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Future increase in temperature may stimulate litter decomposition in temperate mountain streams: evidence from a stream manipulation experiment

VERÓNICA FERREIRA AND CRISTINA CANHOTO

MARE – Marine and Environmental Sciences Centre & IMAR – Institute of Marine Research, University of Coimbra, Coimbra, Portugal

SUMMARY

1. Small woodland streams constitute the majority of water courses in many catchments. Given their generally low water temperature, high surface : volume ratio and primarily heterotrophic nature, such streams can be strongly affected by increases in temperature. It is therefore important to assess how stream communities and processes respond to the global warming projected for this century.
2. We assessed the effects of a *c.* 3 °C experimental warming of stream water on decomposition of *Quercus robur* leaf litter and on the associated fungal biomass (ergosterol concentration), sporulation rates of aquatic hyphomycetes, and total macroinvertebrate and shredder abundance in spring, autumn and winter.
3. A mountain stream reach in central Portugal was divided longitudinally over 22 m with local stones. The study followed a before-after control-impact design, with both stream halves at ambient temperature during 1 year and one stream half being experimentally warmed in the second year.
4. Experimental warming of stream water stimulated litter decomposition only in winter, probably because at that time, the low natural temperature limited microbial activities. The effect of experimental warming did not depend on the presence of macroinvertebrates. Contrary to expectations, no significant effect of experimental warming was found on fungal biomass accrual, sporulation rate of aquatic hyphomycetes or macroinvertebrate abundance on decomposing litter.
5. Although the stimulation of litter decomposition in winter could lead to food depletion, this is unlikely when streams are subsidised by more recalcitrant leaves such as oak, which enter the stream in later winter and decompose slowly.

Keywords: climate change, fresh waters, fungal activity, leaf litter, macroinvertebrates

Introduction

The projected increase in global mean air temperature of up to 4.8°C over this century (IPCC, 2013) will be mirrored by an increase in stream water temperature (Pilgrim, Fang & Stefan, 1998). Such increase is likely to stimulate metabolic activities, provided it does not exceed species optimal temperature (Brown *et al.*, 2004). Laboratory studies have shown higher growth and sporulation rates by aquatic fungal species (Graça & Ferreira, 1995; Chauvet & Suberkropp, 1998; Dang *et al.*, 2009; Duarte *et al.*, 2013) and higher fragmentation rates by stream invertebrate shredders (González & Graça, 2003; Azevedo-Pereira, Graça & González, 2006) when temperatures increase up to the optimal temperature. This enhanced activity can translate into accelerated decomposition rates (Ferreira & Chauvet, 2011a,b). However, many cool water organisms from high latitude/elevation streams are already near their upper thermal tolerance limit, and further increases in temperature can lead to inhibition or suppression of activity (Gaufin & Hern, 1971; Moulton *et al.*, 1993; Graça & Ferreira, 1995). Also, species experience complex biotic interactions and are affected by several abiotic factors under natural settings,

Correspondence: Verónica Ferreira, MARE – Marine and Environmental Sciences Centre, IMAR – Institute of Marine Research, Faculty of Sciences and Technology, University of Coimbra, 3004-517 Coimbra, Portugal. E-mail: veronica@ci.uc.pt

which can affect their thermal optima (Webster, Moran & Davey, 1976).

Correlative large-scale studies across streams over gradients of elevation (Fabre & Chauvet, 1998; Taylor & Chauvet, 2014), latitude (Irons *et al.*, 1994; Boyero *et al.*, 2011b) or geothermy (Friberg *et al.*, 2009) have reported faster litter decomposition with an increase in temperature. The effect of temperature on litter decomposition in these cases can, however, be confounded by parallel changes in other factors (e.g. shredder density; Boyero *et al.*, 2011a). Additionally, the temperature gradient addressed in these studies is often wider than the temperature increase projected from a doubling in atmospheric CO_2 concentration. Thus, it might not be realistic to predict the effects of warming on litter decomposition in a given stream based on these wide range field surveys (Woodward, Perkins & Brown, 2010).

In-stream manipulation of water temperature would permit control of potentially confounding variables while still giving information on the effects of warming at the ecosystem level. However, this approach is logistically challenging, which explains why it has been performed only once with surface (Hogg *et al.*, 1995) and once in hyporheic (Bärlocher *et al.*, 2008) stream water, both in the same stream (Valley Spring, southern Ontario, Canada). The experimental warming of surface water of a 1st order stream by 2–3.5 °C over 2 years led to reduction in densities, increase in growth rates, decrease in size at maturity and earlier emergence of benthic macroinvertebrates in the heated stream half, although the magnitude of the responses depended on species identity (Hogg *et al.*, 1995; Hogg & Williams, 1996). However, the effect of stream warming on ecosystem-level processes was not addressed in this experiment. Bärlocher *et al.* (2008) increased hyporheic stream water temperature by an average of 4.3 °C over 1 year and found lower diversity of aquatic hyphomycetes and lower number of leaf fragments in the heated stream half, suggesting faster decomposition of buried litter with warming.

We experimentally warmed a stream to assess the effect of a *c.* 3 °C increase in water temperature on the decomposition of submerged *Quercus robur* L. leaf litter, in the presence and absence of macroinvertebrates. The experiment was conducted in spring, autumn and winter. We anticipated that (i) experimental warming of stream water would stimulate litter decomposition due to the temperature sensitivity of biological activities (Brown *et al.*, 2004), (ii) stimulation by experimental warming would be stronger in winter than in spring due to the low natural temperature limiting microbial activity (Kirschbaum, 1995) and (iii) stimulation would be stronger in the presence of macroinvertebrates that would profit from enhanced microbial colonisation of litter (Canhoto & Graça, 2008) and would have increased feeding activity (González & Graça, 2003).

Methods

Experimental design

We studied the effect of an increase in water temperature on microbe-induced and overall (microbe- + macroinvertebrate-induced) litter decomposition. The study followed an unreplicated before-after control-impact (BACI) design (Smith, 2002). *Quercus robur* leaves were incubated in fine and coarse mesh (CM) bags over 60-day periods in spring, autumn and winter in both halves of a mountain stream reach that was divided longitudinally. During the first year (February 2010–March 2011; ambient year), both stream halves were at ambient water temperature, while during the second year (March 2011–February 2012; warmed year), one stream half (experimental half) was warmed *c.* 3 °C above the ambient temperature registered in the other half (control half) (Table 1). Leaf litter was not incubated during summer because discharge was very low, which in the warmed year prevented operation of the heating system.

