ecol. Environ.* 8, 461–466. doi:10.1890/090037
- Kominoski, J.S., Hoellein, T.J., Leroy, C.J., Pringle, C.M., Swan, C.M., 2010. Beyond species richness: Expanding biodiversity-ecosystem functioning theory in detritus-based streams. *River Res. Appl.* 26, 67–75.
- Kominoski, J.S., Rosemond, A.D., 2012. Conservation from the bottom up: forecasting effects

- of global change on dynamics of organic matter and management needs for river networks. *Freshw. Sci.* 31, 51–68.
- Kominoski, J.S., Shah, J.J.F., Canhoto, C., Fischer, D.G., Giling, D.P., González, E., Griffiths, N.A., Larrañaga, A., LeRoy, C.J., Mineau, M.M., others, 2013. Forecasting functional implications of global changes in riparian plant communities. *Front. Ecol. Environ.* 11, 423–432.
- Lake, P.S., 2011. *Drought and aquatic ecosystems: effects and responses*. John Wiley & Sons.
- Lecerf, A., Richardson, J.S., 2010. Biodiversity-Ecosystem Function research: insights gained from streams. *River Res. Appl.* 45–54. doi:10.1002/rra
- Ledger, M.E., Brown, L.E., Edwards, F.K., Milner, A.M., Woodward, G., 2013. Drought alters the structure and functioning of complex food webs. *Nat. Clim. Chang.* 3, 223–227. doi:10.1038/nclimate1684
- Leigh, C., Bonada, N., Boulton, A.J., Hugueny, B., Larned, S.T., Vander Vorste, R., Datry, T., 2016. Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. *Aquat. Sci.* doi:10.1007/s00027-015-0427-2
- Leopold, L.B., Wolman, M.G., Miller, J.P., 1964. *Fluvial processes in geomorphology*. San Francisco, CA.
- Malmqvist, B., Rundle, S.D., Covich, A.P., Hildrew, A.G., Robinson, C.T., Townsend, C.R., others, 2008. Prospects for streams and rivers: an ecological perspective., in: Polunin, N. (Ed.), *Aquatic Ecosystems: Trends and Global Prospects*. Cambridge University Press, Cambridge, UK, pp. 19–29.
- Martínez, A., Larrañaga, A., Miguélez, A., Yvon-Durocher, G., Pozo, J., 2016. Land use change affects macroinvertebrate community size spectrum in streams: The case of *Pinus radiata* plantations. *Freshw. Biol.* 61, 69–79. doi:10.1111/fwb.12680
- Martínez, A., Larrañaga, A., Pérez, J., Descals, E., Pozo, J., 2014. Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. *FEMS Microbiol. Ecol.* 87, 257–267.
- Menzel, A., Sparks, T.I.M.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K., Bissolli, P., Braslavská, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, Å., Defila, C., Donnelly, A., Filella, Y., Jatzcak, K., Måge, F., Mestre, A., Nordli, Ø., Peñuelas, J., Pirinen, P., Remišová, V., Scheifinger, H., Striz, M., Susnik, A., Van

- Vliet, A.J.H., Wielgolaski, F.-E., Zach, S., Zust, A.N.A., 2006. European phenological response to climate change matches the warming pattern. *Glob. Chang. Biol.* 12, 1969–1976. doi:10.1111/j.1365-2486.2006.01193.x
- Meyer, J.L., Strayer, D.L., Wallace, J.B., Eggert, S.L., Helfman, G.S., Leonard, N.E., 2007. The Contribution of Headwater Streams to Biodiversity in River Networks1. *JAWRA J. Am. Water Resour. Assoc.* 43, 86–103. doi:10.1111/j.1752-1688.2007.00008.x
- Millennium Ecosystem Assessment, 2005. *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington, DC.
- Millennium Ecosystem Assessment, 2003. *Ecosystems and human well-being: A framework For assessmen*. Island Press, Washington, D.C.
- Milly, P.C.D., Dunne, K.A., Vecchia, A. V, 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* 438, 347–350. doi:10.1038/nature04312
- Molinero, J., Larrañaga, A., Pérez, J., Martínez, A., Pozo, J., 2016. Stream temperature in the Basque Mountains during winter: thermal regimes and sensitivity to air warming. *Clim. Change* 134, 593–604. doi:10.1007/s10584-015-1546-4
- Norby, R.J., Cotrufo, M.F., Ineson, P., O’Neill, E.G., Canadell, J.G., 2001. Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia* 127, 153–65. doi:10.1007/s004420000615
- Norby, R.J., Zak, D.R., 2011. Enrichment (FACE) Experiments. *Annu. Rev. Ecol. Evol. Syst.* 42, 181–203. doi:10.1146/annurev-ecolsys-102209-144647
- Papadaki, C., Soulis, K., Muñoz-Mas, R., Martinez-Capel, F., Zogaris, S., Ntoanidis, L., Dimitriou, E., 2016. Potential impacts of climate change on flow regime and fish habitat in mountain rivers of the south-western Balkans. *Sci. Total Environ.* 540, 418–428.
- Pascoal, C., Cássio, F., 2004. Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River. *Appl. Environ. Microbiol.* 70, 5266 – 5273. doi:10.1128/AEM.70.9.5266
- Pereira, H.M., Leadley, P.W., Proença, V., Alkemade, R., Scharlemann, J.P.W., Fernandez-Manjarrés, J.F., Araújo, M.B., Balvanera, P., Biggs, R., Cheung, W.W.L., Chini, L., Cooper, H.D., Gilman, E.L., Guénette, S., Hurtt, G.C., Huntington, H.P., Mace, G.M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R.J., Sumaila, U.R., Walpole, M., 2010. Scenarios for global biodiversity in the 21st century. *Science* (80-.). 330, 1496–

1501. doi:10.1126/science.1196624

- Perkins, D.M., Reiss, J., Yvon-Durocher, G., Woodward, G., 2010. Global change and food webs in running waters. *Hydrobiologia* 657, 181–198.
- Perkins, D.M., Yvon-Durocher, G., Demars, B.O.L., Reiss, J., Pichler, D.E., Friberg, N., Trimmer, M., Woodward, G., 2012. Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. *Glob. Chang. Biol.* 18, 1300–1311. doi:10.1111/j.1365-2486.2011.02597.x
- Pozo, J., Gonzalez, E., Díez, J., Molinero, J., Elósegui, A., 1997. Inputs of particulate organic matter to streams with different riparian vegetation. *J. North Am. Benthol. Soc.* 16: 602–611.
- Pozo, J., Gonzalez, J., Elosegi, A., 1997. Leaf-litter budgets in two contrasting forested streams. *Limneti* 13, 77–84.
- Reiss, J., Bridle, J.R., Montoya, J.M., Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends Ecol. Evol.* 24, 505–14. doi:10.1016/j.tree.2009.03.018
- Rolls, R.J., Leigh, C., Sheldon, F., 2012. Mechanistic effects of low-flow hydrology on riverine ecosystems: ecological principles and consequences of alteration. *Freshw. Sci.* 31, 1163–1186. doi:10.1899/12-002.1
- Sabater, S., 2008. Alterations of the global water cycle and their effects on river structure, function and services. *Freshw. Rev.* 1, 75–88. doi:10.1608/FRJ-1.1.5
- Sabater, S., Tockner, K., 2010. Effects of hydrologic alterations on the ecological quality of river ecosystems, in: Sabater, S., Barceló, D. (Eds.), *Water Scarcity in the Mediterranean: Perspectives under Global Change*. *The Handbook of Environmental Chemistry*, pp. 15–39. doi:10.1016/0143-1471(82)90111-8
- Sala, O., Chapin, F.S., Armesto, J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L., Jackson, R., Kinzing, A., Leemans, R., Lodge, D., Mooney, H., Oesterheld, M., LeRoy Poof, N., Sykes, M., Walker, B., Walker, M., Wall, D., 2000. Biodiversity : global biodiversity scenarios for the year 2100. *Science* (80-.). 287, 1770. doi:10.1126/science.287.5459.1770
- Tank, J., Rosi-Marshall, E., Griffiths, N. a., Entekin, S. a., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. North Am. Benthol.*

Soc. 29, 118–146. doi:10.1899/08-170.1

- Taylor, G., Tallis, M.J., Giardina, C.P., Percy, K.E., Miglietta, F., Gupta, P.S., Gioli, B., Calfapietra, C., Gielen, B., Kubiske, M.E., Scarascia-Mugnozza, G.E., Kets, K., Long, S.P., Karnosky, D.F., 2008. Future atmospheric CO₂ leads to delayed autumnal senescence. *Glob. Chang. Biol.* 14, 264–275. doi:10.1111/j.1365-2486.2007.01473.x
- Tonin, A.M., Boyero, L., Monroy, S., Basaguren, A., Pérez, J., Pearson, R.G., Cardinale, B.J., Gonçalves, J.F., Pozo, J., 2017. Stream nitrogen concentration, but not plant N-fixing capacity, modulates litter diversity effects on decomposition. *Funct. Ecol.* doi:10.1111/1365-2435.12837
- Tuchman, N.C., Wetzel, R.G., Rier, S.T., Wahtera, K.A., Teeri, J.A., 2002. Elevated atmospheric CO₂ lowers leaf litter nutritional quality for stream ecosystem food webs. *Glob. Chang. Biol.* 8, 163–170. doi:10.1046/j.1365-2486.2002.00460.x
- Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The River Continuum Concept. *Can. J. Fish. Aquat. Sci.* 37, 130–137. doi:10.1139/f80-017
- Vitousek, P., 1994. Beyond global warming: Ecology and global change. *Ecology* 75, 1861–1876.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, a, Green, P., Glidden, S., Bunn, S.E., Sullivan, C. a, Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *Nature* 467, 555–561. doi:10.1038/nature09549
- Wallace, J.B., Eggert, S.L., Meyer, J.L., Webster, J.R., 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* (80-.). 277, 102–104. doi:10.1017/CBO9781107415324.004
- Woodward, G., Perkins, D.M., Brown, L.E., 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2093–106. doi:10.1098/rstb.2010.0055
- Yvon-Durocher, G., Caffrey, J.M., Cescatti, A., Dossena, M., Giorgio, P. Del, Gasol, J.M., Montoya, J.M., Pumpanen, J., Staehr, P. a., Trimmer, M., Woodward, G., Allen, A.P., 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature* 1–5. doi:10.1038/nature11205

CHAPTER 2

Elevated atmospheric CO₂ interacts with drought and competition to produce complex responses in plant quality and subsequent microbial aquatic decomposition

This chapter was published with the following reference: Monroy S., Miranda-Apodaca J., Pérez-López U., Mena-Petite A., Elozegi A., Muñoz-Rueda A. & Pozo J. 2016. Elevated atmospheric CO₂ interacts with drought and competition to produce complex responses in plant quality and subsequent microbial aquatic decomposition. *Canadian Journal of Fisheries and Aquatic Sciences*, 73:1770-1776, 10.1139/cjfas-2016-0034. Q1

Abstract

Human activities enhance the atmospheric CO₂ concentration and modify climatic patterns, which could change the growth and quality of plant materials, as well as their subsequent decomposition, thus altering stream ecosystem functioning. These effects could be modulated by interactions between plant species differing in biological traits and competitive capacity. We cultivated the forb *Trifolium pratense* and the grass *Agrostis capillaris* under different CO₂ concentration (ambient or elevated), water availability (control and drought) and competition (monoculture or mixture). The material thus grown was conditioned in a stream for microbial colonization, and its subsequent decomposition was measured in laboratory microcosms. Elevated CO₂ reduced the quality of *T. pratense* but not that of *A. capillaris*. Water shortage limited plant quality, but did not interact with CO₂ concentration. Interspecific competition affected nitrogen concentration in *A. capillaris*. Elevated CO₂ did not affect decomposition rate, and *A. capillaris*, the species richer in nutrients decomposed slower. Our results show that decomposition rates of plant materials grown under elevated CO₂ are difficult to predict due to species-specific responses and interactions with other factors.

Keywords: climate change, stream, microbial decomposition, atmospheric CO₂, biological traits, grassland

Introduction

Future scenarios of global environmental change forecast a doubling of the atmospheric CO₂ concentration by the end of the 21st century (IPCC 2013). Increased CO₂ concentration is expected to trigger profound changes in vegetation, altering phenology (Taylor et al. 2008), stimulating plant growth by enhanced photosynthetic activity, along with alterations in plant tissue composition (Cotrufo et al. 1998; Tuchman et al. 2002; Norby and Zak 2011). However, the response of plants to elevated CO₂ might be influenced by other factors such as water shortage (AbdElgawad et al. 2014; Miranda-Apodaca et al. 2015), which will become more prevalent in many regions. Therefore, the interaction between elevated CO₂ and water stress may alter plant quality, which is important for consumers, and modify key ecological processes such as decomposition and carbon (C) cycling (Aerts 1997; Hladysz et al. 2009).

Elevated atmospheric CO₂ tends to promote an increase of C-rich structural compounds such as lignin and cellulose, enhance the synthesis of secondary phenolic compounds, and increase carbon:nutrient ratios (Rier et al. 2005; Lindroth 2012; Sardans et al. 2012). On the other hand, water shortage often increases soluble carbohydrates (Walter et al. 2012; Urbina et al. 2015) and reduces nitrogen (N) and phosphorus (P) concentrations (Urbina et al. 2015). Therefore, the combination of elevated CO₂ and water stress could produce a large decrease in the quality of plant tissues. However, it has also been suggested that the detrimental effects of

water stress could be partially mitigated by the elevated CO₂ (Pérez-López et al. 2014), as it would increase the efficiency of water use, thus enhancing soil moisture, nutrient availability, plant growth and N uptake (Morgan et al. 2004; Dijkstra and Cheng 2008). For example, it has been shown that high CO₂ and drought increase C:N ratios in isolation, but not when acting together (Larsen et al. 2011).

Additionally, there is controversy about these interactions (Norby and Zak 2011) because the response to CO₂ could depend on plant species or functional types (Novotny et al. 2007; Dray et al. 2014). Besides, the response of plants to elevated CO₂ or water shortage could depend on community composition (Strengbom et al. 2008; Walter et al. 2012; Urbina et al. 2015). For example, it has been suggested that elevated CO₂ reduces C:N ratio less in more diverse plant mixtures (Novotny et al. 2007).

The potential changes in plant tissue quality resulting from increased atmospheric CO₂ concentration, altered water availability and different competitive conditions could be highly relevant for ecosystem functioning, as substrate quality exerts a strong influence on organic matter decomposition (Melillo et al. 1982; Aert 1997) and consequently on nutrient and C cycling in both terrestrial and aquatic ecosystems. Freshwater ecosystems may be particularly vulnerable to alterations in plant chemical composition, as their food webs depend heavily on terrestrial subsidies (Woodward et al. 2010), especially in forested headwater streams where primary production is light-limited (Tank et al. 2010). Organic matter rich in nutrients and with lower concentrations of recalcitrant C is more readily used by microbial decomposers and detritivores (Hladyz et al. 2009), and decomposes faster. Therefore, reduction in plant quality will likely affect its use by biota, altering the decomposition process and the energy flow through stream food webs.

Previous studies on the effects of increased atmospheric CO₂ on aquatic decomposition yielded mixed results. Kelly et al. (2010) found leaves grown at elevated CO₂ leaves to have lower fungal biomass and bacterial production, whereas Tuchman et al. (2002) found not such differences. It has also been reported that the effects on decomposition are higher in early stages of the decay process (Tuchman et al. 2003). Nevertheless, most studies were focused on single plant species, and thus, little is still known on more complex assemblages or on interactions with other stressors. Therefore, to predict the effects of oncoming environmental change on decomposition and C and N cycling, it is necessary to improve our understanding of the interactions between different factors such as enhanced CO₂, drought and competition. Moreover, most studies so far focused on leaf litter from deciduous tree species, neglecting the fact that in some systems, such as open-canopy streams, riparian herbs and grasses also provide significant quantities of detritus to stream food webs (Menninger and Palmer 2007; Leberfinger

et al. 2011), and that they are expected to expand as a consequence of the clearing of riparian vegetation for pasture (Allan and Castillo, 2007).

The objective of this study was to assess the effect of material quality of plants grown under different combinations of atmospheric CO₂ concentration, water availability and competition on their microbial decomposition in streams. For this purpose, two common grassland species of different functional groups (a legume and a grass) were grown under two levels of CO₂ concentration, two of water availability, and with or without interspecific competition. Materials obtained in these cultures were submerged in a stream for microbial colonization. Then, these plant tissues were incubated in laboratory microcosms for their microbial decomposition. We hypothesized (1) elevated CO₂ to reduce tissue quality of both species by decreasing N and P concentrations; (2) decreased quality to be more pronounced under low water availability; (3) interspecific competition to affect the quality response of both species; and (4) microbial decomposition of plant tissues to vary according to their resulting quality under the growing conditions, being lowest in tissues grown under high CO₂ and drought.

Materials and methods

Growing conditions

We performed a factorial experiment in which two plant species were subject to two levels of CO₂ concentration and two levels of water availability while growing either in monoculture or mixture, thus totaling 8 treatments per plant species. The plant material grown in these treatments (3 replicates per treatment) was sorted according to species, analyzed for tissue characteristic, and used to perform a decomposition experiment in stream water. We used two common grassland species, the legume *Trifolium pratense* L. and the grass *Agrostis capillaris* L., which were grown in 6-L pots with a 1:1 mixture of peat/vermiculite, with twelve seeds per pot in a controlled environmental growth chamber (Conviron PGR15, Manitoba, Canada). Although *T. pratense* is a N-fixing species, the plants used for the present experiment were not inoculated with *Rhizobium* and under these conditions plants were not nodulated, i.e., they did not fix atmospheric N. The photoperiod was 14-h light/10-h dark, with day/night temperature of 24/20 °C and relative humidity of 70-80%. During the light period, the photosynthetic photon flux density (PPFD) was 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$, provided by a combination of incandescent bulbs and warm-white fluorescent lamps (Sylvania F48T12SHO/VHO; Sylvania, USA). We used two CO₂ concentrations (ambient, 400 ppm or elevated, 700 ppm), two levels of water availability (control and drought, see below) and grew the plants either in monoculture (12 seeds of the same species per pot), or in mixture (6 seeds of each species per pot). Plants

were watered with Hoagland's solution (Arnon and Hoagland 1940) twice per week and were also watered with deionized water between each application of Hoagland's solution until the beginning of the drought treatment (28 d old). For water availability, drought treatment was imposed for 16 d by withholding water until the 15% of the maximum soil volumetric water content was reached; then, the treatments were maintained at 15% of the maximum soil volumetric water content. The control plants were watered with the 100% of their daily evapotranspiration. During the drought period the watering was alternated daily between Hoagland's solution and deionized water. Plants were collected after 44 d and separated into leaves and stems for analysing their chemical composition. For the determination of C, N and P, plant material was oven-dried, weighed, and milled. C and N were determined on 2 mg dry mass using an elemental analyser (FlashEA 1112; ThermoFinnigan, Germany). P was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Horiba YobinYvon Activa). Results were expressed as a percentage elemental content (C, N and P) of plant dry mass.

Microbial decomposition

The plant material from the three replicates per treatment was first cut to separate stems and leaves, air-dried, and then pooled by mixing thoroughly (stems and leaves independently). The 16 types of pooled materials so obtained (8 treatments per species) were used for performing a microbial decomposition experiment. For each type, 4 fine mesh bags (0.5 mm mesh size) were filled with plant litter fragments, incubated for 7 d in a natural stream to allow microbial colonization but not macroinvertebrates, and then transferred to laboratory microcosms for 28 d to measure microbial decomposition. Approximately 0.5 g ($\pm 3\%$, air-dry weight) of stems and leaves of *T. pratense* in a proportion 60% stem and 40% leaf (similar to those observed in individual plants) were placed into each of 32 bags. Another set of 32 bags was filled with amounts of *A. capillaris* ranging from 0.5 g to 0.02 g ($\pm 3\%$), depending on the material produced under the different treatments, in a proportion of 71% leaf and 29% stem. The initial concentration of C, N and P in the material used in the decomposition experiment was estimated based on the proportion of stem and leaf used for each treatment. Additional samples of each material type were used to determine oven-dry mass (72 h at 70 °C) and ashed (12 h at 500 °C) to estimate initial ash free dry mass (AFDM) introduced in each bag.

The 64 bags (four replicates per treatment and species) were immersed in Peñalar Stream (Guriezo, northern Spain) on 31th March 2014, tied by nylon string to iron bars anchored randomly to the streambed. After 7 d of incubation bags were collected and transported to the laboratory in a cooler.

The plant material from each bag was placed in a microcosm in a refrigerated room (10 °C) under a 12:12 h light/dark regime. Each microcosm consisted of a glass beaker containing 200 mL of filtered water (0.7 µm, Whatman GF/F) from Peñalar Stream, aerated continuously by an air pump, and kept in tanks where temperature was controlled by a Julabo EH 17 heater circulator. Water in the microcosms was renewed every 7 d.

During the experiment, water physicochemistry in Peñalar Stream was characterized on five occasions. We measured dissolved oxygen, conductivity and pH (WTW multiparametric sensor; Multi 350i) *in situ*. Water was carried in 20 L canisters to the laboratory, where it was filtered and used for the microcosms experiment; subsamples were used to determine alkalinity (titration to an end pH of 4.5), nitrate concentration (capillary ion electrophoresis, Agilent CE), ammonium (salicylate-hypochlorite method), nitrite (sulphanylamide method) and soluble reactive P (SRP, molybdate method) (APHA 2005). Stream water (mean ± SD of five measurements) was circumneutral (pH 7.52 ± 0.02), low in conductivity (76.8 ± 3.2 µS/cm) and alkalinity (0.24 ± 0.03 meq/L), saturated in oxygen (saturation 96.8 ± 1.8 %) and had an average temperature of 11.16 °C (± 0.45). Dissolved inorganic nutrient concentrations averaged 554.7 µg N/L (Dissolved Inorganic Nitrogen ± 15.1) and 2.2 µg P/L (SRP ± 0.3).

The experiment was terminated on 5th May 2014, after 28 d of microcosm incubation. The plant material from each microcosm was oven-dried to obtain the dry mass (72 h at 70 °C) and ashed (12 h at 500 °C) to determine ash-free dry mass remaining (AFDMr; decomposition was expressed as percentage of initial ash-free dry mass remaining, % AFDMr).

Statistical analyses

Effects of CO₂ concentration, water stress and competition on initial concentrations of C, N and P of plant tissues and remaining mass (% AFDMr) of plant materials were tested individually for each species by three-way ANOVA (factors: CO₂ concentration, water stress and competition). Bivariate relationships between the averaged % AFDMr and the estimated initial nutrient content for each treatment and species were tested by simple linear regression. A $p < 0.05$ was considered statistically significant. The data were arcsin square-root transformed for equal variance and normal distribution when necessary (Zar 2010). All statistical analyses were performed with R statistical software (version 3.0.3; R Development Core Team, 2014).

Results

Quality of harvested plant tissue

The C concentration was similar in both species, around 40%, and was not affected by any of the factors considered. N concentration was higher in *A. capillaris* (4.53-5.53%) than in *T. pratense* (2.78-4.47%) in all treatments (Fig. 1A and B). There was a general decrease of this element in *T. pratense* under elevated CO₂ (Fig. 1A, Table 1). N also decreased under water stress in a similar magnitude at both CO₂ concentrations, but was not affected by interspecific competition (Fig. 1A, Table 1). On the other hand, nitrogen concentration of *A. capillaris* was not affected by elevated CO₂ but decreased under water stress and under interspecific competition (Fig. 1B, Table 1). As for N, P concentration was higher in *A. capillaris* (0.67-0.90%) than in *T. pratense* (0.49-0.69%) (Fig. 1C and D). This element decreased significantly under water stress in both species (Fig. 1C and D, Table 2). For *T. pratense*, phosphorus concentration tended to decrease under elevated CO₂ ($p = 0.092$; Fig. 1C, Table 2) whereas for *A. capillaris* it tended to be higher at elevated CO₂ under competition (negative CO₂ effect in monoculture and positive CO₂ effect in mixture; Fig. 1D, Table 2).

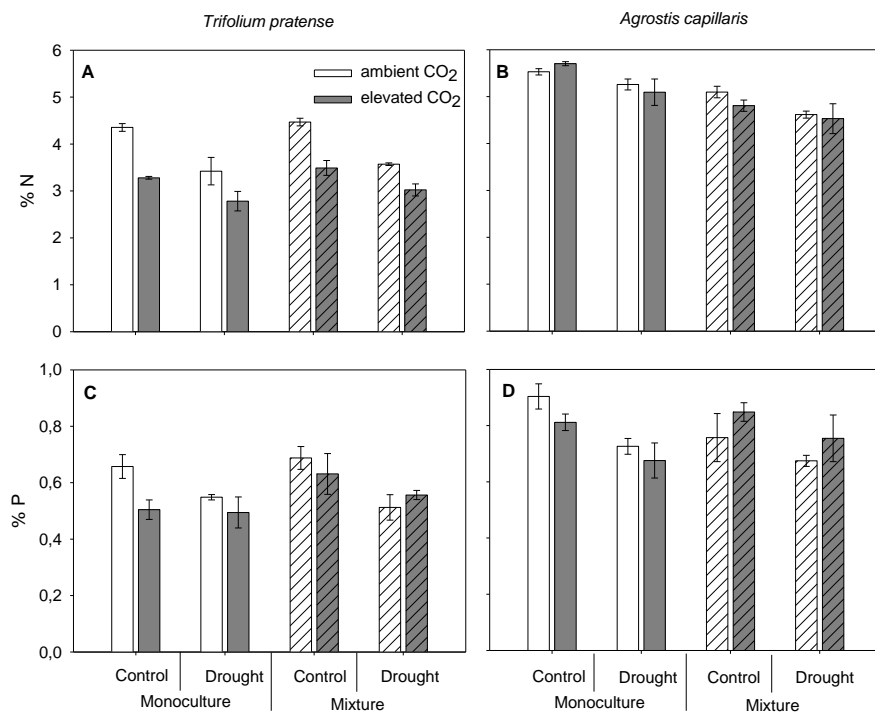


Fig 1. Nitrogen (A and B) and phosphorus (C and D) concentration in *T. pratense* (left) and *A. capillaris* (right) grown under different conditions of CO₂ levels (ambient, elevated), water availability (control, drought) and competition (monoculture, mixture). Values presented as mean \pm SE (n=3).

Table 1. Summary table of three-way ANOVA for the effects of CO₂ concentration (ambient/elevated), water stress (control/drought) and competition (monoculture/mixture) on nitrogen concentration of *T. pratense* and *A. capillaris*. Statistical significance ($p < 0.05$) is highlighted in bold. Stressor effect is shown by vertical arrows.

Factors	<i>Trifolium pratense</i>			<i>Agrostis capillaris</i>		
	Df	F	<i>p</i>	Df	F	<i>p</i>
CO ₂	1	50.56	< 0.001 ↓	1	0.84	0.374
Water stress	1	37.91	< 0.0001 ↓	1	13.14	0.003 ↓
Competition	1	2.89	0.109	1	27.85	< 0.0001 ↓
CO ₂ x Water stress	1	1.93	0.183	1	0.08	0.784
CO ₂ x Competition	1	0.27	0.608	1	0.79	0.387
Water stress x Competition	1	0.11	0.749	1	0	0.998
CO ₂ x Water stress x Competition	1	0	0.992	1	1.089	0.313
Residuals	16			15		

Table 2. Summary table of three-way ANOVA for the effects of CO₂ concentration (ambient/elevated), water stress (control/drought) and competition (monoculture/mixture) on P concentration of *T. pratense* and *A. capillaris*. Statistical significance ($p < 0.05$) is highlighted in bold. Stressor effect is shown by vertical arrows.

Factors	<i>Trifolium pratense</i>			<i>Agrostis capillaris</i>		
	Df	F	<i>p</i>	Df	F	<i>p</i>
CO ₂	1	3.21	0.092	1	0.01	0.942
Water stress	1	8.67	0.010 ↓	1	11.26	0.005 ↓
Competition	1	2.17	0.160	1	0.10	0.759
CO ₂ x Water stress	1	2.56	0.129	1	0.11	0.746
CO ₂ x Competition	1	2.67	0.122	1	4.23	0.059
Water stress x Competition	1	1.06	0.319	1	0.75	0.401
CO ₂ x Water stress x Competition	1	0.00	0.949	1	0.12	0.736
Residuals	16			14		

Decomposition

T. pratense decomposed faster than *A. capillaris* (Fig. 2A and B). Neither growth under elevated CO₂ concentration nor competition during growth affected eventual mass loss in the

former species (Table 3), whereas plant material grown under water stress eventually lost mass more readily (Fig. 2A, Table 3). On the other hand, *A. capillaris* material grown under elevated CO₂ concentrations or under interspecific competition tended to show (non-significant) lower mass loss (Fig. 2B, Table 3).

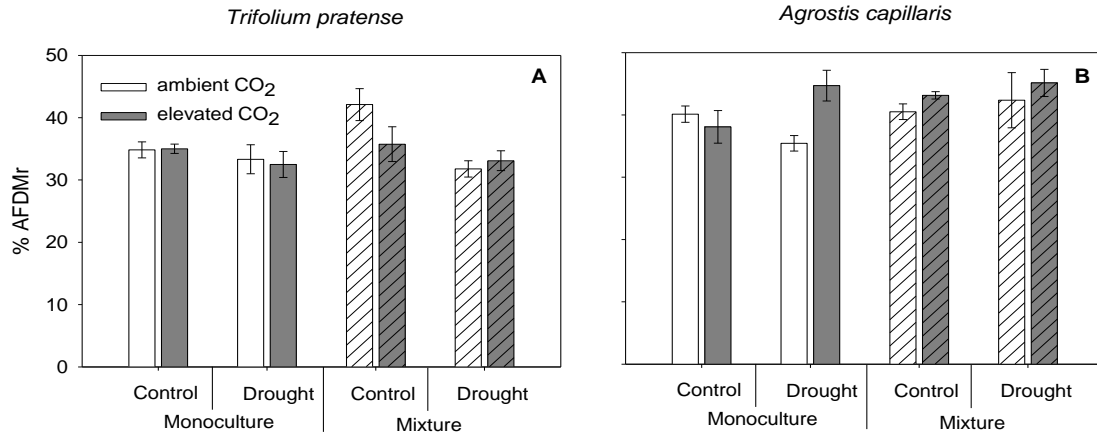


Fig 2. Percentage of ash-free dry mass remaining (% AFDMr) at the end of the decomposition experiment for *T. pratense* (A) and *A. capillaris* (B) grown under different conditions of CO₂ concentration (ambient, elevated), water availability (control, drought) and competition (monoculture, mixture). Values presented as mean \pm SE (n= 4).

Table 3. Summary table of three-way ANOVA performed on percentage of initial ash-free dry mass remaining of *T. pratense* and *A. capillaris* produced under different conditions of CO₂ concentration (ambient/elevated), water stress (control/drought) and competition (monoculture/mixture). Statistical significance ($p < 0.05$) is highlighted in bold. Stressor effect is shown by vertical arrows.

Factors	<i>Trifolium pratense</i>			<i>Agrostis capillaris</i>		
	Df	F	<i>p</i>	Df	F	<i>p</i>
CO ₂	1	0.88	0.357	1	3.76	0.064
Water stress	1	9.37	0.006 ↑	1	0.74	0.397
Competition	1	1.45	0.241	1	3.88	0.060
CO ₂ x Water stress	1	1.42	0.245	1	3.15	0.089
CO ₂ x Competition	1	0.57	0.457	1	0.07	0.792
Water stress x Competition	1	2.54	0.125	1	0.08	0.780
CO ₂ x Water stress x Competition	1	2.32	0.141	1	2.94	0.099
Residuals	23			24		

Relationship between nutrients and mass loss

The remaining mass of *T. pratense* was positively correlated with its initial P concentration ($r^2 = 0.578$, $p = 0.029$; Fig. 3C) and it showed a positive tendency with its initial N concentration ($r^2 = 0.496$, $p = 0.051$; Fig. 3A). In contrast, the remaining mass of *A. capillaris* only showed a negative correlation with the initial N concentration ($r^2 = 0.644$, $p = 0.017$; Fig. 3B).

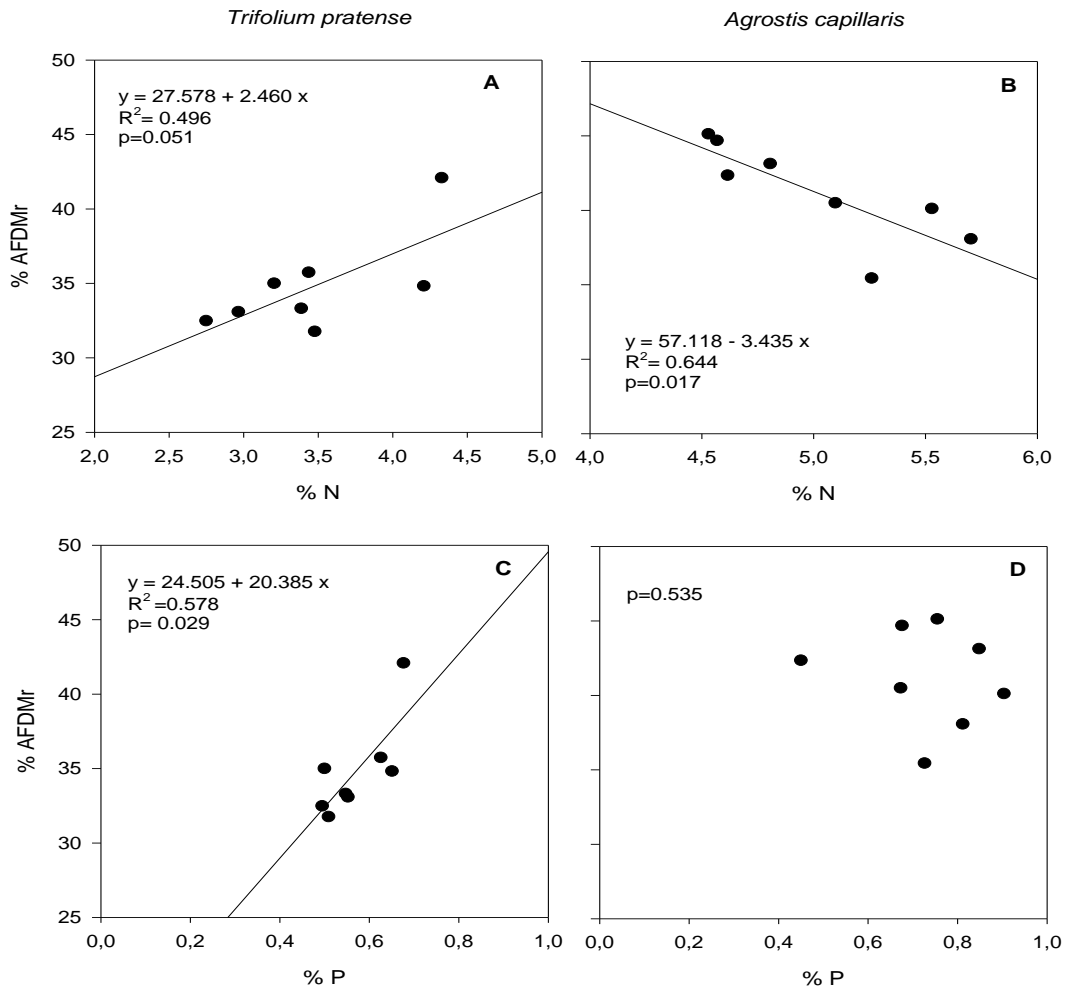


Fig 3. Relationships between average % AFDMr after decomposition experiment and estimated initial nutrient concentrations (% N; % P) for *T. pratense* (A and C) and *A. capillaris* (B and D) according to each treatment. The p -values of the relationship and fitted lines are also shown.

Discussion

Increased atmospheric CO_2 concentration is expected to modify the chemical composition of plant tissue (Cotrufo et al. 1998), although the response depends on species (Lüscher and Nosberger 1997; Rier et al. 2005). In our case, *A. capillaris* showed higher N concentration than *T. pratense* irrespective of the growing condition, but enhanced CO_2 resulted in a 20% decrease of *T. pratense* N, whereas it did not change *A. capillaris* N. Although the

mechanisms by which elevated CO₂ causes reductions in N concentration are still debated (Taub and Wang 2008), the reduction observed in *T. pratense* could be due to a dilution effect as a consequence of the accumulation of non-structural carbohydrates, as was reported by Norby and Zak (2011) and Gifford et al. (2000). According to Miranda-Apodaca et al. (2015), the growth of the legume, higher than that of the grass, due to its higher concentration of photosynthetic pigments, higher photosynthetic photochemical efficiency, and higher photosynthetic rates, points in this direction. Another possible explanation for this decrease could be the detected reduction in N uptake rate (see below). Concerning plant P concentration, enhanced CO₂ has been reported to result in variable responses (Gifford et al. 2000). In our experiment, both species differed clearly in P and, although neither responded significantly to elevated CO₂, P concentration in *T. pratense* tended to drop at elevated CO₂. Therefore, environmental changes do not seem to affect equally all nutrients, as has been shown before (Pérez-López et al. 2015).

Water scarcity has been reported to reduce nutrient uptake by plants (Garg 2003; Larsen et al. 2011) and, consequently, plant tissue nutrient concentration. In our study, drought strongly reduced N and P concentration in both species, likely as a consequence of reductions in nutrient uptake rate by 60 and 80% for *T. pratense* and by 65 and 75% for *A. capillaris*, respectively (Miranda-Apodaca, in prep.). However, this decline was not buffered by high CO₂, as suggested by Pérez-López et al. (2014), probably because of the severity of our drought treatment (Xu et al. 2013).

The effect of competition on nutrient content of plant materials was species-specific and mainly resulted in decreased N concentration of the grass species, probably because its reduced the rate of N uptake (around 65%), as already has been found elsewhere (Dijkstra et al. 2010). However, competition seemed not to interact with either CO₂ concentration or water availability to determine N content in plants.

Plant chemical composition greatly influences decomposition (Hladyz et al. 2009). Therefore, modifications in plant composition such as those detected in the present experiment in response to elevated CO₂, water availability and competition could be relevant for the functioning of open-canopy stream ecosystems, where herbaceous plants can be a food source for stream organisms (Leberfinger et al. 2011). Nevertheless, CO₂ growing conditions, as a single factor, did not result in differential decomposition, and only drought-affected *T. pratense* decayed faster than the other materials. Nevertheless, a trend to lower decomposition of materials grown at elevated CO₂ and in competition was observed for *A. capillaris*.

Elevated CO₂ resulted in N reduction in *T. pratense*, but water availability also changed plant N and P. N concentration and C:N ratio have been reported to drive decomposition rates of organic matter (Hladyz et al. 2009). Nevertheless, the contrasting correlations between mass remaining and N concentration in *T. pratense* (positive) and *A. capillaris* (negative) along with

the slower decomposition observed in *A. capillaris* despite its higher N and P concentration, suggest that other characteristics drive mass loss (Cotrufo et al. 1994). The positive relationship between mass remaining and initial phosphorus concentration in *T. pratense* also points in this direction. The concentration of structural carbohydrates (cellulose, hemicellulose, among others), which is higher in *A. capillaris*, could be another reason, as was reported by Picon-Cochard et al. (2004), who found that grasses have a higher content of structural carbohydrates than forbs. In the same direction but for P, Ferreira et al. (2010) and Ferreira and Chauvet (2011) observed that, although alder leaves grown at elevated CO₂ had reduced P concentration, they decomposed faster than control leaves. Similarly, in terrestrial ecosystems, changes in plant composition induced by elevated CO₂ have been shown to have no effects on litter decomposition (Norby et al. 2001). The lack of correlation between nutrient content and decomposition rate has been often attributed to leaching of soluble compounds (Tuchman et al. 2003; Rier et al. 2005; Dray et al. 2014), which can reduce or amplify initial differences in substrate composition (Rier et al. 2002; Tuchman et al. 2002).

Our experiment was focused on microbial decomposition, as materials were conditioned in the stream inside fine mesh bags, which excluded invertebrate colonization. Elevated CO₂ accentuated the differences in decomposition rate due to a slight decrease in the decomposition rate of *A. capillaris*. Data on decomposition of these species are scarce in the literature, but other studies indicate that, although fungal biomass and communities do not seem to be affected by litter quality changes induced by increased atmospheric CO₂ (Rier et al. 2002; Kelly et al. 2010), leaves grown at elevated CO₂ may decay more slowly (e.g., Rier et al. 2002).

The ongoing increase in atmospheric CO₂ is accompanied by rising temperatures, which could produce stronger effects on leaf decomposition (Ferreira and Chauvet 2011). Drought will be another stressor for plant growth (LeRoy et al. 2014), and our results show that its effects on chemical composition of plant materials can surpass those resulting from elevated CO₂. The decay rate of materials grown under drought conditions increased in *T. pratense*, but did not change in *A. capillaris*, which suggests, as pointed by LeRoy et al. (2014), that although litter quality can change under different climatic conditions, the overall decay of plant material will not be dramatically modified by droughts.

In short, although elevated CO₂ reduced nutrient concentration as stated by hypothesis 1, this reduction only occurred in *T. pratense*. Second, the nutrient concentration of plant materials was more affected by drought than by CO₂ concentration, irrespective of plant species. Third, interspecific competition only affected N concentration in *A. capillaris*. Finally, although we hypothesized decomposition rate to decrease with reduced nutrient content, our results point in other direction, since declines in N and P concentration by drought in *T. pratense* resulted in higher decomposition rate, whereas plant tissues of *A. capillaris*, the species richest in N and P,

decomposed slower. Perhaps, as suggested by Ferreira and Chauvet (2011), changes in quality were not strong enough to determine decomposition. In fact, N and P values at the beginning of the decomposition experiment were higher than those found in many riparian trees that usually feed stream ecosystem microbial communities (Menninger and Palmer 2007). A point worth discussing is the influence of fresh versus senescent material in breakdown rates. Most studies on decomposition use senescent leaf litter from deciduous species, which have lower quality than their green leaf counterparts (Tuchman et al. 2002, 2003), as trees re-absorb most of the nutrients before shedding the leaves. None of the herbaceous species we studied undergoes a senescence process comparable to those of deciduous trees, although following senescence they also show a decline in leaf quality caused by remobilization of their nutrients (Sanaullah et al. 2010). Anyway, streams do not receive only abscised leaves; instead, freshly fallen and herbaceous plant inputs occur throughout the year (Leberfinger et al. 2011; Menninger and Palmer 2007).

The present experiment showed CO₂ concentration during growth not to consistently modify plant decomposition, but its interactions with other factors (species, water availability and competition) to determine decomposition rate. Ecosystem functioning in the future will be subject to multiple stressors. Species-specific responses to growing conditions, together with significant interactions between species and stressors, make the response hard to forecast. Therefore, many efforts are still needed to predict microbial decomposition rates and their repercussion on biogeochemical cycles in complex and changing aquatic environments.

REFERENCES

- AbdElgawad, H., Peshev, D., Zinta, G., Van den Ende, W., Janssens, I. A., and Asard, H. 2014. Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO₂: a comparison of fructan and non-fructan accumulators. *PLoS ONE* **9**(3): e92044. doi:10.1371/journal.pone.0092044.
- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos*. **79**: 439-449.
- Allan, J.D., and Castillo, M.M. 2007. *Stream ecology: structure and function of running waters*. Springer, Dordrecht.
- APHA. 2005. *Standard methods for the examination of water and wastewater*. 21st ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- Arnon, D.I., and Hoagland, D.R. 1940. Crop production in artificial culture solutions and in

- soils with special reference to factors influencing yields and absorption of inorganic nutrients. *Soil Sci.* **50**: 463-485.
- Cotrufo, M.F., Inesson, P., and Rowland, A.P. 1994. Decomposition of tree leaf litters grown under elevated CO₂: Effect of litter quality. *Plant Soil.* **163**: 121–130.
- Cotrufo, M.F., Ineson, P., and Scott A. 1998. Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Glob. Change Biol.* **4**: 43–54.
- Dijkstra, F.A., Blumenthal, D., Morgan, J.A., LeCain, D.R., and Follett, R.F. 2010. Elevated CO₂ effects on semi-arid grassland plants in relation to water availability and competition. *Funct. Ecol.* **24**: 1152–1161.
- Dijkstra, F.A., and Cheng, W. 2008. Increased soil moisture content increases plant N uptake and the abundance of 15N in plant biomass. *Plant Soil.* **302**: 263–271.
- Dray, M.W., Crowther, T.W., Thomas, S.M., A'Beare, A. D., Godbold, D.L., Ormerod, S.J., et al. 2014. Effects of elevated CO₂ on litter chemistry and subsequent invertebrate detritivore feeding responses. *PLoS ONE.* **9**: 1–7.
- Ferreira, V., and Chauvet, E. 2011. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia.* **167**: 279–91.
- Ferreira, V., Gonçalves, A.L., Godbold, D.L., and Canhoto, C. 2010. Effect of increased atmospheric CO₂ on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Glob. Change Biol.* **16**: 3284–3296.
- Garg, B.K. 2003. Nutrient uptake and management under drought: nutrient-moisture interaction. *Curr. Agric.* **27**: 1–8.
- Gifford, R.M., Barrett, D.J., and Lutze, J.L. 2000. The effects of elevated [CO₂] on the C: N and C: P mass ratios of plant tissues. *Plant Soil.* **224**: 1–14.
- Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J., and Woodward, G. 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biol.* **54**: 957–970.
- IPCC. 2013. Summary for policymakers. In *Climate change 2013: The physical science basis. Contribution of the Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Edited by T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley. Cambridge University Press, Cambridge, UK, and New York, N.Y.

