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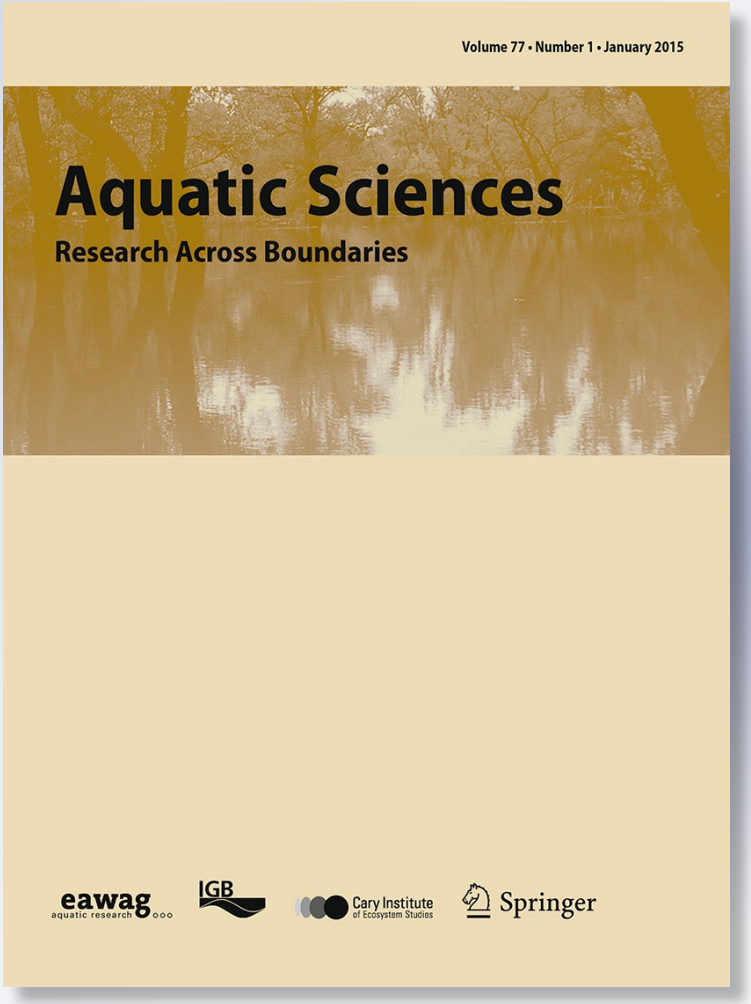

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RESEARCH ARTICLE

Warming, and the presence of a dominant shredder, drive variation in decomposer communities in a mountain stream

Cátia Domingos · Verónica Ferreira · Cristina Canhoto · Christopher Swan

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Abstract We assessed the effects of rising temperature and presence of a dominant detritivore (*Allogamus laureatus;* Trichoptera, Limnephilidae) on the decomposition of submerged oak litter (*Quercus robur* L.) and associated detritivore and fungal communities in a mountain stream in central Portugal. It was divided longitudinally, with one half maintained at ambient temperature (mean = 12.4 °C) while the other was warmed ~3 °C above ambient temperature. Oak leaves in litter bags were incubated in both stream halves, with half of the bags containing one *A. laureatus* larva. Replicate bags were collected over 6 weeks to determine litter mass remaining and the detritivore and fungal communities. *A. laureatus* stimulated decomposition of oak litter and colonization by other shredders at ambient temperature. It also increased fungal biomass at increased temperature, and changed the community of fungi. Higher temperature inhibited *A. laureatus* activity, resulting in a substantial change in the strength of interactions within both fungal and detritivore assemblages, with important consequences for leaf litter decomposition.

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Introduction

Biotic interactions, environmental constraints and dispersal combine to determine ecological communities (Begon et al. 2006; Wisz et al. 2013). Species interactions can govern species coexistence, and thus influence diversity in space and time (Araújo and Luoto 2007; Montoya and Raffaelli 2010; Wiens 2012). Such interactions are expected to change with the predicted rise in global temperatures and it is important to understand how such interactions influence community structure and any associated ecosystem processes.

Warming is one of several stresses associated with climate change. It is expected that the mean global air temperature will increase by 1.5–4.6 °C through 2100 (IPCC 2014). Such a high rate of temperature change has not been experienced by organisms in the past (Raven 2003) and it is expected that rising temperatures will lead to changes in many ecosystems through alterations in life history patterns, the strength of biotic interactions, and rates of key ecosystem processes (Traill et al. 2010).

Warming can directly alter ecosystems through changes in phenology (Cotton 2003; Edwards and Richardson 2004; Cleland et al. 2007), geographic range (e.g. displacement towards the poles or higher altitude; Parmesan and Yohe 2003; Walther 2010) and the physiology of individuals (e.g. increased metabolism; Brown et al. 2004; Rumbos et al. 2010). Such changes are likely to alter the strength and the type of species interactions (Gilman et al. 2010): spatial mismatches between species (Stenseth and

Mysterud 2002; Schweiger et al. 2008), increases in predation rates in response to higher energy requirements (Gotthard 2000), increases in herbivory and pathogenicity (Roy et al. 2004), changes in the size of food webs (Petchey et al. 1999), and shifts in species dominance (Tylianakis et al. 2008; Traill et al. 2010) are some of the known consequences of global warming.

In freshwater ecosystems, where most organisms are ectotherms whose metabolic processes are therefore strongly dependent on temperature (Parmesan and Yohe 2003; Heino et al. 2009; Perkins et al. 2010), warming has been shown to alter species ranges, phenology, and body size distributions across food webs (Hogg and Williams 1996; Friberg et al. 2009; Woodward et al. 2010). These changes may cause decreases in the energetic efficiency of predators or lead to local extinctions (Beisner et al. 1997; Abrahams et al. 2007), decreases in host abundances (Mouritsen et al. 2005) and higher mortality rates of less competitive species (Taniguchi and Nakano 2000). The consequences of these changes include shifts in species traits, trophic structure (Petchey et al. 1999) and, consequently, the magnitude of the biological contribution of these species to ecosystem processes.

