eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

Effects of flow regulation, land-use changes and temperature on stream ecosystem functioning

by

Aingeru Martínez Gómez

January 2013

eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

AUTORIZACION DEL/LA DIRECTOR/A DE TESIS PARA SU PRESENTACION

Dr. Jesús Pozo Martínez con N.I.F. 22700120-V y Dr. Aitor Larrañaga Arrizabalaga con N.I.F. 44170706-B como Directores de la Tesis Doctoral: “Effects of flow regulation, land-use changes and temperature on stream ecosystem functioning” realizada en el Departamento de Biología Vegetal y Ecología por el Doctorando Don Aingeru Martínez Gómez, autorizamos la presentación de la citada Tesis Doctoral, dado que reúne las condiciones necesarias para su defensa.

En Leioa, a 23 de enero de 2013

LOS DIRECTORES DE LA TESIS

Fdo.: Jesús Pozo Martínez

Fdo.: Aitor Larrañaga Arrizabalaga

Universidad
del País Vasco

Euskal Herriko
Unibertsitatea

CONFORMIDAD DEL DEPARTAMENTO

El Consejo del Departamento de en reunión celebrada el día Biología Vegetal y Ecología en reunión celebrada el día 23 de enero de 2013 ha acordado dar la conformidad a la admisión a trámite de presentación de la Tesis Doctoral titulada "Effects of flow regulation, land-use changes and temperature on stream ecosystem functioning" dirigida por el Dr. Jesús Pozo Martínez y el Dr. Aitor Larrañaga Arrizabalaga y presentada por Don Aingeru Martínez Gómez ante este Departamento.

En Leioa, a 23 de enero de 2013

Vº Bº DIRECTOR DEL DEPARTAMENTO

Fdo.: Jesús Pozo Martínez

SECRETARIA DEL DEPARTAMENTO

Fdo.: Itziar García Mijangos

eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

ACTA DE GRADO DE DOCTOR O DOCTORA
ACTA DE DEFENSA DE TESIS DOCTORAL

DOCTORANDO DON Aingeru Martínez Gómez

TITULO DE LA TESIS: "Effects of flow regulation, land-use changes and temperature on stream ecosystem functioning".

El Tribunal designado por la Subcomisión de Doctorado de la UPV/EHU para calificar la Tesis Doctoral arriba indicada y reunido en el día de la fecha, una vez efectuada la defensa por el doctorando y contestadas las objeciones y/o sugerencias que se le han formulado, ha otorgado por ____________________ la calificación de:

unanimidad ó mayoría

APTO o NO APTO

Idioma/s de defensa (en caso de más de un idioma, especificar apartados o porcentaje defendido en cada idioma): ____________________

En ____________________ a ______ de ____________________ de ______

EL/LA PRESIDENTE/A,

EL/LA SECRETARIO/A,

Fdo.:

Fdo.:

Dr/a: ____________________

Dr/a: ____________________

VOCAL 1º,

VOCAL 2º,

VOCAL 3º,

Fdo.:

Fdo.:

Fdo.:

Dr/a: ____________________

Dr/a: ____________________

Dr/a: ____________________

EL/LA DOCTORANDO/A,

Fdo.: Aingeru Martínez Gómez

Don´t gain the world and lose your soul, wisdom is better than silver and gold


I WAS READING ABOUT HOW COUNTLESS SPECIES ARE BEING PUSHED TOWARD EXTINCTION BY MAN'S DESTRUCTION OF FORESTS.
SOMETIMES I THINK THE SUREST SIGN THAT INTELLIGENT LIFE EXISTS ELSEWHERE IN THE UNIVERSE IS THAT NONE OF IT HAS TRIED TO CONTACT US.

Agradecimientos

Gracias a la ayuda de Dios y un sinfín de personas ha sido posible la culminación de este proyecto. Tanto es así, que el hecho de que sólo figure el nombre de este humilde servidor como autor me llena de remordimientos. Es por ello que este apartado de agradecimientos es un intento de hacer justicia y limpiar mi conciencia.

Esta tesis nunca hubiese tenido un comienzo sin Jesús Pozo, quien confió en mí para dirigir este trabajo y me ha guiado a lo largo de esta travesía científica. Y nunca hubiese tenido un final sin su paciencia y saber hacer a la hora de enfrentarse a mis manuscritos, incrementando enormemente su calidad.

Durante los cuatro años que ha durado este periplo he tenido la suerte de contar en todo momento con la inestimable ayuda de Aitor Larrañaga, quien pasó a ocupar el puesto de co-director en la segunda mitad de esta andadura. Las muchas horas de monte y sobre todo de laboratorio a su lado han servido para ampliar mis conocimientos no sólo sobre la ecología de ríos sino sobre un buen número de aspectos del mundo científico.

Ana Basaguren ha sido otra gran influencia, sobre todo durante el primer año de tesis ejerciendo como directora del trabajo de máster, transmitiéndome todo su conocimiento sobre macroinvertebrados fluviales. Además, durante este año de máster tuve la fortuna de compartir faenas y experiencias con Clara. La incorporación de Jon Molinero en nuestro grupo de investigación fue posterior a la realización de los experimentos plasmados en esta tesis, sin embargo, con él he pasado muy buenos momentos, y algunos menos buenos también, viviendo aventuras y desventuras por montes y ríos durante días y días de muestreos.

Todo esto hubiese sido inimaginable sin el trabajo incansable de los alumnos de máster y alumnos internos: Ana, Liseth, Andrea (identificar y medir más de 14000 invertebrados no es tarea fácil), Alberto, Iker (gran portero, mejor persona) y Mikel. En serio, vuestro esfuerzo y dedicación no se paga con dinero y me siento realmente en deuda con todos vosotros. Me gustaría acordarme también de los últimos tres alumnos de máster que han pasado por el laboratorio: Sandra, Silvia y Unai. A pesar de que la labor que realizaron no se incluye dentro de la presente tesis, su ayuda no quedó sólo en el plano profesional, sino que anímicamente representaron un gran refuerzo alentándome a finiquitar el presente trabajo.

El proyecto IMPARIOS me permitió conocer por primera vez a científicos que estudiaban este tipo de ríos, mostrándome que no estábamos solos en esto (¡hasta entonces pensaba que éramos los únicos!). Este hecho fue un gran espaldarazo a mi confianza al ver que nuestro esfuerzo interesaba a más personas. Gracias a Margarita Menéndez, Jesús Casas, Enric Descals, Carmen Casado, Salvador Mollá y Jose M. González. De entre ellos, me gustaría resaltar la figura de Enric, eminencia mundial en la taxonomía de hifomicetos acuáticos. Al contrario de lo imaginable, no fui yo quien le ayudó a él en su trabajo, sino que él dedicó gran parte de su tiempo y esfuerzo a la identificación de esporas fúngicas en mis experimentos, acrecentando en gran medida el interés de los mismos.

Agradecer a Arturo Elosegi la ampliación de mi conocimiento, o al menos la de mi carpeta de bibliografía, con sus periódicas distribuciones de artículos científicos. El compartir el espacio y los materiales de trabajo con los integrantes del grupo de investigación de Arturo más que un problema ha sido verdaderamente un placer, aunque dudo que este sentimiento sea recíproco. Pese a su ojeriza inicial hacia mí, surgida en clases de ficología, Lorea ha terminando siendo una gran amiga. Desde el principio de la tesis, y durante todo este tiempo, he podido contar con su ayuda, hemos compartido trabajos, experiencias, desilusiones, alegrías, viajes a congresos… y hemos arreglado el mundo en más de una ocasión. Ibon, polifacético donde los haya (bertsolari, monitor, socorrista… aparte de doctorando), es un ejemplo de valentía a la hora de enfrentarse a cualquier trabajo por duro que pueda ser (no sé si ha dejado algún río de la península sin muestrear). A Maite, siempre agradable y dispuesta a ayudar en todo aquello que sea, no sé si debo incluirle en este apartado de agradecimientos o en uno especial de disculpas por todas las tonterías que le he dedicado día tras día. Ya sabes que todas mis feas palabras son en verdad un antagonismo de la realidad. Aparte de becarios/as, dos alumnas de máster, Carmen y Anna, han pasado por este grupo. La forma de ser de Carmen y sobre todo sus surrealistas aventuras (recordadas durante los años posteriores) contribuyeron a que los peores momentos del primer año de tesis se vieran desde una óptica más optimista. Durante el último año hemos tenido la suerte de contar entre nosotros con Anna. Al ser italiana, y excusándose en la diferencia de idioma (tampoco hay mucha) podría haber decidido trabajar a su bola y quedarse un poco al margen del resto (creo que es lo que hubiese hecho la mayoría de gente al conocernos). Sin embargo, se integró rápidamente a la dinámica del laboratorio y no tardó mucho tiempo en participar en las bromas y vaciles.
Con todo esto quiero decir que, independientemente del grupo de trabajo al que perteneciéramos, durante este tiempo he tenido la sensación de formar parte de un único equipo.

Me gustaría hacer una mención especial a Mª Jesús, la cual era capaz de devolver a un estado normal el desorden generado a mi paso por el laboratorio, especialmente tras las jornadas de muestreo.

Gracias a todas las personas que en mayor o menor medida han pasado por el laboratorio por aguantar mis rarezas, manías, chorradas y sobre todo horas y horas de (excelente) reggae.

Fuera del laboratorio, una de las cosas que más me ha ayudado a sobrellevar con buen ánimo este trabajo (en ocasiones no muy agradecido) ha sido compartir experiencias y comida alrededor de una mesa con un magnífico grupo de personas: Alberto, Ane, Dani, Elena, Endika, Haizea, Iñaki, Jone, Miriam, Tamara… Vuestra compañía ha servido para desahogar mis preocupaciones y olvidarme, al menos por un rato, de los quehaceres cotidianos, lo cual significaba una inyección de moral diaria para enfrentarme de nuevo a las obligaciones laborales. También me gustaría acordarme del resto de compañeros de máster (Edorta, Etxepe, Eva, Gorka, Izaskun, Joana, Mireia…), los cuales contribuyeron a que ese año (primero de la tesis) fuera el mejor de mi paso por la universidad, en la que llevo más de nueve, recargándome de energía para aguantar el resto de la tesis.

Durante la recta final de esta carrera contrarreloj, el deporte me ha servido como válvula de escape para no estallar. Por eso, me gustaría dar las gracias a todas las personas que han hecho posible la organización de las “patxangas” semanales de fútbol sala.

Agradecer también el aguante de mi familia y amigos. Al contrario de la mayoría de personas que han aparecido anteriormente, ellos no han tenido que sufrirme únicamente durante cuatro años, sino que llevan haciéndolo un poco más de tiempo. No creo que estos últimos cuatro años se les hayan hecho más duros que los anteriores, pero sí que han tenido que soportar mis chapas sobre la magnificencia de los ríos que estudio, a los que consideran como simples hilillos de agua que hay por el monte. De entre este grupo quisiera resaltar a Naiara y Vane. Al estar durante este tiempo en una situación parecida a la mía desarrollando sus respectivas tesis, creo que son las que mejor han podido entender mis neuras y preocupaciones, apoyándome moralmente en todo momento.

La financiación de este trabajo estuvo constituida por una beca predoctoral del Gobierno Vasco, por tres proyectos del Ministerio de Ciencia e Innovación (IMPARIOS, CGL2007-66664-C04-01; TEMPERIOS, CGL2010-22129-C04-01; RIOTEMP, CGL2011-23984-C04-01) y por dos cofinanciaciones del Gobierno Vasco (IT-422-07; IT-302-10). En todo momento se ha contado con la colaboración de todos los integrantes del Departamento de Biología Vegetal y Ecología. Es de agradecer también el apoyo desde la administración y los gestores: Parque Natural de Gorbeia, Euskalmet, Consorcio de Aguas de Bilbao Bizakaia, Servicio de Cartografía Digital del Gobierno Vasco, Confederación Hidrográfica del Norte y Ministerio de Agricultura, Alimentación y Medio Ambiente.

Será interesante observar la cara de Javi la primera vez que lea este apartado. Atónito e incluso con una pizca de enfado y desconfianza en su interior al no encontrar alusión alguna a su persona. De hecho, soy plenamente consciente de mencionarte en última instancia reservándote un lugar destacado. Simplemente es un gesto para agradecerte la ayuda brindada durante estos años ya que toda palabra me resulta insuficiente.

Contents

Summary

The central idea of this thesis is that environmental factors, most of them modulated by human activities, can affect structural and functional properties of headwater forested streams. We focused the thesis on three specific impacts or variables: 1) flow regulation by small surface release dams, 2) land-use changes related to pine plantations and 3) temperature. We tried to test if these factors can condition decomposition of organic matter (functioning), assessed through the study of leaf-litter processing experiments, and biotic communities (structure), with special emphasis on the study of macroinvertebrates and aquatic hyphomycetes.

In the first chapter we observed that flow regulation changed the trophic structure of the macroinvertebrate communities, being detritus consumers, mainly shredders, principally affected. This alteration helped to explain the affectation on leaf-litter decomposition and was related to a reduction of the trait diversity of shredders. In the second chapter we ratified that regardless of the stream, nitrogen content of leaf-litter was observed to be able to stimulate the processing rates of leaves. This suggested that detritus quality can determine the structure of the community of consumers. We tested this idea in the third chapter in which we focused on the effects of pine plantations on macroinvertebrate communities and leaf-litter decomposition. The replacement of native deciduous forest by pine plantations altered the structure of macroinvertebrate community, being shredders the most affected, with lower richness, diversity and density in streams under monocultures. This alteration helped to explain the reduced leaf-litter processing capability of pine streams. Nevertheless, the differences in density of shredders were not paralleled with differences in biomass, suggesting that the average body mass and body mass distribution of individuals was altered in streams receiving pine needles. We specifically addressed the differences in body mass distribution between streams receiving native deciduous leaves and pine needles in the fourth chapter. We ratified that body size distribution of shredders was altered by the difference in resource inputs, being the smallest individuals more affected than the biggest ones. We attribute this difference to a single trait, pine needles toughness, which can limit the accessibility of small individuals to this resource. Moreover, we observed that predators mirrored the alterations in body size distribution of shredders, what strongly supports the link between basal resources and higher trophic levels. Finally, in the fifth chapter, we tested the effects of temperature on leaf litter decomposition mediated by microbial activity and on the performance of fungal assemblages colonizing the material in streams. Temperature enhanced leaf-litter processing rates and influenced spore production. Moreover, the fungal assemblages differed among temperature regimes, being the assemblages adapted to cold environments the most affected by the temperature variation.

This thesis proves that the studied modifications of environmental factors alter forested stream functioning due to changes in structure and/or activity of biotic communities.

General introduction

Streams and rivers are among the most threatened habitats in the world due to human activities (Malmqvist & Rundle, 2002). Thus, a major need is to understand the consequences of the human disturbances on aquatic ecosystems. One of the affectations that face streams and rivers is flow regulation, important worldwide and very conspicuous. Rivers have been dammed throughout the history of humanity for various purposes: drink water supply, land irrigation, flood control, and more recently for industrial use and energy generation. The number of dams has increased uninterruptedly, especially in the last 100 years (World Commission on Dams, 2000; Poff & Hart, 2002) due to growing population and the higher water demands. In fact, approximately 66% of the world's total freshwater flow is controlled by reservoirs (World Commission on Dams, 2000). The impacts of dams on fluvial ecosystems depend on their size, its use and management (Gore & Petts, 1989), but every dam constitutes an artificial barrier that entails certain river degradation (Ward, 1998) as changes in flow regimes and substrate size distribution, and reductions in the heterogeneity of environmental conditions (Stanford &Ward, 2001). The effects of large dams and those destined to hydroelectric production have been widely studied (e.g. Armitage, 1984; Pozo *et al*., 1997a; Cortes *et al*., 2002; Robinson *et al*., 2003; Jackson *et al*., 2007; Rehn, 2008), however, in spite of their numerical importance, the ecological effects of small dams (< 1 hm^3) have been much less studied (but see Cortes *et al*., 1998; Lessard & Hayes, 2003; Mendoza-Lera *et al*., 2012). Furthermore, the studies of the effects of flow regulation on biota, mainly benthic macroinvertebrates, have focused traditionally on structural components of the community (e.g. Maxted *et al*., 2005; Nichols *et al*., 2006; Rehn, 2008). However, most structural metrics consider species on an equal basis without considering their biological characteristics and ecological role, what can lead to misinterpret the potential impacts of environmental disturbances as some affected species might be keystone species for certain important processes (Cardinale *et al*., 2006; Woodward *et al*., 2008). A way to assess impacts on ecosystems, which integrates information about biological and ecological traits of macroinvertebrate species, has been developed (Usseglio-Polatera *et al*., 2000a; b). Using these functional metrics, some studies in aquatic systems have demonstrated their response to ecosystem alterations (*i.e.* Dolédec *et al*., 2006; Péru & Dolédec, 2010) and some other address the affectation of large dams on traits (Usseglio-Polatera *et al*., 2000b), although there is none that studies the effect of small dams on the functional diversity of traits of stream macroinvertebrates.

The food web of low order forested streams is based almost entirely on allocthonous detritus from terrestrial ecosystem (Wallace *et al*., 1997). In this sense, dams can disturb food sources by trapping dead coarse particulate organic matter and increasing the availability of fine particulate organic matter below the dam (Stanford & Ward, 2001), what can alter the energy fluxes associated to the detritic pathway. Within this pathway decomposition of the detritus plays a critical role (Polis & Strong, 1996). In freshwater ecosystems, the organisms responsible for the processing of detritus are microbial decomposers, primarily fungi (Mille-Lindblom & Tranvik, 2003; Abelho & Graça, 2006; Ferreira & Chauvet, 2011a), and invertebrate detritivores (Graça, 2001; Merrit & Cummins, 2007), both of them acting as transfers of energy to higher trophic levels (Wallace *et al.*, 1997; 1999). The physical properties of leaf litter, the main detritus inputs to streams (Abelho, 2001), as toughness, surface structure and roughness or the abundance of extracellular substances determine the fungal colonization and activity (Dang *et al*., 2007; Kearns & Bärlocher, 2008). Fungal growth on leaves increments the palatability of the resource for subsequent consumers (Graça & Cressa, 2010) since fungal mycelium presents higher nutritional quality than leaf litter (Cross *et al.*, 2005). On the other hand, invertebrate performance is driven by the elemental composition of the resource and the microbial conditioning (Hladyz *et al*., 2009; Kominoski & Pringle, 2009; Graça & Cressa, 2010), which can narrow the big stoichiometric gap between leaf-litter and consumers (see Cross *et al*., 2005). Detritus entering lotic waters shows a very wide range of elemental composition and physicochemical properties depending mainly on the species in the surrounding forest (Swan & Palmer, 2006). This disparity creates big differences in the utilization of detritus as food resource by consumers (Enríquez *et al*., 1993; Tibbets & Molles, 2005), and can affect ultimately its conversion to secondary production (Friberg & Jacobsen, 1994; González & Graça, 2003; Campos & Gozález, 2009).

Land-use changes replacing native vegetation by monocultures inflict great changes in the quantity, quality and seasonality of leaf litter inputs to the streams (Pozo *et al*., 1997b, Molinero & Pozo, 2004). Although at a global scale other monocultures have received much more attention of scholars, studies dealing effects of conifer monocultures are scarce, as pointed out by Riipinen *et al*. (2010). Specifically, in the Atlantic region of the Iberian Peninsula, the effects of *Eucalyptus globulus* Labill. on streams have been widely studied (e.g. Molinero *et al*., 1996; Chauvet *et al*., 1997; Pozo *et al*., 1997a; Pozo *et al*., 1998; Molinero & Pozo, 2004; Larrañaga *et al*., 2006; 2009a; b). However, in spite of the much higher cover of *Pinus radiata* D. Don monocultures in some parts of the northern Iberian Peninsula (e.g., 62% of the forested surface in the province of Biscay, www.bizkaia.net), their effects on benthic macroinvertebrates communities and stream functioning are yet unknown.

Detritus processing and therefore energy incorporation into food-webs is not only driven by its properties, environmental factors such as the presence of dissolved nutrients in water (Greenwood *et al*., 2007; Woodward *et al*., 2012), the degree of oxygen saturation (Medeiros *et al*., 2009) or pH (Dangles *et al*., 2004), for instance, influencing this process. Temperature is obviously an important factor since warming accelerates chemical reactions and modulates biological activities (Brown *et al*., 2004; Davidson & Janssens, 2006; Davidson *et al*., 2006), community structure (Mouthon & Daufresne, 2006), species distribution (Castella *et al*., 2001), interspecific relationships (Jiang & Morin, 2007) and ecological processes (Baulch *et al*., 2005). The Intergovernmental Panel on Climate Change predicts an increase of 1.1–6.4 ºC in global air temperature by the end of this century (IPCC, 2007), with an expected parallel increase of the stream water temperature (Eaton & Scheller, 1996). Thus, it is fundamental to go further in the understanding of the role that temperature plays in stream ecosystem functioning, and specifically in decomposition. There are some studies that assess the effects of temperature on detritus decomposition mediated by microbial activity in microcosm experiment (Dang *et al*., 2009; Fernandes *et al*., 2009; Ferreira & Chauvet, 2011a; b), mainly by using artificial communities with few species selected from pure cultures, what makes their results difficult to scale up to extrapolate implications in nature.

This work tries to gain understanding on the effects of three environmental alterations on structural and functional properties of streams: 1) the flow regulation by small dams, 2) the substitution of natural forest by exotic species and 3) the shift of water temperature (Fig. 1).

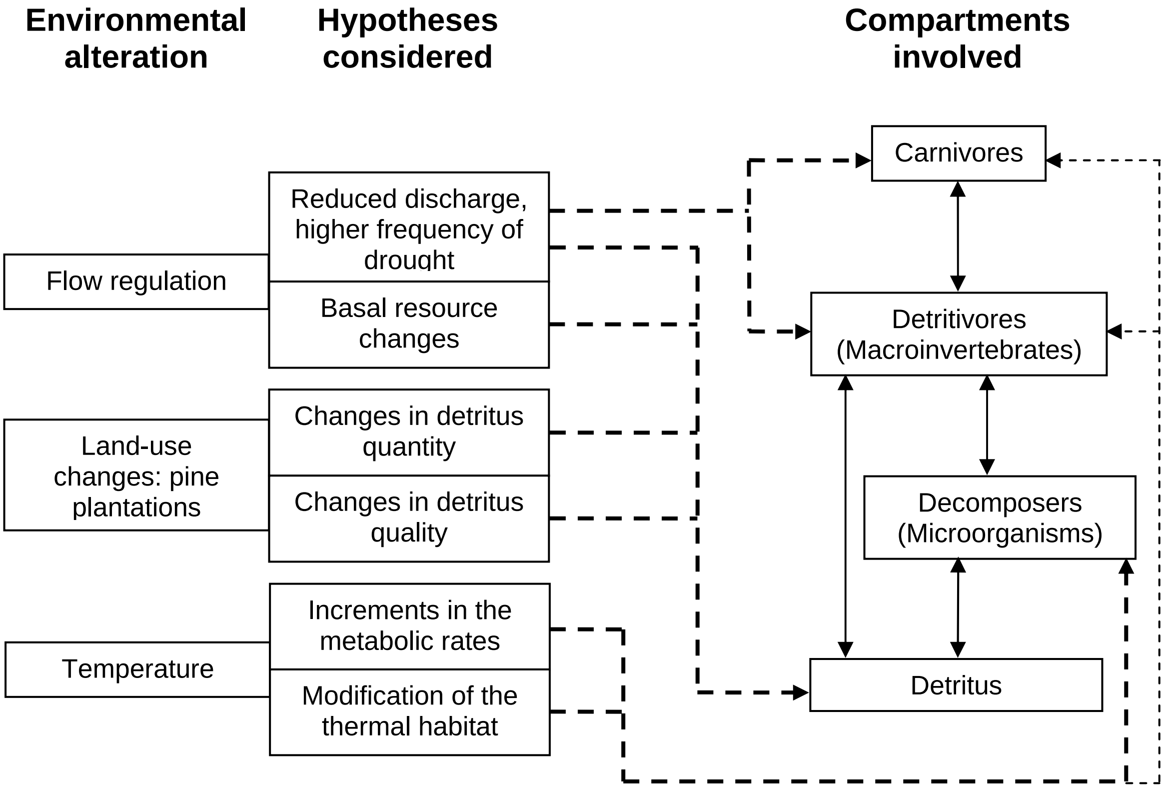


Figure 1. Schematic representation linking environmental alterations studied, main hypotheses in relation to its alterations in freshwater ecosystems and biotic and abiotic compartments affected by the environmental alteration in low order forested streams. Bold dashed lines indicate paths studied in the present thesis, simple dashed lines refer to paths not studied.

The thesis has been structured in five chapters. The first chapter focuses on the effects of flow regulation on the structure and functional diversity (based on traits) of benthic macroinvertebrate communities. The three following chapters (II–IV) deal with the effects of land-use changes. In chapter II we compare the capability of five leaf types with very different nutritional quality for being processed. Chapter III tackles the effects of pine plantations on biotic community structure (aquatic hyphomycetes and macroinvertebrates) and ecosystem functioning (leaf-litter breakdown). In chapter IV the effects of pine plantations on body-size spectra of entire macroinvertebrate communities and feeding guilds are studied. Finally, chapter V relates water temperature to leaf litter decomposition mediated by microbial activity and the performance of fungal assemblages colonizing the material.

References

Abelho M, 2001. From litterfall to breakdown in streams: A review. The Scientific World Journal 1: 656–680.

Abelho M & Graça MAS, 2006. Effects of nutrient enrichment on decomposition and fungal colonization of sweet chestnut leaves in an Iberian stream (Central Portugal). Hydrobiologia 560: 239–247.

Armitage PD, 1984. Environmental changes induced by stream regulation and their effect on lotic macroinvertebrate communities. In Regulated Rivers, Lillehammer A, Saltveit SJ (eds). Oslo University Press: Oslo, Norway; 139–165.

Baulch HM, Schindler DW, Turner MA, Findlay DL, Paterson MJ & Vinebrooke RD, 2005. Effects of warming on benthic communities in a boreal lake: implications of climate change. Limnology and Oceanography 50: 1377–1392.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB, 2004. Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Campos J & González JM, 2009. *Sericostoma vittatum* (Trichoptera) larvae are able to use pine litter as an energy source. International Review of Hydrobiology 94: 472–483.

Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M & Jouseau C, 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443: 989–992.

Castella E, Adalsteinsson H, Britain JE, Gislason GM, Lehmann A, Lencioni V, Lods–Crozet B, Maiolini B, Milner AM, Olafsson JS, Saltveit SJ & Snook DL, 2001. Macrobenthic invertebrate richness and composition along a latitudinal gradient of European glacier–fed streams. Freshwater Biology 46: 1811–1831.

Chauvet E, Fabre E, Elosegui E & Pozo J, 1997. The impact of eucalypt on the leaf associated aquatic hyphomycetes in Spanish streams. Canadian Journal of Botany 75: 880–888.

Cortes RMV, Ferreira MT, Oliveira SV & Godinho F, 1998. Contrasting impact of small dams on the macroinvertebrates of two Iberian mountain rivers. Hydrobiologia 389: 51–61.

Cortes RMV, Ferreira MT, Oliveira SV & Oliveira D, 2002. Macroinvertebrate community structure in a regulated river segment with different flow conditions. River Research and Applications 18: 367–382.

Cross WF, Benstead JP, Frost PC & Thomas SA, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50: 1895–1912.

Dang CK, Gessner MO & Chauvet E, 2007. Influence of conidial traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. Mycologia 99: 24–32.

Dang CK, Schindler M, Chauvet E & Gessner MO, 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90: 122–132.

Dangles O, Gessner MO, Guerold F & Chauvet E, 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. Journal of Applied Ecology 41: 365–378.

Davidson EA & Janssens IA, 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173.

Davidson EA, Janssens IA & Luo YQ, 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q10. Global Change Biology 12: 154–164.

Dolédec S, Phillips N, Scarsbrook M, Riley RH & Townsend CR, 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25: 44–60.

Eaton JG & Scheller RM, 1996. Effects of climate warming on fish thermal habitat in streams on the United States. Limnology and Oceanography 41: 1109–1115.

Enríquez S, Duarte CM & Sand–Jensen K, 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457–471.

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: 410–419.

Ferreira V & Chauvet E, 2011a. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia 67: 279–291.

Ferreira V & Chauvet E, 2011b. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17: 551–565.

Friberg N & Jacobsen D, 1994. Feeding plasticity of two detritivores shredders. Freshwater Biology 32: 133–142.

González JM & Graça MAS, 2003. Conversion of leaf litter to secondary production by a shredding caddis–fly. Freshwater Biology 48: 1578–1592.

Gore JA & Petts GA, 1989. Alternatives in regulated river management. CRC Press: Boca Raton – Florida, USA; 344 pp.

Graça MAS, 2001. The role of invertebrates on leaf litter decomposition in streams – A review. International Review of Hydrobiology 86: 383–393.

Graça MAS & Cressa C, 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. International Review of Hydrobiology 95: 27–41.

Greenwood JL, Rosemond AD, Wallace JB, Cross WF & Weyers HS, 2007. Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. Oecologia 151: 637–649.

Hladyz S, Gessner MO, Giller PS, Pozo J & Woodward G, 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. Freshwater Biology 54: 957–970.

IPCC (Intergovernmental Panel on Climate Change), 2007. Climate Change 2007: The Physical Science Basis. Contribution of the Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Jackson HM, Gibbins CN & Soulsby C, 2007. Role of discharge and temperature variation in determining invertebrate community structure in a regulated river. River Research and Applications 23: 651–669.

Jiang L & Morin PJ, 2007. Temperature fluctuation facilitates coexistence of competing species in experimental microbial communities. Journal of Animal Ecology 76: 660–668.

Kearns SG & Bärlocher F, 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. Fungal Ecology 1: 13–18.

Kominoski JS & Pringle CM, 2009. Resource–consumer diversity: testing the effects of leaf litter species diversity on stream macroinvertebrate communities. Freshwater Biology 54: 1461–1473.

Larrañaga A, Larrañaga S, Basaguren A, Elosegi A & Pozo J, 2006. Assessing impact of eucalyptus plantations on benthic macroinvertebrate communities by a litter exclusion experiment. International Journal of Limnology 42: 1–8.

Larrañaga A, Basaguren A, Elosegi A and Pozo J, 2009a. Impacts of Eucalyptus globulus plantations on Atlantic streams: changes in invertebrate density and shredder traits. Fundamental and Applied Limnology 175: 151–160.

Larrañaga A, Basaguren A & Pozo J, 2009b. Impacts of *Eucalyptus globulus* plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. International Review of Hydrobiology 94: 497–511.

Lessard JL & Hayes DB, 2003. Effects of elevated water temperature on fish and macroinvertebrate communities below small dams. River Research and Applications 19: 721–732.

Malmqvist B & Rundle S, 2002. Threats to the running water ecosystems of the world. Environmental Conservation 29: 134–153.

Maxted JR, McCready CH & Scarsbrook MR, 2005. Effects of small ponds on stream water quality and macroinvertebrate communities. New Zealand Journal of Marine and Freshwater Research 39: 1069–1084.

Medeiros AO, Pascoal C & Graça MAS, 2009. Diversity and activity of aquatic fungi under low oxygen conditions. Freshwater Biology 54: 142–149.

Mendoza–Lera C, Larrañaga A, Pérez J, Descals E, Martínez A, Moya O, Arostegui I & Pozo J, 2012. Headwater reservoirs weaken terrestrial–aquatic linkage by slowing leaf–litter processing in downstream regulated reaches. River Research and Applications 28: 13–22.

Merritt RW & Cummins KW, 2007. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company: Dubuque; 1158 pp.

Mille–Lindblom C &Tranvik LJ (2003) Antagonism between bacteria and fungi on decomposing aquatic plant litter. Microbial Ecology 45:173–183.

Molinero J & Pozo J, 2004. Organic matter, nitrogen and phosphorus fluxes associated with leaf litter in two small streams with different riparian vegetation: a budget approach. Archiv Fur Hydrobiologie 528: 143–165.

Molinero J, Pozo J & González E, 1996. Litter breakdown in streams of the Agüera catchment: influence of dissolved nutrients and land use. Freshwater Biology 36: 745–756.

Mouthon J & Daufresne M, 2006. Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: a large lowland river and of its two main tributaries (France). Global Change Biology 12: 441–449.

Nichols S, Norris R, Maher W & Thoms M, 2006. Ecological effects of serial impoundment on the Cotter River, Australia. Hydrobiologia 572: 255–273.

Péru N & Dolédec S, 2010. From compositional to functional biodiversity metrics in bioassessment: A case study using stream macroinvertebrate communities. Ecological Indicators 10: 1025–1036.

Poff NL & Hart DD, 2002. How dams vary and why it matters for the emerging science of dam removal. BioScience 52: 659–738.

Polis GA & Strong DR, 1996. Food web complexity and community dynamics. The American Naturalist 147: 813–846.

Pozo J, Basaguren A, Elósegui A, Molinero J, Fabre E & Chauvet E. 1998. Afforestation with Eucalyptus globulus and leaf litter decomposition in streams of northern Spain. Hydrobiologia 373/374: 101–109.

Pozo J, Orive E, Fraile H & Basaguren A, 1997a. Effects of the Cernadilla–Valparaiso reservoir system on the river Tera. Regulated Rivers: Research & Management 13: 57–73.

Pozo J, González E, Díez JR, Molinero J & Elósegui A, 1997b. Inputs of particulate organic matter to streams with different riparian vegetation. Journal of North American Benthological Society 16: 602–611.

Rehn AC, 2008. Benthic macroinvertebrates as indicators of biological condition below hydropower dams on west slope Sierra Nevada streams, California, USA. River Research and Applications 25: 208–228.

Riipinen MP, Fleituch T, Hladyz S, Woodward G, Giller P & Dobson M, 2010. Invertebrate community structure and ecosystem functioning in European conifer plantation streams. Freshwater Biology 55: 346–359.

Robinson CT, Uehlinger U & Monaghan MT, 2003. Effects of a multi–year experimental flood regime on macroinvertebrates downstream of a reservoir. Aquatic Sciences 65: 210–222.

Stanford JA, Ward JV, 2001. Revisiting the Serial Discontinuity concept. Regulated Rivers: Research and Management 17: 303–310.

Swan CM & Palmer MA, 2006. Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. Oecologia 147: 469–478.

Tibbets TM & Molles MC, 2005. C:N:P stoichiometry of dominant riparian trees and arthropods along the Middle Rio Grande. Freshwater Biology 50: 1882–1894.