Study site

The study stream reach was located in Ribeira do Candal (Lousã mountains, central Portugal; 40°4′44″N, 8°12′10″W, 620 m a.s.l.), a 2nd order stream that drains an area of 0.8 km^2 covered by mixed deciduous forest dominated by *Castanea sativa* Mill. and *Q. robur*. Human activity in the catchment was low. The bedrock is schist, and the stream substratum is mainly cobbles, pebbles and sand. This stream was selected for this study because it meets three criteria needed to carry out the experimental warming: (i) it is a near-pristine stream and yet (ii) is relatively close to a small mountain village with electrical supply and (iii) is easily accessible.

The study stream was divided longitudinally into two halves for a distance of 22 m using local stones driven into the stream bed. The water provided to the study reach was collected upstream and transported by gravity through pipes to two 260-L stainless steel tanks that delivered the water to both stream halves at 1.5–3.0 L s^{-1} (Table 2) through two valves inserted in a dam constructed upstream of the study reach. One of the

Table 1 Mean water temperature (°C, ±SD) and thermal sum (cumulative daily mean temperature over each 60-day study period) during the seasonal decomposition experiments. The 2-week delay in litter incubation in autumn between the ambient and the warmed year was due to the fact that the stream dried out for a longer period in summer 2011 than in summer 2010; comparison between stream halves and years for each season was made by two-way ANOVA; treatments with the same letter do not significantly differ (Fisher's test, $P > 0.050$)

Season	Year	Stream half	Month/Year	Start (day 0)	End (day 60)	Mean ± SD	Thermal sum
Spring	Ambient	Control	April/2010–May/2010	2 April 2010	1 June 2010	10.8 ± 1.3 a	658
		Experimental	April/2010–May/2010	2 April 2010	1 June 2010	11.1 ± 1.3 a	679
	Warmed	Control	April/2011–May/2011	31 March 2011	30 May 2011	11.8 ± 0.9 b	719
		Experimental	April/2011–May/2011	31 March 2011	30 May 2011	14.8 ± 1.4 c	903
Autumn	Ambient	Control	October/2010–December/2010	15 October 2010	14 December 2010	9.8 ± 1.2 a	599
		Experimental	October/2010–December/2010	15 October 2010	14 December 2010	10.1 ± 1.1 a	614
	Warmed	Control	November/2011–December/2011	28 October 2011	27 December 2011	9.7 ± 1.1 a	592
		Experimental	November/2011–December/2011	28 October 2011	27 December 2011	12.0 ± 1.8 b	730
Winter	Ambient	Control	January/2011–February/2011	28 December 2010	26 February 2011	8.4 ± 1.4 b	510
		Experimental	January/2011–February/2011	28 December 2010	26 February 2011	8.7 ± 1.4 bc	533
	Warmed	Control	January/2012–February/2012	27 December 2011	25 February 2012	6.4 ± 1.2 a	388
		Experimental	January/2012–February/2012	27 December 2011	25 February 2012	9.2 ± 2.4 c	560

tanks was equipped with 30 electrical resistors (2000 ; Crussel, Alberto Lindo da Cruz, Lda, Mealhada, Portugal) supplied by a constant 42 kW, which warmed the water above ambient temperature during the warmed year. A detailed description of the hydraulic and heating systems can be found in Canhoto, Lima & Almeida (2013). The target increase in water temperature was 3 °C, based on predictions for air temperature in Portugal by the end of this century (Miranda *et al.*, 2002) and on the relationship between water and air temperature reported for similar streams (Pilgrim *et al.*, 1998). However, mainly due to electrical shutoffs during thunderstorms, the mean warming was 2.3 °C (autumn 2011)–3.0 °C (spring 2011) (Table 1).

Water variables

Water temperature was recorded hourly during the study period in both stream halves using submersed data loggers (Hobo Pendant UA-001-08; Onset Computer Corp., Cape Cod, MA, U.S.A.). Hourly temperature records were averaged to produce daily means, and daily means were accumulated by the sampling dates to generate thermal sums (degree-days). Weekly, discharge was determined by recording the time needed to fill in a 10-L bucket at the outlet of the valves that delivered the water to each stream half (Gore, 1996), and electrical conductivity (LF 330; WTW, Weilheim, Germany), dissolved oxygen (Oxi 3210; WTW) and pH (pH 3110; WTW) were recorded *in situ* in both stream halves. At the same time, 300 mL of water was collected from each stream half, filtered through glass fibre filters (47 mm ∅, nominal pore size 0.7 μm; Millipore APFF04700; Millipore Corp., , Billerica, MA, U.S.A.), transported on ice to the laboratory and frozen until analysed for nutrient concentrations. Nitrate concentration was determined by ion chromatography (Dionex DX-120, Sunnyvale, CA, U.S.A.), and soluble reactive phosphorus (SRP) concentration was determined by the ascorbic acid method (APHA, 1995). Alkalinity was determined by titration with sulphuric acid to an endpoint of pH 4.2 (APHA, 1995).

Leaf litter decomposition

Castanea sativa and *Q. robur* contribute most to the benthic litter standing stock in Candal stream. However, due to differences in phenology between species, *C. sativa* trees shed most of their leaves earlier than *Q. robur* trees (Pozo *et al.*, 1997), and *C. sativa* leaves decompose faster than *Q. robur* leaves (Ferreira, Encalada & Graça, 2012). Also, *Q. robur* is a marcescent species, i.e. it retains dead leaves that can be dropped throughout the year (Dios, Benoti-Garzón & Sainz-Ollero, 2009). Therefore, the presence of *C. sativa* leaves in the stream bed in spring and summer is much reduced compared with that of *Q. robur*. *Quercus robur* leaves were thus selected for this study. Leaves were collected just after abscission in the Lousã mountains (autumn 2006), air-dried at room temperature and stored in paper boxes in the dark until needed. All the leaf litter needed for this study was collected in the same period (autumn 2006) to decrease the variability in litter quality that could arise from collecting the litter in different periods. Storing for 3.5–5 years did not noticeably change the litter. Batches of air-dried leaves (2.24–2.54 g; approximately

five leaves) were enclosed in bags of fine mesh (FM; 10 × 12 cm, 0.5 mm mesh) and of coarse mesh (CM; 10 × 12 cm, 10 mm mesh). FM bags were to prevent the access of macroinvertebrates to the litter so that decomposition is mostly microbial, while CM bags allowed macroinvertebrates to contribute to decomposition. Fifteen litter bags of each mesh size were deployed in each stream half in spring, autumn and winter of the ambient year and then again during the warmed year (Table 1). Due to low water depth (<10 cm), *Q. robur* litter bags were in close contact with the stream bed and naturally submerged litter; during peak litter fall, bags were also completely covered with litter. On day 0, five extra litter bags of each mesh size were taken to the field, submerged for *c.* 10 min, returned to the laboratory, oven-dried at 105 °C for 48 h, weighed (±0.1 mg) and ground (<500 μm powder size; ZM 100; Retsch, Haan, Germany). A subsample of litter powder was oven-dried, weighed, ignited and reweighed to calculate the initial ash-free dry mass (AFDM) that was used to create a conversion factor between initial air-dry mass and initial AFDM taking into account mass loss due to handling.