- Kelly, J.J., Bansal, A., Winkelman, J., Janus, L.R., Hell, S., Wencel, M., Belt, P., Kuehn, K.A., Rier, S.T., and Tuchman, N.C. 2010. Alteration of microbial communities colonizing leaf litter in a temperate woodland stream by growth of trees under conditions of elevated atmospheric CO₂. *Appl. Environ. Microbiol.* **76**: 4950–9.
- Larsen, K.S., Andresen, L.C., Beier, C., Jonasson, S., Albert, K.R., Ambus, P., et al. 2011. Reduced N cycling in response to elevated CO₂, warming, and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments. *Glob. Change Biol.* **17**: 1884–1899.
- Leberfinger, K., Bohman, I., and Herrmann, J. 2011. The importance of terrestrial resource subsidies for shredders in open-canopy streams revealed by stable isotope analysis. *Freshwater Biol.* **56**: 470–480.
- LeRoy, C.J., Wymore, A.S., Davis, R., and Marks, J.C. 2014. Indirect influences of a major drought on leaf litter quality and decomposition in a southwestern stream. *Fundam. Appl. Limnol.* **184**: 1–10.
- Lindroth, R.L. 2012. Atmospheric change, plant secondary metabolites and ecological interactions. In *The Ecology of Plant Secondary Metabolites: From Genes to Global Processes*. Edited by G.R. Iason, M. Dicke and S.E. Hartley. Cambridge: Cambridge University Press. pp. 120–153.
- Lüscher, A., and Nosberger, J. 1997. Interspecific and intraspecific variability in the response of grasses and legumes to free air CO₂ enrichment. *Acta Oecologica.* **18**: 269–275.
- Melillo, J.M., Aber, J.D., and Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology.* **63**: 621–626.
- Menninger, H.L., and Palmer, M.A. 2007. Herbs and grasses as an allochthonous resource in open-canopy headwater streams. *Freshwater Biol.* **52**: 1689–1699.
- Miranda-Apodaca, J., Pérez-López, U., Lacuesta, M., Mena-Petite, A., and Muñoz-Rueda, A. 2015. The type of competition modulates the ecophysiological response of grassland species to elevated CO₂ and drought. *Plant Biol.* **17**: 298–310.
- Morgan, J.A, Pataki, D.E., Korner, C., Clark, H., Del Grosso, S.J., Grun- zweig, J.M., Knapp, A.K., Mosier, A.R., Newton, P.C.D., Niklaus, P.A., Nippert, J.B., Nowak, R.S., Parton, W.J., Polley, H.W., and Shaw, M.R. 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia.* **140**: 11–25.

- Norby R.J., Cotrufo M.F., Ineson P., O'Neill E.G. & Canadell J.G. (2001) Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**: 153–65.
- Norby, R.J., and Zak, D.R. 2011. Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Annu. Rev. Ecol. Evol. Syst.* **42**: 181–203.
- Novotny, A.M., Schade, J.D., Hobbie, S.E., Kay, A.D., Kyle, M., Reich, P.B., and Elser, J.J. 2007. Stoichiometric response of nitrogen-fixing and non-fixing dicots to manipulations of CO₂, nitrogen, and diversity. *Oecologia*. **151**: 687–696.
- Pérez-López, U., Miranda-Apodaca, J., Mena-Petite, A., and Muñoz-Rueda, A. 2014. Responses of nutrient dynamics in barley seedlings to the interaction of salinity and carbon dioxide enrichment. *Environ. Exp. Bot.* **99**: 86-99.
- Pérez-López, U., Miranda-Apodaca, J., Lacuesta, M., Mena-Petite A., and Muñoz-Rueda, A. 2015. Growth and nutritional quality improvement in two differently pigmented lettuce cultivars grown under elevated CO₂ and/or salinity. *Sci. Hortic.* **195**: 56-66.
- Picon-Cochard, C., Teyssonneyre, F., Besle, J.M., and Soussana, J.F. 2004. Effects of elevated CO₂ and cutting frequency on the productivity and herbage quality of a semi-natural grassland. *Eur. J. Agron.* **20**: 363-377.
- R Development Core Team 2014 (version 3.0.3). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Rier, S.T., Tuchman, N.C., Wetzel, R.G., and Teeri, J.A. 2002. Elevated-CO₂-induced changes in the chemistry of quaking aspen (*Populus tremuloides* Michaux) leaf litter: subsequent mass loss and microbial responses in a stream ecosystem. *J. N. Am. Benthol. Soc.* **21**: 16-27.
- Rier, S.T., Tuchman, N.C., and Wetzel, R.G. 2005. Chemical changes to leaf litter from trees grown under elevated CO₂ and the implications for microbial utilization in a stream ecosystem. *Can. J. Fish. Aquat. Sci.* **62**: 185–194.
- Sanaullah, M., Chabbi, A., Lemaire, G., Charrier, X., Rumpel, C. 2010. How does plant leaf senescence of grassland species influence decomposition kinetics and litter compounds dynamics? *Nutr. Cycling Agroecosyst.* **88**: 159-171.
- Sardans, J., Rivas-Ubach, A., and Peñuelas, J. 2012. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. *Perspect Plant Ecol Evol Syst.* **14**: 33–47.

- Strengbom, J., Reich, P.B., and Ritchie, M.E. 2008. High plant species diversity indirectly mitigates CO₂- and N-induced effects on grasshopper growth. *Acta Oecol.* **34**: 194–201.
- Tank, J., Rosi-Marshall, E., Griffiths, N.A., Entekin, S.A., and Stephen, M.L. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. N. Am. Benthol. Soc.* **29**: 118–146.
- Taub, D.R., and Wang, X. 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO₂? A critical examination of the hypotheses. *J. Integr. Plant Biol.* **50**: 1365–1374.
- Taylor, G., Tallis, M.J., Giardina, C.P., Percy, K.E., Miglietta, F., Gupta, P.S., et al. . 2008. Future atmospheric CO₂ leads to delayed autumnal senescence. *Glob. Change Biol.* **14**: 264–275.
- Tuchman, N.C., Wahtera, K.A., Wetzel, R.G., and Teeri, J.A. 2003. Elevated atmospheric CO₂ alters leaf litter quality for stream ecosystems: an in situ leaf decomposition study. *Hydrobiologia.* **495**: 203–211.
- Tuchman, N.C., Wetzel, R.G., Rier, S.T., Wahtera, K.A., and Teeri, J.A. 2002. Elevated atmospheric CO₂ lowers leaf litter nutritional quality for stream ecosystem food webs. *Glob. Change Biol.* **8**: 163–170.
- Urbina, I., Sardans, J., Beierkuhnlein, C., Jentsch, A., and Backhaus, S. 2015. Shifts in the elemental composition of plants during a very severe drought. *Environ. Exp. Bot.* **111**: 63–73.
- Walter, J., Hein, R., Auge, H., Beierkuhnlein, C., Löffler, S., Reifenrath, K., Shcädler, M., Weber, M., and Jentsch, A. 2012. How do extreme drought and plant community composition affect host plant metabolites and herbivore performance? *Arthropod-Plant Interactions.* **6**: 15–25.
- Woodward, G., Perkins, D. M., & Brown, L. E. 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philos. Trans. R. Soc., B.* **365**: 2093-2106.
- Xu, Z., Shimizu, H., Yagasaki, Y., Ito, S., Zheng, Y., and Zhou, G. 2013. Interactive Effects of Elevated CO₂, Drought, and Warming on Plants. *J. Plant Growth Regul.* **32**: 692–707.
- Zar, JH. 2010. *Biostatistical Analysis*. 5th ed. Pearson Prentice Hall, Upper Saddle River.

CHAPTER 3

Temperature sensitivity of leaf litter decomposition in headwater forested streams: potential role of litter quality and stream environmental characteristics

Abstract

Ongoing climate change and anthropogenic activities are expected to increase water temperature and strongly compromise ecosystem processes due to temperature influence on chemical reactions and biological activities of organisms that support them. The effects on plant litter decomposition in streams are still unclear because drivers other than the direct temperature effect on metabolic rate can influence the temperature sensitivity of this process.

We examined and quantified (based on theory metabolic of ecology) the temperature sensitivity of microbial-mediated decomposition and associated functional variables (i.e. respiration, fungal growth, leaf-nutrient changes) of two leaf litter species contrasting in quality and explored the influence of the thermal histories of stream communities and water characteristics on this response. Alder (*Alnus glutinosa*) and eucalypt (*Eucalyptus globulus*) litter discs were incubated in three headwater forested streams differing in the autumn-winter mean water temperature (range 4.6- 8.9 °C). Simultaneously, in laboratory, leaf discs microbially conditioned in these streams were incubated at 5, 10 and 15 °C in their respective waters (stream water) as well as in a common water from fourth stream (control). Litter decomposition increased with temperature, but the response in the field was less predictable and depended on litter species. Alder and eucalypt litter showed similar temperature sensitivity i.e. a similar activation energy. The response of decomposition rate to temperature increase was consistent across streams. Microbial activities from streams with different thermal regimes responded similarly to temperature. Although water dissimilarities did play a role, the effect of temperature was always more clear and relevant for the responses observed. The patterns of respiration, fungal growth and nutrient change were less clear and fitted worse. Our results suggest that leaf processing of contrasting quality could be similarly affected by temperature rises and microbial communities adapted to different thermal regimen could respond similarly.

Keywords: climate change, activation energy, temperature sensitivity, leaf chemistry, fungi

Introduction

Global mean air temperature is predicted to increase by 1.5 - 4.6 °C by the end of the 21st century (IPCC 2013) and it is expected to be translated into increasing in water temperature of streams due to their correlation (Langan *et al.* 2001; Kaushal *et al.* 2010; Molinero *et al.* 2016). In addition, increasing in stream water temperature could be also promoted by anthropogenic activities such as riparian-canopy removal for agricultural or forest practices (Kominoski *et al.* 2013) or damming and water abstraction (Poff & Zimmerman 2010). Temperature is an important factor influencing chemical reactions and metabolic rates of organisms (Gillooly *et al.* 2001; Brown *et al.* 2004). Therefore, temperature increases can strongly compromise ecosystem processes in freshwater systems (Tank *et al.* 2010).

Plant litter decomposition in streams is a pivotal ecosystem-level process since it constitutes a major pathway of energy transfer across food webs and nutrient recycling in these

systems (Wallace *et al.* 1997; Perkins *et al.* 2010; Tank *et al.* 2010). This is particularly important in forested streams (Vannote *et al.* 1980; Wallace *et al.* 1997) in which primary production is limited by riparian shading (Vannote *et al.* 1980; Acuña *et al.* 2005) and the primary source of organic matter consist of leaf litter from the terrestrial ecosystem (Pozo *et al.* 1997). Litter decomposition is a complex process that involves physical fragmentation, leaching of soluble compounds, and degradation by microbial decomposers and by invertebrate consumers (Abelho 2001; Graça 2001; Tank *et al.* 2010). These abiotic and biotic processes are governed by an array of both intrinsic (e.g. litter quality) and extrinsic (e.g. temperature, nutrient availability) factors (Tank *et al.*, 2010) that ultimately determine the rate of conversion of plant litter to inorganic forms (mineralization) or its storage in the long term as other organic forms. Microbial decomposers, mainly fungi, are primary actors in the leaf litter decomposition (Pascoal & Cássio 2004). They convert organic matter into new fungal biomass and inorganic compounds (Gessner & Chauvet 1994; Gessner 1999) and make detritus more palatable food resource (microbial conditioning) for detritivores (Pascoal & Cássio 2004; Gessner *et al.* 2007). All these characteristics make litter decomposition to be sensitive to many anthropogenic impacts, among them, the temperature.

As litter decomposition is a primarily biological process, litter decomposition could be highly sensitive to increasing temperature through its effect on biota (Taylor & Chauvet 2014; Ferreira, Chauvet & Canhoto 2015). Temperature influences the metabolic rates of organisms, their physiology and biological activities (Friberg *et al.* 2009; Bergfur & Friberg 2011; Perkins *et al.* 2012; Fernandes *et al.* 2014; Canhoto, Gonçalves & Bärlocher 2016), as well as their inter- or intraspecific relationships and community structure (Dang *et al.* 2009; Ferreira & Chauvet 2011a; Duarte *et al.* 2013; Martínez *et al.* 2014). Therefore, decomposition rate could be partially determined by the response of these actors to temperature increase in addition to by directly promoting leaching of soluble compounds (Chergui & Pattee 1990).

However, the temperature effects on functional indicators such as decomposition rate is still unclear because drivers other than the direct temperature effect on metabolic rate can influence the temperature sensitivity of these processes (Follstad Shah *et al.* 2017). Some processes might need that temperature increases surpass certain threshold to make observable its effects whereas effects would go unnoticed if that threshold is exceeded (Dang *et al.* 2009; Bergfur & Friberg 2011; Ferreira, Encalada & Graça 2012; Geraldés, Pascoal & Cássio 2012; Canhoto *et al.* 2016), which would be related to temperature tolerance range of microbial assemblages. Litter chemistry is another important factor influencing activities of organisms and organic matter decomposition in freshwater ecosystems (García-Palacios *et al.* 2016; Boyero *et al.* 2016). Litter with a higher nutrient concentration and a lower content of structural and secondary compounds (labile substrate) is preferably colonized and decomposes faster than

recalcitrant litter (Hladyz *et al.* 2009). Evidence from terrestrial ecosystem suggests that the quality of substrate may modulate the temperature sensitivity of its decomposition and low-quality substrates (high carbon to nutrient ratio, structurally complex C compounds) may be more sensitive to temperature increase than high-quality ones (Fierer *et al.* 2005; Conant *et al.* 2008, 2011; Wetterstedt, Peterson & Agren 2010). It is likely to be because microbial enzymatic reactions require a higher net activation energy to metabolise more recalcitrant substrates (Fierer *et al.* 2005). In aquatic ecosystems, studies addressing this issue are scarce and contradictory, since temperature sensitivity of microbial decomposition seems not to be affected by organic-matter quality (Sand-Jensen, Pedersen & Søndergaard 2007; Ferreira & Chauvet 2011b) or is inversely related to lability of substrate (Fernandes *et al.* 2012; Bärlocher *et al.* 2013; Gonçalves, Graça & Canhoto 2013). In addition, temperature sensitivity of litter decomposition may also be influenced by the trophic status of streams, and result in different sensitivity depending on nutrient concentration in water and the range of temperature increase (Ferreira & Chauvet 2011a; Gonçalves *et al.* 2013; Fernandes *et al.* 2014).

It is thus critical to understand how temperature and its interaction with other factors govern plant litter decomposition and functional processes associated with it, as well as quantify their temperature sensitivity to anticipate future consequences of temperature increases under future global-change scenarios.

The aim of this study was to assess the temperature sensitivity of microbial-mediated decomposition as well as the associated functional variables (i.e. respiration, fungal growth, leaf-nutrient changes) of leaf litter species contrasting in quality and to explore whether the thermal histories of stream communities influence this response. We also wanted to quantify the apparent temperature sensitivity of the measured functional variables by the Metabolic Theory of Ecology approach (Brown *et al.* 2004). We performed a decomposition experiment with leaf litter of the high-quality *Alnus glutinosa* (L.) Gaertner (alder), and the exotic low-quality *Eucalyptus globulus* Labill (eucalypt), in three headwater forested streams that differed in the autumn-winter mean water temperature (range from 4.6 to 8.9 °C). We also explore these issues in a simultaneous laboratory experiment to isolate temperature effect from potentially confounding local environmental factors. For this purpose, laboratory incubations were carried out in water from the respective stream and in water from an additional stream used as control to rule out differences in water physicochemical characteristics of streams (hereafter, stream water and control water, respectively). We hypothesised that (1) as biological activities are temperature-dependent, litter microbial decomposition rate will increase with water temperature, (2) low-quality detritus will decompose slower because it can limit microbial growth and activities, but the stimulation of the decomposition by the temperature will be stronger than that of high-quality detritus because of higher sensitive to temperature of low-

quality substrates, (3) the litter decomposition-temperature relationship will be influenced by microbial assemblages adapted to different thermal regimes, (4) according to metabolic theory of ecology (MTE) the apparent activation energy of litter decomposition will be close to the inherent activation energy of 0.65 eV, but as detritus quality appears to influence the temperature sensitivity, low-quality litter will show higher activation energy than high-quality one, (5) the relationship between litter decomposition rate and temperature will be more clear in the laboratory, as other factors (e.g. discharge patterns, chemical characteristics...) are also driving this process in the field, and (6) respiration, fungal growth and the change of nutrients will mimic the patterns observed for decomposition rate.

Methods

Study sites and stream water

For the study, we selected four headwater streams with siliceous substrate located in northern Spain (Cordillera Cantábrica; Table 1). Three of them (S1, S2 and S3) were used in field and laboratory experiment whereas one of them (Control) was only used in the laboratory experiment.

The field experiment was conducted in three headwater streams that differed in their daily mean water temperature (ranging from 4.5 to 8.9 °C; Table 1), which was recorded hourly with ACR Smart-Button temperature loggers (ACR Systems Inc., Surrey, BC, Canada). The three streams drain forested watersheds: La Calzada (S1) run through *Fagus sylvatica* L. forest, and Peñaranda (S2) and Peñalar (S3) through mixed deciduous forest dominated by *Quercus robur* L., although some upland areas of the S3 catchment is occupied by *Eucalyptus globulus* Labill plantations.

During the study period (from 4th December 2013 to 29th January 2014), water physico-chemistry was characterised on 6 occasions. Each time, conductivity, pH and oxygen saturation were measured with a multiparametric sensor (WTW Multi 350i; Weilheim, Germany), and discharge was estimated from instantaneous water velocity measured by a current meter (Martin Marten Z30, Current Meter). Water samples were collected at all streams on each sampling date for nutrient analyses. In laboratory, water samples were filtered (0.70 µm pore- size glass fibre filters, Whatman GF/F). Nitrate concentration was determined by capillary ion electrophoresis (Agilent CE, Agilent Technologies, Waldbronn, Germany), and the rest of the nutrients were analysed colorimetrically: nitrite by the sulphanilamide method, ammonium by the salicylate method and dissolved reactive phosphorus (SRP) by the molybdate method (APHA 2005).

Leaves colonization and processing in streams

In November 2013, leaves of two species contrasting in quality, *A. glutinosa* and *E. globulus*, were collected from the forest floor following natural abscission (Agüera basin; on the border line of Cantabria and Basque Country, northern Spain, 43°12' 50" N, 3°16'10" W). The leaves were punched out with a cork borer (20 mm diameter) and air dried. Approximately 1.0 g (± 0.1 g) of air-dried leaf-discs were enclosed in fine mesh bags (12 \times 15 cm, 0.5 mm mesh size) and deployed in the three streams on 4th December 2013. At each site, 4 iron bars were anchored randomly to the streambed in riffle sections and 8 bags (2 species \times 4 sampling) were tied to each bar by nylon lines, making a total of 32 bags per site. After 8, 22, 36 and 56 days of incubation, four bags per stream and species were removed and transported to the laboratory. Leaf discs were rinsed with filtered stream water (100 μ m) over a 0.5-mm mesh sieve to remove sediments. For each bag, a set of four leaf discs was punched out with a cork borer (12 mm diameter) for respiration measurements and another set of three discs was frozen at -80 °C for later fungal biomass determination. The rest of the remaining material was oven-dried (70 °C, 72 h) and weighed to determine leaf dry mass. A portion of leaf material from each bag and species was stored (-20 °C) for elemental analysis (C, N, P) and the rest was combusted (500 °C, 12 h) and weighed to determine the remaining ash-free dry mass (AFDM).

42 extra bags per species and stream were tied to additional bars for microbial conditioning of leaf discs during 8 days that were later used for the laboratory experiment (see below). In addition, four extra bags per species and stream were incubated to estimate the mass lost, C:N:P, respiration rates and fungal biomass during this conditioning time.

Microcosms assay

Within a controlled-temperature room at 10°C, three 36-L tanks were set up as water bath at each experimental temperature (5, 10 and 15 °C), making a total of 9 tanks (one tank per temperature and stream). A recirculating cooler (HL-160CA) was used to cool water of the tanks to 5 °C and a heater circulator (Julabo EH-17) to heat up water temperature of the tanks to 15 °C. Each tank contained 28 microcosms (14 microcosms for alder and 14 for eucalypt). Each microcosm consisted of glass jars containing 200 mL of filtered stream water (0.7 μ m) from the studied streams (S1, S2 or S3) or from an additional stream (S4) used as control to rule out differences in water physicochemical characteristic of the three streams. Therefore, each tank contained 4 replicates with stream water and 10 replicates with control water for each litter species. The previously conditioned leaf discs (see above) were added to microcosms, the discs of one bag to each microcosm. Microcosms were constantly aerated under a light/dark regime of 12:12 hours. The experiment run for 50 days and the water was renewed every 4 days. After 50 days, four microcosms from each litter species and temperature treatment incubated with water from each respective stream and another four with control water were sampled. Additionally,

after 6 and 27 days, three microcosms from each litter species and temperature treatment incubated with water from control stream were sampled. At each sampling, the remaining ash-free dry mass and C:N:P were determined (see above). A set of three leaf discs was punched out with a cork borer (12 mm diameter) for respiration measurements and another set of discs was at $-80\text{ }^{\circ}\text{C}$ for later fungal biomass determination.

Leaf-litter stoichiometry

Carbon (C) and nitrogen (N) concentrations in the leaf materials were determined with a Perkin Elmer II CHNS/O elemental analyser, and phosphorus (P) was measured after acid digestion using a spectrophotometer by molybdenum blue method (Allen *et al.* 1974). Results were expressed as a percentage of leaf dry mass (% C, % N, % P).

Fungal biomass

The sets of leaf disks were freeze-dried and weighed ($\pm 0.1\text{ mg}$) to determine disc dry mass and later to determine ergosterol concentration as a measure of mycelial biomass (Gessner & Chauvet 1993). Lipid extraction and saponification were performed using KOH/methanol (8 g L^{-1}) at $80\text{ }^{\circ}\text{C}$ for 30 min in a shaking bath. Extracted lipids were then purified by solid-phase extraction (Oasis HLB cartridge, barrel size 3 cc, particle size $30\text{ }\mu\text{m}$, pore size 80 \AA ; Waters Corp., Massachusetts, USA). Ergosterol was quantified by HPLC (Dionex DX-120, Sunnyvale, California, USA) by measuring absorbance at 282 nm. The HPLC detector was equipped with the Thermo Scientific Synchronis C18 ($250 \times 4\text{ mm}$, $5\text{ }\mu\text{m}$ particle size) column (Thermo, Waltham, MA USA) and the Thermo Universal Uniguard holder for 4/4.6 mm ID3 + Synchronis C18 ($10 \times 4\text{ mm}$, $5\text{ }\mu\text{m}$ particle size) drop in guard precolumn (Thermo, Waltham, MA USA), maintained at $33\text{ }^{\circ}\text{C}$. The mobile phase was 100% methanol, flowing at 1.4 mL min^{-1} . Ergosterol was detected at $33\text{ }^{\circ}\text{C}$ and converted into mycelial biomass assuming $5.5\text{ }\mu\text{g ergosterol mg}^{-1}$ mycelial DM (Gessner & Chauvet 1993). The results were expressed as $\text{mg fungal biomass g}^{-1}$ leaf litter DM.

Respiration

The microbial oxygen consumption rates were measured using a closed dissolved oxygen measurement system (Strathkelvin 928 System, North Lanarkshire, Scotland). Leaf discs were incubated in chambers with 3 mL 100% dissolved O_2 saturated filtered stream water (at $10\text{ }^{\circ}\text{C}$, 40 min). An extra chamber without discs was used as control for respiration of the water. Oxygen consumption rates were determined by the difference in the oxygen concentration in the sample and the control over a 20-min interval and were corrected for the time and disc mass (DM determination). The results were expressed as $\text{mg O}_2\text{ g}^{-1}\text{ DM h}^{-1}$.

Data analysis

Comparisons for water temperature during the experimental period were performed with one-way ANOVA (factor: stream) considering mean daily temperature ($n = 174$) as replicate. Differences in other physicochemical characteristics were analysed by one-way ANOVA with stream as factor. Pairwise comparisons were performed by Tukey's HSD test (Zar 2010).

The first sampling (after 8 days of incubation allowing microbial conditioning) was used for estimating initial values of the different variables and as date of starting both field and laboratory experiments. In doing this we do not consider the part in which the organic matter processing is less mediated by the biota and more by physicochemical drivers as the leaching (Abelho 2001). Decomposition rates of alder and eucalypt were calculated assuming an exponential model, $M_t = M_o \times e^{-kt}$ (where k is the decomposition rate, M_o is the initial mass, M_t is the remaining final mass -%AFDM-, and t is the time). Using this equation, a k value was calculated for each replicate assuming an initial value of 100. Separate analyses were performed for field and laboratory experiments. Average water temperature slightly changed from one sampling to the next and this allowed us to have a range of values for the field experiment. Therefore, we used field experiment data to test if the relationship of the variables with temperature were linear or non-linear. To do this we constructed first order (linear) and second order (quadratic) models and compared the overall fit by using Akaike's Information Criterion (AIC) and tested the significance of the first and second order coefficients using ANOVA. In the laboratory, materials were incubated at three temperatures (5, 10 and 15°C) and we did not test for the non-linearity and only fitted linear models. We fitted all the replicates from the laboratory experiment in a single model per variable by means of linear mixed models (LME) using *lme4* package from R software (Bates *et al.* 2015). To consider the correlation among samples and to deal with the non-linear temporal pattern of some variables the sampling was adjusted as a random factor in all analyses. Leaf species (alder vs eucalypt), site where materials were conditioned (S1 vs S2 vs S3) and water used (stream water vs control water) were all included as fixed sources of variation. The temperature was included in all analyses as a covariate. We fitted maximal models (those including all 3 factors and the temperature, and all the interactions among them) and proceed to model simplification (Crawley 2007) sequentially removing non-significant sources of variation starting from the most complex interaction terms (4-way interaction in our analyses). We used restricted maximum likelihood (REML) to estimate the components of the variance, and P values and d.f were estimated with log likelihood ratio tests (Pinheiro & Bates 2000).

We also performed mixed-model analyses to explore the data based on the Metabolic Theory of Ecology (MTE), which allows assessing the relationship between temperature and biological activities in quantitative terms (Brown *et al.* 2004; Griffiths & Tiegs 2016; Follstad Shah *et al.* 2017). The MTE describes the temperature sensitivity as the slope (in eV) of the natural logarithm of biological activity (in our case litter decomposition, respiration, fungal growth, litter nutrient change) vs the inverse of the product of the absolute temperature in degrees Kelvin (K) and Boltzmann constant (8.617×10^{-5} eV K⁻¹) (Brown *et al.* 2004). Litter decomposition and fungal biomass were converted to rates before analyses. Respiration was used directly in the analyses because its measure is already a rate. Fungal biomass was converted to growth rate, r , by means of an exponential equation $FB_t = FB_o \times e^{rt}$, in which initial and final fungal biomass (FB) enable the computation of the growth rate, r . Fungal biomass at day 8 was considered the initial amount and different values were considered for alder and eucalypt, as they displayed significantly different fungal biomasses. We realize that fungal biomass does not follow an exponential pattern when studying its amount along the decomposition (Pozo *et al.* 1998; Ferreira & Chauvet 2011b). Nevertheless, we think it is a valid approach, as microbial growth can be modelled with an exponential function (Suberkropp 2001; Artigas *et al.* 2011) and fungal biomass accumulates as microorganism growth. Positive rates for decomposition and respiration allowed Ln transformation. For the rate of increase of fungal biomass were obtained negative values and non-transformed data were used. We calculated MTE slopes and 95% confidence intervals for each species and treatment to examine whether the temperature dependence (slope) varied among all studied cases.

All statistical analyses were conducted using R statistical software version 3.2.2 (R Development Core Team 2015).

Results

Stream water characteristics

Water temperature ranged approximately 4 °C from the coldest to the warmest stream (Table 1) and differed significantly among the three studied streams (ANOVA: $F_{2, 174} = 195.96$, $p < 0.0001$). The four streams presented circum-neutral pH, low mineralisation and well oxygenated waters (Table 1). SRP concentration was low but it was higher in control stream than in S1 and S2 (Table 1; ANOVA: $F_{3,19} = 4.54$, $p = 0.01$). Nitrate concentration differed among streams (Table 1; ANOVA: $F_{3,19} = 7.63$, $p = 0.002$), with S3 presenting higher values than the other streams.

Litter decomposition

The decomposition rates after incubation in the field for 48 days (the first 8 days eliminated from the analysis, see above) ranged from 0.0063 d⁻¹ at the coldest stream (S1) to

0.0094 d⁻¹ in the warmest one (S3) for alder litter, whereas for eucalypt, the highest rate, 0.0043 d⁻¹, was observed at the stream with intermediate temperature (S2) and the lowest, 0.0023 d⁻¹, at the warmest stream (S3) (Fig. 1). Eucalypt decomposition was slower than that of alder (Fig. 1; Table 2). The decomposition rate of alder increased with temperature, but eucalypt decomposition was lowered at higher temperature (Fig. 1; Table 2), with the quadratic equation describing better the relationship between temperature and decomposition rate than a linear one (Fig. S1; Table S1).

Table 1. Location and physicochemical characteristics (mean \pm SE) of the selected streams (n=6) and the control stream (n=5) during the study period (4th December 2013- 29th January 2014). For water temperature, daily mean values (n = 174) and their range (in parenthesis) are shown. Superscripts indicate among-stream differences (Tukey's HSD, $p < 0.05$).

	S1	S2	S3	Control
Latitude (N)	43°07'05''	43°10'01''	43°18'33''	43°13'01''
Longitude (W)	03°25'59''	03°21'31''	03°15'36''	03°16'15''
Altitude (ma. s. l)	960	460	100	265
Catchment area (km ²)	1.05	3.60	1.89	8.01
Water temperature (°C)	4.6 (0.0-7.5) ^a	6.7 (4.0-10.3) ^b	8.9 (6.0-11.8) ^c	-
Discharge (L s ⁻¹)	105.9 \pm 70.9 ^a	206.7 \pm 167.3 ^a	104.1 \pm 98.0 ^a	-
pH	7.53 \pm 0.46 ^a	7.05 \pm 0.65 ^a	7.27 \pm 0.36 ^a	7.32 \pm 0.48 ^a
Conductivity (μ S cm ⁻¹)	44.0 \pm 11.2 ^a	79.7 \pm 11.1 ^b	70.2 \pm 9.9 ^b	103.6 \pm 27.1 ^b
Oxygen saturation (%)	102.9 \pm 3.6 ^a	100.6 \pm 2.9 ^a	106.4 \pm 4.61 ^a	101.0 \pm 6.29 ^a
NO ₃ -N (μ g N L ⁻¹)	284.4 \pm 43.9 ^a	250.6 \pm 213.5 ^a	632.8 \pm 114.3 ^b	427.3 \pm 191.6 ^{ab}
NO ₂ -N (μ g N L ⁻¹)	1.82 \pm 0.58 ^a	2.37 \pm 1.15 ^a	2.19 \pm 0.89 ^a	2.65 \pm 1.3 ^a
NH ₄ -N (μ g N L ⁻¹)	20.2 \pm 7.1 ^a	14.15 \pm 6.02 ^a	17.3 \pm 8.58 ^a	21.39 \pm 7.31 ^a
SRP (μ g P L ⁻¹)	1.75 \pm 0.24 ^a	1.48 \pm 0.88 ^a	2.67 \pm 1.23 ^{ab}	4.16 \pm 2.29 ^b

In the laboratory, the decomposition rate of eucalypt was again lower than that of alder and its rates were more variable than those for alder (Fig. 1 and Fig. S1b, c; Table 2), but in general, no significant effect of water was detected on decomposition rate (Table 2 and Table S1). The origin stream of the microbiota influenced statistically the decomposition rate, particularly that of eucalypt litter, but it also depended on type of water (Fig. 1 and Fig. S1b, c; Table 2). The rate of the two species in the incubations carried out with control and stream water increased with temperature independently of the origin of the microbiota (Fig. 1 and Fig. S1b, c; Table 2), but temperature \times leaf interaction was significant (Table 2), with alder litter showing higher rates with temperature (Fig. 1 and Fig. S1b, c). The apparent sensitivity to temperature (expressed here as the activation energy, E_a) of the decomposition rates for alder in the field was -0.71 eV, but for eucalypt was positive, i.e. higher temperatures were related to an inhibition of the rate (Fig. 6). Nevertheless, in the laboratory experiment, the values for the

activation energy for alder and eucalypt were all negative as expected (positive relationship between temperature and rate) and averaged -0.48 eV taking into account all treatments, with average values of -0.52 eV in stream water and -0.47 eV in control water for alder, and, -0.44 eV and -0.49 eV for eucalypt respectively. However, the E_a was not significantly different among treatments nor they were not significantly different from the predicted activation energy of -0.65 eV stated in the literature (Fig. 6).

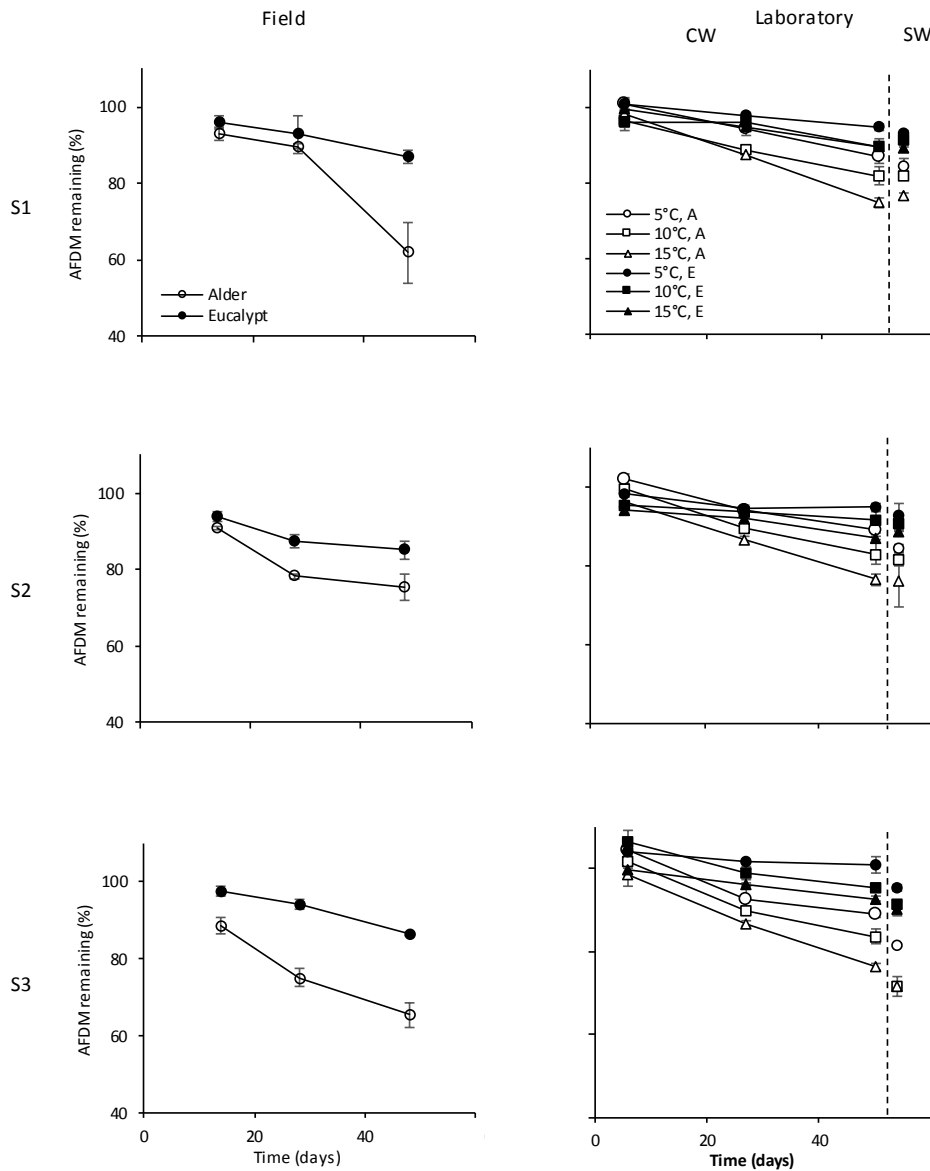


Fig. 1. AFDM remaining (%) in alder (white; A) and eucalypt (black; E) leaf discs incubated in the field (left) and in the laboratory (right) at 5°C (circle), 10°C (square) and 15°C (triangle). In the laboratory experiment, the points to the left of the dashed line show the AFDM remaining dynamic in leaf discs incubated in control water (CW) and, the right ones show the AFDM remaining dynamic in leaf discs incubated in stream water (SW) at the end of the experiment. S1, coldest stream; S2, intermediate stream; S3, warmest stream. Mean \pm ES.

Table 2. Results of linear models performed on leaf decomposition, respiration, fungal biomass, and leaf nutrients change (% N, % P) associated with alder and eucalypt leaf discs incubated in the field and laboratory. Models have been simplified and significant interactions removed (see methods). Site = S1, S2 and S3; Leaf = alder, eucalypt; Water = control water or stream water. Significant values are highlighted in bold.

	Df	F value	p-value
Leaf decomposition-Field			
Temperature	1	3.45	0.0881
Leaf	1	45.77	< 0.0001
Temperature:Leaf	1	6.34	0.0113
Leaf decomposition-Laboratory			
Temperature	1	47.40	< 0.0001
Leaf	1	22.99	< 0.0001
Site	2	10.86	< 0.0001
Water	1	0.84	0.3773
Temperature:Leaf	1	5.43	0.0249
Leaf:Site	2	6.75	0.0020
Site:Water	2	6.54	0.0018
Respiration-Field			
Temperature	1	0.01	0.7950
Leaf	1	136.35	< 0.0001
Temperature:Leaf	1	4.57	0.0304
Respiration-Laboratory			
Temperature	1	39.27	< 0.0001
Leaf	1	626.02	< 0.0001
Site	2	0.91	0.4640
Water	1	0.31	0.6032
Temperature:Leaf	1	15.00	< 0.0001
Temperature:Site	2	0.34	0.7319
Leaf:Site	2	3.14	0.0568
Temperature:Water	1	0.30	0.5768
Leaf:Water	1	2.23	0.1379
Site:Water	2	3.40	0.0412
Leaf:Site:Water	2	3.13	0.0457

(Continue in the next page)

	Df	F value	p-value
Fungal biomass-Field			
Temperature	1	2.45	0.1209
Leaf	1	41.74	< 0.0001
Temperature:Leaf	1	1.28	0.2462
Fungal biomass-Laboratory			
Temperature	1	4.71	0.0231
Leaf	1	200.29	< 0.0001
Site	2	1.48	0.2355
Water	1	8.94	0.0059
Temperature:Site	2	5.70	0.0039
% Nitrogen-Field			
Temperature	1	1.74	0.3650
Leaf	1	915.76	< 0.0001
Temperature:Leaf	1	2.26	0.1301
% Nitrogen-Laboratory			
Leaf	1	4213.50	< 0.0001
% Phosphorus-Field			
Temperature	1	0.02	0.8130
Leaf	1	49.00	< 0.0001
Temperature:Leaf	1	8.69	0.0036
% Phosphorus-Laboratory			
Temperature	1	0.01	0.6544
Leaf	1	376.97	< 0.0001
Site	2	0.41	0.7197
Water	1	23.18	< 0.0001
Temperature:Leaf	1	5.13	0.0403
Temperature:Site	2	0.75	0.4248
Leaf:Site	2	6.48	0.0037
Temperature:Water	1	5.95	0.0213
Leaf:Water	1	0.42	0.5450
Site:Water	2	2.93	0.0646
Temperature:Site:Water	2	7.61	< 0.0001

Respiration

In the leaf discs from the field, respiration rate was lower for eucalypt than alder leaf (on average, 0.12 vs 0.26 mg O₂ g⁻¹ DM h⁻¹, respectively; Fig. 2 and Fig. S1d; Table 2). The respiration rates of both species were unrelated to water temperature (Table 2), but for eucalypt it tended to be lower at the lowest temperature (Fig. 2 and Fig. S1d; Table 2). In the laboratory experiment, the respiration in the eucalypt discs was also lower than in the alder discs (on average, 0.10 vs 0.28 mg O₂ g⁻¹ DM h⁻¹, respectively; Fig. 2 and Fig. S1e and f; Table 2), and was significantly higher in eucalypt discs from S3 incubated in their respective stream water (Fig. 2 and Fig. S1f; Table 2). No significant differences were found between stream and control water nor among origin site of leaf discs (Fig. 2; Table 2). In all cases, there was a negative relationship between the respiration rate and the temperature, that is, leaf discs incubated at 5 °C respired more than discs incubated at 15 °C when the respiration was measured at standard temperature of 10 °C (Fig. 2 and Fig. S1e and f; Table 2 and Table S1), which was higher for alder species (Table 2; Fig S1e and f). The apparent activation energy of the respiration rates in the field was not significant statistically ($p > 0.05$). In the laboratory experiment it was on average - 0.27 eV for both leaf species independently of type of water or origin of the microbiota (Fig. 6).

Fungal biomass

In the field, fungal biomass generally increased until a peak was reached, attaining maximum values of 257 mg g⁻¹ DM) on alder leaf and 123 mg g⁻¹ DM on eucalypt at S2, the stream with intermediate temperature (Fig. 3). There was not significant statistically relationship between fungal biomass and temperature (Fig. 3 and Fig. S1g; Table 2). In the laboratory, alder leaf also exhibited higher fungal biomass than eucalypt (Fig.3 and Fig. S1h and i; Table 2). Fungal biomass differed significantly between water types (Fig. 3; Table 2). In general, there was a positive significant relationship between fungal biomass and temperature for both leaf species, but the slope of this relationship was different among origin stream (Fig. 3; Table 2), if we considered raw data, and differed depending on the water used (Fig. S1h and I; Table S1), if we focus on values transformed to rates. The temperature sensitivity of fungal growth was not statistically significant in the field experiment (Fig. 6). In the laboratory experiment the temperature sensitivity showed differences between water types, with leaf discs incubated in control water showing significantly higher sensitivity to temperature than those incubated in stream water (Fig. 6; Table S1).

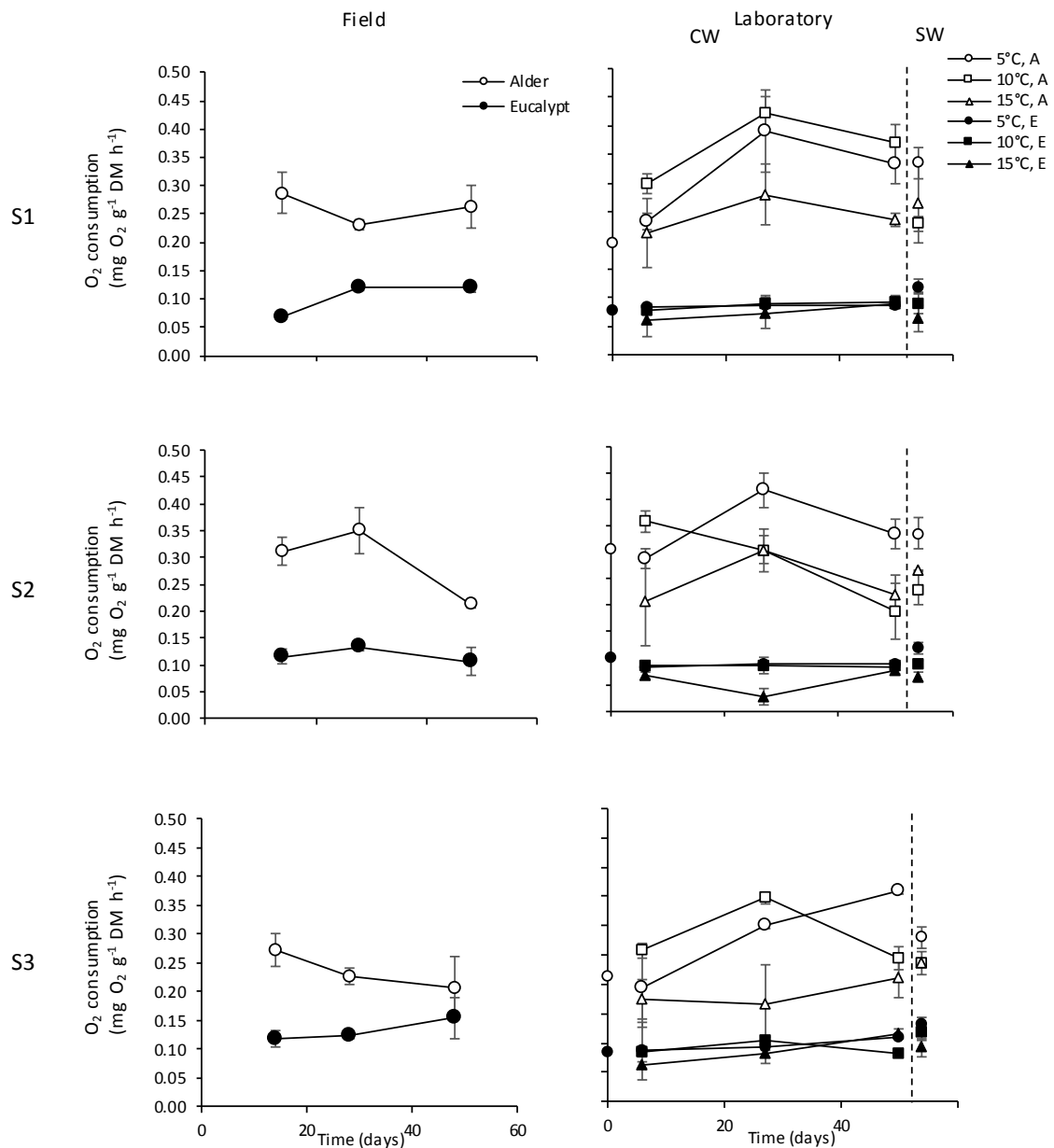


Fig. 2. Oxygen consumption ($\text{mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$) in alder (white; A) and eucalypt (black; E) leaf discs incubated in the field (left) and in the laboratory (right) at 5°C (circle), 10°C (square) and 15°C (triangle). In the laboratory experiment, the points to the left of the dashed line show the oxygen consumption dynamic in leaf discs incubated in control water (CW) and, the right ones show the oxygen consumption in leaf discs incubated in stream water (SW) at the end of the experiment. S1, coldest stream; S2, intermediate stream; S3, warmest stream. Mean \pm ES.