Usseglio–Polatera P, Bournaud M, Richoux P & Tachet H, 2000a. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biology 43: 175–205.

Usseglio–Polatera P, Bournaud M, Richoux P & Tachet H, 2000b. Biomonitoring through biological traits of benthic macroinvertebrates: how to use species trait databases? Hydrobiologia 422/423: 153–162.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277: 102–104.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1999. Effects of resource limitation on a detrital–based ecosystem. Ecological Monographs 69: 409–442.

Ward JV, 1998. Riverine landscapes: biodiversity patterns, disturbance regimes, and aquatic conservation. Biological Conservation 83: 269–278.

Woodward G, Gessner MO, Giller PS, Gulis V, Hladyz S, Lecerf A, Malmqvist B, McKie BG, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graça MA, Fleituch T, Lacoursière JO, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A, Vought LB & Chauvet E, 2012. Continental–scale effects of nutrient pollution on stream ecosystem functioning. Science 336: 1438–1440.

Woodward G, Papantoniou G, Edwards F & Lauridsen RB, 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117: 683–692.

World Commission on Dams, 2000. Dams and development: a new framework for decision making. World Commission on Dams Nov.16 2000.

Flow regulation

Chapter I

Stream regulation by small dams affects benthic macroinvertebrate communities: from structural changes to functional implications

Abstract

We studied benthic macroinvertebrate communities upstream and downstream of five small reservoirs (surface-release in autumn-winters) (north Spain) to assess the effect of flow regulation on structural and functional characteristics of stream ecosystems. We based our approach on the use of structural metrics (density, biomass, richness and diversity) in combination with two functional diversity indices based on biological and ecological traits: FD_{PG} index, related to species richness, and FD_Q, which incorporates evenness across taxa. Although water physicochemical parameters were unaffected by the reservoirs during the study period (autumn-winter), macroinvertebrate metrics were lower below the dams, with detritivores (shredders and collector-gatherers) being the most affected. Alder leaf breakdown rate estimated by the litter-bag technique was related to the density, biomass, richness, diversity and FD_{PG} index of shredders, compromising the ecosystem functioning. The most plausible origin for the observed differences in macroinvertebrate metrics between upstream and downstream reaches was the change of the flow regime caused by the impoundments at downstream sites, leading to droughts in summer in those naturally permanently flowing streams. The observed functional diversity loss might reduce the chances of the community to override natural or man-induced fluctuations in their environment with possible repercussions on important ecosystem functions and services.

Key words: Headwater streams, Small dams, Benthic macroinvertebrates, Functional diversity, Ecosystem functioning.

Introduction

Ecological effects of the flow regulation have become an important subject in environmental research (Rosenberg *et al.*, 2000). All human societies have dammed rivers with different objectives: water supply, land irrigation, flood control, industrial use and energy generation. In Spain alone about 1500 reservoirs are documented, of which 35% can be considered small, *i.e.* dams of less than 15 m of wall height (Spanish Ministry for Agriculture, Alimentation and the Environment; http://www.magrama.gob.es/). These numbers have historically positioned Spain as one of the nations with more dams in the world (see World Commission on Dams; http://www.dams.org/). Impacts created by river regulation through large dams have received much attention (Petts, 1988), particularly in temperate regions. These reservoirs may suffer thermal stratification and, through hypolimnetic release, generate substantial changes in the physicochemistry (temperature, oxygen, nutrients, etc.) of the water in the streams below. These changes are responsible for alterations in the biological communities downstream (Haxton & Findlay, 2008). In contrast to their numerical importance, the ecological effects of small dams (< 1 hm^3, following Martin & Hanson, 1966) have been much less studied than those caused by large dams or hydroelectric production dams. These small dams are usually older and lack any management plan (Poff & Hart, 2002), leading to an uncontrolled release of epilimnetic water. Since the damming impact on fluvial ecosystems depends on the size of the dam, but also on its use and management (Gore & Petts, 1989), these small dams might create undocumented effects on macroinvertebrate communities.

The Serial Discontinuity Concept (Ward & Stanford, 1983) not only predicts changes in water physicochemistry caused by the flow regulation, but also changes in the transport of materials (sediments and organic matter), and in fact, the whole ecosystem functioning. The effects on downstream fluvial biota altered by water chemistry (Fairchild & Velinsky, 2006) and hydrological regime (Marty *et al*., 2009) have been frequently reported by studies of benthic macroinvertebrate communities (Rehn, 2008). The majority of these studies have focused on structural components of the communities, as density, biomass or taxa richness (Maxted *et al*., 2005; Nichols *et al*., 2006). However, most structural metrics consider species on an equal basis without considering their biological characteristics and ecological role. A common initial approach to study the community structure from a functional point of view consists in the classification of macroinvertebrates in functional feeding groups (Vallania & Corigliano, 2007; Príncipe, 2010) in order to look for trophic ecosystem alterations. A further way to assess impacts on ecosystems, which integrates information about biological and ecological traits as well, has been developed (Usseglio-Polatera *et al*., 2000). Although many studies in aquatic systems have demonstrated the response of biological traits to ecosystem alterations (Dolédec *et al*., 2006; Péru & Dolédec, 2010), studies of the effect of small dams on macroinvertebrates traits are lacking so far.

Our study addresses the impact of water regulation by small dams on structural and functional metrics of the benthic macroinvertebrates. Our main hypotheses are: 1) density and biomass of macroinvertebrates will be lower below the dam, mainly due to changes in hydrology and habitat structure, 2) taxa richness, diversity and functional diversity will also be affected negatively in downstream sites, and 3) these structural and functional losses will correlate to the processing rate of organic matter, which have been previously shown to be reduced in the downstream sites of the same systems (Mendoza-Lera *et al*., 2012).

Material and methods

Study sites

The study was carried out in five low order (2^{nd}–3^{rd}) streams affected by small (0.14–0.64 hm^3), surface-releasing water supply reservoirs (Artiba, Lekubaso, Lingorta, Regato and Zollo) in the Nerbioi-Ibaizabal drainage basin (Northern Spain, mean latitude 43º 13' N; mean longitude 3º 7' W), which flows into the Gulf of Biscay (Atlantic Ocean). The climate is temperate with an average annual air temperature of around 14 ºC and a mean annual precipitation of 1200 mm, without droughts in summer (Fig. 1). Water from the entire drainage basin flows through siliceous rock formations and forested watersheds (native *Quercus robur* L. forests and plantations of *Pinus radiata* D. Don and *P. pinaster* Ait.), with alder (*Alnus glutinosa* (L) Gaertner) dominating the riparian gallery; other anthropological land uses are negligible. The reservoirs do not maintain any ecological flow and, consequently, downstream reaches show a year-round variation in water discharge (see Fig. 1, average water level for a 10-year period is shown for Artiba Reservoir).

Autumn seasonal precipitations completely fill the reservoir and it keeps the level until the beginning of summer when the water level gradually drops, whereas with the beginning of autumn the water level starts to rise again until winter, when the reservoir is completely full with water (Fig. 1). During the period when the water level is below the maximum level of the reservoir, downstream sites suffer from droughts (confirmed by the authors for the period of the study) and water level is only recovered by groundwater and inputs from the catchment below the dam.

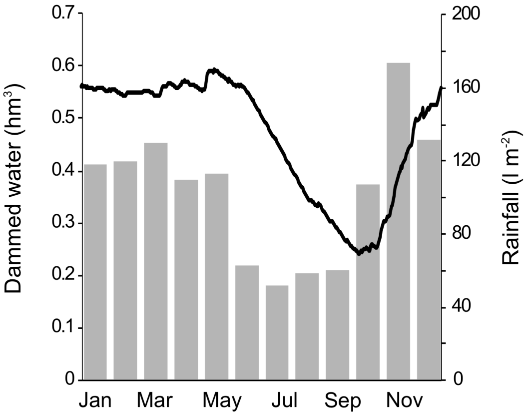


Figure 1. Average (1999–2008) dammed volume of water (hm^3; line) and monthly rainfall (mm; columns) in Artiba reservoir.

At each stream, two similar 50 m-long reaches were selected: one above (Up), in the main tributary of the reservoir, and another one below the dam (Down) (see Table 1 for characteristics of each site; site-specific details in Mendoza-Lera *et al*., 2012). Due to the accessibility of the

Table 1. Characterization of reservoirs and studied stream reaches. Range of values for watershed surface, reservoir surface, storage capacity and reservoir average depth are shown for the five reservoirs. Range of altitude, reach slope, width, granulometric composition, IHF and QBR and ranges of site-average values for temperature, flow, SRP, DIN, alkalinity, conductivity, pH, O_2 saturation, FPOM, FPIM, FPM and CPOM for upstream and downstream sites are also shown (period sampled 19th November–2nd February).

		Reservoir	
Watershed surface (ha)		259–930	
Reservoir surface (ha)		2.3–3.9	
Storage capacity (hm^3)		0.14–0.64	
Average depth (m)		6.1–16.5	
		Up	Down
Altitude (m)		100–340	60–190
Reach slope (%)		3.7–24.4	3.7–27.4
Width (m)		2.8–7.5	4.9–10.9
Water temperature (ºC)		7.2–8.8	7.1–7.8
Flow ($L\ s^{-1}$)		90–250	86–675
SRP ($\mu g\ P\ L^{-1}$)		13.2–18.3	13.4–16.9
DIN ($\mu g\ N\ L^{-1}$)		196.2–493.0	179.6–597.4
Alkalinity ($meq\ L^{-1}$)		0.4–1.2	0.3–1.2
Conductivity ($\mu S\ cm^{-1}$)		94.1–256.8	98.1–254.2
pH		7.5–8.0	7.5–8.0
% O_2 Saturation		101.7–109.3	102.4–111.0
FPOM ($mg\ L^{-1}$)		0.4–1.3	0.9–2.5
FPIM ($mg\ L^{-1}$)		0.4–5.3	1.0–9.9
FPM ($mg\ L^{-1}$)		0.8–6.5	1.9–12.4
CPOM ($g\ m^{-2}$)		2.7–20.6	3.3–35.1
Granulometric composition (%)	>25 cm	29.10–46.67	37.49–53.30
	6–25 cm	29.17–49.17	24.15–34.16
	<6 cm	20.00–33.33	19.16–30.88
IHF index		75.0–84.0	63.0–79.0
QBR index		67.5–97.5	55.0–85.0

streams, downstream reaches were located at different distances from the dam (Artiba 675 m, Lekubaso 220 m, Lingorta 86 m, Regato 285 m and Zollo 660 m). In all ten reaches, riparian vegetation quality was determined using the Riparian Quality Index (QBR; Munné *et al.*, 2003) that is based on four descriptors of the riparian vegetation (total riparian vegetation cover, cover structure, cover quality and channel alterations). Benthic habitat quality was estimated using IHF index (Pardo *et al.*, 2002), which measures the habitat heterogeneity of the substrate and other physical variables of the stream channel. In addition, streambed granulometric composition was visually estimated and three categories roughly corresponding to the Wenworth grain size scale (Allan

& Castillo, 2007) were used: percentage of 'boulders' (>25 cm), 'cobbles' (6–25 cm) and 'gravel-sand' (<6 cm).

This study coincided with an alder leaf-litter decomposition experiment (litter-bag technique) in the same streams (see Mendoza-Lera *et al*., 2012). During the time that the decomposition experiment was carried out (71 days from November 2008 to February 2009), water temperature was continuously monitored in all sites (data recorded every hour) with SmartButton temperature data loggers (ACR Systems Inc., Surrey, BC, Canada). Conductivity, pH, dissolved oxygen (WTW multiparametric sensor) and river flow (Martin Marten Z30, Current Meter) were measured at 5 sampling dates (ca. fortnightly). In every field visit, water samples were collected, transported to the laboratory and filtered (0.7 mm pore size glass fiber filter, Whatman GF/F). Filters were oven-dried (70 ºC, 72 h) and weighed to obtain fine particulate matter (FPM). After combusting the resultant material (500 ºC, 12h) and re-weighing it, fine particulate inorganic matter (FPIM) was estimated; fine particulate organic matter (FPOM) was calculated as the difference. Alkalinity was determined on filtered water by titration to an end pH of 4.5 (APHA, 2005). Nitrate concentration was determined by the sodium salicylate method, ammonium by the manual salicylate method, nitrite by the sulphanilamide method and soluble reactive phosphorus (SRP) by the molybdate method (APHA, 2005).

Benthic standing stock and macroinvertebrates

In January 2009, when the remaining alder leaf mass in the bags of the breakdown experiment was approximately 50%, five benthic samples (Surber 0.09 m^2, 0.5 mm mesh size) were taken at each study site. Location for each sample was chosen randomly within the available riffles with no predefinition of the substrate to be sampled, which nonetheless showed to be very similar among sites (see Table 1). Coarse particulate organic matter (CPOM) was sorted out from macroinvertebrates in the field on an 8 mm sieve, oven-dried and combusted in the laboratory to determine the ash free dry mass (AFDM). We chose the 8-mm mesh to sieve CPOM as it enabled the separation of the fauna from the organic matter in the field, and as the organic matter within the 1–8 mm interval is usually a quite small fraction of the total. Macroinvertebrates, preserved in 70% ethanol, were identified mainly to genus (Oligochaeta to order), counted and sorted into functional feeding groups (FFG; after Tachet *et al.*, 2002; Merritt & Cummins, 2007): shredders, collectors-gatherers, collector-gatherer-scrapers, collectors-filterers, scrapers and predators. Individuals from each FFG were altogether oven-dried and weighed for dry mass determination. For each sample we calculated taxa richness, EPT taxa richness, Shannon diversity index and two functional metrics based on 12 selected biological and ecological traits of macroinvertebrates (maximum body size, life cycle length, number of generations per year, dispersion type, consumed food resource, temperature preference, pH preference, trophic status preference, longitudinal distribution, microhabitat preference, locomotion type and current preference). We considered the other 10 traits defined in Tachet *et al.* (2002) not relevant for our study because, 1) we were studying very similar reaches located in a very narrow geographic area, and 2) the variation of those traits in our streams was expected to be negligible. The affinity scores for macroinvertebrate traits were obtained from Tachet *et al.* (2002), and converted into percentages prior to the calculation of the functional metrics. We computed functional diversity indices because the values obtained combine information about the distribution of modalities within traits together with the distribution of modalities among species that can thereafter be compared with simple univariate statistics. The two functional diversity metrics computed were Petchey & Gaston's FD_{PG} (2002) and Rao's quadratic entropy (FD_Q; Rao, 1982; Botta-Dukát, 2005). Both indices are based on the pairwise difference matrix between taxa of our communities, for which values of trait modalities are taken into account. FD_{PG} is based on the presence-absence of taxa and measures functional diversity by the summed branch lengths of the dendrogram constructed from differences among traits, which blends the diversity caused by species richness, number of functional groups, community composition, and species identity (Petchey & Gaston, 2002). On the other hand, FD_Q is an index of functional diversity that incorporates both the relative abundances of species and a measure of the pairwise functional differences between species. Whereas FD_{PG} ignores the abundance of species, which might undervalue the functional implications of abundant species (Mason *et al.*, 2005), FD_Q is influenced by the most abundant species (Rao, 1982), thus the loss of key species can go unnoticed by it. The comparison of the results obtained by these two metrics can help to discern whether changes in functional diversity are due to changes in taxa richness or/and in the evenness of the abundance. Indices were computed for the entire macroinvertebrate assemblage as well as for each FFG separately since the reservoirs could differently affect the resources sought by the taxa of each FFG.

Statistical analyses

For physicochemical variables, FPOM, FPIM, QBR, IHF and alder leaf-litter decomposition rates, comparisons between upstream and downstream sites were presented in Mendoza-Lera *et al.* (2012). In the present study, these comparisons were related with the faunal variables. Benthic CPOM, density, biomass, taxa richness, EPT richness, Shannon diversity, FD_{PG} and FD_{Q} for total invertebrates and for each FFG (except EPT) were compared using two-way mixed-model ANOVAs (factors: ‘stream’, ‘site location’). Relationships between biological parameters and structural indices, FPOM, FPIM, CPOM and leaf-litter breakdown rates were tested by ordinary least square linear regressions. Relationships between QBR and macroinvertebrate metrics were tested for each type of site (Up or Down) separately and for the ten sites together; with this approach we wanted to see if the relationships observed for the upstream reference sites was followed by the impaired downstream sites. When necessary, data were log-transformed to meet normality. All statistical analyses were conducted with R (version 2.11.1; R Development Core Team, 2010).

Results

During the study period the dams did not lead to great changes in water physicochemical characteristics; only a slight but significant lower temperature (0.3 ºC) in downstream sites was found. However, reaches below the dams showed a structural simplification as QBR and IHF values were lower downstream (see Mendoza-Lera *et al.*, 2012). Total benthic CPOM and deciduous leaves were similar in both types of sites ($\text{ANOVA}_{\text{CPOM}}$: $F_{1,44} = 0.113$, $p = 0.739$; $\text{ANOVA}_{\text{Deciduous leaves}}$: $F_{1,44} = 0.001$; $p = 0.979$). Although we did not find statistical differences in discharge between upstream and downstream sites in the winter sampling campaign, we confirmed that the downstream sites were completely dry in summer.

A total of 68 taxa were identified, 57 upstream and 55 downstream (Table 2).

Table 2. Mean and range of density (ind m^{-2}) of each macroinvertebrate taxon for upstream and downstream reaches. Lower densities highlighted in bold.

FFG	Order	Family	Genus	Up		Down	
Shredders							
	Coleoptera	Dryopidae		0	0–0	0.4	0–2.2
	Coleoptera	Haliplidae	*Haliplus*	0.4	0–2.2	0	0–0
	Crustacea	Gammaridae	*Echinogammarus*	383.6	0–1651.1	212.4	0–724.4
	Diptera	Limoniidae	*Hexatomini*	8.0	0–20	0.4	0–2.2
	Diptera	Limoniidae	*Limoniini*	4.9	0–17.8	2.2	0–11.1
	Plecoptera	Capniidae	*Capnia*	4.9	0–17.8	0	0–0
	Plecoptera	Leuctridae	*Leuctra*	108.4	2.2–300	13.8	0–37.8
	Plecoptera	Nemouridae	*Amphinemura*	1.3	0–4.4	0	0–0
	Plecoptera	Nemouridae	*Nemoura*	7.6	4.4–13.3	4.0	0–8.9
	Plecoptera	Nemouridae	*Protonemura*	19.6	2.2–64.4	5.3	0–17.8
	Trichoptera	Lepidostomatidae	*Lepidostoma*	0	0–0	4.0	0–17.8
	Trichoptera	Limnephilidae		1.3	0–4.4	2.7	0–8.9
	Trichoptera	Sericostomatidae	*Sericostoma*	3.6	0–11.1	0	0–0
Collector-Gatherers							
	Diptera	Chironomidae (sF. Orthocladinae)		158.2	51.1–424.4	75.1	4.4–164.4
	Diptera	Chironomidae (Tr. Chironomini)		12.9	0–62.2	1.3	0–6.7
	Diptera	Chironomidae (Tr. Tanytarsini)		28.0	0–86.7	20.0	0–47.2
	Diptera	Psychodidae		1.8	0–8.9	0	0–0
	Ephemeroptera	Caenidae	*Caenis*	17.8	0–66.7	3.1	0–8.9
	Ephemeroptera	Ephemerellidae	*Ephemerella*	1.8	0–6.7	1.3	0–4.4
	Ephemeroptera	Ephemeridae	*Ephemera*	1.8	0–4.4	6.2	0–26.7
	Ephemeroptera	Heptageniidae	*Ecdyonorus*	36.0	2.2–77.8	81.3	0–193.3
	Ephemeroptera	Heptageniidae	*Epeorus*	2.2	0–8.9	0.9	0–2.8
	Ephemeroptera	Heptageniidae	*Heptagenia*	129.8	4.4–273.3	24.9	0–46.7
	Ephemeroptera	Leptophlebiidae	*Habroleptoides*	129.8	48.9–331.1	85.8	2.2–311.1
	Ephemeroptera	Leptophlebiidae	*Habrophlebia*	0	0–0	1.8	0–11.1
	Oligochaeta			296.0	82.2–615.6	103.6	26.7–213.3
	Trichoptera	Brachycentridae		4.4	0–20	1.8	0–11.1
	Trichoptera	Hydroptilidae		0	0–0	0.4	0–2.2
Collector-Filterers							
	Diptera	Simuliidae (Tr. Prosimuliini)		1.8	0–6.7	1.3	0–5.6
	Diptera	Simuliidae (Tr. Simuliini)		20.0	0–31.1	17.3	0–53.3
	Trichoptera	Hydropsychidae	*Diplectrona*	3.6	0–8.9	4.0	0–25
	Trichoptera	Hydropsychidae	*Hydropsyche*	12.0	0–44.4	12.4	0–48.9
	Trichoptera	Philopotamidae		0.9	0–2.2	0	0–0

(*Continues in next page*)

(*Comes from previous page*)

FFG	**Order**	**Family**	**Genus**	**Up**		**Down**	
Scrapers							
	Coleoptera	Scirtidae	*Elodes*	5.8	0–24.4	0	0–0
	Coleoptera	Scirtidae	*Hydrocyphon*	49.8	0–171.1	13.8	0–42.2
	Diptera	Blephariceridae		0.4	0–2.2	0	0–0
	Mollusca	Ancylidae	*Ancylus*	1.8	0–4.4	6.7	0–33.3
	Mollusca	Hydrobiidae	*Potamopyrgus*	25.8	4.4–97.8	64.4	4.4–155.6
	Mollusca	Planorbidae		0.4	0–2.2	0	0–0
	Trichoptera	Glossosomatidae		1.3	0–4.4	0	0–0
	Trichoptera	Goeridae		0	0–0	0.9	0–5.6
Col-Gath-Scrap							
	Coleoptera	Elmidae	*Elmis*	20.4	2.2–73.3	23.6	0–102.2
	Coleoptera	Elmidae	*Esolus*	37.8	4.4–68.9	23.1	0–77.8
	Coleoptera	Elmidae	*Limnius*	7.1	0–20	6.2	0–22.2
	Coleoptera	Elmidae	*Potamophilus*	0	0–0	0.4	0–2.8
	Coleoptera	Elmidae	*Riolus*	0.0	0–0	0.4	0–2.8
	Coleoptera	Hydraenidae	*Hydraena*	0.9	0–4.4	0.4	0–2.2
	Ephemeroptera	Baetidae	*Baetis*	167.1	11.1–457.8	154.2	0–473.3
Predators							
	Diptera	Athericidae	*Atherix*	28.4	11.1–57.8	15.6	2.2–42.2
	Diptera	Athericidae	*Atrichops*	0.4	0–2.2	0	0–0
	Diptera	Empididae	*Clinocerinae*	0	0–0	2.7	0–11.1
	Diptera	Empididae	*Hemerodromiinae*	1.8	0–4.4	0.4	0–2.2
	Diptera	Rhagionidae		0.4	0–2.2	2.2	0–11.1
	Heteroptera	Corixidae	*Cymatia*	0	0–0	0.4	0–2.8
	Hirudinea	Glossiphoniidae		0.9	0–4.4	1.3	0–6.7
	Odonata	Aeshnidae		0.4	0–2.2	0	0–0
	Odonata	Calopterygidae		0	0–0	7.6	0–37.8
	Odonata	Gomphidae		0	0–0	1.8	0–8.3
	Odonata	Libellulidae		0.4	0–2.2	8.0	0–33.3
	Plecoptera	Chloroperlidae	*Chloropela*	0.9	0–4.4	0	0–0
	Plecoptera	Chloroperlidae	*Siphonoperla*	30.2	0–100	0.4	0–2.2
	Plecoptera	Perlidae	*Perla*	4.9	0–20	0	0–0
	Plecoptera	Perlodidae	*Isoperla*	22.7	0–75.6	0	0–0
	Trichoptera	Polycentropodidae	*Holocentropus*	2.7	0–11.1	0.4	0–2.2
	Trichoptera	Rhyacophilidae	*Hyporhyacophila*	7.1	2.2–17.8	13.3	2.2–33.3
	Trichoptera	Rhyacophilidae	*Pararhyacophila*	0.4	0–2.2	0.9	0–2.8
	Trichoptera	Rhyacophilidae	*Rhyacophila*	0.4	0–2.2	2.7	0–8.9
	Turbellaria	Planariidae	*Dugesia*	16.0	0–40	3.6	0–11.1

Table 3. Richness and diversity of total macroinvertebrates and each Functional Feeding Group (FFG), and EPT index (mean ± SE) for upstream and downstream reaches. Results of two-way ANOVA are shown. The mean and the SE values are based on untransformed data, whereas the statistical output refers to the analysis of the log-transformed data.

	Up	Down	*df*	*F*	*p*	Comparison
Richness						
Total	16.76 ± 2.54	12.60 ± 3.24	1,44	14.692	<0.001	U>D
Shredders	3.56 ± 0.64	1.76 ± 0.46	1,44	26.005	<0.001	U>D
Collector-Gatherers	5.56 ± 0.39	4.64 ± 0.92	1,44	6.661	0.013	U>D
Collector-Filterers	1.04 ± 0.34	0.84 ± 0.31	1,44	0.496	0.485	
Scrapers	1.56 ± 0.38	1.16 ± 0.40	1,44	1.849	0.181	
Col-Gath-Scrap	2.16 ± 0.37	1.84 ± 0.69	1,44	5.277	0.026	U>D
Predators	2.88 ± 1.00	2.24 ± 0.67	1,44	1.091	0.302	
Diversity						
Total	2.76 ± 0.26	2.43 ± 0.39	1,44	4.427	0.041	U>D
Shredders	1.22 ± 0.26	0.51 ± 0.17	1,44	18.123	<0.001	U>D
Collector-Gatherers	1.83 ± 0.10	1.51 ± 0.32	1,44	5.804	0.020	U>D
Collector-Filterers	0.30 ± 0.19	0.16 ± 0.09	1,44	1.582	0.215	
Scrapers	0.52 ± 0.21	0.28 ± 0.19	1,44	3.27	0.077	
Col-Gath-Scrap	0.72 ± 0.19	0.40 ± 0.29	1,44	20.488	<0.001	U>D
Predators	1.00 ± 0.39	0.84 ± 0.26	1,44	0.279	0.599	
EPT	8.20 ± 1.51	5.28 ± 1.34	1,44	22.619	<0.001	U>D

The average number of taxa per sample was higher in the upstream sites (17) than in the downstream ones (13) (Table 3, ANOVA: $F_{1,44} = 14.692$, $p < 0.001$). Streams below dam presented lower taxa richness of shredders (50.6% lower; ANOVA: $F_{1,44} = 26.005$, $p < 0.001$), collector-gatherers (16.5% lower; ANOVA: $F_{1,44} = 6.661$, $p = 0.013$) and collector-gatherers-scrapers (10.6% lower; ANOVA: $F_{1,44} = 5.277$, $p = 0.026$) than streams above dam. Shannon diversity of the entire invertebrate assemblages was lower downstream (Table 3; ANOVA: $F_{1,44} = 4.427$, $p = 0.041$), and also shredders diversity (ANOVA: $F_{1,44} = 18.123$, $p < 0.001$), collector-gatherers diversity (ANOVA: $F_{1,44} = 5.804$, $p = 0.020$) and collector-gatherer-scrapers diversity (ANOVA: $F_{1,44} = 20.488$, $p < 0.001$). EPT richness was 35.6% lower downstream (ANOVA: $F_{1,44} = 22.619$, $p < 0.001$).

Total macroinvertebrate density was highly variable among the ten different reaches, ranging from 82.2 individuals m^{-2} in Lingorta Down to 2940

Table 4. Density and biomass of total macroinvertebrates and each FFG (mean ± SE) for upstream and downstream reaches. Results of two-way ANOVA are shown. The mean and SE values are based on untransformed data, whereas the statistical output refers to the analysis of the log-transformed data.

	Up	Down	*df*	*F*	*p*	Comparison
Density (ind m^{-2})						
Total	1840.4 ± 455.7	1044.4 ± 380.1	1, 44	17.616	<0.001	U>D
Shredders	543.6 ± 316.5	245.3 ± 134.0	1,44	11.249	0.002	U>D
Collector-Gatherers	820.4 ± 156.3	40.6 ± 118.4	1,44	15.388	<0.001	U>D
Collector-Filterers	38.2 ± 11.1	35.1 ± 17.2	1,44	0.366	0.549	
Scrapers	85.3 ± 38.5	85.8 ± 35.7	1,44	0.110	0.742	
Col-Gath-Scrap	245.3 ± 91.9	220.9 ± 118.8	1,44	5.041	0.030	U>D
Predators	119.6 ± 58.5	62.2 ± 29.5	1,44	1.938	0.171	
Biomass (mg m^{-2})						
Total	678.1 ± 150.8	891.8 ± 226.0	1, 44	0.899	0.348	
Shredders	201.1 ± 146.2	211.3 ± 85.1	1,44	1.354	0.251	
Collector-Gatherers	232 ± 44.8	369.2 ± 135.8	1,44	0.458	0.502	
Collector-Filterers	28.2 ± 15.7	30.7 ± 19.1	1,44	0.850	0.362	
Scrapers	47.8 ± 22.6	71.9 ± 38.7	1,44	0.027	0.870	
Col-Gath-Scrap	66.1 ± 25.5	59.1 ± 27.3	1,44	4.048	0.051	
Predators	102.9 ± 38.9	149.5 ± 81.6	1,44	0.312	0.579	

individuals m^{-2} in Artiba Up. On average, it was 43.2% lower below the dams (Table 4, ANOVA: $F_{1,44} = 17.616$, $p < 0.001$). Communities were dominated by detritivores (shredders and any kind of gatherers), which represented per site on average 73.36 to 92.82% for density, and 48.30 to 99.92% for biomass. There was a decrease in density of functional feeding groups within detritivores from Up to Down sites: shredders (ANOVA: $F_{1,44} = 11.25$, $p = 0.002$), collector-gatherers ($F_{1,44} = 15.39$, $p < 0.001$) and collector-gatherer-scrapers ($F_{1,44} = 5.04$, $p = 0.030$). Nevertheless, biomass data did not confirm the observed density reduction (Table 4), attributable to the presence of a few large individuals of Trichoptera for shredders and of Oligochaeta for gatherers in the downstream sites. From the 68 taxa found, a total of 44 showed a lower average density in the downstream sites, although not all differences were statistically significant. The 18 most abundant taxa (those representing > 1% of the total density) comprised collectively 91.43% of the total invertebrate abundance. Among them, only four detritivores and one predator

Table 5. Functional diversity indexes, FD_{PG} and FD_Q, of total macroinvertebrates and each FFG (mean ± SE) for upstream and downstream reaches. Results of two-way ANOVA are shown.

	Up	Down	*df*	*F*	*p*	Comparison
FD_{PG}						
Total	0.31 ± 0.04	0.24 ± 0.05	1,44	11.375	0.002	U>D
Shredders	0.36 ± 0.06	0.26 ± 0.03	1,44	19.540	<0.001	U>D
Collector-Gatherers	0.44 ± 0.02	0.36 ± 0.07	1,44	5.536	0.023	U>D
Collector-Filterers	0.16 ± 0.08	0.12 ± 0.06	1,44	0.878	0.354	
Scrapers	0.19 ± 0.09	0.18 ± 0.08	1,44	0.005	0.944	
Col-Gath-Scrap	0.47 ± 0.07	0.24 ± 0.15	1,44	16.743	<0.001	U>D
Predators	0.18 ± 0.07	0.16 ± 0.05	1,44	0.582	0.449	
FD_Q						
Total	0.41 ± 0.03	0.39 ± 0.07	1,44	0.392	0.535	
Shredders	0.19 ± 0.05	0.09 ± 0.04	1,44	9.233	0.004	U>D
Collector-Gatherers	0.46 ± 0.03	0.39 ± 0.09	1,44	2.921	0.094	
Collector-Filterers	0.06 ± 0.04	0.03 ± 0.03	1,44	0.319	0.575	
Scrapers	0.08 ± 0.04	0.09 ± 0.06	1,44	0.055	0.815	
Col-Gath-Scrap	0.22 ± 0.09	0.19 ± 0.12	1,44	0.297	0.589	
Predators	0.21 ± 0.07	0.25 ± 0.08	1,44	0.718	0.402	

were significantly and negatively affected by the reservoir: the shredders *Leuctra* and *Protonemura* (87.3 and 72.7% less abundant downstream; ANOVA: $F_{1,44} = 23.635$, $p < 0.001$; $F_{1,44} = 8.183$, $p = 0.006$, respectively), the collector-gatherers *Habroleptoides*, *Heptagenia* and Orthocladiinae (31.9, 80.8 and 52.5% lower; ANOVA: $F_{1,44} = 12.779$, $p < 0.001$; $F_{1,44} = 22.605$, $p < 0.001$; $F_{1,44} = 4.267$, $p = 0.045$, respectively), and the predator *Siphonoperla* (98.5% lower; ANOVA: $F_{1,44} = 23.405$, $p < 0.001$). Regarding the functional diversity indices, FD_{PG} (based on species richness) values were lower below the dam (ANOVA: $F_{1,44} = 11.375$, $p = 0.002$; Table 5). It was also lower downstream in the case of shredders (ANOVA: $F_{1,44} = 19.540$, $p < 0.001$) and collector-gatherers-scrapers (ANOVA: $F_{1,44} = 16.743$, $p < 0.001$; Table 5). FD_Q (evenness included) for total invertebrates did not show differences between Up and Down reaches (ANOVA: $F_{1,44} = 0.392$, $p = 0.535$), however, FD_Q for shredders was lower downstream (Table 5, ANOVA: $F_{1,44} = 9.233$, $p = 0.004$).

No physicochemical variable was related to the estimated benthic macroinvertebrate metrics. Considering resource-consumer relationships, suspended FPOM was not related to collector-filterer variables. On the other hand, a positive relationship was found between shredder biomass and the amount of deciduous leaves (Pearson's $R^2 = 0.743$, $p = 0.001$, $n = 10$). The abundance, biomass, richness, and FD_{PG} index of benthic shredders showed a significant positive correlation with alder leaf-litter breakdown rates (Fig. 2). The quality of the riparian forest (QBR index) was the variable showing most correlations with the structural and functional metrics based on the total macroinvertebrate community or shredders, but relationships appeared depending on the type of site (altogether, only Up or only Down sites) considered for the regression analysis. When the ten sites were considered together, seven positive relationships appeared. Total density, taxa richness and FD_{PG} for both total invertebrates and shredders (Fig. 3) were following a linear trend with respect to QBR. Considering only Up sites, six significant positive relationships could be seen: both density and biomass of both total invertebrates and shredders and FD_Q of total invertebrates (Fig. 3) were related to QBR. Down sites did not show any significant relationship with QBR index. The relationships between macroinvertebrate metrics and IHF index were significant only in three cases: EPT richness, shredder richness and shredder FD_{PG} (Pearson's R: $R^2 = 0.401$, 0.448, 0.455, $p = 0.049$, 0.034, and 0.032, respectively, $n = 10$).