Litter bags ($n = 3$) were sampled after 11, 20, 31, 45 and 60 days incubation, transported to the laboratory on ice and promptly processed. Leaves from FM bags were gently rinsed with distilled water, and two sets of five leaf discs were removed with a cork borer (12 mm ∅) for microbial analyses (see below). The five leaf discs for each set were removed from individual leaves when possible, and discs for both sets were generally taken symmetrically in relation to the main vein. Leaves from CM bags were rinsed with tap water over a 500-μm mesh sieve, and the macroinvertebrates retained were recovered (see below). The remaining litter was oven-dried, weighed and ground. A subsample of litter powder was oven-dried, weighed, ignited and reweighed as above to calculate ash fraction and AFDM remaining (after correction for the mass of discs removed from FM bags). Results were expressed as AFDM remaining (%) at each sampling date.

Fungal biomass and conidial production

One set of five leaf discs was used for the determination of ergosterol concentration, as a proxy of fungal biomass. Leaf discs were frozen at −20 °C until ergosterol extraction, and ergosterol was extracted, purified and quantified following Graça, Bärlocher & Gessner (2005). Ergosterol was converted into mycelial biomass assuming 5.5 μg ergosterol mg^{-1} mycelial dry mass (DM) (Gessner & Chauvet, 1993). Disc DM was converted into AFDM using the ash fraction derived from the discs used for sporulation (see below), and results were expressed as mg mycelial biomass g^{-1} leaf litter AFDM.

The second set of five leaf discs was used to induce conidial production by aquatic hyphomycetes. Conidial production indicates fungal reproductive activity and is usually highly sensitive to environmental changes (Castela, Ferreira & Graça, 2008; Lecerf & Chauvet, 2008). Leaf discs were incubated in 100-mL Erlenmeyer flasks with 25 mL of filtered (47 mm ∅, pore size 0.7 μm, Millipore APFF04700; Millipore Corp.) stream water from the corresponding stream half. Incubations took place over 48 h on an orbital shaker (100 rpm), at 15 °C and under a 12 h light : 12 h dark photoperiod. Inducing fungal sporulation at the same temperature throughout the study and for both stream halves allowed us to assess differences in conidial production that are due to differences in the number of conidiophores on the surface of decomposing litter induced by differences in temperature during the incubation in the field. The conidial suspensions were fixed with 37% formalin and stored until use. The suspensions were mixed with 150 μL Triton X-100 (0.5%), and an aliquot was filtered through membrane filters (2.5 mm ∅, pore size 5 μm, Millipore SMWP; Millipore Corp.). Filters were stained with trypan blue in lactic acid, and conidia were counted under a compound microscope at 320× magnification (Graça *et al.*, 2005). The remaining leaf discs were oven-dried, weighed, ignited and reweighed as above to calculate discs' ash fraction and AFDM. Sporulation rates were expressed as number of conidia mg^{-1} leaf litter AFDM day^{-1}.

Macroinvertebrates

Macroinvertebrates retrieved from CM bags were preserved in 96% ethanol. Individuals were counted and grouped as shredders and non-shredders (Tachet *et al.*, 2010). Abundance was expressed as total number of macroinvertebrates g^{-1} leaf litter AFDM and number of shredders g^{-1} leaf litter AFDM.

Data analysis

Water variables (log [$x + 1$] transformed, except for temperature) were compared within each season by two-way analyses of variance (ANOVA), with year (ambient versus warmed) and stream half (control versus experimental) as categorical variables. Due to equipment malfunctioning, conductivity was not determined in winter

of the ambient year and SRP was determined only once in autumn of the ambient year; therefore, comparisons between stream halves were made only for the warmed year by two-tail *t*-tests.

Exponential decomposition rates (k, day^{-1}) were calculated as the slope of linear regressions of ln-transformed fraction of remaining AFDM against time (days), with the intercept fixed at ln(1). Fraction of remaining AFDM (ln-transformed) was compared between years, stream halves and mesh sizes for each season by repeated-measures analyses of variance (RM ANOVA), with year (ambient versus warmed) and date (d11, d20, d31, d45 versus d60) as within-subject factors, and stream half (control versus experimental) and mesh size (CM versus FM) as between-subject factors. Given the low statistical power of our analyses, effect sizes were also calculated to indicate the percentage of variance in the dependent variable (litter decomposition) that can be attributed to a factor or interaction. As our data are non-independent, effect sizes could not be calculated as the classical η^2 values but as partial η^2 values. Partial η^2 values were calculated as the ratio between the variance explained by a factor or interaction and the variance explained by that factor or interaction plus its error variance and can add up to more than one in multifactorial ANOVA design (Pierce, Block & Aguinis, 2004). To facilitate interpretation, partial η^2 values were converted into percentage by multiplying by 100.

The sensitivities to temperature of decomposition rates during the warmed year were calculated as Q_{10-q} values: $Q_{10-q} = (t_c/t_w)^{(10/(T_w-T_c))}$, where t_c and t_w are the time (day) to decompose 50% AFDM at the cooler (T_c) and warmed temperature (T_w), respectively (Conant *et al.*, 2008a). The time to decompose 50% initial AFDM under 10 °C warming was calculated as time (day) to decompose 50% initial AFDM under ambient temperature/Q_{10-q}.

Dynamics of fungal biomass (log [x + 1] transformed), rate of sporulation by aquatic hyphomycetes (log [x] transformed), and total macroinvertebrate and shredder abundance (log [x + 1] transformed) were compared between treatments for each season by RM ANOVA, with year (ambient versus warmed) and date (d11, d20, d31, d45, d60) as within-subject factors and stream half (control versus experimental) as the between-subject factor. Partial η^2 values were calculated as above.