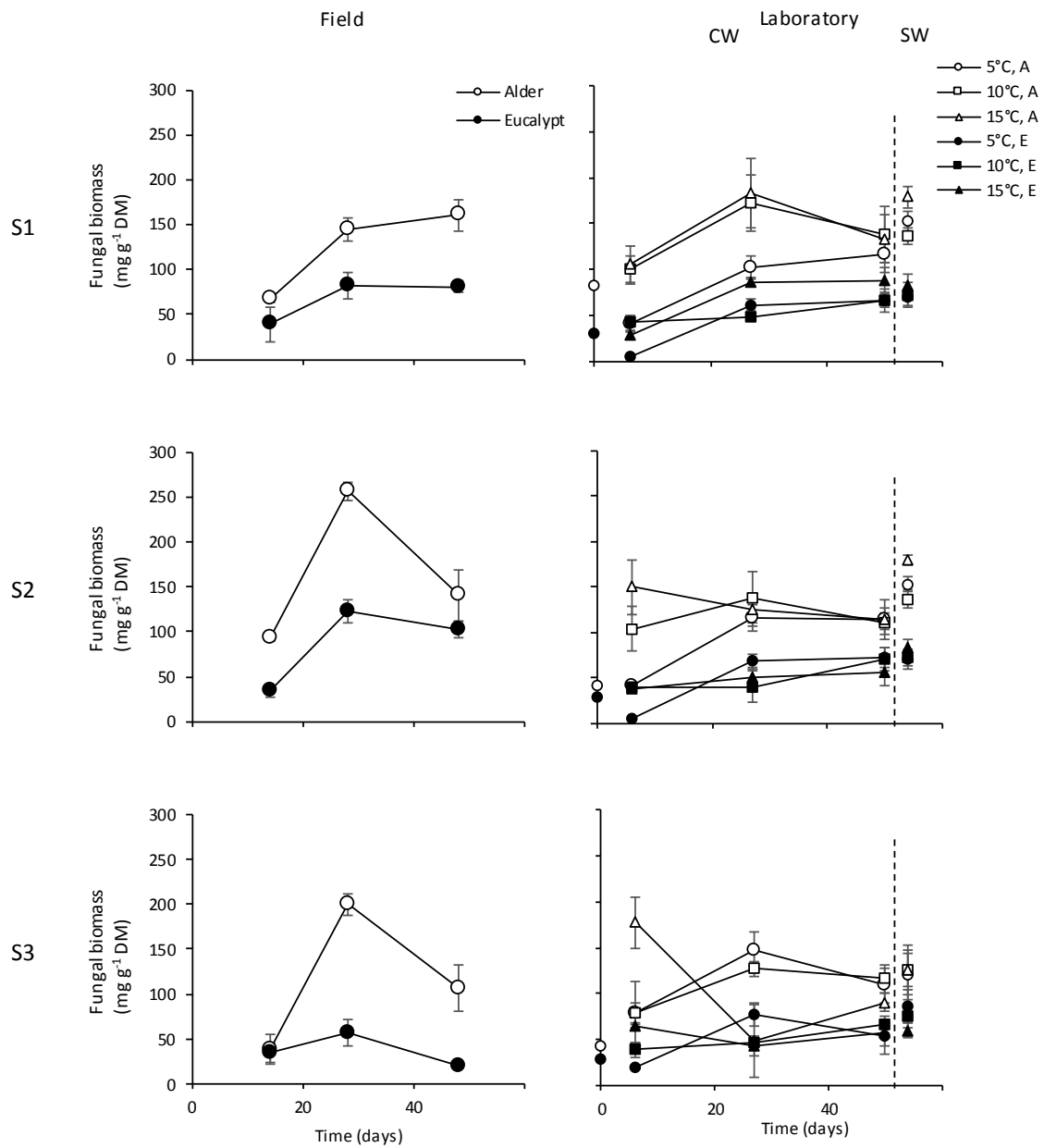


Fig. 3. Fungal biomass (mg g^{-1} DM) in alder (white; A) and eucalypt (black; E) leaf discs incubated in the field (left) and in the laboratory (right) at 5°C (circle), 10°C (square) and 15°C (triangle). In the laboratory experiment, the points to the left of the dashed line show the fungal biomass dynamic on leaf discs incubated in control water (CW) and, the right ones show the fungal biomass on leaf discs incubated in stream water (SW) at the end of the experiment. S1, coldest stream; S2, intermediate stream; S3, warmest stream. Mean \pm ES.

Nitrogen and phosphorus

In the field experiment, % N was significantly different between leaf species with concentrations being on average 3.51 % in alder leaf and 1.55 % in eucalypt leaf (Fig. 4; Table 2). The % P also differed statistically between leaf species, it was on average 0.06 % in alder leaf and 0.05 % in eucalypt leaf (Fig. 5; Table 2). Neither in alder nor in eucalypt leaves nutrient concentration (%N and %P) was significantly related to water temperature (Fig. 4 and 5; Table 2).

As in the field, % N and % P significantly differed between leaf species in the laboratory (Table 2). % N and % P were on average 3.42 % and 0.07 %, respectively, in alder and 1.47 % N and 0.05 % P in eucalypt (Fig. 4 and 5). The temperature increase did not affect the % N nor % P (Fig. 4 and 5; Table 2), but it appeared to depend on type of water for % P (Table 2), with leaf discs incubated in control water being higher (Fig. 5). In general, the % P in eucalypt tend to be more variable among origin streams (Fig. 5; Table 2).

Discussion

Field and laboratory experiments enabled to examine how different factors interact with temperature to influence microbial-mediated decomposition. As expected, microbial decomposition of high-quality species (alder) was faster than that of low-quality leaf (eucalypt) (Canhoto & Graça 1996; Pérez *et al.* 2014) and it was related to differences in microbial colonization and activity (i.e. microbial metabolism, fungal growth and leaf nutrient change - indirect proxy-). It was consistent with the findings of many previous studies about the role played by leaf quality on decomposition and associated biological variables which showed tight relationships between them (Hladyz *et al.* 2009; Bergfur & Friberg 2011). In particular, the slower decomposition of eucalypt litter can be attributed to its low nutrient, high secondary compounds (oils, polyphenols) and waxy cuticle which hinder microbial degradation initially (Canhoto & Graça 1999; Graça *et al.* 2002).

As chemical reactions and biological activities depend on temperature (Brown *et al.* 2004), we expected the decomposition rate of two leaf species, in both field and laboratory approach, to be enhanced by temperature increase. In fact, in our study, the decomposition rate depended strongly on temperature in the laboratory (temp. range: 5-15 °C) what is in agreement with the outcome often reported by other studies carried out in microcosms and natural systems (Boyer *et al.* 2011; Ferreira & Chauvet 2011a; Fernandes *et al.* 2014; Martínez *et al.* 2014; Griffiths & Tiegs 2016). The effect of temperature was not so clearly observed in the field experiment as it was dependent on species, with only alder showing the predicted pattern.

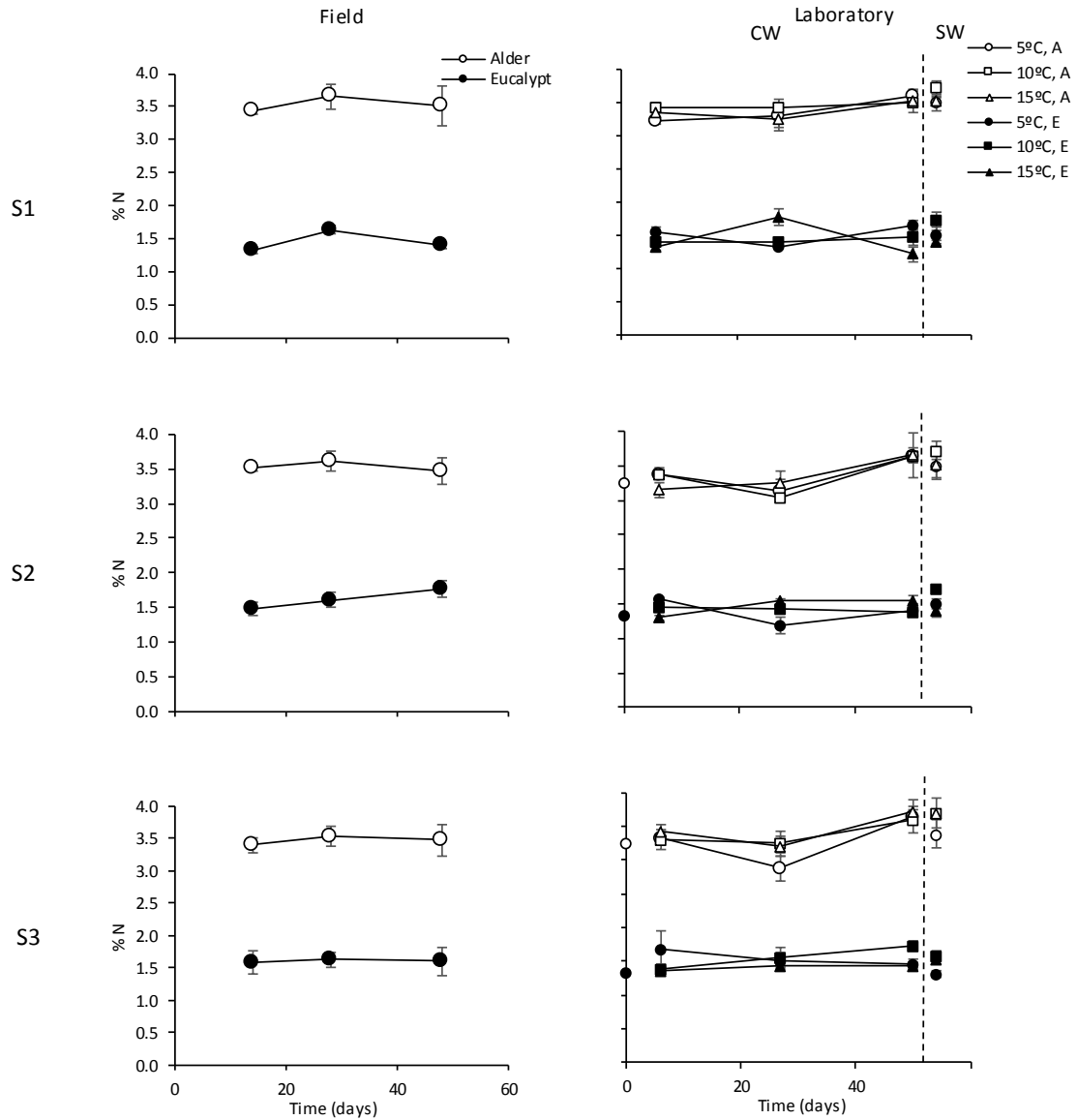


Fig. 4. Nitrogen concentration (% DM) in alder (white; A) and eucalypt (black; E) leaf discs incubated in the field (left) and in the laboratory (right) at 5°C (circle), 10°C (square) and 15°C (triangle). In the laboratory experiment, the points to the left of the dashed line show the N dynamic of leaf discs incubated in control water (CW) and, the right ones show the N of leaf discs incubated in stream water (SW) at the end of the experiment. S1, coldest stream; S2, intermediate stream; S3, warmest stream. Mean \pm ES.

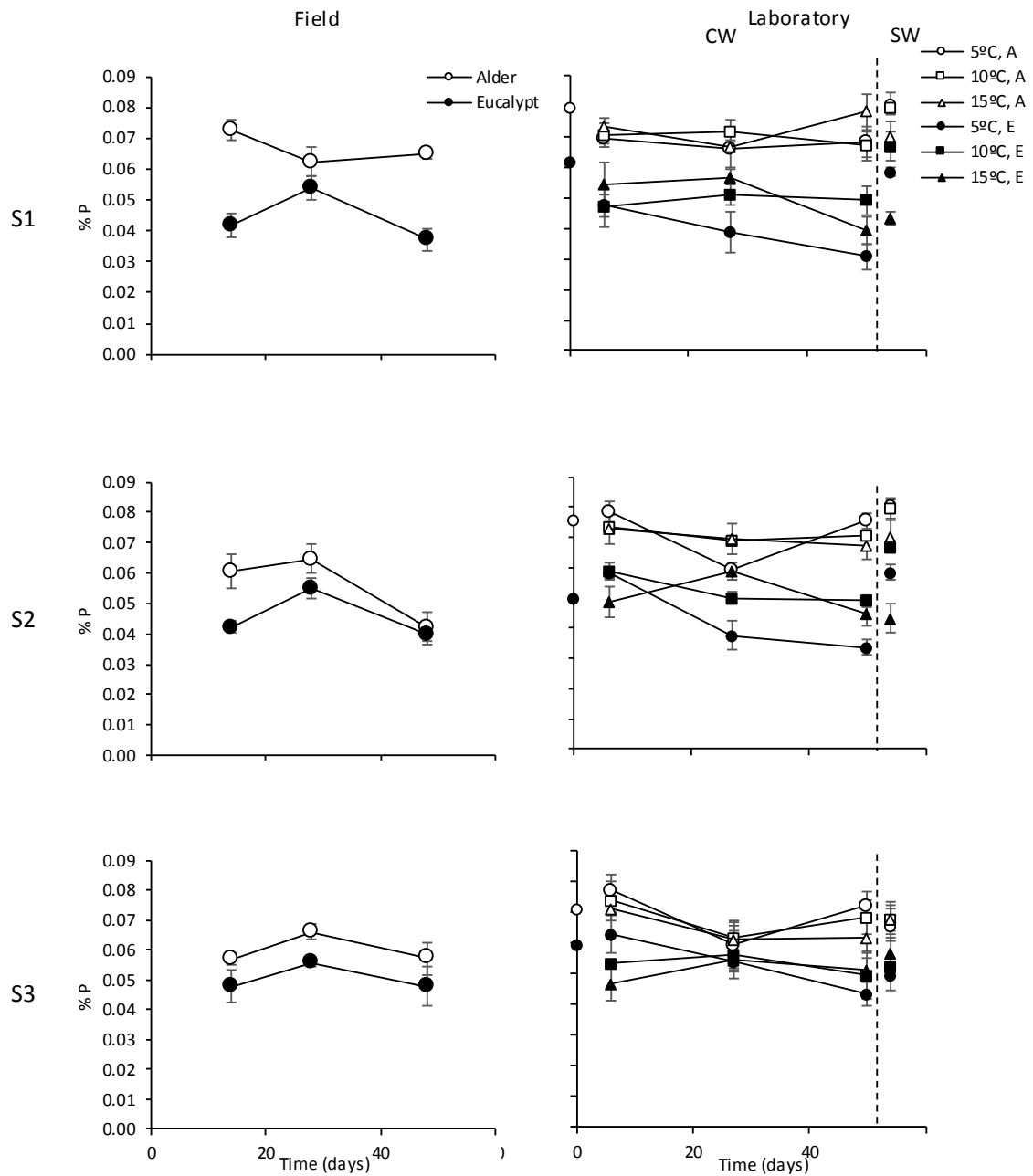


Fig. 5. Phosphorus concentration (% DM) in alder (white; A) and eucalypt (black; E) leaf discs incubated in the field (left) and in the laboratory (right) at 5°C (circle), 10°C (square) and 15°C (triangle). In the laboratory experiment, the points to the left of the dashed line show the P dynamic of leaf discs incubated in control water (CW) and, the right ones show the P of leaf discs incubated in stream water (SW) at the end of the experiment. S1, coldest stream; S2, intermediate stream; S3, warmest stream. Mean \pm ES.

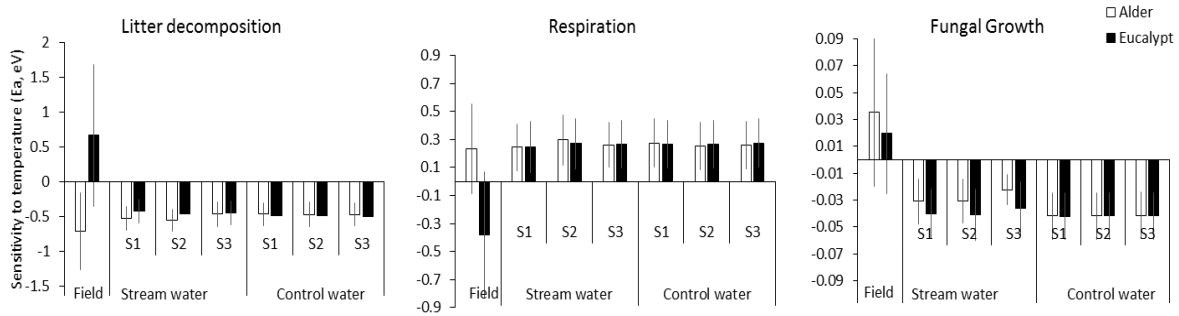


Fig. 6. Sensitivity to temperature (energy of activation, eV) of litter decomposition, respiration and fungal biomass growth for alder (white) and eucalypt (black) in field and laboratory experiment (stream water vs control water). Streams are identified as: S1 (colder), S2 (intermediate), S3 (warmer). Slope \pm Confidence interval. Slope of the relationship between the natural logarithm of rate and water temperature expressed as the inverse of absolute temperature in Kelvin and the Boltzmann constant. Note the different scale. Positive values indicate negative relationship with the temperature.

The lower gradient of temperature in the field (temp. range: 4.6-8.9 °C) could have made difficult to find strong correlations, but the materials incubated in the field also face other factors that are idiosyncratic for each site. In this study, the clear relationship of the decomposition of alder with temperature shows that the capacity to process organic matter of the warmest site follows the expected pattern, but this stream also had the highest dissolved nutrient which would not allow to assume the temperature as the only factor determining decomposition (Martínez *et al.* 2014). Nevertheless, the availability of nutrients does not appear explain the patterns in the field, particularly for eucalypt, the species of lower quality, which in precedent studies in the area has shown to be strongly dependent on nutrient in the water (i.e. faster rates in nutrient-rich water). Furthermore, even showed lower differences in processing rates with alder in streams with higher dissolved nutrients (Molinero, Pozo & Gonzalez 1996; Pozo *et al.* 1998; Pérez *et al.* 2014). Additionally, the incubations in the laboratory were carried out both in water from each stream and in a water brought from a fourth stream to rule out any possible interference due to dissimilarity in water chemistry of the three selected streams, and in general, the decomposition of both species seemed not to be influenced by the water quality. Moreover, temperature increases may interact with the dissolved nutrients resulting in synergistic effects and modulate the response of litter decomposition and associated biological activities to temperature (Ferreira & Chauvet 2011a; Fernandes *et al.* 2014). However, although the nitrogen range among streams can be considered large enough to influence decomposition (range 284- 632 $\mu\text{g N L}^{-1}$), the water quality did not influence the decomposition response to temperature, perhaps because the availability of phosphorus was very low ($< 4.16 \mu\text{g P L}^{-1}$).

Ruling out the nutrients as drivers of the unimodal response in the field, it seems that sensitivity to the quality of the organic substrate could be behind that differential response.

We also hypothesize that streams with different thermal regimes could support different fungal composition and respond in different way to temperature in relation to their thermal tolerance (Dang *et al.* 2009; Geraldles *et al.* 2012; Canhoto *et al.* 2016), and consequently result in different decomposition rate. However, the effect of temperature was consistent independently of microbial community origin. Although neither origin of microbial community nor water quality influenced the decomposition-temperature relationship in the laboratory experiment, it should be noted that eucalypt decomposition was less uniform in relation to these two factors, suggesting that the response of this low-quality species is more variable but without conditioning its response to temperature.

Temperature sensitivity did not differ between the two types of litter species, suggesting a weaker effect of the organic substrate quality on the temperature sensitivity of microbially mediated decomposition than in terrestrial ecosystems where decomposition of low-quality resources (structurally complex C substrates) is more sensitive to temperature increases (Fierer *et al.* 2005; Conant *et al.* 2008, 2011). Follstad Shah *et al.* (2017) also reported weak evidence of a negative relationship between litter quality and sensitivity to temperature in freshwater ecosystems and suggest that stream attributes (e.g. moisture, leaching of secondary compounds for water flow) could remove or mitigate constraints operating on terrestrial ecosystems. The temperature sensitivity (E_a , activation energy) of litter decomposition estimated in this study was nearly always within the range estimated by metabolic theory of ecology (0.6 -0.7 eV; Gillooly *et al.*, 2001; Brown *et al.*, 2004), and although the estimate in laboratory was on average slightly lower (0.48 eV), it was similar to those estimated for microbial decomposition rates in a study carried out across a broad latitudinal gradient by Boyero *et al.* (2011) and closer to the lower value stated by Follstad Shah *et al.* (2017) in a recent global synthesis of decomposition rates across the globe.

Increases in decomposition rates should be linked to the stimulation of other biological processes such as the increase of fungal biomass or activity. Both microbial growth and their metabolism are positively related to temperature until the optimal temperature is reached within the physiological range (Chauvet & Suberkropp 1998; Dang *et al.* 2009; Fernandes *et al.* 2009; Ferreira & Chauvet 2011a; Padfield *et al.* 2016). Leaf-associated respiration rate, a measure of overall microbial activity, was higher on high-quality litter (alder) than on low-quality one (eucalypt), as already reported (Gonçalves *et al.* 2013; Martínez *et al.* 2016). We also observed a relationship between respiration and temperature, but it was negative. This might be seen as contradictory to general prediction (Brown *et al.* 2004; Canhoto *et al.* 2016), but we need to

bear in mind that, in our study, the target was to measure the potential leaf-associated respiration rates and the respiration was measured at standard temperature of 10 °C, temperature included within the range of water variation of the 3 selected streams. In the laboratory, the potential respiration rate on both species was higher on leaf discs from the incubation at lower temperature, suggesting a higher metabolic activity at low temperatures. This is in agreement to previous studies (Padfield *et al.* 2016) that show that species are able to adapt to new thermal regimes and drift their optimum temperature accordingly. It seems that our incubation allowed the microbial assemblages to adapt to either 5, 10 or 15 °C, and that an incubation in 10 °C forces a higher metabolic effort to the assemblages incubated at 5 °C and, contrarily, diminish the metabolism of assemblages incubated at 15 °C. Nevertheless, in agreement with previous studies, respiration of microbial assemblages from different thermal regimes (i.e. conditioned in different sites) responded to temperature in a similar way (Sand-Jensen *et al.* 2007; Perkins *et al.* 2012) and the response was independent on water chemistry. Moreover, the sensitivity to temperature of alder and eucalypt litter was similar.

Increasing rate of biological processes with temperature was confirmed in our study by the stimulation of fungal biomass (Ferreira & Chauvet 2011a; Fernandes *et al.* 2014; Ferreira *et al.* 2015) on both species in the laboratory experiment, but not in the field, where the response was less predictable. Moreover, the stimulation of fungal growth with temperature was more evident in incubations with control water, suggesting the need to consider water characteristics when assessing of temperature effects on biomass. As well as temperature, nutrients can stimulate mycelial growth (Ferreira & Chauvet 2011a), but although we had more nutrients in the warmest stream we did not observed such a synergetic effect in the field incubation, particularly on eucalypt. The change in leaf nutrient concentration can be seen as a proxy of microbial colonization (Pozo *et al.* 2011; Casas *et al.* 2013), but it showed no strong pattern with temperature, neither in the field nor in the laboratory with stream water. Nevertheless, taking into account the control water, we observed that fungal growth was stimulated more in the warmest treatment (15 °C) whereas the nitrogen accumulation was stimulated at the lowest temperatures (5 °C). This result could be an adaptation of microbial assemblages inhabiting the coldest, but also the nutrient poorest stream to maximize the fixation of nutrients. We can, nevertheless, assert that the variety of patterns and temperature sensitivities shown by nutrient enrichment and fungal biomass, more related to the structure of the detrital compartment, are all translated into an extremely good fit between the predictions for organic matter decomposition and our observations.

In summary, our result shows that: (1) both substrate quality and temperature are important drivers of microbial-mediated litter decomposition and associated biological activities, (2) the increase in water temperature may lead to faster microbial mediated litter decomposition (3) the similar temperature sensitivity (i.e., activation energy) between alder - high-quality litter- and eucalypt- low-quality- suggests that leaf processing of contrasting quality could be similarly affected by temperature rises, (4) the response of decomposition rate to temperature increase was consistent across streams. Microbial activities from streams with different thermal regimes responded similarly to temperature. Although water dissimilarities did play a role, the effect of temperature was always more clear and relevant for the responses observed, (5) the patterns of decompositions were clearer and fitted better to those reported by previous studies than those of respiration, fungal growth and leaf nutrient. Our results showed that temperature response in the field was less predictable, underlining the difficulty of direct extrapolation of the results obtained under controlled laboratory conditions due to complex interactions that can occur in natural ecosystems. Understanding how water warming will affect microbial activities is crucial for forecast changes in plant litter processing, and more studies combining field and laboratory approaches would allow explore the potential responsible mechanisms.

References

- Abelho M. (2001) From Litterfall to Breakdown in Streams: A Review. *The Scientific World JOURNAL* **1**, 656–680.
- Acuña V., Muñoz I., Giorgi A., Omella M., Sabater F. & Sabater S. (2005) Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. *Journal of the North American Benthological Society* **24**, 919–933.
- Allen S.E., Grimshaw H.M., Parkinson J.A., Quarmby C. & others (1974) *Chemical analysis of ecological materials*. Blackwell Scientific Publications.
- APHA (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C.
- Artigas J., Gaudes A., Munoz I., Romani A.M. & Sabater S. (2011) Fungal and Bacterial Colonization of Submerged Leaf Litter in a Mediterranean Stream. *International Review of Hydrobiology* **96**, 221–234.
- Bärlocher F., Kebede Y.K., Gonçalves A.L. & Canhoto C. (2013) Incubation Temperature and Substrate Quality Modulate Sporulation by Aquatic Hyphomycetes. *Microbial Ecology* **66**, 30–39.
- Bates D., Maechler M., Bolker B. & Walker S. (2015) Fitting Linear Mixed-Effects Models

- Using lme4. *Journal of Statistical Software*. *Journal of Statistical Software* **67**, 1–48.
- Bergfur J. & Friberg N. (2011) Trade-offs between fungal and bacterial respiration along gradients in temperature, nutrients and substrata: Experiments with stream derived microbial communities. *Fungal Ecology* **5**, 46–52.
- Boyero L., Pearson R.G., Gessner M.O., Barnuta L.A., Ferreira V., Graça M.A.S., *et al.* (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters* **14**, 289–294.
- Boyero L., Pearson R.G., Hui C., Gessner M.O., Pérez J., Alexandrou M.A., *et al.* (2016) Biotic and abiotic variables influencing plant litter breakdown in streams: a global study. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20152664.
- Brown J.H., Gillooly J.F., Allen A.P., Savage V.M. & West G.B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
- Canhoto C., Gonçalves A.L. & Bärlocher F. (2016) Biology and ecological functions of aquatic hyphomycetes in a warming climate. *Fungal Ecology* **19**, 201–218.
- Canhoto C. & Graça M.A.S. (1999) Leaf Barriers to Fungal Colonization and Shredders (*Tipula lateralis*) Consumption of Decomposing *Eucalyptus globulus*. *Microbial Ecology* **37**, 163–172.
- Canhoto C. & Graça M. a. S. (1996) Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. *Hydrobiologia* **333**, 79–85.
- Casas J., Larrañaga A., Menéndez M., Pozo J., Basaguren A., Martínez A., *et al.* (2013) Leaf litter decomposition of native and introduced tree species of contrasting quality in headwater streams: How does the regional setting matter? *Science of the Total Environment* **458–460**, 197–208.
- Chauvet E. & Suberkropp K. (1998) Temperature and Sporulation of Aquatic Hyphomycetes. *Applied and Environmental Microbiology* **64**, 1522–1525.
- Chergui H. & Pattee E. (1990) THE INFLUENCE OF SEASON ON THE BREAKDOWN OF SUBMERGED LEAVES. *ARCHIV FUR HYDROBIOLOGIE* **120**, 1–12.
- Conant R., Drijber R.A., Haddix M.L., Parton W.J., Paul E.A., Plante A.F., *et al.* (2008) Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology* **14**, 868–877.
- Conant R.T., Ryan M., Birge H.E., Davidson E.A., Eliasson P.E., Evans S.E., *et al.* (2011) Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. *Global Change Biology* **17**, 3392–3404.
- Crawley M.J. (2007) *The R Book*, 1st edn. Wiley Publishing.
- Dang C.K., Schindler M., Chauvet E. & Gessner M.O. (2009) Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition.

- Ecology* **90**, 122–131.
- Duarte S., Fernandes I., Nogueira M.J., Cássio F. & Pascoal C. (2013) Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecology* **6**, 187–191.
- Fernandes I., Pascoal C., Guimaraes H., Pinto R., Sousa I. & Cássio F. (2012) Higher temperature reduces the effects of litter quality on decomposition by aquatic fungi. *Freshwater Biology* **57**, 2306–2317.
- Fernandes I., Seena S., Pascoal C. & Cássio F. (2014) Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. *Freshwater Biology* **59**, 2390–2399.
- Fernandes I., Uzun B., Pascoal C. & Cássio F. (2009) Responses of aquatic fungal communities on leaf litter to temperature-change events. *International Review of Hydrobiology* **94**, 410–418.
- Ferreira V. & Chauvet E. (2011a) Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia* **167**, 279–91.
- Ferreira V. & Chauvet E. (2011b) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology* **17**, 551–564.
- Ferreira V., Chauvet E. & Canhoto C. (2015) Effects of experimental warming, litter species, and presence of macroinvertebrates on litter decomposition and associated decomposers in a temperate mountain stream. *Canadian Journal of Fisheries and Aquatic Sciences* **72**, 206–216.
- Ferreira V., Encalada A.C. & Graça M.A.S. (2012) Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science* **31**, 945–962.
- Fierer N., M C.J., McLauchlan J. & Schimel J.P. (2005) Litter quality and the temperature sensitivity of decomposition. *Ecology* **86**, 320–326.
- Follstad Shah J.J., Kominoski J.S., Ardón M., Dodds W.K., Gessner M.O., Griffiths N.A., *et al.* (2017) Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. *Global change biology*.
- Friberg N., Dybkjær J.B., Olafsson J.S., Gislason G.M., Larsen Sjø.E. & Lauridsen T.L. (2009) Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology* **54**, 2051–2068.
- García-Palacios P., McKie B.G., Handa I.T., Frainer A. & Hättenschwiler S. (2016) The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology* **30**, 819–829.
- Geraldes P., Pascoal C. & Cássio F. (2012) Effects of increased temperature and aquatic fungal

- diversity on litter decomposition. *Fungal Ecology* **5**, 734–740.
- Gessner M.O. (1999) A perspective on leaf litter breakdown in streams. *OIKOS* **85**, 377–384.
- Gessner M.O. & Chauvet E. (1993) Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. *Applied and Environmental Microbiology* **59**, 502–507.
- Gessner M.O. & Chauvet E. (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology* **75**, 1807–1817.
- Gessner M.O., Gulis V., Kuehn K.A., Chauvet E. & Suberkropp K. (2007) Fungal decomposers of plant litter in aquatic ecosystems. In: *The Mycota: Environmental and Microbial Relationships*. (Eds C.P. Kubicek & I.S. Druzhinin), pp. 301–321. Springer, Berlin.
- Gillooly J.F., Brown J.H., West G.B., Savage V.M. & Charnov E.L. (2001) Effects of Size and Temperature on Metabolic Rate. *Science* **293**, 2248–2251.
- Gonçalves A.L., Graça M.A.S. & Canhoto C. (2013) The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. *Fungal Ecology* **6**, 546–553.
- Graça M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams—a review. *International Review of Hydrobiology* **86**, 383–393.
- Graça M.A.S., Pozo J., Canhoto C. & Elozegi A. (2002) Effects of Eucalyptus Plantations on Detritus, Decomposers, and Detritivores in Streams. *The Scientific World JOURNAL* **2**, 1173–1185.
- Griffiths N.A. & Tiegs S.D. (2016) Organic-matter decomposition along a temperature gradient in a forested headwater stream. *Freshwater Science* **35**, 518–533.
- Hladyz S., Gessner M.O., Giller P.S., Pozo J. & Woodward G. (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology* **54**, 957–970.
- IPCC (2013) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (Eds T.F. Stocker, D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, et al.), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Kaushal S.S., Likens G.E., Jaworski N.A., Pace M.L., Sides A.M., Seekell D., et al. (2010) Rising stream and river temperatures in the United States. *Frontiers in Ecology and the Environment* **8**, 461–466.
- Kominoski J.S., Shah J.J.F., Canhoto C., Fischer D.G., Giling D.P., González E., et al. (2013) Forecasting functional implications of global changes in riparian plant communities. *Frontiers in Ecology and the Environment* **11**, 423–432.
- Langan S.J., Johnston L., Donaghy M.J., Youngson A.F., Hay D.W. & Soulsby C. (2001) Variation in river water temperatures in an upland stream over a 30-year period. *Science of The Total Environment* **265**, 195–207.

- Martínez A., Larrañaga A., Pérez J., Descals E. & Pozo J. (2014) Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. *FEMS microbiology ecology* **87**, 257–67.
- Martínez A., Monroy S., Pérez J., Larrañaga A., Basaguren A., Molinero J., *et al.* (2016) In-stream litter decomposition along an altitudinal gradient: does substrate quality matter? *Hydrobiologia* **766**, 17–28.
- Molinero J., Larrañaga A., Pérez J., Martínez A. & Pozo J. (2016) Stream temperature in the Basque Mountains during winter: thermal regimes and sensitivity to air warming. *Climatic Change* **134**, 593–604.
- Molinero J., Pozo J. & Gonzalez E. (1996) Litter breakdown in streams of the Agüera catchment: influence of dissolved nutrients and land use. *Freshwater Biology* **36**, 745–756.
- Padfield D., Yvon-Durocher G., Buckling A., Jennings S. & Yvon-Durocher G. (2016) Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecology Letters* **19**, 133–142.
- Pascoal C. & Cássio F. (2004) Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River. *Applied and environmental microbiology* **70**, 5266–5273.
- Pérez J., Galán J., Descals E. & Pozo J. (2014) Effects of Fungal Inocula and Habitat Conditions on Alder and Eucalyptus Leaf Litter Decomposition in Streams of Northern Spain. *Microbial Ecology* **67**, 245–255.
- Perkins D.M., Reiss J., Yvon-Durocher G. & Woodward G. (2010) Global change and food webs in running waters. *Hydrobiologia* **657**, 181–198.
- Perkins D.M., Yvon-Durocher G., Demars B.O.L., Reiss J., Pichler D.E., Friberg N., *et al.* (2012) Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. *Global Change Biology* **18**, 1300–1311.
- Pinheiro J.C. & Bates D.M. (2000) Linear mixed-effects models: basic concepts and examples. *Mixed-effects models in S and S-Plus*, 3–56.
- Poff N.L. & Zimmerman J.K.H. (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. *Freshwater Biology* **55**, 194–205.
- Pozo J., Basaguren A., Elosegui A., Molinero J., Fabre E. & Chauvet E. (1998) Afforestation with Eucalyptus globulus and leaf litter decomposition in streams of northern Spain. *Hydrobiologia* **373**, 101–110.
- Pozo J., Casas J., Menéndez M., Mollá S., Arostegui I., Basaguren A., *et al.* (2011) Leaf-litter decomposition in headwater streams: a comparison of the process among four climatic regions. *Journal of the North American Benthological Society* **30**, 935–950.
- Pozo J., Gonzalez E., Díez J., Molinero J. & Elósegui A. (1997) Inputs of particulate organic

- matter to streams with different riparian vegetation. *Journal North American Benthological Society*, 16: 602-611.
- R Development Core Team (2015) R: A Language and Environment for Statistical Computing.
- Sand-Jensen K., Pedersen N.L. & Søndergaard M. (2007) Bacterial metabolism in small temperate streams under contemporary and future climates. *Freshwater Biology* **52**, 2340–2353.
- Suberkropp K. (2001) Fungal Growth , Production , and Sporulation during Leaf Decomposition in Two Streams Fungal Growth , Production , and Sporulation during Leaf Decomposition in Two Streams. *Applied and Environmental Microbiology* **67**, 5063–5068.
- Tank J.J.L., Rosi-Marshall E.J.E., Griffiths N. a., Entekin S. a. & Stephen M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* **29**, 118–146.
- Taylor B.R. & Chauvet E.E. (2014) Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient. *Hydrobiologia* **721**, 239–250.
- Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137.
- Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102–104.
- Wetterstedt J.A., Peterson T. & Agren G.I. (2010) Temperature sensitivity and substrate quality in soil organic matter decomposition : results of an incubation study with three substrates. *Global Change Biology* **16**, 1806–1819.
- Zar J.H. (2010) *Biostatistical Analysis*, 5th edn. Prentice Hall, Upper Saddle River, USA.

Supporting Information

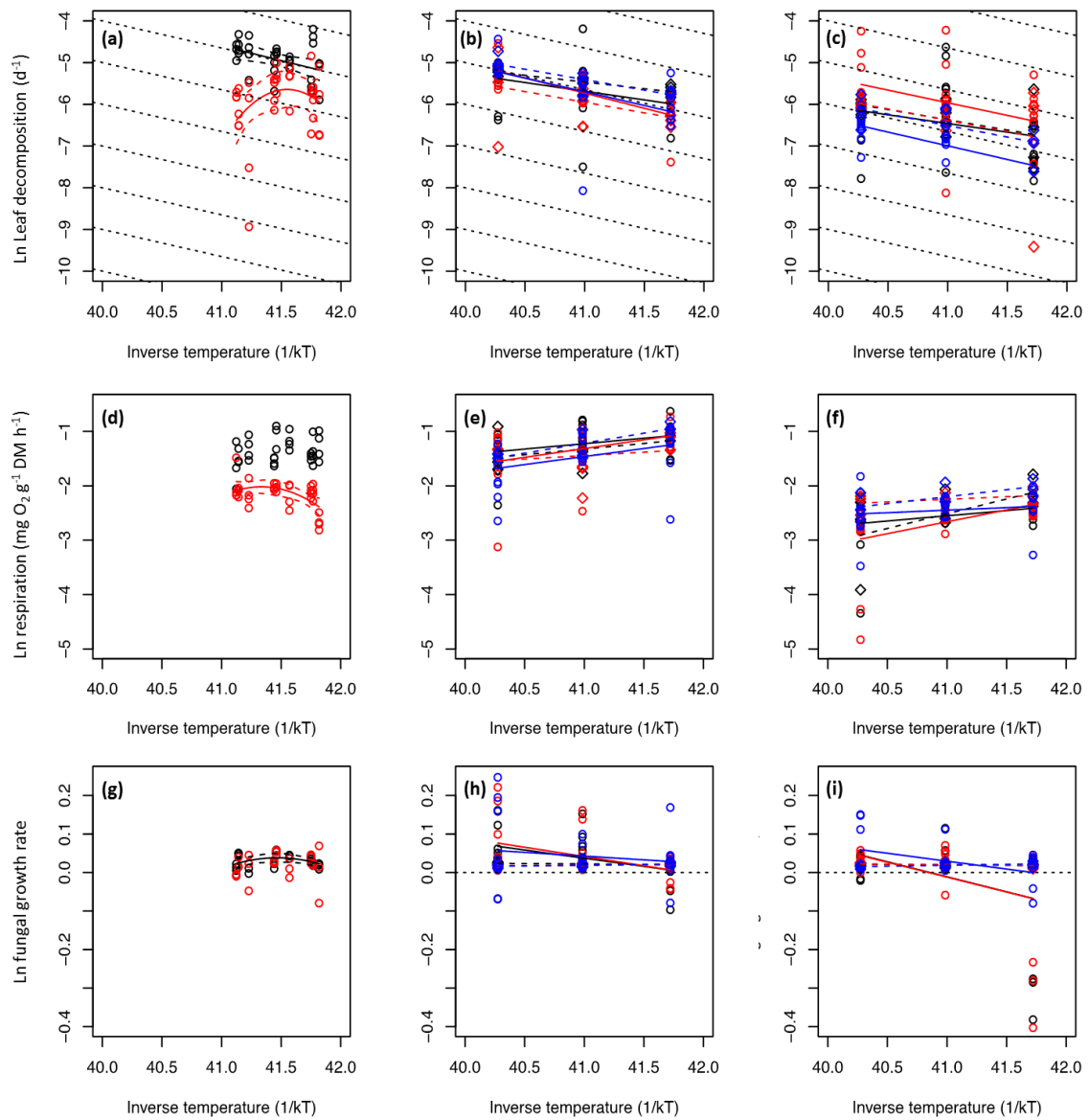


Figure S1. Relationships between the natural logarithm of leaf decomposition rate (a, b, c), respiration rate (d, e, f), fungal growth rate (g, h, i) and water temperature expressed in terms of metabolic theory of ecology (Brown *et al.* 2004) as the inverse of absolute temperature (T) in Kelvin and the Boltzman constant (k). Data from field experiment (a, d, g) for alder (black symbols) and eucalypt litter (red symbols). Data from laboratory experiment for alder (b, e, h) and eucalypt litter (c, f, i), preconditioned in the three streams (S1: black; S2: red; S3: blue) and incubated in stream water (discontinuous lines) or control water (continuous lines). Regression lines are showed.

Table S1. Results of linear mixed models performed on leaf decomposition, respiration and fungal biomass of alder and eucalypt leaf discs incubated in the field and the laboratory in microcosms at three temperatures (5, 10 and 15°C). Site = S1, S2 and S3; Leaf = alder, eucalypt; Water = control water or stream water. Significant values are highlighted in bold. InvTemp = temperature expressed in terms of metabolic theory of ecology (Brown *et al.* 2004) as the product of the inverse of temperature (in Kelvin) and the Boltzman constant.

		Df	F value	p-value
Leaf decomposition-Field				
Alder	InvTemp	1	6.22	0.014
Eucalypt	InvTemp2*	1	4.43	0.033
Leaf decomposition-Laboratory				
	InvTemp	1	54.28	< 0.0001
	Leaf	1	87.54	< 0.0001
	Site	2	2.61	0.100
	Water	1	0.17	0.552
	Leaf:Site	2	10.60	< 0.0001
	Site:Water	2	6.14	0.003
Respiration-Field				
Alder	InvTemp	1	2.05	0.144
Eucalypt	InvTemp	1	8.49	0.008
	InvTemp2*	1	5.94	0.017
Respiration-Laboratory				
	InvTemp	1	33.29	< 0.0001
	Leaf	1	469.55	< 0.0001
	Site	2	0.59	0.556
	Leaf:Site	2	3.15	0.045
Fungal biomass-Field				
Alder	InvTemp2*	1	6.39	0.013
Eucalypt	InvTemp	1	0.72	0.418
Fungal biomass-Laboratory				
	InvTemp	1	23.54	< 0.0001
	Leaf	1	10.17	0.001
	Water	1	0.02	0.876
	InvTemp:Water	1	8.55	0.005

CHAPTER 4

Structural and functional recovery of macroinvertebrate communities and leaf litter decomposition after a marked drought: does vegetation type matter?