Discussion

Although not all the studied variables were affected by the dams, structural and functional aspects of the stream always showed significantly lower values below the dam. Changes in water quality caused by river regulation can directly affect the abundance and diversity of aquatic organisms downstream (Bredenhand & Samways, 2009). Nevertheless, we did not observe any noticeable change of physicochemical parameters between upstream and downstream reaches during the study period (Mendoza-Lera *et al.*, 2012). This can be explained by the overflow water release, the small size of the studied dams and their location in oligotrophic headwaters (Cortes *et al.*, 1998; Príncipe, 2010). Water temperature is one of the most frequently

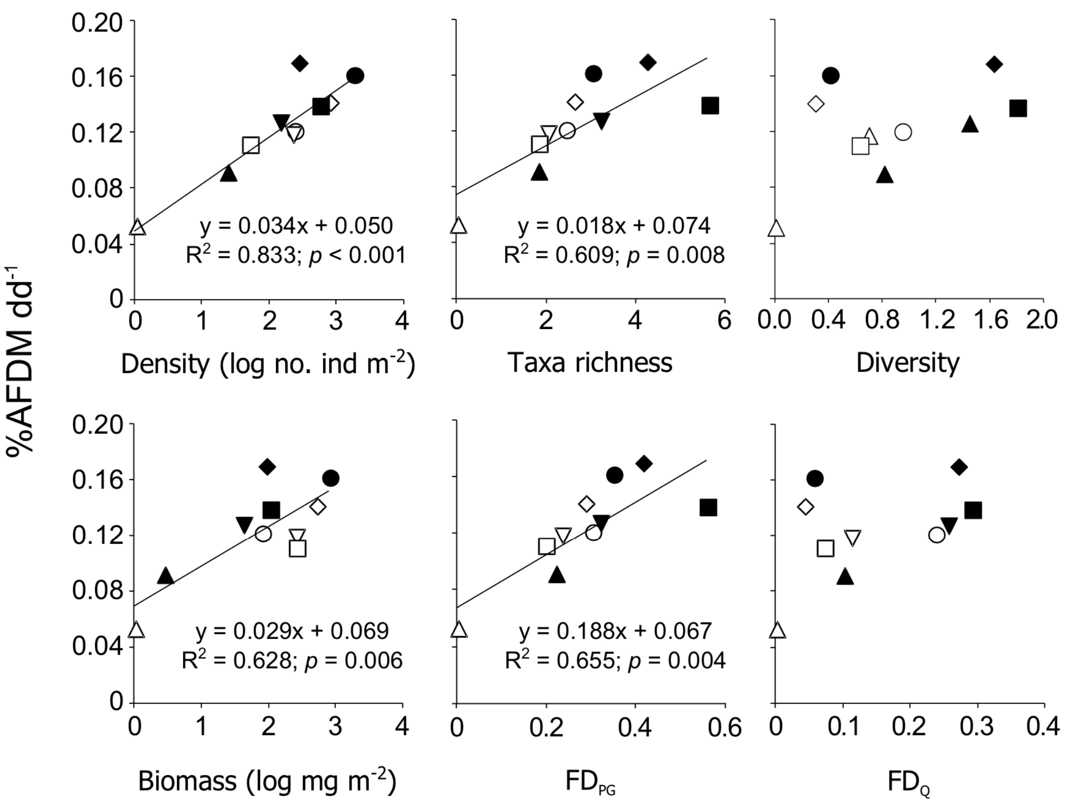
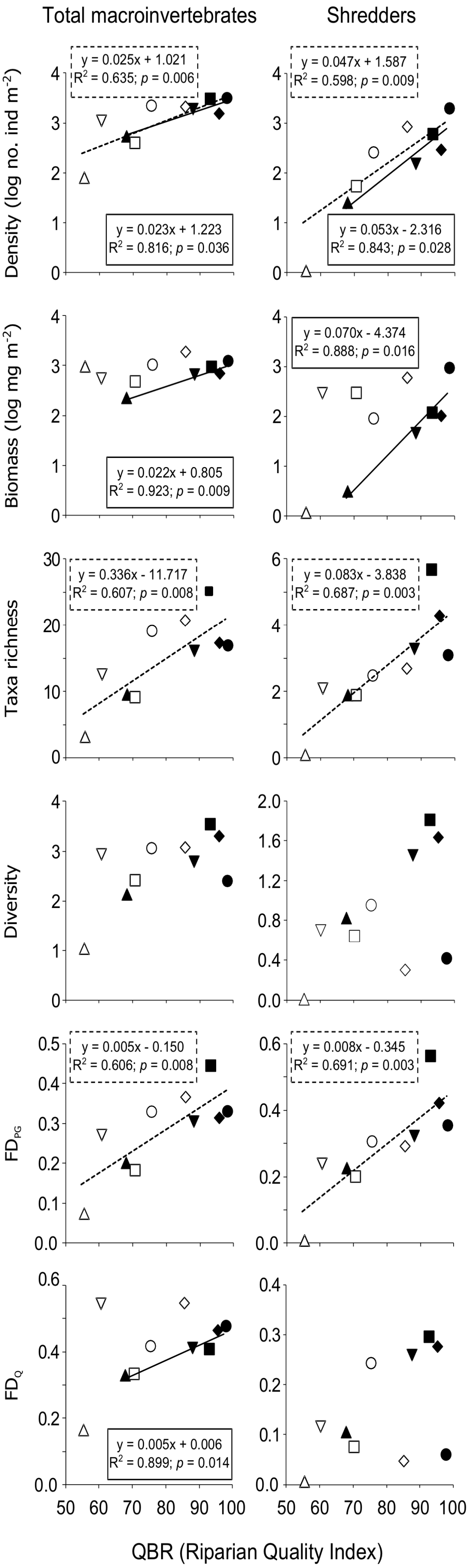


Figure 2. Relationship between structural and functional variables for shredders and alder leaf-litter breakdown rate. Filled symbols represent upstream sites and open symbols represent downstream sites. Streams are identified as follows: Artiba (circle), Lekubaso (inverted pyramid), Lingorta (pyramid), Regato (diamond) and Zollo (square). Note the different scales.

affected variables by river impoundment (Pozo *et al.*, 1997; Bredenhand & Samways 2009), and it is a very important factor for the biology and the evolutionary ecology of stream insects (Ward & Stanford, 1982). We reject the idea that minimal changes in water temperature and in other physicochemical parameters can explain the observed differences in invertebrate communities but state that other factors are more important determinants for invertebrates. Independently of the stream, the CPOM to FPOM ratio suffers a natural decline as the detritus is transported downstream, but impoundments generate steep reductions of the ratio due to coarse detritus entrapment (Ward & Stanford, 1983). This disruption, as Bredenhand & Samways (2009) observed, causes alterations in the functional trophic structure of the macroinvertebrate community below dams leading to an increase of the number of filterers and a decrease of shredders. Nevertheless, in our study, despite of the higher amount of FPOM in suspension downstream we did not observe higher densities of collector-filterers below dams.



◄ **Figure 3.** Relationship between riparian vegetation quality index (QBR) and structural and functional variables for total macroinvertebrate assemblage and shredders. Filled symbols represent upstream sites and open symbols represent downstream sites. Streams are identified as follows: Artiba (circle), Lekubaso (inverted pyramid), Lingorta (pyramid), Regato (diamond) and Zollo (square). Significant linear regressions are shown for the 5 upstream sites (continuous line) and for the 10 studied sites (dashed line). No significant relationship was observed using only the 5 downstream sites. Note the different scales.

On the other hand, although dams did not affect the amount of CPOM shredders and collector-gatherers richness and densities were reduced downstream.

Our downstream sites suffer a year-round flow fluctuation that approximates to that observed in Mediterranean streams: with flows similar to temperate streams throughout autumn–winter–spring when the reservoirs are filled, and droughts during summer (observed by the authors), since the dams in the present study retain all the water without ensuring any ecological flow downstream. Navarro-Llácer *et al.* (2010) noted that these alterations of the annual flow regime induced a loss in riparian forest quality downstream of impoundments under Mediterranean climate. In low-order forested streams, riparian forests define the functional feeding structure of macroinvertebrate communities (Sandin, 2009) by determining the quality and quantity of the allochthonous resources (Sweeney, 1993). Flow alteration is also able to homogenize the benthic habitat structure (Hart & Finelli, 1999), which can determine the distribution of benthic biota (Sandin, 2009), as many taxa require specific habitat types (see Tachet *et al*., 2002). Substratum homogenization was observed in our downstream sites (Mendoza-Lera *et al*., 2012), but it was not related to the expected increase of generalist taxa recorded by Townsend *et al*. (1997). However, the significant correlations found between QBR and macroinvertebrates indicate that structural changes in the riparian corridor might influence macroinvertebrate communities. The positive and significant relationship between deciduous leaves and shredder biomass are also pointing out the importance of allochthonous inputs for the invertebrates. Nevertheless, the correlation between QBR and macroinvertebrates was only evident in the upstream sites, with no significant correlation if we only consider the downstream sites. This contrasting result between the upstream and the downstream sites might indicate that the riparian forest is an important driver of macroinvertebrate communities in natural streams (our upstream sites) and that the impact on downstream sites have its origin on the observed flow alteration. Actually, water shortage caused by summer droughts can by itself define the structure of communities (Muñoz, 2003). After dry periods, the taxonomic composition depends on the recolonization capacity that varies among taxa (Otermin *et al*., 2002), and notably the scarcity of shredders in comparison with other FFG at these first stages (Boulton, 1991). Instead of temporal changes, some authors have studied streams with different flow regimes. For instance, Sabater *et al*. (2008) compared a permanent Atlantic stream with an intermittent Mediterranean stream and found lower detritivore and predator biomass in the intermittent stream. We have not observed differences between upstream and downstream sites in terms of biomass, but abundance results are comparable to Sabater's study with a significantly lower shredder density in reaches below the dam, and also lower densities of *Leuctra* and *Protonemura* (shredders), *Habroleptoides*, *Heptagenia* and Orthocladiinae (collector-gatherers) and *Siphonoperla* (predator); most of them described as slow colonizers (Gore, 1982; McArthur & Barnes, 1985; Otermin *et al*., 2002). We lack macroinvertebrate data for summer months, but the patterns of upstream-downstream differences in macroinvertebrate communities on winter and the visually corroborated droughts in downstream reaches on summer suggest Up-Down differences might become even higher.

Macroinvertebrate assemblages from most of the streams contain many species that are redundant (Lawton, 1991) in the sense that ecosystem functions can proceed even when some of the taxa are absent (Wallace *et al.*, 1986). Nevertheless, our trait-based results of functional diversity indices support that alterations in the structural metrics of the community, are accompanied by a change at the functional level as shown by the FD_{PG} index which was lower downstream and which is strongly correlated with taxa richness (Petchey & Gaston, 2002). The effects were, nevertheless, undetected by the FD_Q index. This index includes evenness in the computation, and is further strongly influenced by abundant taxa (Rao, 1982), hence, changes of functionally singular but rare taxa (among them keystone species) can go unnoticed. Although it is difficult to consider any stream macroinvertebrate a keystone species (Paine, 1966; Mills *et al.*, 1993), there are some ecosystem processes in which some taxa seem to be cornerstones (McKie & Cranston, 1998; Woodward *et al.*, 2008). One of the most important processes in low order forested streams is the coarse organic matter processing, for which shredders are mainly responsible. In our streams, this function was negatively affected by river regulation as we observed a reduction of above 20% in leaf-litter decomposition rates from upstream to downstream sites (Mendoza-Lera *et al.*, 2012). This reduction in the decay could be more easily explained by structural metrics of benthic shredders than by metrics of shredders associated with bags. In fact, we obtained higher R^2 values using benthic data than those reported by Mendoza-Lera *et al.* (2012) using fauna of litter bags. Leaf-litter processing is just one among many other functions that are essential for freshwater ecosystems. The loss of species and the consequent loss of trait diversity would also weaken the capability of the community to satisfactorily develop other functions. Considering stream regulation by small dams, we have observed that the taxa involved in the detritic pathway seem to be the most affected (shredders and gatherers have suffered significant reductions in both functional diversity indexes, FD_{PG} and FD_Q). If there are functions, apart from leaf-litter decomposition, in which shredder and collector gatherer taxa are especially important (*e.g.* secondary production, recolonization, etc.), regulated headwater streams might also suffer from deficiencies in those other processes. Nevertheless, caution should be taken when stating that small dams weaken the resistance of a given community because of two reasons. Firstly, the functional redundancy among taxa (Lawton, 1991) can lead to adaptations of the communities to the loss of species, filling the gaps left by missing taxa by others with similar characteristics. Since density and biomass of shredders were good predictors of the rates, being irrelevant the inter-site taxonomic differences on shredder assemblages, redundancy might be playing for the leaf-litter decomposition. Secondly, we have not observed upstream-downstream differences for the biomass of macroinvertebrates. Biomass correlates stronger than density to other important properties (*i.e.* metabolism) and functions (*i.e.* secondary production) of the macroinvertebrate community and the lack of differences in biomass might be understood as a limited impact.

To sum up, the regulation by surface release small reservoirs negatively affects density, richness and diversity of macroinvertebrates in the reaches below the dam. The main driver is probably the variability of the flow regime due to the absence of any ecological flow, which creates droughts below the dam in summer. Furthermore, downstream reaches are characterized by loss of riparian forest quality and reduction of benthic habitat heterogeneity. The changes in the trophic structure of the macroinvertebrate communities are strongly related to stream processes, such as leaf-litter decomposition, and are accompanied by a loss of functional diversity, which in turn could alter functions of the system.

References

Allan JD & Castillo MM, 2007. Stream ecology–Structure and function of running waters. 2nd edition. Springer: Dordrecht; 436 pp.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation: Washington, DC; 1368 pp.

Botta-Dukát Z, 2005. Rao’s quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science 16: 533–540.

Boulton AJ, 1991. Eucalypt leaf decomposition in an intermittent stream in south eastern Australia. Hydrobiologia 211: 123–136.

Bredenhand E & Samways MJ, 2009. Impact of a dam on benthic macroinvertebrates in a small river in a biodiversity hotspot: Cape Floristic Region, South Africa. Journal of Insect Conservation 1: 297–307.

Cortes RMV, Ferreira MT, Oliveira SV & Godinho F, 1998. Contrasting impact of small dams on the macroinvertebrates of two Iberian mountain rivers. Hydrobiologia 389: 51–61.

Dolédec S, Phillips N, Scarsbrook M, Riley RH & Townsend CR, 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25: 44–60.

Fairchild GW & Velinsky DJ, 2006. Effects of small ponds on stream water chemistry. Lake and Reservoir Management 22: 321–330.

Gore JA, 1982. Benthic invertebrate colonization: source distance effects on community composition. Hydrobiologia. 94: 183–193.

Gore JA & Petts GA, 1989. Alternatives in regulated river management. CRC Press: Boca Raton – Florida, USA; 344 pp.

Hart DD & Finelli CM, 1999. Physical-biological coupling in streams: the pervasive effect of flow on benthic organisms. Annual Review of Ecology and Systematics 30: 363–395.

Haxton TJ & Findlay CS, 2008. Meta-analysis of the impacts of water management on aquatic communities. Canadian Journal of Fisheries and Aquatic Sciences 65: 437–447.

Lawton J, 1991. Are species useful? Oikos 62: 3–4.

Martin ROR & Hanson RL, 1966. Reservoirs in the United States, Water Supply Paper 1838, U.S. Geological Survey: Washington, D.C.; 115 pp.

Marty J, Smokorowski K & Power M, 2009. The influence of fluctuating ramping rates on the food web of boreal rivers. River Research and Applications. 25: 962–974.

Mason NWH, Mouillot D, Lee WG & Wilson JB, 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 111: 112–118.

Maxted JR, McCready CH & Scarsbrook MR, 2005. Effects of small ponds on stream water quality and macroinvertebrate communities. New Zealand Journal of Marine and Freshwater Research 39: 1069–1084.

McArthur JV & Barnes JR, 1985. Patterns of macroinvertebrate colonization in an intermittent rocky mountain stream in Utah. Great Basin Naturalist 45: 117–123.

McKie BGL & Cranston PS, 1998. Keystone coleopterans? Colonization by wood-feeding elmids of experimentally immersed woods in south-eastern Australia. Marine and Freshwater Research 49: 79–88.

Mendoza–Lera C, Larrañaga A, Pérez J, Descals E, Martínez A, Moya O, Arostegui I & Pozo J, 2012. Headwater reservoirs weaken terrestrial-aquatic linkage by slowing leaf-litter processing in downstream regulated reaches. River Research and Applications 28: 13–22.

Merritt RW & Cummins KW, 2007. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company: Dubuque; 1158 pp.

Mills LS, Soule ME & Doak DF, 1993. The keystone-species concept in ecology and conservation. BioScience 43: 219–224.

Munné A, Prat N, Sola C, Bonada N & Rieradevall M, 2003. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. Aquatic Conservation: Marine and Freshwater Ecosystems 13: 147–163.

Muñoz I, 2003. Macroinvertebrate community structure in an intermittent and a permanent Mediterranean streams (NE Spain). Limnetica 22: 107–16.

Navarro-Llácer C, Baeza D & de las Heras J, 2010. Assessment of regulated rivers with indices based on macroinvertebrates, fish and riparian forest in the southeast of Spain. Ecological Indicators 10: 935–942.

Nichols S, Norris R, Maher W & Thoms M, 2006. Ecological effects of serial impoundment on the Cotter River, Australia. Hydrobiologia 572: 255–273.

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: 117–28.

Paine RT, 1966. Food web complexity and species diversity. American Naturalist 100: 65–75.

Pardo I, Álvarez M, Casas J, Moreno JL, Vivas S, Bonada N, Alba-Tercedor J, Jáimez-Cuéllar P, Moyà G, Prat N, Robles S, Suárez ML, Toro M & Vidal-Abarca MR, 2002. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21: 115–133.

Péru N & Dolédec S, 2010. From compositional to functional biodiversity metrics in bioassessment: A case study using stream macroinvertebrate communities. Ecological Indicators 10: 1025–1036.

Petchey OL & Gaston JG, 2002. Functional diversity (FD), species richness and community composition. Ecology Letters 5: 402–411.

Petts GE, 1988. Impounded rivers: perspectives for ecological management. John Wiley & Sons Ltd.: West Sussex; 326 pp.

Poff NL & Hart DD, 2002. How dams vary and why it matters for the emerging science of dam removal. BioScience 52: 659–738.

Pozo J, Orive E, Fraile H & Basaguren A, 1997. Effects of the Cernadilla-Valparaiso reservoir system on the River Tera. Regulated Rivers: Research and Management 13: 57–73.

Príncipe RE, 2010. Ecological effects of small dams on benthic macroinvertebrate communities of mountain streams (Córdoba, Argentina). Annales de Limnologie-International Journal of Limnology 46: 77–91.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org.

Rao CR, 1982. Diversity and dissimilarity coefficients—a unified approach. Theoretical Population Biology 21: 24–43.

Rehn AC, 2008. Benthic macroinvertebrates as indicators of biological condition below hydropower dams on west slope Sierra Nevada streams, California, USA. River Research and Applications 25: 208–228.

Rosenberg DM, McCully P & Pringle CM, 2000. Global-scale environmental effects of hydrological alterations: introduction. BioScience 50: 746–751.

Sabater S, Elosegi A, Acuña V, Basaguren A, Muñoz I & Pozo J, 2008. Effect of climate on the trophic structure of temperate forested streams. A comparison of Mediterranean and Atlantic streams. Science of the Total Environment 390: 475–484.

Sandin L, 2009. The effects of catchment land-use, near-stream vegetation, and river hydromorphology on benthic macro invertebrate communities in a south-Swedish catchment. Fundamental and Applied Limnology 174: 75–87.

Sweeney BW, 1993. Effects of streamside vegetation on macroinvertebrates communities of White Clay Creek in eastern North America. Proceedings of the Academy of Natural Sciences of Philadelphia 144: 291–340.

Tachet H, Richoux P, Bournaud M & Usseglio-Polatera P, 2002. Invertébrés d'eau douce: systématique, biologie et écologie. CNRS: Paris; 587 pp.

Townsend CR, Dolédec S & Scarsbrook MR, 1997. Species traits in relation to temporal and spatial heterogeneity in streams: a test of habitat templet theory. Freshwater Biology 37: 367–387.

Usseglio-Polatera P, Bournaud M, Richoux P & Tachet H, 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biology 43: 175–205.

Vallania A & Corigliano MC, 2007. The effect of regulation caused by a dam on the distribution of the functional feeding groups of the benthos in the sub basin of the Grande river (San Luis, Argentina). Environmental Monitoring and Assessment 124: 201–209.

Wallace JB, Vogel DS & Cuffney TF, 1986. Recovery of a headwater stream from an insecticide-induced community disturbance. Journal of North American Benthological Society 5: 115–126.

Ward, J. V. & J. A. Stanford, 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27: 97–117.

Ward JV & Stanford JA, 1983. The serial discontinuity concept of lotic ecosystems. In: Dynamics of lotic ecosystems, Fontaine TD, Bartell SM (eds). Ann Arbor Science Publishers: Michigan; 29–42.

Woodward G, Papantoniou G, Edwards F & Lauridsen RB, 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117: 683–692.

Land-use changes

Chapter II

Consistency of leaf-litter stoichiometry *vs*. processing relationships among three forested streams

Abstract

We examined effects of leaf quality on leaf-litter processing and invertebrate colonization in stream ecosystems. Leaves from five tree species with different nutritional quality (*Alder glutinosa*, *Populus nigra*, *Quercus pyrenaica*, *Platanus* x *hispanica*, *Pinus radiata*) were incubated (litter bags) in three unaltered forested streams in Northern Spain. Leaf-litter breakdown rates, nitrogen concentration, C:N ratio and invertebrate community in benthos and bags were analyzed. Breakdown rates differed among litter species and were positively related with initial nitrogen concentration and negatively with C:N ratio. After incubation period (6–7 weeks), the differences in nutritional quality among species remained and the relationship between breakdown rates and quality persisted. The density and biomass of macroinvertebrates, mainly shredders, associated with leaf-litter bags were related to leaf quality and helped to explain the differences in breakdown rates. A change in surrounding forest composition could alter quality of leaf-litter inputs to stream and could affect organic matter processing and the subsequent transference of energy throughout the food web.

Key words: Leaf-litter decomposition, Leaf-litter quality, Nitrogen concentration, C:N ratio, Macroinvertebrates.

Introduction

Detrital processing constitutes a decisive component in the functioning of any ecosystem (Moore *et al.*, 2004). This detritic pathway has been, over the last three decades (Webster & Benfield, 1986), the focus of functional studies in forested low order streams (Woodward, 2009), as the inputs of death organic matter, mainly in form of leaves (Abelho, 2001), represent the principal source of matter and energy to these systems (Webster *et al.*, 1999). The organisms responsible for allochthonous detritus processing are microbial decomposers, primarily aquatic hyphomycete fungi (Bärlocher, 1992; Abelho & Graça, 2006), and invertebrate detritivores (Graça, 2001), both acting as transfers of energy to higher trophic levels (Wallace *et al.*, 1997; 1999). Microorganisms use leaves as carbon source obtaining nutrients from water column (Suberkropp & Chauvet, 1995; Canhoto & Graça, 2008). However, invertebrates are not able to incorporate dissolved nutrients, their detritivorous activity being governed by elemental composition of resource (Hladyz *et al.*, 2009). Benthic leaf materials show diverse elemental composition, intrinsic properties depending on species of the surrounding forest (Swan & Palmer, 2006) and differences in quality as food resource to consumers (Enríquez *et al.*, 1993; Tibbets & Molles, 2005). There is a wide stoichiometric gap between leaf-litter and consumers (Cross *et al*., 2005), but invertebrates are obliged to feed on resources with low nutritional quality to supply their energetic demands (Sterner & Elser, 2002; Woodward, 2009). The physical characteristics of leaves, as toughness, surface structure and roughness or extracellular substances, determine the decomposer colonization and activity (Bärlocher & Oertli, 1978; Dang *et al*., 2007; Kearns & Bärlocher, 2008) and also the palatability of the resource for ulterior consumers (Graça & Cressa, 2010).

Although some studies have look for the relationship between leaf-litter elemental composition and its breakdown rate (Ostrofsky, 1997; Leroy & Marks, 2006) a further understanding is required, as some quality-breakdown rate relationships are still unclear (Ardón *et al*., 2009; Alonso *et al*., 2010), and stream ecosystems functioning could be affected by the replacement of riparian vegetation and the quality of leaf-litter entering these systems. Thus, our goal is to gain an insight into the linkage between leaf-litter quality and its in-stream breakdown by studying the decomposition rates and associated macroinvertebrates of five leaf types (one pine and four broadleaf species) with different nutritional quality (based on leaf-N concentrations and C:N ratio) in three undisturbed streams.

Material and methods

Study area

The study was carried out in three low-order (2nd–3rd) streams (S1, S2 and S3) with siliceous substrata flowing into the Atlantic Ocean (Cordillera Cantábrica, Northern Spain). The streams drain native deciduous forests, the dominant tree species being *Quercus robur* L. and *Fagus sylvatica* L. Anthropic impacts in the basins are negligible.

During the study period (November 2009 to January 2010) water temperature was continuously monitored (every 1 hour) with SmartButton data loggers (ACR Systems Inc., Surrey, BC, Canada). In each field visit (*n* = 5–6) conductivity, pH, dissolved oxygen (WTW multiparametric sensor) and river flow (Martin Marten Z30, Current-meter) were measured and water samples were collected and filtered in the laboratory (0.7 mm pore size glass fiber filter, Whatman GF/F). An aliquot of the filtered water was used to determine alkalinity by titration to an end pH of 4.5 (APHA, 2005) and the rest was stored (-20 ºC) for later analyses. Nutrient analyses of the water were conducted using the capillary ion electrophoresis for nitrate (Agilent CE), the manual salicylate method for ammonium, the sulphanilamide method for nitrite and the molybdate method for soluble reactive phosphorus (SRP) (APHA, 2005). The water of the three streams was well oxygenated, with neutral pH, cold temperatures and low nutrient concentrations. The only remarkable difference was the higher nitrate concentration in S1 than in the other two streams (Table 1).

In order to characterize macroinvertebrate community of each stream, five benthic samples (Surber 0.09 m^2, 0.5 mm mesh size) were taken from randomly chosen riffles in January 2010. Fauna was preserved in 70% ethanol until the ulterior study. In the laboratory, macroinvertebrates were identified to family (Oligochaeta to order, Chironomidae to tribe) using Tachet *et al.* (2002), counted and sorted into functional feeding groups, FFG (after Tachet *et al.*, 2002; Merritt and Cummins, 2007): shredders (Sh), collector-gatherers (CG), collector-gatherer-scrapers (CGS), collector-filterers (CF), scrapers (Sc) and predators (P). The dry mass, as an estimation of the biomass, was determined oven-drying altogether the individuals sorted in two clusters: shredders and the rest of the FFG. For each benthic macroinvertebrate sample we calculated the taxa richness and Shannon diversity index.

Leaf types, decomposition and associated macroinvertebrates

For the study, leaves of five tree species with different elemental quality were used: common alder (*Alnus glutinosa* (L) Gaertner), black poplar (*Populus nigra* L.), Pyrenean oak (*Quercus pyrenaica* Willd.), plane tree (*Platanus* x *hispanica* Münchh.) and radiata pine needles (*Pinus radiata* D. Don). All these species are present in the Iberian Peninsula. Alder and poplar are typical of riparian forest but also the latter and its hybrids as riparian fast-growing plantations. Pyrenean oak forms natural forests, plane trees are commonly planted in riparian zones and pine plantations cover large areas. In October–November 2009 leaves were collected from the floor immediately after natural abscission except for pine needles, which were collected from dry branches. Leaf-litter was air dried, and approximately 5 g (± 0.25) of each species were placed separately into mesh bags (15 × 20 cm, 5 mm mesh size to allow access to stream biota). Pine

Table 1. Location and characterization of studied streams. Physicochemical characteristics of the study sites (mean ± SE; *n* = 5–6). For water temperature, daily mean values and its range are shown. Flow values correspond to those obtained during the study period (November 2009–January 2010).

	S1	S2	S3
Latitude	43º 12' 32" N	42º 59' 48" N	42º 59' 59" N
Longitude	3º 16' 03" W	2º 52' 47" W	2º 53' 00" W
Basin (ha)	225	361	357
Altitude (m a.s.l)	315	420	400
Reach slope (%)	20.5	14.2	13.3
Width (m)	3.52	3.27	3.73
Tree canopy cover (%)	42.2	50.5	55.05
Flow (L s^{-1})	72.7 ± 15.5	46.1 ± 10.4	98.2 ± 32.6
Water temperature (ºC)	8.03 (3.20–14.20)	7.24 (2.80–12.10)	7.66 (3.60–12.40)
SRP (µg PL^{-1})	14.4 ± 1.2	16.9 ± 1.3	15.8 ± 0.8
Nitrate (µg NL^{-1})	600.1 ± 59.2	33.1 ± 6.8	35.3 ± 8.3
Nitrite (µg NL^{-1})	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Ammonium (µg NL^{-1})	34.0 ± 5.4	34.1 ± 7.7	32.9 ± 5.7
pH	7.10 ± 0.12	7.45 ± 0.13	7.58 ± 0.08
Alkalinity (meq L^{-1})	0.43 ± 0.04	1.23 ± 0.09	0.69 ± 0.10
Conductivity (µg S cm^{-1})	134.0 ± 7.7	171.2 ± 7.3	120.4 ± 10.2
FPIM (mg L^{-1})	1.22 ± 0.38	0.92 ± 0.21	1.35 ± 0.38
FPOM (mg L^{-1})	1.21 ± 0.15	0.51 ± 0.13	0.50 ± 0.14
% O_2 saturation	103.5 ± 4.2	99.9 ± 3.9	107.2 ± 2.4

needles for each bag were grouped in 5 branches tied with a bridle (ca. 1 g each) in order to avoid whole needle washing out of the bag but without limiting biota access. In each site, five iron bars were anchored to the streambed and one bag of each of five species per bar were fastened with nylon lines (25 bags per site). Five extra bags of each species were used to estimate initial ash free dry mass (AFDM); an additional bar in one of the streams (S2) was used to tie 5 extra bags of each species to determine mass loss by leaching (initial material). Bags were placed between the 23th and the 26th of November. After 24 h, bags for the leaching estimation were collected. The remaining was retrieved at the same time in each stream (6–7 weeks, coinciding with benthic sampling), and transported refrigerated to the laboratory for later processing.

Bag contents were rinsed with filtered (filter: 0.2 mm) stream water on a 0.5

mm sieve and macroinvertebrates were separated from leaf-litter and preserved in 70% ethanol until the ulterior study. Leaf material was oven-dried to obtain dry mass (70 ºC, 72 h); subsamples of each bag were used for nutrient analyses (C and N) and the rest ashed (500 ºC, 4h) to determine remaining AFDM. The leaf nutrient content analyses were carried out on initial material (post-leaching) and incubated material. Leaf carbon and nitrogen were determined using a Perkin Elmer series II CHNS/O elemental analyzer. Results were expressed as percentage of elemental contents (C and N) of leaf-litter dry mass and in molecular elemental ratios (C:N). Associated macroinvertebrates were studied using the same methodologies as used for benthic fauna.

Statistical analyses

The leaf mass losses were transformed into rates considering the incubation period, (M_0 - M_t/t), where M_0 is the initial mass and M_t the remaining mass at time t. As there were slight temperature differences between streams, processing rates were also calculated with degree-days (dd, the sum of mean daily temperatures during the time period considered) in order to remove the effect of temperature on the process. Analyses of differences in breakdown rates, leaf elemental ratios and associated macroinvertebrate variables were performed with two-way ANOVA (stream; leaf type) followed by post-hoc testing with Tukey test (Zar, 2010). Bivariate relationships were tested by ordinary least square linear regressions. When necessary data were transformed (log (x+1)) to obtain requirements for parametric analyses. All statistical analyses were conducted using R statistical program (version 2.11.1; R Development Core Team, 2010).

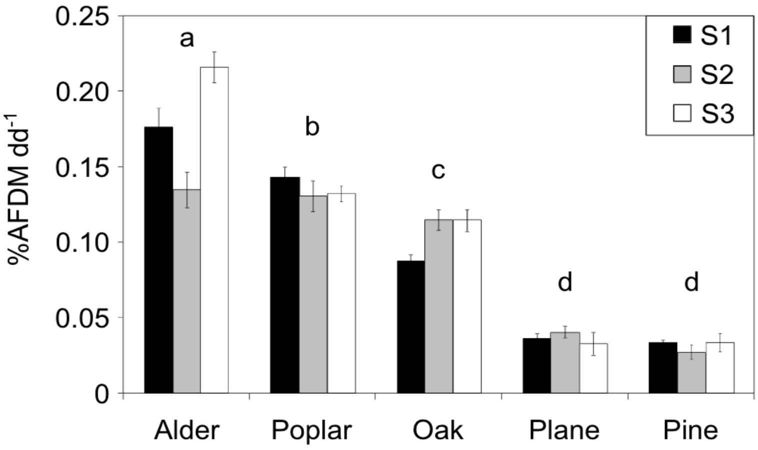
Results

Breakdown rates and resource quality

The breakdown rates differed among leaf types (Fig. 1; ANOVA: $F_{4,60} = 205.57$, $p < 0.001$). Alder leaves presented the fastest rates, followed by poplar, oak and finally plane and pine. Only alder and oak rates presented differences among streams.

The leaves used in this study showed differences in their elemental contents (C and N), C:N ratio and percentage of ash, alder presenting the highest and plane and pine the lowest overall nutritional quality (Table 2).