Normality was checked by Shapiro–Wilk's test, and homoscedasticity was checked by the Bartlett chi-square test. Fisher's least significant difference test was used for multiple comparisons. All statistical analyses were performed with Statistica 7 software (StatSoft Inc., Tulsa, OK, U.S.A.).

Results

Water variables

Water temperature was similar between stream halves in the ambient year (Fisher's test, $P > 0.467$; Table 1). During the warmed year, the experimental warming successfully increased the water temperature of the experimental half by an average of 3.0 °C in spring, 2.3 °C in autumn and 2.8 °C in winter above the temperature observed in the control half (Fisher's test, $P < 0.001$). This resulted in thermal sums 138–184 degree-days higher in the experimental half than in the control half after the 60-day incubation periods (Table 1). The water temperature in the experimental half during the warmed year was also significantly higher than that observed in both halves during the ambient year (Fisher's test, $P < 0.028$), except in winter when it was similar to the experimental half in the ambient year ($P = 0.409$). Due to inter-annual temperature variation, the water temperature in the control half in the warmed year was warmer (spring; Fisher's test, $P < 0.033$), similar (autumn; $P > 0.420$) or colder (winter; $P < 0.001$) than in both halves in the ambient year (Table 1).

During the study period, stream water had low conductivity and alkalinity and was well oxygenated, circumneutral and nutrient poor (Table S1). Within each year, stream halves did not significantly differ in any measured water variables. However, there was inter-annual variation for all measured variables, at least in one season and one stream half (Table S1).

Leaf litter decomposition

Quercus robur leaf litter lost 43–73% initial AFDM over 60 days in water in spring, 31–48% in autumn and 24–42% in winter, across mesh sizes, stream halves and years (Fig. 1). This resulted in decomposition rates of 0.0101–0.0204 day^{-1} in spring, 0.0065–0.0115 day^{-1} in autumn and 0.0051–0.0098 day^{-1} in winter (Fig. 1). Decomposition was significantly faster in CM than in FM bags, especially in spring and winter (RM ANOVA, $P < 0.001$ for mesh size), independently of the stream half ($P > 0.361$ for stream half × mesh size) (Fig. 1, Table S2). In winter, litter decomposition was significantly faster in the experimental half than in the control stream half in the warmed year and significantly faster

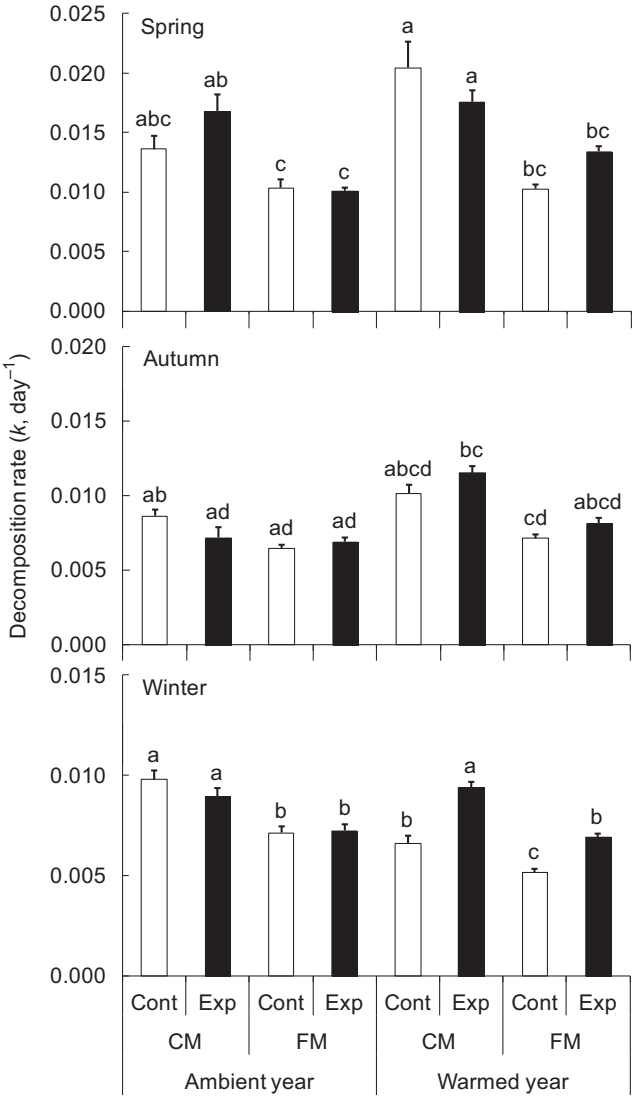

Fig. 1 Decomposition rates of *Quercus robur* leaf litter incubated in coarse (CM) and fine (FM) mesh bags in the control (Cont) and experimental (Exp) stream halves over 60 days in spring, autumn and winter in the ambient and warmed year. Values are means + SE. Decomposition within each season was compared between treatments by RM ANOVA; treatments with the same letter do not differ significantly (Fisher's test, $P > 0.050$).

in the ambient year than in the warmed year for the control stream half (RM ANOVA, $P = 0.003$ for stream half, $P = 0.001$ for year, and $P = 0.005$ for year × stream half; Fig. 1, Table S2). In spring, the effect of experimental warming on litter decomposition was not significant (RM ANOVA, $P = 0.105$ for stream half), but still 38% of the variance on litter decomposition was accounted for by stream half.

Sensitivity of litter decomposition to temperature in the warmed year was higher in winter than in spring and autumn, which resulted in faster litter decomposition in winter when considering a 10 °C increase in temperature (Table 2). Warming reduced or even inverted the difference in litter decomposition rate among seasons. Under ambient conditions, litter decomposed faster in spring (2.0- to 3.1-fold) and autumn (1.4- to 1.5-fold) than in winter, while when considering a 10 °C increase in temperature, decomposition was 1.1- to 1.9-fold slower in the warmer seasons than in winter (except for litter in FM that decomposed 40% faster in spring) (Table 2).

Fungal biomass and sporulation by aquatic hyphomycetes

Biomass build-up on litter decomposing in spring and autumn generally peaked by day 31–45; in winter, fungal biomass increased over the incubation period (warmed year) or peaked by day 20 and then again by the last sampling date (ambient year) (Fig. 2). Maximum values ranged between 193 and 235 mg g^{-1} AFDM in spring, 135 and 169 mg g^{-1} AFDM in winter and 106 and 152 mg g^{-1} AFDM in autumn across years and stream halves (Fig. 2). In autumn, fungal biomass was significantly higher in the experimental half than in the control stream half in both the ambient and the warmed years (RM ANOVA, $P = 0.003$ for stream half and $P = 0.201$ for year × stream half; Fig. 2, Table S3). There were no significant differences in fungal biomass concentration between years for any season (RM ANOVA, $P > 0.342$ for year and $P > 0.201$ for year × stream half; Fig. 2, Table S3). However, the year × stream half interaction accounted for 27–64% of the year × stream half plus error variance across seasons.