This chapter was published with the following reference: Monroy S., Martínez A., López-Rojo N., Pérez-Calpe A.V., Basaguren A. & Pozo J. 2017. *Science of the Total Environment*, 599/600: 1241–1250. Q1

Abstract

Climate change and anthropogenic disturbances are expected to lead to more intense and frequent droughts, with potentially severe effects on structure and function of perennial temperate streams. However, more information is required on whether streams flowing through basins already affected by exotic plantations will respond to droughts in the same way as streams under native forests. The recolonisation dynamics of benthic macroinvertebrate communities and leaf litter decomposition rates were examined in nine streams of oceanic-temperate climate that differed in catchment vegetation (three streams draining native deciduous forest, three in pine plantations and three in eucalypt plantations) after a marked drought. In each stream, five benthic samples were collected three times (ca. 1.5 months between sampling dates) after flow recovery, and the taxonomic and functional trait compositions of the macroinvertebrate communities were analysed. The decomposition rate of *Alnus glutinosa* was measured in fine- and coarse-mesh litter bags. Benthic macroinvertebrate density, richness and diversity increased with time after flow recovery but only richness and diversity differed among stream types, with eucalypt streams showing the lowest values. Both the taxonomic and functional compositions of the macroinvertebrate community were dependent on vegetation type and time, with the differences among stream types diminishing over time. While leaf-litter decomposition rate did not depend on catchment vegetation after drought, detritivore activity was the lowest under eucalypt streams and it was positively correlated to benthic shredder density. Our results indicated that in these perennial temperate streams the catchment vegetation influenced the recovery of benthic macroinvertebrate communities after a period of drought, although the decomposition rate of leaf litter was not strongly affected. Greater understanding of the structural and functional responses of stream ecosystems to different stressors is required before the effects of expected more intense and frequent hydrological changes caused by climate change can be adequately forecast.

Keywords: drought, temperate streams, exotic plantations, macroinvertebrates, functional traits, litter decomposition

Introduction

By the end of the 21st century, as predicted by IPCC (2014), shifts in climate by alteration of temperature and precipitation patterns may lead to changes in hydrological features of freshwater ecosystems (Papadaki et al., 2016; Vicente-Serrano et al., 2014), including higher frequency and intensity of extreme events such as droughts or floods (Huang et al., 2016), with major consequences for aquatic communities and the processes they support (Lake, 2011). Within this scenario, temperate areas are expected to become more similar to Mediterranean regions (Milly et al., 2005), which are characterised by cold and relatively humid winters and hot-dry summers (Gasith and Resh, 1999), thereby increasing the possibility of summer droughts. In Mediterranean streams, physicochemical and biological characteristics result from seasonal and predictable drought and flood events (Bonada et al., 2007a; Hershkovitz and Gasith, 2013). In fact, because of evolutionary adaptations of in-stream biota, macroinvertebrate communities show traits that enable them to resist (resistance traits; e.g., behaviours to seek shelter) or recover quickly after droughts (resilience traits; e.g., shortlife cycles) (Bonada et al., 2007a; Bond et al., 2008; Leigh et al., 2016). By contrast, the expected extreme drought events in oceanic-temperate streams could trigger important alterations in the structure and functioning of these ecosystems (Lake, 2011) because those biological communities do not have such inherent physiological-ecological adaptations (Bonada et al., 2007a; Leigh et al., 2016).

In addition to drought-related disturbances, freshwater ecosystems are subjected to other anthropogenic stressors. Among them, the replacement of native forests by exotic monospecific plantations is one of the most widespread worldwide (Kominoski et al., 2013). These practices affect low order forested streams because organic matter input from surrounding vegetation is the basis of trophic food webs with microbial decomposers and invertebrate detritivores incorporating it into consumer biomass (Tank et al., 2010). In temperate regions, the replacement of autochthonous broadleaf forests with coniferous and eucalypt monocultures implies changes in quality, quantity and timing of leaf litter inputs (Casas et al., 2013; Martínez et al., 2016; Pozo et al., 1997). While such changes often negatively alter feeding rates, growth, density, richness and diversity of detritivores (Ferreira et al., 2015; Larrañaga et al., 2009a; Martínez et al., 2013; Wallace et al., 1997), the resource colonisation and decomposer activity of microbial assemblages are affected to a lesser extent (Bärlocher and Graça, 2002; Kominoski et al., 2010). However, because of functional redundancy among taxa (Bogan et al., 2013), whether these types of plantations trigger changes in the total set of biological and ecological traits of these macroinvertebrate communities remain unknown. Several studies reported shifts in the density of macroinvertebrate trophic groups (Ferreira et al., 2015; Larrañaga et al., 2009b; Martínez et al., 2013) and in the range of body sizes (Albariño and Balseiro, 2002; Larrañaga et al., 2009a; Martínez et al., 2016), which suggest that exotic plantations alter macroinvertebrate

communities at a functional level. Therefore, depending on catchment vegetation, these communities could differ in the prevalence of traits that might be indirectly related to drought vulnerability, which in turn could influence the recolonisation capacity of these freshwater ecosystems. Changes in the prevalence of traits might have consequences not only for the recovery of macroinvertebrate communities but also for ecosystem functioning due to the key role that they play in organic matter processing and secondary production (Graça, 2001).

In forested streams, the process of leaf litter decomposition is fundamental in transferring energy and matter across trophic levels (Perkins et al., 2010), controlling nutrient cycling (Cheever et al., 2012) and contributing significantly to the global carbon cycle with the release of CO₂ (Battin et al., 2009). Droughts are relatively well known to slowdown litter decomposition rates (Datry et al., 2011; Martínez et al., 2015; Monroy et al., 2016). This effect is driven by the alteration of macroinvertebrate communities (e.g., decline in abundance and activity) rather than alteration of microbial activity (Acuña et al., 2005; Corti et al., 2011) since microbial assemblages can persist in moist substrata (Sridhar and Bärlocher, 1993) and recover their activity very fast after flow restoration (Langhans and Tockner, 2006). Nevertheless, information is not available on whether the recolonisation dynamic of macroinvertebrate communities and organic matter decomposition after drying depend on catchment vegetation.

The recolonisation of the macroinvertebrate community, which was analysed for taxonomic (structural) and biological and ecological traits (functional), and leaf litter decomposition were studied in temperate streams after a drought event. Our goal was to assess whether these communities and processes were dependent on the catchment vegetation type by examining oceanic-temperate streams that differed in catchment vegetation (native deciduous forest, *Pinus radiata* D. Don plantation and *Eucalyptus globulus* Labill. plantation) after a marked drought. The expectations were the following: 1) density, richness and diversity of benthic macroinvertebrate communities are lower in streams under exotic plantations during the recolonisation period; 2) stream communities under deciduous forest have higher functional variability and therefore show traits that facilitate the recolonisation process and, 3) despite the drought stress, decomposition rates are slower in streams under exotic plantations than those under deciduous forest, with the effect more related to alterations in macroinvertebrate communities than in microbial activity.

Methods

Study Area

The study was conducted in nine low order streams with siliceous substrates located in the Cordillera Cantábrica (northern Spain). In this region, the climate is temperate oceanic

with mean annual air temperature of approximately 14 °C and mean annual precipitation of ca. 1500 mm distributed throughout the year with a winter maximum of approximately 450 mm (Elosegi et al., 2006). Although precipitation is low in summer, droughts are not common. Unusual temperature and precipitation conditions during summer-autumn 2015 (Fig.1; <http://www.bizkaia.net>) caused low levels of flow, reaching temporary cessation of flow in the nine selected streams (from August to October). Three of the streams drain native deciduous forests catchments (deciduous streams, D) dominated by oak (*Quercus robur* L.), three run through *P. radiata* plantations (pine streams, P) and the other three streams drain *E. globulus* plantations (eucalypt streams, E) (Table 1). Streams located within pine and eucalypt plantations showed some riparian deciduous trees (e.g., alder, *Alnus glutinosa* L. Gaertner). The native riparian vegetation was more developed for the pine streams because of the relatively long harvesting cycle of approximately 30-35 years compared with 12-15 years for the eucalyptus plantations. Deciduous streams showed well developed riparian forest dominated by alder. Anthropogenic impacts in the basins, apart from the plantations, were negligible.

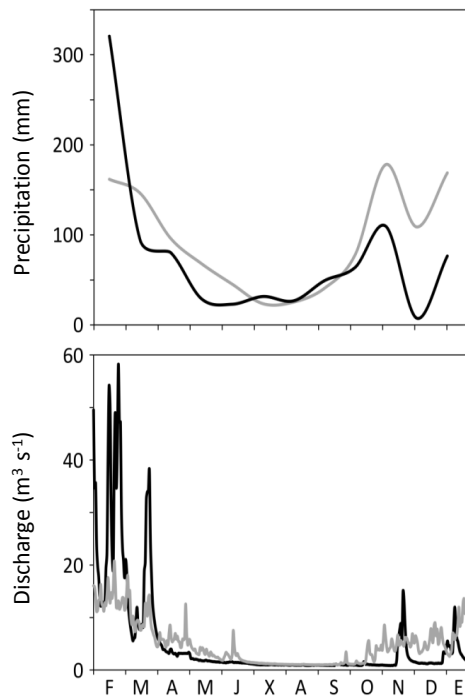


Fig. 1. Mean monthly-accumulated rain (mm) and mean daily discharge ($\text{m}^3 \text{s}^{-1}$) from February 2005 to January 2015 (grey line, historical 10-y period) and from February 2015 to January 2016 (black line). Data recorded at the Balmaseda station located downstream of the pine streams.

Table 1. Location, catchment vegetation and physiochemical characteristics of the selected streams during the study period (mean \pm SE; n = 6). D = deciduous streams, E= *Eucalypt* streams, P= *Pine* streams, SRP = soluble reactive phosphorus.

	D1	D2	D3	E1	E2	E3	P1	P2	P3
Latitude (N)	43°12'44''	43°12'30''	43°14'37''	43°18'59''	43°18'57''	43°19'09''	43°11'10''	43°11'19''	43°11'25''
Longitude (E)	3°16'18''	3°15'48''	3°16'43''	3°16'08''	3°16'17''	3°16'41''	3°10'13''	3°09'23''	3°08'57''
Altitude (ma.s.l.)	306	306	251	100	90	90	312	271	271
Catchment area (km²)	0.83	0.37	0.37	0.89	0.66	0.77	0.59	0.39	0.68
Catchment land use (%)									
Deciduous forest	100	100	100	0	0	0	0	0	0
<i>E.globulus</i> plantation	0	0	0	100	100	100	0	0	0
<i>P. radiata</i> plantation	0	0	0	0	0	0	100	100	100
Width (m)	0.29 \pm 0.13	0.27 \pm 0.07	0.31 \pm 0.12	0.27 \pm 0.07	0.32 \pm 0.07	0.26 \pm 0.05	0.31 \pm 0.13	0.17 \pm 0.06	0.27 \pm 0.07
Discharge (L s⁻¹)	2.87 \pm 1.80	3.96 \pm 1.57	2.47 \pm 1.43	4.40 \pm 1.92	2.89 \pm 1.16	4.78 \pm 2.02	1.64 \pm 1.34	0.47 \pm 0.37	0.97 \pm 0.95
Water temperature (°C)	10.8 \pm 0.9	10.6 \pm 0.9	10.8 \pm 1.3	11.9 \pm 0.9	12.6 \pm 1.0	12.3 \pm 0.9	10.1 \pm 1.1	10.1 \pm 0.9	9.7 \pm 1.2
pH	8.04 \pm 0.12	7.92 \pm 0.11	7.91 \pm 0.03	7.91 \pm 0.11	7.17 \pm 0.37	8.05 \pm 0.10	8.08 \pm 0.07	8.04 \pm 0.04	7.89 \pm 0.03
Conductivity (μS cm⁻¹)	79.8 \pm 3.9	85.4 \pm 1.2	130.2 \pm 6.3	66.8 \pm 2.9	64.5 \pm 1.5	84.7 \pm 2.4	124.7 \pm 12.0	209.0 \pm 24.4	126.0 \pm 6.4
Oxygen saturation (%)	94.8 \pm 1.2	93.4 \pm 2.5	78.0 \pm 17.3	89.8 \pm 2.6	75.6 \pm 7.4	95.3 \pm 1.4	94.0 \pm 1.7	91.5 \pm 2.9	93.1 \pm 2.9
Dissolved oxygen (mg L⁻¹)	10.2 \pm 0.3	10.0 \pm 0.4	9.4 \pm 1.3	9.6 \pm 0.4	8.0 \pm 0.9	10.1 \pm 0.3	10.3 \pm 0.4	10.0 \pm 0.5	10.5 \pm 0.6
Nitrate (μg N L⁻¹)	92.8 \pm 21.3	283.3 \pm 41.5	361.9 \pm 83.4	176.5 \pm 48.4	66.5 \pm 33.5	171.0 \pm 35.0	275.1 \pm 23.7	313.6 \pm 122.0	161.3 \pm 34.0
Nitrite (μg N L⁻¹)	2.17 \pm 0.31	2.81 \pm 0.48	2.24 \pm 0.45	3.13 \pm 0.86	2.63 \pm 0.80	2.30 \pm 0.55	2.62 \pm 0.65	3.19 \pm 0.84	2.62 \pm 0.94
Ammonium (μg N L⁻¹)	25.1 \pm 2.2	23.3 \pm 2.7	21.9 \pm 2.4	22.0 \pm 2.4	26.9 \pm 3.5	26.2 \pm 4.1	34.1 \pm 2.5	28.8 \pm 3.2	27.5 \pm 2.9
SRP (μg P L⁻¹)	9.03 \pm 1.79	9.74 \pm 1.15	8.12 \pm 1.14	8.79 \pm 1.09	9.84 \pm 1.54	9.98 \pm 1.03	10.94 \pm 0.86	9.74 \pm 1.76	9.98 \pm 1.76

Stream water characterization

Water parameters were monitored (six sampling dates) during the study period at each site (October 2015 to January 2016). Water temperature, conductivity, pH and oxygen saturation were measured with a multiparametric sensor (WTW Multi 350i; WTW, Weilheim, Germany). Discharge was estimated from instantaneous water velocity measured by a current meter (MiniAir 2; Schiltknecht Co, Gossau, Switzerland). Water samples for nutrient analyses were filtered (Millipore, 0.45 µm pore) in the field and frozen (-20 °C) in laboratory until further analyses. Nitrate concentration was determined by capillary ion electrophoresis (Agilent CE, Agilent Technologies, Waldbronn, Germany), and the other nutrients were analysed colorimetrically: nitrite by the sulphanilamide method, ammonium by the salicylate method and dissolved reactive phosphorus (SRP) by the molybdate method (APHA, 1998).

Benthic macroinvertebrates: structural and functional attributes

On 28th September all streams were dried. Rainfall started on 1st October, and on 8th October all streams showed flows between 0.13 L/s (E2) and 2.68 L/s (E1). In each stream, five benthic samples (Surber 0.09 m², 0.5 mm mesh size) were taken from random riffles along a 50 m reach on three sample dates (13th October 2015, T1; 2nd December 2015, T2; and 12th January 2016, T3). The frequency of sampling (ca. 1.5 months between sampling dates after flow recovery) was established in relation to the change in the communities throughout the autumn-winter after drought event in the same region (Otermin et al., 2002). Macroinvertebrates were separated from benthic materials on an 8-mm sieve and then preserved in 70 % ethanol. In the laboratory, macroinvertebrates were identified to genus (except Oligochaeta, identified to order, and Chironomidae, identified to tribe) and sorted into shredders and non-shredders following Tachet *et al.* (2002). For each benthic macroinvertebrate sample, the density (no. ind m⁻²), the taxa richness and the Shannon diversity index were calculated. The biological and ecological attributes of each taxon were also characterised using 10 traits with a total of 55 modalities (Table 2) following Tachet *et al.* (2002). These traits were related to drought (Bonada et al., 2007a, 2007b; Chessman, 2015; García-Roger et al., 2013) and food resource and described features of the lifecycle (lifecycle duration, reproductive cycles per year), resilience or resistance (dispersal, locomotion, substrate relation, resistant forms), physiology and morphology (respiration, maximum size), feeding behaviour (food, feeding habits) and current flow. Each taxon was coded according to its affinity to each modality of a trait (score from 0 to 5), and the mean distribution frequency of the affinities of each subgroup for each modality of the 10 traits was used to build the trait matrix.

Table 2. List of biological and ecological traits of benthic macroinvertebrates and their respective trait modalities considered in this study. An *a priori* rationale justification of the expected differences in traits is shown. The assigned code corresponds to the numbering of these traits according to Tachet *et al.* (2002).

Traits	Code	Modalities	Justification
Maximum size	1.1	≤ 0.25 cm	Better resilience capacity of smaller sizes after drought.
	1.2	0.25-0.5 cm	
	1.3	0.5-1 cm	
	1.4	1-2 cm	
	1.5	2-4 cm	
	1.6	4-8 cm	
	1.7	> 8 cm	
Life-cycle duration	2.1	≤1 year	Better resilience capacity through shorter life cycles after drought.
	2.2	>1 year	
Reproductive cycles/year	3.1	<1	More frequent reproduction favours the resilience capacity.
	3.2	=1	
	3.3	>1	
Dispersal	6.1	Aquatic passive	Flight capability favours flying to other less-dry sites and rapid recolonisation after a drought event.
	6.2	Aquatic active	
	6.3	Aerial passive	
	6.4	Aerial active	
Resistance forms	7.1	Eggs, statoblasts, gemmules	Resistant forms increase resistance against drought and will facilitate the recolonisation.
	7.2	Cocoons	
	7.3	Cells against desiccation	
	7.4	Diapause or dormancy	
	7.5	None	
Food type	8.1	Fine sediment/microorganisms	The organisms that feed on other invertebrates are the last to reach the system because they require prey to survive.
	8.2	Detritus < 1 mm	
	8.3	Plant detritus ≥ 1 mm	
	8.4	Living microphytes	
	8.5	Living macrophytes	
	8.6	Dead animals ≥ 1 mm	
	8.7	Living microinvertebrates	
	8.8	Living macroinvertebrates	
	8.9	Vertebrates	
Feeding habits	9.1	Absorber	Predators are the last to reach the system because they require other invertebrates as food. Filterers require currents to feed. Shredder response to more abundant/nutritive input of larger litter.
	9.2	Collector	
	9.3	Shredder	
	9.4	Scraper	
	9.5	Filter feeder	
	9.6	Piercer	
	9.7	Predator	
	9.8	Parasite or parasitoid	
Respiration	10.1	Tegument (skin)	Stagnant conditions or drought increase the difficulty of oxygen uptake (e.g., lower dissolved oxygen), specialized structures that maximise the uptake and aerial respiration could be favoured.
	10.2	Gill	
	10.3	Plastron	
	10.4	Spiracle (aerial)	
	10.5	Hydrostatic vesicle (aerial)	
Locomotion and substrate relation	21.1	Flier	Flow cessation harms swimmers. Interstitial and burrower forms increase resistance. Crawlers favour recolonization.
	21.2	Surface swimmer	
	21.3	Swimmer	
	21.4	Crawler	
	21.5	Burrower (epibenthic)	
	21.6	Interstitial (endobenthic)	
	21.7	Temporarily attached	
	21.8	Permanently attached	

Leaf litter decomposition

In October 2015, freshly fallen leaves of black alder (*A. glutinosa*) were collected from the ground at a single site (Agüera basin; on the border line of Cantabria and Basque Country, northern Spain, 43°12'50''N, 3°16'10''W). Leaves were air-dried in the laboratory, weighed (3.50 ± 0.10 g) and enclosed in either fine (12 × 15 cm, 0.5 mm mesh size) or coarse (20 × 25 cm, 5 mm mesh size) mesh bags. Litterbags were deployed in the nine streams on 2nd December 2015. At each site, 5 iron bars were anchored randomly to the streambed in riffle sections along 50 m of the channel, and one fine bag and one coarse bag were tied to each bar by nylon lines a total of 10 bags per site (5 replicates × 2 mesh sizes; single sampling). An extra set of five fine mesh bags were incubated for 24 h in one of the streams and used to correct initial air-dry mass for leaching loss and to estimate air-dry to oven-dry (72 h at 60 °C) and to ash-free dry mass (AFDM, 4 h at 500 °C) conversion factors.

The litterbags were retrieved after 45 days, enclosed individually in zip-lock bags and transported in a refrigerated cooler to the laboratory. The leaf litter material from each bag was rinsed with distilled water on a 500- μ m sieve to remove sediments and associated macroinvertebrates. The remaining material was oven-dried (70°C, 72h), weighed to determine leaf dry mass and then was combusted (500 °C, 4h) and weighed to determine the remaining ash-free dry mass (AFDM). The macroinvertebrates from coarse bags were preserved in 70 % ethanol and then density (no. inv bag⁻¹) and biomass (mg bag⁻¹, after oven-dried at 70 °C for 72 h) of total and shredder macroinvertebrates were calculated.

Data analyses

Differences in physicochemical characteristics of water among the three stream types were analysed by a two-way linear mixed model (fixed factor: “vegetation type”; random factor: “stream”). Density, richness, and Shannon diversity of total macroinvertebrates and shredders and functional traits of total benthic macroinvertebrates were compared using a three-way mixed model (fixed factors: “vegetation type” and “sampling time”; random factor: “stream”). Tukey’s test was used for post hoc comparisons (Zar, 2010). For the functional traits, the differences among stream types for each sampling time were also tested using a two-way linear mixed model (fixed factor: “vegetation type”; random factor: “stream”). To analyse community structure, based on taxonomic abundance and on affinity for the modalities of functional traits, a non-metric multidimensional scaling (NMDS) based in the Bray-Curtis dissimilarity matrix followed by PERMANOVA (10^5 perm) with the *adonis* function in the “vegan” R package was performed. To prevent noise caused by rare taxa, only those taxa representing > 1% of the total density were used in the analyses.

The decomposition rate was calculated with a linear model as follows: $b = (M_0 - M_t)/t$, where b is the decomposition rate, M_0 is the initial mass after correcting for leaching, M_t is the

remaining mass at the end of incubation, and t is the incubation time (45 days). Detritivore activity was estimated as the difference of mass loss between coarse and fine bags for each stream (Flores et al., 2013). Differences in decomposition rates (fine, total and detritivore activity) and benthic and leaf litter associated macroinvertebrates (density and biomass) among stream types were compared using a two-way linear mixed model (fixed factor: “vegetation type”; random factor: “stream”). Bivariate relationships between breakdown rates and macroinvertebrate variables were tested by linear regression analyses.

Density and biomass data were log ($x+1$) transformed to meet the statistical requirements of parametric analyses. All mixed model parameters were estimated by means of restricted maximum likelihood using *lme* function in the “nlme” R package. All statistical analyses were conducted using the R statistical program version 3.3.2 (R Development Core Team, 2010).

Results

Water quality

All streams presented well-oxygenated water, circumneutral pH, low conductivity and nutrient concentrations, and water temperature of approximately 10 °C (Table 1). None of the physicochemical characteristics differed significantly among stream types ($p > 0.05$).

Benthic macroinvertebrates: structural and functional attributes

A total of 20862 macroinvertebrates belonging to 88 taxa were identified in the benthic samples from the nine streams: 7284 individuals in deciduous streams, 4834 in eucalypt streams and 8744 in pine streams. Although lower values were recorded in eucalypt streams, mean density of macroinvertebrates did not differ significantly among the stream types (Fig. 2; $F_{2,6} = 1.86$, $p = 0.234$). Density increased with time ($F_{2,120} = 5.85$, $p = 0.003$) for all stream types. Taxa richness ($F_{2,124} = 19.26$, $p < 0.001$) and Shannon diversity ($F_{2,124} = 15.01$, $p = 0.002$) were significantly different among stream types, with eucalypt streams showing the lowest values (Fig. 2). Density, richness ($F_{2,124} = 6.80$, $p = 0.002$) and Shannon diversity ($F_{2,124} = 6.26$, $p = 0.001$) increased with time (Fig. 1). For shredders, density ($F_{2,120} = 17.72$, $p = 0.003$) and richness ($F_{2,120} = 5.15$, $p = 0.049$) differed among stream types (Fig. 2), with higher densities in pine streams and eucalyptus streams with the lowest richness. Only shredder richness ($F_{2,120} = 14.29$, $p < 0.0001$) and diversity ($F_{2,120} = 17.75$, $p < 0.0001$) increased significantly with time (Fig. 2).

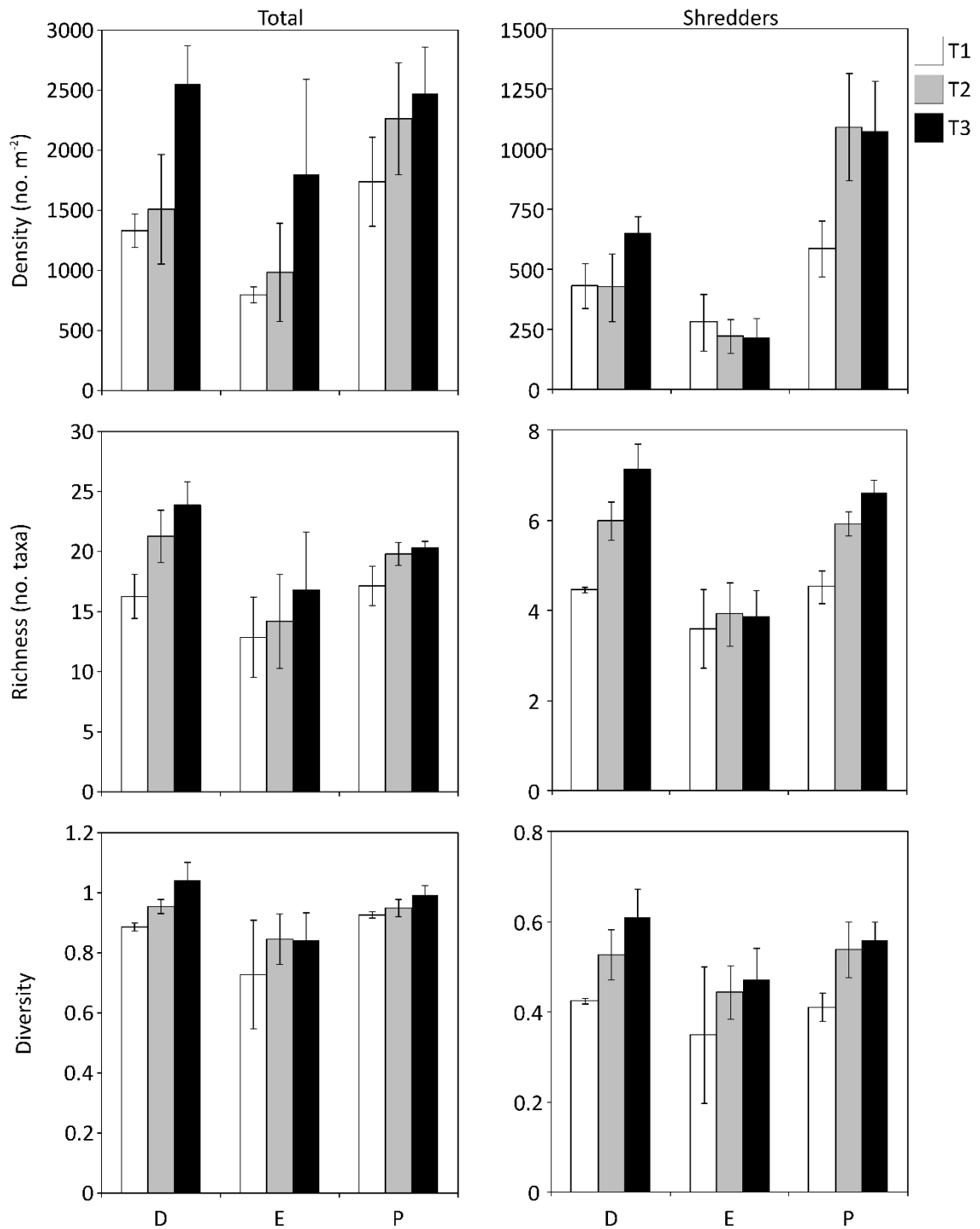


Fig. 2. Density, richness and Shannon diversity (mean \pm SE) of benthic total invertebrates and shredders for each vegetation type (D, deciduous forest; E, eucalypt plantation; P, pine plantation and samplings date (T1, T2 and T3).

Community structure based on taxonomic composition was significantly different among stream types (PERMANOVA, Pseudo- $F_{2,134} = 30.76$, $p < 0.001$; Fig. 3a), with pine streams different from the other catchment vegetation types (Fig. 3a, axis 1). The community

structure always changed through time (PERMANOVA, Pseudo- $F_{2,134} = 14.18$, $p < 0.001$), with the primary difference occurring between T1 and the other sampling dates (Fig. 3a, axis 2). The differences among stream types diminished over time (PERMANOVA, Pseudo- $F_{4,134} = 1.95$, $p < 0.001$). At T1, deciduous and eucalypt streams were characterised by the crustacean *Asellus* and pine streams by *Echinogammarus*, although oligochaetes were highly abundant in the three stream types (Table S1). Over time, the plecopteran *Capnioneura* and oligochaetes were abundant in deciduous and eucalypt streams, whereas detritivores of fine particles, such as the ephemeropterans *Habroleptoides* and *Heptagenia*, were more abundant in the pine streams (Table S1).

Although the spatial distribution of the structure of communities based on biological and ecological traits was different from the one based on taxonomy (Fig. 3a vs 2b), the macroinvertebrate community based on these traits also showed differences among stream types (PERMANOVA, Pseudo- $F_{2,134} = 28.22$, $p < 0.001$) and sampling times (PERMANOVA, Pseudo- $F_{2,134} = 18.69$, $p < 0.001$), with the differences among the stream types also more noticeable at the beginning of the study period (T1) (PERMANOVA, Pseudo- $F_{2,134} = 3.70$, $p < 0.001$), particularly between pine streams and the other two types.

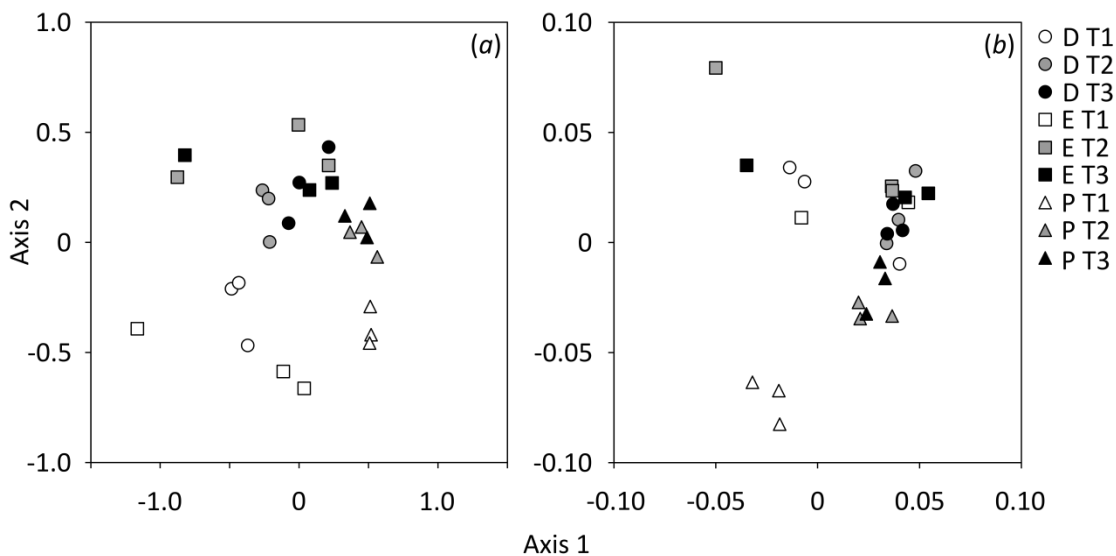


Fig. 3. Non-metric multidimensional scaling ordination (NMDS) of stream types and sampling dates (T1, T2 and T3) based on taxonomic (a) and biological and ecological traits (b) of benthic macroinvertebrate communities. The stream types are identified as follows: deciduous streams (D, circle), *Eucalyptus* streams (E, square) and *Pinus* streams (P, triangle). The sampling dates are identified as follows: T1 (white), T2 (grey) and T3 (black). One data of E T1 was excluded to facilitate NMDS visualization.

Of the 55 modalities included in the 10 traits assessed, 36 showed significant statistical differences ($p < 0.05$) among the stream types and/or a stream type \times sampling time interaction (Fig. 4). At T1, 15 modalities showed differences among stream types (Fig. 4). Stream communities under deciduous forest showed organisms with higher affinity for parasitic feeding habits, presence of a spiracle as a respiration form and aerial active dispersion than those from plantations. In the eucalypt streams, organisms with affinity for shorter lifecycles were highlighted. Under pine monocultures, the organisms showed affinity for traits poorly adapted to drought (e.g., absence of resistant forms) compared with deciduous and eucalypt streams. At T2, 14 modalities showed differences among the three types of streams (Fig. 4). Under deciduous forest, the organisms showed higher affinity for predatory feeding habits than those from streams under plantations. At T3, only 10 modalities differed among the three types of streams (Fig. 4). Streams under deciduous forests showed more affinities for small size organisms. Organisms with affinity for swimmer locomotion were higher in streams under pine plantations and lower under eucalypt plantations. Independent of vegetation type, all stream communities showed low affinity for slow current velocity.

With time, although the general trend was towards convergence of traits among the three stream types, four modalities remained differentiated from the other types for pine streams at all times (Fig. 4), which were higher affinity for medium size organisms ($F_{2,6} = 11.28$, $p = 0.009$) to the detriment of those of small size ($F_{2,6} = 70.24$, $p < 0.001$) and higher affinity for crawler organisms ($F_{2,6} = 16.60$, $p = 0.003$) as locomotion form to the detriment of those interstitial forms ($F_{2,6} = 9.54$, $p < 0.013$).

Organic matter processing and associated biota

In the fine mesh litterbags, the decomposition rate ranged from 0.58 % AFDM d^{-1} at P1 to 1.13 % AFDM d^{-1} at E1 (Fig. 5) and differed significantly among stream types ($F_{2,6} = 7.57$, $p = 0.023$), with the lowest rate in pine streams (Fig. 5). The total decomposition rate (due to both decomposers and detritivores) ranged from 1.43 % AFDM d^{-1} at E1 and E2 to 2.34 % AFDM d^{-1} at D2 (Fig. 5) and tended to be lower in eucalypt and higher in pine streams (Fig. 5), although the differences were not significant ($F_{2,6} = 4.45$, $p = 0.065$). Based on detritivore activity, the differences among stream types were statistically significant ($F_{2,6} = 5.63$, $p = 0.042$), with the lowest rate in eucalypt streams.

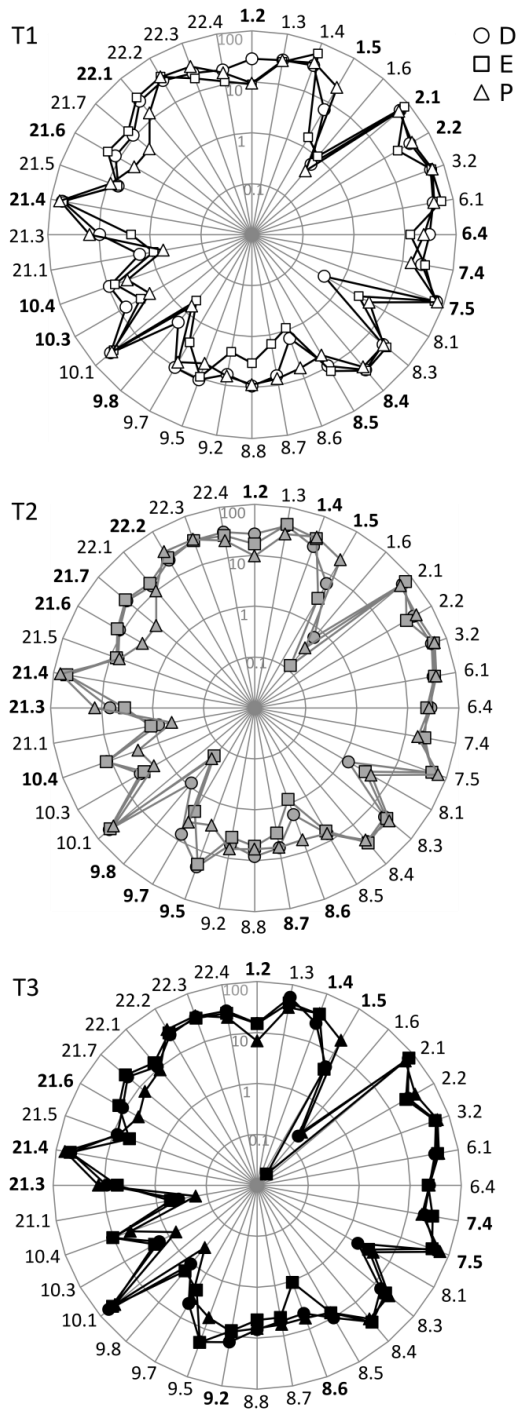


Fig.4. Mean relative frequency of the modalities of macroinvertebrate traits that present statistical differences ($p < 0.05$) among stream types or significant stream type \times sampling time interaction. The stream types are identified as follows: deciduous (D, circle), *Eucalyptus* (E, square) and *Pinus* (P, triangle). The sampling time are identified as follows: T1 white, T2 grey and T3 black. The modalities that present statistical differences among stream types are highlighted in bold (see Table 2 for code dictionary). Note the logarithmic scale.

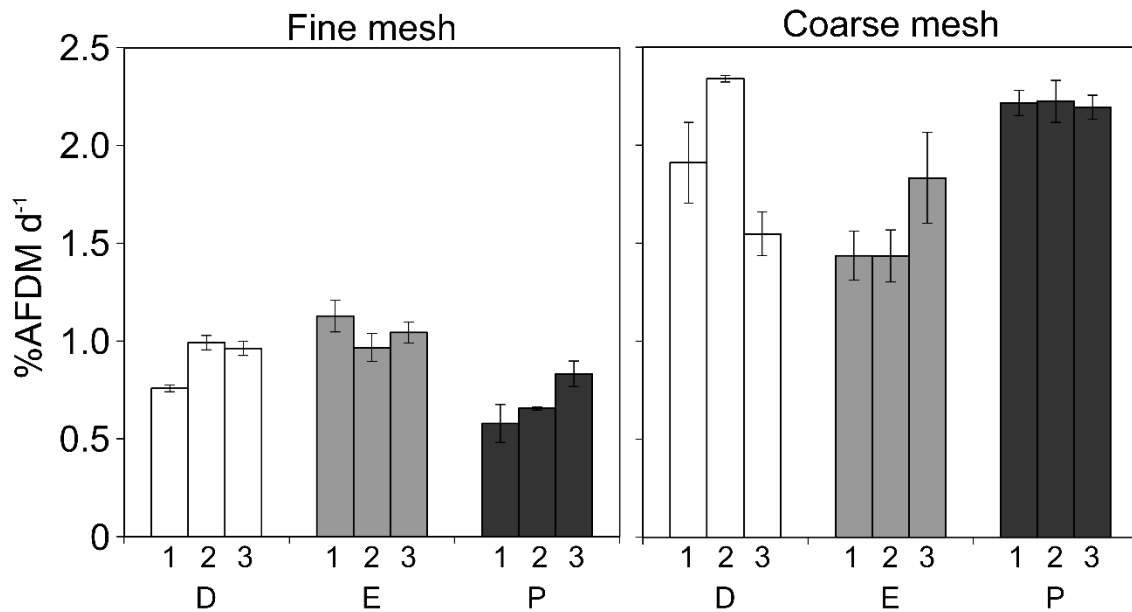


Fig.5. Decomposition rate (%AFDM d⁻¹, mean ± SE) in fine (left) and coarse mesh bags (right) for each studied stream. D, deciduous forest; E, eucalypt plantation; P, pine plantation. Numbers represent the three streams for each vegetation type.

A total of 1008 individuals were associated with coarse litterbags representing 43 taxa. Mean total density ranged from 8 ind bag⁻¹ at P1 to 51.6 ind bag⁻¹ at E3 (Fig. 6), and shredder density ranged from 3 ind bag⁻¹ at P1 to 12.2 ind bag⁻¹ at D3 (Fig. 6). However, these differences among stream types were not statistically significant ($F_{2,6} = 2.87$, $p = 0.134$; $F_{2,6} = 0.38$, $p = 0.702$, respectively). Total macroinvertebrate and shredder biomass ranged from 3.67 mg bag⁻¹ at D3 to 68.67 mg bag⁻¹ at E3 and from 0.84 mg bag⁻¹ at D3 to 56.58 mg bag⁻¹ at E3, respectively (Fig. 6), but these differences were not significant among stream types (total biomass: $F_{2,6} = 1.35$, $p = 0.327$; shredder biomass: $F_{2,6} = 1.39$, $p = 0.319$).

The total decomposition rate and the estimated rate due to detritivores were positively correlated with shredder density in the benthos (total rate: $R^2 = 0.53$, $p = 0.024$; detritivore rate: $R^2 = 0.55$, $p = 0.014$) but did not show any correlation with macroinvertebrates colonising bags.

Discussion

After the drought event, the recolonisation dynamics of the taxonomic and functional attributes of the benthic macroinvertebrate communities were dependent on the catchment vegetation. However, the expected differences in density, richness and diversity among stream types were not clearly evident. Nevertheless, eucalypt streams tended to show lower values for these variables. Moreover, contrary to our hypothesis, litter decomposition rate was apparently not dependent on vegetation type after the drought event, although rates due to microbial activity were the lowest in pine streams and detritivore activity diminished in eucalypt streams.

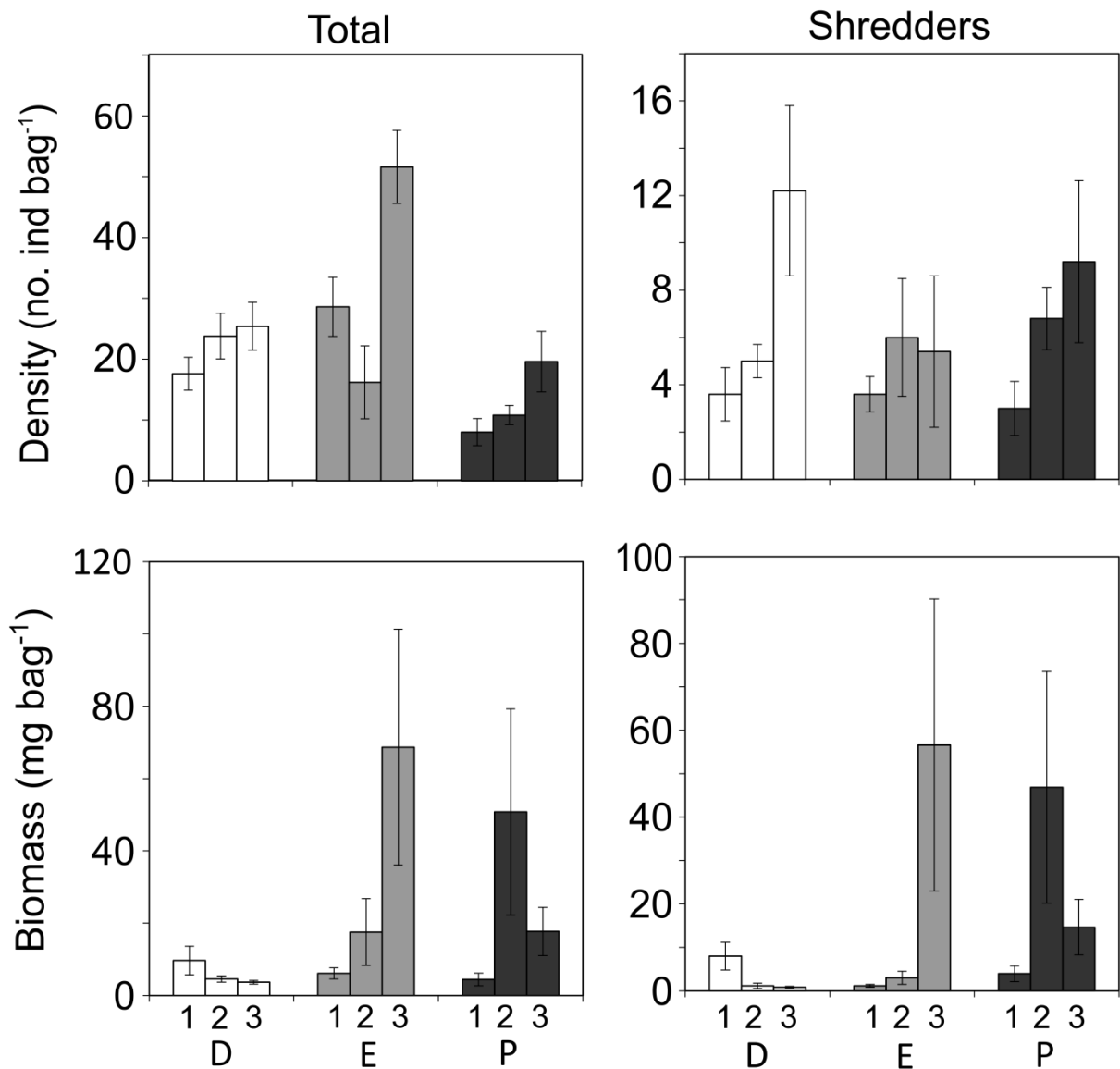


Fig. 6. Density and biomass (mean \pm SE) of total macroinvertebrates and shredders, respectively, associated with alder litter in coarse mesh litterbags. D, deciduous forest; E, eucalypt plantation; P, pine plantation. Numbers represent the three streams for each vegetation type.

