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Effect of temperature and presence of a dominant shredder (*Allogamus laureatus*) in the variability patterns of macroinvertebrate assemblages associated with decomposing leaf litter

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Resumo

Vários modelos climáticos prevêem, durante este século, um aumento da temperatura média do ar até 4,7ºC, a temperatura da água irá acompanhar esta subida. As consequências do aumento de temperatura na composição das comunidades e interacções biológicas estabelecidas dentro delas, ainda são pouco conhecidas nos ecossistemas lóticos. Neste estudo foi avaliado o efeito, individual e em conjunto, do aumento de temperatura e da presença de um detritívoro dominante (*Allogamus laureatus*) na decomposição de folhas de carvalho, e nas comunidades de detritívoros associados à folhada. Para tal, um pequeno ribeiro foi dividido longitudinalmente em duas secções; numa das secções a água foi mantida á temperatura ambiente (12,4ºC, média), na outra secção a água foi aquecida cerca de 3ºC acima da temperatura ambiente. Os sacos de carvalho foram incubados durante a primavera de 2011 em ambos os lados do ribeiro, metade dos sacos continham uma larva de *A. laureatus.* Os sacos de folhada foram removidos do rio a cada duas semanas durante seis semanas, para posterior determinação da massa final da folhada de carvalho e identificação dos detritívoros.

Verificou-se que a presença do *A. laureatus* estimulou a decomposição das folhas de carvalho e o aparecimento de outros detritívoros à temperatura ambiente. O aumento da temperatura, na presença do *A. laureatus,* causou uma diminuição na taxa de decomposição da folhada e a relação positiva entre *A. laureatus* e os outros detritívoros desapareceu. O padrão natural de variabilidade da comunidade de detritívoros não diferiu entre tratamentos, contudo a riqueza específica dos macroinvertebrados que colonizaram as folhas foi significativamente superior à temperatura elevada.

Estes resultados sugerem que o *A. laureatus* desempenha uma função importante nos ecossistemas lóticos, quer pelas suas elevadas taxas de consumo quer através mecanismos de facilitação para com outros invertebrados. O aumento da temperatura causou uma inibição da actividade do *A. laureatus* alterando substancialmente a força da interacção entre ele e outros detritívoros, consequentemente limitando a taxa de decomposição foliar. Apesar de não terem sido encontradas diferenças significativas no padrão natural de variabilidade dos detritívoros, parece que o aumento de temperatura aumenta a variabilidade em termos da composição da comunidade e que a presença do *A. laureatus* tende a aumentar a variabilidade agregada.

Este estudo mostra que os efeitos do aquecimento global no funcionamento dos ecossistemas são complexos pois este é moderado por factores bióticos. Entender a susceptibilidade de interacções biológicas ao aumento da temperatura da água pode fornecer pistas importantes e melhorar previsões sobre as alterações nos ecossistemas lóticos sob o cenário do aquecimento global, podem ainda constituir uma importante ferramenta em procedimentos de remediação.

Abstract

Climate models predict an increase in mean global air temperature up to 4.7ºC during this century and water temperature should follow this increase. The consequences of this temperature increase on communities' composition and biotic interactions establish within them are mostly unknown for stream ecosystems. In this study I assessed the individual and combined effects of rising temperature and presence of a dominant detritivore (*Allogamus laureatus*) on the decomposition of submerged oak litter and associated shredder communities. For this, a headwater stream was divided longitudinally into two sides; one side was kept at ambient temperature (12.4ºC, mean) while the other side was warmed ~3ºC above ambient temperature. Oak litter bags were incubated in spring 2011 on both stream sides, with half of bags having one *A. laureatus* larvae. Replicate litter bags were collected every two weeks over six weeks for determination of oak litter remaining mass and detritivores identity.

The presence of *A. laureatus* stimulated decomposition of oak litter and the appearance of other shredders at ambient stream side. Temperature increase, in the presence of *A. laureatus*, caused a decrease on decomposition rate and the positive relationship of *A. laureatus* and the other shredders disappeared. The natural variability patterns of shredder communities did not differ among treatments, but the total macroinvertebrate richness was significant higher at elevated temperature.

These results suggest that *A. laureatus* play an important role on stream ecosystems either by high consumption rates or through facilitation mechanisms for other shredders. The temperature increase caused an inhibition of *A. laureatus* activity thus cause a substantial change on strength of interactions between him and other shredders, limiting leaf litter decomposition. In spite no significant differences on

variability patterns it seems that rising temperature, increases compositional variability and the presence of *A. laureatus* increases aggregate variability.

This study highlights that the effects of global warming on ecosystem functioning are complex as they are moderated by biotic factors. Understanding the susceptibility of biological interactions to increased water temperature may give important clues to make better predictions on stream ecosystems changes under global warming scenarios and may constitute an important tool for remediation procedures.

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Chapter I - General introduction

1.1 Streams: an overview

Lotic ecosystems are one of the most impaired systems in the world (Malmqvist & Rundle 2002; Strayer & Dudgeon 2010). These systems are primarily vulnerable to local and global changes largely due to their isolation, important role as water source for human activities and close dependence on their catchment (Malmqvist & Rundle 2002; Perkins et al. 2010; Strayer & Dudgeon 2010).

Small streams represent more than 75% of total length of river network in a catchment basin (Benda et al. 2005; Clarke et al. 2008). They are located at the head of the *continuum* (*River Continuum Concept*; Vannote et al. 1980) and constitute very specific environments (Richardson & Moore 2007). Forested headwaters in temperate areas usually present overshadowed channels, low primary productivity, local microclimate gradients and a high edge to surface area ratio. One of the main features of these systems is the strong stream-riparian area relationships, guarantee of supply of organic matter as a source of nutrients and energy to the stream biota. A complex stream-riparian coupling largely explains the vulnerability of these systems to anthropogenic disturbances (Richardson & Moore 2007; Kominoski & Rosemond 2012).

Woodland low order streams (Strahler 1957) are heterotrophic systems and largely dependent on leaflitter inputs from the riparian area (Abelho 2001; Graça & Canhoto 2006). Once in the stream, these leaves are decomposed and converted into secondary production (González & Graça 2003). Decomposition is a key ecosystem process carried out by microbes, primarily aquatic hyphomycetes, and invertebrate detritivores (Gessner et al. 1999; Hieber & Gessner 2002); the dynamics of this process depends on intrinsic (e.g. leaf chemistry) and extrinsic (e.g. temperature, water nutrient contents, oxygen dissolved, pH, light) factors (e.g. Canhoto & Graça 1999; Franken et al. 2005; Simon et al. 2009; Ferreira & Chauvet 2011)

Leaf litter decomposition is usually subdivided into three sequential phases that can overlap: leaching, conditioning, and fragmentation. Right after immersion, leaves start to leach soluble compounds. This abiotic phase usually lasts about 48 hours determining a mass loss that can reach 42 % (Abelho 2001). Conditioning (Boling et al. 1975) is characterized by the colonization of leaves by microorganisms (aquatic fungi and bacteria) that promote a nutrient enrichment of leaf litter and increase its palatability to invertebrate detritivores (Cummins 1974; Vannote et al. 1980; Graça 2001). Finally, leaves suffer fragmentation either by physical abrasion or by invertebrate activity (Gessner et al. 1999).