Rates of sporulation by aquatic hyphomycetes on decomposing litter peaked by day 20–31, with values of 2074–3194 conidia mg^{-1} AFDM day^{-1} in winter, 1009–3142 conidia mg^{-1} AFDM day^{-1} in spring and 1125–2103 conidia mg^{-1} AFDM day^{-1} in autumn across years and stream halves (Fig. 2). Sporulation rates did not significantly differ between stream halves or years in any

Table 2 Temperature sensitivity (Q_{10-q}) of litter decomposition and time to decompose 50% of the initial ash-free dry mass under 10 °C warming for *Quercus robur* litter incubated in coarse and fine mesh bags in spring, autumn and winter in the warmed year

Season	Mesh size	Q_{10-q}	Time (days)
Spring	Coarse	0.6	56
	Fine	2.4	28
Autumn	Coarse	1.7	39
	Fine	1.8	54
Winter	Coarse	3.5	30
	Fine	2.8	48

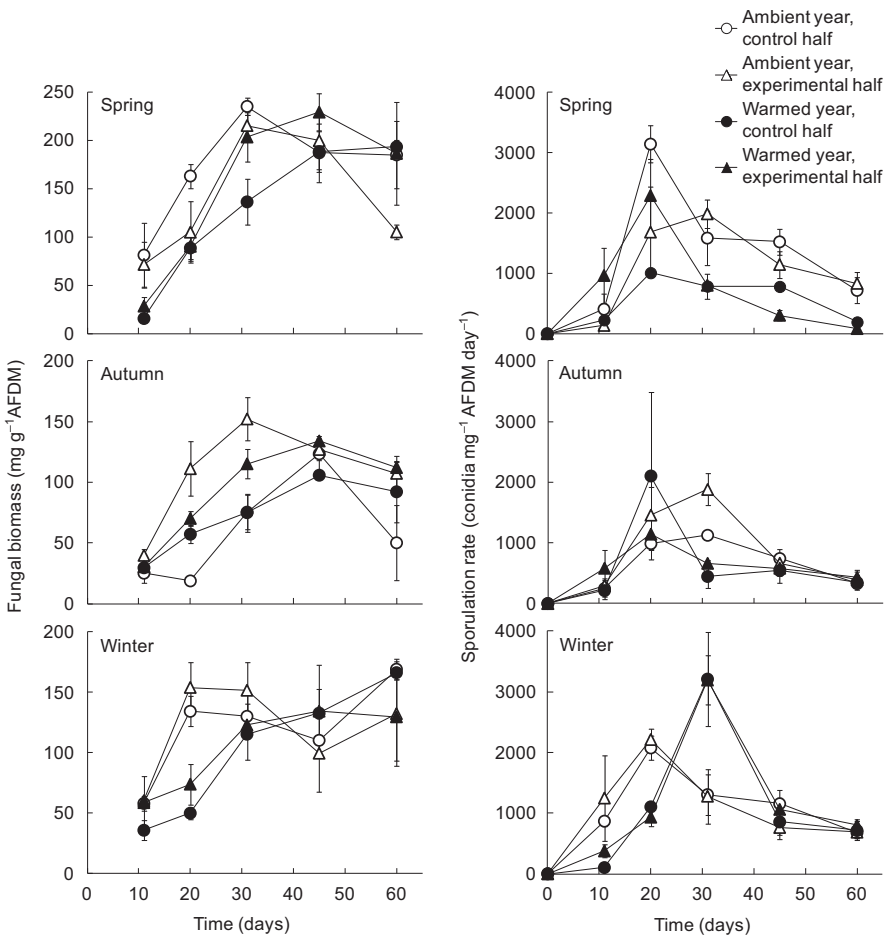

Fig. 2 Fungal biomass concentration and sporulation rates of aquatic hyphomycetes associated with *Quercus robur* leaf litter incubated in fine mesh bags in the control and experimental stream halves over 60 days in spring, autumn and winter in the ambient and warmed year. Values are means ± SE.

season (RM ANOVA, $P > 0.266$ for stream half, $P > 0.102$ for year, and $P > 0.233$ for year × stream half; Fig. 2, Table S4). However, 38 and 49% of the variance in sporulation rates was accounted for by stream half in winter and autumn, respectively. Also, the year × stream half interaction accounted for 35 and 59% of the year × stream half plus error variance in winter and spring, respectively.

Macroinvertebrates

Colonisation of litter bags by macroinvertebrates increased until the last sampling date or peaked by day 31–45, and maximum abundance ranged between 51 and 79 macroinvertebrates g^{-1} AFDM in spring, 15 and 33 macroinvertebrates g^{-1} AFDM in autumn and 16 and 24 macroinvertebrates g^{-1} AFDM in winter across years and stream halves (Fig. 3). There was no significant difference in total macroinvertebrate abundance between stream halves for any season (RM ANOVA, $P > 0.136$ for stream half and $P > 0.141$ for year × stream half; Fig. 3, Table S5). However, 29 and 47% of the variance in total macroinvertebrate abundance was accounted for by stream half in spring and winter, respectively. Also, the year × stream half interaction accounted for 57% of the year × stream half plus error variance in autumn.

Colonisation of litter bags by shredders increased until the last sampling date or peaked by day 31–45, and maximum abundance ranged between 3 and 12 macroinvertebrates g^{-1} AFDM in spring, 3 and 11 macroinvertebrates g^{-1} AFDM in autumn and 1 and 4 macroinvertebrates g^{-1} AFDM in winter across years and stream halves (Fig. 3). In spring, shredder abundance was significantly higher in the warmed year than in the ambient year in the control stream half (RM ANOVA, $P = 0.004$ for year × stream half; Fig. 3, Table S6). The variance in shredder abundance accounted for by stream half varied between 5 and 26% across seasons. The year × stream half interaction accounted for 31 and 95% of the year × stream half plus error variance in autumn and spring, respectively.