After in-stream incubation time, in general the % C diminished, especially



◄ **Figure 1.** Breakdown rates (% AFDM dd^{-1}; mean ± SE) of different leaf types. Results of Tukey post-hoc among leaf species are shown.

in oak. The incubated leaves showed differences among leaf types in the % C (Fig. 2; ANOVA: $F_{4,60}$ = 51.08, p < 0.001) being lowest in oak and highest in pine. The leaf-N concentrations after incubation were higher than tinitial ones, particularly on alder, and maintained the initial observed differences among leaf types, alder showing the highest and pine the lowest concentrations (Fig. 2; ANOVA: $F_{4,60}$= 630.09, p < 0.001). The leaf-N increase was more evident in the stream with more dissolved nitrogen availability (S1). However, only oak and pine showed a net nitrogen immobilization (134.1% and 106.1% of initial N, respectively). Thus, after incubation time, leaf types showed differences in C:N ratio among leaves (ANOVA: $F_{4,60}$ = 318.75, p < 0.001; Pine > Plane > Poplar = Oak > Alder) and among streams (Fig. 2; ANOVA: $F_{2,60}$ = 19.41, p < 0.001; S2 = S3 > S1). The ash content increased also in all leaves. The oak presented three times higher values than other species, varying from 10.0% of the initial material to a mean of 35.1% after incubation time (Fig. 2). Breakdown rates were positively correlated with the % N and negatively with C:N ratio of material after incubation time (Fig. 3).

Table 2. Initial percentage of C, N and ash content and molecular ratio C:N of leaf-litter. Letters represent statistical differences (ANOVA and Tukey post-hoc).

Leaf	% C	% N	C:N	% ash content
Alder	51.2^{a}	3.05^{a}	19.66^{c}	4.54^{c}
Poplar	43.9^{c}	0.74^{b}	69.25^{b}	$4.56b^{c}$
Oak	46.0^{bc}	0.79^{b}	68.61^{b}	10.02^{a}
Plane	48.8^{ab}	0.68^{bc}	84.45^{ab}	6.21^{b}
Pine	49.5^{a}	0.59^{c}	99.93^{a}	2.53^{d}

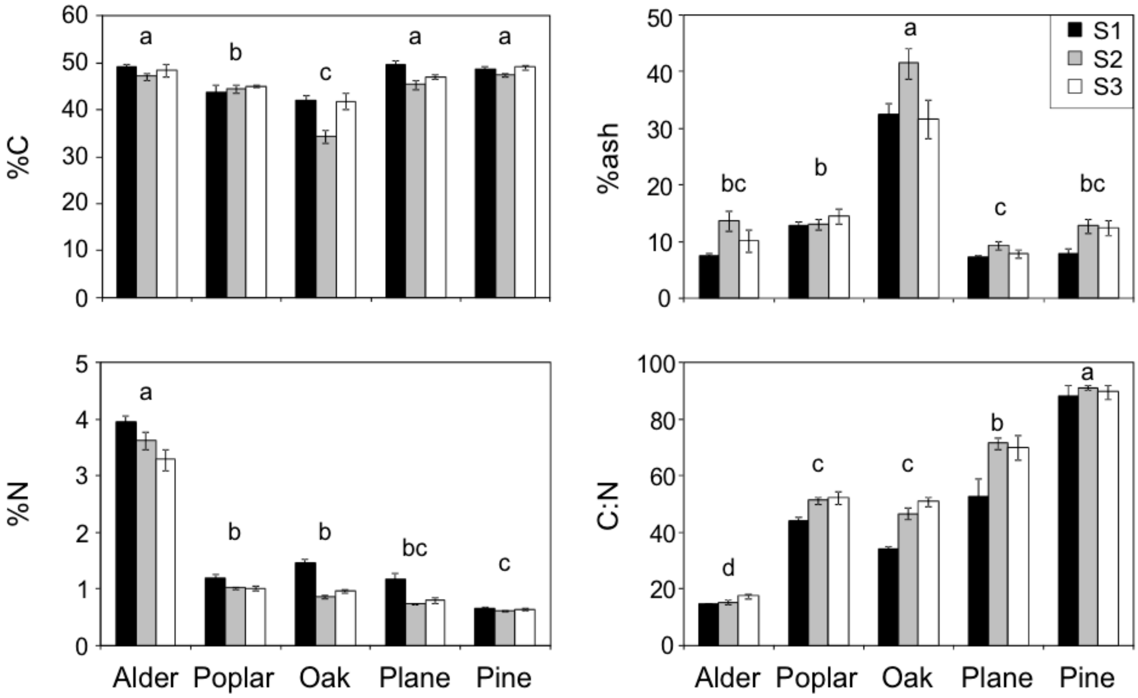


Figure 2. Percentage of C, N and ash content and C:N molecular ratio of leaf-litter after the incubation time in the three streams (mean ± SE). Results of Tukey post-hoc among leaf species are shown.

Benthic and leaf-litter associated macroinvertebrates

Benthic macroinvertebrate communities of the three streams showed similar values of taxa richness and diversity. However, the density and biomass in S1 were much higher than in the other two streams (Table 3). These communities were dominated by shredders and collector-gatherers. Of the 48 benthic taxa only 39 were identified in bags. Nevertheless, the 11 shredder taxa found in the benthic samples were also present in the leaf-litter bags. The average taxa richness per bag of total invertebrates (range 6.6–11.2) and shredders (range 2.4–4.2) was similar among different leaf types and streams. In the same way the diversity of the entire invertebrate assemblages (range 1.87–2.85) and shredders (range 0.79–1.55) did not show great differences. The densities of total macroinvertebrates and shredders showed variability among leaf types (Fig. 4; ANOVA: $F_{4,60} = 4.09$, $p < 0.001$; ANOVA: $F_{4,60} = 9.38$, $p < 0.001$) and streams (ANOVA: $F_{2,60} = 3.29$, $p = 0.044$; ANOVA: $F_{2,60} = 5.94$, $p = 0.004$). In general the bags in S2 were more densely colonized than in the other two streams, although this site presented the lowest number of benthic macroinvertebrates (Table 3). Alder and

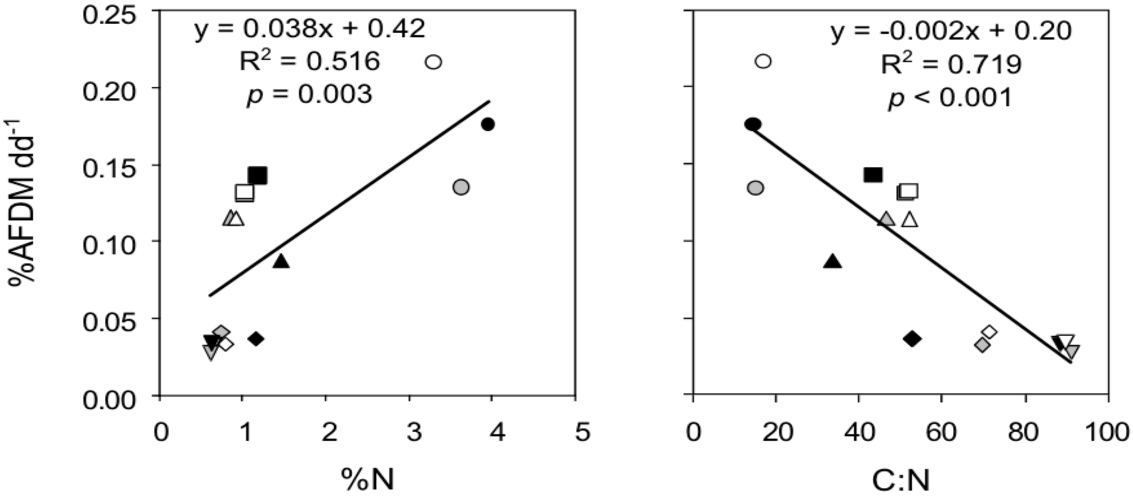


Figure 3. Breakdown rate *vs*. leaf %N or C:N ratio (mean values) after incubation period. Leaves are identified as follows: alder (circle), poplar (square), oak (pyramid), plane (diamond) and pine (inverted pyramid). Streams are identified as follows: S1 black, S2 grey and S3 white.

oak bags were the most densely colonized by total macroinvertebrates and by shredders, pine and plane being the less colonized ones (Fig. 4). There were differences also in terms of biomass of total macroinvertebrates and shredders among leaf types (ANOVA: $F_{4,60}$ = 6.01, p < 0.001; ANOVA: $F_{4,60}$ = 4.41, p = 0.003) and streams (ANOVA: $F_{2,60}$ = 5.82, p = 0.005; ANOVA: $F_{2,60}$ = 6.11, p = 0.004), showing the same trend than in terms of density (Fig. 4). In relation to macroinvertebrate community structure, leaf-litter associated communities showed a higher relative abundance in numbers and in biomass of shredders than benthic ones (Fig. 5). Among leaf types, alder bags showed higher values than plane ones in terms of density (ANOVA: $F_{4,60}$ = 2.75, p = 0.036). Shredders density and biomass were negatively correlated with C:N ratio (Fig. 6) and helped explain significantly the observed breakdown rates (Fig. 7).

Table 3. Benthic macroinvertebrate variables for each site (Jaunary 2010, mean ± SE; n = 5).

	S1	S2	S3
Total richness	23.8 ± 2.1	21.0 ± 2.3	22.2 ± 2.1
Shredders richness	7.0 ± 0.5	5.6 ± 0.6	6.8 ± 0.9
Total diversity	3.11 ± 0.21	3.59 ± 0.13	3.73 ± 0.04
Shredders diversity	1.92 ± 0.12	1.68 ± 0.13	1.88 ± 0.17
Total density m^{-2}	4457.8 ± 703.2	1117.8 ± 109.0	1531.1 ± 355.9
Shredders density m^{-2}	791.1 ± 105.7	360.0 ± 63.4	451.1 ± 171.1
Total biomass mg m^{-2}	1184.2 ± 265.6	345.5 ± 121.9	295.6 ± 91.5
Shredders biomass mg m^{-2}	286.9 ± 85.0	31.7 ± 5.7	87.7 ± 55.5

Discussion

Independently of the stream, breakdown rates differed among leaf species. Despite the slight inter-stream variability in rates of alder and oak, species ranking remained constant in the three streams. Thus, as Leroy & Marks (2006) pointed out, leaf species seems to be more important than stream differences in determining processing rates in the present study. In the same geographical region, Arroita *et al*. (2012) observed that although the breakdown rates of diverse species varied between two streams with different characteristics, the differences among species remained constant.

Based on results from previous studies demonstrating that initial nitrogen concentration and/or C:N ratio determined greatly the processing (e.g. Enríquez *et al*., 1993; Richardson *et al*., 2004; Hladyz *et al*., 2009; Muto *et al*., 2011), the differences in initial nutritional quality on N concentration and C:N ratio among the five leaf types used in our study make us expect the

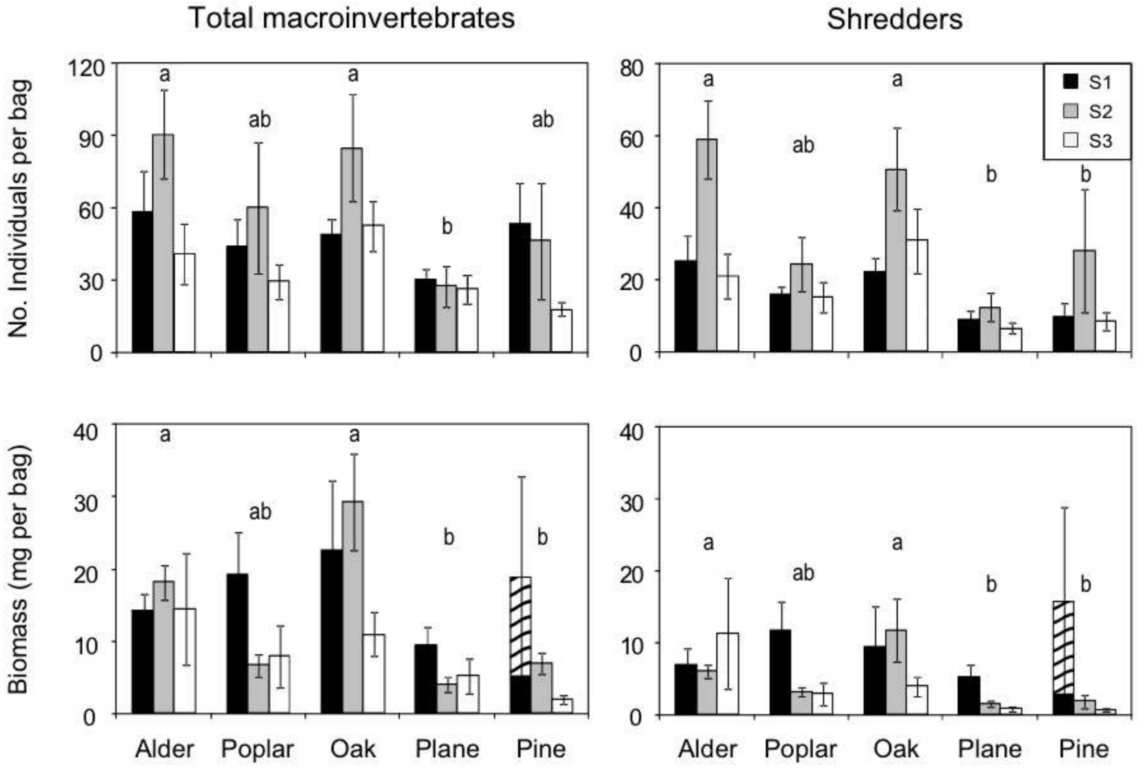
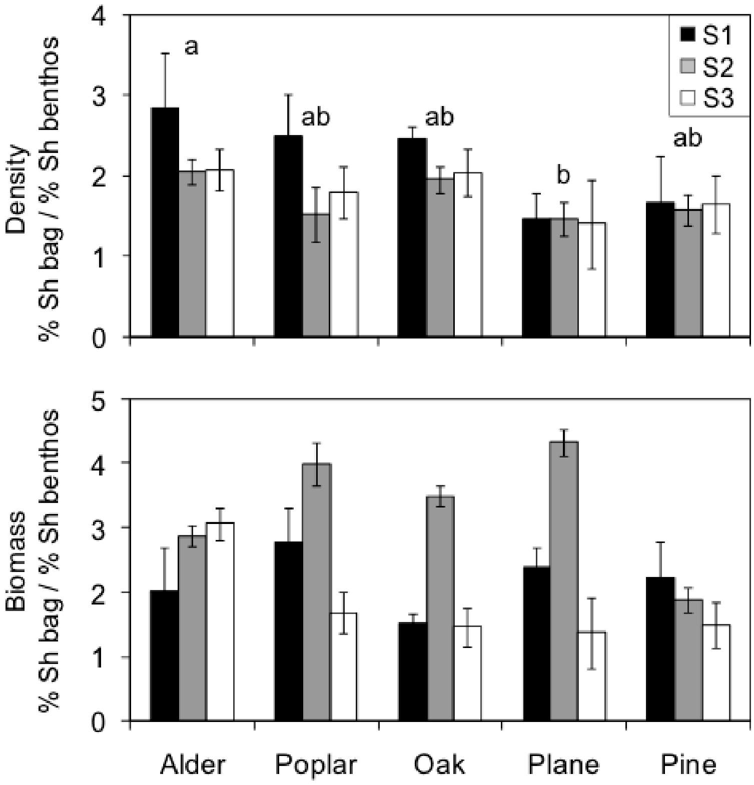


Figure 4. Density and biomass of total macroinvertebrates and shredders per bag (mean ± SE). Results of Tukey post-hoc among leaves are shown. Note the different scales. Biomass in pine bags of stream S1 is very influenced by a single big Tipulidae that appeared in one of the five replicas (cross lined bars). In order to avoid its effect, average biomass value considering the other four replicas is displayed (black bars). Statistical results do not change considering 4 or 5 replicas.



◄ **Figure 5.** Relationships between the relative abundance of shredders in the macroinvertebrate community associated with leaf litter bags and that of benthic shredders in terms of density (top) and biomass (bottom).

observed variability in breakdown rates. Effectively, leaves with higher nitrogen content and/or lower C:N ratio broke down faster than those with lower nitrogen content and/or higher ratio. However, during breakdown, the nutritional quality of leaves often varies, increasing the nitrogen concentration and decreasing the C:N ratio by the middle of the process (Pozo, 1993; Ferreira *et al.*, 2006; Pérez *et al.*, 2012), as we observed in all leaf types. This modification has been often attributed to microbial growth (Pozo *et al.*, 1998; Canhoto & Graça, 2008; Webster *et al.*, 2009), since the microbial decomposers present better nutritional quality than leaves (compare elemental ratios in Cross *et al.*, 2005). In this sense, alder showed the highest absolute increase in nitrogen concentration due probably to a higher fungal colonization since this material presents characteristics that favor microbial colonization such as non-cereous soft cuticles and low concentration of inhibitory compounds (Canhoto & Graça, 1999). However, the relative changes in alder nitrogen concentration were proportionally lower than in the other broadleaf species (poplar, oak and plane) with initial lower concentrations of this nutrient, confirming previous observations (Molinero *et al.*, 1996; Pozo *et al.*, 1998). In pine needles, the absence of remarkable change in nitrogen

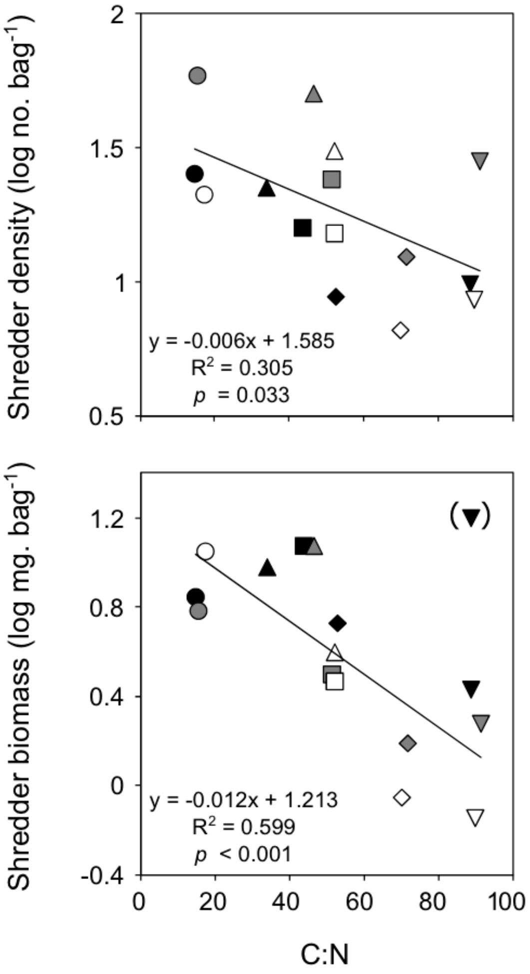


Figure 6. Log. shredder density or biomass in bags *vs*. leaf C:N ratio after incubation period. Leaves are identified as follows: alder (circle), poplar (square), oak (pyramid), plane (diamond) and pine (inverted pyramid). Streams are identified as follows: S1 black, S2 grey and S3 white. Shredder biomass in pine bags of stream S1 is bracketed as it was very influenced by a single big Tipulidae that appeared in one of the five replicas. In order to avoid its effect, average biomass value considering the other four replicas is displayed and used for the regressions.

concentration suggests a lower fungal colonization due to the hard cuticle and antifungal agents present in conifer needles (Bärlocher & Oertli, 1978). Despite these changes in N concentration and C:N ratio during incubation, the relative differences in nutritional quality among species remained, and therefore the relationship between nutritional quality and processing rates persisted. Nevertheless, some leaf specific response of the processing rate was also observed: despite the similar nutritional quality between poplar and oak, decomposition was slower in oak, probably related with the facilitation of the consumer activity (see below). On the contrary, plane and pine showed different nutrient content, but similar rates. Other studies (Gessner & Chauvet, 1994; Quinn *et al.*, 2000; Royer & Minshall, 2001; Lecerf & Chauvet, 2008) did not observe a relationship between breakdown rate and nitrogen content, attributing the variability of processing to other leaf characteristics as toughness, lignin or phosphorous content. Furthermore, although the leaf nitrogen enrichment occurred more notoriously in the stream with the highest dissolved nitrogen (S1), breakdown rates in this stream were not stimulated, contrary to what is usually observed (Greenwood *et al.*, 2007; Suberkropp *et al.*, 2010; Pérez *et al.*, 2012), suggesting that, according to other authors (Ostrofsky, 1997; Ardón *et al.*, 2009; Schindler & Gessner, 2009), rather than only one variable of nutrient

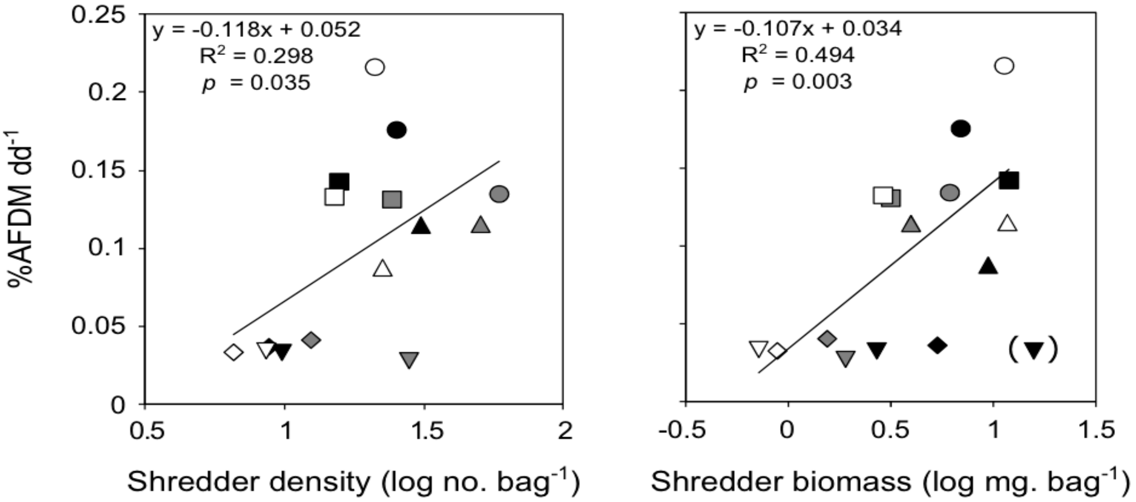


Figure 7. Breakdown rates *vs*. log. shredder density or biomass. Leaves are identified as follows: alder (circle), poplar (square), oak (pyramid), plane (diamond) and pine (inverted pyramid). Streams are identified as follows: S1 black, S2 grey and S3 white. Shredder biomass in pine bags of stream S1 is bracketed as it was very influenced by a single big Tipulidae that appeared in one of the five replicas. In order to avoid its effect, average biomass value considering the other four replicas is displayed and used for the regressions.

content, a combination of diverse factors, the biotic community involved included, could explain better the processing rates among different resources.

The total invertebrate and shredder assemblages colonizing the leaf-litter bags reflected a simplification of the benthic community, but the relative contribution of shredders to macroinvertebrate assemblage was higher in any kind of bag than in the benthos. This concentration of shredders on bags has an inevitable explanation coming from the method itself, i.e. the incubation of litter in bags promotes shredder colonization due to food resource concentration (Tiegs *et al*., 2008). The density and biomass of this feeding group were positively related with leaf-litter quality, as other studies reported (Haapala *et al*., 2001; Lecerf *et al*., 2005; Hladyz *et al*., 2009; Kominoski & Pringle, 2009), and this variability in shredder aggregating (resource selection) helped explain the observed breakdown rates. Nevertheless, the similar density and biomass of invertebrates (total and shredders) between alder and oak was not in accordance with the differences in their nutritional quality and processing rates. It is often unclear if invertebrates found in litter bags are consuming leaves or finding refuge (Hofer & Richardson, 2007). Moreover, as other authors pointed out (Zweig & Rabeni, 2001; Niyogi *et al*., 2003; Rabeni *et al*., 2005), the particle sedimentation, great in oak leaves as ash content indicates, could limit macroinvertebrate activity slowing down leaf processing rates. In any case, the great C:N imbalance between shredders and leaf resource (see Cross *et al*., 2003; 2005; Hladyz *et al*., 2009) might explain the shredder preference for high quality leaf species to supply their stoichiometric demands (Sterner & Elser, 2002; Woodward, 2009). Thus, in streams affected by the replacement of native vegetation by other with lower nutritional quality, the feeding rates, growth, densities and survival of detritivores are often altered (Graça, 2001; Albariño & Balseiro, 2002; Larrañaga *et al*., 2009a; b), and the effects of this alteration could affect higher trophic levels due to the constriction of energy transfer throughout the trophic web (Woodward, 2009).

Our results indicate that the nutritional quality of leaves based on N concentration and C:N ratio are important factors to control breakdown rate in forested streams, although some leaf specific response of the processing rate can be observed.

The abundance of shredders associated with leaf-litter responds to the nutritional quality of leaves and helps explain their breakdown rates. The replacement of forests at watershed or at riparian scale by tree species with lower quality leaf-litter, as plane and pine, could translate in a lower entry of leaf-nutrients to streams, with implications on the basal resources and the associated detritivore assemblage, and the subsequent transference of energy to other functional groups and trophic levels.

References

Abelho M, 2001. From litterfall to breakdown in streams: A review. The Scientific World Journal 1: 656–680.

Abelho M & Graça MAS, 2006. Effects of nutrient enrichment on decomposition and fungal colonization of sweet chestnut leaves in an Iberian stream (Central Portugal). Hydrobiologia 560: 239–247.

Albariño RJ & Balseiro EG, 2002. Leaf litter breakdown in Patagonian streams: native versus exotic trees and the effect of invertebrate size. Aquatic Conservation: Marine and Freshwater Ecosystems 12: 181–192.

Alonso A, Gozález-Muñoz N & Castro-Díez P, 2010. Comparison of leaf decomposition and macroinvertebrate colonization between exotic and native trees in a freshwater ecosystem. Ecological research 25: 647–653.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation: Washington, DC; 1368 pp.

Ardón M, Pringle CM & Eggert SL, 2009. Does leaf chemistry differentially affect breakdown in tropical versus temperate streams? Importance of standardized analytical techniques to measure leaf chemistry. Journal of North American Benthological Society 28: 440–453.

Arroita M, Aristi I, Flores L, Larrañaga A, Díez J, Mora J, Romaní AM & Elosegi A, 2012. The use of wooden sticks to assess stream ecosystem functioning: Comparison with leaf breakdown rates. Science of the Total Environment 440: 115–122.

Bärlocher F & Oertli JJ, 1978. Inhibitors of aquatic hyphomycetes in dead conifer needles. Archiv für Hydrobiologie 81, 462–474.

Bärlocher F, 1992. The Ecology of Aquatic Hyphomycetes. Springer: Berlin, Germany.

Canhoto C & Graça MAS, 1999. Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. Microbial ecology 37: 163–172.

Canhoto C & Graça MAS, 2008. Interactions between fungi and stream invertebrates: back to the future. In: Novel Techniques and Ideas in Mycology (eds. Sridhar S, Bärlocher F & Hyde KD). Fungal Diversity Research Series 20: 205–325.

Cross WF, Benstead JP, Frost PC & Thomas SA, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50: 1895–1912.

Cross WF, Benstead JP, Rosemond AD & Wallace JB, 2003. Consumer–resource stoichiometry in detritusbased streams. Ecology Letters 6: 721–732.

Dang CK, Gessner MO & Chauvet E, 2007. Influence of conidial traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. Mycologia 99: 24–32.

Enríquez S, Duarte CM & Sand-Jensen K, 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94: 457–471.

Ferreira V, Elosegi A, Gulis V, Pozo J & Graça MAS, 2006. Eucalyptus plantations affect fungal communities associated with leaf-litter decomposition in Iberian streams. Archiv fur Hydrobiologie 166: 467–491.

Gessner MO & Chauvet E, 1994. Importance of stream microfungi in controlling breakdown rates of leaf-litter. Ecology 75:1807–1817.

Graça MAS, 2001. The role of invertebrates on leaf litter decomposition in streams – A review. International Review of Hydrobiology 86: 383–393.

Graça MAS & Cressa C, 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. International Review of Hydrobiology 95: 27–41.

Greenwood JL, Rosemond AD, Wallace JB, Cross WF & Weyers HS, 2007. Nutrients stimulate leaf breakdown rates and detritivores biomass: bottom-up effects via heterotrophic pathways. Oecologia 151: 637–649.

Haapala A, Muotka T & Markkola A, 2001. Breakdown and macroinvertebrate and fungal colonization of alder, birch, and willow leaves in a boreal forest stream. Journal of the North American Benthological Society 20: 395–407.

Hladyz S, Gessner MO, Giller PS, Pozo J & Woodward G, 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. Freshwater Biology 54: 957–970.

Hofer N & Richardson JS, 2007. Comparisons of the colonisation by invertebrates of three species of wood, alder leaves, and plastic “leaves” in a temperate stream. International Review of Hydrobiology 6: 647–55.

Kearns SG & Bärlocher F, 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. Fungal Ecology 1: 13–18.

Kominoski JS & Pringle CM, 2009. Resource–consumer diversity: testing the effects of leaf litter species diversity on stream macroinvertebrate communities. Freshwater Biology 54: 1461–1473.

Larrañaga A, Basaguren A & Pozo J, 2009a. Impacts of Eucalyptus globulus plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. International Review of Hydrobiology 94: 497–511.

Larrañaga A, Basaguren A, Elosegi A and Pozo J, 2009b. Impacts of Eucalyptus globulus plantations on Atlantic streams: changes in invertebrate density and shredder traits. Fundamental and Applied Limnology 175: 151–160.

Lecerf A, Dobson M, Dang CK & Chauvet E, 2005. Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. Oecologia 146: 432–442.

Lecerf A & Chauvet E. 2008. Intra-specific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic and Applied Ecology 9: 598–607.

Leroy CJ & Marks JC, 2006. Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshwater Biology 51: 605–617.

Merritt RW & Cummins KW, 2007. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company: Dubuque; 1158 pp.

Molinero J, Pozo J & González E, 1996. Litter breakdown in streams of the Agüera catchment: influence of dissolved nutrients and land use. Freshwater Biology 36: 745–756.

Moore JC, Berlow EL, Coleman DC, de Ruiter PC, Dong Q, Hastings A, Collins Johnson N, MacCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ & Wall DH. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7: 584–600.

Muto EA, Kreutzweiser DP & Sibley PK, 2011. Over-winter decomposition and associated macroinvertebrate communities of three deciduous leaf species in forest streams on the Canadian Boreal Shield. Hydrobiologia 658: 111–126.

Niyogi, DK, Simon KS & Townsend CR, 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. Freshwater Biology 48: 1698–1708.

Ostrofsky ML, 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. Journal of the North American Benthological Society 16: 750–759.

Pérez J, Descals E & Pozo J, 2012. Aquatic hyphomycete communities associated with decomposing alder leaf litter in reference headwater streams of the Basque Country (northern Spain). Microbial Ecology 64: 279–290.

Pozo J, 1993. Leaf litter processing of alder and eucalyptus in the Agüera stream system (North Spain) I.Chemical changes. Archiv für Hydrobiologie 127: 299–317.

Pozo J, Basaguren A, Elosegi A, Molinero J, Fabre E & Chauvet E, 1998. Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. – Hydrobiologia 374: 101–109.

Quinn JM, Burrell GP & Prkyn SM, 2000. Influences of leaf toughness and nitrogen content on in-stream processing and nutrient uptake by litter in a Waikato, New Zealand, pasture stream and streamside channels. New Zealand Journal of Marine and Freshwater Research 34: 253–271.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rabeni CF, Doisy KE & Zweig LD, 2005. Stream invertebrate community functional responses to deposited sediment. Aquatic Sciences 67: 395–402.

Richardson JS, Shaughnessy CR & Harrison PG, 2004. Litter breakdown and invertebrate association with three types of leaves in a temperate rainforest stream. Archiv Fur Hydrobiologie 159: 309–325.

Royer TV & Minshall GW, 2001. Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. Freshwater Biology 46: 603–610.

Schindler MH & Gessner MO, 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology 90: 1641–1649.

Sterner RW & Elser JJ, 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, NJ.

Suberkropp K & Chauvet E, 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433–1445.

Suberkropp K, Gulis V, Rosemond AD & Benstead JP, 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. Limnology and Oceanography 55: 149–160.

Swan CM & Palmer MA, 2006. Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. Oecologia 147: 469–478.

Tachet H, Richoux P, Bournaud M & Usseglio-Polatera P, 2002. Invertébrés d'eau douce: systématique, biologie et écologie. CNRS: Paris; 587 pp.

Tibbets TM & Molles MC, 2005. C:N:P stoichiometry of dominant riparian trees and arthropods along the Middle Rio Grande. Freshwater Biology 50: 1882–1894.

Tiegs SD, Peter FD, Robinson CT, Uehlinger U & Gessner MO, 2008. Leaf decomposition and invertebrate colonization responses to manipulated litter quantity in streams. Journal of the North American Benthological Society 27: 321–331.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277: 102–104.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1999. Effects of resource limitation on a detrital-based ecosystem. Ecological Monographs 69: 409–442.

Webster JR & Benfield EF, 1986. Vascular plant breakdown in fresh-water ecosystems. Annual Review of Ecology and Systematics 17: 567–594.

Webster JR, Benfield EF, Ehrman TP, Schaeffer MA, Tank JL, Hutchens JJ, D’Angelo DJ. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. Freshwater Biology 41: 687–705.

Webster JR, Newbold JD, Thomas SA, Valett HM & Mulholland PJ, 2009. Nutrient uptake and mineralization during leaf decay in streams – a model simulation. International Review of Hydrobiology 94: 372–390.

Woodward G, 2009. Biodiversity, ecosystem functioning and freshwater food webs: assembling the jigsaw puzzle. Freshwater Biology 54: 2171–2187.

Zar JH, 2010. Biostatistical Analysis. 5th ed. Prentice Hall: Upper Saddle River.

Zweig LD & Rabeni CF, 2001. Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. Journal of the North American Benthological Society 20: 643–657.

Land-use changes

Chapter III

Effects of pine plantations on structural and functional attributes of forested streams

Abstract

In low-order forested streams, watershed-scale land-use modifications in vegetation can affect energy inputs into streams and trophic interactions within these donor-controlled food webs. We examined the effects of *Pinus radiata* D. Don plantations on structural (benthic macroinvertebrate communities) and functional (alder and pine leaf-litter breakdown) attributes of headwater streams. We compared three streams draining pine plantations with three draining natural deciduous forests. We found differences in the structure of benthic macroinvertebrate communities, namely lower densities of shredders in streams flowing through pine plantations. Breakdown rate of alder (*Alnus glutinosa* (L.) Gaertner) leaves was lower in streams draining pine plantations, and the breakdown of pine (*P. radiata*) needles, a much lower quality material, was slower than that of alder and did not differ between stream types. Although fungal activity was similar in all streams, lower detritivore densities in streams under pine plantations could explain the lower rate of alder breakdown in this environment. The replacement of native deciduous forests by pine monocultures reduces the overall nutritional quality of terrestrial inputs and reduces energy transfer through the detrital pathway.