Stream invertebrates that consume decaying leaves are known as shredders. This functional feeding group (Cummins 1974) includes a wide range of invertebrates such as larvae of Diptera, Trichoptera and Plecoptera and crustaceans (Tachet et al. 2002). It is commonly accepted (but see Gonçalves et al. 2007) that they participate in the decomposition process and that their feeding behavior accelerates litter decomposition (Graça 2001; Alemanno et al. 2007). Shredders present high ingestion rates and low assimilation efficiencies (Wallace et al. 1982); their feeding activities convert coarse particulate organic matter (CPOM; > 1mm) into fine particulate organic matter (FPOM; 0.45µm-1mm) as feces, and lower size fragmented material - dissolved organic matter (DOM; <0.45 µm). FPOM becomes a food resource available for other functional feeding groups, namely the collectors (Jonsson & Malmqvist 2005). The density, diversity and identity of shredders associated with leaf decomposition, as well their behavior (Jonsson & Malmqvist 2003; Alemanno et al. 2007), play a key role in the flux of organic matter, and as a structuring force in composition of lotic food webs (Allan &

Castillo 2007). Along with the less abundant scrapers and predators, shredders are part of the stream *brown* food webs (Kaspari 2004).

1.2 Water temperature

Temperature increase is one of the main physical threats associated with global change. Warming can directly threaten the ecological integrity of freshwater ecosystems (many life processes are temperature-dependent) and indirectly through changes in the hydrological conditions, oxygen solubility, intensity of anthropogenic stress, etc. (reviewed by Perkins et al. 2010). Several studies suggest important effects of increases in stream water temperature on the structure of the biotic communities through changes in individuals life histories (e.g., emergence patterns, metabolism, behavior; Harper & Peckarsky 2006; Dillon et al. 2010) and species interactions (e.g., competition, predation; Nilsson & Otto 1977; Nicola et al. 2010), with important consequences on ecosystem functioning (Gilman et al. 2010; Traill et al. 2010; Woodward et al. 2010). However, a gap still exists on the recognition of the main determinants of community structure (see Townsend 1989; Perkins et al. 2010), along with the clarification of the real importance of diversity, and species identity (Jonsson & Malmqvist 2003b; Vos et al. 2010), particularly within the shredders group, on stream processes, such as decomposition. This seems of paramount importance in global warming scenarios considering that “species interactions seem to shape the effects of climate-change” (Gilman et al. 2010)

1.3 Main objectives

In this work I tried to contribute to fill this gap by assessing the combined effects of a ~3ºC increase in water temperature and the presence of a common competitively dominant shredder (*Allogamus laureatus*) on the composition of shredder communities associated with decomposing oak litter in a woodland stream in Central Portugal over a 6 week time period.

Chapter II –

Effect of temperature and presence of a dominant shredder (*Allogamus laureatus*) in the variability patterns of macroinvertebrate assemblages associated with decomposing leaf litter

2.1 Introduction

Headwater streams are a critical component of hydrological basins as they represent more than 75% of total length of river network (Benda et al. 2004; Clarke et al. 2008) and play an important role in supporting biodiversity and ensuring the functioning of the entire ecosystem (Meyer et al. 2007; Clarke et al. 2008). Many headwater streams flow through forests and can be heavily shaded, which limits in-stream primary production. The organic matter provided by the surrounding vegetation is, therefore, the main source of energy and carbon for aquatic food webs in these systems (Abelho 2001). This organic matter is incorporated into the food web by the activities of shredders, a guild of invertebrates that uses coarse organic matter directly, establishing the link between dead organic matter and higher trophic levels (Cummins & Klug 1979; Abelho 2001; Graça 2001; Hieber & Gessner 2002; Graça & Canhoto 2006). The abundance and identity of shredders can regulate the leaf litter decomposition rate (Jonsson & Malmqvist 2005; Kobayashi & Kagaya 2005) and also determine the growth rate of other macroinvertebrates (Graça 2001; Jonsson & Malmqvist 2005). So, any perturbation that cause a shift in shredder communities can have several impacts on the food web and ecosystem functioning.

Climate models predict that the mean maximum air temperature in Portugal will increase by up to 4.7°C (A2 scenario) until 2100 (Ramos et al. 2011). Water temperature should follow this increase closely (Eaton & Scheller 1996; Webb & Nobilis 1997; Morrill et al. 2005). This magnitude of changes was not experienced by organisms before (Raven 2002) and it is likely that such global warming will cause changes in many ecosystems through changes in life history, biotic interactions, and ecosystem processes (Traill et al. 2010).

The responses to global warming will be species-specific and will be more severe in ectotherms (Heino et al. 2009; Perkins et al. 2010). In a recent review, Woodward et al. (2010) stated that an increase in temperature will affect all levels of biological organization. At the organismal level, an increase in temperature, up to species optimal temperature, will stimulate individual metabolism, consumption and growth rates and lead to reduced adult body size, which might alter organismal effects on ecosystem processes (Sweeney & Vannote 1986; Hogg & Williams 1996; González & Graça 2003; Dillon et al. 2010; Rumbos et al. 2010; Ferreira et al. 2010). Population densities will be modulated by temperature through direct and indirect effects (Hogg & Williams 1996; Beveridge et al. 2010), emergence patterns may be altered in warmer environment (Langford 1975; Hogg & Williams 1996; Harper & Peckarsky 2006) and population range shifts will likely occur under global warming (Sweeney et al. 1992; Parmesan & Yohe 2003).

Numerous studies have highlighted the effect of temperature on macroinvertebrate communities (Hogg et al. 1995; Lessard & Hayes 2002; Daufresne et al. 2007; Friberg et al. 2009; Lawrence et al. 2010; Lecraw & Mackereth 2010). Some authors argue that water temperature increases will lead to a shift in species traits that provide them more resistance to this perturbation. For instance, life cycle duration, and number of reproductive cycles per year will shift in the way communities function (Bonada et al. 2007; Tobin et al. 2008; Lawrence et al. 2010; Poff et al. 2010). Petchey et al. (1999) found that trophic structure in microcosms was severely changed with increases in temperature, with a loss of 30-40% species, which translated into altered ecosystem functioning (primary production). However, our knowledge of the effect of warming on stream community composition, biologic interactions and ecosystem processes remains poor (Friberg et al. 2009).