In rivers, leaf litter is the primary source of carbon and nutrients to shaded streams (Petersen and Cummins 1974; Gessner et al. 1999). Macroinvertebrates, specifically the leaf shredding detritivores, and aquatic fungal communities contribute substantially to leaf litter decomposition (Gessner et al. 1999; Hieber and Gessner 2002). Changes in species interactions within these decomposer communities can lead to changes in the rates of organic matter decomposition and, therefore, the flow of energy to higher trophic levels. Since species interactions are an indirect pathway by which the effects of global warming can influence community dynamics, it is important to include them in enhancing our understanding of the implications of global warming (Montoya and Raffaelli 2010).

We performed an experiment to understand the roles of warming and species interactions in the community structure of detritivores and aquatic hyphomycetes in a small stream. We further investigated the implications of such changes on organic matter processing. We raised water temperature by $\sim$3 °C above ambient temperature in spring, in the presence or absence of a dominant detritivorous insect (*Allogamus laureatus*), and we followed *Quercus robur* decomposition and community assembly of fungal and detritivore communities associated with the decomposing litter.

We predicted that the presence of *A. laureatus* would stimulate litter decomposition at ambient temperature due to its high consumption rates, and that raised temperature would increase litter decomposition in the absence of *A. laureatus* through an increase of shredder abundance and accrual of fungal biomass. We hypothesized that the effects of increased temperature on *A. laureatus* and its consequences for other species would depend on the thermal tolerance of this species.

We anticipated that the increase in temperature would stimulate increase in fungal biomass through increases of biological activity. We expected an interaction between raised temperature and the presence of *A. laureatus* on fungi, with the outcome contingent on whether shredder feeding is selective for certain fungi, or if feeding (or lack thereof) facilitated fungal growth (Graça and Canhoto 2006). We further hypothesized an interaction between increased temperature and the presence of *A. laureatus* on detritivore community structure. Often, the presence of an aggressive competitor can reduce local biodiversity by eliminating competitive inferiors either by higher consumption rates or by an increase in the frequency of aggressive encounters (Creed et al. 2009). Should increased temperatures not exceed the thermal tolerance of this dominant competitor, interspecific interactions should be more intense, resulting in a reduction in both total abundance and local diversity of detritivores. However, if this species' thermal tolerance is exceeded, this could lead to competitive release, with a subsequent increase in total detritivore biomass and diversity over time.

Materials and methods

Experimental conditions

Study area. The study took place in Candal stream, Lousã mountain, Central Portugal (40°04′48.10″N, 8°12′11.16″W, 634 m a.s.l.). This second order stream (sensu Strahler 1957) runs through native mixed deciduous forest dominated by chestnut (*Castanea sativa* Mill.) and oak (*Quercus* spp), in an area of low human activity. The substratum is dominated by schist pebbles and coarse sand. The study section was divided longitudinally along 22 m with schist stones, thus preventing water mixing. Each half of the study section was $\sim$50 cm wide and 5–10 cm deep. One half of the study section was warmed by $\sim$3 °C (elevated half) above the ambient temperature registered in the other half (ambient half).

Warming began about one month before the experiment started (28 March 2011) and continued until 14 June 2011. Stream water from upstream was diverted into a 260-L reservoir equipped with electric heaters, supplied with a continuous power of 42 kW, that warmed the water before discharging it to the elevated half at a rate of $\sim$2.0 L s^{-1}. The flow rate was controlled manually at the outlet of the reservoir. A similar system was used to provide water to the ambient stream half, except that the water was

delivered at ambient temperature. The increase in temperature of the elevated half in relation to the ambient half was designed to simulate the expected increase in water temperature in the region (3 °C; Eaton and Scheller 1996; Miranda et al. 2002). Water temperature in each stream half was recorded hourly with immersed data loggers (Hobo Pendant UA-001-08, Onset Computer Corp.) placed upstream and downstream in each half of the study section. For a detailed description of the warming and hydraulic systems see Canhoto et al. (2013).

Ten times over the study period (42 days), 300 mL of water from each stream half were collected, filtered through fibre glass filters (47 mm diameter, pore size 0.7 µm; Millipore APFF04700, Millipore, MA, USA), and frozen at −20 °C for determination of nutrient concentrations and alkalinity. Nitrate concentration (NO_3^-) was determined by catalyzed reaction and quantified by colourimetry (with an Hach nitrate kit LCK 339, Hach Lange DR3900, Germany), soluble reactive phosphorus (SRP) concentration by the ascorbic acid method (APHA 1995), and alkalinity by titration with H_2SO_4 (0.02 N) to an end point pH of 4.2 (American Public Health Association (APHA) 1995). Conductivity, total dissolved solids (TDS) (portable conductivity/TDS meter, model LF330 315, WTW, Weilheim, Germany), pH (portable pH meter, model pH 3110, WTW, Weilheim, Germany), and dissolved oxygen (portable Oxygen meter, model Oxi 3210, WTW, Weilheim, Germany) were recorded in situ in both stream sides.