Key words: *Alnus glutinosa*, Fungal colonization, Leaf-litter decomposition, Macroinvertebrates, *Pinus radiata*.

Introduction

Natural terrestrial ecosystems are altered by human activities encompassing broad-scale land-use changes related to natural resource acquisition (forestry, agriculture and mining) or urban and industrial development (settlements and transport infrastructures). These alterations can have ecological impacts on running waters (Zhang *et al.*, 2009; Magbanua *et al.*, 2010). In low-order forested streams, modifications in watershed vegetation can alter the quantity, quality and seasonality of leaf-litter inputs (Pozo *et al.*, 1997), affecting the feeding rates, growth, densities and survival of detritivores (Graça, 2001; Albariño & Balseiro, 2002; Campos & González, 2009; Larrañaga *et al.*, 2009a; b). As a consequence, important ecosystem processes, such as leaf-litter decomposition, can be affected (Lecerf & Richardson, 2010; Hladyz *et al.*, 2011).

Replacement of temperate deciduous forests with monocultures of exotic evergreen species is among the most important land-use modifications in the Atlantic region of the Iberian Peninsula, with *Eucalyptus globulus* Labill. and *P. radiata* being two of the most commonly planted species (Spanish Ministry of Agriculture, Food and Environment: www.magrama.gob.es). The effects of *E. globulus* plantations on stream ecosystems have been widely studied, revealing their impact on fluvial processes and macroinvertebrate communities (e.g. Pozo *et al*., 1997; Larrañaga *et al*., 2006; Larrañaga *et al*., 2009a; b). In contrast, as Riipinen *et al*. (2010) pointed out, studies on the effects of conifers, planted or natural, on both structural and functional attributes of streams are scarce (but see Whiles & Wallace, 1997; Goodman *et al*., 2006; Riipinen *et al*., 2010; Kominoski *et al*., 2011). Moreover, despite the extremely high cover of *P. radiata* monocultures in some parts of the northern Iberian Peninsula (e.g., 62% of the forested surface in the province of Biscay, www.bizkaia.net), their effects on streams are unknown. *P. radiata* litter is considered a poor quality resource for microbial decomposers and invertebrate detritivores due to its toughness, low nitrogen concentration and elevated levels of lignin and polyphenols (Bärlocher & Oertli, 1978; Girisha *et al*., 2003). Deciduous oak (*Quercus* sp.) and beech (*Fagus sylvatica* L.) forests, however, yield higher quality detrital inputs into streams (Girisha *et al*., 2003; Lecerf *et al*., 2007), especially when nitrogen-rich alder (e.g. *A. glutinosa*; Hladyz *et al*., 2009) dominates the riparian zone. However, the natural spread of alder is constrained or even completely absent when watersheds are subjected to plantations.

The objective of the present study was to determine the effects of pine plantations on the structure of stream macroinvertebrate communities and ecosystem functioning (i.e., breakdown of *A. glutinosa* leaves and *P. radiata* needles). We hypothesized that streams flowing through pine plantations 1) would have lower densities of benthic macroinvertebrates (specifically shredders) due to the impoverishment of the detrital resources, 2) would show lower breakdown rates of a high-quality leaf-litter due to the lower activity of decomposers and detritivores, and 3) would present similar or faster breakdown rates of *P. radiata* needles than those in streams under deciduous forests, as the communities would have adapted to the consumption of pine needles.

Materials and methods

Study sites

This study was conducted in six low-order (2nd–3rd) streams with siliceous substrata flowing into the Atlantic Ocean (Cordillera Cantábrica, Northern Spain). Three of these streams drain native deciduous forests (deciduous sites, D), *Q. robur* L. and *F. sylvatica* being the dominant tree species; the other three drain mature *P. radiata* pine plantations (pine sites, P). Anthropic impacts in the basins, apart from the pine plantations, are negligible (Table 1). The riparian forest and streambed substrate were described in a 50 m-long reach in each stream. Riparian tree species were identified and densities estimated in five 10 m^2 areas along both stream margins (100 m^2 in total). At streams under pine plantations, the riparian conditions permit to appear a narrow strip of native deciduous trees with some scattered pines (mean tree density of 0.46 trees m^{-2}), which abruptly becomes a densely planted pine monoculture further from the stream. Moreover, the catchments dominated by pine plantations did not reveal recent clear-cuttings or other works done by heavy machinery. At deciduous sites, there was a mean density of 0.34 native trees m^{-2} in their riparian forest (Table 1), followed by mature deciduous forest. The granulometric composition of the streambed was estimated visually and three categories incorporating Wentworth grain size classes (Allan & Castillo, 2007) were used: percentage of ‘boulders’ (>256 mm), ‘cobbles’ (64–256 mm) and ‘gravel-sand’ (<64 mm) (Table 1).

Water quality

Water parameters were monitored during the study period at each site (November 2009 to April 2010). Water temperature was continuously measured (every hour) using SmartButton temperature data loggers (ACR Systems Inc., Surrey, BC, Canada). Every site was visited 8–9 times and conductivity, pH, dissolved oxygen (WTW multiparametric sensor) and river flow (Martin Marten Z30, Current Meter) were measured. Water samples were collected and filtered in the laboratory (preweighed 0.7 µm pore size glass fiber filters, Whatman GF/F) on each occasion. An aliquot of the filtered water was used to determine alkalinity by titration to an end pH of 4.5 (APHA, 2005) and the remainder was stored (-20 ºC) for later analyses. The filters were oven-dried (70 ºC, 72 h), burned (500 ºC, 12 h) and weighed to obtain fine particulate matter (FPM) and fine particulate inorganic matter (FPIM). Fine particulate organic matter (FPOM) was estimated by the difference between the two weights. Nutrient analyses of the water were conducted using capillary ion electrophoresis for nitrate (Agilent CE), the manual salicylate method for ammonium, the sulphanilamide method for nitrite and the molybdate method for

Table 1. Location and characterization of catchment area of studied streams. Physicochemical characteristics of the study sites (mean ± SE; *n* = 8–9). For water temperature, daily mean values and its range are shown (*n* = 147). Flow values correspond to those obtained during the study period (November 2009–April 2010).

	D1	D2	D3	P1	P2	P3
Latitude	43º 12' 32'' N	42º 59' 48'' N	42º 59' 59'' N	43º 05' 39'' N	43º 56' 49'' N	43º 05' 57'' N
Longitude	3º 16' 3'' W	2º 52' 47'' W	2º 53' 00'' W	2º 53' 54'' W	2º 59' 49'' W	2º 55' 15'' W
Basin (Ha)	225	361	357	137	220	404
Altitude (m)	315	420	400	251	190	225
Reach slope (%)	20.5	14.2	13.3	19.1	16.0	12.0
Width (m)	3.52	3.27	3.73	3.23	3.63	4.52
Land-use (%)						
Native vegetation	99.7	97.4	97.8	3.4	3.2	33.0
P. radiata plantations	0.3	2.6	2.2	88.1	96.3	65.3
Other plantations	0.0	0.0	0.0	4.2	0.0	0.0
Farming	0.0	0.0	0.0	4.3	0.5	1.7
Tree canopy cover (%)	42.2	50.5	55.05	45.5	62.8	48.5
Flow (Ls^{-1})	72.7 ± 15.5	46.1 ± 10.4	98.2 ± 32.6	23.6 ± 4.4	20.3 ± 7.5	75.6 ± 25.1
Water temperature (ºC)	8.03 (3.20–14.20)	7.24 (2.80–12.10)	7.66 (3.60–12.40)	8.34 (4.20–11.10)	7.81 (3.80–13.30)	8.49 (4.10–13.50)
SRP (µg PL^{-1})	14.4 ± 1.2	16.9 ± 1.3	15.8 ± 0.8	14.0 ± 0.8	15.7 ± 0.9	14.6 ± 1.5
NO_3 (µg NL^{-1})	600.1 ± 59.2	33.1 ± 6.8	35.3 ± 8.3	596.1 ± 57.8	282.0 ± 33.9	257.4 ± 48.4
NO_2 (µg NL^{-1})	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.5	0.8 ± 0.1	0.9 ± 0.1
NH_4 (µg NL^{-1})	34.0 ± 5.4	34.1 ± 7.7	32.9 ± 5.7	28.5 ± 4.3	36.8 ± 5.1	35.4 ± 5.3
pH	7.10 ± 0.12	7.45 ± 0.13	7.58 ± 0.08	6.97 ± 0.11	7.18 ± 0.13	7.15 ± 0.09
Alkalinity (meq L^{-1})	0.43 ± 0.04	1.23 ± 0.09	0.69 ± 0.10	0.22 ± 0.04	0.53 ± 0.06	0.40 ± 0.07
Conductivity (µg S cm^{-1})	134.0 ± 7.7	171.2 ± 7.3	120.4 ± 10.2	76.8 ± 3.0	156.0 ± 9.7	111.7 ± 7.3
FPIM (mg L^{-1})	1.22 ± 0.38	0.92 ± 0.21	1.35 ± 0.38	3.80 ± 2.41	4.12 ± 0.89	2.10 ± 0.34
FPOM (mg L^{-1})	1.21 ± 0.15	0.51 ± 0.13	0.50 ± 0.14	1.30 ± 0.52	1.18 ± 0.15	1.13 ± 0.2
% O_2 Saturation	103.5 ± 4.2	99.9 ± 3.9	107.2 ± 2.4	101.2 ± 0.9	95.3 ± 2.6	98.1 ± 2.8
Riparian forest						
Tree density (no. m^{-2})	0.61	0.23	0.18	0.43	0.33	0.64
Tree richness	5	6	2	6	5	6
% pine	0	0	0	7	27	2
Granulometric composition (%)						
>256 mm	85.8	66.5	55.0	78.3	49.3	86.7
64–256 mm	10.8	12.7	13.4	15.3	20.8	9.2
<64 mm	3.4	20.8	31.6	6.4	29.9	4.1

soluble reactive phosphorus (SRP) (APHA, 2005).

Structural attributes: benthic standing stock and macroinvertebrate communities

In January 2010, five benthic samples (Surber 0.09 m^2, 0.5 mm mesh size) were taken from randomly chosen riffles at each study site. Very coarse particulate organic matter was separated from macroinvertebrates on an 8-mm sieve. This sieve enabled *in situ* separation of the fauna from the organic matter in the same sample, facilitating further consumer–resource correlations. The rest of the sample was preserved in 70% ethanol. The organic matter was subsequently sorted in the laboratory into deciduous leaves, pine needles and the remainder; each group was oven-dried and burned to determine its ash-free dry mass (AFDM). Macroinvertebrates were identified to family level (Oligochaeta to order, Chironomidae to tribe) according to Tachet *et al.* (2002), and then counted and sorted into functional feeding groups (FFG) as follows (after Tachet *et al.*, 2002; Merritt & Cummins, 2007): shredders (Sh), collector-gatherers (CG), collector-gatherer-scrapers (CGS), collector-filterers (CF), scrapers (Sc) and predators (P). Each FFG was oven-dried to determine its dry mass. For each benthic macroinvertebrate sample we calculated the taxa richness and Shannon diversity index.

Functional attributes: organic matter processing and associated biota

In October–November 2009 we collected alder leaves from the forest floor immediately after natural abscission and pine needles from dry branches. This material was air-dried and approximately 5 g (± 0.25) of alder leaves or 4 g (± 0.25) of pine needles were placed into coarse mesh bags (15 × 20 cm, 5-mm mesh size). Needles were grouped into four bunches and tied with plastic ties in order to prevent whole needles washing out of the bag. At each site, five iron bars were anchored to the streambed and five bags of each species were fastened to each bar with nylon lines (50 bags in total per site). Five extra bags were used to estimate initial AFDM; an additional set of five bags was incubated in one of the streams to determine loss by leaching. Bags were placed in position between the 23rd and the 26th of November. Bags that were to be used to estimate leaching loss were collected after 24 h. Sets of five bags were retrieved after 7 days (t7) and, thereafter, when losses roughly corresponded to 20% (t20), 35% (t35), 50% (t50) and 70% (t70) of the initial mass (corrected for leaching) for each species, which on average corresponded to 15, 28, 50 and 69 days for alder leaves, and to 32, 50, 105 and 147 days for pine needles. Leaf remnants were rinsed with filtered (0.2-mm sieve) stream water on a 0.5-mm sieve and oven-dried to determine dry mass (70 ºC, 72 h); subsamples of each bag were used for elemental analyses (C, N, P) and the remainder was ashed (500 ºC, 4 h) to estimate the remaining AFDM. Leaf carbon and nitrogen were determined using a Perkin Elmer series II CHNS/O elemental analyser (Perkin Elmer, Norwalk, Connecticut, USA), and phosphorus concentration was measured using a spectrophotometer after mixed acid digestion (molybdenum blue method; Allen *et al*., 1974). The results were expressed both as a percentage of leaf-litter dry mass and as molecular elemental ratios (C:N, C:P and N:P).

At t20 (2–4 weeks of incubation), sporulation rates on alder were estimated from five leaf discs (19.7–32.7 mg) that were punched out with a cork borer (12 mm diameter) from five different leaves from each bag. Sporulation rates on pine needles were estimated from five needles (28.4–168.7 mg). Discs and needles were incubated with 25 ml of filtered stream water (0.7 µm, Whatman GF/F) on a shaker (60 rpm, 10 ºC, 48 h). The resulting conidial suspensions were fixed with 2 ml of formalin (37%) and pre-stained with two drops of trypan blue in lactic acid (0.05%). An aliquot of the suspension was filtered (Millipore SMWP 5 µm pore size) and filters were stained again to ease identification and counting of spores under the compound microscope. Leaf disc and needle dry mass was determined as above. Sporulation rates were expressed as the number of conidia per mg of dry mass and days of incubation (no. mg^{-1} day^{-1}).

At the same time as benthic sampling (approximately day 50), macroinvertebrates from alder and pine bags were sorted out on a 0.5-mm sieve and preserved in 70% ethanol. They were identified, counted and assigned to functional feeding groups, as for benthic invertebrates. Dry mass was determined for shredders and all macroinvertebrates combined.

Statistical analyses

Physicochemical characteristics of water and structural metrics (density, biomass, richness and diversity) of total benthic invertebrates and distributed in FFG were compared using a two-way mixed-model ANOVA (fixed factor ‘land use’; random factor ‘stream’). In the comparison of water temperature daily mean temperature as a replicate was considered (n = 147 days). Although breakdown rate was estimated both by a linear ($M_t = M_o – b \cdot t$; where b is loss rate, M_t remaining % AFDM at time t and M_o is the initial % AFDM after correcting for leaching) and an exponential model ($M_t = M_o \cdot e^{-kt}$ where k is the exponential loss rate), the correction of data for the initial leaching made linear rates fit better and only these are presented herein. Degree-days were used rather than time in order to standardize the rates in view of slight differences in water temperature. For comparison purposes we constructed a linear-exponential rate conversion equation for alder ($k = 0.033 \cdot b - 0.002$, $r^2 = 0.93$, $P = 0.002$) and for pine needles ($k = 0.018 \cdot b - 0.0002$, $r^2 = 0.96$, $P < 0.001$). A three-way mixed-model ANCOVA (fixed factor ‘land use’ and ‘leaf type’; random factor ‘stream’), with degree-days as the covariate, was used to compare the decay rates of alder and pine. Leaf nutrient content was compared using a three-way mixed-model ANOVA (stream, land-use and sampling date) for each leaf type, with post-hoc testing using the Tukey HSD test (Zar, 2010). Fungal sporulation rates, hyphomycetes richness, hyphomycetes diversity and metrics of associated macroinvertebrates (total and distributed in FFG) were compared using three-way mixed-model ANOVA (fixed factor ‘land use’ and ‘leaf type’; random factor ‘stream’). Bivariate relationships were tested by ordinary least square linear regressions. When necessary data were log-transformed and all mixed model parameters were estimated by means of restricted maximum likelihood. All statistical analyses were conducted using the R statistical program (version 2.11.1; R Development Core Team, 2010).

Results

Water quality

All streams contained well-oxygenated water with a neutral pH, low temperature and low nutrient levels (Table 1) and no one of the physico-chemical characteristics differed significantly between deciduous and pine streams.

Structural attributes: benthic organic matter standing stock and macroinvertebrate communities

The amount of total coarse particulate organic matter (CPOM) was on average 68.6 g m^{-2} in deciduous streams and 40.8

g m^{-2} in pine streams, although the large variation precluded finding statistically significant differences between stream types. Deciduous leaf-litter accounted for 81.8% of the total benthic organic matter in the deciduous streams, together with 0.1% of pine needles and 18.1% of other detritus. In pine streams, deciduous leaves accounted for 46.0% of the organic matter, pine needles for 24.5%, and other detritus for 29.5%.

A total of 56 taxa of macroinvertebrates were identified from the benthos of all streams: 48 taxa in deciduous and 50 in pine streams. The average taxa richness was 22.3 in deciduous streams and 20 in pine streams. Taxa richness and the Shannon diversity index for the entire macroinvertebrate community did not differ significantly between deciduous and pine streams. Among the FFG, shredders presented lower richness (ANOVA: $F_{1,4}$ = 10.28; p = 0.033) and diversity (ANOVA: $F_{1,4}$ = 9.75; p = 0.035) in pine streams.

Although the total benthic macroinvertebrate abundance was 27.7% lower in pine than in deciduous streams, the difference was not statistically significant (Fig. 1). However, shredder density was lower in pine streams (58.5% lower; ANOVA: $F_{1,4}$ = 9.49; p = 0.037; Fig. 1). This change in absolute density reduced the relative contribution of shredders to the community (45.9% lower), close to reach statistical differences (ANOVA: $F_{1,4}$ = 5.84; p = 0.073). Among the 16 most common taxa (those representing >1% of the total density, and comprising collectively 89.3% of the total invertebrates on average) the shredder Leuctridae and the predator Chloroperlidae presented lower density in pine streams (60.4% lower, ANOVA: $F_{1.4}$ = 9.58, p = 0.036; and 82.7% lower, ANOVA: $F_{1.4}$ = 20.94, p =

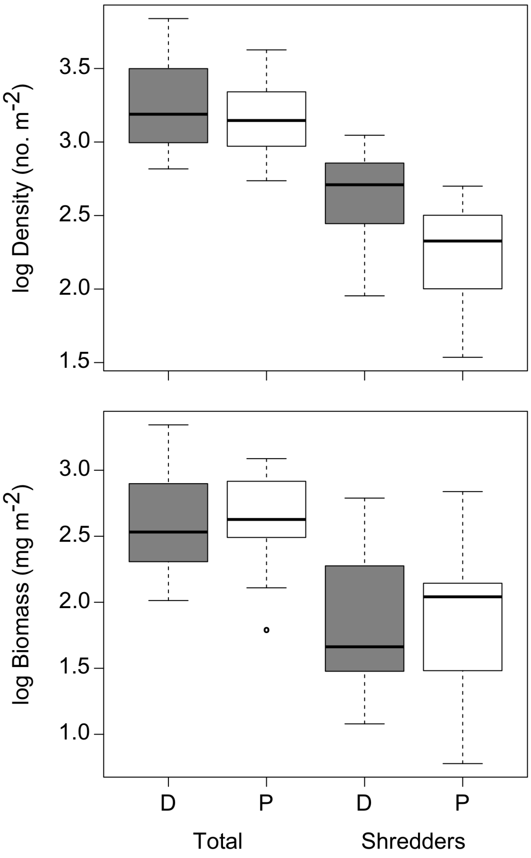


Figure1. Density (top) and biomass (bottom) of total benthic macroinvertebrates and shredders in deciduous (D, filled boxes) and pine (P, open boxes) streams. Dark bars represent the median and the boxes denote the interquartile range.

0.010, respectively). Although the density of shredders Nemouridae and Sericostomatidae and the predator Athericidae was 49.5%, 88.5% and 63.8% lower in pine streams, did not reach statistical differences. In terms of biomass, the differences were less noticeable (10% lower total macroinvertebrate biomass in pine streams, Fig. 1) and were not statistically different, even when distributing taxa into FFG.

Functional attributes: organic matter processing and associated biota

Alder leaves initially (pre-leaching) contained 50.8% C, 2.93% N and 0.057% P. The average mass loss associated with leaching (after 24 h of incubation) was 14.3%. The post-leaching material contained 51.2% C, 3.05% N and 0.057% P. Alder breakdown was significantly slower in pine than in deciduous streams (ANCOVA: $F_{1,139} = 7.79$; $p = 0.006$) and an average reduction of 21.3% in the linear rates was observed (Fig. 2). Pine needles initially (pre-leaching) contained 50.9% C, 0.84% N and 0.014% P. Leaching averaged 6.8% of the initial mass. The post-leaching material contained 49.5% C, 0.59% N and 0.013% P, i.e. it was much poorer in nutrients than alder. The breakdown rate of pine needles (0.035% AFDM dd^{-1}) was 76.4% slower than that of alder (0.148% AFDM dd^{-1}) (ANCOVA: $F_{1,272} = 418.85$; $p < 0.001$) and did not differ between pine and deciduous sites (ANCOVA: $F_{1,129} = 1.69$; $p = 0.196$). No physicochemical variable was correlated with the breakdown rates of alder and pine.

The nutrient content of alder leaves changed throughout breakdown, showing the highest nitrogen concentrations at intermediate phases of the process, increasing from 3.05% (C:N = 19.66) after leaching to 3.78% (C:N = 15.23) on average after 45–53 days of incubation (ANOVA: $F_{4,132} = 3.99$; $p = 0.004$). The initial post-leaching phosphorus content (0.057%) showed a significant reduction to 0.033% by the end of the study (ANOVA: $F_{4,128} = 5.02$; $p < 0.001$), leading to increments in the C:P ratio (ANOVA: $F_{4,128} = 2.13$; $p = 0.081$) and N:P ratio (ANOVA: $F_{4,128} = 2.58$; $p = 0.040$) over time. No differences in nutrient content were observed in alder leaves between deciduous and pine streams. Pine needles underwent changes in nitrogen and phosphorus content during incubation ($\text{ANOVA}_{N\%}$: $F_{4,125} = 9.83$; p

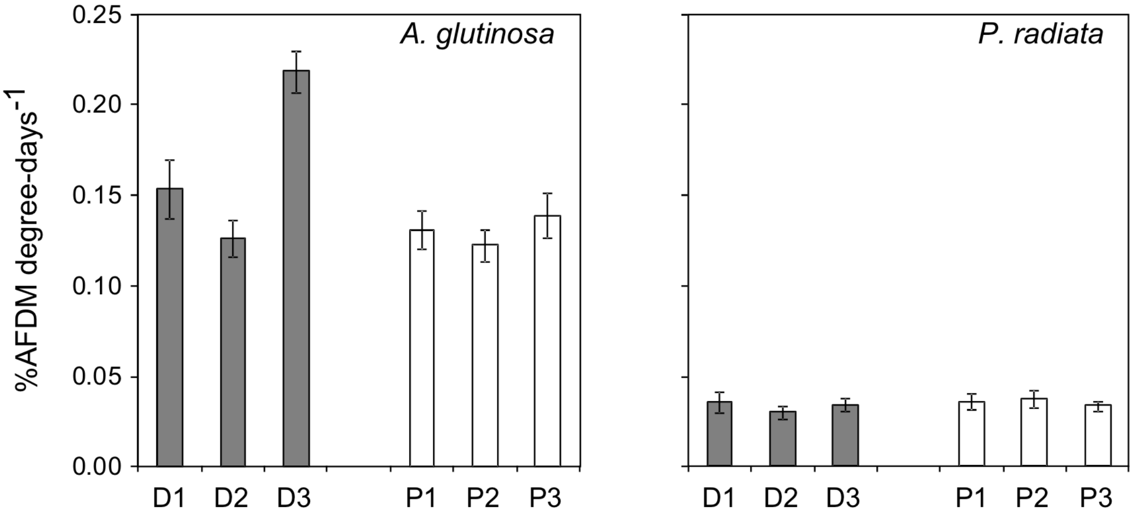


Figure 2. Breakdown rates (% AFDM dd^{-1}; Mean ± SE) of alder leaves and pine needles in deciduous (D, filled bars) and in pine streams (P, open bars).

< 0.001; $ANOVA_{P\%}$: $F_{4,120} = 3.24$; $p = 0.017$), but without any clear temporal dynamics or differences between deciduous and pine sites.

Fungal sporulation rates on alder leaves ranged from 1755 conidia mg $leaf^{-1}$ d^{-1} at site D2 to 7238 conidia mg $leaf^{-1}$ d^{-1} at site D1 and did not differ significantly between deciduous and pine sites (Fig. 3; ANOVA: $F_{1,4} = 0.01$; $p = 0.918$). *Flagellospora curvula* was the dominant species at five sites (relative abundance of 93–97%), while at P1 it was codominant with *Anguillospora filiformis* (53% and 36%, respectively). Sporulation rates on pine needles were much lower than on alder (ANOVA: $F_{1,52} = 236.15$; $p < 0.001$), ranging from 0.5 conidia mg $needle^{-1}$ d^{-1} at site P3 to 16.2 conidia mg $needle^{-1}$ d^{-1} at site D1, and without significant differences between deciduous and pine streams (Fig. 3; ANOVA: $F_{1,4} = 0.82$; $p = 0.416$). Although *F. curvula* was again the most abundant species, it was not as dominant as in alder leaves, showing relative abundances of 64.2–73.1% in four of the streams (two pine and two deciduous). In these four streams, different conidial forms of *Alatospora acuminata* were present (11.5–23.3%). Even though species richness was lower on pine than on alder (ANOVA: $F_{1,52} = 236.15$; p <0.001), the diversity was higher on pine (ANOVA: $F_{1,52} = 11.27$; $p = 0.002$) as a result of greater evenness among species (Fig. 3). Neither sporulation rates nor fungal richness were correlated with the physicochemical characteristics of stream water, nor did they show any correlation with alder or pine breakdown rates.

Out of the 56 benthic macroinvertebrate

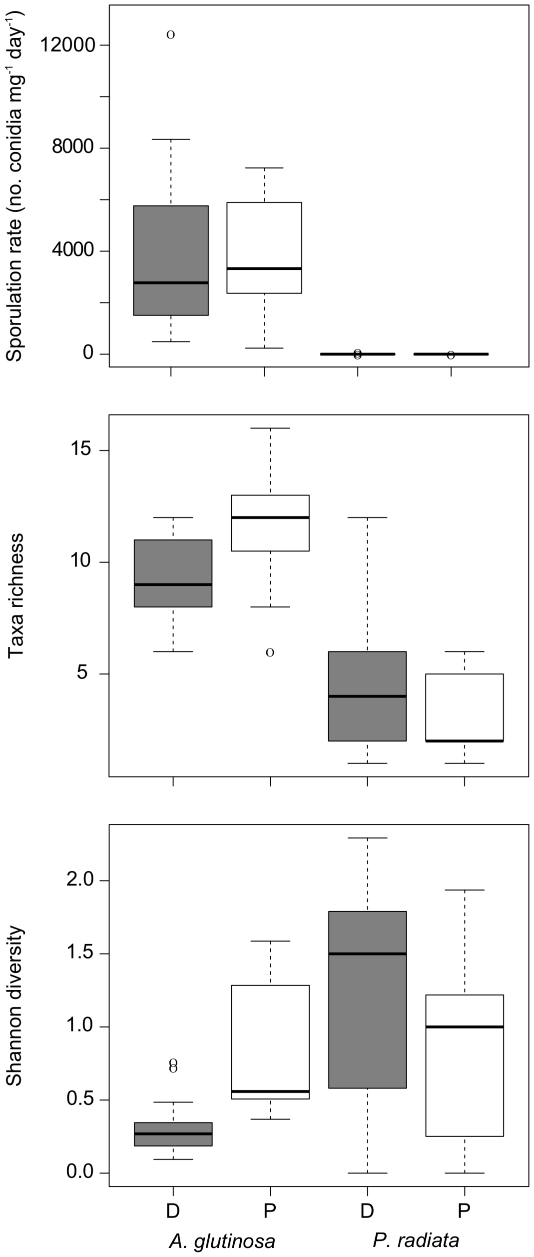


Figure 3. Sporulation rates, taxa richness and Shannon diversity of aquatic hyphomycetes in alder and pine bags in deciduous (D, filled boxes) and pine (P, open boxes) streams. Dark bars represent the median and the boxes denote the interquartile range.

taxa, 45 families were identified in leaf-litter bags, 35 in alder leaves (deciduous sites: 26; pine sites: 28) and 38 in pine needles (deciduous: 29; pine: 31). Community diversity did not vary between land uses or leaf type. Although distributing the taxa into FFG did not create diversity differences between land uses, difference between leaf types arose: shredders were less diverse (ANOVA: $F_{1,52} = 5.72$; $p = 0.020$) and collector-gatherer-scrapers more diverse (ANOVA: $F_{1,52} = 4.80$; $p = 0.033$) in pine bags. The total macroinvertebrate density in alder bags was 43.7% higher than in pine bags (ANOVA: $F_{1,52} = 17.81$; $p < 0.001$), but there were no differences between deciduous and pine sites (Fig. 4). Bags in pine streams contained lower densities of shredders than those in deciduous streams, with reductions of 71.9% in alder and 58.2% in pine bags, close to reach statistical differences (Fig. 4; overall ANOVA: $F_{1,4} = 5.62$; $p = 0.077$). Regarding the incubated species, the densities of shredders and collector-gatherers were 51.2% and 58.0% higher in alder than in pine bags ($\text{ANOVA}_{\text{Shr}}$: $F_{1,52} = 18.98$; $p < 0.001$; ANOVA_{CG}: $F_{1,52} = 10.59$; $p = 0.002$). The biomass of entire community or of any FFG did not differ significantly between leaf species or land uses. There was no significant correlation between the macroinvertebrates found in the bags and alder or pine needle breakdown rates.

Discussion

This study demonstrates that the replacement of deciduous forests by pine plantations affects the structure of macroinvertebrate communities and the functioning of low-order forested streams. Riipinen *et al.* (2010) attributed the differences in leaf-litter processing between streams under deciduous forest and pine plantations to water chemistry (specifically, to a lower pH under plantations) rather than vegetation type. In fact, water acidification is a common effect of conifer plantations on stream ecosystems (Clenaghan *et al*., 1998), which can be attributed to the leaching of organic acids produced from mycorrhizal roots and to nutrient uptake (Sollins *et al*., 1981). A low pH reduces litter breakdown rates in streams (Dangles & Chauvet, 2009) and has negative effects on the density and composition of macroinvertebrate communities (Larrañaga *et al*., 2010). Nevertheless, in our study, there were not significant differences in water pH or in other physicochemical parameter between streams under deciduous forest and pine plantations. Thus, rather than responding to water chemistry, the studied structural and functional attributes seem to respond to changes in detrital resources and to alterations in the cycles of matter and energy in these ecosystems.

Structural attributes: benthic standing stock and macroinvertebrate communities

Pine plantations alter the functional feeding group structure of stream macroinvertebrates, with shredders being the most affected. Changes in native vegetation modify the quantity and quality of leaf-litter inputs (Pozo *et al*., 1997), which are the primary resource for shredders (Graça, 2001). In our case, deciduous leaves were replaced by pine needles, a material with a tough cuticle, very low nutrient content (Girisha *et al*., 2003) and high levels of toxic compounds (Bärlocher & Oertli, 1978). These characteristics make pine needles a recalcitrant resource for benthic macroinvertebrates (Valdovinos, 2001; Collen *et al*., 2004, but see Campos & González, 2009). Nevertheless, we would have expected a stronger negative impact on shredders if pine was their only energy source (Collen *et al*., 2004). However, despite the observed differences in density between the two land uses (58.5% lower in pine streams), there was no impact on shredder biomass, contrary to the effect associated with *Eucalyptus globulus* plantations on

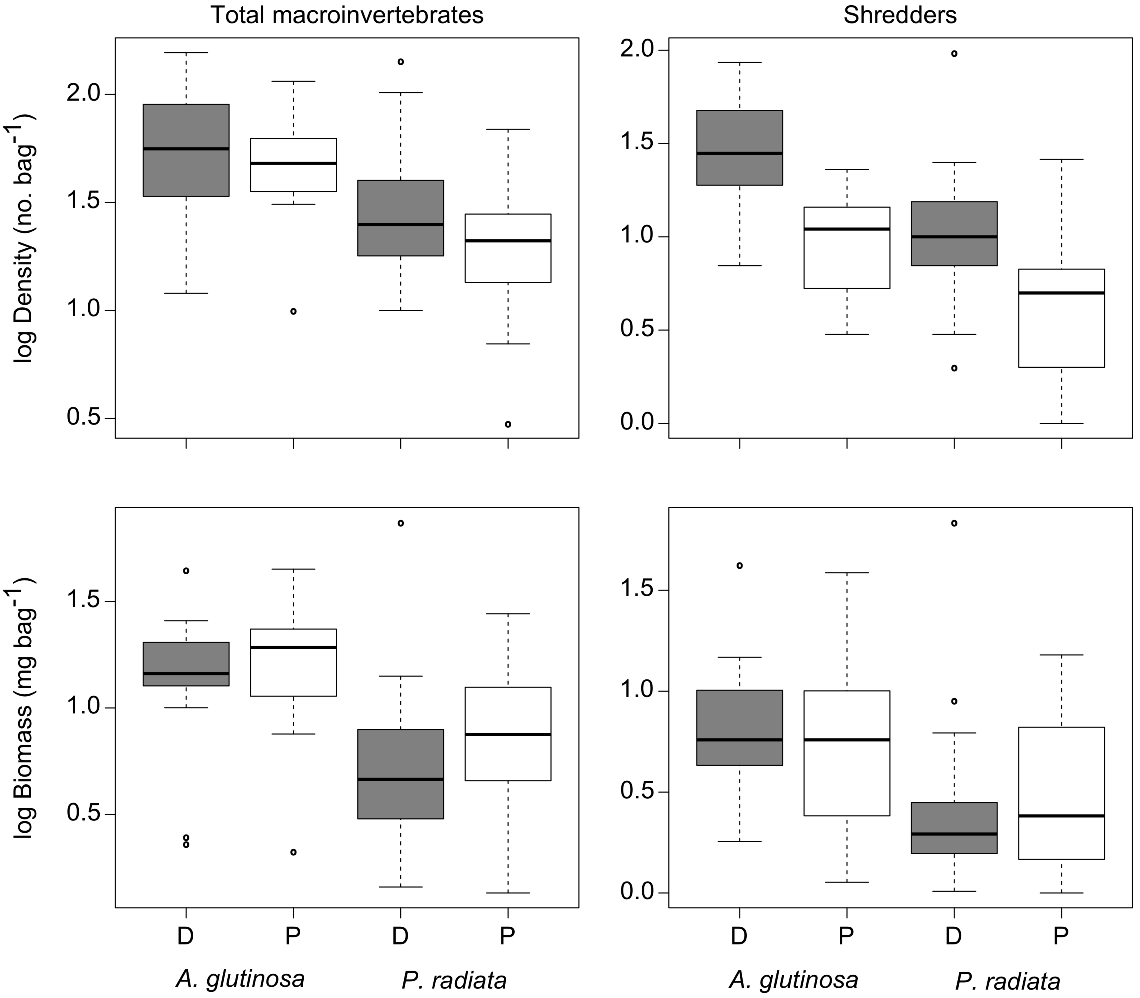


Figure 4. Density (top) and biomass (bottom) of total macroinvertebrates (left) and shredders (right) in alder and pine bags in deciduous (D, filled boxes) and pine (P, open boxes) streams. Dark bars represent the median and the boxes denote the interquartile range. Note the different scales.

streams in the same region (84.3% lower shredder biomass in streams under eucalyptus; Larrañaga *et al.*, 2009a). *Eucalyptus* leaves are also low quality resources for macroinvertebrates (Canhoto & Graca, 1999), but the processing of *Eucalyptus* litter in streams is faster (Pozo *et al.*, 1998) than that of pine needles (present study). The weaker effects of pine compared to those of eucalyptus on macroinvertebrates may be due to the differences in the development of the riparian buffer, which is less well developed in streams draining eucalyptus plantations (Larrañaga *et al.*, 2009a). Riparian buffers provide higher-quality leaf-litter inputs into streams (Goodman *et al.*, 2006), control soil disturbance and reduce sediment and nutrient levels in the runoff from the basin. In our case, the riparian buffer might contribute to the lack of significant differences in the total amount of benthic CPOM between deciduous and pine streams. Nevertheless, a change in quality was evident, as needles were very abundant in the standing stock of pine streams. The high settling rate of conifer needles in stream water (Hoover *et al.*, 2010) can facilitate the retention of this kind of material over deciduous leaves.