Biological interactions are strong modulators of community structure and they should be incorporated in experimental studies to allow us a deeper understanding of the effect of global warming on communities and ecosystem function. Biological interactions are the indirect pathway by which the effects of global warming spread through communities (Gilman et al. 2010). For instance, raising temperature can alter the predator-prey relationships by decreasing energetic efficiency of predators or by driving them locally extinct (Beisner et al. 1997; Abrahams et al. 2007; Vucic-Pestic et al. 2011). The parasite-host relationship can change under warmer conditions. Mouritsen et al. (2005) verified a model prediction of gradual decline in host population abundance with rising temperature due to stimulation of parasite infection, which caused change on community structure. Temperature effects was also observed by Nilsson & Otto (1977) on the densities of two detritivores species (*Potamophylax cingulatus* Steph. and *Gammarus pulex* L.). The authors demonstrated that elevated temperature enhanced interspecific competition between them, resulting in higher mortality in the less competitive species. Taniguchi & Nakano (2000) argued that competitive ability is an important mechanism that structure communities and showed that competition between fish species was strongly mediated by temperature. Also, Jiang & Morin (2007) reported results from a microcosm study in which they tested random fluctuations on temperature *versus* temperature fluctuation correlated with time series and observed that random fluctuations favored coexistence of two competing freshwater bacterivorous species, whereas temperature fluctuations correlated with time series enhance competitive exclusion.

In this study I assessed the effects of experimental warming (~3ºC above ambient temperature in spring) and of the presence of a competitively dominant shredder (*Allogamus laureatus*), individually and in combination, on oak litter decomposition and on the composition of the shredder communities associated with decomposing litter, over 6 weeks in a mountain temperate stream. I expected that the presence of *A. laureatus* would stimulate litter decomposition rate at ambient temperature. Increase in temperature was expected to stimulate litter decomposition in the absence of *A. laureatus*. It is more difficult to anticipate the response of litter decomposition rates at elevated temperature in the presence of *A. laureatus* because this will depend on the shredder thermal tolerance and on it interaction with the other members of the shredder guild. Finally I hypothesized that the increase in temperature, as well the presence of a dominant shredder, would decrease the abundance and specific richness of shredder communities, changing therefore the natural aggregate and compositional variability patterns of macroinvertebrate communities.

2.2 Materials and methods

Experimental conditions

Study area. The study took place in Candal stream, Lousã mountain, Central Portugal (40º04’48.10’’N, 8º12’11.16’’W, 634 m a.s.l.). This is a second order stream (Strahler 1957) that runs through schistose substrate, and is bordered by native mixed deciduous forest, dominated by chestnut (*Castanea sativa* Mill.) and oak (*Quercus* spp) trees. The study section was divided longitudinally along 22 m with schistose stones, each side ~50 cm wide and 5–10 cm deep. One side of the study section was warmed up by 2.8 ºC (elevated side) above the ambient temperature registered in the other side (ambient side) (Fig 1.) Warming was initiated two months before the experiment started (28 March 2011) and continued during the study period. Stream water was derived from upstream into a 260L reservoir provided with 30 electrical resistances (2000 W; Crussel, Portugal), supplied with a continuous power of 40kW, that warmed the water and discharged it into the elevated side at a rate of 2.0 L/s ± 0.2 (mean, SE). The flow rate was controlled manually at the outlet of the reservoir. A similar system was used to provide water to the ambient stream side, except that the reservoir did not have any electrical resistances and therefore the water was delivered at ambient temperature (more details in Canhoto et al., *in prep.*). The increase in temperature simulates the expected increase in water temperature in the area. Expected water temperature was calculated by multiplying the mean air temperature increase predicted over this century (3.75ºC; IPCC 2007) by a conversion factor (0.9) proposed by Eaton & Scheller (1996). Ten times over the study period (42 days), 300 mL of water from each stream side were collected, filtered through fiber glass filters (47 mm ∅, pore size 0.7 µm; Millipore APFF04700, Millipore, MA, USA), and frozen at –20 ºC for later determination of nutrient concentrations and alkalinity. Nitrate concentration (NO_3^-) was determined by catalyzed reaction and quantification by colorimetry (MI I.LB.I2 – LCK 339), soluble reactive phosphorus (SRP) concentration was determined by the ascorbic acid method (APHA 1995), and alkalinity was determined by titration with H_2SO_4 0.02N up to an end point pH of 4.2 (APHA 1995). Conductivity, total dissolved solids (TDS) (LF330 315, WTW, Weilheim, Germany), pH (pH 3110, WTW, Weilheim, Germany), and dissolved oxygen (Oxi 3210, WTW, Weilheim, Germany) were recorded *in situ* in both stream sides. Temperature was recorded hourly during the study period with submersed data loggers (Hobo® Pendant, Onset Computer Corp., MA, USA).

Initial litter quality. Oak (*Quercus robur* L.) leaves used in this experiment were collected after senescence in November 2010, air dried in the dark at ambient temperature and stored until needed. Before the beginning of the experiment, the chemical and physical properties of leaves were assessed: phosphorous using four replicates (Graça et al. 2005), carbon and nitrogen using three replicates (IRMS Thermo Delta V advantage with a Flash EA 1112 series) and polyphenolic concentration using four replicates (Graça et al. 2005). Oak leaf composition was: 0.03% ± 0.01 (mean ± SE) of phosphorous/g DM; 49.86% (± 0.66, SE) of carbon /g DM; 0.83% (± 0.05) of nitrogen /g DM and 7.47% (± 0.23) of polyphenols /g DM. Leaf toughness was also determined on leaves previously soaked in water for 30 min using a penetrometer (Graça et al. 2005). Three oak discs (12 mm ∅) were cut out of tree replicates with a cork borer, removed from leaves and fixed between two acrylic sheets. The mass required to force an iron rod (0.5 mm ∅) through the leaf (avoiding major and secondary veins) was determined as g of water + cup + rod weight : 147.61 ± 6.59 (mean ± SE) (Graça et al. 2005).

Shredders. The caddisfly *Allogamus laureatus* (Trichoptera: Limnephilidae) was chosen for this study because it is a dominant detritivore in aquatic systems. Limnephilidae, in general, are reported as aggressive with high growth rates (Wissinger et al. 1996), and are functionally dominant in the decomposition process (Creed et al. 2009). To avoid interfering with the invertebrate communities in the experimental reach, the individuals were collected in a nearby stream (Cerdeira, Lousã mountain, 40°05'21.39"N, 8°12'06.67"W). All the individuals were inspected using a binocular microscope (6.4 x, Wild M38, Heerbrugg, Switzerland) and the linear distance between the eyes (interocular distance) was measured using a micrometer. Forty-two individuals with interocular distance of 10 micrometer divisions (1.43 mm) and case opening diameter > 4 mm were selected. Then, 24 individuals were randomly chosen and individually allocated into tetrahedral mesh bags, according to the treatments (see below). The remaining individuals (18) were dried (105 ºC, 24 h) and then weighed (± 0.01 mg) to estimate the mass of individuals used in experiment (1.5 ± 0.12 g dry mass, mean ± SE).