Discussion

This study was not replicated at the stream reach level due to budget and logistic constraints. To deal with this

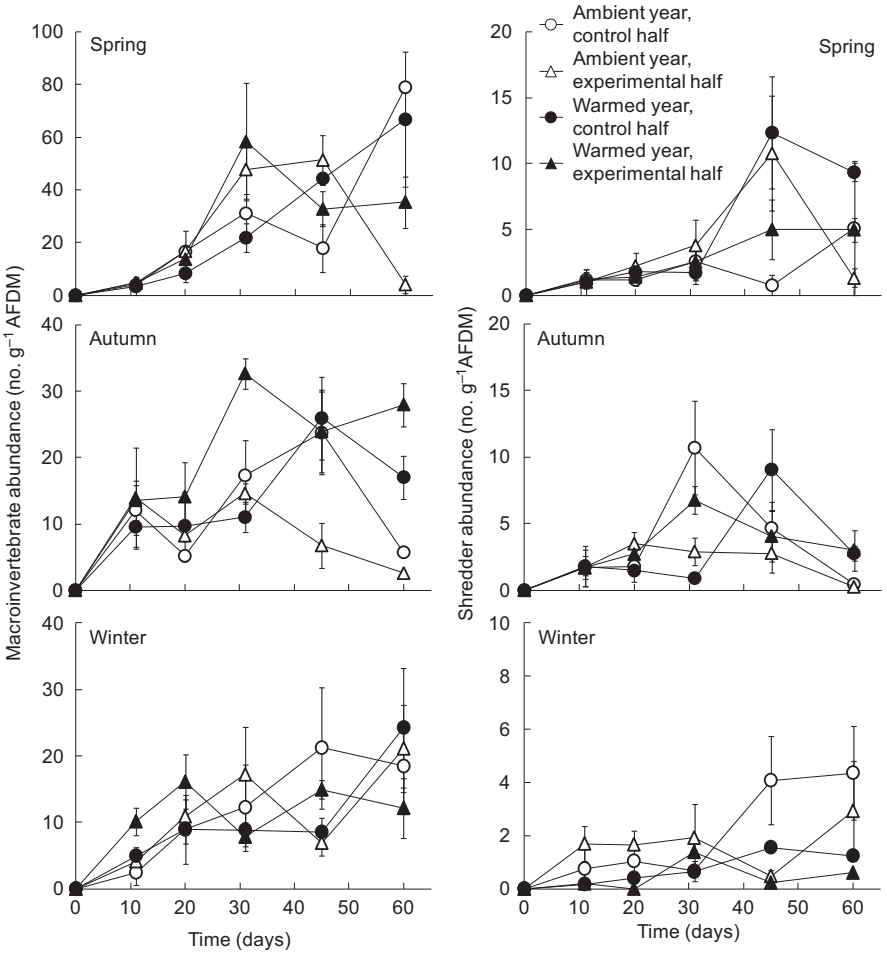

Fig. 3 Total macroinvertebrate abundance and shredder abundance associated with *Quercus robur* leaf litter incubated in coarse mesh bags in the control and experimental stream halves over 60 days in spring, autumn and winter in the ambient and warmed year. Values are means ± SE.

limitation, we followed a BACI design coupled with repeated measures analyses. Given the high number of factors and interactions considered, the analyses were performed for each season individually to allow the biological interpretation of results. Although this ecosystem-level approach provides new insights and a realistic picture of the effects of warming on stream ecosystems, this study has low statistical power and its ability to detect an effect of warming on litter decomposition and associated biota is limited.

By performing a stream manipulation experiment, we found that warming by *c.* 3 °C significantly stimulated litter decomposition in winter, but not in spring or autumn, in the presence and absence of macroinvertebrates. The higher temperature sensitivity of litter decomposition in the coldest season (ambient temperature: 6.4 °C) than in warmer conditions (spring: 11.8 °C; autumn: 9.7 °C) was expected (Kirschbaum, 1995) and is explained by 'a declining relative increase in the fraction of molecules with sufficient energy to react' as temperature increases (Davidson & Janssens, 2006). The observed acceleration of litter decomposition in winter in this study under warmer conditions supports a previous correlational study that suggested a decrease in carbon sequestration with increased temperature (Boyero *et al.*, 2011b).

However, since *Q. robur* litter naturally decomposes slowly (Gulis, Ferreira & Graça, 2006; Ferreira *et al.*, 2012), the faster decomposition under a *c.* 3 °C warming might still allow litter to remain in the stream bed for long periods. Thus, although there is the possibility of a mismatch between life histories of shredders and microbial decomposers and the peak in litter abundance and quality (Durant *et al.*, 2007), food shortage might not occur in streams supplied with low quality, slowly decomposing litter. Moreover, the study stream had low nutrient concentration (Gulis *et al.*, 2006; Woodward *et al.*, 2012), and therefore, the effect of increases in temperature on decomposition of *Q. robur* litter might have been mild (Berggren *et al.*, 2010; Ferreira & Chauvet, 2011a). Many streams worldwide are nutrient enriched (Woodward *et al.*, 2012), and therefore, the effect of

warming on litter decomposition might be exacerbated in these scenarios (Friberg *et al.*, 2009; Ferreira & Chauvet, 2011a). Also, if increases in temperature go beyond the *c.* 3 °C tested here, litter decomposition might be accelerated (Bärlocher *et al.*, 2008).

Changes in decomposition rates caused by modifications of environmental conditions are generally mediated by changes in microbial and invertebrate colonisation and activity (Suberkropp & Chauvet, 1995; Gulis *et al.*, 2006). Surprisingly, however, the patterns found for the effect of changes in temperature on litter decomposition, within each season, were not explained by similar patterns in the assessed biotic variables. The lack of an effect of warming on fungal biomass accumulation and sporulation rates was unexpected, as both variables are usually sensitive to environmental changes (Gulis *et al.*, 2006; Lecerf & Chauvet, 2008). In particular, increases in temperature up to an optimal temperature (20–30 °C) have been shown to stimulate fungal growth and conidial production in the laboratory (Webster *et al.*, 1976; Chauvet & Suberkropp, 1998; Rajashekhar & Kaveriappa, 2000; Dang *et al.*, 2009; Ferreira & Chauvet, 2011a,b; Duarte *et al.*, 2013), although previously tests were of higher temperature increases than those applied in our present study. The lack of an effect of warming on sporulation rates might also be partially due to conidial production in the laboratory being induced at 15 °C and not at the temperature observed in the field. However, in a manipulative field experiment in a freshwater marsh, Flury & Gessner (2011) found an inhibition of fungal biomass build-up on decomposing *Phragmites australis* with warming by 3.2 °C. When considering effect sizes, stream half accounts for a medium fraction of the variance in sporulation rates in autumn and winter, which matches the higher sensitivity of litter decomposition to warming in the colder seasons.