Functional attributes: organic matter processing and associated biota

Breakdown rate of pine needles was much lower than for alder leaves, as already observed when comparing other pine species *vs.* deciduous leaves (Whiles & Wallace, 1997; Collen *et al.*, 2004), in accordance with the nutrient levels in the leaves (Hladyz *et al.*, 2009). The fungal spore production was very low in pine needles pointing out a poor colonization and consumption of this material by aquatic hyphomycetes. The physical characteristics of leaves as toughness, surface structure and roughness or the abundance of extracellular substances determine the fungal colonization and activity (Dang *et al.*, 2007; Kearns & Bärlocher, 2008). Thus, we expected this result as pine needles present a tough cuticle and anti-fungal agents (Bärlocher & Oertli, 1978).

Regarding invertebrates in bags, shredders were less abundant in pine bags than in alder bags. Moreover, shredder densities in pine bags under pine plantations were lower than under deciduous forests. In contrast, Whiles & Wallace (1997) noticed that shredders, trichopterans in particular, were more abundant in pine needle bags in streams under pine plantations, than in deciduous streams, leading to the more rapid processing of needles in pine streams. These authors pointed out that the lack of an alternative resource induces detritivores to concentrate on pine needles. Similarly, Campos & González (2009) observed that the trichopteran *Sericostoma vittatum* could consume and grow on pine needles probably due to the low mobility, large mandibles and low respiration rates of this taxon (Friberg & Jacobsen, 1994). However, in our study, we found very low densities of trichopterans in the benthos of pine streams, especially *Sericostoma* (absent in two of them). Moreover, the colonization of pine bags under pine plantations by caddisflies was minimal. Although small plecopteran shredders colonized pine litter, they can also feed on FPOM (Tachet *et al.*, 2002) and might use these bags as habitat rather than as a food source (Albariño & Balseiro, 2002). Thus, we reject the hypothesis that the community has adapted to the consumption of pine needles under these plantations, as breakdown rates of pine needles were similar in the two stream types.

We observed that pine plantations reduce the capacity of streams to process high-quality resources, with a reduction of 21.3% in the breakdown rate of alder. Due to the similarity in sporulation rates between the two stream types, we believe that invertebrate feeding activity rather than fungal performance could explain the differences in processing rates. We failed to detect a significant relationship between decomposition and shredders, although colonization of alder bags in streams under pine plantations was lower than in streams under deciduous forests, in line with the lower density of shredders in the benthos of pine streams. Nevertheless, similarities in shredder biomass between the two land uses may be attributed to the presence of shredders of different sizes depending on land use (bigger in pine streams); in turn, this may be responsible in part for the lack of a shredder *vs.* breakdown rate relationship, reflecting the different metabolic rates of large and small animals (Brown *et al.*, 2004). Another explanation for the lack of the shredder *vs.* processing rate relationship may be associated with key species that are critical for maintaining various processes (Swan & Palmer, 2006; Woodward *et al.*, 2008), including leaf-litter breakdown (Pomeroy *et al.*, 2000; Creed *et al.*, 2009). In our case, the density reduction pattern of two small plecopterans (Leuctridae and Nemouridae) and a large trichopteran (Sericostomatidae) at streams under pine plantations might account for most of the loss in the detritivore capacity of the community.

Our results demonstrate that pine plantations alter the structure of macroinvertebrate communities, reducing shredder densities and hence affecting the leaf-litter processing capability of streams. These structural and functional effects on the detritic pathway would result in a reduction in the capacity of the ecosystem to sustain in-stream food webs and secondary production.

References

Albariño RJ & Balseiro EG, 2002. Leaf litter breakdown in Patagonian streams: native versus exotic trees and the effect of invertebrate size. Aquatic Conservation: Marine and Freshwater Ecosystems 12: 181–192.

Allan JD & Castillo MM, 2007. Stream ecology–Structure and Function of Running Waters, 2nd edn. Springer, Dordrecht.

Allen SE, Grimshaw HM, Parkinson,JA & Quarmby C, 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater. 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation: Washington, DC; 1368 pp.

Bärlocher F & Oertli JJ, 1978. Inhibitors of aquatic hyphomycetes in dead conifer needles. Archiv für Hydrobiologie 81, 462–474.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB, 2004. Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Campos J & González JM, 2009. *Sericostoma vittatum* (Trichoptera) larvae are able to use pine litter as an energy source. International Review of Hydrobiology 94: 472–483.

Canhoto C & Graça MAS, 1999. Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. Microbial ecology 37: 163–172.

Clenaghan C, O'Halloran JO, Giller P & Roche N, 1998. Longitudinal and temporal variation in the hydrochemistry of streams in an Irish conifer afforested catchment. Hydrobiologia 389: 63–71.

Collen P, Keay EJ & Morrison BRS, 2004. Processing of pine (*Pinus sylvestris*) and birch (*Betula pubescens*) leaf material in a small river system in the northern Cairngorms, Scotland. Hydrology and Earth System Sciences 8: 567–577.

Creed RP, Cherry RP, Pflaum JR & Wood CJ, 2009. Dominant species can produce a negative relationship between species diversity and ecosystem function. Oikos 118: 723–732.

Dang CK, Gessner MO & Chauvet E, 2007. Influence of conidial traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. Mycologia 99: 24–32.

Dangles O & Chauvet E, 2009. Effects of stream acidification on fungal biomass in decaying beech leaves and leaf palatability. Water Research 37: 533–538.

Friberg N & Jacobsen D, 1994. Feeding plasticity of two detritivores shredders. Freshwater Biology 32: 133–142.

Girisha GK, Condron LM, Clinton PW & Davis MR, 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. Forest Ecology Management 179: 169–181.

Goodman KJ, Hershey AE & Fortino K, 2006. The effect of forest type on benthic macroinvertebrate structure and ecological function in a pine plantation in the North Carolina Piedmont. Hydrobiologia 559: 305–318.

Graça MAS, 2001. The role of invertebrates on leaf litter decomposition in streams – A review. International Review of Hydrobiology 86: 383–393.

Hladyz S, Åbjörnsson K, Giller PS & Woodward G, 2011. Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. Journal of Applied Ecology 48: 443–452.

Hladyz S, Gessner MO, Giller PS, Pozo J & Woodward G, 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. Freshwater Biology 54: 957–970.

Hoover TM, Marczak LB, Richardson JS & Yonemitsu N, 2010. Transport and settlement of organic matter in small streams. Freshwater Biology 55: 436–449.

Kearns SG & Bärlocher F, 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. Fungal Ecology 1: 13–18.

Kominoski JS, Marczak LB & Richardson JS, 2011. Riparian forest composition affects stream litter decomposition despite similar microbial and invertebrate communities. Ecology 92:151–159.

Larrañaga A, Larrañaga S, Basaguren A, Elosegi A & Pozo J, 2006. Assessing impact of eucalyptus plantations on benthic macroinvertebrate communities by a litter exclusion experiment. International Journal of Limnology 42: 1–8.

Larrañaga A, Basaguren A, Elosegi A and Pozo J, 2009a. Impacts of Eucalyptus globulus plantations on Atlantic streams: changes in invertebrate density and shredder traits. Fundamental and Applied Limnology 175: 151–160.

Larrañaga A, Basaguren A & Pozo J, 2009b. Impacts of *Eucalyptus globulus* plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. International Review of Hydrobiology 94: 497–511.

Larrañaga A, Layer K, Basaguren A, Pozo J & Woodward G, 2010. Consumer body composition and community structure in a stream is altered by pH. Freshwater Biology 55: 670–680.

Lecerf A, Risnoveanu G, Popescu C, Gessner MO & Chauvet E, 2007. Decomposition of diverse litter mixtures in streams. Ecology 88: 219–227.

Lecerf A & Richardson JS, 2010. Litter decomposition can detect effects of high and moderate levels of forest disturbance on stream condition. Forest Ecology and Management 259: 2433–2443.

Magbanua FS, Townsend CR, Blackwell GL, Phillips N & Matthaei CD, 2010. Responses of stream macroinvertebrates and ecosystem function to conventional, integrated and organic farming. Journal of Applied Ecology 47: 1014–1025.

Merritt RW & Cummins KW, 2007. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company: Dubuque; 1158 pp.

Pomeroy KE, Shannon JP & Blinn DW, 2000. Leaf breakdown in a regulated desert river: Colorado River, Arizona, U.S.A. Hydrobiologia 434: 193–199.

Pozo J, González E, Díez JR, Molinero J & Elósegui A, 1997. Inputs of particulate organic matter to streams with different riparian vegetation. Journal of North American Benthological Society 16: 602–611.

Pozo J, Basaguren A, Elosegi A, Molinero J, Fabre E & Chauvet E, 1998. Afforestation with *Eucalyptus globulus* and leaf litter decomposition in streams of northern Spain. – Hydrobiologia 374: 101–109.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical

Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Riipinen MP, Fleituch T, Hladyz S, Woodward G, Giller P & Dobson M, 2010. Invertebrate community structure and ecosystem functioning in European conifer plantation streams. Freshwater Biology 55: 346–359.

Sollins P, Cromack JrK, Fogel RD & Li CY, 1981. Role of low molecular-weight organic acids in the inorganic nutrition of fungi and higher plants. In: Wicklow DT and Carroll GC (Eds.), The Fungal Community. Marcel Decker, New York, pp. 607–619.

Swan CM & Palmer MA, 2006. Composition of speciose leaf litter alters stream detritivore growth, feeding activity and leaf breakdown. Oecologia 147: 469–478.

Tachet H, Richoux P, Bournaud M & Usseglio-Polatera P, 2002. Invertébrés d'eau douce: systématique, biologie et écologie. CNRS: Paris; 587 pp.

Valdovinos C, 2001. Riparian leaf litter processing by benthic macroinvertebrates in a woodland stream of central Chile. Revista Chilena de Historia Natural 74: 445–453.

Whiles MT & Wallace JB, 1997. Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments Hydrobiologia 353: 107–119.

Woodward G, Papantoniou G, Edwards F & Lauridsen R, 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117: 683–692.

Zar JH, 2010. Biostatistical Analysis. 5th ed. Prentice Hall: Upper Saddle River.

Zhang Y, Richardson JS & Pinto X, 2009. Catchment-scale effects of forestry practices on benthic invertebrate communities in Pacific coastal streams. Journal of Applied Ecology 46:1292–1303.

Land-use changes

Chapter IV

Resource quality affects size spectrum of selected feeding guilds in streams

Abstract

We examined the effects of *Pinus radiata* D. Don plantations on the taxonomic composition and size-body structure of macroinvertebrate communities in low order forested streams. We compared three streams draining pine plantations with three draining natural deciduous forests without significant differences in water physicochemical characteristics. Pine plantations had negative effects on density, richness and diversity of shredders, and they altered the size-body structure of this group. Pine streams exhibited lower density of small shredder individuals than deciduous streams, driving the size spectra to become shallower under these plantations than in native deciduous forests. We suggest that a single trait, as toughness of pine needles, may difficult the smallest individuals to use it as resource forcing a reduction of their density. There are evidences that the alteration detected on shredders might have scaled up in the trophic level, as the predators size spectra also tended to be shallower in streams under pine streams, again due to lower density of small individuals. Our results point out that the change of resource quality due to the replacement of deciduous vegetation by pine plantations can alter the size structure of specialized consumers of detritus in the stream and that this modification might scale up to higher trophic levels.

Key words: Pine plantations, Forested streams, Benthic macroinvertebrates, Body-size.

Introduction

Since Sheldon *et al.* (1972) pioneer work, macroecological patterns have been widely studied through abundance–body mass relationships of organisms (e.g. Damuth, 1981; Brown & Maurer, 1989; Bourassa & Morin, 1995, Gaston & Blackburn, 2000; Jennings & Mackinson, 2003; Blanchard *et al.*, 2009). The existing negative relationship between mass and abundance is referred to as the “size spectrum” (Kerr & Dickie, 2001), and multiple competing hypotheses about the distribution of species body mass have been advanced (see Allen *et al.*, 2006). Changes in the regression slope of this relationship can be used to assess the effects of impacts at community level (Reuman *et al.*, 2008; Petchey & Belgrano, 2010). Although understanding how size spectrum might be altered by human impacts is an important contemporary challenge for ecology, there are few studies about specific impacts on freshwater benthic invertebrate community (but see Yvon-Durocher *et al.*, 2011a; Dossena *et al.*, 2012).

In addition to their numerical scarcity, these works only determine the size spectra of the community as a whole. However, individuals within different functional groups might respond differently to an impact due to their biological, physiological and ecological traits. As some groups are key for certain processes, changes in size distributions of specific groups might translate in altered ecosystem functioning.

One of the most noticeable anthropogenic alterations affecting forested headwater streams is the replacement of native vegetation by exotic plantations. As the benthic invertebrate food web is based on leaf litter inputs from surrounding forest, the feasible changes in the quantity, quality and seasonality of these inputs are expected to be responsible for altering the structure of stream biotic communities (Wallace *et al*., 1997). Most of the studies about effects of plantations on streams are focused on structural metrics of benthic macroinvertebrates communities (e.g. Whiles & Wallace, 1997; Collen *et al*., 2004; Riipinen *et al*., 2010; Kominoski *et al*., 2011). There is, nevertheless, evidence that the replacement of basal resource affects size of organisms due to changes in feeding rates, growth and/or maximum body size of principal consumers (Albariño & Balseiro, 2002; Campos & González, 2009; Larrañaga *et al*., 2009). Specifically, in the province of Biscay, 62% of forested surface is covered by *Pinus radiata* D.Don plantations (www.bizkaia.net), which litter is considered a poor quality resource for microbial decomposers and invertebrates detritivores due to its low nutrients and toxic compounds (Bärlocher & Oertli, 1978; Quinn *et al*., 2000; Girisha *et al*., 2003). A previous study by us (Chapter III) supports that the replacement of deciduous vegetation by pine plantations (main resource change) impacts negatively the structure of stream benthic macroinvertebrate communities, showing streams under pine monocultures lower density of shredder. In that study we observed differences between reference and impaired streams in terms of density but not in biomass, what points out to shallower slopes of size spectra due to the lowered density of smaller individuals. Nevertheless, it is known that larger organisms tend to be more susceptible to anthropogenic perturbations due to their lower population densities and greater energetic demands (Woodward *et al*., 2005a; Yvon-Durocher *et al*., 2011b), and reduced resource availability can bring down individual growth rates of consumers (Boersma & Kreutzer, 2002). These two reasons predict steeper size spectra in streams under pine monocultures in accordance with the suggestion by Petchey & Belgrano (2010) for impacted communities, but opposes to what have been inferred in our previous study. Nevertheless, we have not directly measured the size spectra yet and we lack actual information about the magnitude of its differences between native deciduous forests and pine monocultures. Thus, in this work we will test if pine plantations affect the size spectra of benthic macroinvertebrate community as a whole or of selected feeding guilds. Contrary to expected, our previous results make us hypothesize that the main consumers of leaf-litter inputs (shredders) will present shallower size spectra slopes.

Material and methods

Study area

The study was conducted in six low order (2^{nd}–3^{rd}) streams with siliceous substrata flowing into the Atlantic Ocean (Northern Spain, 43º 12’ N / 2º 58’ W). Three of those streams drain native deciduous forests (D1, D2, D3), being the dominant tree species the English oak (*Quercus robur* L.) and the common beech (*Fagus sylvatica* L.). The other three streams drain pine plantations (P1, P2, P3) of *P. radiata*. Streams under pine plantations present a strip of riparian forest composed by deciduous trees and pines. Any other anthropic impact in the basins, aside from the pine plantations, is negligible. All streams presented similar water characteristics, well oxygenated, neutral pH, cold temperatures and low nutrient concentrations (see Chapter III for more extensive information).

Benthic organic matter stock and macroinvertebrate sampling

From July 2010 to June 2011, at each study site, 8 Surber benthic samples (0.09 m^2 each) were taken monthly from randomly chosen riffles. Organic matter was taken from each sample on an 8-mm sieve. In the laboratory, it was sorted into alder, other deciduous leaves (oak and beech fundamentally), pine needles and others (fruits, branches, etc.); each category was oven-dried and burned to determine its ash free dry mass (AFDM). For each sample, we estimated the overall nitrogen concentration from concentration measured on leaves in our previous studies (alder: 3.05%, other deciduous: 1.36% and pine needles: 0.56%). On the 20th December 2010, in addition to organic matter, macroinvertebrates were sorted on a 0.5 mm mesh and were preserved in 70% ethanol until the ulterior study. They were identified to the lowest practical taxonomic level using Tachet *et al.* (2002), counted and sorted into feeding guilds (after Tachet *et al.*, 2002; Merritt

& Cummins, 2007): predators, basal consumers (i.e. non-predators), and shredders, a subgroup within the basal consumers which depend specifically on leaf litter. For each benthic macroinvertebrate sample we calculated the taxa richness and Shannon diversity index for each mentioned feeding guild.

Body mass estimation

Total body length of all macroinvertebrates was measured under a binocular microscope with an accuracy of 0.5 mm. Individual body mass was estimated from body length following equations from the literature (Meyer, 1989; Burgherr & Meyer, 1997; Benke *et al*., 1999; Baumgartner & Rothhaupt, 2003; Larrañaga *et al*., 2009). Total body length was considered to be the distance from the anterior of the head to the posterior of the last abdominal segment, excluding tails and antennas. In gastropod molluscs the maximum length of the shell was considered (Meyer, 1989) and for the crustaceans of the genus *Echinogammarus* the total length of the extended body was measured (Larrañaga *et al*., 2009).

Size spectra

Size spectra were constructed by dividing the total range of $\log_{10}(M)$ values, where M is body mass, into n logarithmic bins of equal width, and the logarithm of the abundance of organisms ($\log_{10}N$) in each bin was regressed against the centre of the bin (after White *et al*., 2008). There is no consensus on the number of bins to use when constructing size spectra. An increment in the number of bins increases the power of the statistical comparisons, but also the number of empty bins, i.e. body mass intervals with no individuals. On the other hand, a reduction in the number of bins solves the problem of empty bins but compromising statistical power. We decided to approach the issue by selecting the number of bins that reduced the overall error of the estimate of the size spectra slopes. The number of bins (6) was chosen as it maximizes the linearity of the size spectra and minimizes the occurrence of empty bins. Size spectra were constructed for each feeding guild considered and for the total macroinvertebrates. The lower boundary of body mass to construct size spectra was set in 0.1 mg as we have estimated that under this mass the organisms tend to be undersampled, as they were able to be washed through the 500 µm sieve used, and introduce an artifact in the size spectra if they are considered in the analyses. For the rest of the variables (density, biomass, richness and diversity) all individuals were taken into account.

Statistical analysis

Total leaf amount and the leaf-nitrogen concentration were compared using three-way mixed-model ANOVA (‘stream’: random factor; ‘land use’ -deciduous or pine- and ‘month’ fixed). Density, biomass (the sum of individual body masses per replica), taxa richness and Shannon diversity of total benthic macroinvertebrates, predators, basal consumers and shredders were computed for each sample and compared using two-way mixed-model ANOVA (‘stream’: random factor; ‘land use’ fixed). Slopes of the size spectra were compared by two-way mixed-model ANCOVA (‘stream’: random factor; ‘land use’ fixed) with body-mass as covariant. When necessary, data were transformed (log (x+1)) to improve normality of data and all mixed model parameters were estimated by means of restricted maximum likelihood (REML). All statistical analyses were conducted using the R statistical program (version 2.11.1; R Development Core Team, 2010).

Results

From July 2010 to June 2011, on average 68.19 g m^{-2} of organic matter (AFDM) was collected in streams flowing through native deciduous forests and 48.35 g m^{-2} in those under pine plantations. Non-leaf material (others: fruits, branches…) was similar between two stream types representing on average the 43.6% in deciduous and 47.8% in pine streams. On average, leaf quantity in deciduous streams tended to be higher than those in pine ones (39.43 g m^{-2} in deciduous *vs.* 25.17 g m^{-2} in pine streams), being differences close to statistical significance (Fig. 1; ANOVA $F_{1,4} = 6.80$; $p = 0.059$). In streams under pine plantations needles represented on average the 41.5% of the leaves. Moreover, the leaf quality was higher in the deciduous streams with a significantly higher nitrogen concentration than in pine streams (1.62% in deciduous *vs.* 1.23% in pine streams on average) (Fig. 1; ANOVA $F_{1,4} = 9.94$; $p = 0.034$).

Although total abundance of macroinvertebrates was 42.1% lower in pine than in deciduous streams, there were not statistical differences (Table 1, Fig. 2). Basal consumers represented a high percentage of total community in both density (85.5% in deciduous and

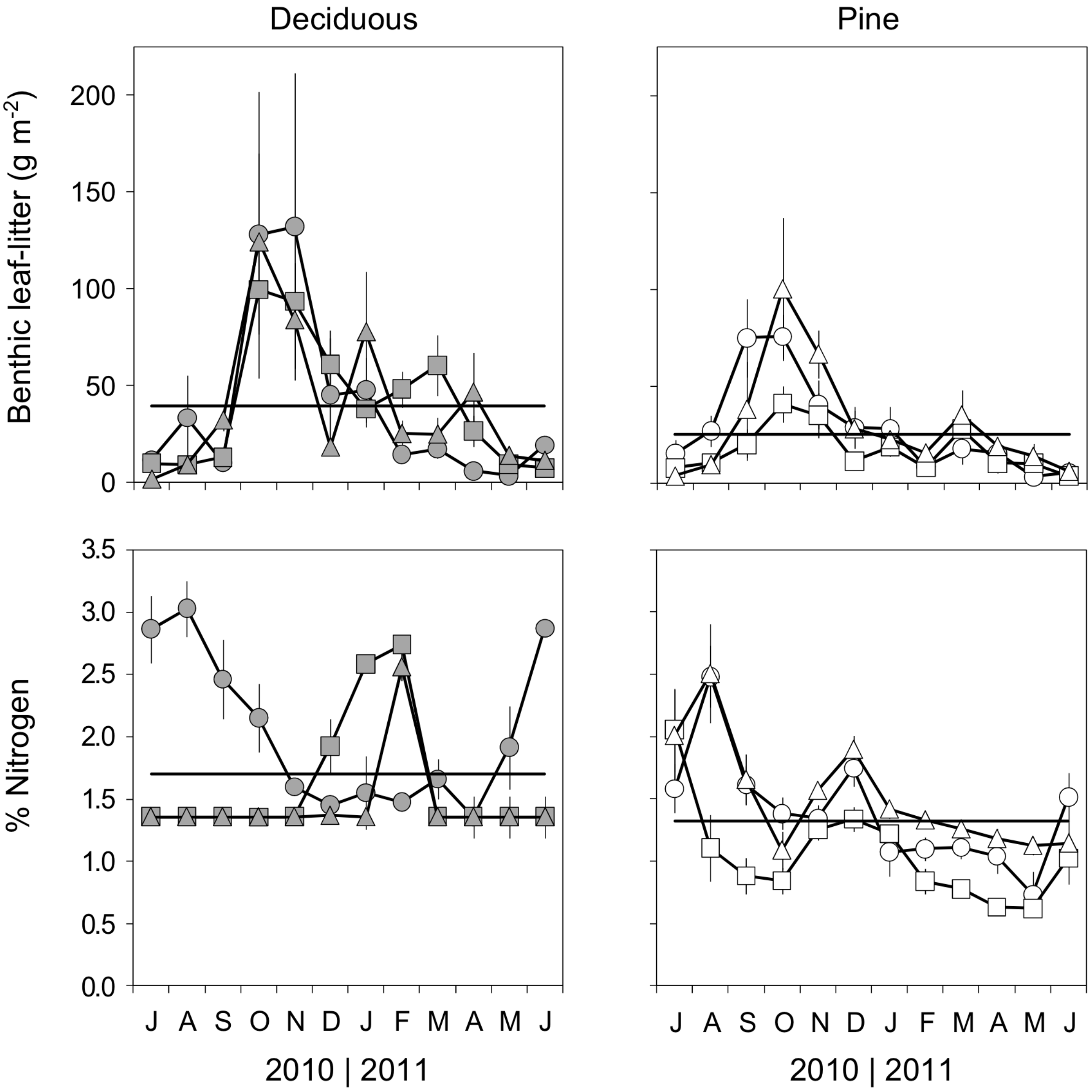


Figure 1. Benthic leaf-litter quantity (upper panels) and estimated leaf nitrogen concentration (bottom panels) in deciduous (left panels; filled symbols: D1 circle, D2 square and D3 pyramid) and pine streams (right panels; open symbols: P1 circle, P2 square and P3 pyramid). Symbols does not mean pairment between deciduous and pine streams.

93.0% in pine streams) and biomass (71.0% in deciduous and 79.6% in pine streams), but differences between types of site were not significant for density (Table 1, Fig. 2). Contrastingly, the mean abundance of shredders was statistically lower in pine (294.0 ind. m^{-2}) than in deciduous streams (1216.7 ind. m^{-2}) (Table 1, Fig. 2). Predator density did not statistically differ between stream types. Although average reduction in biomass represented 31.9% for total macroinvertebrates, 23.6% for basal consumers, 32.3% for shredders and 52.2% for predators from deciduous to pine streams, in no case these

Table 1. Results from the linear mixed effect model analyses. ANOVA was used to test for density, biomass, taxa richness and Shannon diversity, and ANCOVA to test size spectra slopes. Every model was fitted with land use (deciduous *vs*. pine) as fixed factor and stream as random factor, with the body-mass as covariant in ANCOVAs.

		df	*F*	*p*
Total invertebrates	Density	1,4	5.10	0.087
	Biomass	1,4	2.07	0.224
	Richness	1,4	1.56	0.280
	Diversity	1,4	1.60	0.275
	Size spectrum	1,27	3.84	0.061
Basal consumers	Density	1,4	3.24	0.146
	Biomass	1,4	1.17	0.341
	Richness	1,4	1.85	0.246
	Diversity	1,4	2.66	0.179
	Size spectrum	1,27	0.36	0.553
Shredders	Density	1,4	10.66	**0.031**
	Biomass	1,4	4.61	0.098
	Richness	1,4	11.29	**0.028**
	Diversity	1,4	12.11	**0.025**
	Size spectrum	1,27	5.31	**0.029**
Predators	Density	1,4	4.07	0.114
	Biomass	1,4	4.34	0.106
	Richness	1,4	0.34	0.591
	Diversity	1,4	0.02	0.890
	Size spectrum	1,27	4.06	0.054

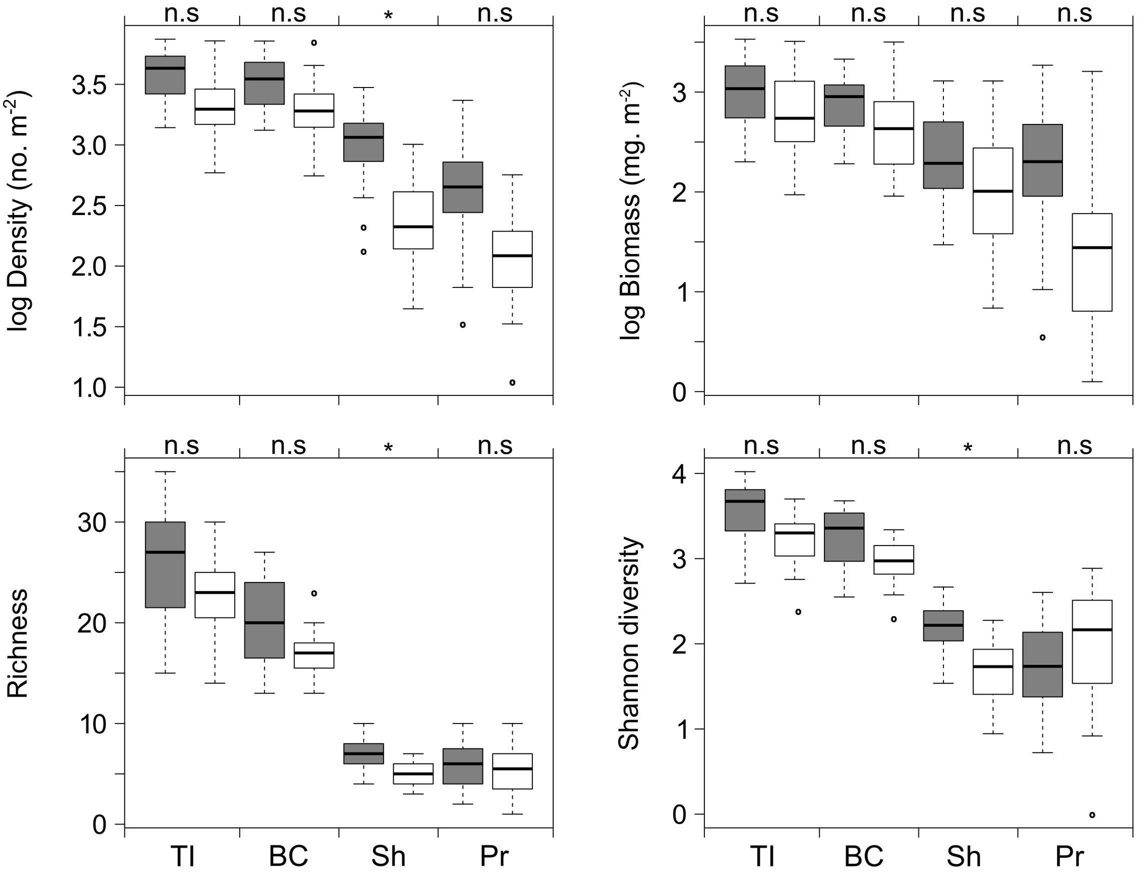


Figure 2. Boxplots of density, biomass, taxa richness and Shannon diversity of total invertebrates (TI), basal consumers (BC), shredders (Sh) and predators (Pr) in deciduous streams (filled boxes) and pine streams (open boxes). Horizontal dark bars represent the median and the boxes denote the interquartile range. Statistical output (*p*-values) for the fixed effect (deciduous *vs*. pine) of the fitted linear mixed models are shown above each plot (n.s.: non significant, $p > 0.05$; *: $0.05 > p > 0.01$).

differences were supported by statistical significance (Table 1, Fig. 2). For taxa richness and Shannon diversity for the entire invertebrate community and the three feeding guilds, a tendency for lower values was observed again in pine streams (Table 1, Fig. 2). Nevertheless, these differences were only backed up with statistical significance in the case of shredders (Table 1, Fig. 2).

In total, 14254 organisms were measured. The 95th percentile for the body mass of total macroinvertebrates was 1.36 mg in deciduous streams and 2.05 mg in pine streams. The slopes of the size spectra taking into account all invertebrates ranged from -0.99 to -1.21 in deciduous sites and from -0.80 to -1.02 in pine sites (Fig. 3a), i.e. small individuals tended to be relatively less abundant than big ones in pine streams. These differences in slopes of the size spectra for the total macroinvertebrates were near to statistical significance (p = 0.061; Table 1). The slopes of size spectra for basal resource consumers were very similar between the two stream types (Table 1, Fig. 3b). However, considering only shredders, the slopes of the size spectra were significantly shallower in pine streams due to the lower abundance of smaller shredders (Table 1, Fig. 3c). Similarly, the slope for predator size spectra was shallower in pine streams (Fig. 3d), but differences were only just close to statistical significance (p = 0.054; Table 1).

Discussion

In low order forested streams, invertebrate food-web is based on detritus (Wallace *et al.*, 1997), and organisms of different sizes share a common non-size-dependent resource, i.e. individuals of different sizes feed on the same resource and most of the community is not chained in size-structured predator-prey interactions (Rosi-Marshall & Wallace, 2002). In unstructured food-webs as those of the present study, due to body-size scales allometrically with metabolic rate, a slope of size spectrum of about -3/4 has been suggested (Damuth, 1981, 1987; Peters, 1983; Brown *et al.*, 2004), although it is still a matter of intense debate (see Reuman *et al.*, 2009; Glazier, 2010). However, the entire macroinvertebrate communities of all our streams showed similar slope values to size-structured food-webs (around -1) typical of pelagic systems (Jennings & Mackinson, 2003; Blanchard *et al.*, 2009) where predator and prey sizes are interdependent.