Litter bags. A total of 48 tetrahedral mesh bags (12 × 12 cm, 0.4 mm mesh) were prepared with 2.01 ± 0.01 g (mean ± SE) air dried oak leaves (rehydrated with distilled water to avoid breakage). One single *A. laureatus* larvae was added to half the bags just before immersion in both stream sides (ambient and elevated), according to the treatment (see below). (Fig. 2)

Experimental design

The individual and combined effects of the presence of a dominant detritivore and of increase in water temperature on the shredder communities associated with decomposing oak litter were assessed by incubating litter bags with and without one *A. laureatus* larvae, in the study stream, at ambient and elevated temperature, for 42d (3rd, May – 14th, June). The two factors, detritivore presence and water temperature, were crossed in a complete factorial design resulting in four treatments: 1) Bags with *A. laureatus* at ambient temperature (LimnA); 2) Bags with *A. laureatus* at elevated temperature (LimnE); 3) Bags without *A. laureatus* at ambient temperature (NoLimnA) and 4) Bags without *A. laureatus* at elevated temperature (NoLimnE). Twelve litter bags per treatment were distributed into four blocks and fixed to the streambed with nails; each block received three litter bags from each treatment. After 14d, 27d, and 42d, four litter bags from each treatment (one from each block) were retrieved, enclosed in individual zip-lock bags, and transported to the laboratory in a cooler (Fig. 3).

In the laboratory, leaves were carefully rinsed with distilled water on top of a 500 μm mesh sieve to remove adhering invertebrates and sediment. The remaining litter material was oven dried (105ºC, 48h), weighed (± 0.1 mg) to allow determination of dry mass (DM), ignited (550ºC, 4h) and reweighed (± 0.1 mg) to allow determination of the ash fraction and ash free dry mass remaining (AFDMr). The proportion of AFDM mass remaining at each sampling date was calculated by dividing AFDMr per initial AFDM.

Ten extra litter bags were prepared in the same way as the samples, taken to the stream on d0, submerged for ~10 min, brought back to the laboratory and processed as above. These were used to calculate an air DM to AFDM conversion factor, taking into account mass loss due to handling, to be applied to the samples and estimate initial AFDM.

Macroinvertebrates

The macroinvertebrates retained on top of the 500 μm mesh sieve were recovered, stored in 20 mL scintillation vials, and preserved with 95 % ethanol. Invertebrates were sorted and identified under a binocular microscope (50x; Leica M80, Singapore). Identification was carried to the lowest taxonomic level possible, generally genus or species, following Lanero (2000) and Tachet et al. (2002). Invertebrates were classified into functional feeding groups following Lanero (2000) and Tachet et al. (2002). The results were expressed by number of individuals per bag and number of individuals per mass (AFDMr, g) and number of taxa per bag. During the experiment, several *A. laureatus* larvae entered in the bags due to their small size (case opening ∅ < 4mm), and the number of *A. laureatus* were no longer statistically different between treatments initially with and without *A. laureatus* (Presence of *A. laureatus* x Temperature interaction; two-way ANOVA, $F_{0.05(1)1,36} = 1.46$, p= 0.24).

Statistical analysis

Litter breakdown rates (*k*/d) were calculated assuming an exponential decay with a fixed intercept: k = -Ln(AFDMr/AFDMi)/t, where t is the incubation time in days (d), AFDMi is the initial mass and AFDMr is the final mass. Remaining mass over time was compared among treatments by analysis of covariance (ANCOVA), with presence/absence of *A. laureatus* and temperature regime (ambient and elevated) as categorical variables, and time as continuous variable. Tukey´s honest significant difference (HSD) test was used for post hoc multiple comparisons if there was an effect of presence of *A. laureatus,* temperature or their interaction ($\alpha = 0.05$). Given that both stream sides differed in temperature, litter breakdown rates per degree day (*k*/dd) were also calculated by replacing time (t) by the sum of mean daily temperatures accumulated by the sampling day (ºC).

Total macroinvertebrate abundance, total species richness, shredder abundance and shredder richness per bag, and total abundance and shredder abundance per AFDMr were compared among treatments with a three-way ANOVA (presence/absence of *A. laureatus*, temperature and time as categorical variables). Total macroinvertebrate abundance, total species richness, shredder abundance and shredder richness per bag, and total abundance and shredder abundance expressed per AFDMr were also compared among treatments with a two-way ANCOVA (presence of *A. laureatus* and temperature as categorical variables and time as continuous variable). Only shredders were used in the analysis described below.

Principal component analysis (PCA) was used to evaluate the compositional variability of detritivores in each sample (CANOCO for Windows 4.5; Biometrics-Plant Research International, Wageninger, The Netherlands). The Euclidean distances (EuD) were calculated between samples that belong to the same block and treatment. Assuming that

P1(x_1,y_1,z_1) represents one sample of first sampling date with x_1,y_1,z_1 as coordinates of the three first axis of PCA, P2(x_2,y_2,z_2) and P3 (x_3,y_3,z_3) represent, respectively, the samples of second and third sampling date for that same treatment and block, then $\text{EuD}(P1,P2,P3) = \text{EuD}(P1,P2) + \text{EuD}(P2,P3)$, with $\text{EuD}(P1,P2) = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}$. Euclidean distances were compared among treatments by two-way ANOVA with presence/absence of *A. laureatus* and temperature regime as categorical variables.

The coefficient of variation (CV%, standard deviation/mean) was determined for total macroinvertebrate and shredder abundance, were calculated per treatment and per block, only CV% of shredder abundance was used to calculate aggregate variability. The CVs were compared among treatments by a two-way ANOVA (presence/ absence of *A. laureatus* and temperature regime as categorical variables). The relationships between number of *A. laureatus* per bag, number of detritivores per bag and remaining AFDM were assessed by linear regression.

Tukey´s honest significant difference (HSD) test was used for post hoc multiple comparisons if the ANCOVAs or ANOVAs above detected an effect of presence of *A. laureatus,* temperature or their interaction ($\alpha = 0.05$). The assumptions of normality and homocedasticity were confirmed with Shapiro-Wilk and Bartlett tests, respectively. All statistical analyses were performed with STATISTICA 7 software (StaSoft, OK, USA), except when indicated otherwise.