Shredders. The caddisfly *Allogamus laureatus* (Trichoptera: Limnephilidae) was chosen for this study because Limnephilidae are known to be dominant consumers of detritus in some forest streams (Creed et al. 2009). Limnephilidae, in general, are reportedly aggressive towards other macroinvertebrates, have high growth rates (Wissinger et al. 1996), and are functionally important contributors to decomposition (Creed et al. 2009). To avoid interfering with the invertebrate communities in the experimental reach, test individuals were collected from a nearby stream in the same catchment area with similar environmental conditions (Cerdeira stream, Lousã mountain, 40°05′21.39″N, 8°12′06.67″W; Gulis et al. 2006) and where *A. laureatus* is also an important detritivore (personal observation). All individuals were inspected using a binocular microscope (6.4×, Wild M38, Heerbrugg, Switzerland) and the linear interocular distance was measured using a micrometer. Forty-two individuals with interocular distance of 1.43 mm and case opening diameter >4 mm were selected. From these, 24 individuals were randomly chosen and individually placed in tetrahedral mesh bags, and assigned to treatments (see below). The remaining 18 individuals were removed from their cases, oven dried (105 °C, 24 h) and weighed (±0.01 mg) to estimate the dry mass of individuals used in the experiment (15 ± 1.2 mg).

Litter bags. Forty-eight tetrahedral mesh bags (12 × 12 cm, 4 mm mesh) were prepared with 2.01 ± 0.01 g (mean ± SE) air-dried unconditioned oak (*Quercus robur* L.) leaves (rehydrated with distilled water to avoid breakage). Oak leaves were collected after senescence in November 2010, air dried at ambient temperature and stored in the dark until needed. This species decays slowly (Ferreira et al. 2012) and occurs naturally in the local streams (Ferreira and Canhoto 2014). At the start of the experiment, the chemical composition of leaves, as percentages of dry mass (mean ± SE, $n = 3$ or 4) was: phosphorus, 0.03 ± 0.01 % (Graça et al. 2005); carbon, 49.9 ± 0.66 %; nitrogen, 0.83 ± 0.05 % (both analyzed by isotope-ratio mass spectrometry; Thermo Delta V advantage with a Flash Elemental Analyser 1112 series); and polyphenolics, 7.47 ± 0.23 % (Graça et al. 2005). One *A. laureatus* larvae was added just before immersion in the stream halves, according to the treatments (see below).

Experimental design

Litter bags were placed in the stream on 3 May and incubation lasted up to 42 days. 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). Forty-eight litter bags were distributed into four blocks, in both halves of the study reach, and attached to the streambed with nails, with each block having three litter bags from each appropriate treatment (two temperatures × presence or absence of *A. laureatus* × 4 blocks × 3 bags). After 14, 27, and 42 days, four litter bags from each treatment (one from each block) were retrieved, enclosed in individual re-sealable plastic bags, and transported in a cooler to the laboratory.

In the laboratory, leaves were carefully rinsed with distilled water into a 500 µm mesh sieve to separate invertebrates and litter from sediment. Two sets of five leaf discs (12 mm diameter) were cut out with a cork borer to be used for microbial determinations (see below). The remaining litter was oven-dried (105 °C, 48 h), weighed (±0.1 mg) to determine dry mass, ignited (550 °C, 4 h), and reweighed (±0.1 mg) to determine the ash fraction and ash free dry mass (AFDM) remaining. The proportion of AFDM remaining on each sampling date was calculated by dividing AFDM remaining by the initial AFDM (see below).

Ten extra litter bags were prepared in the same way as the samples, taken to the stream on day zero, submerged for ~10 min, brought back to the laboratory, and processed as above. These bags were used to calculate an initial air dry mass to initial AFDM conversion factor (0.83), taking into account mass loss due to handling.

Fungal biomass, sporulation rate and species richness of aquatic hyphomycetes. One set of leaf discs was used for ergosterol determination as a surrogate for fungal biomass (Gessner and Chauvet 1993; Graça et al. 2005). Leaf discs were frozen at −20 °C, freeze-dried (LY3TTE, Snijders Scientific, Tilburg, Netherlands) overnight, weighed (±0.01 mg), and transferred into glass tubes with 10 mL KOH/methanol (8 g L^{-1}). Lipids were extracted in a water bath (80 °C) for 30 min. Extracts were purified by solid phase extraction (Waters Oasis® HLB 3 cc cartridges, Waters Corp., Milford, MA, USA). The concentration of ergosterol was measured using high performance liquid chromatography (HPLC; Dionex, Sunnyvale, CA, USA). The HPLC was equipped with a LiChroCART 250-4 LiChrospher 100 RP-18 (5 mm) column (Merck, Darmstadt, Germany), maintained at 33 °C. The methanol served as the mobile phase, at 1.4 mL min^{-1}. Ergosterol was converted to fungal biomass assuming 5.5 µg ergosterol per mg of fungal dry mass (Gessner and Chauvet 1993). The dry mass of the leaf discs was converted into AFDM using the ash fraction determined for the discs used to induce sporulation (see below). Results were expressed as mg fungal biomass g^{-1} AFDM.

The second set of leaf discs was used to induce sporulation by aquatic hyphomycetes to determine conidial production rates and species richness of aquatic hyphomycetes communities (Appendix 2) associated with the litter. Discs were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (glass fibre filters; Millipore APFF04700, Millipore, MA, USA) on an orbital shaker (100 rpm) for 48 h at 15 °C. Sporulation was not measured in situ due to logistic constraints but was measured in the laboratory by determining conidia production from the conidiophores previously produced in the field. Conidial suspensions were poured into 50-mL Falcon tubes, fixed with 2 mL of 37 % formalin, and the final volume was adjusted to 35 mL with distilled water. Leaf discs were recovered, dried, and ashed to determine ash fraction and AFDM.

To prepare the slides for conidia counts and identification, 100 µL of 0.5 % Triton X-100 were added to the conidial suspension, and mixed with a magnetic stirring bar to ensure homogeneous distribution of conidia. An aliquot of the suspension was passed through a membrane filter (2.5 mm diameter, pore size 5 µm; Millipore SMWP, Millipore Corp., Massachusetts, USA). Filters were stained with 0.05 % Trypan blue in 60 % lactic acid, and conidia were counted and identified under a compound microscope at 250× (Graça et al. 2005). Sporulation rates were expressed as number of conidia per mg AFDM per hour and species richness was expressed as number of species per bag.