In our study the size spectrum slope of communities from streams under pine plantations tended to be shallower than those of communities from unaltered streams, contrary to what was suggested by Petchey & Belgrano (2010) for impacted systems. Looking for the most affected feeding strategy altered by the substitution of resource (native deciduous leaves by pine needles) we subdivided the community (similar as in Schmid *et al.*, 2000) on feeding groups (basal consumers, shredders and predators) to compare their taxa richness, diversity, density, biomass and size spectrum. As in a previous study (Chapter III), the present one shows that, in forested streams, pine plantations affect mainly shredders: richness, diversity and density were lower under pine plantations than in streams under native deciduous forests. For size spectrum of shredders we observed that the slope was significantly different between streams flowing through native deciduous forests and through pine plantations. In the former the shredder

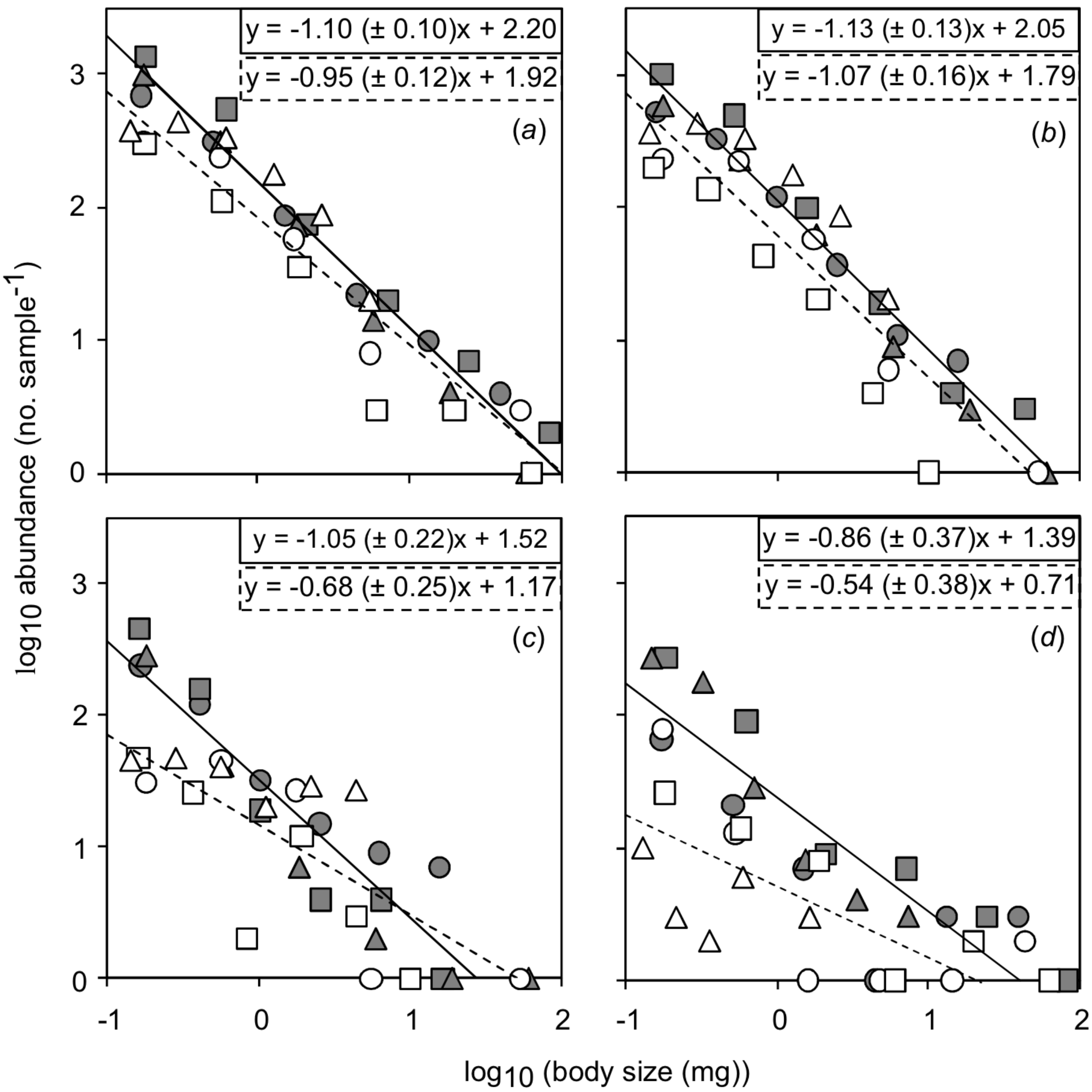


Figure 3. Size spectra for total invertebrates (*a*), basal consumers (*b*), shredders (*c*) and predators (*d*) in deciduous streams (filled symbols and solid lines; D1 circle, D2 square and D3 pyramid) and pine streams (open symbols and dashed lines; P1 circle, P2 square and P3 pyramid). Regression lines and equations shown derive from the fixed effects (deciduous or pine) of the fitted linear mixed models. Confidence intervals of 95% for the slope are shown within brackets.

size spectrum was stepper (-1.05 ± 0.22) than in latter ones (-0.68 ± 0.25), due to the lower density of smaller animals in pine streams. Nevertheless, according to the higher per capita energetic and material demands of big individuals (Brown *et al.*, 2004), it would be expectable them to be less abundant in pine plantations. Previous studies in the area linking resource quality change and body size of consumers (Larrañaga *et al.*, 2009) pointed that big taxa were more sensitive to resource impoverishment (by eucalypt plantations in that case). They explained this sensitivity by their higher demands of food quantity and quality they need to ingest. Nevertheless, although quality and quantity changes (decrease) of the resource are common to eucalypt and pine plantations (Molinero & Pozo, 2004 and the present study), the resultant effect on the size spectra of the consumers is the opposite. Even if energy or nutrient-based modification does not seem to be a valid explanation for the observed size spectra changes, the impairment is clearly focused on shredders and, thus, we should search an explanation in the resource quality. Moreover, the response of shredders by means of the size spectra shows that the nature of the impact needs to have a relationship with a trait of the invertebrates. Many biological or ecological traits of species scale with body mass of the animals (Peters, 1983; Calder, 1984). A trait that we can easily assume to be related with the body mass range in the present study (from 0.1 mg to nearly 100 mg) is the strength of the mandibles. In relation with this, a tentative explanation for the observed changes in size spectra might be that a leaf litter trait, the hardness of pine needles, can limit the resource acquisition to small animals. For their part, big shredders may be able to use needles as resource due to their large mandibles, their low mobility and low respiration rate (Friberg & Jacobsen, 1994; Campos & González, 2009). As Dossena *et al.* (2012) reported, this difference in shredder size structure may explain the functional alteration (lower capability to process leaf-litter in pine streams) observed previously (Chapter III) due to the lower consumption rates per unit of biomass by the large organisms than by small ones (Reiss *et al.*, 2011). Moreover, shredders, as other detritivores, can also support a big part of the secondary production of higher trophic levels (Vetter, 1994; Polis *et al.*, 1997; Woodward *et al.*, 2008). Since freshwater invertebrate predators are about an order of magnitude larger than their preys (Brose *et al.*, 2006) the shifts in shredders' size spectrum may be related with the alteration in the size structure of predators. The changes in the size spectra of these two feeding guilds are reflected in the entire invertebrate community and may affect animals out of the body mass range of the invertebrates studied, specifically affecting fish assemblages of streams. It is unsurprising to see no differences in the size spectra of basal consumers between deciduous and pine streams as we consider that limitations imposed by the strength of pine needles disappear when it is converted to fine particulate organic matter. Moreover, even if these consumers are mostly dependent on organic matter, their ability to exploit multiple energy pathways based on either living (e.g. benthic algae) and/or non-living (e.g. detritus) organic matter (Woodward *et al.*, 2005b) can enhance the stability of the assemblage.

Our study points out that the replacement of deciduous vegetation by pine plantations affects taxa richness, diversity, density and size spectrum of shredders in streams, proportional to the functional alteration (leaf litter processing) observed previously in these same streams (Chapter III). Although the current literature supports an effect of the lower nutrient concentration of the resource on the largest individuals (as suggested by Brown *et al.*, 2004), small individuals were more affected than big ones by the food changes caused by pine plantations. We suggest that a single trait, toughness of the material, may difficult the smallest individuals to use pine needles as resource and force a reduction of their density. We foresee that other single traits of resources might also be important shaping size spectra of consumers, with consequences to entire assemblages.

References

Albariño RJ & Balseiro EG, 2002. Leaf litter breakdown in Patagonian streams: native versus exotic trees and the effect of invertebrate size. Aquatic Conservation: Marine and Freshwater Ecosystems 12: 181–192.

Allen CR, Gaemestani AS, Havlicek TD, Marquet PA, Paterson GD, Restrepo C, Stow CA & Weeks BE, 2006. Patterns in body mass distributions: sifting among alternative hypotheses. Ecology Letters 9: 630–643.

Bärlocher F & Oertli JJ, 1978. Inhibitors of aquatic hyphomycetes in dead conifer needles. Archiv für Hydrobiologie 81: 462–474.

Baumgartner D & Rothhaupt KO, 2003. Predictive lengthdry mass regressions for freshwater invertebrates in a prealpine lake littoral. Internationale Revue der Gesamten Hydrobiologie 88: 453–463.

Benke AC, Huryn AD, Smock LA & Wallace JB, 1999. Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18: 308–343.

Blanchard J, Jennings S, Law R, Castle MD, McCloghrie P, Rochet M & Benoit E, 2009. How does abundance scale with body size in coupled size–structured food webs? Journal of Animal Ecology 78: 270–280.

Boersma M, & Kreutzer C, 2002. Life at the edge: Is food quality really of minor importance at low quantities? Ecology 83: 2552–2561.

Bourasa N & Morin A, 1995. Relationships between Size Structure of Invertebrate Assemblages and Trophy and Substrate Composition in Streams. Journal of the North American Benthological Society, 14: 193–403.

Brose U, Jonsson T, Berlow EL, Warren P, Banasek–Richter C, Bersier LF, et al., 2006. Consumer–resource body–size relationships in natural food webs. Ecology 87: 2411–2417.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB, 2004. Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Brown JH & Maurer BA, 1989. Macroecology: the division of food and space among species on continents. Science 243: 1145–1150.

Burgherr P & Meyer EI, 1997. Regression analysis of linear body dimensions *vs*. dry mass in stream macroinvertebrates. Archiv für Hydrobiologie 139: 101–112.

Calder WA, 1984. Size, function and life history. Harvard University Press, Cambridge, MA, USA.

Campos J & González JM, 2009. *Sericostoma vittatum* (Trichoptera) larvae are able to use pine litter as an energy source. International Review of Hydrobiology 94: 472–483.

Collen P, Keay EJ & Morrison BRS, 2004. Processing of pine (*Pinus sylvestris*) and birch (*Betula pubescens*) leaf material in a small river system in the northern Cairngorms, Scotland. Hydrology and Earth System Sciences, 8: 567–577.

Damuth J, 1981. Population density and body size in mammals. Nature, 290: 699–700.

Damuth J, 1987. Interspecific allometry of population density in mammals and other animals: the independence of body mass and population energy use. Biological Journal of the Linnean Society 31: 193–246.

Dossena M, Yvon–Durocher G, Grey J, Montoya JM, Perkins DM, Trimmer M & Woodward G, 2012. Warming alters community size structure and ecosystem functioning. Proceedings of the Royal Society B–Biological Sciences 279: 3011–3019.

Friberg N & Jacobsen D, 1994. Feeding plasticity of two detritivores shredders. Freshwater Biology 32: 133–142.

Gaston KJ & Blackburn TM, 2000. Pattern and Process in Macroecology. Blackwell Science, Oxford.

Girisha GK, Condron LM, Clinton PW & Davis MR, 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. Forest Ecology Management 179: 169–181.

Glazier D, 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biological Reviews 85: 111–138.

Jennings S & Mackinson S, 2003. Abundance–body mass relationships in size–structured food webs. Ecology Letters 6: 971–974.

Kerr SR & Dickie LM, 2001. The biomass spectrum: a predator–prey theory of aquatic production. New York, NY: Columbia University Press.

Kominoski JS, Marczak LB & Richardson JS, 2011. Riparian forest composition affects stream litter decomposition despite similar microbial and invertebrate communities. Ecology 92:151–159.

Larrañaga A, Basaguren A & Pozo J, 2009. Impacts of *Eucalyptus globulus* plantations on physiology and population densities of invertebrates inhabiting Iberian Atlantic streams. International Review of Hydrobiology 94: 497–511.

Merritt RW & Cummins KW, 2007. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company: Dubuque; 1158 pp.

Meyer E, 1989. The relationship between body length parameters and dry mass in running water invertebrates. Archiv für Hydrobiologie 117: 191–203.

Molinero J & Pozo J, 2004. Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation on the nutrient content and dynamics of coarse particulate organic matter (CPOM) in a small stream. Hydrobiologia, 528: 143–165.

Petchey OL & Belgrano A, 2010. Body–size distributions and size–spectra: universal indicators of ecological status? Biology Letters 6: 434–437.

Peters RH, 1983. The Ecological Implications of Body Size. Cambridge University Press, Cambridge.

Polis GA, Anderson WB & Holt RD, 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology, Evolution, and Systematics 28: 289–316.

Quinn JM, Smith BJ, Burrell GP and Parkyn SM, 2000. Leaf litter characteristics affect colonisation by stream invertebrates and growth of *Olinga feredayi* (Trichoptera: Conoesucidae). New Zealand Journal of Marine and Freshwater Research 34: 273–287.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07–0, URL http://www.R–project.org.

Reiss J, Bailey RA, Perkins DM, Pluchinotta A & Woodward G, 2011. Testing effects of consumer richness, evenness and body size on ecosystem functioning. Journal of Animal Ecology 80: 1145–1154.

Reuman DC, Mulder C, Banasek–Richter C, Blandenier MFC, Breure AM, Den Hollander H, Kneitel JM, Raffaelli D, Woodward G & Cohen JL, 2009. Allometry of Body Size and Abundance in 166 Food Webs. Advances In Ecological Research 41: 1–44.

Reuman DC, Mulder C, Raffaelli D & Cohen JE, 2008. Three allometric relations of population density to body mass: theoretical integration and empirical tests in 149 food webs. Ecology Letters 11: 1216–1228.

Riipinen MP, Fleituch T, Hladyz S, Woodward G, Giller P & Dobson M, 2010. Invertebrate community structure and ecosystem functioning in European conifer plantation streams. Freshwater Biology 55: 346–359.

Rosi–Marshall EJ & Wallace B, 2002. Invertebrate food webs along a stream resource gradient. Freshwater Biology 47: 129–141.

Schmid PE, Tokeshi M & Schmid–Araya JM, 2000. Relationship between population density and body size in stream communities. Science 389: 1557–1560.

Sheldon RW, Prakesh A & Sutcliffe WHJ, 1972. The size distribution of particles in the ocean. Limnology and Oceanography 17: 327–340.

Tachet H, Richoux P, Bournaud M & Usseglio–Polatera P, 2002. Invertébrés d'eau douce: systématique, biologie et écologie. CNRS: Paris; 587 pp.

Vetter EW, 1994. Hotspots of benthic production. Nature 372: 47–47.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277: 102–104.

Whiles MT. & Wallace JB, 1997. Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments. Hydrobiologia, 353: 107–119.

White EP, Enquist BJ & Green J, 2008. On estimating the exponent of power–law frequency distributions. Ecology 4: 905–912.

Woodward G, Ebenman B, Emmerson MC, Montoya JM, Olese, JM, Valido A & Warren P, 2005a. Body mass in ecological networks. Trends in Ecology and Evolution 20, 402–409.

Woodward G, Papantoniou G, Edwards F, & Lauridsen RB, 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117: 683–692.

Woodward G, Speirs DC & Hildrew AG, 2005b. Quantification and resolution of a complex, sizestructured food web. Advances in Ecological Research 36: 85–135.

Yvon-Durocher G, Montoya JM, Trimmer M & Woodward G, 2011a. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. Global Change Biology 17: 1681–1694.

Yvon-Durocher G, Reiss J, Blanchard J, Ebenman B, Perkins DM, Reuman DC, Thierr, A, Woodward G & Petchey O, 2011b. Across ecosystem comparisons of size structure: methods, approaches and prospects. Oikos 120: 550–563.

Temperature

Chapter V

Effects of temperature on leaf-litter decomposition in forested streams: in-stream and microcosm approaches

Abstract

Despite the predicted global warming to the end of the century, the effects of temperature on headwater streams functioning are yet scarcely studied. We tested the effects of temperature on leaf litter decomposition mediated by microbial activity and on the performance of fungal assemblages colonizing the material in streams. For this purpose, leaves of alder (*Alnus glutinosa*) were incubated in three streams differing in average winter water temperature (8.3, 10.5 and 11.6 ºC) to determine mass loss. Simultaneously, in the laboratory, leaf discs of *A. glutinosa*, preincubated in these three streams for fungal colonization, were incubated at three different temperatures (5, 10 and 15 ºC). We determined mass loss, nitrogen concentration, fungal sporulation rates, richness, diversity and evenness from these discs. We observed that leaf-litter processing rates correlated positively with water temperature both in the field and in the laboratory. Sporulation rates correlated also with temperature but not with decomposition rates. Fungal assemblage from the coldest stream differed from those in the other two streams with higher taxa richness, diversity and evenness. This coldest stream was the only that reduced the richness and diversity of the sporulating fungal assemblage with a rise in temperature. Our results point out that temperature is an important factor determining leaf litter processing and fungal assemblages. The response of the fungal assemblage suggests that temperature rises could affect more directly the fungal communities adapted to cold environments.

Key words: Temperature, Decomposition, Aquatic hyphomycetes, *Alnus glutinosa*.

Introduction

Detritus is a source of energy and nutrients for live organisms (Moore *et al.*, 2004) and its decomposition allows for the recycling of carbon and nutrients, thus fuelling new primary production (Swift *et al.*, 1979) and playing a critical role in ecosystem functioning (Polis & Strong, 1996). Some food webs are based almost entirely on detritus, such as those in low-order forested streams where leaf-litter inputs from surrounding forests represent the primary source of matter and energy (Wallace *et al.*, 1997). In these systems, the organisms responsible for the processing of detritus are microbial decomposers, primarily fungi (Bärlocher, 1992; Abelho & Graça, 2006), and invertebrate detritivores (Graça, 2001), which transfer the energy to higher trophic levels (Wallace *et al.*, 1997; 1999). Microorganisms use detritus as a carbon and nutrient source, but are able to complement their needs extracting nutrients from the water column (Suberkropp & Chauvet, 1995; Canhoto & Graça, 2008). The fixation of nutrients from the water to the detritus renders it more palatable for detritivores (Graça & Cressa, 2010). Microbial activity responds to several environmental conditions, such as the concentration of dissolved nutrients in water (Suberkropp & Chauvet, 1995; Gulis & Suberkropp, 2003a; b), the degree of oxygen saturation (Medeiros *et al.*, 2009) and pH (Dangles *et al.*, 2004). Temperature is obviously another important condition (Chauvet & Suberkropp, 1998; Ferreira & Chauvet, 2011a; b), since warming accelerates chemical reactions and enhances biological activities (Brown *et al.*, 2004; Davidson & Janssens, 2006; Davidson *et al.*, 2006), decomposition included (Knorr *et al.*, 2005; Cornelissen *et al.*, 2007; Pérez *et al.*, 2011). Decomposition releases CO_2 back into the atmosphere and, together with primary production, controls the carbon fluxes between the biosphere and the atmosphere (Cadish & Giller, 1997; Cornwell *et al.*, 2008). Therefore, relative changes in global decomposition and primary production rates might have implications on climate (Davidson & Janssens, 2006). Nevertheless, some processes might need to surpass certain thresholds of temperature rise to be noticed, and as the prediction of temperature rise by the end of this century is of 1.1–6.4 ºC in global temperature (IPCC, 2007), it is unclear the consequences that this increment can create on microbial communities and on decomposition.

Thus, the aim of this study was to assess the effects of temperature on leaf-litter decomposition mediated by microbial activity in headwater forested streams. For this, alder leaf litter was preincubated in three streams that differed in winter average temperatures (range: 4.9–9.8 ºC), and incubation was followed either in the field (in the same conditions of the preincubation) or in the laboratory in microcosms at three temperatures (5, 10 and 15 ºC). Apart from decomposition rates, we measured conidial production of aquatic hyphomycetes throughout the process. Although there are studies with a similar objective carried out in microcosms (Chauvet & Suberkropp, 1998; Dang *et al.*, 2009; Ferreira & Chauvet, 2011a; b), most of them use artificial microbial assemblages with a few species selected from pure cultures (but see Fernandes *et al.*, 2009). Moreover, we are not aware of studies that, using the same inoculum replicating simultaneously the decomposition experiment in the laboratory and in the field. We hypothesize that: 1) temperature will enhance leaf-litter decomposition and microbial activity; 2) the relationship of decomposition and microbial activity with temperature will be more difficult to detect in the field, as other environmental factors can also play an important role; 3) the response of decomposition and microbial activity to temperature will vary in accordance to the adaptations of the microbial communities derived from the thermal regime they are subject in the field.

Material and methods

Incubation of leaves and processing in streams

The incubation of alder leaves in the field was carried out in three headwater streams (S1, S2 and S3) with siliceous substrata flowing into the Atlantic Ocean (Cordillera Cantábrica, Northern Spain). They showed differences in their water temperature monitored from October 2010 to April 2011 (mean ± SE; S1: 4.9 ± 0.3 ºC; S2: 8.2 ± 0.6 ºC; S3: 9.8 ± 0.7 ºC; $n = 199$), inversely proportional to the altitude of each site (S1: 900 m; S2: 400 m; S3: 90 m above sea level). The three sites featured well oxygenated waters (average of 101–103%), a circum-neutral pH (6.8–7.3), low conductivity (< 100 µS cm^{-1}) and low concentrations of dissolved phosphorus (S1: 19 ± 5 µg P L^{-1}; S2: 12 ± 4 µg P L^{-1}; S3: 18 ± 5 µg P L^{-1}) and nitrogen (S1: 159 ± 54 µg N L^{-1}, S2: 786 ± 75 µg N L^{-1}; S3: 744 ± 64 µg N L^{-1}).

In October 2010 leaves of *Alnus glutinosa* (L.) Gaertner were collected from the forest floor just after natural abscission. Approximately 4 g (± 0.25) of air-dried leaves were placed into mesh bags (dimensions 15 x 20 cm, 0.5-mm mesh size). On the 23th of February 22 bags were fastened to anchored bars in the stream benthos at each site. After 27 days of incubation, 17 bags per site were transported to the laboratory. Leaves were rinsed with stream water (filtered through a 0.2-mm mesh sieve) on a 0.5-mm mesh sieve. The material from 5 bags per stream was oven-dried to obtain dry mass (70 ºC, 72 h); subsamples from each bag were used for elemental analyses (C, N) and the rest ashed (500 ºC, 4 h) to determine the remaining ash free dry mass (AFDM). Leaf carbon and nitrogen were determined using a Perkin Elmer series II CHNS/O elemental analyzer. From the other 12 bags per stream, 20 pairs of leaf-discs (one pair per leaf; 20 mm diam.) per bag were punched out with a cork borer for later utilization in the microcosm experiment (see below). A set of 20 discs was used to estimate the initial AFDM (15 discs) and elemental composition (5 discs) included in each glass jar of the microcosm experiment (see below). After incubating 54 days in the streams, 27 days after the first bags were retrieved, the remaining 5 bags were collected from each stream. In the laboratory, the same methodologies as used for the 5 bags of the first sampling was used to obtain dry mass, AFDM and elemental composition.

Microcosm assay

Three 36-L tanks were set up as water baths at three different temperatures (5, 10 and 15 ºC). Twelve microcosms (4 replicas from each stream) were placed (Fig. 1) in each tank. The microcosms consisted in 370-mL glass jars containing 200-mL filtered (0.2 µm) water from a different siliceous stream (pH: 7.8 ± 0.17; conductivity: 159 µS cm^{-1} ± 3; SRP: 18 ± 7 µg P L^{-1}; DIN: 204 ± 46 µg N L^{-1}), which were constantly aerated under a light/dark regime of 12:12. All glass and plastic material used was previously autoclaved. In each microcosm the second set of 20 leaf-discs was introduced and incubated for 27 days. Water was renewed every 3 days. On three occasions (9, 18 and 27 days of incubation in microcosm) a water volume of 25 mL was collected from each jar to sample the hyphomycete conidia suspension, fixed with 2 mL 37% formalin and stained with a couple of drops of trypan blue in lactic acid (0.05%). An aliquot of the suspension was filtered (Millipore SMWP 5 µm pore size) and stained for conidial identification and quantification under microscope (500X magnification).

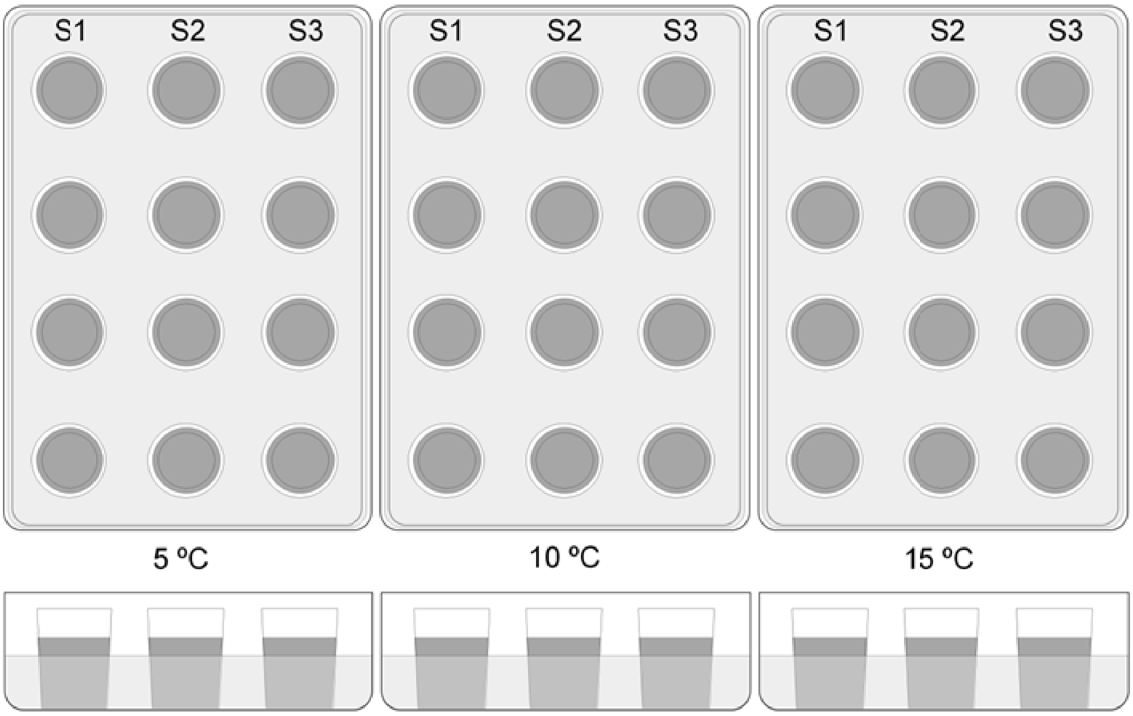


Figure 1. Top and lateral view of microcosms.

Sporulation rates were expressed as number of conidia per mg dry mass and day of incubation (no. conidia mg dry mass^{-1} day^{-1}). After 27 days of incubation, coinciding with the retrieval of bags in the field, each set of 20 leaf-discs were oven-dried to obtain dry mass (70 ºC, 72 h). Five dried discs were preserved (-20 ºC) for elemental analyses following the procedure explained above and the others were ashed (500 ºC, 4h) to determine remaining AFDM.

Statistical analyses

Water temperature comparison among streams was performed with one-way ANOVA considering daily mean temperature as replica. The leaf mass losses in the stream were transformed into linear rates by the equation (M_0 - M_t/t), where M_0 is the remaining mass estimated after the first 27 days of incubation, M_t the remaining mass at the end of incubation (54 days since implantation) and t the time of the incubation (27 days). The leaf-disc decomposition rate in the microcosms were computed with the same equation, in which the initial AFDM, M_0, was obtained for each microcosm from the 20 discs set aside at the beginning of the trial; M_t was the final mass and t the time of incubation in microcosms (27 days). The rates were calculated also in terms of degree-days in order to standardize them by incubation temperature. Differences of breakdown rates (in terms of days and degree-days) and leaf nitrogen concentration of the material incubated in the three streams were tested with one-way ANOVA, with stream as factor. In microcosms, differences of decomposition rates in terms of days and leaf nitrogen concentration of the material incubated in the different streams were tested with one-way ANCOVA (factor: stream), including the temperature of incubation as covariate to test for its effect on decomposition and leaf nitrogen concentration. Differences in decomposition rates based in degree-days among temperature treatments in the microcosms were compared with two-way ANOVA (factors: stream and treatment – cold, intermediate and warm–). Bivariate relationships were tested by ordinary least square linear regressions. Sporulation rates were compared with two-way ANCOVA (factors: stream and sampling date) with water temperature as covariant. To search for general differences in fungal assemblages among treatments in the microcosm study, a non-metric multidimensional scaling (NMDS) was performed based in the Bray-Curtis dissimilarity matrix, followed by PERMANOVA (10^6 permutations) to test if there were differences between source stream and/or between incubation temperature in microcosm. When necessary, data were transformed (log (x+1)) to obtain requirements for parametric analyses. All statistical analyses were conducted using R statistical software (version 2.11.1; R Development Core Team, 2010).

Results

Leaf-litter processing in the streams

During the 27 days of in-stream incubation (24th March–19th April 2011, coinciding with the microcosm assays), only a range of 3.2 ºC was observed for the average temperature among streams, but it still differed significantly (mean ±SE; S1: 8.3 ± 0.3 ºC; S2: 10.5 ± 0.3 ºC; S3: 11.6 ± 0.3 ºC; ANOVA: $F_{2,78}$ = 31.93, $p < 0.001$). The slowest decomposition rate was measured in the coldest stream (S1) and the fastest rate in the warmest one (S3) (Fig. 2; ANOVA: $F_{2,11}$ = 12.303, $p = 0.002$), with a significant positive relationship between decomposition rate and water temperature (Fig. 2; R = 0.99; $p = 0.006$; $n = 3$). These differences did not disappear after standardizing decomposition rates by accumulated heat, calculated on a degree-days basis (ANOVA: $F_{2,11}$ = 9.44, $p = 0.004$); S1 still showed the slowest (0.019% AFDM degree-days^{-1}) and S3 the fastest rate (0.089% AFDM degree-days^{-1}). Although the nutritional quality of leaf-litter incubated (Day 0) in the three streams was the same (Table 1), the N concentration in the leaves recovered after 27 days varied among streams (Table 1; ANOVA: $F_{2,12}$ = 4.872, p = 0.028). The material from S2 showed the highest nitrogen percentage and S1 the lowest. In the second sampling (Day 54), the material from S3 showed higher increases in N than those from the other two streams, and S1 remained with the lower values (Table 1; ANOVA: $F_{2,11}$ = 9.954, $p = 0.003$). In general, leaf-N

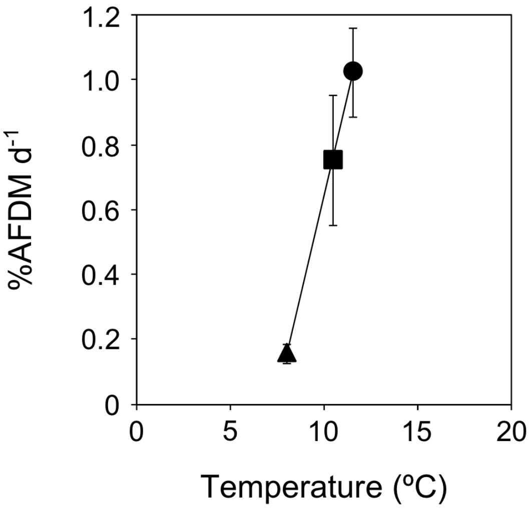


Figure 2. Relationship of decomposition rates (% AFDM d^{-1}; mean ± SE) with temperature in the field experiment. Streams are identified as follows: S1 pyramids, S2 squares and S3 circles.

concentration showed a positive trend with water temperature and the availability of dissolved inorganic nitrogen. Decomposition rate showed a similar positive trend with dissolved nitrogen. In the same way, decomposition rates were positively correlated with leaf-N concentration at the end of incubation period in stream (R = 0.99; p = 0.009; n = 3).

Table 1. Percentage of leaf-N during in-stream incubation. Letters represent statistical differences (ANOVA and Tukey post-hoc).