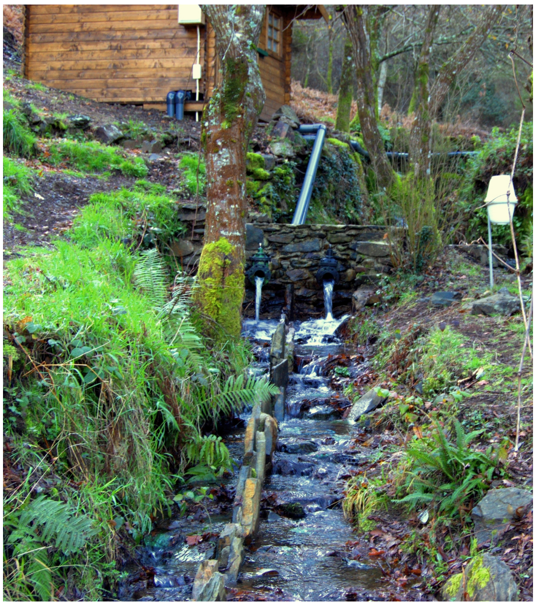

Fig. 1 Study area (Candal stream, Lousã mountain, Central Portugal 40°04'48.10''N, 8°12'11.16''W, 634 m a.s.l.). The study section was divided longitudinally along 22 m with schistose stones, each side being ~50 cm wide and 5–10 cm deep. One side of the study section was warmed up by 2.8 °C (elevated side) above the ambient temperature registered in the other side (ambient side).

Fig. 2 Litter bags (12 × 12 cm; 4mm mesh) were prepared with ~2 g of air dried oak leaves. One *A. laureatus* larvae was added to half of the bags. Half of the bags were incubated at ambient and half at elevated temperature.

Fig. 3 After 14d, 27d, and 42d, four litter bags from each treatment (one from each block) were retrieved, enclosed in individual zip-lock bags, and transported to the laboratory in a cooler.

2.3 Results

Water variables

Experimental warming effectively increased the water temperature in the elevated stream side by a mean of 2.8°C above ambient temperature during the study period (t = -36.9, df = 1922, p < 0.0001). This translated into a slightly lower dissolved oxygen concentration in the elevated side (9.4 mg/L) compared with the ambient side (10 mg/L) (t = 4.1, df = 18, p > 0.001). In general, the water variables did not statistically differ between sides (ambient and elevated); however, SRP was significant higher in the ambient side (t = -2.3, df = 12, p = 0.042) and conductivity was significant higher in the elevated side (t = -3.2, df = 16, p = 0.005) (Table 1).

Table 1 Water variables during the experimental period in both stream sides (mean ± SE).

	Ambient side	Elevated side
Water properties		
Temperature (C°) *	12.4 ± 0.1	15.1 ± 0.3
pH	7.1 ± 0.1	7.1± 0.1
Conductivity (μS/cm) *	27.2 ± 0.1	27.5 ± 0.1
TDS (mg/L)	30.0 ± 0.2	30.1 ±0.1
Oxygen dissolved (mg/L)*	10.0 ± 0.1	9.4 ± 0.1
Oxygen dissolved (%)	99.8 ± 1.0	98.8 ± 0.9
SRP (µg/L) *	34.8 ± 9.1	11.8 ± 4.4
Nitrates (mg/L)	0.08 ± 0.01	0.11 ±0.02
Alkalinity (mg $CaCO_3$/L)	4.7 ± 0.1	4.6 ± 0.2
Discharge (L/s)	2.3 ± 0.3	2.0 ± 0.2

*, variables that significantly differed between stream sides (t test, p>0.05)

Litter decomposition

After the 6 week incubation period, LimnA treatment had the lowest percentage of AFDMr (36.9 % ± 4.1, mean ± SE), followed by NoLimnE (40.5% ± 4.0, SE), LimnE (46.3% ± 4.7) and lastly NoLimnA (48.4% ± 2.6). The litter decomposition rates were significantly different among treatments (Fig. 4, Table 2). In the ambient temperature side the decomposition rate of oak leaves in the presence of *Allogamus laureatus* was higher (0.023/d) than in its absence (0.017/d) (Tukey HSD, p = 0.04). In the elevated temperature side there was no effect of the presence of *A. laureatus* in the decomposition rate, however the temperature increase, in the presence of *A. laureatus*, caused a significant decrease on decomposition rate suggesting that warming may cause a negative effect in *A. laureatus* activity (Fig. 4). When considering decomposition rates expressed per degree days (*k*/dd) significant differences were found among treatments (Table 2). LimnA had the highest decomposition rate (0.0017/dd) and it was statistically different from both LimnE (0.0011/dd; Fisher LSD, p = 0.03) and NoLimnA (0.0013/dd; Fisher LSD, p = 0.01) (Fig. 4).

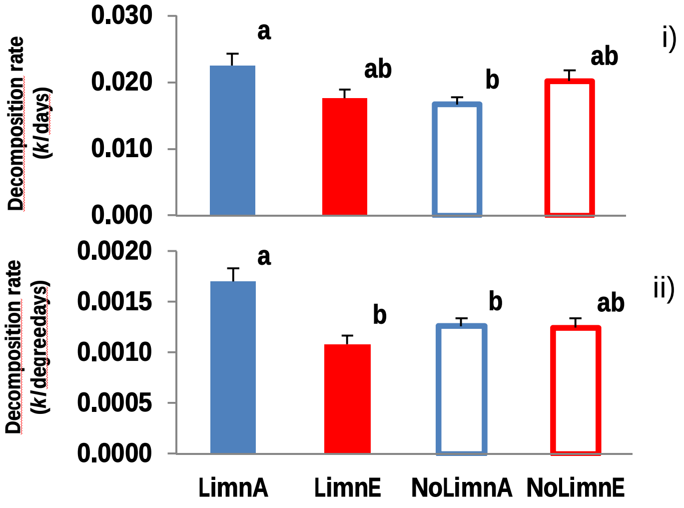

Fig. 4 Decomposition rates of oak leaf litter incubated in the ambient (A) and elevated (E) stream side, in the presence (Limn) and absence (NoLimn) of *A. laureatus* for 42d. i) *k*/day ii) *k*/degree days. Bars represent the mean ± standard error (SE). Different letters indicate statistical differences among treatments (ANCOVA followed by Tukey HDS and Fisher LSD respectively, p ≤ 0.05).

Table 2 Summary table for the ANCOVA performed on fraction of mass remaining over time (days) or degree days of oak litter incubated in the ambient and elevated stream side over 42 days, in the presence/absence of *A. laureatus*.