Abundance and richness of macroinvertebrates. Macroinvertebrates were separated from the leaves with distilled water in a 500 µm mesh sieve; they were recovered, stored in 20-mL vials, and preserved with 95 % ethanol. Macroinvertebrates were sorted and identified under a binocular microscope (50×; Leica M80, Singapore). Identification was to the lowest taxonomic level possible, generally genus or species, following Vieira-Lanero (2000) and Tachet et al. (2002). Macroinvertebrates were classified into functional feeding groups following Tachet et al. (2002) (Appendix 1). Abundance was expressed as number of individuals per bag and richness was expressed as number of taxa per bag.

Statistical analyses

Water variables were compared between stream halves by *t* test and significant differences were recorded at $P \leq 0.100$. The relationship between ambient and elevated water temperature was assessed by Pearson Correlation and it was considered significant at $P \leq 0.100$.

Decomposition rates (k $days^{-1}$) were calculated assuming an exponential decay: $k = -\ln$ (AFDMr/AFDMi)/t, where AFDMi is the initial mass (g) and AFDMr is the mass remaining (g) at time t (days); the intercept was fixed at 0 in all treatments. Given that the stream halves differed in temperature, litter decomposition rates per degree-day (k dd^{-1}) were also calculated by replacing time in days in the equation above by the sum of mean daily temperatures accumulated by the sampling day. Ln-transformed fractions of the remaining mass over time (in days or degree-days) were compared among treatments with two general linear models (i.e., an ANCOVA relaxing the assumption of parallel slopes), with presence/absence of *A. laureatus* and temperature regime (ambient and elevated) as categorical variables, and time (days or degree days) as the continuous variable.

Fungal biomass, sporulation rate (log $(x + 1)$) and species richness, total macroinvertebrate abundance and taxa richness, and shredder abundance and taxa richness were compared among treatments by three-way analysis of variance (ANOVA), with presence/absence of *A. laureatus*, temperature, and time as categorical variables (Zar 1996). The relationships between shredder abundance and *A. laureatus* abundance and between AFDM remaining and shredder abundance in each stream half were assessed by linear regression.

Several *A. laureatus* larvae and other invertebrates entered the bags during the experimental period (Appendix

1). The number of *A. laureatus* (dependent variable) that entered into the bags (temperature and shredder presence as categorical variables) was compared by a two-way ANOVA. A two-way ANOVA was also performed with number of *A. laureatus* as the dependent variable and the presence/absence of *A. laureatus* and temperature as categorical variables. A three-way ANOVA was performed with *A. laureatus* mean dry mass as the dependent variable instead, and presence/absence of *A. laureatus*, temperature, and time as categorical variables.

As the abundance of *A. laureatus* was no longer different between treatments, regressions were performed between detritivore abundance and number of *A. laureatus* and between AFDM remaining and number of detritivores, for each temperature treatment. Tukey's honest significant difference (HSD) test was used for post hoc multiple comparisons when the ANCOVAs or ANOVAs above detected a significant effect of presence of *A. laureatus*, temperature or their interaction ($P \leq 0.100$). In cases where Tukey's test was not able to identify the significant differences detected by the ANOVA/ANCOVA, Fisher's lowest significant difference (LSD) test was used instead.

The assumptions of normality and homocedasticity were confirmed with Shapiro–Wilk and Bartlett tests, respectively. All statistical analyses were performed with STATISTICA 7 software (StatSoft, OK, USA).

Results

Water temperature and chemistry

During the study period, stream water was circumneutral, well oxygenated, and low in conductivity, nutrient concentrations (nitrate and SRP), and alkalinity (Table 1). Experimental warming significantly increased the water temperature in the elevated stream half by a mean of 2.8 °C above ambient temperature (hourly values; $t = -37$, d.f. = 1922, $P < 0.0001$); elevated and ambient temperature were positively correlated ($R = 0.31$, $N = 45$, $P < 0.0001$). Dissolved oxygen concentration ($t = 4$, d.f. = 18, $P < 0.001$), and SRP ($t = -2$, d.f. = 12, $P = 0.042$) were significantly lower in the elevated than in the ambient half, while the opposite pattern was observed for conductivity ($t = -3$, d.f. = 16, $P = 0.005$).

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

Variable	Ambient half	Elevated half	Number of observations
Temperature (°C)*	12.4 ± 0.1	15.1 ± 0.3	1928
pH	7.1 ± 0.1	7.1 ± 0.1	10
Conductivity (μS cm^{-1})*	27.2 ± 0.1	27.5 ± 0.1	9
TDS (mg L^{-1})	30.0 ± 0.2	30.1 ± 0.1	9
Oxygen dissolved (mg L^{-1})*	10.0 ± 0.1	9.4 ± 0.1	10
Oxygen dissolved (%)	99.8 ± 1.0	98.8 ± 0.9	10
SRP (μg L^{-1})*	34.8 ± 9.1	11.8 ± 4.4	7
Nitrates (μg L^{-1})	84.7 ± 11.0	107.4 ± 25.0	7
Alkalinity (mEq L^{-1})	0.094 ± 0.002	0.092 ± 0.004	4
Discharge (L s^{-1})	2.3 ± 0.3	2.0 ± 0.2	6

Asterisks denote variables that significantly differed between stream halves (*t* test, $P \leq 0.100$)

Litter decomposition

After the 6 week incubation period, mass remaining ranged from 36.9 % ± 4.1 to 48.4 % ± 2.6. There was no significant main effect of temperature or the presence of the detritivore on litter decomposition rates expressed per day, but a significant interaction between them was observed (Fig. 1; Table 2). In the ambient half, decomposition rate of oak leaves was faster in the presence of *A. laureatus* (0.023 d^{-1}) than in its absence (0.017 d^{-1}) (Tukey HSD, $P = 0.041$), while the presence of *A. laureatus* did not induce faster decomposition under elevated temperature (Tukey HSD, $P = 0.648$). When considering decomposition rates corrected for temperature (k dd^{-1}), significant differences were identified for the temperature treatment and for the shredder presence × temperature interaction (Table 2). Significant differences among treatments were similar to those found when considering k d^{-1}, except that litter decomposition was significantly slower, in the presence of *A. laureatus,* in the elevated half than in the ambient half (Fisher LSD, $P = 0.032$; Fig. 1b).