Stream	Day 0	Day 27	Day 54
S1	3.08	3.35^{b}	3.44^{b}
S2	3.08	3.68^{a}	3.82^{a}
S3	3.08	3.58^{ab}	4.00^{a}

Leaf-disc processing and fungal assemblages in the microcosms

During the 27 days of incubation in the microcosm (24th March–19th April 2011) leaf-disc decomposition rates did not differ statistically among the materials preconditioned in the three streams (Fig. 3a; ANCOVA: $F_{2,30}$ = 0.28, p = 0.756). In general, rates were slowest at 5 ºC showing a significant positive relationship with incubation temperature (Fig. 3b; ANCOVA: $F_{1,30}$ = 6.15, p = 0.019), although the slope of the relationship was much flatter for the incubation in microcosms than the incubation in the field (see Figs. 2 and 3b). The relationship between temperature and decomposition did not change significantly among sites (ANCOVA, interaction: $F_{2,30}$ = 0.37, p = 0.691). The decomposition rates standardized by accumulated heat (degree-days) were again different among the three temperatures (ANOVA: $F_{2,27}$ = 9.61, p < 0.001), but contrarily to the rates on a day basis, the rates were higher at 5 ºC (0.090% AFDM degree-days^{-1}) than at 10 ºC (0.062% AFDM degree-days^{-1}) and 15 ºC (0.045% AFDM degree-days^{-1}). The initial nitrogen content of leaf discs (after 27 days of incubation in streams) incubated in microcosms was different among sets from the three streams (Fig. 3c; ANOVA: $F_{2,11}$ = 12.03, p = 0.002). Discs from S2 presented higher N content (4.50%) than those from S3 and S1 (4.10 and 3.87% respectively). After incubation period in microcosms, the N concentrations of discs from S1 at the three temperatures and discs from S3 at 15 ºC were higher than the initial one. Discs from S1 showed the highest N concentration and those from S3 the

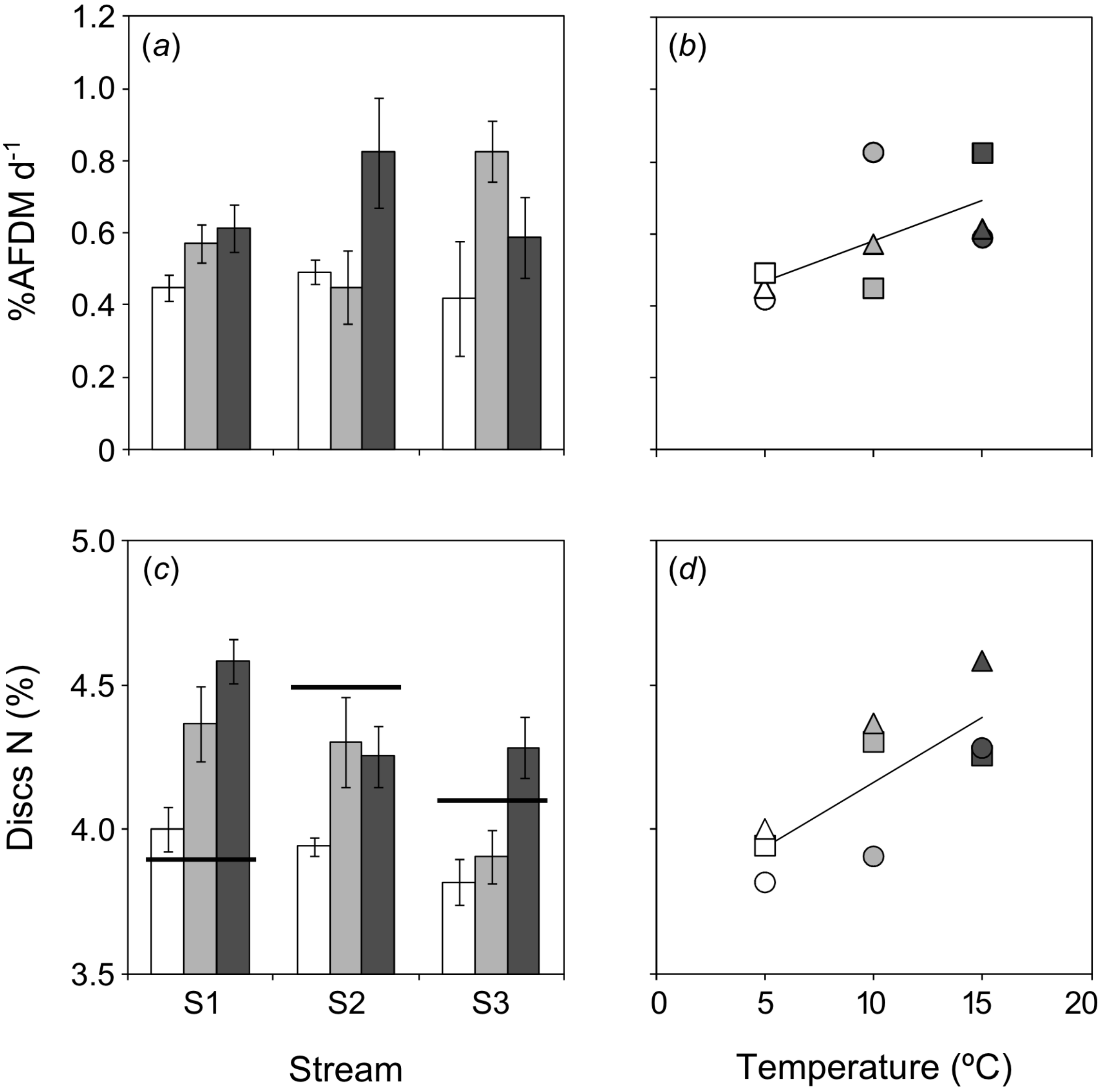


Figure 3. Decomposition rates (% AFDM d^{-1}; mean ± SE) (*a*), and N-concentration of leaf-discs at the beginning (black lines) and at the end of incubation period (bars) in microcosms (*c*) for each stream of origin and each temperature of incubation. Relationships between processing rates (*b*), and N-concentration of leaf-discs incubated in microcosms (*d*) with temperature are also shown. Incubation temperatures in microcosms are identified as follows: 5 ºC white, 10 ºC grey and 15 ºC dark grey, whereas streams are identified as follows: S1 pyramid, S2 square and S3 circle.

lowest (Fig. 3c; ANCOVA: $F_{2,30}$ = 7.01, p = 0.003). The N concentration of the material at the end of the incubation period in microcosms related positively to temperature (Fig. 3d; ANCOVA: $F_{1,30}$ = 28.98, p < 0.001), with no significant interaction detected in this relationship (ANCOVA: $F_{2,30}$ = 0.87, p = 0.428). However, in contrast with that observed in the field, decomposition rates did not correlate with nitrogen concentration of discs (R = 0.16; p = 0.679; n = 9).

The sporulation rates were different among streams (Table 2; ANCOVA: $F_{2,90}$ = 17.88, p < 0.001), the assemblage from S3 presenting the lowest values.

Conidia production differed also among sampling dates (Fig. 4; ANCOVA: $F_{2,90} = 61.90$, $p < 0.001$), peaking in day 9 of the incubation. Conidia production covaried significantly with temperature in an analysis including the three samplings (ANCOVA: $F_{1,90} = 7.55$, $p = 0.007$), however the direction of the relationship varied among the three sampling dates. While in the first sampling (after 9 days of incubation in the microcosms) the relationship was positive, in the other two sampling dates (18 and 27 days) it was negative (Fig. 4). Mean sporulation rates of the three sampling dates were not correlated with decomposition rates. Only when considering the first sampling date alone (day 9) the same relationship ($R = 0.87$, $p = 0.002$, $n = 9$) was observed. A total of 34 species of aquatic hyphomycetes were identified in the microcosms (Table 2). Independently of the incubation temperature, the material preincubated in the coldest stream, S1, showed more species than that from S2 and S3 (Table 2). Seven species were exclusively found in S1, only one was exclusive from S2 and three from S3. The most evident relationship between species richness and temperature was found in the discs coming from S1, in which richness seemed to display a negative relationship with incubation temperature (Table 2). Aditionally, in material preincubated in this stream the diversity and evenness were highest and there was not a clear dominant species, i.e. five species (*Articulospora tetracladia*, *Flagellospora curvula*, *Heliscus lugdunensis*, *Lemonniera alabamensis* and *Lemonniera aquatica*) were co-dominant in the conidial assemblage, representing each one >10% of the total sporulation rates. In contrast, in material from S2 and S3 only two species displayed higher than 10% of total sporulation, *F. curvula* being the species that highest number of spores produced, followed by *A. tetracladia*. Only in discs preconditioned in the warmest stream, S3, and incubated at 5 ºC this trend was the opposite, *A. tetracladia* being dominant (Table 1). The two-dimensional non-metric MDS (Fig. 5) and the PERMANOVA analyses showed higher differences in the assemblages among the three different source streams (PERMANOVA: $PseudoF_{2,27} = 9.31$, $p < 0.001$) than among incubation temperature in microcosm (PERMANOVA: $PseudoF_{2,27} = 2.25$, $p = 0.017$). The coldest stream, S1, differed most and appeared separated from the samples of the other two streams (Fig. 5).

Table 2. Contribution (%) of aquatic hyphomycete taxa to mean total conidial production, sporulation rate, taxa richness, Shannon diversity and Pielou evenness in microcosm. Data for the five predominant taxa (>10%) for each treatment are highlighted in bold. Segregated forms * and aggregated taxa entries ** (see explanation in Pozo *et al*., 2011).

Stream	**S1**			**S2**			**S3**		
Temperature (ºC)	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Alatospora acuminata “subulate” *	0.7	0.6	0.1	0.2	0.4	0.2		0.1	<0.1
A. acuminata “pulchelloid” *	0.8	0.4	1.2	0.4	0.5	0.2	0.5	0.8	0.1
A. acuminata (neotype) *	2.1	1.0	0.5	1.3	4.2	1.0	2.5	1.0	0.6
A. pulchella Marvanová	0.2	<0.1	<0.1				0.1		
Anguillospora crassa Ingold			<0.1						
A. filiformis Greathead	0.6	1.2	0.6	1.2	2.4	1.0	9.4	9.7	0.9
A. longissima (Sacc. & Syd.) Ingold		<0.1			<0.1	0.1	0.1	0.0	<0.1
A. tetracladia Ingold	**24.1**	**20.0**	**30.1**	**14.3**	**25.3**	**10.2**	**51.6**	**16.2**	**15.9**
Clavariopsis aquatica De Wildeman	0.9	3.2	0.3	0.1	0.3	<0.1	0.0	0.1	
Culicidospora aquatica R.H. Petersen	1.4	1.1	0.6				0.1		0.1
Dendrospora erecta Ingold	<0.1								
Flagellospora curvula Ingold	**30.9**	**22.6**	**17.1**	**79.7**	**60.6**	**79.9**	**25.6**	**58.2**	**62.3**
Fontanospora eccentrica (R.H. Petersen) Diko	0.2	0.1					<0.1	0.1	<0.1
Goniopila/Margaritispora **							<0.1	0.2	<0.1
Gyoerffyella oxalidis Vanev	<0.1								
Heliscella stellata (Ingold & Cox) Marvanová								0.3	
Heliscus lugdunensis Sacc. & Théry	**11.2**	**18.3**	**22.7**	1.0	3.4	2.4	2.0	1.7	1.1
Lemonniera alabamensis Sinclair & Morgan-Jones	**14.0**	5.1	**14.0**	0.2				<0.1	
L. aquatica De Wildeman	**10.5**	**25.3**	**11.5**	0.2	<0.1		0.2		<0.1
L. terrestris Tubaki							<0.1		<0.1
Lunulospora curvula Ingold		<0.1				0.4		5.5	9.9
Mycofalcella calcarata Marvanová, Om-Kalth. & Webster	0.9	0.4	0.1				<0.1	<0.1	

Stenocladiella neglecta Marvanová & Descals	0.1	0.1	<0.1						
Tetrachaetum elegans Ingold	<0.1			0.8	2.4	3.9	0.3	3.4	4.8
Tetracladium furcatum Descals	0.1		0.1						
T. maxilliforme (Rostr.) Ingold			<0.1						
T. setigerum (Grove) Ingold	0.2	<0.1	0.1						
Tricladium angulatum Ingold						<0.1			
T. attenuatum Iqbal	0.1	0.1		0.2			0.1		
T. biappendiculatum (Arnold) Vanev	<0.1	<0.1							
T. chaetocladium Ingold	<0.1			0.2	0.4	0.6	7.4	2.7	4.2
T. splendens Ingold	0.1	<0.1	0.1		<0.1	<0.1			
Varicosporium elodeae Kegel	0.4	0.2	0.9	0.1		<0.1			
Variocladium giganteum (Iqbal) Descals & Marvanová	0.1	<0.1	0.1						<0.1
Mean sporulation rate (no. conidia g^{-1} d^{-1})	39.3	85.4	99.4	49.8	122.0	58.6	18.6	25.2	65.9
Richness	26	23	21	14	13	15	18	17	17
Shannon diversity	2.74	2.67	2.55	1.09	1.69	1.17	1.98	2.04	1.79
Pielou evenness	0.58	0.59	0.58	0.29	0.46	0.30	0.47	0.50	0.44

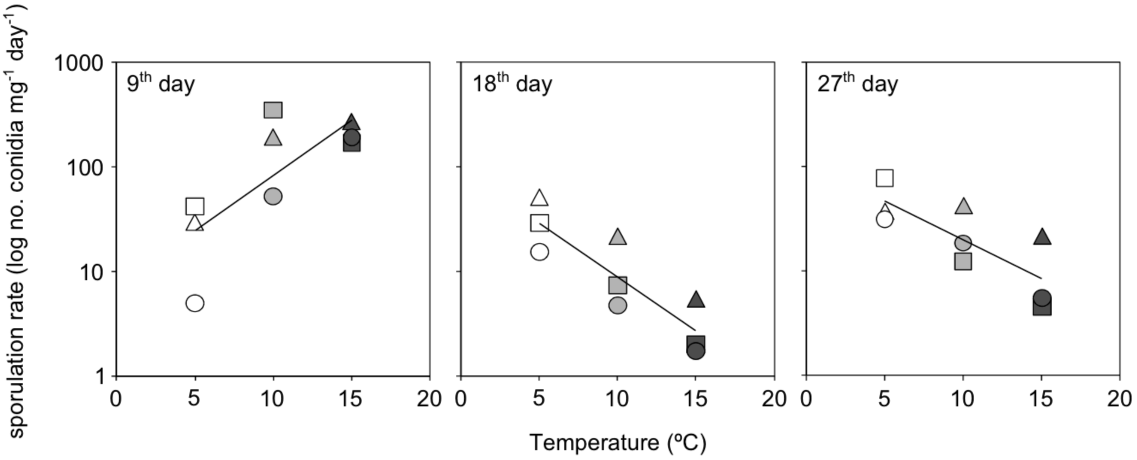
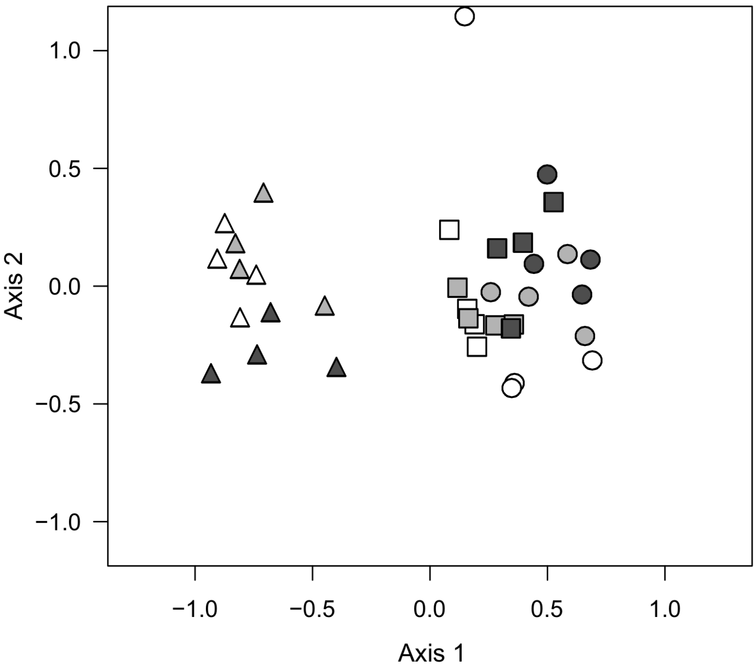


Figure 4. Relationship between sporulation rates ($\log_{10}$ no. conidia mg^{-1} day^{-1}) and temperature (5 ºC: white, 10 ºC: grey and 15 ºC: dark grey) on leaf-discs incubated in microcosms for the three sampling dates. Streams are identified as follows: S1: pyramids, S2: squares and S3: circles.

Discussion

In the present work, temperature seems to drive leaf-litter processing rates, as very good positive fits were obtained between both variables. Microbial decomposition rates increments have been linked to higher temperature (Ferreira & Chauvet, 2011a; Ferreira *et al.*, 2012) that stimulated fungal production (Chauvet & Suberkropp, 1998; Dang *et al.*, 2009). Besides, the still patent differences among the rates of the different streams after the standardization with degree-days suggest the intervention of other factors in the process. Many other environmental factors, such as the physico-chemical properties of the water could affect leaf-litter processing and fungal performance (Duarte *et al.*, 2009; Pérez *et al.*, 2012). In fact, the relationship with temperature may not be so direct and could be conditioned by synergetic effects of other factors such as dissolved nutrients (Ferreira & Chauvet, 2011b). It is well known that fungal activity is enhanced by nutrient availability in waters (Suberkropp & Chauvet, 1995; Abelho & Graça, 2006; Benstead *et al.*, 2009), even under oligotrophic conditions (Pérez *et al.*, 2012) as occurs in the present study. In fact, since materials had a similar quality at the beginning of the experiment and fungal mycelium seems to show a higher nitrogen concentration than the leaves themselves



◄**Figure 5.** Non-metric multidimensional scaling ordination (NMDS) of fungal assemblages taking into account the average spore production of all the taxa (average of three samplings). Incubation temperatures in microcosms are identified as follows: 5 ºC white, 10 ºC grey and 15 ºC dark grey, whereas streams are identified as follows: S1 pyramid, S2 square and S3 circle.

(Cross *et al.*, 2005), the increase of leaf-litter quality (N concentration) after the field incubation in the present work might be attributed to the fungal growth promotion (Canhoto & Graça, 2008; Webster *et al.*, 2009), related to the N availability in water. In the field experiment, the dissolved nitrogen roughly covaried with the average water temperature in our sites, and thus, we cannot discard that, dissolved nutrients in water might also have played an important role in determining the N-concentration and the decomposition rates. In the microcosms, we detected a clear sign of response by microbiota to nutrient availability in the final N-concentration measured: the materials coming from the poorest stream in terms of dissolved nutrients (S1, also the coldest) enhanced their N-concentration more than the other two sites.

Sporulation rates in our study (after 5–8 weeks incubation) presented lower values than those usually reported for sporulation peaks (after 2–4 weeks incubation; Gessner, 1997; Schlickeisen *et al.*, 2003; Pérez *et al.*, 2012). The sporulation rates of hyphomycetes seem to be affected by temperature, as clear relationships were observed between both variables in the three samplings. Other authors (Chauvet & Suberkropp, 1998; Rajashekar & Kaveriappa, 2000; Ferreira & Chauvet, 2011a) have reported that fungal sporulation rates are stimulated by an increase in temperature, a result only observed in the sampling after 9 days of incubation in microcosms of the present study. Nevertheless, in the consecutives sampling dates (18 and 27 days) the effect was the opposite. Thus, low temperatures seem to maintain a constant spore production through the decomposition process. This might have functional consequences in the field, as the success in the colonization of new substrata can be a function of spore production and the time interval in which a relatively high production of spores is maintained. A positive relationship between conidial production and decomposition rate has been reported in some cases (e.g. Maamri *et al*., 2001; Pérez *et al*., 2012) but not in others (e.g. Pozo *et al*., 2011; Mendoza-Lera *et al*., 2012). In the present study, where fungal sporulation was examined on leaves incubated in microcosms, mean sporulation rate did not present significant relationship with the corresponding decomposition rate.

Temperature influences metabolic activity (Brown *et al*., 2004), but also plays a key role determining community structure (Mouthon & Daufresne, 2006), species distribution (Castella *et al*., 2001) and interspecific relationships (Jiang & Morin, 2007). Hyphomycete assemblages in microcosms were more different among source streams than among incubation temperature in richness, evenness and diversity. Together with water chemistry (Duarte *et al*., 2009), the differences in altitude and water temperature among the three studied sites seem to determine or at least influence hyphomycete assemblages, as other authors suggested (Suberkropp, 1984; Gonczol *et al*., 2003; Rajashekhar & Kaveriappa, 2003; Belwal *et al*., 2008). Bärlocher *et al.* (2008) observed that warming affected negatively fungal richness. Efectively, in the present study, the coldest site presented the richest assemblage of fungal taxa. This same negative relationship between temperature and fungal richness was evident in microcosms, but only in assemblages from the coldest stream (S1). This different response among assemblages from the three streams could be due to temperature regime since, as Wallenstein & Hall (2012) suggested, it could play a role in how microbial communities can adapt to rapid environmental shifts. However, caution is needed, as changes operate on species with low spore production and in both directions: some of them disappeared but other appeared at higher temperature incubations (see Table 2). The difference in species response to warming could critically influence community structure (Walther *et al*., 2002; Thuiller *et al*., 2005) and have consequences not only for species distributions but also for critical ecosystem processes such as litter decomposition (Dang *et al*., 2009). Nevertheless, contrary to what was observed by Duarte *et al.* (2006) and Pérez *et al.* (2012), taxa richness was not followed by leaf-litter decomposition rates, which can be explained by the attributed high ecological redundancy of hyphomycete species (Dang *et al*., 2005; Pascoal *et al*., 2005). As usual, few taxa were responsible for most of the conidial production (e.g. Chauvet *et al*., 1997; Fernandes *et al*., 2009; Pérez *et al*., 2012). Although changes in dominance induced by temperature have been already observed in hyphomycete assemblages (Bärlocher *et al*., 2008; Dang *et al*., 2009), we did not find a remarkable alteration in microcosms. Independently of incubation temperature, the fungal assemblage from the coldest stream (S1) showed a higher diversity, evenness and number of co-dominant species than those from the other two streams. This manifests the importance of early fungal colonizers determining the composition of fungal assemblages on decomposing leaves (Sridhar *et al*., 2009; Ferreira & Chauvet, 2011b). Additionally, the incubation in microcosms at higher temperatures (10 and 15 ºC) only reduced the richness and the diversity of the fungal assemblages of the coldest stream. This suggests that fungal assemblages from cold headwater streams are more sensitive to the predicted global rise of water temperature. Since detritivore activity may be enhanced by the presence or dominance of certain fungal species (Suberkropp *et al*., 1983; Arsuffi & Suberkropp, 1984), a change of the structure of fungal assemblages might affect detritus decomposition.

Our results support that temperature enhances leaf-litter decomposition by the mediation of microbial activity. However, this must be stated carefully since synergisms with other factors, such as nutrients in the water, have also shown to contribute to the observed patterns. Moreover, it seems that temperature affects more directly to communities adapted to cold environments, with shifts in the structure of fungal communities if the predicted global temperature rises happen. Ultimately, changes in water temperature could have repercussions in the carbon flux at global scale.

References

Abelho M & Graça MAS, 2006. Effects of nutrient enrichment on decomposition and fungal colonization of sweet chestnut leaves in an Iberian stream (Central Portugal). Hydrobiologia 560: 239–247.

Arsuffi TL & Suberkropp K, 1984. Leaf processing capabilities of aquatic hyphomycetes: interspecific differences and influence on shredder feeding preferences. Oikos 42: 144–154.

Bärlocher F, 1992. The Ecology of Aquatic Hyphomycetes. Springer: Berlin, Germany.

Bärlocher F, Seena S, Wilson KP, Williams DD, 2008. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. Freshwater Biology 53: 368–379.

Belwal M, Sati SC & Arya P, 2008. Temperature tolerance of water borne conidial fungi in freshwater streams of Central Himalaya. National Academy Science Letters–India 31: 175–179.

Benstead JP, Rosemond AD, Cross WF, Wallace JB, Eggert SL, Suberkropp K, Gulis V, Greenwood JL & Tant CJ, 2009. Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. Ecological Society of America 90: 2556–2566.

Brown JH, Gillooly JF, Allen AP, Savage VM & West GB, 2004. Toward a metabolic theory of ecology. Ecology 85: 1771–1789.

Cadish G & Giller KE, 1997. Driven by Nature: Plant Litter Quality and Decomposition. Wallingford: CAB Int. 432 pp.

Canhoto C & Graça MAS, 2008. Interactions between fungi and stream invertebrates: back to the future. In: Novel Techniques and Ideas in Mycology (eds. Sridhar S, Bärlocher F & Hyde KD). Fungal Diversity Research Series 20: 205–325.

Castella E, Adalsteinsson H, Britain JE, Gislason GM, Lehmann A, Lencioni V, Lods–Crozet B, Maiolini B, Milner AM, Olafsson JS, Saltveit SJ & Snook DL, 2001. Macrobenthic invertebrate richness and composition along a latitudinal gradient of European glacier–fed streams. Freshwater Biology, 46, 1811–1831.

Chauvet E, Fabre E, Elosegui E & Pozo J, 1997. The impact of eucalypt on the leaf associated aquatic hyphomycetes in Spanish streams. Canadian Journal of Botany 75: 880–888.

Chauvet E & Suberkropp K, 1998. Temperature and sporulation of aquatic hyphomycetes. Applied and Environmental Microbiology 64: 1522–1526.

Cornelissen JHC, van Bodegom PM, Aerts R, Callaghan TV, van Logtestijn RSP, Alatalo J, Chapin FS, Gerdol R, Gudmundsson J, Gwynn–Jones D, Hartley AE, Hik DS, Hofgaard A, Jónsdóttir IS, Karlsson S, Klein JA, Laundre J, Magnusson B, Michelsen A, Molau U, Onipchenko VG, Quested HM, Sandvik SM, Schmidt IK, Shaver GR, Solheim B, Soudzilovskaia NA, Stenström A, Tolvanen A, Totland Ø, Wada N, Welker JM, Zhao X & Team OL., 2007. Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. Ecology Letters 10: 619–627.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez–Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Díaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV & Westoby M, 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecologu Letters 11: 1065–1071.

Cross WF, Benstead JP, Frost PC & Thomas SA, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology 50: 1895–1912.

Dang CK, Chauvet E & Gessner MO, 2005. Magnitude and variability of process rates in fungal diversity–litter decomposition relationships. Ecology Letters 8: 1129–1137.

Dang CK, Schindler M, Chauvet E & Gessner MO, 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90: 122–132.

Dangles O, Gessner MO, Guerold F & Chauvet E, 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. Journal of Applied Ecology 41: 365–378.

Davidson EA & Janssens IA, 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440: 165–173.

Davidson EA, Janssens IA & Luo YQ, 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q10. Global Change Biology 12: 154–164.

Duarte S, Pascoal C, Cássio F & Bärlocher F, 2006. Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. Oecologia 147: 658–667.

Duarte S, Pascoal C, Garabétian F, Cássio F & Charcosset JY, 2009. Microbial decomposer communities are mainly structured by trophic status in circumneutral and alkaline streams. Applied and Environmental Microbiology 75: 6211–6222.

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: 410–419.

Ferreira V & Chauvet E, 2011a. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia 67: 279–291.

Ferreira V & Chauvet E, 2011b. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17: 551–565.

Ferreira V, Gonçalves AL & Canhoto C, 2012. Aquatic hyphomycete strains from metal–contaminated and reference streams might respond differently to future increase in temperature. Mycologia 104: 613–622.

Gessner MO, 1997. Fungal biomass, production and sporulation associated with particulate organic matter in streams. Limnetica 13: 33–45.

Gonczol J, Csontos P & Revay A, 2003. Catchment scale patterns of aquatic hyphomycetes. The role of physicochemical variables and substrate composition in structuring conidial communities. Archiv Fur Hydrobiologie 157: 249–266.

Graça MAS, 2001. The role of invertebrates on leaf litter decomposition in streams – A review. International Review of Hydrobiology 86: 383–393.

Graça MAS & Cressa C, 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. International Review of Hydrobiology 95: 27–41.

Gulis V & Suberkropp K, 2003a. Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. Microbial Ecology 45: 11–19.

Gulis V & Suberkropp K, 2003b. Leaf litter decomposition and microbial activity in nutrient enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123–134.

IPCC (Intergovernmental Panel on Climate Change), 2007. Climate Change 2007: The Physical Science Basis. Contribution of the Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Jiang L & Morin PJ, 2007. Temperature fluctuation facilitates coexistence of competing species in experimentalmicrobial communities. Journal of Animal Ecology 76: 660–668.

Knorr W, Prentice IC, House JI & Holland EA, 2005. Long–term sensitivity of soil carbon turnover to warming. Nature 433: 298–301.

Maamri A, Bärlocher F, Pattee E & Chergui H, 2001. Fungal and bacterial colonization of Salix pedicellata leaves decaying in permanent and intermittent streams in Eastern Morocco. International Review of Hydrobiology 86:337–349.

Medeiros AO, Pascoal C & Graça MAS, 2009. Diversity and activity of aquatic fungi under low oxygen conditions. Freshwater Biology 54: 142–149.

Mendoza–Lera C, Larrañaga A, Pérez J, Descals E, Martínez A, Moya O, Arostegui I and Pozo J, 2012. Headwater reservoirs weaken terrestrial–aquatic linkage by slowing leaf–litter processing in downstream regulated reaches. River Research and Applications 28: 13–22.

Moore JC, Berlow EL, Coleman DC, de Ruiter PC, Dong Q, Hastings A, Collins Johnson N, MacCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ & Wall DH. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7: 584–600.

Mouthon J & Daufresne M, 2006. Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: a large lowland river and of its two main tributaries (France). Global Change Biology 12: 441–449.

Pascoal C, Cássio F & Marvanová L, 2005. Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low–order stream. Archiv Fur Hydrobiologie 162:481–497.

Pérez J, Menéndez M, Larrañaga S & Pozo J, 2011. Inter– and Intra–Regional Variability of Leaf Litter Breakdown in Reference Headwater streams of Northern Spain: Atlantic versus Mediterranean streams. International Review of Hydrobiology 96: 105–117.

Pérez J, Descals E & Pozo J, 2012. Aquatic Hyphomycete Communities Associated with Decomposing Alder Leaf Litter in Reference Headwater Streams of the Basque Country (northern Spain). Microbial Ecology 64: 279–290.

Polis GA & Strong DR, 1996. Food web complexity and community dynamics. The American Naturalist 147: 813–846.

Pozo J, Casas J, Menéndez M, Mollá S, Arostegui I, Basaguren A, Casado C, Descals E, García–Avilés J, González JM, Larrañaga A, López E, Lusi M, Moya O, Pérez J, Riera T, Roblas N & Salinas MJ, 2011. Leaf–litter decomposition in headwater streams: a comparison of the process among four climatic regions. Journal of North American Benthological Society 30: 935–950.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajashekhar M & Kaveriappa KM, 2003. Diversity of aquatic hyphomycetes in the aquatic ecosystems of the Western Ghats of India. Hydrobiologia 501: 167–177.

Schlickeisen E, Tietjen TE, Arsuffi TL, Groeger AW (2003) Detritus processing and microbial dynamics of an aquatic macrophyte and terrestrial leaf in a thermally constant, spring–fed stream. Microb Ecol 45:411–419.

Sridhar KR, Duarte S, Cássio F & Pascoal C, 2009. The role of early fungal colonizers in leaf–litter decomposition in Portuguese streams impacted by agricultural runoff. International Review of Hydrobiology 94: 399–409.

Suberkropp, K., 1984. Effect of temperature on seasonal occurrence of aquatic hyphomycetes. Transactions of the British Mycological Society 82: 53–62.

Suberkropp K, Arsuffi TL, Anderson JP (1983) Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Applied Environmental Microbiology 46: 237–244.

Suberkropp K & Chauvet E, 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76: 1433–1445.

Swift MJ, Heal OW & Anderson JM, 1979. Decomposition in Terrestrial Ecosystems. University of California Press, Berkeley, CA, USA.

Thuiller W, Lavorel S, Araujo MB, Sykes MT & Prentice IC, 2005. Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences of the USA 102: 8245–8250.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277: 102–104.

Wallace JB, Eggert SL, Meyer JL & Webster JR, 1999. Effects of resource limitation on a detrital–based ecosystem. Ecological Monographs 69: 409–442.

Wallenstein MD & Hall EK, 2012. A trait–based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109: 35–47.

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh–Guldberg O & Bairlein F, 2002. Ecological responses to recent climate change. Nature 413: 389–395.

Webster JR, Newbold JD, Thomas SA, Valett HM & Mulholland PJ, 2009. Nutrient uptake and mineralization during leaf decay in streams – a model simulation. International Review of Hydrobiology 94: 372–390.

Relevant conclusions

1. Flow regulation by small reservoirs, which can create droughts below dams, modifies the trophic structure of benthic macroinvertebrate communities simplifying the detritivore assemblages. The shifts in benthic communities explain the functional alteration (reduction of leaf-litter decomposition rate) in streams below dams.

2. Leaf quality, as indicated by nitrogen concentration, controls leaf breakdown in streams, through the differential colonization of leaf-litter by shredders, being more determinant of decomposition rate than factors of inter-stream variability.

3. The replacement of native deciduous forests by pine plantations reduces the quantity and quality of leaf-litter inputs in forested streams. The change in the basal resource simplifies the shredder invertebrate community, small individuals being more affected than large ones, with consequences on the individual size structure of shredders under pine plantations. This alteration translates in a lower leaf-litter processing capability of streams under pine monocultures than those under deciduous forest.

4. Temperature controls microbial decomposer activity, independently on the temperature regime the fungal assemblage is naturally subjected to. Furthermore, the fungal assemblages inhabiting coldest environments appear to be more sensitive to temperature increases by simplifying their community structure.

5. Overall, flow regulation by small dams, the replacement of native forest by pine plantations and the water temperature appear as impact factors that can modify structural attributes of stream ecosystems as microbial decomposers and detritivore invertebrates. Due to the implication of these organisms in a key function such as leaf-litter decomposition, these alterations in biotic communities compromise the correct functioning of the ecosystem. Thus, the observed narrow linkage between structure and functioning leads us to consider leaf-litter processing as indicator of ecosystem conservation status.

New directions

Due to the large number of human activities, diverse alterations on environmental factors act simultaneously on structure and functioning of natural ecosystems. Thus, from the results obtained in this thesis some further questions about synergistic interactions emerge:

- Does land use condition the structural and functional response of streams to flow regulation?

- How will affect the predicted temperature rise by climate change on structure and functioning of regulated streams?

- How will affect the predicted temperature rise by climate change on structure and functioning of streams under exotic plantations?

- Does temperature regulate the resource quality effect on detritus processing?

Publication status:

Chapter I: Published in Hydrobiologia

Martínez A, Larrañaga A, Basaguren A, Pérez J, Mendoza-Lera C & Pozo J, 2013. Stream regulation by small dams affects benthic macroinvertebrate communities: from structural changes to functional implications. DOI: 10.1007/s10750-013-1459-z.

Chapter II: Submitted to Aquatic Sciences

Martínez A, Larrañaga A, Pérez J, Basaguren A & Pozo J. Consistency of leaf-litter stoichiometry *vs*. processing relationships among three forested streams.

Chapter III: Submitted to Freshwater Biology

Martínez A, Larrañaga A, Pérez J, Descals E, Basaguren A & Pozo J. Effects of pine plantations on structural and functional attributes of forested streams.

Chapter IV: In preparation

Resource quality affects size spectrum of selected feeding guilds in streams.

Chapter V: In preparation

Effects of temperature on leaf-litter decomposition in forested streams: in-stream and microcosm approaches.