	Mass loss (days)			Mass loss (degree days)		
	df	F	p	df	F	p
ANCOVA						
Intercept	1	15.37	<0.01	1	12.79	<0.00
Time	1	231.74	0.00	1	215.85	0.00
Presence shredder	1	1.25	0.27	1	1.18	0.28
Temperature	1	0.20	0.66	1	15.33	<0.01
Presence shredder*temperature	1	7.70	0.01	1	7.26	0.01
Error	43			43		

Macroinvertebrates

There were no statistically significant differences among treatments in terms of macroinvertebrates abundance (three-way-ANOVA interaction presence shredder x temperature, $p = 1.00$), total species richness ($p = 0.86$), shredder abundance ($p = 0.85$) and shredder richness ($p = 0.56$) per bag, if the variability of the three factors (time, temperature and presence of *A. laureatus*) are considered (Table 3; Fig. 5). The two-way ANCOVA detected differences in total macroinvertebrate richness between temperatures in either the presence and absence of *A. laureatus* (Tukey test, $p < 0.05$; Table 3). The coefficient of variability (CV%) was compared among treatments by two-way ANOVA and no significant differences were detected for total macroinvertebrate abundance (interaction presence shredder x temperature, $p = 0.52$), species richness ($p = 0.56$), shredder abundance ($p = 0.55$) and shredder richness ($p = 0.33$) (Table 3). When total and shredder abundance were expressed per litter mass no differences were found among treatments (three-way ANOVA, interaction Presence shredder x temperature: $F_{0.05(1)1,28} = 3.39$, $p = 0.67$ and $F_{0.05(1)1,28} = 0.60$, $p = 0.63$, respectively). In general, neither the presence of *A. laureatus* nor the increase in temperature generated different patterns of macroinvertebrates colonization, but the global macroinvertebrate richness responded positively to the increase of temperature (Table 3).

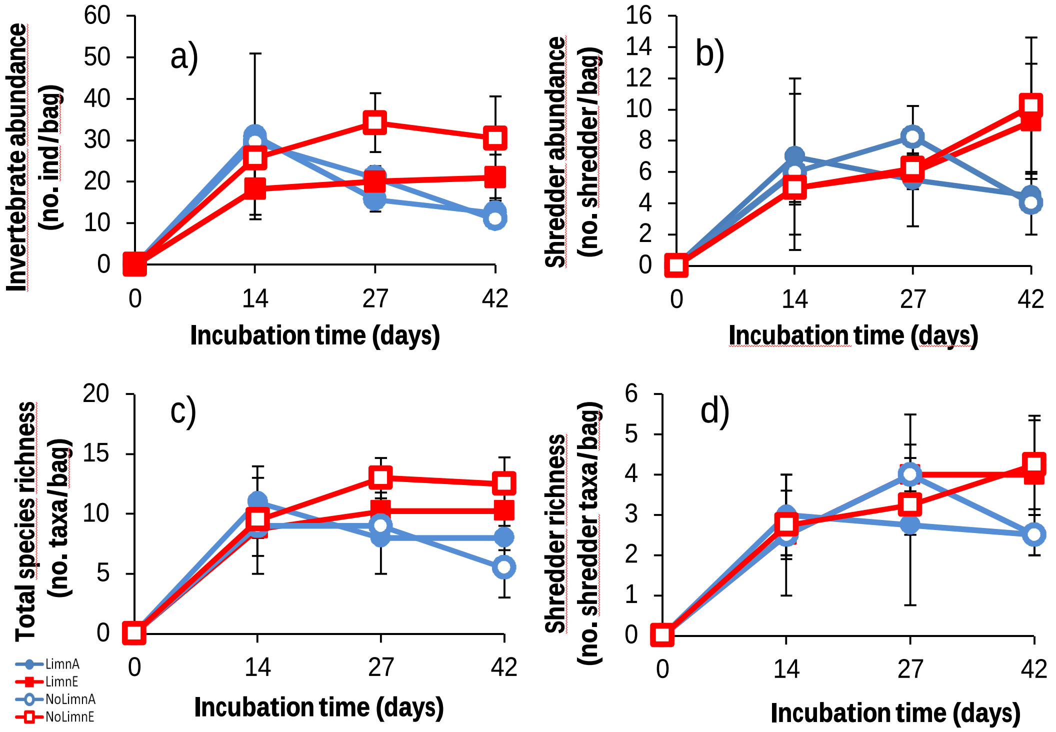

Fig. 5 Macroinvertebrate community response associated with oak leaf litter incubated in the ambient (A) and elevated (E) stream side, in the presence (Limn) and absence (NoLimn) of *A. laureatus* for 42d. a) Total invertebrate abundance b) shredder abundance c) total species richness and c) shredder species richness per bag. Values are means ± SE.

Table 3 Summary table for the three-way-ANOVA and two-way-ANCOVA, performed on macroinvertebrate abundance, macroinvertebrate richness, shredder abundance and shredder richness, associated with oak litter incubated at ambient or elevated side stream, in the presence or absence of *A. laureatus* for 42 days. p value ≤ 0.05 indicate statistical differences among treatments.

	Macroinvertebrate abundance			Macroinvertebrate richness			Shredder abundance			Shredder richness		
	df	F	p	df	F	p	df	F	p	df	F	p
3-way- ANOVA												
Intercept	1	92.97	<0.01	1	270.50	<0.01	1	60.81	<0.01	1	115.52	<0.01
Time (days)	1	1.43	0.24	1	0.10	0.75	1	0.06	0.80	1	0.02	0.89
Presence shredder	1	1.07	0.31	1	3.88	0.06	1	0.43	0.52	1	0.98	0.33
Temperature	2	0.74	0.49	2	0.27	0.77	2	0.17	0.84	2	0.70	0.51
Presence shredder*temperature	1	1.07	0.31	1	1.76	0.20	1	0.00	1.00	1	0.08	0.78
Presence shredder*time	2	0.23	0.80	2	0.49	0.62	2	0.14	0.87	2	0.04	0.96
Temperature*time	2	1.88	0.17	2	1.80	0.18	2	1.69	0.20	2	0.74	0.48
Presence shredder*temperature*time	2	0.00	1.00	2	0.15	0.86	2	0.17	0.85	2	0.58	0.56
Error	28			28			28			28		
2-way- ANCOVA												
Intercept	1	19.84	<0.01	1	38.95	<0.01	1	4.15	<0.05	1	10.53	<0.01
Time (days)	1	0.45	0.50	1	0.07	0.79	1	1.58	0.22	1	1.69	0.20
Presence shredder	1	2.00	0.17	1	0.34	0.56	1	0.22	0.64	1	0.15	0.70
Temperature	1	1.48	0.23	1	4.25	<0.05	1	0.29	0.59	1	0.70	0.41
Presence shredder *temperature	1	0.97	0.33	1	1.34	0.26	1	0.04	0.85	1	0.29	0.59
Error	35			35			35			35		
Coefficient of variability (Cv%)												
2-way-ANOVA												
Intercept	1	53,04	<0.01				1	35,39	<0.01			
Presence shredder	1	0,41	0,54				1	2,93	0,13			
Temperature	1	2,23	0,17				1	0,18	0,68			
Presence shredder *temperature	1	0,45	0,52				1	0,40	0,55			
Error	8						8					

The Euclidean distances and the coefficient of variability of shredder abundance were similar among treatments (two-way-ANOVA interaction Presence shredder x Temperature, p = 0.80 and p = 0.55, respectively; Table 4). Therefore, all treatments have the same pattern in both aggregate and compositional variability through time, suggesting that macroinvertebrate colonization was not influenced by the presence/absence of *A. laureatus* nor elevated temperature (Fig.6)

Table 4 Summary of two-way-ANOVA´s performed on coefficient of shredder abundance and Euclidean distances calculated from shredder communities associated with oak litter incubated in the ambient or elevated stream side, in the presence/absence of *A. laureatus* for 42 days. p value ≤ 0.05 indicates statistical differences among treatments.