Nor did the differences found in macroinvertebrate abundance between stream halves and years explain the differences in decomposition rates. However, the number of macroinvertebrates colonising decomposing litter was low, especially in autumn and winter, which may have prevented detection of any response to warming. The low number of invertebrates could also explain the absence of differences in the response of litter decomposition to warming between mesh sizes.

Microbial metabolism (respiration; Conant *et al.*, 2008b; Ferreira & Chauvet, 2011a,b), invertebrate feeding (Azevedo-Pereira *et al.*, 2006) and enzymatic activities (Baldrian *et al.*, 2012) are generally stimulated by increases in temperature. Stimulation of these activities, which may not be correlated with increases in biomass or abundance, can have contributed to enhance litter mass loss in winter in our study. Studies addressing the individual effects of increases in temperature on microbial and invertebrate metabolism could clarify this issue.

Within each season, species identity, species interactions and thermal tolerance might have played a key role in determining the sensitivity of litter decomposition to experimental warming. For instance, in spring, experimental warming led to a maximum temperature of 20.0 °C, which was above the maximum temperature registered in this stream year round (the maximum temperature in the stream main channel over 2011, including summer, was 16.1 °C). Such an increase in temperature in the experimental stream half in spring might have been higher than the optimum temperature for some fungal and invertebrate species, especially considering that species thermal optima may depend on species interactions (Webster *et al.*, 1976).

Our study contributes to the assessment of the effects of global warming on stream ecosystems. First, by experimentally manipulating water temperature in a small mountain stream, it addresses the effects of warming at the stream level, under realistic environmental conditions, while controlling for factors associated with biogeography (e.g. differences in community composition between streams). The continuous heating of flowing water presents several challenges, primarily related to the large amount of power required (constant 42 kW in this study). This explains why such an approach has not been attempted more often and also the lack of replication at the stream level when it has been performed, as in the present study. Secondly, our study evaluates the effects of warming on litter decomposition over a seasonal gradient to take into consideration the interaction between ambient temperature and warming. The realistic simulation of warming under field conditions in a mountain temperate oligotrophic stream increases our ability to foresee future effects of climate change on the functioning of small headwater streams. Similar manipulative studies are necessary to evaluate possible interactions between warming and other environmental stressors, since most streams are no longer in their original oligotrophic state (Woodward *et al.*, 2012) and warming is likely to co-vary with changes in other environmental variables (e.g. decrease in litter quality due to increases in atmospheric CO_2; Ferreira *et al.*, 2010). Long-term studies should be encouraged if we want to be able to grasp the consequences for aquatic food webs of faster litter decomposition.

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References

American Public Health Association (APHA) (1995) *Standard Methods for the Examination of Water and Wastewater*. APHA, Washington, DC, USA.

Azevedo-Pereira H.V.S., Graça M.A.S. & González J.M. (2006) Life history of *Lepidostoma hirtum* in an Iberian stream and its role in organic matter processing. *Hydrobiologia*, **559**, 183–192.

Baldrian P., Šnajdr J., Merhautová V., Dobiášová P., Cajthaml T. & Valášková V. (2012) Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. *Soil Biology and Biochemistry*, **56**, 60–68.

Bärlocher F., Seena S., Wilson K.P. & Williams D.D. (2008) Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshwater Biology*, **53**, 368–379.

Berggren M., Laudon H., Jonsson A. & Jansson M. (2010) Nutrient constraints on metabolism affect the temperature regulation of aquatic bacterial growth efficiency. *Microbial Ecology*, **60**, 894–902.

Boyero L., Pearson R.G., Dudgeon D., Graça M.A.S., Gessner M.O., Albariño R.J. *et al.* (2011a) Global distribution of a key trophic guild contrasts with common latitudinal diversity patterns. *Ecology*, **92**, 1839–1848.

Boyero L., Pearson R.G., Gessner M.O., Barmuta L.A., Ferreira V., Graça M.A.S. *et al.* (2011b) A global experiment suggests climate warming will not accelerate litter decomposition in streams but may reduce carbon sequestration. *Ecology Letters*, **14**, 289–294.

Brown J.H., Gillooly J.F., Allen A.P., Savage V.M. & West G.B. (2004) Towards a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Canhoto C. & Graça M.A.S. (2008) Interactions between fungi (aquatic hyphomycetes) and invertebrates. In: *Novel Techniques and Ideas in Mycology* (Eds K.R. Sridhar, F. Bärlocher & K.D. Hyde), pp. 305–325. University of Hong Kong, Pokfulam, Hong Kong.

Canhoto C., Lima J.L.M.P. & Almeida A.T. (2013) Warming up a stream reach: design of a hydraulic and heating system. *Limnology and Oceanography: Methods*, **11**, 410–417.

Castela J., Ferreira V. & Graça M.A.S. (2008) Evaluation of stream ecological integrity using litter decomposition and benthic invertebrates. *Environmental Pollution*, **153**, 440–449.

Chauvet E. & Suberkropp K. (1998) Temperature and sporulation of aquatic hyphomycetes. *Applied and Environmental Microbiology*, **64**, 1522–1525.

Conant R.T., Drijber R.A., Haddix M.L., Parton W.J., Paul E.A., Plante A.F. *et al.* (2008a) Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology*, **14**, 868–877.

Conant R.T., Steinweg J.M., Haddix M.L., Paul E.A., Plante A.F. & Sixs J. (2008b) Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. *Ecology*, **89**, 2384–2391.

Dang C.K., Schindler M., Chauvet E. & Gessner M.O. (2009) Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology*, **90**, 122–131.

Davidson E.A. & Janssens I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.

Dios R.S., Benoti-Garzón M. & Sainz-Ollero H. (2009) Present and future extension of the Iberian submediterranean territories as determined from the distribution of marcescent oaks. *Plant Ecology*, **204**, 189–205.

Duarte S., Fernandes I., Nogueira M.J., Cássio F. & Pascoal C. (2013) Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecology*, **6**, 187–191.

Durant J.M., Hjermann D.Ø., Ottersen G. & Stenseth N.C. (2007) Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, **33**, 271–283.

Fabre E. & Chauvet E. (1998) Leaf breakdown along an altitudinal stream gradient. *Archiv für Hydrobiologie*, **141**, 167–179.