Drought greatly reduces and modifies the stream habitat negatively affecting the density, richness and diversity of macroinvertebrate communities (Datry, 2012; Drummond et al., 2015; Stubbington et al., 2009). With the resumption of flow, invertebrates must recolonise the system, and the success of this process is dependent on the resistance and resilience of taxa and a “population source” from hyporheos (Vander Vorste et al., 2015, 2016) and nearby zones with favourable conditions (Chester and Robson, 2011; Sundermann et al., 2011). Nearby streams to those examined in this study flow through similar vegetation type and therefore the “community reservoir” both from hyporheos and nearby zones is conditioned by the same type of vegetation. The replacement of deciduous forest by monocultures of pine and eucalypt

implies a poorer quality of food source (hardness, low nutrients and high-toxic compounds) for stream macroinvertebrate detritivores (Casas et al., 2013; Girisha et al., 2003), which can often reduce the density, richness and diversity (Ferreira et al., 2015; Larrañaga et al., 2009b; Martínez et al., 2013). However, contrary to our hypothesis, this pattern was not consistent for all structural variables after the drought event. The three stream types had similar total macroinvertebrate densities, although pine streams showed the highest density of shredders. Increases in the storage of detritus during and immediately after drought events (Acuña et al., 2005) could promote higher macroinvertebrate densities because of the positive relationship between resource quantity and consumer density (Graça et al., 2004). Because the peak of leaf fall in this region occurs during autumn (González and Pozo, 1996), a prolonged summer period could result in an accumulation of benthic organic matter that would favour higher densities of macroinvertebrates after flow recovery. Although pine leaf litter is not a high-quality resource compared with broadleaves (Casas et al., 2013), the streams draining through these plantations were bordered by a narrow riparian strip sprinkled with native deciduous trees (e.g., alder) that, together with the narrow width of the streams, could have promoted the storage of this high-quality litter and partially mitigated the plantation effects (Ferreira et al., 2015). Similarly, richness and diversity were negatively affected by *Eucalyptus* plantations but not by those of *Pinus*, which could be related to the species composition of the riparian strip (Ferreira et al., 2016b; Swan and Palmer, 2006), because a more diverse strip can support a richer and more diverse invertebrate community (Ferreira et al., 2016b). In fact, riparian deciduous trees were less developed in eucalypt than in pine plantations likely because of the relatively shorter harvesting cycle of eucalypt compared with that of pine in the area (approximately 12-15-years vs 30-35 years, respectively). The community structure also reflected the vegetation effects on the taxonomic composition; with predominant taxa differing among stream types as also occurs in other perennial streams (Dolédec et al., 2011; Martínez et al., 2013). However, at the initial stage of flow recovery (T1), oligochaetes were abundant independent of the stream type, which is commonly observed because many species of these worms can resist long periods in the sediment as cocoons or cysts (Stubbington et al., 2016). Despite increasing values during the recolonisation period as the stream conditions were restored (Otermin et al., 2002; Stubbington et al., 2009), the densities of total macroinvertebrates remained similar and the differences in shredder density and total richness or diversity among the three stream types also remained. However, community structure changed with time attenuating differences among the stream types except for pine in which a different community remained throughout the process of recolonisation. Therefore, these differences suggested that the early stages of recolonisation dynamics in these streams were more influenced by stream types than later stages.

The recolonisation dynamics based on functional traits were also vegetation type- and time-dependent, with the differences among stream types diminishing over time. However, the

functional traits in stream types differed less than the species composition, which could be explained by functional redundancy (Boersma et al., 2014; Bogan et al., 2013) among the three stream types. For example, at the T3 sampling, organisms adapted to a fast current were predominant in the three stream types but the taxa composition was different, with the plecopteran *Capnioneura* dominating in deciduous and eucalypt streams and *Leuctra* in pine streams. Nevertheless, differences were also detected in some traits among stream types. At the early stage (T1), deciduous streams showed taxa characterised by traits related to high resistance and resilience capacity such as aerial dispersion, which facilitates the arrival of organisms after drought (Robson et al., 2013), or the presence of specialised structures such as a plastron or spiracle that maximise oxygen uptake and aerial respiration when flow ceases (Statzner and Bêche, 2010) (e.g., coleopterans *Hydraena* and *Elodes*). In eucalypt streams, macroinvertebrate communities were characterised by resilient taxa with short lifecycles (Bonada et al., 2007b; Datry, 2012), highlighted by the crustacean *Asellus*. Communities from pine streams showed traits *a priori* not advantageous to recolonise, with organisms without apparent resistant forms (early presence of the crustacean *Echinogammarus* and ephemeropterans *Habroleptoides* and *Heptagenia*), which suggests that the conditions of the hyporheic zone were more suitable than those of the other streams or the existence of nearly isolated pools (corroborated by personal observation) that could have served as shelter. However, deciduous streams showed signs of more complex community due to the presence of predators at T2, which may be related to the greater presence of preys than under the other two catchment vegetation types. At the T3 sampling, swimmer taxa increased under plantations and organisms adapted to a faster current predominated in the three stream types. Although a general trend towards the convergence of traits over time was observed among the three stream types, some traits in pine streams remained differentiated from the other streams during the recolonisation process. Pine streams were characterised by many crawling forms and few small size and interstitial taxa. Large size organisms tend to have longer lifecycles than small ones (Tachet et al., 2002), which could be a disadvantage in the recolonisation dynamics. Moreover, the lack of interstitial forms excavating to find shelter in the hyporheic zone reduces the resistance capacity of these communities against a severe drought period because interstitial form it is one of the primary mechanisms to resist these stress events (Bonada et al., 2007a; Robson et al., 2013). These aspects all highlight again that nearly isolated pools, rather than suitable conditions of hyporheic zone, acted as refuge areas for the selected pine streams favouring a more rapid re-establishment of the macroinvertebrate community.

The importance of detritivores and decomposers as drivers of the litter decomposition process changed among stream types, but notably, the total rate (due to both decomposers and detritivores) did not differ among the stream types after the drought event. This finding is contrast to our hypothesis, because *E. globulus* and *P. radiata* monocultures are documented

with slower leaf litter decomposition rates in permanent streams not affected by a drought event(Ferreira et al., 2015; Martínez et al., 2013).

Microbial-mediated decomposition differed among the stream types, with a lower rate in pine streams and with no differences between deciduous and eucalypt streams, as observed in previous works(Ferreira et al., 2016a). Moreover, the relative contribution of microorganisms to total decomposition rate was also low in pine streams (31%) compared with eucalypt streams (66.88 %), suggesting that detritivore-mediated decomposition was less important in eucalypt streams, consistent with the lower shredder density observed. However, by contrast, no major difference was expected in the litter decomposition rate from fine bags, because microbial activity is not greatly affected by plantations compared with deciduous streams(Ferreira et al., 2015) and apparently recovered quickly after the drought event(Datry et al., 2011). As highlighted above, decomposition in coarse bags was not vegetation type dependent, despite structural and functional differences in macroinvertebrate communities among vegetation types. However, the density of the total macroinvertebrates was similar among vegetation types, as already reported(Martínez et al., 2013), with shredders (litter consumers) the primary alteration at a lower density under monocultures(Larrañaga et al., 2009a; Martínez et al., 2013). In the present study, only *E. globulus* plantations negatively affected the density of benthic shredders, which was consistent with the lower detritivore activity in decomposition mentioned above for *E. globulus* streams. The importance of shredders to leaf litter decomposition was clear; both the total and detritivore decomposition rates were positively correlated with benthic shredder density, as frequently reported(Monroy et al., 2016; Pozo et al., 2011).

In general, our results indicated that the recolonisation dynamic of stream macroinvertebrates, based on both structural and functional attributes,after a drought event in perennial temperate streams differs depending on catchment vegetation type. Although the differences in density, richness and macroinvertebrate diversity were not as high as expected among vegetation types, eucalypt streams were more affected. By contrast, leaf-litter decomposition rate was clearly not dependent on catchment vegetation after drought, but detritivore activity was lowest under *E. globulus* monocultures, and in general, the abundance of shredders in the streambeds was the determinant of rates. Our results suggest a resilient capacity of these temperate streams after drought events, consistent with results reported elsewhere for streams subjected to drought(Mariluan et al., 2015), although streams draining through *E. globulus* plantations were affected to a greater extent. Nevertheless, these results are reported in streams under a temperate climate in which environmental moisture is high even during periods without precipitation and in which isolated pools can remain during droughts (in this case, particularly in pine streams). Future drought events are expected to be more recurrent and longer(Huang et al., 2016), which imply a likely greater effect on in-stream communities that

typically cope with a relatively less severe drought (Stubbington et al., 2016). Moreover, due to the lower diversity in streams affected by monocultures, structural and functional characteristics of in-stream communities could determine their capacity to cope with both drought and land-use change. Furthermore, because storm events are also likely to increase in the future, the recovery of the benthic community following drought disturbance could also be conditioned by previous flood episodes, despite the common occurrence of flood events in the studied streams and the ability of communities to recover from them. A greater understanding of the structural and functional responses to these stressors is necessary to forecast the effects in stream ecosystems affected by changes that are expected to be more intense and frequent under future climate change.

References

- Acuña, V., Muñoz, I., Giorgi, A., Omella, M., Sabater, F., Sabater, S., 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. *J. North Am. Benthol. Soc.* 24, 919–933.
- Albariño, R.J., Balseiro, E.G., 2002. Leaf litter breakdown in Patagonian streams: native versus exotic trees and the effect of invertebrate size. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 12, 181–192. doi:10.1002/aqc.511
- APHA, 1998. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC.
- Bärlocher, F., Graça, M.A.S., 2002. Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. *Freshw. Biol.* 47, 1123–1135. doi:10.1046/j.1365-2427.2002.00836.x
- Battin, T.J., Luysaert, S., Kaplan, L.A., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. *Nat. Geosci.* 2, 598–600.
- Boersma, K.S., Bogan, M.T., Henrichs, B.A., Lytle, D.A., 2014. Invertebrate assemblages of pools in arid-land streams have high functional redundancy and are resistant to severe drying. *Freshw. Biol.* 59, 491–501. doi:10.1111/fwb.12280
- Bogan, M.T., Boersma, K.S., Lytle, D.A., 2013. Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network. *Freshw. Biol.* 58, 1016–1028. doi:10.1111/fwb.12105

- Bonada, N., Dolédec, S., Stanzner, B., 2007a. Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Glob. Chang. Biol.* 13, 1658–1671. doi:10.1111/j.1365-2486.2007.01375.x
- Bonada, N., Rieradevall, M., Prat, N., 2007b. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia* 589, 91–106. doi:10.1007/s10750-007-0723-5
- Bond, N.R., Lake, P.S., Arthington, A.H., 2008. The impacts of drought on freshwater ecosystems: An Australian perspective. *Hydrobiologia* 600, 3–16. doi:10.1007/s10750-008-9326-z
- Casas, J., Larrañaga, A., Menéndez, M., Pozo, J., Basaguren, A., Martínez, A., Pérez, J., González, J.M., Mollá, S., Casado, C., Descals, E., Roblas, N., López-González, J.A., Luis Valenzuela, J., 2013. Leaf litter decomposition of native and introduced tree species of contrasting quality in headwater streams: How does the regional setting matter? *Sci. Total Environ.* 458-460, 197–208. doi:10.1016/j.scitotenv.2013.04.004
- Cheever, B.M., Kratzer, E.B., Webster, J.R., 2012. Immobilization and mineralization of N and P by heterotrophic microbes during leaf decomposition. *Freshw. Sci.* 31, 133–147.
- Chessman, B.C., 2015. Relationships between lotic macroinvertebrate traits and responses to extreme drought. *Freshw. Biol.* 60, 50–63. doi:10.1111/fwb.12466
- Chester, E.T., Robson, B.J., 2011. Drought refuges, spatial scale and recolonisation by invertebrates in non-perennial streams. *Freshw. Biol.* 56, 2094–2104. doi:10.1111/j.1365-2427.2011.02644.x
- Corti, R., Datry, T., Drummond, L., Larned, S.T., 2011. Natural variation in immersion and emersion affects breakdown and invertebrate colonization of leaf litter in a temporary river. *Aquat. Sci.* 73, 537–550.
- Datry, T., 2012. Benthic and hyporheic invertebrate assemblages along a flow intermittence gradient: effects of duration of dry events. *Freshw. Biol.* 57, 563–574. doi:10.1111/j.1365-2427.2011.02725.x
- Datry, T., Corti, R., Claret, C., Philippe, M., 2011. Flow intermittence controls leaf litter breakdown in a French temporary alluvial river: the “drying memory.” *Aquat. Sci.* 73, 471–483.
- Dolédec, S., Phillips, N., Townsend, C., 2011. Invertebrate community responses to land use at a broad spatial scale: Trait and taxonomic measures compared in New Zealand rivers.

- Freshw. Biol. 56, 1670–1688. doi:10.1111/j.1365-2427.2011.02597.x
- Drummond, L.R., McIntosh, A.R., Larned, S.T., 2015. Invertebrate community dynamics and insect emergence in response to pool drying in a temporary river. *Freshw. Biol.* n/a–n/a. doi:10.1111/fwb.12591
- Elosegi, A., Basaguren, A., Pozo, J., 2006. A functional approach to the ecology of Atlantic Basque streams. *Limnetica* 25, 123–134.
- Ferreira, V., Castela, J., Rosa, P., Tonin, A.M., Boyero, L., Graça, M.A.S., 2016a. Aquatic hyphomycetes, benthic macroinvertebrates and leaf litter decomposition in streams naturally differing in riparian vegetation. *Aquat. Ecol.* 50, 1–15. doi:10.1007/s10452-016-9588-x
- Ferreira, V., Koricheva, J., Pozo, J., Graça, M.A.S., 2016b. A meta-analysis on the effects of changes in the composition of native forests on litter decomposition in streams. *For. Ecol. Manage.* 364, 27–38. doi:10.1016/j.foreco.2016.01.002
- Ferreira, V., Larrañaga, A., Gulis, V., Basaguren, A., Elosegi, A., Graça, M.A.S., Pozo, J., 2015. The effects of eucalypt plantations on plant litter decomposition and macroinvertebrate communities in Iberian streams. *For. Ecol. Manage.* 335, 129–138.
- Flores, L., Díez, J.R., Larrañaga, A., Pascoal, C., Elosegi, A., 2013. Effects of retention site on breakdown of organic matter in a mountain stream. *Freshw. Biol.* 58, 1267–1278.
- García-Roger, E.M., Sánchez-Montoya, M.D.M., Cid, N., Erba, S., Karaouzas, I., Verkaik, I., Rieradevall, M., Gómez, R., Suárez, M.L., Vidal-abarca, M.R., Demartini, D., Buffagni, A., Skoulikidis, N., Bonada, N., Prat, N., 2013. Spatial scale effects on taxonomic and biological trait diversity of aquatic macroinvertebrates in Mediterranean streams. *Fundam. Appl. Limnol.* 183, 89–105. doi:10.1127/1863-9135/2013/0429
- Gasith, A., Resh, V.H., 1999. Streams in mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Evol. Syst.* 51–81.
- Girisha, G.K., Condrón, L.M., Clinton, P.W., Davis, M.R., 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. *For. Ecol. Manage.* 179, 169–181. doi:10.1016/S0378-1127(02)00518-2
- González, E., Pozo, J., 1996. Longitudinal and temporal patterns of benthic coarse particulate organic matter in the Agüera stream (northern Spain). *Aquat. Sci.* 58, 355–366.
- Graça, M. a. S., Pinto, P., Cortes, R., Coimbra, N., Oliveira, S., Morais, M., Carvalho, M.J., Malo, J., 2004. Factors Affecting Macroinvertebrate Richness and Diversity in Portuguese

- Streams: a Two-Scale Analysis. *Int. Rev. Hydrobiol.* 89, 151–164.
doi:10.1002/iroh.200310705
- Graça, M.A.S., 2001. The role of invertebrates on leaf litter decomposition in streams—a review. *Int. Rev. Hydrobiol.* 86, 383–393. doi:10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D
- Hershkovitz, Y., Gasith, A., 2013. Resistance, resilience, and community dynamics in mediterranean-climate streams. *Hydrobiologia* 719, 59–75.
- Huang, S., Huang, Q., Chang, J., Leng, G., 2016. Linkages between hydrological drought, climate indices and human activities: a case study in the Columbia River basin. *Int. J. Climatol.* 36, 280–290.
- IPCC, 2014. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK, and New York, NY, USA.
- Kominoski, J.S., Marczak, L., Richardson, J.S., 2010. Riparian Forest Composition Affects Stream Litter Decomposition Despite Similarities in Microbial and Invertebrate Communities. *Ecology* 92, 151–159. doi:10.1890/10-0028.1
- Kominoski, J.S., Shah, J.J.F., Canhoto, C., Fischer, D.G., Giling, D.P., González, E., Griffiths, N.A., Larrañaga, A., LeRoy, C.J., Mineau, M.M., others, 2013. Forecasting functional implications of global changes in riparian plant communities. *Front. Ecol. Environ.* 11, 423–432.
- Lake, P.S., 2011. *Drought and aquatic ecosystems: effects and responses.* John Wiley & Sons.
- Langhans, S.D., Tockner, K., 2006. The role of timing, duration, and frequency of inundation in controlling leaf litter decomposition in a river-floodplain ecosystem (Tagliamento, northeastern Italy). *Oecologia* 147, 501–509.
- Larrañaga, A., Basaguren, A., Elozegi, A., Pozo, J., 2009. Impacts of *Eucalyptus globulus* plantations on Atlantic streams: changes in invertebrate density and shredder traits. *Fundam. Appl. Limnol. für Hydrobiol.* 175, 151–160.
- Larrañaga, A., Basaguren, A., Pozo, J., 2009. Impacts of *Eucalyptus globulus* Plantations on Physiology and Population Densities of Invertebrates Inhabiting Iberian Atlantic Streams. *Int. Rev. Hydrobiol.* 94, 497–511. doi:10.1002/iroh.200811156
- Leigh, C., Bonada, N., Boulton, A.J., Hugueny, B., Larned, S.T., Vander Vorste, R., Datry, T.,

2016. Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. *Aquat. Sci.* doi:10.1007/s00027-015-0427-2
- Mariluan, G.D., Villanueva, V.D., Albariño, R.J., 2015. Leaf litter breakdown and benthic invertebrate colonization affected by seasonal drought in headwater lotic systems of Andean Patagonia. *Hydrobiologia* 760, 171–187.
- Martínez, A., Larrañaga, A., Miguélez, A., Yvon-Durocher, G., Pozo, J., 2016. Land use change affects macroinvertebrate community size spectrum in streams: The case of *Pinus radiata* plantations. *Freshw. Biol.* 61, 69–79. doi:10.1111/fwb.12680
- Martínez, A., Larrañaga, A., Pérez, J., Descals, E., Basaguren, A., Pozo, J., 2013. Effects of pine plantations on structural and functional attributes of forested streams. *For. Ecol. Manage.* 310, 147–155.
- Martínez, A., Pérez, J., Molinero, J., Sagarduy, M., Pozo, J., 2015. Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. *Sci. Total Environ.* 503, 251–257.
- Milly, P.C.D., Dunne, K.A., Vecchia, A. V, 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* 438, 347–350. doi:10.1038/nature04312
- Monroy, S., Menéndez, M., Basaguren, A., Pérez, J., Elozegi, A., Pozo, J., 2016. Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain). *Sci. Total Environ.* 573, 1450–1459. doi:10.1016/j.scitotenv.2016.07.209
- Otermin, A., Basaguren, A., Pozo, J., 2002. Re-colonization by the macroinvertebrate community after a drought period in a first-order stream (Agüera Basin, northern Spain). *Limnetica* 21, 117–128.
- Papadaki, C., Soulis, K., Muñoz-Mas, R., Martinez-Capel, F., Zogaris, S., Ntoanidis, L., Dimitriou, E., 2016. Potential impacts of climate change on flow regime and fish habitat in mountain rivers of the south-western Balkans. *Sci. Total Environ.* 540, 418–428.
- Perkins, D.M., Reiss, J., Yvon-Durocher, G., Woodward, G., 2010. Global change and food webs in running waters. *Hydrobiologia* 657, 181–198.
- Pozo, J., Casas, J., Menéndez, M., Mollá, S., Arostegui, I., Basaguren, A., Casado, C., Descals, E., García-Avilés, J., González, J.M., Larrañaga, A., López, E., Lusi, M., Moya, O., Pérez, J., Riera, T., Roblas, N., Salinas, M.J., 2011. Leaf-litter decomposition in headwater streams: a comparison of the process among four climatic regions. *J. North Am. Benthol. Soc.* 30, 935–950. doi:10.1899/10-153.1

- Pozo, J., Gonzalez, E., Díez, J., Molinero, J., Elósegui, A., 1997. Inputs of particulate organic matter to streams with different riparian vegetation. *J. North Am. Benthol. Soc.* 16: 602–611.
- R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Robson, B.J., Chester, E.T., Mitchell, B.D., Matthews, T.G., 2013. Disturbance and the role of refuges in mediterranean climate streams. *Hydrobiologia* 719, 77–91. doi:10.1007/s10750-012-1371-y
- Sridhar, K.R., Bärlocher, F., 1993. Aquatic hyphomycetes on leaf litter in and near a stream in Nova Scotia, Canada. *Mycol. Res.* 97, 1530–1535. doi:10.1016/S0953-7562(09)80229-3
- Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshw. Biol.* 55, 80–119. doi:10.1111/j.1365-2427.2009.02369.x
- Stubbington, R., Gunn, J., Little, S., Worrall, T.P., Wood, P.J., 2016. Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream. *Freshw. Biol.* 61, 1293–1307. doi:10.1111/fwb.12770
- Stubbington, R., Wood, P.J., Boulton, A.J., 2009. Low flow controls on benthic and hyporheic macroinvertebrate assemblages during supra-seasonal drought. *Hydrol. Process.* 23, 2252–2263. doi:10.1002/hyp.7290
- Sundermann, A., Stoll, S., Haase, P., 2011. River restoration success depends on the species pool of the immediate surroundings. *Ecol. Appl.* 21, 1962–1971. doi:10.1890/10-0607.1
- Swan, C.M., Palmer, M. a, 2006. Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. *Oecologia* 147, 469–78. doi:10.1007/s00442-005-0297-8
- Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, F., 2002. *Invertébrés d'eau douce. Systematique, biologie, écologie.* Paris, France.
- Tank, J., Rosi-Marshall, E., Griffiths, N. a., Entekin, S. a., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. North Am. Benthol. Soc.* 29, 118–146. doi:10.1899/08-170.1
- Vander Vorste, R., Malard, F., Datry, T., 2015. Is drift the primary process promoting the resilience of river invertebrate communities? A manipulative field experiment in an intermittent alluvial river. *Freshw. Biol.* doi:10.1111/fwb.12658

- Vander Vorste, R., Mermillod-Blondin, F., Hervant, F., Mons, R., Forcellini, M., Datry, T., 2016. Increased depth to the water table during river drying decreases the resilience of *Gammarus pulex* and alters ecosystem function. *Ecohydrology*. doi:<http://10.1002/2co.1716>
- Vicente-Serrano, S.M., Lopez-Moreno, J.I., Beguería, S., Lorenzo-Lacruz, J., Sanchez-Lorenzo, A., García-Ruiz, J.M., Azorin-Molina, C., Morán-Tejeda, E., Revuelto, J., Trigo, R., Coelho, F., Espejo, F., 2014. Evidence of increasing drought severity caused by temperature rise in southern Europe. *Environ. Res. Lett.* 9. doi:10.1088/1748-9326/9/4/044001
- Wallace, J.B., Eggert, S.L., Meyer, J.L., Webster, J.R., 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* (80-.). 277, 102–104. doi:10.1017/CBO9781107415324.004
- Zar, J.H., 2010. *Biostatistical Analysis*, 5th edn. ed. Prentice Hall, Upper Saddle River, USA.

Supplementary material

Table S1. Density of invertebrate taxa (no. m⁻²; mean ± standard error) for each vegetation type (D = deciduous streams, E= *Eucalypt* streams, P= *Pine* streams) and samplingtime (T1, T2 and T3). Only taxa with densities > 1% are listed.

	D			E			P		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
<i>Capnioneura</i>	3.0 ± 3.0	200.7 ± 28.7	74.8 ± 18.4	0	118.5 ± 27.0	76.3 ± 30.6	0	111.1 ± 24.9	31.1 ± 9
<i>Leuctra</i>	11.9 ± 7.3	20.0 ± 8.8	340.7 ± 64.5	20.0 ± 6.1	12.6 ± 4.5	43.0 ± 14.6	26.7 ± 13.8	16.3 ± 5.7	196.3 ± 100
<i>Protonemura</i>	0	1.5 ± 1.0	21.5 ± 8.7	19.3 ± 8.4	14.8 ± 4.4	25.9 ± 9.9	7.4 ± 4.3	44.4 ± 12.3	60.7 ± 17.5
<i>Nemoura</i>	127.4 ± 51.2	17.8 ± 5.1	58.5 ± 20.4	7.4 ± 2.8	0.7 ± 0.7	8.1 ± 3.8	151.9 ± 32.1	215.6 ± 48.3	217.8 ± 47.7
<i>Asellus</i>	224.4 ± 68.1	105.9 ± 38.1	57.0 ± 18.2	217.8 ± 93.4	59.3 ± 12.5	51.9 ± 16.5	0	1.5 ± 1.0	0
<i>Echinogammarus</i>	1.5 ± 1.0	5.2 ± 3.7	28.9 ± 7.7	0	0	2.2 ± 1.6	348.9 ± 77.1	588.1 ± 198.7	454.1 ± 96.8
<i>Limnephilinae</i>	38.5 ± 10.9	48.9 ± 10.0	44.4 ± 10.7	0.7 ± 0.7	8.9 ± 4.2	6.7 ± 2.1	11.1 ± 5.8	99.3 ± 24.9	34.8 ± 8.0
<i>Siphonoperla</i>	0.7 ± 0.7	5.2 ± 3.7	31.9 ± 11.5	0.7 ± 0.7	11.1 ± 5.4	33.3 ± 9.1	12.6 ± 4.2	17.0 ± 4.7	77.0 ± 21.6
<i>Polycelis</i>	0	0.7 ± 0.7	2.2 ± 1.6	3.7 ± 1.4	2.2 ± 1.6	9.6 ± 5.5	57.0 ± 16.4	51.9 ± 11.7	35.6 ± 11.8
<i>Habroleptoides</i>	1.5 ± 1.0	3.0 ± 1.7	1.5 ± 1.5	125.9 ± 39.4	240.7 ± 120.5	199.3 ± 59.7	389.6 ± 114.6	340.7 ± 70.4	338.5 ± 55.4
<i>Heptagenia</i>	0.7 ± 0.7	40.0 ± 10.5	86.7 ± 32.7	5.2 ± 2.4	40.0 ± 13.5	215.6 ± 48.2	181.5 ± 34.1	357.8 ± 77.7	345.2 ± 46.7
<i>Oligochaeta</i>	380.0 ± 60.5	440.0 ± 103.8	478.5 ± 126.8	113.3 ± 34.2	101.5 ± 23.6	214.1 ± 64.8	174.8 ± 28.1	117.8 ± 40.7	184.4 ± 56.2
<i>Tanytarsini</i>	14.8 ± 6.4	20.7 ± 7.0	111.1 ± 34.8	6.7 ± 2.8	2.2 ± 1.6	4.4 ± 1.8	31.9 ± 12.6	4.4 ± 1.8	3.7 ± 2.6
<i>Orthocladinae</i>	43.7 ± 13.4	27.4 ± 6.9	97.8 ± 33.5	7.4 ± 3.2	6.7 ± 3.4	65.2 ± 21.6	51.9 ± 33.6	9.6 ± 4.0	31.1 ± 10.7
<i>Baetis</i>	0	7.4 ± 2.6	94.1 ± 21.2	1.5 ± 1.0	17.8 ± 6.9	13.3 ± 4.8	8.9 ± 2.9	15.6 ± 5.2	42.2 ± 11.3
<i>Hydraena</i>	66.7 ± 17.6	28.1 ± 6.2	25.9 ± 7.6	13.3 ± 4.4	11.1 ± 3.4	17.0 ± 5.6	14.8 ± 4.9	30.4 ± 20.6	11.9 ± 3.0
<i>Simuliidae</i>	65.2 ± 19.8	262.2 ± 56.3	531.1 ± 155	77.0 ± 20.3	203.0 ± 40.9	584.4 ± 179.6	38.5 ± 20.4	57.0 ± 19.0	155.6 ± 37.2
<i>Agapetus</i>	0	7.4 ± 3.2	117.0 ± 22.7	0	42.2 ± 14.4	111.9 ± 39.2	0	29.6 ± 9.9	72.6 ± 15.3
<i>Ancylus</i>	10.4 ± 4.0	5.9 ± 2.6	7.4 ± 3.2	94.1 ± 23.7	19.3 ± 7.4	21.5 ± 7.1	1.5 ± 1.0	5.2 ± 3.0	0.7 ± 0.7
<i>Elodes</i>	124.4 ± 32.3	72.6 ± 11.6	31.1 ± 5.6	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.7	15.6 ± 5.4	26.7 ± 6.6	14.8 ± 4.4

CHAPTER 5

Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain)

This chapter was published with the following reference: Monroy S., Menéndez M., Basaguren A., Pérez J., Elósegi A. & Pozo J. 2016. Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain). *Science of The Total Environment* (2016) 573: 1450-1459

Abstract

Drought, an important environmental factor affecting the functioning of stream ecosystems, is likely to become more prevalent in the Mediterranean region as a consequence of climate change and enhanced water demand. Drought can have profound impacts on leaf litter decomposition, a key ecosystem process in headwater streams, but there is still limited information on its effects at the regional scale. We measured leaf litter decomposition across a gradient of aridity in the Ebro River basin. We deployed coarse- and fine-mesh bags with alder and oak leaves in 11 Mediterranean calcareous streams spanning a range of over 400 km, and determined changes in discharge, water quality, leaf-associated macroinvertebrates, leaf quality and decomposition rates. The study streams were subject to different degrees of drought, specific discharge ($L s^{-1} km^{-2}$) ranging from 0.62 to 9.99. One of the streams dried out during the experiment, another one reached residual flow, whereas the rest registered uninterrupted flow but with different degrees of flow variability. Decomposition rates differed among sites, being lowest in the 2 most water-stressed sites, but showed no general correlation with specific discharge. Microbial decomposition rates were not correlated with final nutrient content of litter nor to fungal biomass. Total decomposition rate of alder was positively correlated to the density and biomass of shredders; that of oak was not. Shredder density in alder bags showed a positive relationship with specific discharge during the decomposition experiment. Overall, the results point to a complex pattern of litter decomposition at the regional scale, as drought affects decomposition directly by emersion of bags and indirectly by affecting the functional composition and density of detritivores.

Keywords: climate change, litter breakdown, ecosystem functioning, water stress, leaf quality

Introduction

Global climate models forecast widespread shifts in temperature and precipitation patterns in the next decades, including increased temperature, reduced rainfall and higher frequency of extreme climate events in the Mediterranean area (IPCC, 2014). Because of the linkages between climate and hydrological processes (Papadaki et al., 2016), these changes are expected to affect flow regime in different ways, such as reducing average flow or increasing the frequency and magnitude of extreme flow events (Huang et al., 2016).

Flow is a key driver of the structure and function of aquatic ecosystems, as it affects water quality, physical habitat, energy resources and biotic interactions (Allan and Castillo, 2007; Dewson et al., 2007; Poff et al., 1997). Drought, in particular, is an important environmental stressor for freshwater ecosystems (Sabater, 2008). It reduces water velocity and depth, reduces hydrological connectivity, promotes sedimentation (Dewson et al., 2007), alters water physicochemical conditions (Schäfer et al., 2012; von Schiller et al., 2011) and affects the inputs, storage and quality of organic matter (Sanpera-Calbet et al., 2015; Ylla et al., 2010). Therefore, it can affect not only stream biological assemblages (Bonada et al., 2007; Filipe et al., 2013), but also ecosystem processes (Acuña et al., 2005; Martínez et al., 2015). Semiarid regions such as the Mediterranean are particularly vulnerable to drought disturbances (Milly et al., 2005; Sabater and Tockner, 2010). The Mediterranean climate is characterized by high inter-annual variability and pronounced seasonality with hot, dry summers (Gasith and Resh, 1999),

and climate models forecast reduced precipitation and more severe drought events (Milly et al., 2005). Therefore, to predict the consequences of oncoming climate change, it is important to understand the effects of drought on river ecosystem functioning.

Organic matter decomposition is a key process which transfers energy and matter across trophic levels (Perkins et al., 2010), controls nutrient cycling (Cheever et al., 2012) and contributes greatly to the global carbon cycle (Battin et al., 2009; Kominoski and Rosemond, 2012). It is a complex process that includes the leaching of soluble compounds, physical abrasion, microbial conditioning and invertebrate fragmentation, and which depends on a complex array of both intrinsic (e.g. litter quality) and extrinsic (e.g. temperature, dissolved nutrients, discharge) factors (Tank et al., 2010). Therefore, decomposition rate has been proposed as an integrative indicator of ecosystem functional status (Gessner and Chauvet, 2002; Young et al., 2008). Previous studies have shown decomposition in streams to occur more slowly during periods of residual flow (Leberfinger et al., 2010; Mora-Gómez et al., 2016), being also slower in temporary than in perennial streams (Langhans and Tockner, 2006), and the effects of drying events to extend long after flow resumption (Datry et al., 2011). Nevertheless, most information so far existing on the effects of drought on litter decomposition derives from studies at a few sites (Martínez et al., 2015) and there is not yet a clear consensus on whether the patterns observed also hold for larger, regional scales subject to other confounding factors.

Additionally, it is still unclear whether the effects of drought differ among groups of consumers. Reduced decomposition rates in temporary streams have been attributed to reduced macroinvertebrate activity (Langhans and Tockner, 2006; Martínez et al., 2015), whereas microbial decomposition seems to recover from drought faster than invertebrate activities (Datry et al., 2011), although fungi and bacteria also differ in their sensitivity to environmental stress (Mora-Gómez et al., 2016). Also, it is still unclear whether the effects of drought differ among leaf species. Leaf species show a wide range of degradability (Petersen and Cummins, 1974), determined in part by their contents in nutrients and structural molecules such as lignin (Hladyz et al., 2009), although intra-specific variations can be large (Graça and Poquet, 2014). The most palatable leaves can be readily consumed by shredding invertebrates, whereas the decomposition of more recalcitrant species is more driven by microbes (Martínez et al., 2016). Therefore, it is likely that the differential impact of drought on microbes and invertebrates could result in contrasting effects on the decomposition of different leaf species.

The aim of our study was to examine the effect of drought on leaf litter decomposition across a regional gradient of aridity, to check whether microbial or detritivore activities are more affected, and whether the effects are consistent for leaves with contrasting quality. We performed a decomposition experiment with a fast-decaying (alder) and a slow-decaying species

(oak) along an aridity gradient in 11 streams in the Ebro river basin (Spain). We hypothesized that 1) drought reduces the decomposition rate; 2) detritivore activity is more affected than microbial activity; 3) fast-decaying, high-quality leaf litter is more affected than slow-decaying, low-quality one; 4) differences in decomposition rate between species are higher where detritivore activity is most affected.

Methods

Study sites

We performed a first screening of potential sites for experiments in the Ebro basin (Spain) by checking the information on hydrology and ecological status available at the Ebro Hydrographic Confederation (CHE; <http://www.chebro.es>). We selected streams of the Mediterranean calcareous mountain typology, which are characterised by limestone substrate, catchment slope >2%, specific discharge (Q_s) < 16.5 L s⁻¹ km⁻² and conductivity > 320 μS cm⁻¹ (CEDEX, 2004; CHE). In spring 2014, we visited over 40 calcareous mountain streams with good ecological status according to the monitoring network of CHE, where we analyzed the physical habitat and determined basic physico-chemical characteristics. Based on all this information, we performed a principal component analysis from which we selected 11 sites with similar habitat and water quality but spatially distributed along a precipitation gradient, which ranged from 311.2 up to 621.4 mm y⁻¹, with average annual temperature ranging from 8.2 to 14.2 °C (Fig. 1; Table 1). The sites spanned a distance of over 400 km. Given that inter-annual variability of the Mediterranean climate is high and hydrological conditions in a given period can depart markedly from historic averages, we used the accumulated rainfall for a year prior to sampling as a surrogate of the climate conditions at the sampling site. We used the specific discharge (Q_s), calculated as the mean annual discharge per unit catchment area (L s⁻¹ km⁻²) (Munné and Prat, 2004) as a proxy for drought. Moreover, we also calculated the monthly coefficient of variation of water flow (CV_Q) as the standard deviation of monthly flow divided by annual average flow, since it yields information about intra-annual flow variability. All flow variables were calculated using daily mean flows from a common 10-y period (Q_{s10} ; CV_{Qs10}) and from 1-y prior to the study period ($Q_{s13/14}$; $CV_{Qs13/14}$) (Table 1). Daily mean flow values for all study sites were obtained from nearby gauging stations for the period 2005- 2015 (data from CHE; <http://www.chebro.es>).

Environmental variables

During the study period (autumn-winter 2014-2015), water temperature was recorded hourly with two ACR Smart-Button temperature loggers (ACR Systems Inc) at each site placed

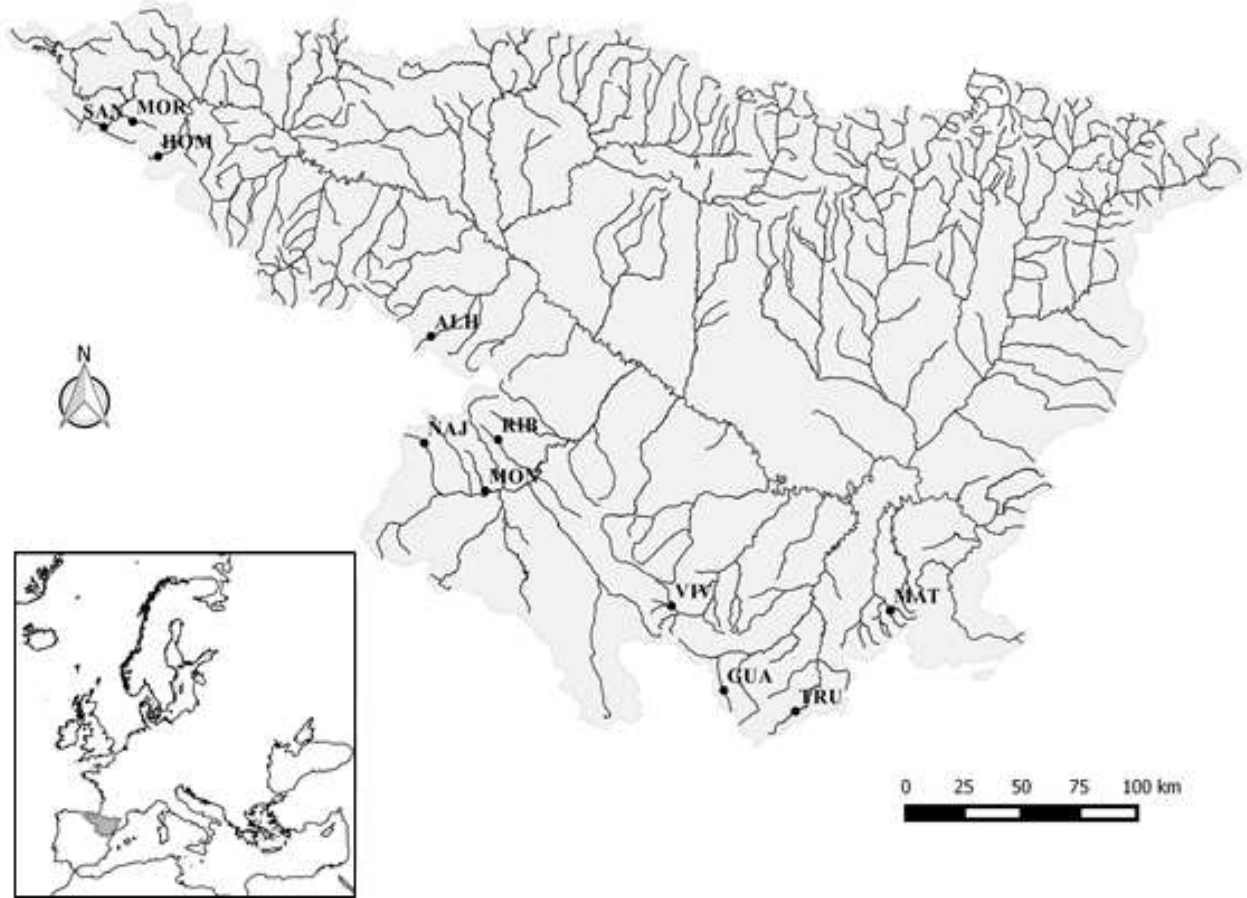


Fig. 1. Location of the study sites.

at different depths, thus also allowing to detect reductions in flow. Conductivity, pH and oxygen saturation were measured on three occasions with a multiparametric sensor (WTW Multi 350i). Discharge was estimated on these occasions from instantaneous water velocity measured by a current metre (MiniAir 2, Schiltknecht Co). Furthermore, following the calculation procedure in the precedent section, Q_s and CV_Q were determined for the experimental period ($Q_{s,exp}$, $CV_{Qs,exp}$) for both total and microbial decomposition periods. Water samples for nutrient analyses were collected at all streams on each sampling date, immediately filtered (Millipore, 0.45 μm pore) and frozen ($-20\text{ }^\circ\text{C}$). Nitrate, chlorine and sulphate concentration were determined by capillary ion electrophoresis (Agilent CE), and the rest of the nutrients were analyzed colorimetrically (Spectrophotometer Jasco V-630): nitrite by the sulphanilamide method, ammonium by the salicylate and dichloroisocyanurate method and dissolved reactive phosphorus (SRP) by the ascorbic acid method (APHA, 1998). Dissolved inorganic nitrogen (DIN) was computed as the sum of nitrate, nitrite and ammonium.

Table 1. Location, reach characterization and hydrological and climatic attributes for all studied streams. Streams arranged along the longitudinal gradient W-E. For air temperature, mean values and their range (in parenthesis) are shown. Data for hydrological variables of SAN and MOR and NAJ and MON were estimated from the same gauging station. Specific discharge (Q_s) and monthly coefficient of variation of water flow (CV_Q) from a common 10-y period (Q_{S10} ; CV_{Qs10}) and from 1-y prior to the study period ($Q_{S13/14}$; $CV_{Qs13/14}$)

	SAN	MOR	HOM	ALH	NAJ	MON	RIB	VIV	GUA	TRU	MAT
Latitude (N)	42° 41'06.5"	42° 42'24.9"	42° 34'36.5"	41° 53'56.5"	41° 29'54.1"	41° 18'57.0"	41° 30'28.3"	40° 52'12.2"	40° 32'52.8"	40° 27' 42.0"	40° 49' 37.0"
Longitude (W)	3° 52'46.3"	3° 43'33.8"	3° 35'20.4"	2° 09'34.5"	2° 11'58.5"	1° 53'11.5"	1° 48'59.9"	0° 56'12.8"	0° 40'48.1"	0° 18' 57.6"	0° 11' 07.8" E
Altitude (m a.s.l.)	764	786	755	920	918	661	822	954	1251	1124	565
Basin area (km ²)	47.9	59.1	55.6	45.0	41.5	88.2	54.0	45.3	55.9	165.0	48.7
Width (m)	3.3 ± 0.7	3.0 ± 0.34	2.9 ± 0.5	3.5 ± 0.9	1.9 ± 0.3	2.1 ± 0.8	2.9 ± 0.5	4.9 ± 1.2	1.7 ± 0.2	6.7 ± 1.8	7.6 ± 0.8
<i>Riparian vegetation</i>											
Tree canopy cover(%)	83	62	93	73	79	33	72	30	46	1.6	24
<i>Substrate composition(%)</i>											
Boulder (>256 mm)	20	5	5	20	(+)		(+)	20	5	30	5
Cobble (64-256 mm)	50	40	10	60	10		30	0	45	45	40
Pebble (16-64 mm)	10	35	5	5	40		50	0	40	20	45
Gravel (2-16 mm)	10	10	5	10	25		10	0	5	5	10
Sand (<2 mm)	10	10	5	5	25	100	10	80	5	0	0
Travertine substrate		70									
<i>Hydrological variables</i>											
Q_{S10} (L s ⁻¹ km ⁻²)	9.99	9.99	3.41	1.3	0.62	0.62	2.96	0.66	5.73	1.09	6.58
CV_{Qs10}	0.72	0.72	0.62	0.81	0.1	0.1	0.46	0.63	0.49	1.19	0.59
$Q_{S13/14}$ (L s ⁻¹ km ⁻²)	10.08	10.08	3.21	0.84	0.69	0.69	2.58	0.67	5.25	0.31	4.18
$CV_{Qs13/14}$	0.94	0.94	0.78	0.71	0.16	0.16	0.27	0.45	0.48	0.74	0.4
<i>Climatic variables</i>											

Annual mean air temperature (°C)	8.2 (3.3-14.4)	10.0 (3.3-14.4)	12.5 (7.3- 17.6)	10.6 (6.9-14.9)	12.4 (5.9- 19.4)	8.9 (5.1- 13.9)	13.4 (4.7 -17.0)	13.8 (8.5-17.6)	10.5 (4.4- 16.4)	9.7 (6.2-21.4)	14.2 (8.1- 19.5)
Annual mean precipitation from 2005 to 2015 (mm)	621.4	543	503.9	379.6	311.2	349	419.3	408.5	451.8	509.5	508.5
Coefficient of variation of monthly precipitation (%)	43.4	49	44.7	35.9	42.9	40.7	37.1	43.5	42.1	43.8	50.3
Annual mean precipitation 2013/14 (mm)	523.2	476	521.4	386.6	266.7	401.8	454.4	238	350.8	411.4	-
Coefficient of variation of monthly precipitation 2013/14 (%)	100	56.2	68.6	55.4	80.7	63.7	44.6	69.8	69.4	88.7	-

SAN: San Antón; MOR: Moradillo; HOM: Homino; ALH: Alhama; NAJ: Nájima; MON: Monegrillo; RIB: Ribota; VIV: Vivel; GUA: Guadalope; TRU: Truchas; MAT: Matarraña

Leaf litter decomposition

In October 2014 we collected freshly-fallen leaves of two native species from the Iberian Peninsula contrasting in quality, the highly palatable black alder, *Alnus glutinosa* (L.) Gaertner, and the recalcitrant pedunculate oak, *Quercus robur* L. Approximately 5.0 g (\pm 0.1 g) of air-dried leaf litter were enclosed in either fine (12 \times 15 cm, 0.5 mm mesh size) or coarse (20 \times 25 cm, 5 mm mesh size) mesh bags and deployed in the 11 streams on 2nd to 4th December 2014. At each site, 5 iron bars were anchored randomly to the streambed in riffle sections along 50 m of the channel and 4 bags (2 species \times 2 mesh size) were tied to each bar by nylon lines, making a total of 20 bags per site (5 samples per species and mesh bag type). An extra set of five bags per species and type of mesh bag were used to correct initial mass values for manipulation loss and estimate air dry to oven-dry (72 h at 60 °C), to ash free dry mass (AFDM, 12 h at 500 °C) conversion factors.