	Coefficient of variability			Euclidean distance		
	df	F	p	df	F	p
2-way- ANOVA						
Intercept	1	35.39	0.00	1	24.67	0.00
Presence shredder	1	2.93	0.13	1	0.17	0.69
Temperature	1	0.18	0.68	1	1.50	0.25
Presence shredder*temperature	1	0.40	0.55	1	0.07	0.80
Error	8			8		

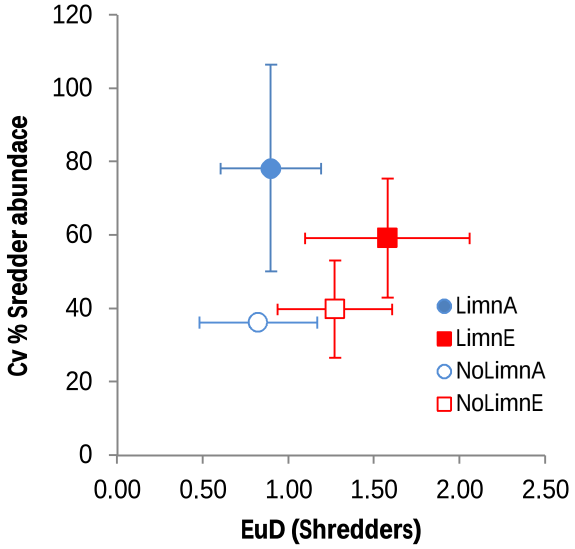

Fig. 6 Variability patterns of shredder communities associated with oak leaf litter incubated in the ambient (A) and elevated (E) stream side, in the presence (Limn) and absence (NoLimn) of *A. laureatus* for 42d. Coefficient of variability (%) versus Euclidean distance of shredder abundance. Values are the means ± SE.

Simple linear regression with number of shredders as the dependent variable and number of *A. laureatus* as the independent variable was significant for ambient temperature ($R^2 = 0.39$, $p = 0.01$), but not for elevated temperature ($R^2 = 0.03$, $p = 0.39$). AFDMr (g) and number of shredders were negatively correlated only for elevated temperature ($R^2 = 0.20$, $p = 0.03$). In spite of no significant differences in variability patterns, it appears that the presence of *A. laureatus* was responsible for the presence of other shredders at ambient temperature and that this effect disappeared when temperature increased (Fig. 7 and Fig. 8).

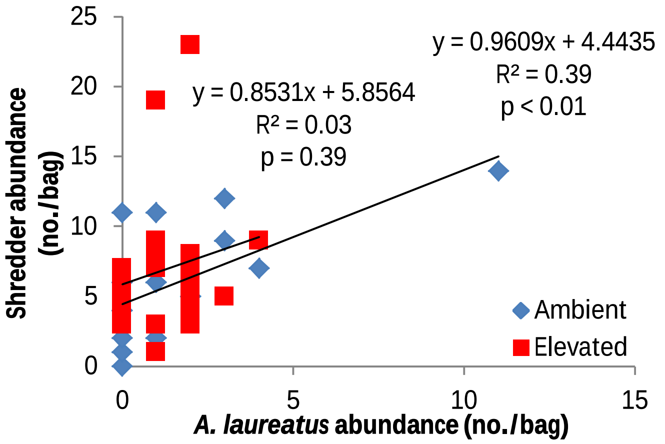

Fig. 7 Plot of simple linear regression between number of shredders and number of *A. laureatus* per bag for ambient and elevated temperature. The linear regression models, R^2 and p values are given.

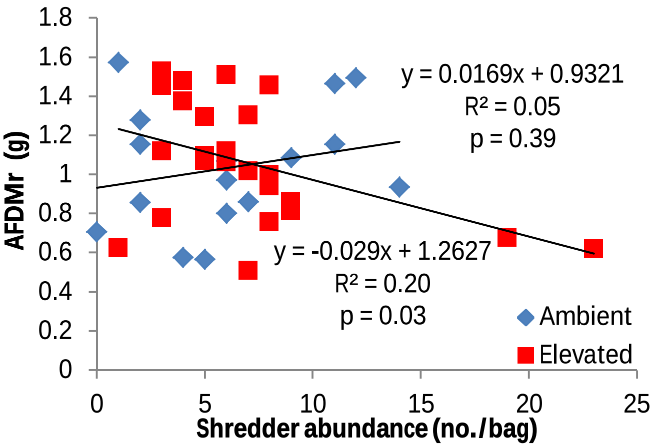

Fig. 8 Plot of simple linear regression between AFDM (g) and number of shredders per bag for ambient and elevated temperature. The linear regression models, R^2 and p values are given.

2.4 Discussion

Previous studies carried out in freshwater ecosystems regarding global warming revealed severe impacts on benthic communities. Some authors found differences on total density, growth patterns and phenology of macroinvertebrates (Hogg et al. 1995; Hogg & Williams 1996) while others showed also changes on structure and communities composition (Burgmer et al. 2007; Daufresne et al. 2007; Friberg et al. 2009). In this study neither the presence of *Allogamus laureatus* nor the raising in temperature caused a significant change in variability patterns of shredder communities. However, there was a slight tendency: the presence of the *A. laureatus* seemed to increase the variability of shredder abundance, and elevated temperature seemed to increase variability in terms of identity of species. Furthermore, another pattern emerged: the presence of *A. laureatus* stimulated the appearance of more shredders in the leaf pack communities at ambient temperature and this effect was eliminated by warming.

Litter decomposition

Leaf litter decomposition is a complex process, which is carried out mainly by aquatic fungi and shredders (Gessner et al. 1999; Jonsson & Malmqvist 2000; Hieber & Gessner 2002; Graça & Canhoto 2006; Costantini & Rossi 2010). In this study, stream water temperature and the presence of a dominant shredder in litter pack were manipulated in a mountain stream. Under these circumstances, oak litter decomposition rate varied between 0.017 day^{-1} and 0.023 day^{-1}. At ambient temperature, the presence of *A. laureatus* stimulated the decomposition rate of oak litter, in accordance with a previous study where the presence of another species of Limnephilidae was manipulated (Creed et al. 2009). At elevated temperature no significant differences were found between presence and absence of *A. laureatus,* nevertheless, the temperature increase, in the presence of *A. laureatus*, led to a significant decrease on decomposition rate Presumably, the increase of temperature caused an inhibition of *A. laureatus* activity. Brandt (2001) showed that the optimal temperature for occurrence of Limnephilidae is about 13.1 ºC (these data were collected from more than four thousands locations on United States of America), and during this experiment the mean temperature in the elevated stream side was approximately 2 ºC above this value. Furthermore, Rumbos et al. (2010) evaluated consumption rates of a Limnephilidae species, at several temperatures, and demonstrated that the highest consumption rate was recorded at 13 ºC in agreement the optimal temperature suggested by Brandt (2001). It is noteworthy that these values are from individuals of Limnephilidae family that is extremely diverse; also, one should consider that temperature tolerance limits vary geographically. Nevertheless, the highest decomposition rate on the LimnA treatment can result from higher consumption rates of *A. laureatus per se* since these larvae can be very competitive (Creed et al. 2009). Alternatively, there may have been a facilitation occurring (i.e. other shredders benefit from presence of *A. laureatus*) as reported in other studies (Cardinale et al. 2002; Jonsson & Malmqvist 2003) since there were no differences on shredder richness or identity between treatments.