Fungal biomass, sporulation rate and species richness of aquatic hyphomycetes

The pattern of fungal colonization was consistent among treatments. Fungal biomass concentration significantly varied over time, reaching a peak at day 27 (Tukey HSD, $P < 0.001$, Fig. 2a). Fungal biomass concentration was significant higher at elevated temperature (three-way ANOVA, $P = 0.078$), but no effect of shredder presence was found (Table 3). The interaction shredder presence × temperature was significant (three-way ANOVA, $P = 0.051$); in the presence of *A. laureatus* the increase of temperature induced a change in fungal biomass (Tukey HSD, $P = 0.048$).

Sporulation by aquatic hyphomycetes reached a peak on day 14 (Tukey HSD, $P < 0.001$, Fig. 2b). Sporulation rates

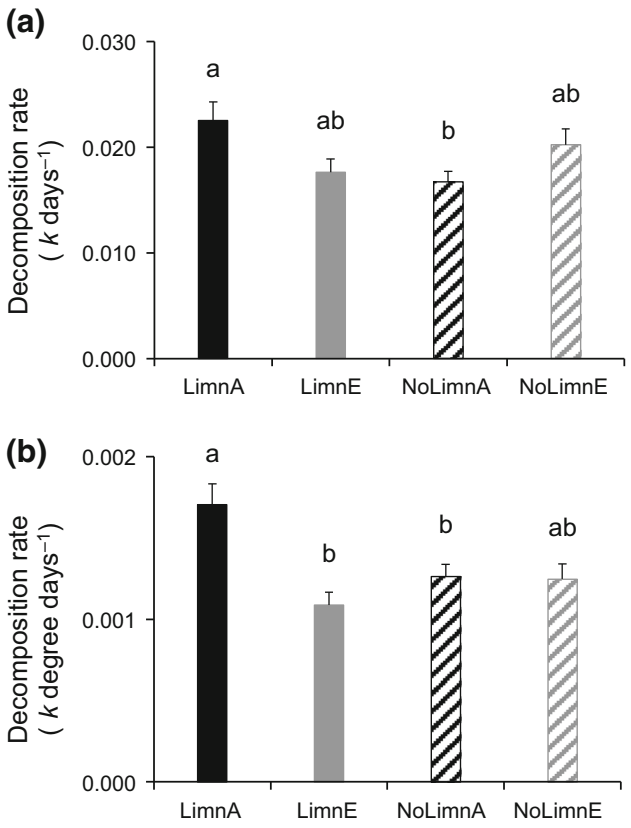

Fig. 1 Decomposition rate per day (**a**) and per degree-day (**b**) of oak leaf litter incubated in the presence (Limn) and absence (NoLimn) of *A. laureatus*, in the ambient (A) and elevated stream half (E) for 42 days. *Bars* represent the mean + standard error (SE). *Different letters* indicate significant differences among treatments, $P \leq 0.100$

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

Source of variation	Remaining mass (days)			Remaining mass (degree-days)		
	d.f.	*F*	*P*	d.f.	*F*	*P*
Intercept	1	15.4	<0.001	1	12.8	<0.001
Time	1	231.7	<0.001	1	215.9	<0.001
Shredder presence	1	1.3	0.270	1	1.2	0.284
Temperature	1	0.2	0.658	1	15.3	<0.001
Shredder presence × temperature	1	7.7	0.008	1	7.3	0.010
Error	43			43		

P values ≤0.100 indicate statistical differences among treatments

were significantly higher at elevated temperature (three-way ANOVA, $P = 0.072$; Table 3), while no effect of the presence of shredders was found (Table 3). Fungal species richness also varied over time with a peak on day 27 (Tukey HSD, $P < 0.001$; Fig. 2c). The interaction shredder