On 13th to 18th January 2015, when alder was expected to have lost approximately 50% of the initial mass, we retrieved all (alder + oak) coarse mesh bags to evaluate total decomposition rate. We retrieved all fine bags on 9th to 11th March 2015 to calculate microbial decomposition. Retrieved litter bags were enclosed individually in zip-lock bags and transported in a refrigerated cooler to the laboratory. The leaf litter material from each bag was rinsed with distilled water on a 200- μ m sieve to remove sediments and associated invertebrates. For each fine-mesh bag, a set of five leaf disks was punched out with a cork borer (12 mm diameter) and frozen at -80 °C for later fungal biomass determination. The remaining material was oven-dried (60 °C, 72 h) and weighed to determine leaf dry mass. A portion of leaf material from each bag retrieved was ground (1 mm pore sieve) and stored (-20 °C) for later nutrient analyses and the rest was combusted (500 °C, 12 h) and weighed to determine the remaining ash free dry mass (AFDMr). The collected fauna was preserved in 70% ethanol for later analyses.

Leaf litter stoichiometry

Carbon (C) and nitrogen (N) concentrations were determined with a Perkin Elmer II CHNS/O elemental analyser and phosphorus (P) colorimetrically after autoclave-assisted extraction (APHA, 1998). Results were expressed as a percentage elemental content of leaf dry mass (%C, %N, %P).

Fungal biomass

The sets of five leaf disks from each fine-mesh bag were freeze-dried and weighed to later determine ergosterol concentration as a measure of fungal biomass (Gessner and Chauvet,

1993). Lipid extraction and saponification were performed using KOH methanol 0.14 M (8 g L⁻¹) at 80 °C for 30 min in a shaking bath. Extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp.), and ergosterol was eluted using isopropanol. Ergosterol was detected and quantified via high pressure liquid chromatography (HPLC) by measuring absorbance at 282 nm. A Jasco HPLC system equipped with a Gemini-NX 5 µm C18 250 × 4.6 mm column was used. The mobile phase was 100% methanol and the flow rate was set to 1.2 mL min⁻¹. Ergosterol was detected at 33 °C and converted to fungal biomass using a conversion factor of 5.5 mg ergosterol per gram of fungal mycelium (Gessner and Chauvet, 1993). The results were expressed in mg of fungal biomass per gram of leaf litter AFDM.

Associated invertebrates

Invertebrates removed from coarse-mesh bags were identified to family level (except Oligochaeta which was identified to the order level), counted and sorted into 2 groups: the functional feeding group of shredder invertebrates according to Tachet et al (2002) and Merritt et al. (2007), and the other invertebrates as non-shredders. Shredders and non-shredders were dried (60 °C, 72 h) and combusted (500 °C, 12 h) to determine AFDM. Results were expressed as number of total invertebrates and shredders per gram of litter AFDM and mg of total invertebrates or shredders per gram of litter AFDM.

Statistical analysis

Differences in physicochemical characteristics were analysed by one-way ANOVA with stream as factor. Leaf litter decomposition rates were estimated by fitting the remaining AFDM to the negative exponential model. The rates were expressed in terms of degree-days to correct for the influence of temperature (Graça et al., 2005). The decomposition rates and final litter nutrient contents were compared separately for each bag type by two-way ANOVA (factors: stream, leaf species). Fungal biomass and invertebrate density and biomass were tested by two-way ANOVA (factors: stream, leaf species). Bivariate relationships between decomposition rates and biological variables and between decomposition rate or biological variables and both hydrological (Qs, CV_{Qs}) and environmental variables (conductivity, pH, SRP, DIN, riparian canopy cover, annual precipitation) were tested by linear regression. When necessary, data were log₁₀ or log₁₀(x+1) transformed to achieve requirements for parametric analyses. All statistical analyses were performed with R statistical program (version 3.0.3; R Development Core, 2014).

Results

Environmental variables

The characteristics of the streams differed markedly (Tables 1 and 2). In general, the riparian vegetation was scarce and dominated by *Populus nigra* L. and *Salix* sp., except stream MON, which was dominated by sedges (*Scirpus* sp.). The canopy cover, an indicator of potential leaf litter inputs to streams, was rather variable, depending mainly on channel width (Table 1).

Rainfall was low during the first part of the study period (from 2nd December 2014 to 18th January 2015), which included the whole coarse mesh bags experiment, followed by intense rain and floods afterwards. Only one site (VIV) was dry when the bags were deployed, and from information recorded by dataloggers, it remained dry for 19 d. Another site (TRU) suffered a strong reduction in water level after the 4th d of incubation, and flow there remained marginal for 87 d. Average discharge during the experimental period ranged from 14.6 to 679.9 L s⁻¹ (Table 2). All streams presented well oxygenated waters and alkaline pH. Water temperature differed about 9 °C from the coldest to the warmest stream, differences being statistically significant (one-way ANOVA, $F_{10,1023}$: 328.4, $p < 0.00001$), and conductivity ranged from 430 to 812 $\mu\text{S cm}^{-1}$ ($F_{10,21}$: 26.2, $p < 0.00001$). DIN concentration ranged from 168 to 11284 $\mu\text{g N L}^{-1}$ (Table 2), differences among sites being statistically significant ($F_{10,21}$: 29.8, $p < 0.00001$); it was dominated by NO_3^- . SRP concentration was relatively low (from 2.3 to 18.8 $\mu\text{g P L}^{-1}$) and showed no significant differences among streams ($p = 0.8$) (Table 2).

Litter decomposition

For alder leaves, the AFDM remaining ranged across streams from 30.3% to 69.2% in fine mesh bags and from 20.1% to 79.4% in coarse bags. For oak, it ranged from 69.8% to 84.3% in fine bags and from 70.1% to 95.8% in coarse bags. Decomposition rates for alder ranged from 0.00062 to 0.00162 dd⁻¹ in fine mesh bags and from 0.00102 to 0.00585 dd⁻¹ in coarse mesh bags; the range for oak was from 0.00025 to 0.00071 dd⁻¹ and from 0.00018 to 0.00082 dd⁻¹, respectively (Fig. 2). Decomposition rates differed significantly among sites for both species and mesh type (two-way ANOVAs; Table 3) the interaction between site and species being statistically significant for coarse but not for fine mesh bags (Table 3).

Decomposition rates in coarse mesh bags were lowest in stream VIV, which dried out, and stream TRU, where flow was marginal for part of the experiment, but no differences were found among these streams and the rest in fine mesh bags (Fig. 2). No general relationship was found between decomposition rate and any hydrologic (Qs) or climatic variables, except for a

Table 2. Water physical and chemical characteristics of the studied streams throughout experiment period (mean \pm SE; n=3). For water temperature, daily mean values and their range (in parenthesis) are shown. $Q_{s \text{ exp}}$ and $CV_{Q_{s \text{ exp}}}$ calculated for the experimental period of the fine mesh ($Q_{s \text{ exp, fine}}$; $CV_{Q_{s \text{ exp, fine}}}$) and coarse mesh ($Q_{s \text{ exp, coarse}}$; $CV_{Q_{s \text{ exp, coarse}}}$)

	SAN	MOR	HOM	ALH	NAJ	MON	RIB	VIV	TRU	GUA	MAT
Discharge (L s ⁻¹)	281.6 \pm 68.4	267.8 \pm 96.5	143.4 \pm 77.7	355.3 \pm 223.1	41.9 \pm 2.2	14.6 \pm 0.9	94.2 \pm 12.0	10.2 \pm 7.4	679.9 \pm 659.4	237.4 \pm 77.9	586 \pm 191.1
$Q_{s \text{ exp, fine}}$ (L s ⁻¹ km ⁻²)	28.75	28.75	8.21	3.76	0.7	0.7	3.11	0.59	1.28	-	6.31
$CV_{Q_{s \text{ exp, fine}}}$	0.8	0.8	0.95	1.29	0.11	0.11	0.75	0.19	1.4	-	0.53
$Q_{s \text{ exp, coarse}}$ (L s ⁻¹ km ⁻²)	14.95	14.95	2.53	4.97	0.75	0.75	4.11	0.53	1.86	-	7.37
$CV_{Q_{s \text{ exp, coarse}}}$	0.49	0.49	0.3	1.34	0.07	0.07	0.8	0.05	1.32	-	0.57
Water temperature (°C)	7.8 (10-5.5)	8.6 (10.2-7.1)	5.7 (10.2 - 3.1)	4.3 (9.4-0.5)	6.6 (9.9 - 3.2)	9.4 (12.2-7.4)	6 (10.6-2.5)	5.8 (9.6-2.9)	3.7 (12.7-1.7)	5.2 (9.2-1.7)	12.9 (14.1-11.3)
Conductivity (μ S cm ⁻¹)	443 \pm 7	430 \pm 8	568 \pm 49	505 \pm 13	659 \pm 21	801 \pm 27	35 \pm 2	812 \pm 73	449 \pm 20	545 \pm 7	460 \pm 6
pH	8.3 \pm 0.1	8.1 \pm 0	8.4 \pm 0	8.5 \pm 0	8.0 \pm 0	8.1 \pm 0	8.1 \pm 0	7.9 \pm 0.3	8.0 \pm 0.2	8.3 \pm 0.1	8.3 \pm 0.1
Oxygen saturation (%)	102.3 \pm 4.4	112.0 \pm 7.6	106.3 \pm 4.8	106.1 \pm 4.7	90.1 \pm 1.7	91.1 \pm 1.3	97.6 \pm 2.4	66.9 \pm 27.1	95.4 \pm 3.1	101.7 \pm 2.5	106.4 \pm 1.2
DIN (μ gN L ⁻¹)	1213.2 \pm 282.5	570.2 \pm 89.7	11284.9 \pm 4460.1	4416.1 \pm 148.3	5667.5 \pm 773.9	835.8 \pm 210.7	4494.4 \pm 676.5	426.0 \pm 164.9	167.8 \pm 14.5	766.2 \pm 277.4	314.9 \pm 68.8
SRP (μ gP L ⁻¹)	6.5 \pm 3.7	4.7 \pm 0.7	14.7 \pm 10.5	6.4 \pm 5.0	2.3 \pm 1.2	12.0 \pm 9.2	18.8 \pm 8.6	14.8 \pm 7.3	15.2 \pm 13.4	15.1 \pm 7.9	13.8 \pm 7.7
Chloride (mg L ⁻¹)	4.9 \pm 0.7	3.0 \pm 0.5	10.9 \pm 0.3	8.8 \pm 0.7	19.8 \pm 0.8	34.3 \pm 4.1	32.6 \pm 5.0	39.0 \pm 0.2	3.7 \pm 0.9	7.9 \pm 2.0	4.1 \pm 0.7
Sulphate (mg L ⁻¹)	5.3 \pm 0.5	2.5 \pm 0.6	15.2 \pm 0.8	27.9 \pm 4.3	28.8 \pm 2.1	82.1 \pm 5.8	33.3 \pm 4.6	77.2 \pm 7.2	16.7 \pm 6.2	51.3 \pm 8.0	11.3 \pm 1.3

positive correlation between oak microbial decomposition and $CV_{Qs,exp}$. The decomposition rate of both litter species in coarse bags was positively correlated with riparian canopy cover (alder: $r^2 = 0.38$; $p = 0.043$; oak: $r^2 = 0.68$; $p = 0.002$).

Nutrients in leaf litter

The initial C, N and P concentrations in alder litter were 51.8%, 2.9% and 0.11%, respectively, and 50.1% C, 1.3% N and 0.05% P for oak litter. The final carbon concentration was around 40.2-51.2% for all mesh and species.

The final N concentration was higher than initial values in both species. In alder litter it ranged from 3.57 to 4.23% in fine bags and from 2.19 to 4.35% in coarse bags; in oak litter it ranged from 1.36 to 1.79% in fine bags and from 1.25 to 1.58% in coarse bags (Fig. 3). Differences among streams were statistically significant for both leaf species and mesh sizes, the interaction between site and species being statistically significant for %N in coarse bags (Table 3).

In general, the final P concentration was lower or similar to initial values in both species. For alder litter, it ranged from 0.04 to 0.08% in fine bags and from 0.06 to 0.11% in coarse bags. For oak litter ranged from 0.04 to 0.08% in fine and from 0.08 to 0.12 in coarse bags (Fig. 3). Differences were statistically significant among sites for both leaf species and mesh sizes, the interaction between site and species being also statistically significant (Table 3).

The final N and P concentrations were not correlated to the hydrologic nor climatic descriptors. Only for oak in fine bags the final N concentration appeared to be positively correlated to dissolved nitrogen availability ($r^2 = 0.58$), but only when excluding the 4 richest sites (NAJ, ALH, RIB and HOM, with $DIN > 1500\mu\text{g-N L}^{-1}$).

In general, the leaf ash content for alder and oak litter was around 15 and 10% in coarse and fine bags respectively, although these values were greater in SAN, HOM and GUA (Fig. 3).

Fungal biomass

Fungal biomass ranged from 2.8 to 68.9 mg g^{-1} AFDM for alder litter and from 15.2 to 65.4 mg g^{-1} AFDM for oak litter (Fig. 4). Differences were statistically significant among sites, values being highest in GUA and lowest in MON, but not between species (Table 3). Fungal biomass was not correlated to any hydrological (Qs and CV_{Qs}) or other environmental variables. There were no relationships neither with decomposition rate nor with litter nutrient content.

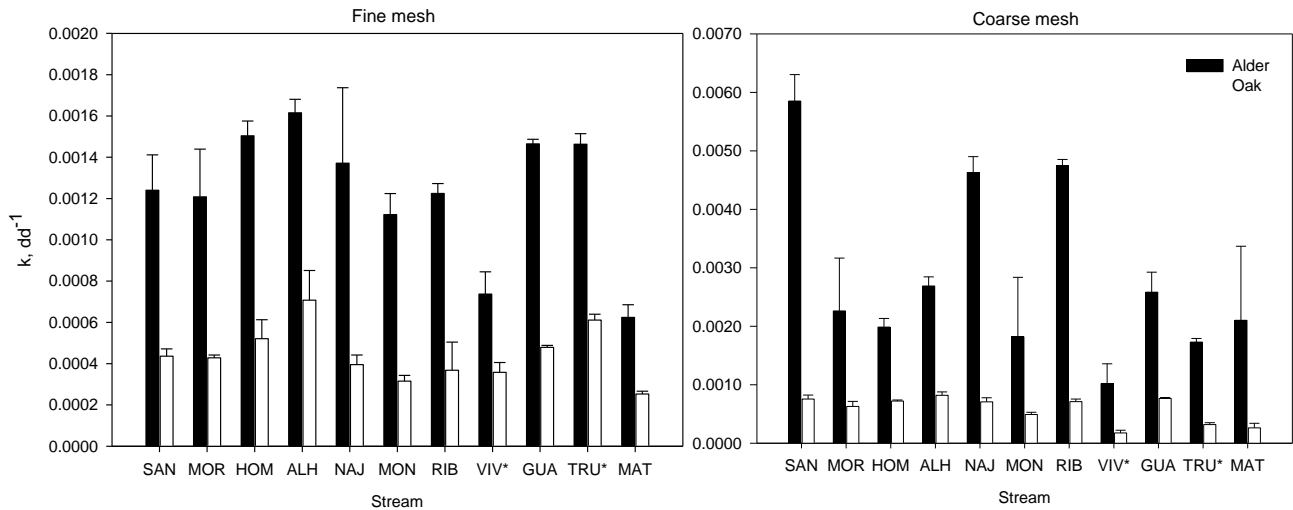


Fig. 2. Decomposition rates (k , dd^{-1}) in fine (left) and coarse mesh bags (right) along longitudinal gradient from west to east (mean \pm SE; $n= 5$). Note the different scales. Streams that suffered strong reduction in water flow throughout experiment are marked with asterisk.

Associated invertebrates

Total invertebrate richness in bags ranged from 6 to 16 taxa; the lowest values were found at TRU and VIV in oak and in TRU, VIV and MON in alder, all them streams with very low Q_s (Table 1). Total invertebrate density ranged from 2.3 to 118.5 individuals g^{-1} AFDM for alder and from 2.2 to 51.6 individuals g^{-1} AFDM for oak, the lowest values occurring at TRU. Total invertebrate biomass ranged from 0.05 to 113.9 $mg g^{-1}$ AFDM for alder and from 1.07 to 23.2 $mg g^{-1}$ AFDM for oak, with the lowest values also occurring at TRU. Invertebrate density and biomass were statistically different among streams for both species (Table 3). Shredder density ranged from zero (VIV, TRU and GUA) to 42.3 individuals g^{-1} AFDM for alder litter and to 11.8 individuals g^{-1} AFDM for oak litter. Shredder biomass ranged from zero in both litter species to 110.8 $mg g^{-1}$ AFDM¹ for alder litter and 18.0 $mg g^{-1}$ AFDM for oak. Shredder density and biomass differed significantly among streams and between species and tended to be more abundant in alder bags, the interaction between site and species being statistically significant (Table 3).

Only shredder density showed a significant relationship with one of the descriptors of drought ($Q_{s_{exp}}$, positive). Among all other environmental variables measured, total invertebrate density (alder: $r^2 = 0.53$; $p = 0.01$; oak: $r^2 = 0.37$; $p = 0.046$) and biomass (alder: $r^2 = 0.68$; $p = 0.002$; oak: $r^2 = 0.38$; $p = 0.044$) in both species only showed a significant relationship with riparian canopy cover. The decomposition rate of alder litter was positively correlated with both density and biomass of shredders in litter bags, whereas that of oak was not correlated with any invertebrate variable (Fig. 5).

Table 3. Summary table for two-way ANOVAs performed on decomposition rate (k , dd^{-1}), final nitrogen and phosphorus concentration (%N, % P), fungal biomass and invertebrate variables (density and biomass of total invertebrates and of shredders). Significant values are highlighted in bold.

		Coarse mesh bags			Fine mesh bags		
		df	F	<i>p</i>	df	F	<i>p</i>
k (dd^{-1})	Stream	10,88	21.0	< 0.0001	10,84	10.0	< 0.0001
	Species	1,88	596.1	< 0.0001	1,84	413.5	< 0.0001
	Stream x Species	10, 88	3.2	0.0016	10,84	0.8	0.583
% N	Stream	10, 87	11.2	< 0.0001	10, 84	2.8	0.005
	Species	1, 87	1608.1	< 0.0001	1, 84	1382.0	< 0.0001
	Stream x Species	10, 87	3.4	0.00086	10, 84	1.0	0.481
% P	Stream	10, 87	23.1	< 0.0001	10, 84	20.4	< 0.0001
	Species	1, 87	473.6	< 0.0001	1, 84	1007.3	< 0.0001
	Stream x Species	10, 87	6.2	< 0.0001	10, 84	5.6	< 0.0001
Fungal biomass	Stream				10, 80	9.5	< 0.0001
	Species				1, 80	2.3	0.1371
	Stream x Species				10, 80	1.1	0.389
Total invertebrate density	Stream	10, 87	12.4	< 0.0001			
	Species	1, 87	12.0	0.001			
	Stream x Species	10, 87	0.9	0.526			
Shredder density	Stream	10, 87	19.2	< 0.0001			
	Species	1, 87	14.7	0.0002			
	Stream x Species	10, 87	3.4	0.0008			
Total invertebrate biomass	Stream	10, 87	9.9	< 0.0001			
	Species	1, 87	19.0	< 0.0001			
	Stream x Species	10, 87	3.3	0.001			
Shredder biomass	Stream	10, 87	14.0	< 0.0001			
	Species	1, 87	20.7	< 0.0001			
	Stream x Species	10, 87	4.0	0.0001			

Discussion

The flow regime in the Mediterranean region can be seriously altered in the coming decades following changes in rainfall and temperature (Milly et al., 2005). Our experiment across a precipitation gradient aimed at gaining knowledge on the likely consequences of such changes for stream ecosystem functioning. We hypothesized drought to reduce decomposition rate, but, although decomposition was slowest at the sites suffering most intense drought, no general pattern could be found between decomposition rate of both litter species and any of the proxies for drought we determined.

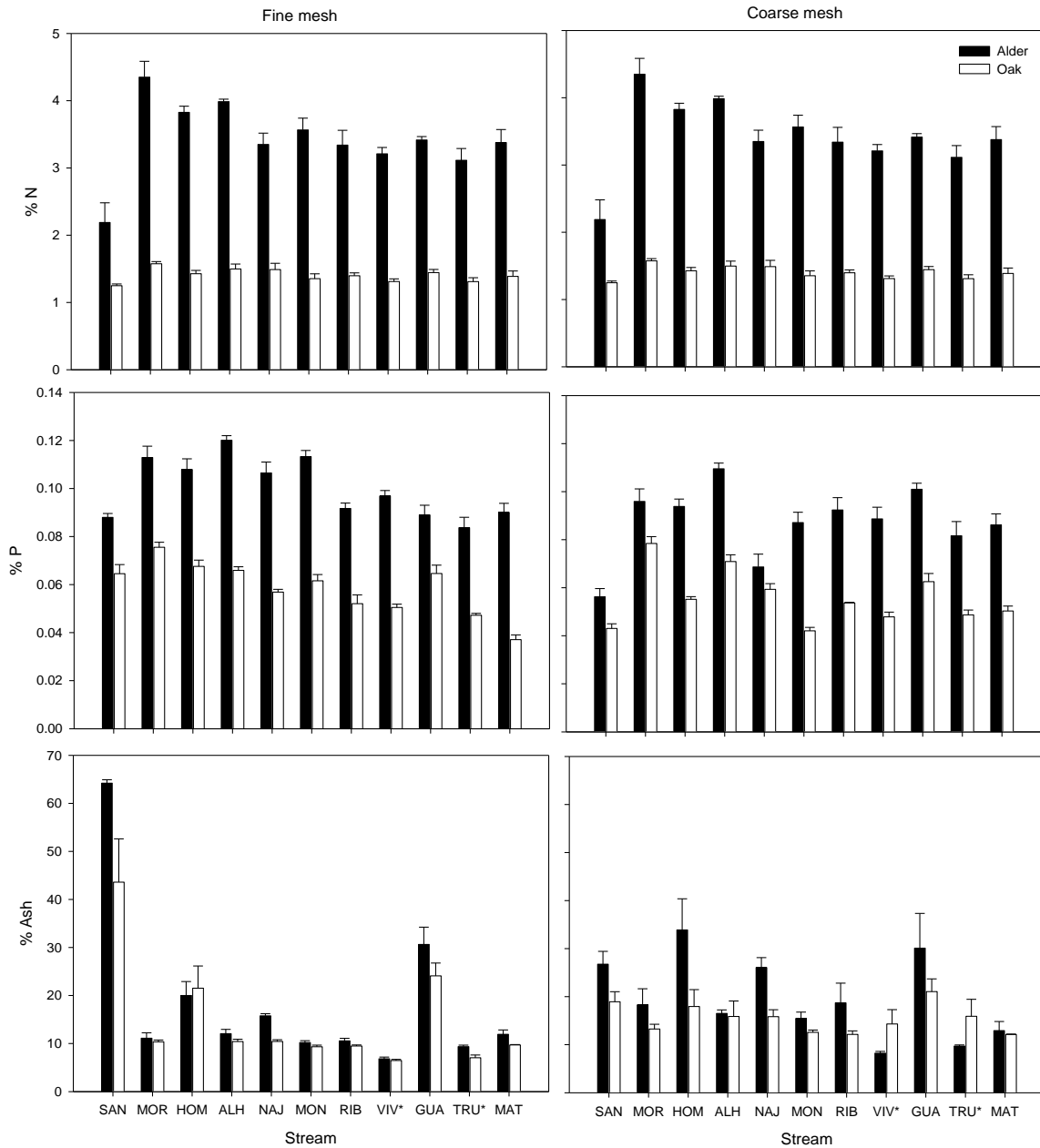


Fig. 3. Leaf nitrogen, phosphorus and ash concentration (% DM) at the end of experiment in fine (left) and coarse mesh bags (right). Streams arranged in longitudinal gradient order from west to east (mean \pm SE; n= 5). Streams that suffered strong reduction in water flow throughout experiment are marked with asterisk.

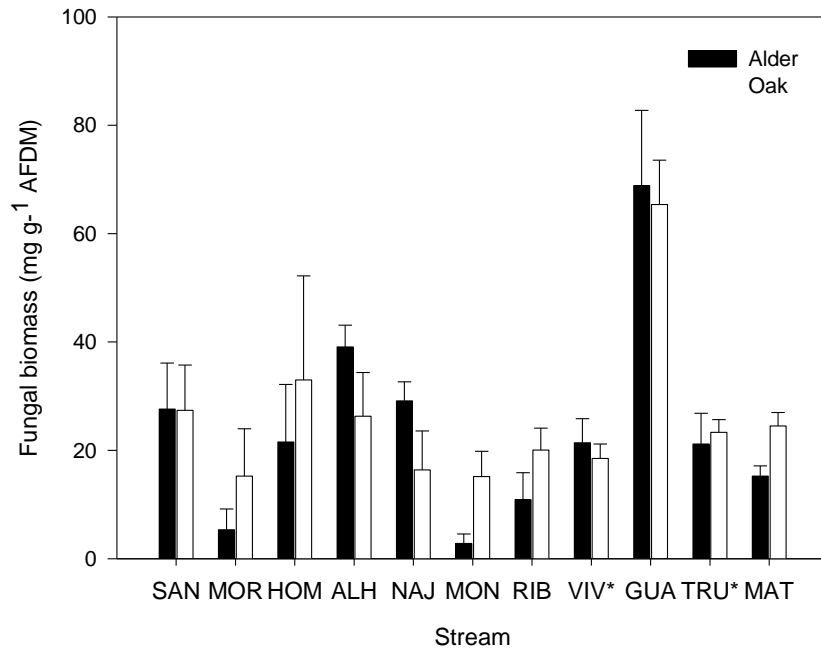


Fig. 4. Fungal biomass on alder and oak leaf litter incubated in fine-mesh bags at the end of the study. Streams arranged in longitudinal gradient order from west to east (mean \pm SE; $n = 4 - 5$). Streams that suffered strong reduction in water flow throughout experiment are marked with asterisk.

In the two streams where drought was most intense (VIV, where the stream dried out during part of the experiment, and TRU, where residual flow occurred) decomposition rates were lower than in the rest of the sites in both species, a result consistent with the literature, that shows decomposition to be slower in temporary than in perennial streams (Richardson, 1990; Datry et al., 2011; Martínez et al., 2015), and also slower under residual flow (Acuña et al., 2005; Leberfinger et al., 2010). Furthermore, this severe level of drought seemed to affect more total than microbial decomposition confirming the second hypothesis and as has also been shown in elsewhere (Martínez et al., 2015). Microbes can survive drought if there is some residual humidity in the field (Abril et al., 2016; Bruder et al., 2011; Sridhar and Bärlocher, 1993), and microbial activity recovers quickly after flow resumption (Langhans and Tockner, 2006), unlike invertebrates, which take longer to recover (Datry et al., 2011). Drying events usually reduce the functional and taxonomic richness of invertebrate communities, and although permanent streams are highly resilient to hydrological fluctuations (Schriever et al., 2015), the recovery of communities in intermittent streams can be slower, as it depends on a few resistant species (Leigh et al., 2016) or on re-colonization from other water bodies. Severe drought events would, thus, show a slower functional recovery, what would explain the lower decomposition rates measured in VIV and TRU.

Microbial decomposition of both species was not correlated with fungal biomass. In the literature there are examples of positive significant relationship (e.g. Foulquier et al., 2015) as well as of lack of correlation (Casas et al., 2011). Nutrient content, which is usually taken as a proxy of nutrient change caused by microbial activity (Cheever et al., 2012; Webster et al., 2009), was also not related to microbial decomposition. However, microbial decomposition of both species tended to increase with $CV_{Qs,exp}$, although the relationship was only statistically significant for oak leaves. Other environmental variables could be driving the microbial decomposition, such as water nutrient availability, as suggested by the relationship between N content of oak litter and dissolved N.

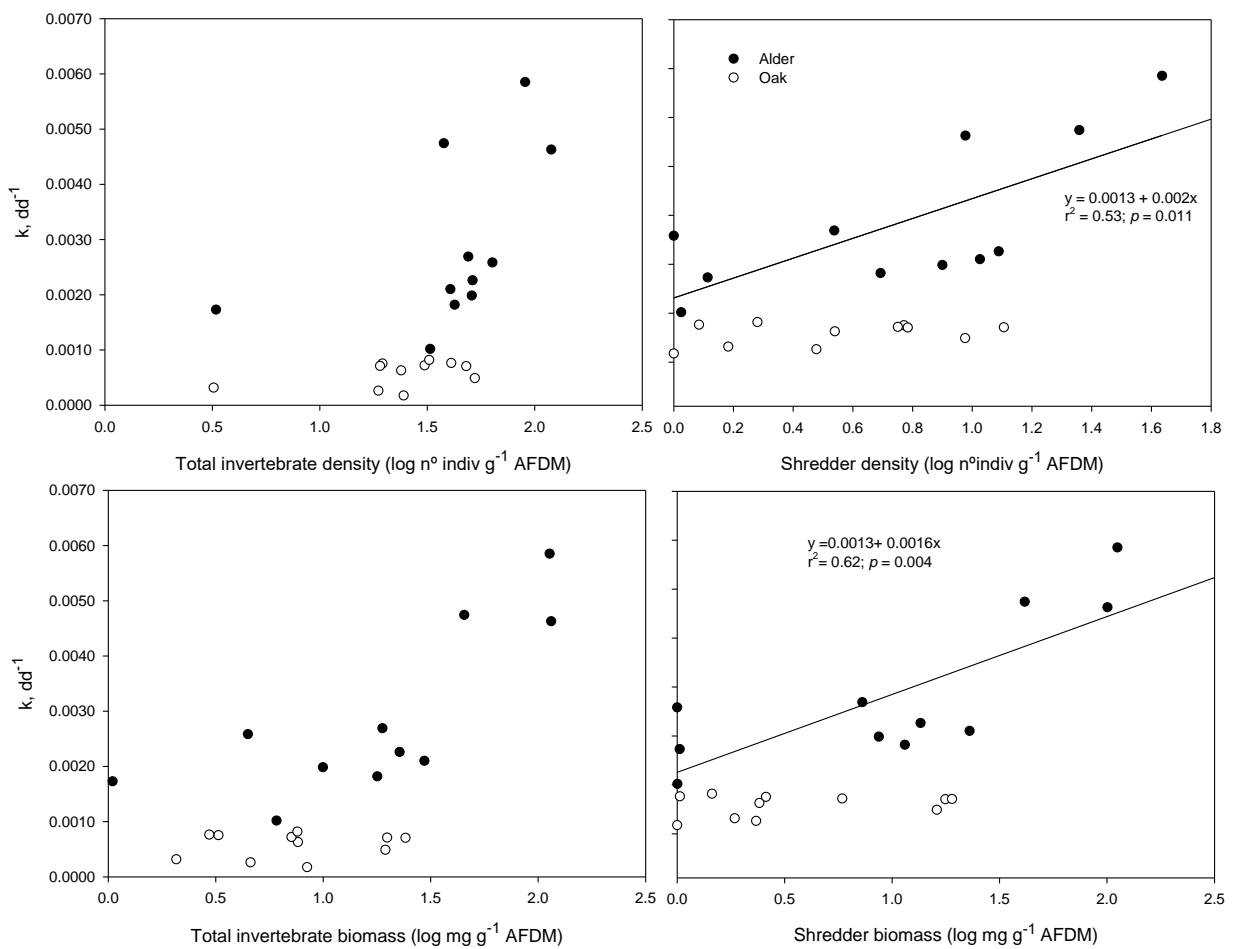


Fig. 5. Relationships between breakdown rates (k , dd^{-1}) in coarse bags and density and biomass of total invertebrates and of shredders g^{-1} AFDM. The equation, r^2 and p -values of the relationship and fitted lines are also shown

Total decomposition rate of alder, the leaf material with the best quality, was mainly determined by detritivore density and biomass. Among the invertebrate variables, only shredders seemed to be limited by specific discharge throughout the experiment. Flow stability,

minimum flow and flow permanence are important drivers of macroinvertebrate assemblages (Belmar et al., 2013; Datry et al., 2011), and affect leaf litter processing. On the other hand, the existence of a clear link between total decomposition of alder and detritivore community suggests that the impact of hydrological conditions on invertebrate community could result in contrasting effects on the decomposition of leaf species with different quality, fast-decaying and high-quality leaf litter being more affected than slow-decaying and low-quality one, as we hypothesized (third hypothesis).

We found a strong positive correlation between total decomposition rate and stream canopy cover, an indirect indicator of litter inputs to streams, which was also correlated with the density and biomass of invertebrates in litter bags. This link suggests the abundance of detritivore invertebrates in Mediterranean streams to be also controlled by the supply of riparian detritus, which is in part dependent on rainfall (Sanpera-Calbet et al., 2015). Changes in the identity and timing of leaf inputs as a consequence of climate change or human activities could, thus, alter decomposition in the near future (Kominoski and Rosemond, 2012; Kominoski et al., 2013).

The lack of statistically significant relationship between decomposition rate and the variables we used to quantify drought could derive from other factors such as human activities, as litter decomposition responds to multiple factors such as nutrient concentration (Woodward et al., 2012), pesticides (Brosed et al., 2016), siltation (Niyogi et al., 2003) or hydraulics (Elosegi and Sabater, 2013). Besides, travertine precipitation, which occurred in some of our streams, can either enhance or slow decomposition, depending on the continuity of the travertine layer (Casas and Gessner, 1999; Miliša et al., 2010). Therefore, the effects of drought could be not easily disentangled given the concomitant variation in other environmental variables and the particular responses of decomposers and detritivores.

Conclusion

In short, our results point to a complex pattern of litter decomposition at the regional scale, as drought affects decomposition directly by emersion of bags and indirectly by affecting the functional composition and density of invertebrates. Decomposition rate was determined by the macroinvertebrate community, which was affected by hydrology as well as by riparian vegetation. Other studies (e.g., Pozo et al., 2011) have also shown leaf decomposition to reflect geographic differences in macroinvertebrate communities, thus highlighting the role of invertebrates as drivers of leaf decomposition. By contrast, microbial-mediated decomposition might be less affected by drought.

Seasonality in precipitation and temperature is expected to increase in oncoming years in the Mediterranean region, resulting in more intense and frequent drought events (e.g. low flows, droughts), as well as an increase in the number of intermittent streams. Future efforts are needed to identify factors and their interactions affecting stream ecosystem processes, such as organic matter decomposition, to predict and monitor the consequences of future hydrologic change under a shifting mosaic of abiotic and biotic conditions.

References

- Abril, M., Muñoz, I., Menéndez, M., 2016. Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. *Sci. Total Environ.* 553, 330–339. doi:10.1016/j.scitotenv.2016.02.082
- Acuña, V., Muñoz, I., Giorgi, A., Omella, M., Sabater, F., Sabater, S., 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. *J. North Am. Benthol. Soc.* 24, 919–933.
- Allan, J.D., Castillo, M.M., 2007. Stream ecology: structure and function of running waters, *Ecology*. doi:10.2307/2265800
- APHA, 1998. Standard methods for the examination of water and wastewater, 20th ed. American Water Works Association., & Water Environment Federation, Washington, D.C.
- Battin, T.J., Luysaert, S., Kaplan, L. a., Aufdenkampe, A.K., Richter, A., Tranvik, L.J., 2009. The boundless carbon cycle. *Nat. Geosci.* 2, 598–600. doi:10.1038/ngeo618
- Belmar, O., Velasco, J., Millán, A., Wood, P.J., 2013. The influence of natural flow regimes on macroinvertebrate assemblages in a semiarid Mediterranean basin 379, 363–379. doi:10.1002/eco.1274
- Bonada, N., Rieradevall, M., Prat, N., 2007. Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia* 589, 91–106. doi:10.1007/s10750-007-0723-5
- Brosed, M., Sylvain, L., Eric, C., 2016. Litter breakdown for ecosystem integrity assessment also applies to streams affected by pesticides. *Hydrobiologia* 773, 87–102.

doi:10.1007/s10750-016-2681-2

- Bruder, A., Chauvet, E., Gessner, M.O., 2011. Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency. *Funct. Ecol.* 25, 1269–1277. doi:10.1111/j.1365-2435.2011.01903.x
- Casas, J., Gessner, M.O., 1999. Leaf litter breakdown in a Mediterranean stream characterised by travertine precipitation. *Freshw. Biol.* 41, 781–793. doi:10.1046/j.1365-2427.1999.00417.x
- Casas, J., Gessner, M.O., López, D., Descals, E., 2011. Leaf-litter colonisation and breakdown in relation to stream typology: Insights from Mediterranean low-order streams. *Freshw. Biol.* 56, 2594–2608. doi:10.1111/j.1365-2427.2011.02686.x
- CEDEX, 2004. Directiva 2000/60/CE. Análisis de las características de las demarcaciones. Caracterización de los tipos de ríos y lagos. v. 1.0. Ministerio de Fomento. Centro de Estudios y Experimentación de Obras Públicas, Madrid.
- Cheever, B.M., Kratzer, E.B., Webster, J.R., 2012. Immobilization and mineralization of N and P by heterotrophic microbes during leaf decomposition. *Freshw. Sci.* 31, 133–147. doi:10.1899/11-060.1
- Datry, T., Claret, R.C.C., Philippe, M., 2011. Flow intermittence controls leaf litter breakdown in a French temporary alluvial river: the “drying memory.” *Aquat. Sci.* 471–483. doi:10.1007/s00027-011-0193-8
- Development, C.R., 2014. R: A Language and Environment for Statistical Computing.
- Dewson, Z.S., James, A.B.W., Death, R.G., 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *J. North Am. Benthol. Soc.* 26, 401–415. doi:10.1899/06-110.1
- Ebro Hydrographic Confederation, 2016. Ebro Hydrographic Confederation [WWW Document]. URL <https://www.chebro.es> (accessed 6.20.02).
- Elosegi, A., Sabater, S., 2013. Effects of hydromorphological impacts on river ecosystem functioning: a review and suggestions for assessing ecological impacts. *Hydrobiologia* 712, 129–143.
- Filipe, A.F., Lawrence, J.E., Bonada, N., 2013. Vulnerability of stream biota to climate change in mediterranean climate regions: A synthesis of ecological responses and conservation challenges. *Hydrobiologia* 719, 331–351. doi:10.1007/s10750-012-1244-4
- Foulquier, A., Artigas, J., Pesce, S., Datry, T., 2015. Drying responses of microbial litter

- decomposition and associated fungal and bacterial communities are not affected by emersion frequency. *Freshw. Sci.* 34, 1233-1244.
- Gasith, A., Resh, V.H., 1999. Streams in mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annu. Rev. Ecol. Evol. Syst.* 51–81.
- Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. *Ecol. Appl.* 12, 498–510. doi:10.1890/1051-0761(2002)012[0498:ACFULB]2.0.CO;2
- Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. *Appl. Environ. Microbiol.*
- Graça, M.A.S., Bärlocher, F., Gessner, M., 2005. Methods to study litter decomposition: a practical guide. Springer Science & Business Media. doi:10.1007/1-4020-3466-0
- Graça, M.A.S., Poquet, J.M., 2014. Do climate and soil influence phenotypic variability in leaf litter, microbial decomposition and shredder consumption? *Oecologia* 174, 1021–1032. doi:10.1007/s00442-013-2825-2
- Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J., Woodward, G., 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshw. Biol.* 54, 957–970. doi:10.1111/j.1365-2427.2008.02138.x
- Huang, S., Huang, Q., Chang, J., Leng, G., 2016. Linkages between hydrological drought, climate indices and human activities: a case study in the Columbia River basin. *Int. J. Climatol.* 36, 280–290.
- IPCC, 2014. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK, and New York, NY, USA.
- Kominoski, J.S., Rosemond, A.D., 2012. Conservation from the bottom up: forecasting effects of global change on dynamics of organic matter and management needs for river networks. *Freshw. Sci.* 31, 51–68. doi:10.1899/10-160.1
- Kominoski, J.S., Shah, J.J.F., Canhoto, C., Fischer, D.G., Giling, D.P., González, E., Griffiths, N.A., Larrañaga, A., Leroy, C.J., Mineau, M.M., McElarney, Y.R., Shirley, S.M., Swan, C.M., Tiegs, S.D., 2013. Forecasting functional implications of global changes in riparian plant communities. *Front. Ecol. Environ.* 11, 423–432. doi:10.1890/120056
- Langhans, S.D., Tockner, K., 2006. The role of timing, duration, and frequency of inundation in

- controlling leaf litter decomposition in a river-floodplain ecosystem (Tagliamento, northeastern Italy). *Oecologia* 147, 501–509. doi:10.1007/s00442-005-0282-2
- Leberfingher, K., Bohman, I., Herrmann, J., 2010. Drought impact on stream detritivores: Experimental effects on leaf litter breakdown and life cycles. *Hydrobiologia* 652, 247–254. doi:10.1007/s10750-010-0337-1
- Leigh, C., Bonada, N., Boulton, A.J., Hugueny, B., Larned, S.T., Vander Vorste, R., Datry, T., 2016. Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. *Aquat. Sci.* doi:10.1007/s00027-015-0427-2
- Martínez, A., Monroy, S., Pérez, J., Larrañaga, A., Basaguren, A., Molinero, J., Pozo, J., 2016. In-stream litter decomposition along an altitudinal gradient: does substrate quality matter? *Hydrobiologia* 766, 17–28. doi:10.1007/s10750-015-2432-9
- Martínez, A., Pérez, J., Molinero, J., Sagarduy, M., Pozo, J., 2015. Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. *Sci. Total Environ.* 503-504, 251–257. doi:10.1016/j.scitotenv.2014.06.018
- Merritt, R.W., Cummins, K.W., Berg, M., 2007. An introduction to the aquatic insects of North America, 4th ed. Kendall/Hunt Publishing Company, Dubuque, Iowa, USA.
- Miliša, M., Belančić, A., Matoničkin Kepčija, R., Sertić-Perić, M., Ostojić, A., Habdija, I., 2010. Calcite deposition in karst waters is promoted by leaf litter breakdown and vice versa. *Ann. Limnol. - Int. J. Limnol.* 46, 225–232. doi:10.1051/limn/2010023
- Milly, P.C.D., Dunne, K.A., Vecchia, A. V, 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* 438, 347–350. doi:10.1038/nature04312
- Mora-Gómez, J., Elosegi, A., Duarte, S., Cássio, F., Pascoal, C., Romaní, A.M., 2016. Differences in the sensitivity of fungi and bacteria to season and invertebrates affect leaf litter decomposition in a Mediterranean stream. *FEMS Microbiol. Ecol.* 1–13. doi:10.1093/femsec/fiw121
- Munné, A., Prat, N., 2004. Defining river types in a Mediterranean area: A methodology for the implementation of the EU water framework directive. *Environ. Manage.* 34, 711–729. doi:10.1007/s00267-003-0098-y
- Niyogi, D.K., Simon, K.S., Townsend, C.R., 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. *Freshw. Biol.* 48, 1698–1708. doi:10.1046/j.1365-2427.2003.01104.x
- Papadaki, C., Soulis, K., Muñoz-Mas, R., Martinez-Capel, F., Zogaris, S., Ntoanidis, L.,

- Dimitriou, E., 2016. Potential impacts of climate change on flow regime and fish habitat in mountain rivers of the south-western Balkans. *Sci. Total Environ.* 540, 418–428.
- Perkins, D.M., Reiss, J., Yvon-Durocher, G., Woodward, G., 2010. Global change and food webs in running waters. *Hydrobiologia* 657, 181–198. doi:10.1007/s10750-009-0080-7
- Petersen, R.C., Cummins, K.W., 1974. Leaf processing in a woodland stream. *Freshw. Biol.* 4, 343–368. doi:10.1111/j.1365-2427.1974.tb00103.x
- Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegard, K.L., Richter, B.D., Sparks, R.E., Stromberg, J.C., 1997. The Natural Flow Regime: A paradigm for river conservation and restoration. *Bioscience* 47, 769–784. doi:10.2307/1313099
- Pozo, J., Casas, J., Menéndez, M., Mollá, S., Arostegui, I., Basaguren, A., Casado, C., Descals, E., García-Avilés, J., González, J.M., Larrañaga, A., López, E., Lusi, M., Moya, O., Pérez, J., Riera, T., Roblas, N., Salinas, M.J., 2011. Leaf-litter decomposition in headwater streams: a comparison of the process among four climatic regions. *J. North Am. Benthol. Soc.* 30, 935–950. doi:10.1899/10-153.1
- Richardson, W.B., 1990. A comparison of detritus processing between permanent and intermittent headwater streams. *J. Freshw. Ecol.* 5, 341–357.
- Sabater, S., 2008. Alterations of the global water cycle and their effects on river structure, function and services. *Freshw. Rev.* 1, 75–88. doi:10.1608/FRJ-1.1.5
- Sabater, S., Tockner, K., 2010. Effects of hydrologic alterations on the ecological quality of river ecosystems, in: Sabater, S., Barceló, D. (Eds.), *Water scarcity in the Mediterranean: perspectives under global change. The Handbook of Environmental Chemistry*, pp. 15–39. doi:10.1016/0143-1471(82)90111-8
- Sanpera-Calbet, I., Acuña, V., Butturini, A., Marcé, R., Muñoz, I., 2015. El Niño southern oscillation and seasonal drought drive riparian input dynamics in a Mediterranean stream. *Limnol. Oceanogr.* 214–226. doi:10.1002/lno.10211
- Schäfer, R.B., Bundschuh, M., Rouch, D.A., Szöcs, E., von der Ohe, P.C., Pettigrove, V., Schulz, R., Nugegoda, D., Kefford, B.J., 2012. Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. *Sci. Total Environ.* 415, 69–78. doi:10.1016/j.scitotenv.2011.05.063
- Schriever, T. a, Bogan, M.T., Boersma, K.S., Cañedo-argüelles, M., Jaeger, K.L., Olden, J.D., Lytle, D. a, Schriever, T. a, Bogan, M.T., Boersma, K.S., Cañedo-argüelles, M., 2015. Hydrology shapes taxonomic and functional structure of desert stream invertebrate

- communities. *Freshw. Sci.* 34, 399–409. doi:10.1086/680518.
- Sridhar, K.R., Bärlocher, F., 1993. Aquatic hyphomycetes on leaf litter in and near a stream in Nova Scotia, Canada. *Mycol. Res.* 97, 1530–1535. doi:10.1016/S0953-7562(09)80229-3
- Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, F., 2002. *Invertébrés d'eau douce. Systematique, biologie, écologie.* Paris, France.
- Tank, J., Rosi-Marshall, E., Griffiths, N. a., Entekin, S. a., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. *J. North Am. Benthol. Soc.* 29, 118–146. doi:10.1899/08-170.1
- von Schiller, D., Acuña, V., Graeber, D., Martí, E., Ribot, M., Sabater, S., Timoner, X., Tockner, K., 2011. Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. *Aquat. Sci.* 73, 485–497. doi:10.1007/s00027-011-0195-6
- Webster, J.R., Newbold, J.D., Thomas, S.A., Valett, H.M., Mulholland, P.J., 2009. Nutrient Uptake and Mineralization during Leaf Decay in Streams – a Model Simulation. *Int. Rev. Hydrobiol.* 94, 372–390. doi:10.1002/iroh.200811158
- Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elozegi, A., Ferreira, V., Graca, M.A.S., Fleituch, T., Lacoursiere, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., 2012. Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning. *Science* (80-.). 336, 1438–1440. doi:10.1126/science.1219534
- Ylla, I., Sanpera-Calbet, I., Vázquez, E., Romaní, A.M., Muñoz, I., Butturini, A., Sabater, S., 2010. Organic matter availability during pre- and post-drought periods in a Mediterranean stream. *Hydrobiologia* 657, 217–232. doi:10.1007/s10750-010-0193-z
- Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *J. North Am. Benthol. Soc.* 27, 605–625. doi:10.1899/07-121.1

CHAPTER 6

Plant functional diversity and leaf-litter decomposition in Mediterranean streams along a drought gradient

Abstract

Recent studies have suggested that plant functional diversity is an important driver of leaf-litter decomposition patterns. Therefore, changes in plant functional diversity could affect freshwater ecosystem processes and have consequences for food webs, as well as for the global carbon cycling. However, its effects on decomposition rates in streams under Mediterranean climate, subject to recurrent water stress, are still poorly understood. We tested the effect of leaf-litter functional trait diversity on the decomposition rate in five Mediterranean calcareous streams along a climate drought gradient by using 3 *Quercus* species with contrasting carbon allocation strategies (*Q. robur*, deciduous; *Q. faginea*, marcescent; and *Q. ilex*, evergreen) to disentangle its role under this environmental stressor. Leaves of each species were incubated in the streams in coarse-mesh bags alone and in all possible combinations of two and three species. Litter decomposition, litter nutrient and invertebrates colonizing bags were determined. We found no consistent relationship between litter diversity and leaf decomposition rate, which rather depended on species mixtures and greatly on stream. Likewise, litter nutrients and invertebrates showed differences among streams but neither of them were related to the decomposition rate. However, the effect of species diversity was more prevalent under drier conditions. Although these results suggest that environmental context might have stronger effects than leaf functional diversity on the decomposition process in calcareous streams from Mediterranean areas, overriding any potential effect of the later one, the plant functional effect could be altered under environmental limiting conditions, suggesting that future drier climate could affect the Biodiversity-Ecosystem Functioning relationships.