Macroinvertebrates community

Communities change naturally through time. Micheli et al. (1999) developed an integrative approach that considers aggregate and compositional variability creating four different natural patterns of communities' variability: synchrony, asynchrony, stasis and compensation. Significant shifts on these natural variability patterns due to stream warming were not demonstrated in this study.

In our study, the increase in temperature and the presence of *A. laureatus* did not significantly change the variability patterns, colonization pattern, richness or abundance of shredders partially in contrast with that previously demonstrated by Nilsson & Otto, (1977). This may be due to the high variability over time by the taxa and the limited number of replicates. If one were to focus on the effect of temperature in this experiment, the pattern suggests that an increase in temperature increases compositional variation in macroinvertebrate communities (but not significantly). The effect of temperature was significant for total macroinvertebrate richness, but not for shredder richness: the increase in temperature caused an increase on invertebrate richness in agreement to what is globally found (Jacobsen et al. 1997). The functional feeding group analyses showed that this result is mainly caused by predators and deposit feeders (results in Annex). Furthermore, *A. laureatus* stimulated the presence of other shredders at ambient temperature, contrary to what was expected. Other researchers showed that Limnephilidae are generally aggressive, strong competitors with elevated activity rates (Wissinger et al. 1996; Creed et al. 2009); so, it was expected that shredder abundance would decrease with their presence. At the elevated reach it appears that *A. laureatus* activity was inhibited and that other shredders played a major role on decomposition, since there was a negative correlation between number of shredders and remaining ash free dry mass. Thus, with the temperature increase, we could detect a substantial change of the strength of interaction between this caddisfly and the other shredders.

Final remarks

The idea that manipulative field experiments allow revealed community level dynamics, reducing confounding effects, is corroborated by our study. In spite of the absence of significant changes in shredder variability patterns due to warming, it is clear that there is a positive relationship between *A. laureatus* and other shredders. Significant changes occurred at leaf litter decomposition rate, either by the presence of *A. laureatus* as by elevated temperature. The decrease in decomposition rate recorded at elevated temperature can result from inhibition of *A. laureatus* activity, and therefore the lack of facilitation for other shredders. This may suggest a change in interspecific relationships established on those leaf pack assemblages. We can point out a shift of dominance of *A. laureatus* to other shredders with the increase of temperature through the decomposition process.

In this work I addressed three important features to understand the potential effect of global warming: changes in community structure and composition, biological interactions and ecosystem processing. I found stronger differences on the last two aspects, suggesting that they are more sensitive to the increase in temperature than structural community attributes. I suggest that understanding the susceptibility of biological interactions to increased water temperature may give important clues to make better predictions on stream ecosystems changes under global warming scenarios and may constitute an important tool for remediation procedures.

Chapter III – Annex

The species richness of each functional feeding group was compared with a three-way-ANOVA (temperature, presence of *A. laureatus* and time categorical variables). When functional feeding groups were tested separately, we found that higher species richness at the elevated stream side were due to predators (temperature: Tukey HSD, p= 0.04) and deposit feeders (temperature x presence of *A. laureatus*: NoLimnA is significant lower than LimnE and NoLimnE, Tukey HSD, p ≤ 0.002 and p ≤ 0.03 respectively) (Table 5, Fig. 9).

Table 5 Summary table for the three-way-ANOVA performed on functional feeding groups richness, associated with oak litter incubated in the ambient or elevated side stream, in the presence or absence of *A. laureatus* for 42 days. p value ≤ 0.05 indicate statistical differences among treatments.

	Grazer richness			Predator richness			Filter feeder richness			Pircer richness			Deposit feeder		
	df	F	p	df	F	p	df	F	p	df	F	p	df	F	p
3-way-ANOVA															
Intercept	1	172.65	<0.01	1	48.72	<0.01	1	26.25	<0.01	1	9.00	<0.01	1	106.11	<0.01
Presence shredder	1	0.15	0.70	1	0.09	0.76	1	0.47	0.50	1	0.25	0.62	1	0.18	0.67
Temperature	1	1.38	0.25	1	4.51	0.04	1	0.00	1.00	1	2.25	0.14	1	11.79	<0.01
Time	2	0.18	0.84	2	3.18	0.06	2	1.00	0.38	2	0.81	0.46	2	1.11	0.35
Presence shredder*temperature	1	0.04	0.85	1	2.30	0.14	1	0.12	0.74	1	0.00	1.00	1	4.61	0.04
Presence shredder*time	2	1.10	0.35	2	0.17	0.85	2	1.71	0.20	2	0.24	0.79	2	0.54	0.59
Temperature*time	2	0.81	0.46	2	2.24	0.13	2	1.00	0.38	2	0.81	0.46	2	0.96	0.39
Presence shredder*temperature*time	2	0.15	0.86	2	0.48	0.62	2	0.11	0.90	2	0.81	0.46	2	0.54	0.59
Error	28			28			28			28			28		

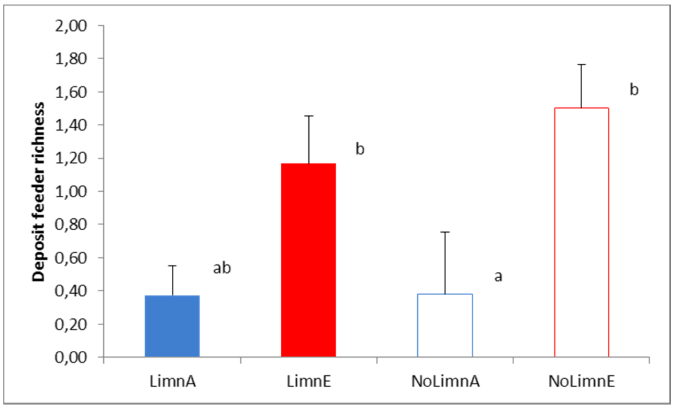

Fig. 9 Deposit feeder richness (mean ± SE) associated with oak leaf litter incubated in the ambient (A) and elevated (E) stream side, in the presence (Limn) and absence (NoLimn) of *A. laureatus* for 42d. Different letters indicate statistical differences among treatments (ANCOVA followed by Tukey HSD, p ≤ 0.05).

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