Ferreira V. & Chauvet E. (2011a) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology*, **17**, 551–564.

Ferreira V. & Chauvet E. (2011b) Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. *Oecologia*, **167**, 279–291.

Ferreira V., Encalada A.C. & Graça M.A.S. (2012) Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science*, **31**, 945–962.

Ferreira V., Gonçalves A.L., Godbold D.L. & Canhoto C. (2010) Effect of increased atmospheric CO_2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Global Change Biology*, **16**, 3284–3296.

Flury S. & Gessner M.O. (2011) Experimentally simulated global warming and nitrogen enrichment effects on microbial litter decomposers in a marsh. *Applied and Environmental Microbiology*, **77**, 803–809.

Friberg N., Dybkaer J.B., Olafsson J.S., Gislason G.M., Larsen S.E. & Laridsen T.L. (2009) Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology*, **54**, 2051–2068.

Gaufin A.R. & Hern S. (1971) Laboratory studies on tolerance of aquatic insects to heated waters. *Journal of the Kansas Entomological Society*, **44**, 240–245.

Gessner M.O. & Chauvet E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502–507.

González J.M. & Graça M.A.S. (2003) Conversion of leaf litter to secondary production by a shredding caddis-fly. *Freshwater Biology*, **48**, 1578–1592.

Gore J.A. (1996) Discharge measurements and streamflow analysis. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 53–74. Academic Press, San Diego, CA, USA.

Graça M.A.S., Bärlocher F. & Gessner M.O. (2005) *Methods to Study Litter Decomposition. A Practical Guide*. Springer, Dordrecht, the Netherlands.

Graça M.A.S. & Ferreira R.C.F. (1995) The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater. *Sydowia*, **47**, 167–179.

Gulis V., Ferreira V. & Graça M.A.S. (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. *Freshwater Biology*, **51**, 1655–1669.

Hogg I.D. & Williams D.D. (1996) Response of stream invertebrates to a global-warming thermal regime: an ecosystem-level manipulation. *Ecology*, **77**, 395–407.

Hogg I.D., Williams D.D., Eadie J.M. & Butt S.A. (1995) The consequences of global warming for stream invertebrates: a field simulation. *Journal of Thermal Biology*, **20**, 199–206.

IPCC (2013) Summary for policymakers. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I t the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Eds T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex & P.M. Midgley), pp. 3–29. Cambridge University Press, Cambridge, UK, and New York, NY, USA.

Irons J.G., Oswood M.W., Stout R.J. & Pringle C.M. (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? *Freshwater Biology*, **32**, 401–411.

Kirschbaum M.U.F. (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming in soil organic C storage. *Soil Biology and Biochemistry*, **27**, 753–760.

Lecerf A. & Chauvet E. (2008) Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshwater Biology*, **53**, 1658–1672.

Miranda P., Coelho F.E.S., Tomé A.R. & Valente M.A. (2002) 20th century Portuguese climate and climate scenarios. In: *Climate Change in Portugal. Scenarios, Impacts and Adaptation Measures. SIAM Project* (Eds F.D. Santos, K. Forbes & R. Moita), pp. 23–83. Gradiva Publications, Lda, Lisbon, Portugal.

Moulton S.R., Beitinger T.L., Stewart K.W. & Currie R.J. (1993) Upper temperature tolerance of four species of caddisflies (Insect: Trichoptera). *Journal of Freshwater Ecology*, **8**, 193–198.

Pierce C.A., Block R.A. & Aguinis H. (2004) Cautionary note on reporting eta- squared values from multifactor ANOVA designs. *Educational and Psychological Measurement*, **64**, 916–924.

Pilgrim J.M., Fang W. & Stefan H.G. (1998) Stream temperature correlations with air temperatures in Minnesota: implications for climate warming. *Journal of the American Water Research Association*, **34**, 1109–1121.

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

Rajashekhar M. & Kaveriappa K.M. (2000) Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. *Hydrobiologia*, **441**, 149–153.

Smith E.P. (2002) BACI design. In: *Encyclopidia of Environmetrics* (Eds A.H. El-Shaarawi & W.W. Piegorsch), pp. 141–148. Wiley, Ltd, Chichester, UK.

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

Tachet H., Richoux P., Bournaud M. & Usseglio-Polatera P. (2002) *Invertébrés d'eau douce.Systématique, biologie, écologie*. CNRS Editions, Paris, France.

Taylor B.R. & Chauvet E. (2014) Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient. *Hydrobiologia*, **721**, 239–250.

Webster J., Moran S.T. & Davey R.A. (1976) Growth and sporulation of *Tricladium chaetocladium* and *Lunulospora curvula* in relation to temperature. *Transactions of the British Mycological Society*, **67**, 491–495.

Woodward G., Gessner M.O., Giller P.S., Gulis V., Hladyz S., Lecerf A. *et al.* (2012) Continental-scale effects of nutri-

ent pollution on stream ecosystem functioning. *Science*, **336**, 1438–1440.

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

Supplementary files

Additional Supporting Information may be found in the online version of this article:

Table S1. Water variables in the control and experimental stream half during the seasonal litter decomposition experiments in the ambient and warmed year.

Table S2. Summary table for RM ANOVAs performed on the proportion of *Quercus robur* litter mass remaining (ln-transformed) in coarse and fine mesh bags (mesh size) incubated over 60 days (time) in the control and experimental stream halves (stream half) in the ambient and warmed years (year), in spring, autumn and winter.

Table S3. Summary table for RM ANOVAs performed on fungal biomass concentration (log [$x + 1$] transformed) associated with *Quercus robur* leaf litter incubated in fine mesh bags in the control and experimental stream half over 60 days in the ambient and warmed year, in spring, autumn and winter.

Table S4. Summary table for RM ANOVAs performed on sporulation rate of aquatic hyphomycetes (log [x] transformed) associated with *Quercus robur* leaf litter incubated in fine mesh bags in the control and experimental stream half over 60 days in the ambient and warmed year, in spring, autumn and winter.

Table S5. Summary table for RM ANOVAs performed on total macroinvertebrate abundance (log [$x + 1$] transformed) associated with *Quercus robur* leaf litter incubated in coarse mesh bags in the control and experimental stream half over 60 days in the ambient and warmed year, in spring, autumn and winter.

Table S6. Summary table for RM ANOVAs performed on shredder abundance (log [$x + 1$] transformed) associated with *Quercus robur* leaf litter incubated in coarse mesh bags in the control and experimental stream half over 60 days in the ambient and warmed year, in spring, autumn and winter.

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