Keywords: functional traits, detritivores, biodiversity, leaf mixing, litter decomposition

Introduction

The current biodiversity loss triggered by global environmental changes such as climate and human-related perturbations (Pereira *et al.* 2010; Ceballos *et al.* 2015) has caused growing concern over the possible consequences of a biodiversity decline for ecosystem processes (Naeem, Duffy & Zavaleta 2012) and an increase in researches to elucidate biodiversity-function relationships (B-EF) (Reiss *et al.* 2009; Cardinale *et al.* 2012). The understanding of relationships between plant diversity and the process of leaf-litter decomposition in freshwater ecosystems is of ecological importance, as many streams are detritus based-food webs and depend on allochthonous leaf litter inputs as primary source of energy and nutrients (Wallace *et al.* 1997). So, changes in plant diversity could affect organic matter processing with subsequent major consequences for stream food webs (Perkins *et al.* 2010) as well as for nutrient and global carbon cycling (Battin *et al.* 2009; Gessner *et al.* 2010; Cheever, Kratzer & Webster 2012). Previous researches on litter diversity effects on organic matter decomposition have resulted in mixed results when litter from different plant species were mixed, with studies suggesting positive, negative and no effects (Srivastava *et al.* 2009; Gessner *et al.* 2010), thus “failing” to show a consistent effect. Functional diversity (e.g. diversity of traits) represents an emerging approach for assessing the effects of biodiversity on ecosystem functioning (Reiss *et al.* 2009) including pivotal processes such as litter decomposition. Recent studies suggest that functional diversity might be an important driver of in-stream leaf-litter decomposition patterns (Handa *et al.* 2014) and it could help to unravel the understanding of biodiversity effects. Mixing leaf-litter from plant with different key functional traits such as resource-acquisition strategies (e.g., nitrogen acquisition and carbon allocation) could result in enhancement leaf litter mass loss in streams (Handa *et al.* 2014). The coexistence of a more diverse plant functional traits could enhance the potential mechanisms for any litter-mixture effects on stream decomposition. Such interactions between mixed-species litter would include, for example, fungi-driver transfer of nutrients or other compounds between litter species (Gessner *et al.*, 2010; Schindler and Gessner, 2009) and positive feedback of fauna due to a relative greater diverse habitat and resource availability (Sanpera-Calbet, Lecerf & Chauvet 2009; Lecerf *et al.* 2011).

However, plant diversity effects on litter decomposition could depend on the environmental context (Cardinale, Nelson & Palmer 2000; Swan & Palmer 2004; Rosemond *et al.* 2010; Lecerf *et al.* 2011) and to be related to abiotic factors (Lima-Fernandes *et al.* 2015; Tonin *et al.* 2017) and differences in biotic community (Lecerf & Richardson 2010; Vos *et al.* 2011). Environmental factors not only have direct effects on litter decomposition but also affect key biotic actors such as the microbial decomposers and invertebrate detritivores (Tank *et al.* 2010) and might differentially influence the effects of diversity. However, the knowledge on effects of litter diversity is still unclear and mainly come from controlled experiment or studies

carried out in a single stream. Therefore, it is important incorporating of comparative field experiments examining the effect of plant litter diversity on the decomposition rate to disentangle its role under the environmental complexity. Further investigations of how species compositional changes interact with other aspects of global change effects, e.g., drought, to affect ecosystem processes are currently lacking (Kominoski *et al.* 2009). Specific characteristics or changing environmental at regional or local-scale determine not only abiotic conditions but also influence microbial decomposers and invertebrate detritivore communities and their activity (Bonada, Dolédec & Statzner 2007a; Boyero *et al.* 2011, 2012), and thus the potential for influence any litter-mixing effect. Particularly interesting, and still poorly understood, would be the effects of plant functional diversity in Mediterranean streams, which are subject to recurrent water stress and are identify as an “Hot-Spots” in the future climate change projections (Giorgi & Lionello 2008; Bonada & Resh 2013). The Mediterranean climate is characterized by high inter-annual variability and pronounced seasonality with hot-dry summers and cold-wet winters (Gasith & Resh 1999). This feature not only induces morpho-functional adaptations in plants against water stress (e.g., thick leaf tissue, high structural or defence compounds; De Micco and Aronne, 2012) but also determines the hydrological regime and the stream biological assemblages (Gasith & Resh 1999; Bonada *et al.* 2007a; Bonada, Rieradevall & Prat 2007b) and constraint the decomposition. For example, within Mediterranean clime, invertebrate community might differ with respect to the local-scale climatic variables in terms of change in precipitation and atmospheric moisture (Pace, Bonada & Prat 2013) and, since the detritivores play a particularly important role to generate non-additive responses in litter decomposition (Swan & Palmer 2006; Jabiol & Chauvet 2015), this could influence litter diversity-decomposition relationships (Vos *et al.*, 2011).

The aim of our study was to examine the effects of leaf-litter diversity from plant functional typed on leaf litter decomposition process and associated invertebrates in streams along a drought gradient. The effect of leaf-litter diversity on decomposition was tested for five Mediterranean calcareous streams by means of 3 *Quercus* species with contrasting carbon allocation strategies: *Q. robur* (deciduous), *Q. faginea* (marcescent) and *Q. ilex* (evergreen). Leaf litter species were incubated in the streams in coarse-mesh bags alone and in all possible two- and three-species combinations. We hypothesized that 1) increasing litter diversity promotes overall litter decomposition (positive non-additive affects) due to positive effects on biota through the use of a greater variety of litter types but 2) the magnitude of these effects is different among streams because of environmental characteristics.

Methods

Study site

The experiment was conducted in five Mediterranean mountain calcareous streams located in the Ebro catchment (Spain; Table 1; for additional information on these sites see Monroy et al., 2016). The selected sites were spatially distributed along a W-E precipitation gradient, which ranged from 621.4 up to 311.2 mm/y, with average annual temperature ranging from 8.2 to 12.5 °C (data from 2005 to 2015 available at the Ebro Hydrographic Confederation; <http://www.chebro.es>; Table 1). We used the specific discharge (Q_s), calculated as the mean annual discharge per unit catchment area ($L s^{-1} km^{-2}$) (Munné & Prat 2004) as a proxy for hydrological drought using daily mean flows from a common 10-y period (hereafter Q_s , <http://www.chebro.es>). It ranged from 10.08 to 0.69 $L s^{-1} km^{-2}$.

Table 1. Location, reach characterization and hydrological and climatic attributes for the 5 studied streams arranged along the longitudinal gradient W–E. Water physicochemical characteristics during the study period are also shown ($n = 4$; mean \pm ES). For air and water temperature, annual mean and daily mean values, respectively, and its ranges (in parenthesis) are shown. Q_s = Specific discharge; SRP = soluble reactive phosphorus.

	San Antón	Moradillo	Homino	Alhama	Nájima
Site code	SAN	MOR	HOM	ALH	NAJ
Latitude (N)	42° 41' 06.5"	42° 42' 24.9"	42° 34' 36.5"	41° 53' 56.5"	41° 29' 54.1"
Longitude (W)	3° 52' 46.3"	3° 43' 33.8"	3° 35' 20.4"	2° 09' 34.5"	2° 11' 58.5"
Altitude (m a.s.l.)	764	786	755	920	918
Basin area (km^2)	47.9	59.1	55.6	45.0	41.5
Mean channel width (m)	3.3 \pm 0.7	3.0 \pm 0.3	2.9 \pm 0.5	3.5 \pm 0.9	1.9 \pm 0.3
Tree canopy cover (%)	83	62	93	73	79
Annual mean air temperature (°C)*	8.2 (3.3-14.4)	10.0 (3.3-14.4)	12.5 (7.3-17.6)	10.6 (6.9-14.9)	12.4 (5.9-19.4)
Annual mean precipitation (mm)*	621.4	543.0	503.9	379.6	311.2
Q_s ($L s^{-1} km^{-2}$) *	10.08	10.08	3.21	0.84	0.69
Discharge ($L s^{-1}$)	255.5 \pm 55.0	237.8 \pm 74.6	135.3 \pm 55.4	290.1 \pm 140.7	46.24 \pm 4.6
Water temperature (°C)	8.2 (5.0-12.1)	9.0 (6.4-12.7)	6.8 (2.6-12.1)	5.5 (0.2-13.9)	7.5 (2.0-13.9)
Conductivity ($\mu S cm^{-1}$)	434.3 \pm 10.1	426.5 \pm 6.9	544.5 \pm 41.5	498.3 \pm 11.2	650.5 \pm 17.3
pH	8.32 \pm 0.04	8.13 \pm 0.03	8.39 \pm 0.02	8.51 \pm 0.02	8.03 \pm 0.01
Oxygen saturation (%)	103.8 \pm 3.4	109.5 \pm 5.9	104.9 \pm 3.7	105.4 \pm 3.4	90.9 \pm 1.5
Nitrate ($mg N L^{-1}$)	1.02 \pm 0.22	0.52 \pm 0.07	9.92 \pm 3.41	4.36 \pm 0.11	6.64 \pm 1.17
Nitrite ($\mu g N L^{-1}$)	1.76 \pm 0.32	1.56 \pm 0.29	4.59 \pm 0.99	4.11 \pm 1.00	5.22 \pm 1.07
Ammonium ($\mu g N L^{-1}$)	73.0 \pm 32.8	46.7 \pm 21.1	33.9 \pm 14.8	46.1 \pm 22.4	39.7 \pm 18.2
SRP ($\mu g P L^{-1}$)	9.18 \pm 3.77	9.01 \pm 4.37	16.00 \pm 7.77	7.23 \pm 3.33	5.79 \pm 3.62

*values calculated based on 10-year record (2005- 2015) from nearby meteorological or gauging stations. Data from the Ebro Hydrographic Confederation (<http://www.chebro.es>)

The surrounding Mediterranean forest was dominated by *Q. faginea*. Riparian vegetation was relative good conserved and consisted of tree species dominated by *Populus nigra sp* and *Salix*

sp. The streambed of the streams was composed of heterogeneous substrate dominated by cobble along with boulder in SAN and ALH and with pebble in MOR. In NAJ it was dominated by pebble, gravel and sand, and in HOM the travertine substrate was dominant (until 70 %).

Experimental design

We selected three plant functional types defined in terms of plant carbon allocation strategies belonging to *Quercus* genus: *Q. robur* (deciduous; R), *Q. faginea* (marcescent; F) and *Q. ilex* (evergreen; I). Litter from these three species varies in several quality traits (Table 2).

Table 2. Initial N and P concentrations (% dry mass) and C:N, C:P and N:P ratios for each single litter species (mean \pm SE; n = 4-5) and mixtures (calculated from its constituent species). Lignin (% dry mass) values were taken from the literature. Different letters indicate statistical differences (ANOVA and Tukey's HSD post-hoc). *Q. robur* = R, *Q. faginea* = F, *Q. ilex* = I.

Species	% C	% N	% P	C:N	C:P	N:P	Lignin
R	50.2 \pm 1.1 ^a	1.30 \pm 0.04 ^a	0.05 \pm 0.004 ^{ab}	44.9 \pm 0.9 ^a	2686.8 \pm 144.2 ^a	59.9 \pm 4.1 ^a	38.50*
F	51.6 \pm 0.6 ^a	1.70 \pm 0.06 ^b	0.04 \pm 0.007 ^a	35.5 \pm 0.9 ^b	3659.4 \pm 780.4 ^a	101.9 \pm 18.7 ^b	14.7 ⁺
I	50.4 \pm 0.8 ^a	1.71 \pm 0.05 ^b	0.06 \pm 0.002 ^b	34.4 \pm 0.9 ^b	2061.3 \pm 63.5 ^a	60.2 \pm 3.3 ^a	15.1 [#]
RF	50.89	1.50	0.05	39.62	2921.2	73.73	
RI	50.28	1.51	0.05	38.95	2593.2	66.57	
FI	50.99	1.71	0.06	34.89	2390.0	68.51	
RFI	50.72	1.57	0.05	37.68	2616.4	69.43	

* Graça and Poquet, 2014; ⁺ Castro-Díez et al., 1997; [#] Jabiol and Chauvet., 2015

In October 2014, we collected leaves of these 3 species at 2 locations from the North of the Iberian Peninsula (42°54'30" N, 2°44'15" W; 43°12'50" N 3°16'10" W). These three-leaf species were air-dried in the laboratory and enclosed in coarse mesh bags (20 \times 25 cm, 5 mm mesh size), which allowed both microbial and invertebrate colonization. Each bag contained approximately 4.5 g (\pm 0.1 g) of air-dried litter that belonged to 1 species (monospecific) and 2 or 3 species (all possible species combination of two and three species, containing 2.25 or 1.50 g per species, respectively) resulting in 7 leaf-litter treatments (R, F, I, RF, RI, FI, RFI). The bags were deployed in the 5 streams on 2nd to 3rd December 2014. At each site, 5 iron bars were anchored randomly to the streambed in riffle sections along 50 m of the channel. One bag per treatment was tied to each bar by nylon lines and each one of them was replicated five times making a total of 35 bags per site (5 samples per species treatment). An extra set of five bags per species was used to correct initial mass values for manipulation loss and estimate air-dried to oven-dry mass (72 h at 60 °C) and to ash free dry mass (AFDM, 12 h at 500 °C) conversion factors.

On 20th to 21st April 2015, all litter-bags were retrieved, enclosed individually in zip-lock bags and transported in a refrigerated cooler to the laboratory for processing. The leaf litter

material from each bag was rinsed with distilled water on a 200- μm sieve to remove sediments and associated invertebrates. Remaining leaves in the mixtures were separated into species for analyses and calculations. The remaining material was oven-dried (60 $^{\circ}\text{C}$, 72 h) and weighed to determine leaf dry mass. A portion of leaf material from each bag and species was ground (1 mm pore sieve) and stored (-20 $^{\circ}\text{C}$) and the rest was combusted (500 $^{\circ}\text{C}$, 12 h) and weighed to determine the remaining ash free dry mass (AFDM). The collected fauna was preserved in 70% ethanol.

Environmental variables

During the study period, water temperature was recorded hourly with ACR Smart-Button temperature loggers (ACR Systems Inc) at each site. Conductivity, pH and oxygen saturation were measured on four occasions with a multiparametric sensor (WTW Multi 350i). Discharge was estimated on these occasions from instantaneous water velocity measured by a current meter (MiniAir 2, Schiltknecht Co). Water samples for nutrient analyses were collected at all streams on each sampling date, immediately filtered (Millipore, 0.45 μm pore) and transported to the laboratory in a cooler, where they were frozen (-20 $^{\circ}\text{C}$). Nitrate concentration was determined by capillary ion electrophoresis (Agilent CE), and the rest of the nutrients were analysed colorimetrically (Spectrophotometer Jasco V-630): nitrite by the sulphanilamide method, ammonium by the salicylate and dichloroisocyanurate method and dissolved reactive phosphorus (SRP) by the ascorbic acid method (APHA 2005).

Leaf-litter stoichiometry

Initial and final (after bags retrieval) concentrations of carbon (C), nitrogen (N) and phosphorus (P) of each species were measured. C and N concentrations were determined using a CHNS/O elemental analyser (Perkin Elmer II) and P colorimetrically after autoclave-assisted extraction (APHA 1998). Results were expressed as a percentage elemental content of leaf dry mass (% C, % N, % P). For assessing litter quality of mixtures both at the start and at the end of the experiment, we calculated the combination nutrient concentrations from individual measurement of the present species as follows:

$$E_m = \left(\sum_{j=1}^n (E_j \times g \text{ DM}_j) \right) / g \text{ DM}_m$$

Where: E , being elemental (C, N or P) nutrient concentration; DM, litter dry mass; j , species; n , number of species in the mixture; m , mixture.

Moreover, we calculated N and P remaining as a proxy of nutrient mineralization and/or immobilization as follows:

$$\text{Nutrient remaining (\% initial)} = (E_f \times M_f) / (E_i \times M_i) \times 100$$

Where: E_i or E_f is initial or final nutrient concentration (% DM), respectively; M_i or M_f is initial or final DM, respectively.

Litter-associated invertebrates

Litter-associated invertebrates were identified to family level (except Oligochaeta, identified to order), counted and sorted into functional feeding groups of shredder and non-shredder invertebrates (Tachet *et al.* 2002). Shredders and non-shredders from each sample were oven-dried (70 °C, 72 h) and weighed (± 0.0001 g). Results were expressed as density (no. inv bag⁻¹) and biomass (mg bag⁻¹) of total and shredder invertebrates.

Data analysis

A principal component analysis (PCA) was performed to discern the main variables responsible for the environmental variability across the streams. It was performed with the average values of pH, conductivity, nitrate, ammonium, SRP, temperature, precipitation, Qs, altitude and canopy cover. Differences in stream water parameters among sites were analysed by one-way ANOVAs followed by Tukey's HSD test.

Litter decomposition was estimated through the relative litter mass loss (LML) during the experiment and calculated as (initial AFDM - final AFDM)/initial AFDM. We calculated LML for each litter species in a bag, and total LML (leaf mass loss of all leaf species in the bag) in both single- and mixed-species bags. We further estimated net diversity effect on litter decomposition as the difference between the observed LML of a mixture and its expected LML based on the average LML of single-species treatment for each stream. Net diversity effect was tested against the null hypothesis that average difference was zero (*t*-test). If the net diversity effect deviates from zero, non-additive leaf litter diversity effects occurred during the decomposition process.

The relative LML, net diversity effects, final litter nutrients, nutrient changes and invertebrate variables were analysed using two-way ANOVA (factors: "species treatment" and "stream"). We further explored if the presence/absence of a species had effect on total LML (ANOVA; factors: "presence/absence" of each litter species and "stream") and whether results for LML depended on litter plant species using LML data for each plant species (three-way ANOVA; factors: "species identity", "species treatment" and "stream"). To analyse invertebrate community among streams and species treatment, a non-metric multidimensional scaling

(nMDS) was performed based on the Bray-Curtis dissimilarity matrix followed by PERMANOVA with the *adonis* function in the “*vegan*” R package. Bivariate relationships between litter decomposition or diversity effect and both biological and litter nutrient variables as well as among these and environmental variables were tested by linear regressions.

All statistical analyses were performed with R software (R Development Core Team 2015). When necessary, data were log-transformed to achieve requirements for parametric analyses.

Results

Stream water characteristics

The PCA based on environmental variables captured 77.4 % of variance among sites (Fig. 1). Axis 1 (45.8%) separated streams along a gradient of drought as follows: SAN, MOR, HOM, ALH, NAJ. Axis 2 (31.6%) was driven positively by SRP and negatively by altitude. All streams presented well oxygenated waters and pH around 8 (Table 1). Conductivity ranged from 426.5 to 650.5 $\mu\text{S cm}^{-1}$ ($F_{4,15}= 18.41$; $p < 0.00001$), NAJ stream showing higher values (post-hoc: $p < 0.05$; Table 1). Water temperature varied about 3.5 °C among streams (Table 1; $F_{4,680}= 73.87$; $p < 0.00001$). SRP concentration was low and similar among streams ($p = 0.63$) but dissolved nitrate differed among them (Table 1; $F_{4,15}= 5.92$; $p = 0.004$).

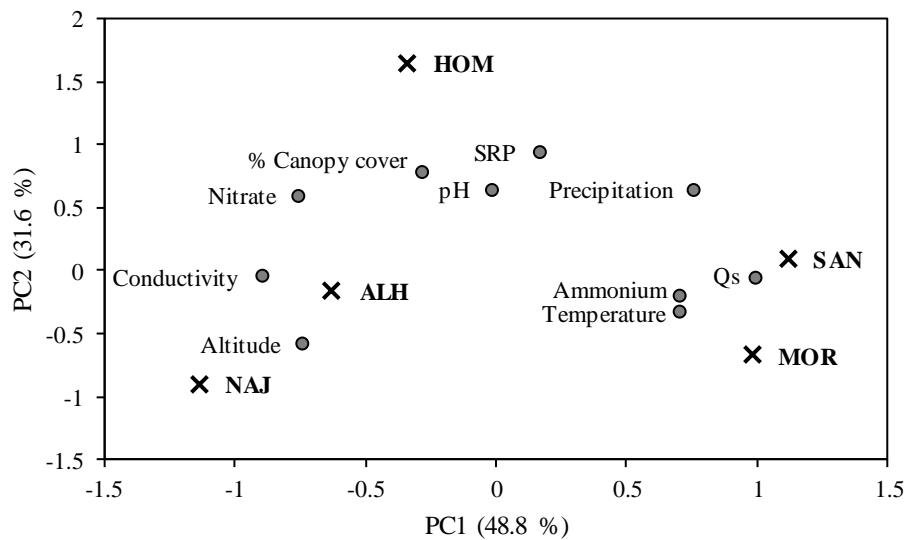


Fig. 1. Principal component analysis of environmental characteristics of the 5 streams. Qs = Specific discharge (see Methods); SRP = soluble reactive phosphorus.

Leaf-litter decomposition

In general, the diversity increase did not seem to influence litter decomposition (Fig. 2A). Looking at the species treatment, only R treatment showed a significant lower mass loss (Table 3, post-hoc: $p < 0.05$; Fig. 2B). The presence/absence of particular species had a significant effect on total LML in the bag (; ANOVA $F_{1,157} = 6.23$, $p = 0.01$): the decomposition was higher when *Q. faginea* (F) was present (Fig. 2B).

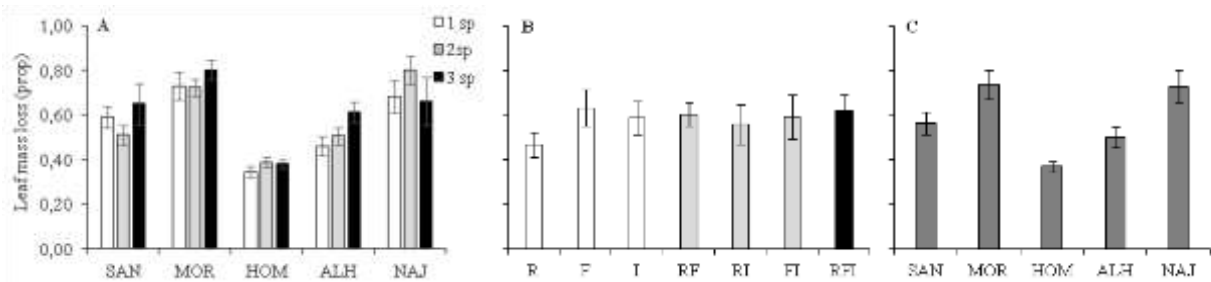


Fig. 2. Leaf mass loss (mean \pm SE) in relation to leaf species diversity (A), leaf treatment (B) and streams (C). R = *Q. robur*, F = *Q. faginea*, I = *Q. ilex*.

Examining the individual LML of the three-litter species when they were incubated alone or in mixture, we found significant differences among species (Fig. S1A; $F_{2,190} = 11.48$, $p < 0.00001$) with lower values for R than for F and I (on average, 50% vs 62 and 60%, respectively). The LML of the three-litter species differed among streams ($p > 0.05$) and all followed the same pattern across streams (Fig. S1B) with the higher decomposition at NAJ and MOR and the lowest at HOM. The three-litter species did not show significant differences in relation to species combination (Fig. S1A; $F_{6,190} = 0.97$, $p = 0.44$).

The average of LML across streams of total LML in a bag (leaf mass loss of all leaf-species in the bag) as well as the individual LML of three-species were negatively related to the scores of axis 2 of PCA (Fig. 3 and S1C, respectively): the stream with the highest nutrient concentrations (HOM) showing the lowest LML.

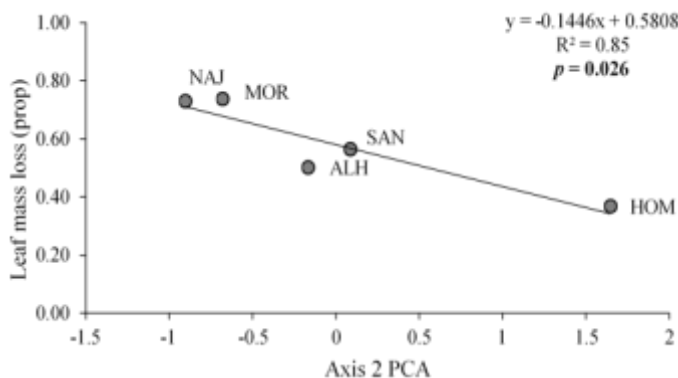


Fig. 3. Relationships between leaf mass loss (LML) and scores on axis 2 of PCA of each stream for overall LML (data pooled across species compositions) (A). The p -value and fitted lines are also shown.

Diversity effects on species mixing

In general, the net diversity effects on litter decomposition did not differ significantly from zero (t -test: $p > 0.05$; Fig. 4). It showed a positive trend with diversity increase (t -test: $p > 0.05$; Fig. 4A). Although net diversity effect was not significant (t -test: $p > 0.05$), it differed depending on species mixture and stream (Table 3 “Net diversity”; Fig. 4B and C). Overall net diversity effect showed a significant negative relationship with drought gradient (Fig. 5) and scores on axis 1 of PCA (data not shown; $r^2 = 0.81$; $p = 0.038$).

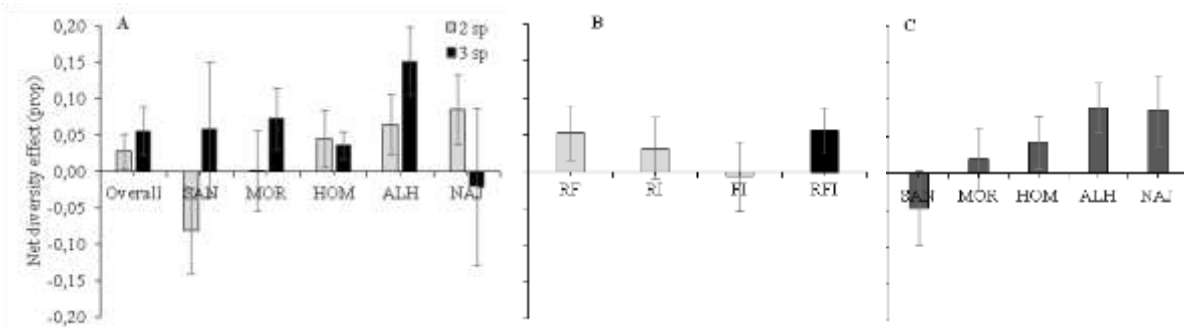


Fig. 4. Net diversity effect of leaf-litter mixtures (mean \pm SE) on LML for each diversity level and stream (A; average across all 2- and 3 species is also shown), species mixture (B) and stream (C; data pooled across all diversity levels). Net diversity calculated as observed minus expected leaf mass loss. Negative deviation from zero value indicates non-additive effect of mixture. Asterisks indicate significant non-additive diversity effect (t -test; $p < 0.05$). R = *Q. robur*, F = *Q. faginea*, I = *Q. ilex*.

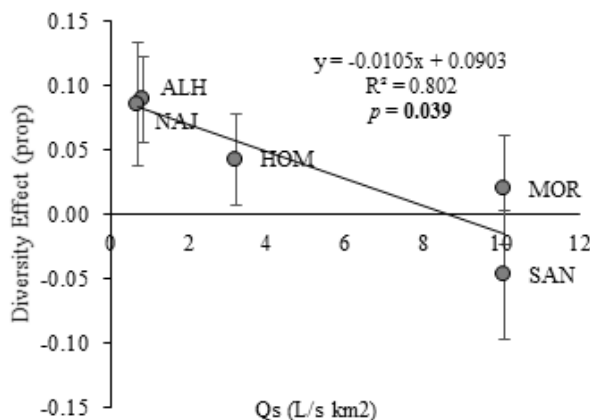


Fig. 5. Relationships between net diversity effect and drought gradient defined as Q_s (left; see Methods) and scores on axis 1 of PCA (right). The p -values and fitted lines are also shown.

Leaf-litter nutrients

The initial % C was around 50.2-51.6 % for all species. N and P concentrations were 1.30 % and 0.05 %, respectively, for R litter, 1.70 % and 0.04 % for F litter, and 1.71 % and 0.06 % for I. In general, R and I treatment showed lower and higher values respectively (Table 2).

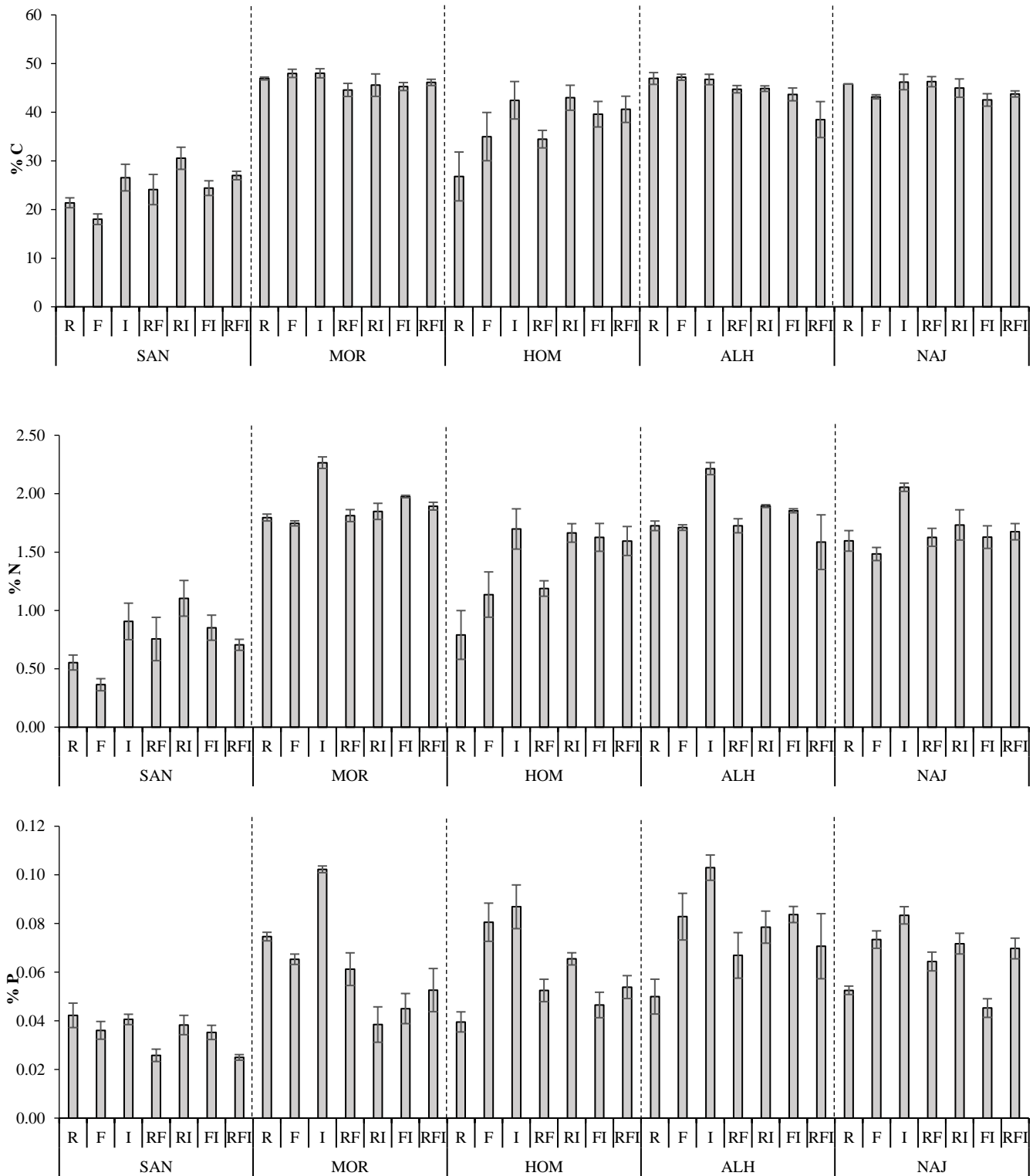


Fig. 6. Leaf carbon (C), nitrogen (N) and phosphorus (P) concentration (%DM) in relation to litter treatment for each stream at the end of the experiment.

The final % C was lower than initial values for all litter treatments in the five streams. It differed significantly among streams ($F_{4,120} = 112.7$; $p < 0.0001$) with SAN and HOM showing

values around 24.5 % and 37.3 % respectively, and approximately of 45 % C in the other streams (Fig. 6). Differences among species treatments only were found at SAN and HOM (treatment \times stream interaction; $F_{24,120} = 2.28$, $p = 0.001$). The final % N was lower (at SAN and HOM) or similar to the initial concentrations (Fig. 6). It was significantly different among species treatments ($F_{6,120} = 16.38$, $p < 0.0001$) and among streams ($F_{4,120} = 127.74$; $p < 0.0001$), with a significant treatment \times stream interaction (Fig.6; $F_{24,120} = 1.99$, $p = 0.008$). In general, final % P was higher than initial values in all streams for all species treatments except at SAN (Fig. 6), which showed statistically significant differences with the others (post-hoc $p < 0.005$). There was also a significant treatment \times stream interaction ($F_{24,120} = 4.76$, $p < 0.0001$) without common pattern for species treatments among streams (Fig. 6).

Neither initial nor final C, N and P concentrations were related to LML ($p > 0.05$).

Changes in nutrient mass

In general, N content decreased for all species and streams (Fig. 7). There were great differences in N changes among streams ($F_{4,120} = 16.11$, $p < 0.0001$) and litter composition ($F_{6,120} = 5.57$, $p < 0.0001$), with a statistically significant litter composition \times stream interaction ($F_{24,120} = 2.14$, $p = 0.004$). HOM and NAJ did not showed significant differences among litter treatments and SAN, MOR and ALH showed lower values for F and RFI treatments. The change in P content showed a similar pattern to N for streams. It decreased in all cases except at SAN and HOM streams, where immobilization was clear, mainly for R and F (Fig. 7). It showed great differences among streams ($F_{4,120} = 20.75$, $p < 0.0001$), with MOR and NAJ showing higher decrease (Fig. 7), and among litter treatments ($F_{6,120} = 2.90$, $p = 0.01$).

Invertebrate communities

Total invertebrate density ranged from 15.6 to 145.0 ind bag⁻¹ and for shredders, from 2.80 to 37.6 ind bag⁻¹. Total and shredder density varied significantly among streams but not among litter composition (Fig. 8; Table 3: “Total density” and “Shredder density”). For total density, the lowest and highest values were observed in NAJ and SAN streams respectively, and for shredders, the highest values in SAN (no differences among the rest of the streams). Total invertebrate biomass ranged from 17.5 to 118.9 mg bag⁻¹ and shredder biomass from 2.78 to 98.1 mg bag⁻¹. On average, total invertebrate biomass did not differ significantly among litter treatments, but depended on the stream (Fig. 8; Table 3 “Total biomass”) with the lowest and highest values in HOM and SAN, respectively. The shredder biomass showed differences among streams, with ALH, HOM and MOR showing the lowest values and SAN the highest (Fig. 8), but not among litter treatments (Table 3 “Shredder biomass”).

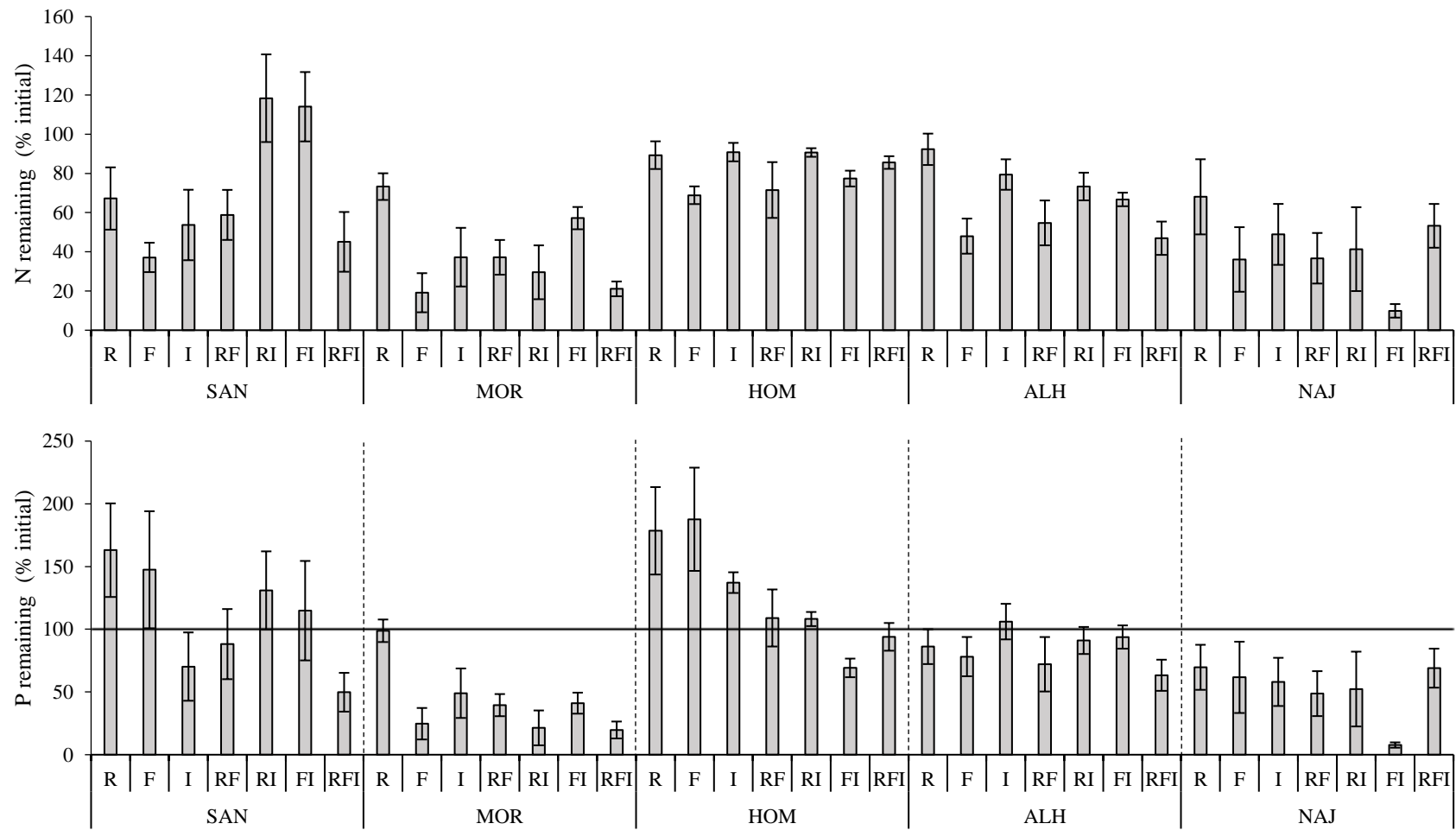


Fig. 7. Nitrogen and phosphorus remaining (% initial) in relation to each leaf treatment for each stream.

Table 3. Results of analyses of variance (factors: stream, leaf species treatment and interactions) on leaf mass loss (LML), net diversity, nutrient concentration, and invertebrate variables (total invertebrate and shredder density and biomass). Significant values are highlighted in bold.