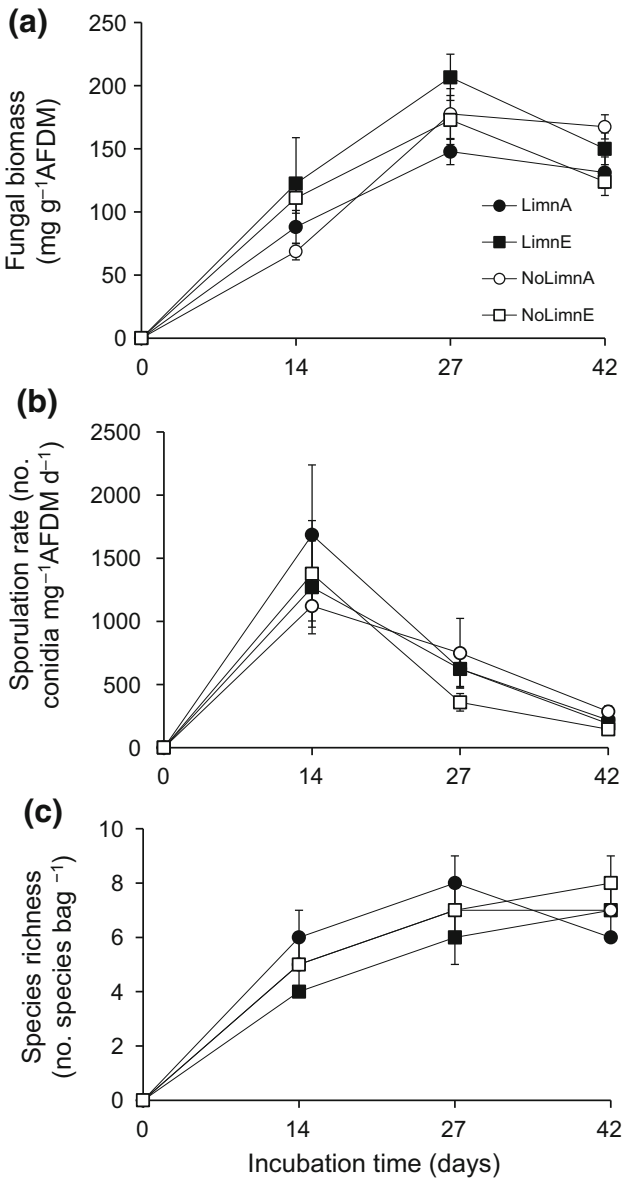

Fig. 2 Fungal biomass (mg g^{-1} AFDM) (**a**), sporulation rate (no. conidia mg^{-1} AFDM d^{-1}) (**b**), and species richness (no. species bag^{-1}) of aquatic hyphomycetes (**c**) associated with oak leaf litter incubated in the presence (Limn) and absence (NoLimn) of *A. laureatus*, in the ambient (A) and elevated stream half (E) for 42 days. Values are mean ± SE

presence × temperature was significant (three-way ANOVA, $P = 0.073$; Table 3): in the presence of *A. laureatus,* the increase of temperature induced a decrease in fungal species richness (Fisher LSD, $P = 0.037$).

Macroinvertebrates

During the experiment, several *A. laureatus* larvae were small enough to enter the bags in a non-differential way in both stream halves (temperature; two-way ANOVA, $F_{1,36} = 0.76$, $P = 0.388$), and the number of *A. laureatus*

Table 3 Summary table for the three-way ANOVA performed on fungal biomass, sporulation rate and species richness of aquatic hyphomycetes associated with oak litter incubated in the presence and absence of *A. laureatus*, in the ambient and elevated stream half over 42 days

Source of variation	Fungal biomass			Sporulation rate			Species richness		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Intercept	1	812.4	<0.001	1	5537.0	<0.001	1	931.5	<0.001
Time	2	21.9	<0.001	2	44.5	<0.001	2	9.8	<0.001
Shredder presence	1	0.2	0.676	1	0.1	0.796	1	0.2	0.684
Temperature	1	3.3	0.078	1	3.4	0.072	1	1.5	0.227
Shredder presence × temperature	1	4.1	0.051	1	0.5	0.508	1	3.4	0.073
Shredder presence × time	2	0.4	0.687	2	0.4	0.676	2	1.7	0.207
Temperature × time	2	2.5	0.097	2	0.6	0.576	2	1.2	0.324
Shredder presence × temperature × time	2	1.5	0.243	2	0.8	0.454	2	0.6	0.555
Error	36			36			36		

P values ≤0.100 indicate statistical differences among treatments

was no longer statistically different between treatments initially with and without *A. laureatus* (presence of *A. laureatus* × temperature interaction; two-way ANOVA, $F_{1,36} = 1.46$, $P = 0.235$). However, the mean dry mass of individuals per bag remained different among treatments: 0.012 ± 0.001 g in the treatment with *A. laureatus* versus 0.006 ± 0.001 g in the treatment without *A. laureatus*, throughout the experiment (Time × shredder presence × temperature; three-way ANOVA, $F_{1,28} = 8.89$, $P = 0.006$).

Invertebrate variables associated with the leaf litter did not differ among sampling dates (Table 4; Fig. 3). Warming had an effect only for macroinvertebrate richness that was significantly higher at the elevated than at the ambient stream half (Table 4; Fig. 3). Shredder presence did not affect invertebrate abundance or richness in litter bags (Table 4).

Detritivore abundance was significantly and positively related to the number of *A. laureatus* at ambient temperature ($R^2 = 0.39$, $N = 16$, $P = 0.010$), but not at elevated temperature ($R^2 = 0.03$, $N = 24$, $P = 0.393$). AFDM remaining (g) and number of shredders were negatively related only for elevated temperature ($R^2 = 0.20$, $N = 24$, $P = 0.027$).

Discussion

In this study, stream water temperature and the presence of a competitively dominant shredder were manipulated in a mountain stream to understand the implications of warming and shredder presence for decomposer community structure and leaf litter decomposition. 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). The higher decomposition rate induced by the presence of this consumer, under ambient temperature, likely resulted from higher feeding activity, given that detritivorous Limnephilidae are known to exhibit very high consumption rates (e.g. Creed et al. 2009). Alternatively, there may have been facilitation of other shredders from the presence of *A. laureatus*, as reported in other studies (Jonsson and Malmqvist 2003), as there were no differences in shredder richness or abundance between treatments. Although the number of *A. laureatus* was no longer significantly different between treatments at the end of experiment, the biomass of *A. laureatus* individuals was significantly higher in the treatments with *A. laureatus* as the colonizing individuals were smaller and less effective consumers. Thus, we assumed that the NoLimn and Limn treatment was maintained.