	Df	F value	<i>p</i>
LML			
Stream	4	23.85	< 0.0001
Treatment	6	2.21	0.04
Stream× Treatment	24	0.68	0.86
Residuals	130		
Net Diversity			
Stream	4	1.66	0.17
Treatment	3	0.44	0.72
Stream× Treatment	12	1.03	0.43
Residuals	73		
% N			
Stream	4	127.74	< 0.0001
Treatment	6	16.39	< 0.0001
Stream× Treatment	24	1.99	0.008
Residuals	120		
% P			
Stream	4	56.99	< 0.0001
Treatment	6	22.86	< 0.0001
Stream× Treatment	24	4.76	< 0.0001
Residuals	120		
Total density			
Stream	4	21.43	< 0.0001
Treatment	6	1.15	0.34
Stream× Treatment	24	0.98	0.50
Residuals	131		
Shredder density			
Stream	4	11.98	< 0.0001
Treatment	6	0.55	0.77
Stream× Treatment	24	0.48	0.98
Residuals	131		
Total biomass			
Stream	4	8.03	< 0.0001
Treatment	6	0.44	0.85
Stream× Treatment	22	1.47	0.10
Residuals	116		
Shredder biomass			
Stream	4	7.29	< 0.0001
Treatment	6	1.16	0.33
Stream× Treatment	24	1.63	0.04
Residuals	131		

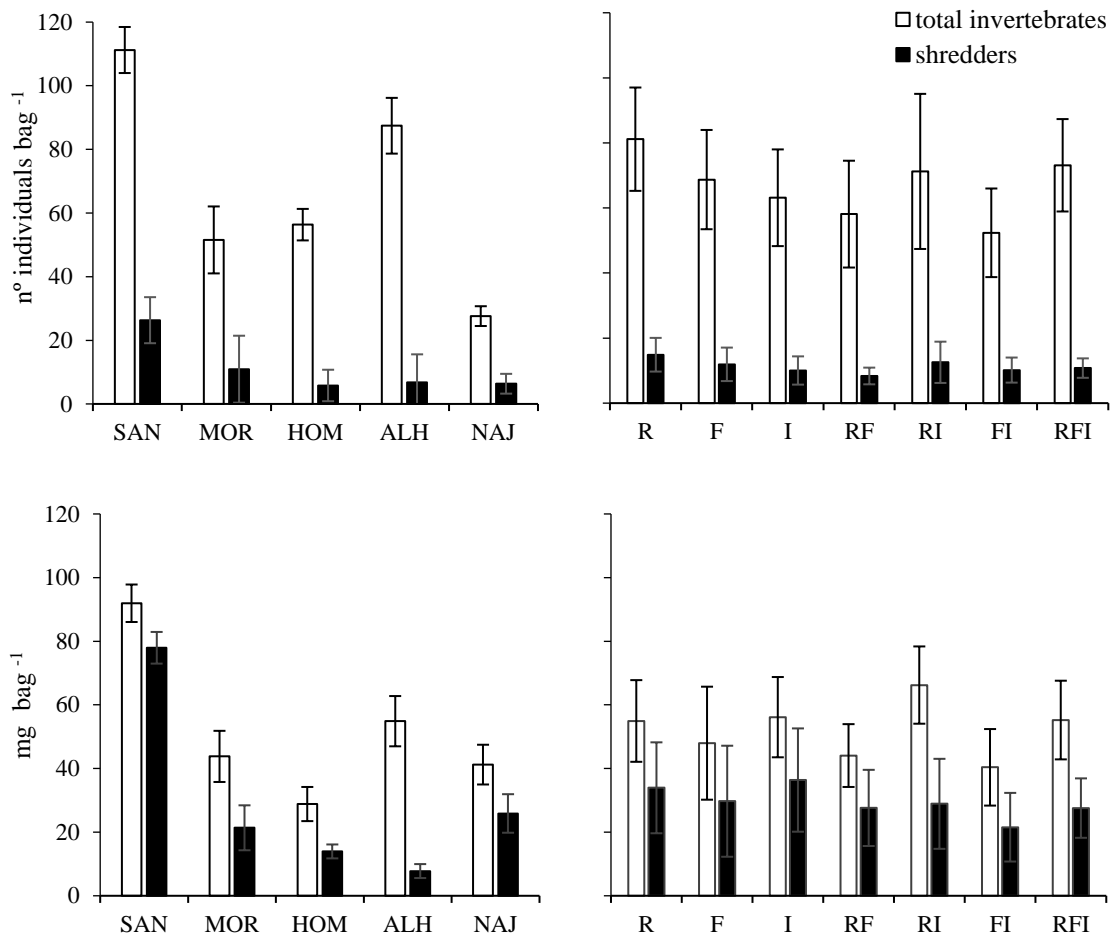


Fig. 8. Density (up) and biomass (down) of total invertebrates and shredders bag⁻¹ (mean \pm SE) for each stream and leaf treatment.

The total invertebrate assemblage did not differ among litter treatments but they were consistently grouped in relation to streams (Fig. 9), pointing out the different structure of leaf-associated invertebrates among them (PERMANOVA, Pseudo- $F_{4,127} = 15.94$, $p < 0.001$). At HOM stream highlights the presence of collector-gatherers and of collector-filterer at ALH (mainly Hydropsychidae), representing up to 75 and 20 % of total invertebrates, respectively. For shredder assemblage, the same pattern as total invertebrate was observed (PERMANOVA, stream: Pseudo- $F_{4,121} = 12.40$, $p < 0.001$). A total of ten families of shredders were identified. They represented from 8% to 26 % of total invertebrate density depending to the stream considered. In general, Gammaridae dominated the assemblage of shredders and represented up to 20% of this one at SAN, but this family was absent at ALH, where together to NAJ stream, shredders were dominated by Nemouridae taxa (6% and 11% of the total shredder density, respectively). In NAJ, also highlights the presence of Tipulidae taxa.

The density of shredders showed a negative trend with drought gradient (Fig. 8; $r^2 = 0.57$; $p = 0.08$) and a negative significant relationship with the estimated net diversity across streams ($r^2 = 0.77$; $p = 0.03$). The litter decomposition was not correlated with any invertebrate variable.

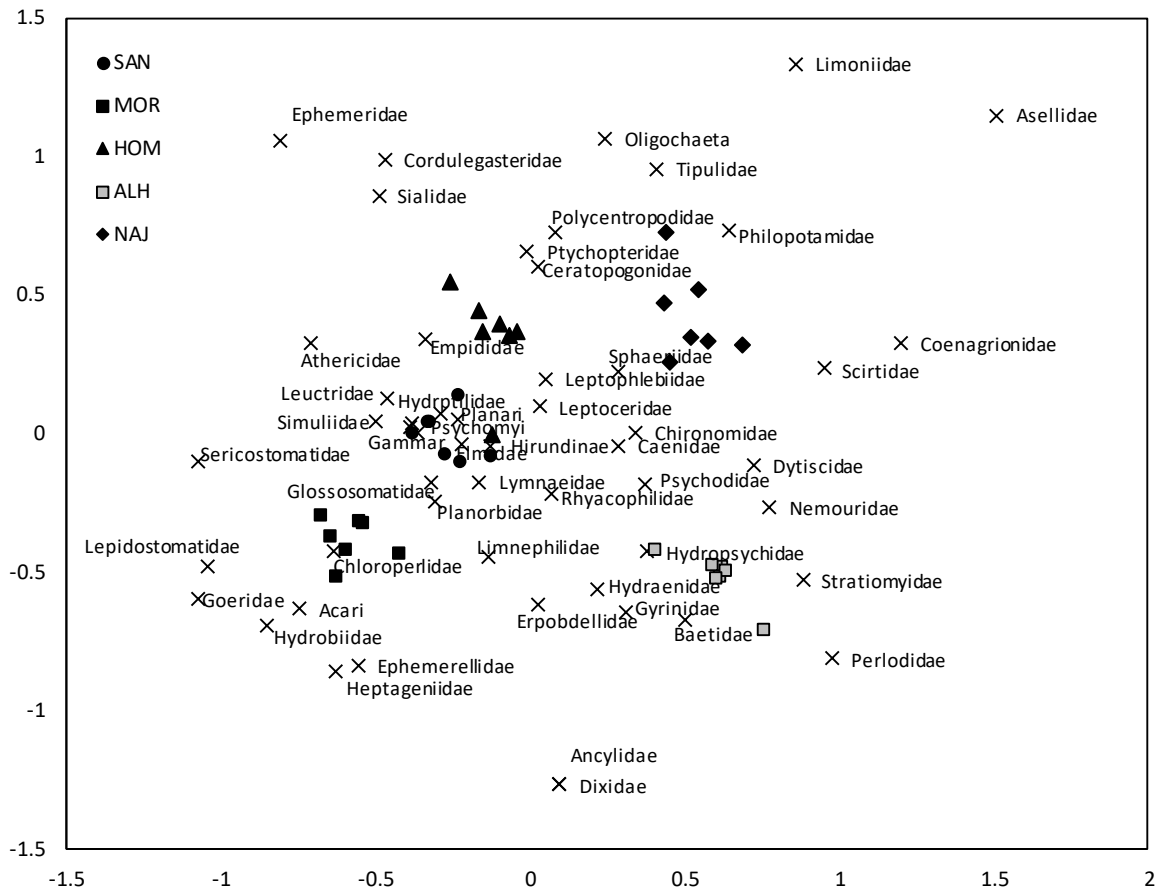


Fig. 9. Non-metric multidimensional scaling ordination (NMDS) of the leaf-litter associated invertebrates in relation to streams (different symbols) based on density data at family level.

Discussion

The increase of plant diversity did not determine the litter decomposition rate, which greatly depended on the stream. There was a general common pattern in leaf decomposition among streams independently of plant diversity level or litter treatment. Further, this pattern across streams was also maintained for the decomposition of each of the three-litter species when they were alone or in mixture. It points out a greater influence of other abiotic and biotic factors on the decomposition than litter diversity. Specifically, the leaf decomposition was negatively related to nutrient content in water stream, with the lower values being found in the stream with the highest nutrient loading, supporting previous findings that indicate an inhibition or restriction of this process at excessive nutrient load (Woodward *et al.* 2012). This result

contrasts with previous B-EF studies in both microcosm and field surveys that in general, suggest positive non-additive effects of mixing of different plant litter types (Lecerf et al., 2011) and a consistent enhancement of decomposition rate across biomes resulting from the higher variability in plant litter and possible interactions between mixed-species litter (Handa *et al.* 2014). In the present study, the differences in leaf decomposition among treatments were subtle, however, the presence of *Q. faginea* seemed to enhance the litter decomposition (the average LML of pooled treatments where it was present was 61% vs 54% where it was absent). Furthermore, attending to litter decomposition of each species, it pointed out that *Q. robur* and *Q. ilex* increased its individual LML when they were incubated with *Q. faginea*. Therefore, it agrees with the assumption that more labile leaves would have positive effect on the decomposition of poorer leaves (Lecerf *et al.* 2005). However, it is possible that the three-litter species selected to test functional diversity (the carbon allocation strategy) effects on decomposition, might have constituted a relatively homogeneous category respect to the pool of litter traits, since the three *Quercus* species are slow-decomposing and low-quality species, hindering to show stronger diversity effect as reported for others (Schindler and Gessner, 2009). Nevertheless, the overall estimated net diversity effect tended to be higher in the streams suffering higher drought stress, suggesting that the effects of the resource diversity could be enhanced under limiting conditions. In the same line, in the terrestrial ecosystems higher positive interactions of diversity on processes when plant species were exposed a stress (Mulder, Uliassi & Doak 2001; Callaway *et al.* 2002). For example, Mulder et al. (2001) observed increases in plant biomass with species richness under drought but not under constant conditions, and suggested that facilitative interactions against competition may be a mechanism linking high diversity to high productivity under stressful environmental conditions. In aquatic ecosystems, some studies also reported that the diversity effect could be modulated by the trophic status of streams and non-additive effect of litter mixing would be reduced in a nutrient-enriched stream where nutrients are not limiting (e.g., Rosemond et al., 2010; Lima-Fernandes et al., 2015). Understanding how stressful or limiting conditions affect the way in which the biodiversity affect to process responses is complicated by potential interactions among abiotic and biotic variables at multiple levels of organisation (Gessner & Hines 2012), therefore, it is difficult disentangle the mechanisms behind the observed pattern in our study. However, the results were variable across litter mixtures and streams and should be considered with caution since there were large deviations in the estimated net diversity values, which were not statistically significant.

Response of nutrient to litter diversity

The leaf decomposition rate is usually related to the litter quality (Melillo, Aber & Muratore 1982; Hladyz *et al.* 2009). Therefore, we expected that the treatment containing beneficial traits

such as higher N or lower lignin content would enhance decomposition rate. However, in this study no relationship between nutrient concentration in the leaf-litter (initial or final %N and %P) and decomposition rate was observed, suggesting that other characteristics beside leaf nutrient could be influencing mass loss, such as the concentration of refractory structural compounds which are high in *Q. robur* and *Q. ilex* (Castro-Díez *et al.* 1997; Gonçalves, Graça & Canhoto 2013; Jabiol & Chauvet 2015). However, there is controversy about the traits involved in the effects of diversity on the decomposition process (e.g., Frainer *et al.* 2015). Some evidences from previous studies inform that heterogeneous litter mixtures affect positively the diversity effects through its action on microorganisms, which could take advantage of the presence of high-N or -P litter mitigating the limitation of both elements in other poorer litters (Gessner *et al.* 2010; Handa *et al.* 2014). Therefore, we should expect that dissimilarity in litter nutrients of the species involved in the mixtures would affect the N and P in the litters and, in turn, would promote positive non-additive effects. However, an inconsistent pattern was observed for both final N and P concentration among diversity levels, species treatments and streams in this study, which highlights the complex behaviour of litter nutrients in relation to biotic and abiotic factors. Nevertheless, in general, litter nutrient content declined during the decomposition and the mineralization of N and P followed the same trend as decomposition across the streams, the decline being more pronounced at MOR and NAJ, the two streams experimenting the higher decomposition. By contrast, neither pattern was observed among treatments across streams. The nitrogen availability in the studied streams was high enough to have been able to override any possible N effect transfer between litters (Rosemond *et al.* 2010; Lima-Fernandes *et al.* 2015; Tonin *et al.* 2017) since fungal species can take it from water column (Gulis & Suberkropp 2003; Cheever *et al.* 2012). Moreover, given the calcareous lithology of the stream basin, some streams were characterised by travertine precipitation on leaf surface, particularly SAN and HOM (values of C lower than 37% at SAN and HOM; see also ash concentration in the leaf-litter in Monroy *et al.*, 2016). This characteristic might have conditioned the observed leaf nutrient content, specially that of phosphorus, and, in turn, the litter microbial conditioning (Casas & Gessner 1999).

Response of invertebrates to litter diversity

There was an important variation in the leaf-litter associated invertebrate community among streams, in both total and detritivore community, but differences in invertebrate community among litter species treatments were not observed. In the same way, although density and biomass of total invertebrates and shredders varied among streams, the leaf-mixtures did not influence the leaf-litter associated invertebrates, contrary to what was expected due to the greater heterogeneity of resource and habitat in the mixtures. More diverse litter species mixing can benefit detritivores because they could use higher variety of resource quality that can be

more appropriate to meet their stoichiometric requirement as well as increase habitat complexity and stability (Sanpera-Calbet *et al.* 2009; Lecerf *et al.* 2011), whereas streams with higher abundance or biomass of invertebrates would lead to faster decomposition rates (Pozo *et al.* 2011; Monroy *et al.* 2016) and to higher non-additive diversity response (Handa *et al.* 2014; Jabiol & Chauvet 2015). However, despite the differences in leaf-litter associated invertebrate among streams, the overall leaf decomposition was not related to density or biomass of litter-associated invertebrates. The absence of relationship between litter-associated invertebrates and the decomposition rate of slow-decaying and low-quality species (oak) was also observed in a parallel study carried out in the same streams (Monroy *et al.* 2016). Further, the main differences in litter-associated invertebrates was observed between SAN and the other streams since at SAN the values of density and biomass were 2- 4-fold higher, respectively, than the others. The relative low decomposition in SAN, in spite of its higher invertebrate density and biomass, was likely because of the travertine deposition on leaf surface (see previous comment section). Microbial conditioning and physical fragmentation could be hampered by travertine (Casas & Gessner 1999), also affecting detritivore activities and leading to a reduction in leaf shredding, and thereby decomposition. By contrast, the negative trend of shredder density colonizing bags with drought gradient and its positive correlation with the enhancement of non-additive suggest that the observed variability in the net diversity effect could be related to in the leaf-consuming invertebrates.

The findings from this study suggest that other ecological factors may be more important than functional diversity on organic matter processing in natural systems (Petchey & Gaston 2006) and could mask the weak litter diversity effect, related to the identity of litter species, in calcareous Mediterranean streams. These finding are consisted with outcomes from other studies in temperate ecosystems showing that litter species richness is less important than composition in controlling litter decomposition rates (Lecerf *et al.*, 2007; Schindler and Gessner, 2009; Swan *et al.*, 2009; Swan and Palmer, 2004). However, our results suggest that future drier climate could affect the B-EF relationships since species diversity-leaf processing relationship could more prevalent under drier conditions. Therefore, although environmental conditions other than leaf functional diversity might have stronger effect on the decomposition process in calcareous streams from Mediterranean areas, it would be necessary to consider the abiotic and biotic differences at local and regional scales to predict the effects of plant diversity loss in riparian forests on organic matter processing and to disentangle the complex interactions between plant species, abiotic factors and litter decomposers.

References

- APHA (1998) *Standard methods for the examination of water and wastewater*, 20th edn. (Ed. American Public Health Association (APHA)), American Water Works Association., & Water Environment Federation, Washington, D.C.
- APHA (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C.
- Battin T.J., Luysaert S., Kaplan L. a., Aufdenkampe A.K., Richter A., Tranvik L.J., *et al.* (2009) The boundless carbon cycle. *Nature Geoscience* **2**, 598–600.
- Bonada N., Dolédec S. & Statzner B. (2007a) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: Implications for future climatic scenarios. *Global Change Biology* **13**, 1658–1671.
- Bonada N. & Resh V.H. (2013) Mediterranean-climate streams and rivers: Geographically separated but ecologically comparable freshwater systems. *Hydrobiologia* **719**, 1–29.
- Bonada N., Rieradevall M. & Prat N. (2007b) Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia* **589**, 91–106.
- Boyero L., Pearson R.G., Dudgeon D., Ferreira V., Graça M. a. S., Gessner M.O., *et al.* (2012) Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. *Global Ecology and Biogeography* **21**, 134–141.
- Boyero L., Pearson R.G., Gessner M.O., Barmuta L.A., Ferreira V., Graça M.A.S., *et al.* (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters* **14**, 289–294.
- Callaway R.M., Brooker R.W., Choler P., Kikvidze Z., Lortie C.J., Michalet R., *et al.* (2002) Positive interactions among alpine plants increase with stress. *Nature* **417**, 844–848.
- Cardinale B.J., Duffy J.E., Gonzalez A., Hooper D.U., Perrings C., Venail P., *et al.* (2012) Biodiversity loss and its impact on humanity. *Nature* **489**, 326–326.
- Cardinale B.J., Nelson K. & Palmer M.A. (2000) Linking species diversity to the functioning of ecosystems: on the importance of environmental context. *Oikos* **91**, 175–183.
- Casas J. & Gessner M.O. (1999) Leaf litter breakdown in a Mediterranean stream characterised by travertine precipitation. *Freshwater Biology* **41**, 781–793.
- Castro-Díez P., Villar-Salvador P., Pérez-Rantomé C., Maestro-Martínez M. & Montserrat-Martí G. (1997) Leaf morphology and leaf chemical composition in three Quercus (Fagaceae) species along a rainfall gradient in NE Spain. *Trees - Structure and Function* **11**, 127–134.
- Ceballos G., Ehrlich P.R., Barnosky A.D., García A., Pringle R.M. & Palmer T.M. (2015)

- Accelerated modern human – induced species losses: entering the sixth mass extinction. *Sciences Advances* **1**, 1–5.
- Cheever B.M., Kratzer E.B. & Webster J.R. (2012) Immobilization and mineralization of N and P by heterotrophic microbes during leaf decomposition. *Freshwater Science* **31**, 133–147.
- Frainer A., Moretti M.S., Xu W. & Gessner M.O. (2015) No evidence for leaf trait dissimilarity effects on litter decomposition fungal decomposers and nutrient dynamics. *Ecology* **96**, 550–561.
- Gasith A. & Resh V.H. (1999) Streams in mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology, Evolution, and Systematics* **30**, 51–81.
- Gessner M.O. & Hines J. (2012) Stress as a modifier of biodiversity effects on ecosystem processes? *Journal of Animal Ecology* **81**, 1143–1145.
- Gessner M.O., Swan C.M., Dang C.K., McKie B.G., Bardgett R.D., Wall D.H., *et al.* (2010) Diversity meets decomposition. *Trends in Ecology and Evolution* **25**, 372–380.
- Giorgi F. & Lionello P. (2008) Climate change projections for the Mediterranean region. *Global and planetary change* **63**, 90–104.
- Gonçalves A.L., Graça M. a. S. & Canhoto C. (2013) The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. *Fungal Ecology* **6**, 546–553.
- Gulis V. & Suberkropp K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater biology* **48**, 123–134.
- Handa I.T., Aerts R., Berendse F., Berg M.P., Bruder A., Butenschoen O., *et al.* (2014) Consequences of biodiversity loss for litter decomposition across biomes. *Nature* **509**, 218–21.
- Hladysz S., Gessner M.O., Giller P.S., Pozo J. & Woodward G. (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology* **54**, 957–970.
- Jabiol J. & Chauvet E. (2015) Biodiversity and litter decomposition: a case study in a Mediterranean stream. *Freshwater Science* **34**, 423–430.
- Kominoski J.S., Hoellein T.J., Kelly J.J. & Pringle C.M. (2009) Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? *Oikos* **118**, 457–463.
- Lecerf A., Dobson M., Dang C.K. & Chauvet E. (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia* **146**, 432–442.
- Lecerf A., Marie G., Kominoski J.S., LeRoy C.J., Bernadet C. & Swan C.M. (2011) Incubation time, functional litter diversity, and ecosystem characteristics predict nonadditive litter mixing effects on decomposition: A synthesis from streams. *Ecology* **92**, 160–169.

- Lecerf A. & Richardson J.S. (2010) Biodiversity-Ecosystem Function research: insights gained from streams. *River research and applications*, 45–54.
- Lecerf A., Risnoveanu G., Popescu C., Gessner M.O. & Chauvet E. (2007) Decomposition of diverse litter mixtures in streams. *Ecology* **88**, 219–227.
- Lima-Fernandes E., Fernandes I., Pereira A., Geraldés P., Cássio F. & Pascoal C. (2015) Eutrophication modulates plant-litter diversity effects on litter decomposition in streams. *Freshwater Science* **34**, 31–41.
- Melillo J.M., Aber J.D. & Muratore J.F. (1982) Nitrogen and Lignin control of hardwood leaf litter decomposition dynamics. *Ecology* **63**, 621–626.
- De Micco V. & Aronne G. (2012) Morpho-Anatomical Traits for Plant Adaptation to Drought. In: *Plant Responses to Drought Stress: From Morphological to Molecular Features*. (Ed. R. Aroca), pp. 37–61. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Monroy S., Menéndez M., Basaguren A., Pérez J., Elosegí A. & Pozo J. (2016) Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain). *Science of the Total Environment* **573**, 1450–1459.
- Mulder C.P., Uliassi D.D. & Doak D.F. (2001) Physical stress and diversity-productivity relationships: the role of positive interactions. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 6704–6708.
- Munné A. & Prat N. (2004) Defining river types in a Mediterranean area: A methodology for the implementation of the EU water framework directive. *Environmental Management* **34**, 711–729.
- Naeem S., Duffy J.E. & Zavaleta E. (2012) The Functions of Biological Diversity in an Age of Extinction. *Science* **336**, 1401–1406.
- Pace G., Bonada N. & Prat N. (2013) Long-term effects of climatic-hydrological drivers on macroinvertebrate richness and composition in two Mediterranean streams. *Freshwater Biology* **58**, 1313–1328.
- Pereira H.M., Leadley P.W., Proença V., Alkemade R., Scharlemann J.P.W., Fernandez-Manjarrés J.F., *et al.* (2010) Scenarios for global biodiversity in the 21st century. *Science* **330**, 1496–1501.
- Perkins D.M., Reiss J., Yvon-Durocher G. & Woodward G. (2010) Global change and food webs in running waters. *Hydrobiologia* **657**, 181–198.
- Petchey O.L. & Gaston K.J. (2006) Functional diversity: Back to basics and looking forward. *Ecology Letters* **9**, 741–758.
- Pozo J., Casas J., Menéndez M., Mollá S., Arostegui I., Basaguren A., *et al.* (2011) Leaf-litter decomposition in headwater streams: a comparison of the process among four climatic regions. *Journal of the North American Benthological Society* **30**, 935–950.
- R Development Core Team (2015) R: A Language and Environment for Statistical Computing.

- Reiss J., Bridle J.R., Montoya J.M. & Woodward G. (2009) Emerging horizons in biodiversity and ecosystem functioning research. *Trends in ecology & evolution* **24**, 505–514.
- Rosemond A.D., Swan C.M., Kominoski J.S. & Dye S.E. (2010) Non-additive effects of litter mixing are suppressed in a nutrient-enriched stream. *Oikos* **119**, 326–336.
- Sanpera-Calbet I., Lecerf A. & Chauvet E. (2009) Leaf diversity influences in-stream litter decomposition through effects on shredders. *Freshwater Biology* **54**, 1671–1682.
- Schindler M. & Gessner M.O. (2009a) Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology* **90**, 1641–1649.
- Schindler M.H. & Gessner M.O. (2009b) Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology* **90**, 1641–1649.
- Srivastava D.S., Cardinale B.J., Downing A.L., Duffy J.E., Jouseau C., Sankaran M., *et al.* (2009) Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology* **90**, 1073–1083.
- Swan C.M., Gluth M.A. & Horne C.L. (2009) Leaf litter species evenness influences nonadditive breakdown in a headwater stream. *Ecology* **90**, 1650–1658.
- Swan C.M. & Palmer M.A. (2006) Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. *Oecologia* **147**, 469–478.
- Swan C.M. & Palmer M. a. (2004) Leaf diversity alters litter breakdown in a Piedmont stream. *Journal of the North American Benthological Society* **23**, 15–28.
- Tachet H., Richoux P., Bournaud M. & Usseglio-Polatera F. (2002) *Invertébrés d'eau douce. Systematique, biologie, écologie.* (Ed. Ed CNRS), Paris, France.
- Tank J., Rosi-Marshall E., Griffiths N. a., Entekin S. a. & Stephen M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* **29**, 118–146.
- Tonin A.M., Boyero L., Monroy S., Basaguren A., Pérez J., Pearson R.G., *et al.* (2017) Stream nitrogen concentration, but not plant N-fixing capacity, modulates litter diversity effects on decomposition. *Functional Ecology* **4**.
- Vos V.C.A., van Ruijven J., Berg M.P., Peeters E.T.H.M. & Berendse F. (2011) Macro-detritivore identity drives leaf litter diversity effects. *Oikos* **120**, 1092–1098.
- Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102–104.
- Woodward G., Gessner M.O., Giller P.S., Gulis V., Hladyz S., Lecerf A., *et al.* (2012) Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning. *Science* **336**, 1438–1440.

Supplemental material

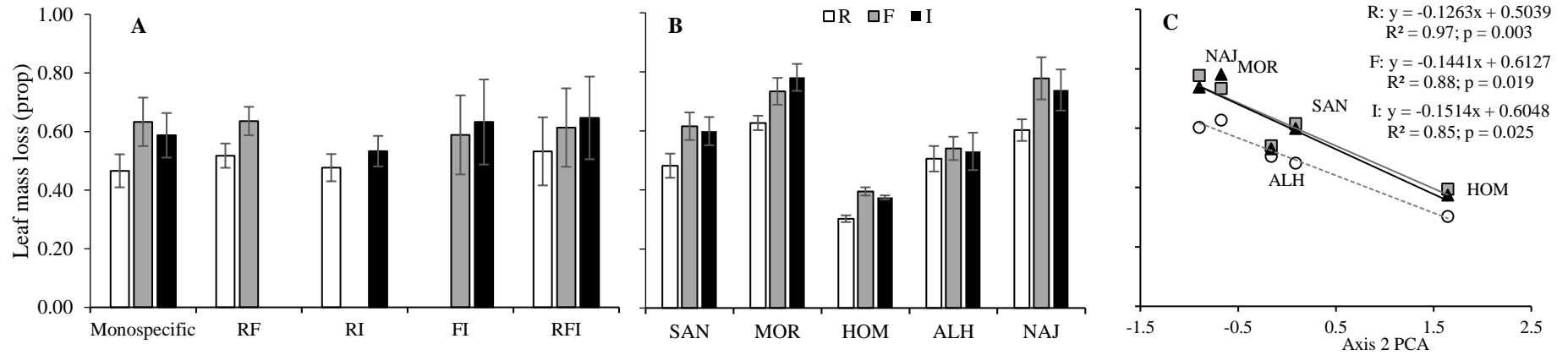


Fig. S1. Relative LML (mean \pm SE) for each litter species in relation to leaf treatment (A) and streams (B). Relationships between leaf mass loss of each litter species for each stream (data pooled across species compositions) and scores on axis 2 of PCA of each stream (C; circle, R; square, F; triangle, I). The p -value and fitted lines are also shown. R = *Q. robur*, F = *Q. faginea*, I = *Q. ilex*

CHAPTER 7

General discussion

This dissertation, through the previous chapters, addresses the effects of various components of Global Environmental Change on stream ecosystem functioning. It is focused on the effects of disturbances related to climate and catchment vegetation change on an important ecosystem-level function sensitive to environmental factors: organic matter decomposition, which is an integrative indicator of stream ecosystem status (Hieber & Gessner 2002; Tank *et al.* 2010).

In this chapter, the potential implications of the results obtained are discussed in the context of the ongoing Global Environmental Change from a broad perspective.

In this dissertation, we examine the effects of elevated atmospheric CO₂ concentration, increasing temperature, catchment vegetation change and drought on leaf litter decomposition. In addition, we explore the role of leaf-litter functional diversity in Mediterranean streams.

Global Change potentially would affect the organic matter processing through (1) altering of availability and quality of the basal resources supplied to the stream and, (2) through changes in the in-stream biotic communities and their activities (Perkins *et al.* 2010).

First, we examine the effects of elevated atmospheric CO₂ on quality of plant materials from species differing in biological traits, under two different scenarios of water availability, and their subsequent microbial decomposition. As shown in Chapter 2, the results from this laboratory experiment suggest that the decomposition rates of plant materials grown under elevated CO₂ are difficult to predict due to species-specific responses and interactions with other factors. Previous studies assessing the effects of increased CO₂ reported mixed results on leaf-litter processing in freshwater ecosystems (Rier, Tuchman & Wetzel 2005; Ferreira *et al.* 2010; Ferreira & Chauvet 2011; Dray *et al.* 2014). Nevertheless, we must take into account that this topic has received little attention from researchers on streams, and the scarce studies have not considered the complexity of different environmental scenarios when examining the effect of CO₂ on plant quality.

There are unequivocal evidences that altered thermal regime promoted by climate change will have substantial consequences for litter decomposition (Boyero *et al.* 2016) and for other ecosystem processes (e.g. food webs) depending on it (Woodward, Perkins & Brown 2010). The effects of that critical component of Global change on leaf decomposition have been addressed through field and laboratory experiments (Chapter 3). As reported in the Chapter 3, leaf processing (microbially mediated) increases with temperature, but the most interesting results are that (1) plant species contrasting in litter quality show similar temperature sensitivity, suggesting that decomposition of contrasting litter quality could be similarly affected by water temperature rises and, (2) microbial communities could respond similarly whatever their

original thermal adaptation. Faster organic matter processing with water temperature increase could lead us to expect a more rapid depletion of resource availability for stream food webs and, consequently, consumers-resource mismatch. In addition, stimulation of microbial-mediated decomposition rate with temperature as reported in this dissertation, could imply a more rapid turnover of leaf material with increases in the CO₂ production and thereby in the amount of carbon returned to the atmosphere (Boyero *et al.* 2011). These effects, in a global context, could be compensated by the slowdown of litter decomposition as a consequence of reduced litter quality inputs (Kominoski *et al.* 2013) and the slower processing related to drought conditions (Mariluan, Díaz Villanueva & Albariño 2015; Abril, Muñoz & Menéndez 2016).

How drought affects litter decomposition have been assessed through field experiments in Temperate and Mediterranean climate areas (Chapter 4 and 5, respectively). The first of these chapters examine structural and functional responses after a drought-period in perennial temperate streams flowing through native forest and catchments already affected by exotic plantations. The results reported in Chapter 4 suggest that the catchment vegetation in perennial temperate streams could influence the recovery of benthic macroinvertebrate communities after dry periods, but the decomposition rate of leaf litter could be less affected. However, when ecosystem functioning after a disturbance is totally recovered? How much time should pass since the disappearance of the stress factor? Greater understanding of the structural and functional responses of stream ecosystems to different stressors is required before the effects of expected more intense and frequent hydrological changes caused by climate change can be adequately forecast.

Increase in water temperature, as well as more frequent drought events, are expected in the context of future climatic scenarios (Milly, Dunne & Vecchia 2005; IPCC 2014), and stream ecosystem may have to face both stressors operating simultaneously. In this sense, Mediterranean region might provide early warning of their potential consequences. Taking advantage of the climatic particularity of the Mediterranean areas, we examine the potential drought effects from broad spatial scale (Chapter 5). The result from the experiment across a regional-scale drought gradient point to a complex pattern of litter decomposition at this spatial scale, as drought affects decomposition directly by emersion of litter and, indirectly, by affecting the macroinvertebrate community inhabiting streams. Therefore, given the higher effect of drying events on leaf-litter processing than those of low-flow, different repercussion for whole stream ecosystem could be expected according to the degree of flow-disturbance (Arroita *et al.* 2016) .

Although the effects of drought have received wide attention from researchers, major efforts have been focused in the Mediterranean climate region, intermittent streams and structural

attributes, lacking information about their effects on ecosystem functioning in temperate climate areas with perennial streams (Lake 2011). The Mediterranean climate is characterized by high inter-annual variability and pronounced seasonality (Gasith & Resh 1999; Bonada & Resh 2013), therefore considering the natural flow fluctuation in the streams located in this area against the higher constancy in temperate areas (Bonada, Dolédec & Statzner 2007), the magnitude of the impact of drought could varied among the different climatic regions (Leigh *et al.* 2016). In addition, global climate models forecast not only reduced precipitation in many areas of the world but also higher frequency and intensity of extreme events (Milly *et al.* 2005; Huang *et al.* 2016). Therefore, the consequences for stream ecosystems could be different depending on duration of the disturbance (long- or short-term change) and its frequency (Reid & Ogden 2006; Woodward *et al.* 2015; Stubbington *et al.* 2016) since all this could affect stream ecosystem in different ways in relation to its resistance and resilience capacity (Chessman 2015; Pinna *et al.* 2016).

Plan litter quality is, together with climate, one of the main determinants of organic matter processing (Aerts 1997). Reduced litter quality could reduce the energy transfer to higher trophic levels, ultimately affecting stream productivity (Vannote *et al.* 1980) and altering the trophic structure of river ecosystem (Wallace *et al.* 1997; Martínez *et al.* 2016). The results from this dissertation highlight the tight relationships between litter quality and the decomposition rates (Chapter 3 and 5), reinforcing the well-known role of the detritus quality in determining leaf-litter processing, and add further evidence that vegetation type could be a key determinant of temperature and drought effect on structural attributes and overall functioning in streams (Chapter 3 and 4). Therefore, additional interactions may occur if the quality of basal resources is altered in response to global changes in climate (e.g. change in plant species distribution; reduced quality by elevated CO₂ and drought -Chapter 2-) or altered vegetation and land use [e.g. replacement of native forest with exotic plantations or conversion to agricultural land (Kominoski *et al.* 2013)]. Consequently, the evaluation of the interaction among temperature, water flow and plant species on litter processing is highly relevant if we want to improve our ability to forecast future effect of Global Change on the stream ecosystem.

Finally, we address the topic of biodiversity-ecosystem functioning theory in Mediterranean streams, which are considered “hot-spot” of biodiversity, and explore the effect of plant functional diversity on decomposition rate (bottom-up diversity effects) in streams along a drought gradient to disentangle its role under this environmental disturbance. Although the results from Chapter 6 suggest that the effects of plant functional diversity could be more prevalent under drier conditions, the results point to stronger effects of the environmental context than leaf functional diversity on the decomposition process in stream from Mediterranean areas, overriding any potential effect of the later one.

The overall results provide evidences of relevant effects of climate change and vegetation change on structural and functional attributes of stream ecosystem and point to the need to evaluate their interaction for improving realistic estimation of stream ecosystem responses to future environmental scenarios (Gieswein, Hering & Feld 2017; Taniwaki *et al.* 2017). However, important questions remain unanswered, particularly if consider other aspects related to anthropogenic disturbances which have not been covered in this dissertation. The finding from this study highlight the utility of litter decomposition as sensitive indicator as other processes (von Schiller *et al.* 2017) of ecosystem status because it integrates all abiotic and biotic processes occurring during processing time.

References

- Abril M., Muñoz I. & Menéndez M. (2016) Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. *Science of the Total Environment* **553**, 330–339.
- Aerts R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* **79**, 439–449.
- Arroita M., Flores L., Larrañaga A., Martínez A., Martínez-Santos M., Pereda O., *et al.* (2016) Water abstraction impacts stream ecosystem functioning via wetted-channel contraction. *Freshwater Biology*.
- Bonada N., Dolédec S. & Statzner B. (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: Implications for future climatic scenarios. *Global Change Biology* **13**, 1658–1671.
- Bonada N. & Resh V.H. (2013) Mediterranean-climate streams and rivers: Geographically separated but ecologically comparable freshwater systems. *Hydrobiologia* **719**, 1–29.
- Boyero L., Pearson R.G., Gessner M.O., Barmuta L.A., Ferreira V., Graça M.A.S., *et al.* (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters* **14**, 289–294.
- Boyero L., Pearson R.G., Hui C., Gessner M.O., Pérez J., Alexandrou M.A., *et al.* (2016) Biotic and abiotic variables influencing plant litter breakdown in streams: a global study. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20152664.
- Chessman B.C. (2015) Relationships between lotic macroinvertebrate traits and responses to

- extreme drought. *Freshwater Biology* **60**, 50–63.
- Dray M.W., Crowther T.W., Thomas S.M., A’Bear a. D., Godbold D.L., Ormerod S.J., *et al.* (2014) Effects of elevated CO₂ on litter chemistry and subsequent invertebrate detritivore feeding responses. *PLoS ONE* **9**, 1–7.
- Ferreira V. & Chauvet E. (2011) Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia* **167**, 279–91.
- Ferreira V., Gonçalves A.L., Godbold D.L. & Canhoto C. (2010) Effect of increased atmospheric CO₂ on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Global Change Biology* **16**, 3284–3296.
- Gasith A. & Resh V.H. (1999) Streams in mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology, Evolution, and Systematics* **30**, 51–81.
- Gieswein A., Hering D. & Feld C.K. (2017) Additive effects prevail: The response of biota to multiple stressors in an intensively monitored watershed. *Science of The Total Environment* **593**, 27–35.
- Hieber M. & Gessner M.O. (2002) Contribution of Stream Detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* **83**, 1026–1038.
- Huang S., Huang Q., Chang J. & Leng G. (2016) Linkages between hydrological drought, climate indices and human activities: a case study in the Columbia River basin. *International Journal of Climatology* **36**, 280–290.
- IPCC (2014) Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the Intergovernmental Panel on Climate Change. In: *Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the Intergovernmental Panel on Climate Change.* (Eds C. Field, V. Barros, D. Dokken, K. Mach, M. Mastrandrea, T. Bilir, *et al.*), Cambridge University Press, Cambridge.
- Kominoski J.S., Shah J.J.F., Canhoto C., Fischer D.G., Giling D.P., González E., *et al.* (2013) Forecasting functional implications of global changes in riparian plant communities. *Frontiers in Ecology and the Environment* **11**, 423–432.
- Lake P.S. (2011) *Drought and aquatic ecosystems: effects and responses.* John Wiley & Sons.

- Leigh C., Bonada N., Boulton A.J., Hugueny B., Larned S.T., Vander Vorste R., *et al.* (2016) Invertebrate assemblage responses and the dual roles of resistance and resilience to drying in intermittent rivers. *Aquatic Sciences* **78**, 291.
- Mariluan G.D., Díaz Villanueva V. & Albariño R.J. (2015) Leaf litter breakdown and benthic invertebrate colonization affected by seasonal drought in headwater lotic systems of Andean Patagonia. *Hydrobiologia*, 171–187.
- Martínez A., Larrañaga A., Miguélez A., Yvon-Durocher G. & Pozo J. (2016) Land use change affects macroinvertebrate community size spectrum in streams: The case of *Pinus radiata* plantations. *Freshwater Biology* **61**, 69–79.
- Milly P.C.D., Dunne K.A. & Vecchia A. V (2005) Global pattern of trends in streamflow and water availability in a changing climate. *Nature* **438**, 347–350.
- Perkins D.M., Reiss J., Yvon-Durocher G. & Woodward G. (2010) Global change and food webs in running waters. *Hydrobiologia* **657**, 181–198.
- Pinna M., Marini G., Cristiano G., Mazzotta L., Vignini P., Cicolani B., *et al.* (2016) Influence of aperiodic summer droughts on leaf litter breakdown and macroinvertebrate assemblages: testing the drying memory in a Central Apennines River (Aterno River, Italy). *Hydrobiologia* **782**, 1–16.
- Reid M.A. & Ogden R.W. (2006) Trend, variability or extreme event? The importance of long-term perspectives in river ecology. *River Research and Applications* **22**, 167–177.
- Rier S.T., Tuchman N.C. & Wetzel R.G. (2005) Chemical changes to leaf litter from trees grown under elevated CO₂ and the implications for microbial utilization in a stream ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 185–194.
- von Schiller D., Acuña V., Aristi I., Arroita M., Basaguren A., Bellin A., *et al.* (2017) River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors. *Science of The Total Environment* **596–597**, 465–480.
- Stubbington R., Gunn J., Little S., Worrall T.P. & Wood P.J. (2016) Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream. *Freshwater Biology* **61**, 1293–1307.
- Taniwaki R.H., Piggott J.J., Ferraz S.F.B. & Matthaei C.D. (2017) Climate change and multiple stressors in small tropical streams. *Hydrobiologia* **793**, 41–53.
- Tank J., Rosi-Marshall E., Griffiths N. a., Entekin S. a. & Stephen M.L. (2010) A review of

allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society* **29**, 118–146.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137.

Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**, 102–104.

Woodward G., Bonada N., Feeley H.B. & Giller P.S. (2015) Resilience of a stream community to extreme climatic events and long-term recovery from a catastrophic flood. *Freshwater Biology* **60**, 2497–2510.

Woodward G., Perkins D.M. & Brown L.E. (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **365**, 2093–2106.

CHAPTER 8

Conclusions

1. The effects of increasing in atmospheric CO₂ concentration on nutrient content of plants are species-specific and do not affect equally all nutrients. Furthermore, decreases in plant quality associated to their growth under low water availability situation surpass to alterations that result from elevated CO₂ levels. These effects are not modulated by interspecific competition. The subsequent microbial decomposition rate of plant materials grown under future scenarios of elevated CO₂ are difficult to predict due to species-specific responses and interaction with other factors.
2. Temperature increase lead to faster microbial-mediated litter decomposition. Leaf litter of high- and low-quality species show a similar temperature sensitivity (i.e. a similar activation energy), therefore species contrasting in litter quality could be similarly affected by temperature rises. The response of decomposition rate and microbial activities to temperature from streams with different thermal regimes follows similar patterns. Water dissimilarities in streams with very low nutrient availability play an unclear and minor role that the temperature. The patterns of respiration, fungal growth and litter nutrient change were erratic and less predictable to temperature rise, suggesting litter decomposition could be a better indicator because it integrate all processes occurring during processing time. The response of litter decomposition and associated biological activities to temperature in the field is less predictable due to complex interactions that can occur in natural ecosystems, underlining the difficulty of direct extrapolation of the results obtained under controlled laboratory conditions.
3. The decomposition rate across a regional gradient of aridity points to a complex pattern at the regional scale, as drought affect decomposition directly by emersion of litter and indirectly by affecting the functional composition and density of detritivores. This response is more evident in high-quality leaf litter species.
4. Although the effect of species diversity could be more prevalent under dry conditions, environmental context have stronger effects than leaf functional diversity on the decomposition process in Mediterranean calcareous streams, overriding any potential effect of the later one.
5. Drought events have more severe effects on functional and taxonomic recovery of benthic macroinvertebrates in perennial temperate streams already affected by exotic plantations than those with native forest. As leaf decomposition after drought period is litter affected by exotic tree plantations, the subsequent repercussion on stream functioning could not be such severe.
6. The overall results provide evidences of relevant effects of climate change and vegetation change on structural and functional attributes of stream ecosystem and point to the need to evaluate their interaction for improving realistic estimation of stream ecosystem responses to

future environmental scenarios. It also highlights the utility of leaf-litter decomposition as a sensitive indicator of ecosystem status.