After adjusting mass loss for temperature by standardizing per degree days, decomposition rates were lower in the elevated temperature half in the presence of *A. laureatus*. The absence of any significant changes in the shredder community suggests that *A. laureatus* played a larger role in changing behaviour at elevated temperature such that other shredders may have been less effective consumers of the leaf litter itself, or of any resident fungi. Our results suggest there was a reduction in feeding activity and an active inhibition of decomposition with raised temperature in the presence of *A. laureatus*. The Limnephilidae are generally aggressive towards other macroinvertebrates, have high growth and consumption rates, and are functionally important contributors to the decomposition process (Wissinger et al. 1996; Creed et al. 2009). A decrease in feeding activity might result from warming above critical thresholds reducing metabolism or

Table 4 Summary table for the three-way ANOVA performed on macroinvertebrate abundance, macroinvertebrate richness, shredder abundance and shredder richness associated with oak litter incubated in the presence and absence of *A. laureatus*, in the ambient and elevated stream half over 42 days

Source of variation	Macroinvertebrate abundance			Macroinvertebrate richness			Shredder abundance			Shredder richness		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Intercept	1	93.0	<0.001	1	271.0	<0.001	1	60.8	<0.001	1	115.5	<0.001
Time	2	0.7	0.486	2	0.3	0.768	2	0.2	0.841	2	0.7	0.506
Shredder presence	1	1.4	0.242	1	0.1	0.749	1	0.1	0.802	1	0.0	0.889
Temperature	1	1.1	0.310	1	3.9	0.059	1	0.4	0.516	1	1.0	0.331
Shredder presence × temperature	1	1.1	0.310	1	1.8	0.196	1	0.0	1.000	1	0.1	0.779
Shredder presence × time	2	0.2	0.796	2	0.5	0.618	2	0.1	0.871	2	0.0	0.965
Temperature × time	2	1.9	0.172	2	1.8	0.183	2	1.7	0.202	2	0.7	0.484
Shredder presence × temperature × time	2	<0.1	0.995	2	0.2	0.863	2	0.2	0.847	2	0.6	0.564
Error	28			28			28			28		

P values ≤0.100 indicate statistical differences among treatments

from the organisms devoting more time to interactions with other individuals. *A. laureatus* may have spent more time and energy interacting with other detritivores than feeding, leading to a lower decomposition rate at elevated temperature, because Limnephilidae species also have been reported to be naturally aggressive (Wissinger et al. 1996; Creed et al. 2009).

Fungal biomass was significantly higher at elevated temperature in agreement with previous studies (Dang et al. 2009; Ferreira and Chauvet 2011a, b). Although a similar pattern was expected for sporulation rates (Ferreira and Chauvet 2011a, b), conidial production was significantly higher in the ambient than in the elevated stream half. However, this was likely due to our sampling schedule that may have missed the peak in sporulation in the elevated half.

Our manipulation resulted in complex patterns in the fungi. The increase in fungal biomass under raised temperature was stronger in the presence of *A. laureatus*. In the same conditions, fungal species richness decreased. We offer two, not necessarily mutually exclusive, interpretations of this result. Selective feeding by the shredder could have led to the decline in species richness. Loss of species preferred by the shredder could then have favoured a compensatory increase in biomass of less preferred fungal species (Arsuffi and Suberkropp 1989). Alternatively, raised temperatures could have constrained colonization of certain fungal species sensitive to temperature changes, while at the same time inducing higher growth in tolerant species. Overall, raising temperature in this experiment led to complex interactions between this leaf-shredding consumer and the fungal community.

The increase in temperature and the presence of *A. laureatus* did not significantly change the colonization patterns or abundance of detritivores, which is, partially, in contrast with that previously demonstrated by Nilsson and Otto (1977). This may be due to the high variability observed over time and to the low number of replicates. However, macroinvertebrates taxa richness was significant higher with increased temperature in agreement to what is frequently found (Jacobsen et al. 1997). Furthermore, *A. laureatus* was associated with the presence of other detritivores at ambient temperature, contrary to our prediction. Other works report that Limnephilidae are generally aggressive, strong competitors with high activity rates (Wissinger et al.1996; Creed et al. 2009). Given this, we expected that detritivore abundance would decrease in the presence of *A. laureatus* at ambient temperature. In the elevated half it seems that *A. laureatus* activity was inhibited, suggesting that other detritivores played a stronger role in decomposition, since there was a negative correlation between detritivore abundance and remaining litter mass. This is revealed by a positive relationship between the abundance of *A. laureatus* and other detritivores that disappeared with temperature increase. Significant changes occurred in leaf litter decomposition rate, either due to the presence of *A. laureatus* or due to warming. The decrease in decomposition rate recorded at elevated temperature can result from inhibition of *A. laureatus* feeding activity, and therefore perhaps discouragement of feeding for other detritivores also. This suggests that warming caused a change in interspecific relationships within the detritivore guild.

Three important features appeared to illustrate the potential effect of global warming in this work: changes in community composition (macroinvertebrates and fungi), biological interactions, and ecosystem functioning. Stronger effects of warming were found for the last two aspects, suggesting that potential food web relationships and ecological processes may change without a significant change

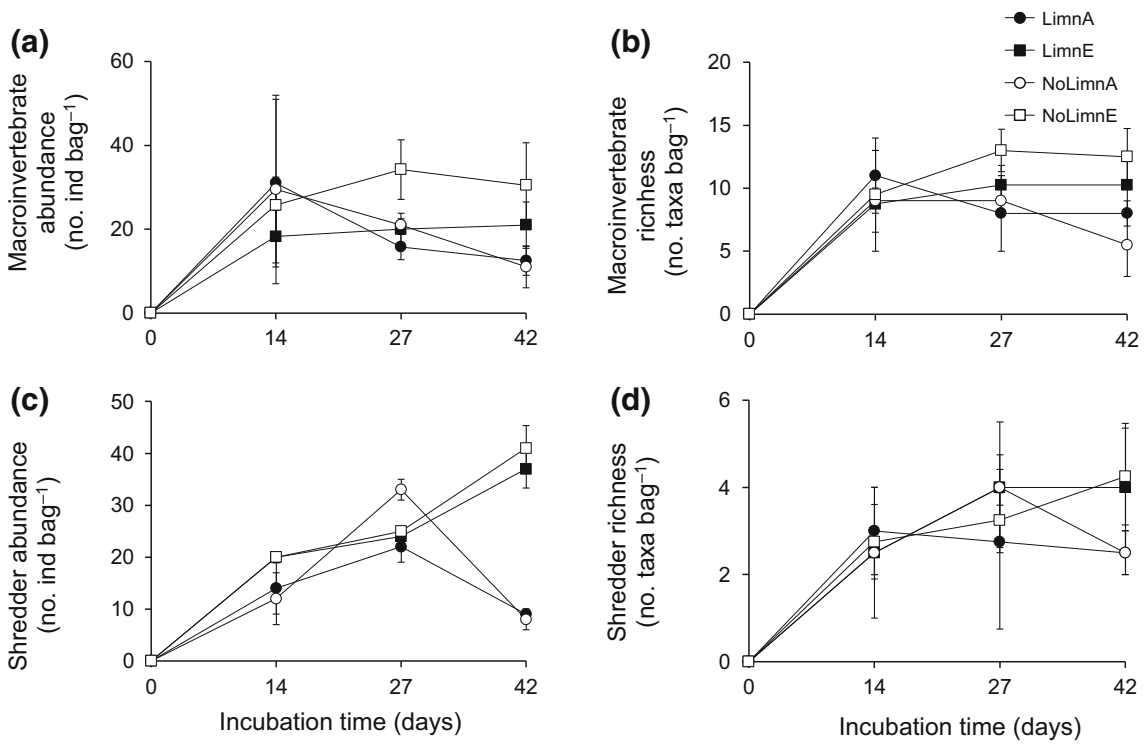

Fig. 3 Macroinvertebrate colonization dynamics: macroinvertebrate abundance (no. individuals bag^{-1}) (**a**), macroinvertebrate richness (no. taxa bag^{-1}) (**b**), shredder abundance (no. individuals bag^{-1}) (**c**) and shredder richness (no. taxa bag^{-1}) (**d**) associated with oak leaf litter incubated in the presence (Limn) and absence (NoLimn) of *A. laureatus*, in the ambient (A) and elevated (E) stream half for 42 days. Values are mean ± SE

in community composition. This result should be considered when assessing ecosystem integrity under environmental change, as many assessments are made based solely on community structure. Assessing the susceptibility of biological interactions to warming is necessary to allow better understanding of how ecosystems will respond to global warming.

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Appendix 1

Taxa recorded in the study area during the experimental period, and their characterization into functional feeding groups (FFG). For Coleoptera, *A* indicates adult individuals and *L* indicates larvae.

Higher level	Species	FFG
Order trichoptera	*Allogamus laureatus*	Shredder
	Catagapetus	Grazer
	Diplectrona felix	Collector
	F. Ecnomidae	Predator
	Glossosoma	Grazer
	Goera pilosa	Grazer
	Helicopsyche helicifex	Grazer
	Hydropsyche ambigua	Collector
	Lepidostoma hirtum	Shredder
	F. Lepidostomatidae	Shredder
	Plectronemia laetabilis	Predator
	F. Polycentropodinae	Predator
	Polycentropus	Predator
	Polycentropus corniger	Predator
	Rhyacophila lusitanica	Predator
	Sericostoma	Shredder
	F. Sericostomatidae	Shredder (Generally)
Order ephemeroptera	*Acentrella sinaica*	Grazer
	Baetis	Grazer
	Centroptilum	Grazer
	Ecdyonurus	Grazer
	Ephemerella	Shredder/grazer
	Habroleptoides	Grazer
	Habrophlebia	Shredder
Order plecoptera	*Capnia*	Shredder
	Chloroperla	Shredder
	Isoperla	Shredder
	Leuctridae	Shredder
	Nemoura	Shredder
	F. Nemouridae	Shredders/ collectors
	Protonemura	Shredder
Order coleoptera	*Chyphon (L)*	Grazer
	Coelambus (A)	Shredder/piercer
	Coelostoma (A)	No information
	Copelatus (A)	Shredder
	Elmis (L)	Grazer
	Elodes (L)	Grazer
	Helophorus	Shredder
	Hydraena (A)	Grazer
	Macroplea (A)	Shredder
	Microcara (L)	Grazer
	Noterus (A)	Shredder/predator
	Octhebius (A)	Grazer
	Oulimnius (A)	Grazer
	Platambus (L)	Shredder/piercer
Odonata	*Condulegaster*	Predator
	Calopterix	Predator
Order diptera	*Atherix*	Piercer
	Tr. Chironomini	Collector
	sF. Clinocerinae	Predator
	Dixa	Collector
	Athichopogon	No information
	sF. Hemerodromiinae	Predator
	sF. Orthocladinae	Grazer
	Tr. Prosimuliini	Collector
	F. Rhagionidae	No information
	Tr Simuliini	Collector
	sF. Tanypodinae	Predator
	Tr. Tanytarsini	Collector
	Tipula	Shredder
Class gastropoda	*Ancylus fluviatilis*	Grazer
	Bythynella	Grazer
Class hirudinea	*Erpobdella octoculata*	Collector
Acari		Parasite
Turbellaria	*Polycelis nigra* and *P. tenuis*	Predator
Class oligochaeta	F. Naididae	Collector
	F. Tubificidae	Collector

Appendix 2

Taxa recorded in the study area during the experimental period.

Fungal species
Alatospora acuminata
Alatospora pulchella
Anguillospora furtiva
Anguillospora filiformis
Articulospora tetracladia
Clavariopsis aquatica
Clavatospora longibrachiata
Culicidospora aquatica
Flagellospora curvula
Heliscus lugdunensis
Lemonniera terrestris
Lunulospora curvula
Margaritispora aquatica/Goniopila monticola
Tetrachaetum elegans
Tricladium chaetocladium
Tricladium splendens
Triscelophorus acuminatus
Triscelophorus monosporus
Unidentified tetraradiate

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