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Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi

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Abstract

In woodland streams, the decomposition of allochthonous organic matter constitutes a fundamental ecosystem process, where aquatic hyphomycetes play a pivotal role. It is therefore greatly affected by water temperature and nutrient concentrations. The individual effects of these factors on the decomposition of litter have been studied previously. However, in the climate warming scenario predicted for this century, water temperature and nutrient concentrations are expected to increase simultaneously, and their combined effects on litter decomposition and associated biological activity remains unevaluated. In this study, we addressed the individual and combined effects of water temperature (three levels) and nutrient concentrations (two levels) on the decomposition of alder leaves and associated aquatic hyphomycetes in microcosms. Decomposition rates across treatments varied between 0.0041 day^{-1} at 5 °C and low nutrient level and 0.0100 day^{-1} at 15 °C and high nutrient level. The stimulation of biological variables at high nutrients and temperatures indicates that nutrient enrichment of streams might have a higher stimulatory effect on fungal performance and decomposition rates under a warming scenario than at present. The stimulation of fungal biomass and sporulation with increasing temperature at both nutrient levels shows that increases in water temperature might enhance fungal growth and reproduction in both oligotrophic and eutrophic streams. The stimulation of fungal respiration and litter decomposition with increasing temperature at high nutrients indicates that stimulation of carbon mineralization will probably occur at eutrophied streams, while oligotrophic conditions seem to be 'protected' from warming. All biological variables were stimulated when both factors increased, as a result of synergistic interactions between factors. Increased water temperature and nutrient level also affected the structure of aquatic hyphomycete assemblages. It is plausible that if water quality of presently eutrophied streams is improved, the potential stimulatory effects of future increases in water temperature on aquatic biota and processes might be mitigated.

Keywords: aquatic hyphomycetes, ecosystem functioning, global change, interactions, litter decomposition, nutrient enrichment, streams, temperature

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Introduction

Earth is presently going through a warming period, and simulations considering a doubling in atmospheric CO_2 predict a 1.1–6.4 °C increase in air temperature by the year 2100 (IPCC, 2007). Water temperature of streams and rivers is expected to mirror this increase (Stefan & Sinokrot, 1993; Eaton & Scheller, 1996), which may lead to altered community structure (Hogg & Williams, 1996; Mouthon & Daufresne, 2006), species distribution (Winterbourn, 1969; Eaton & Scheller, 1996; Castella *et al*., 2001), interspecific relationships (Webster *et al*., 1976; Beisner *et al*., 1997; Mouritsen *et al*., 2005; Jiang & Morin, 2007), biodiversity (Petchey *et al*., 1999; Castella *et al*., 2001) and ecological processes (Petchey *et al*., 1999; Baulch *et al*., 2005).

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Small, moderate to high latitude/altitude forest streams, where water temperature is generally low, are particularly sensitive to temperature increases (Stefan & Sinokrot, 1993). In these streams, the primary source of carbon and energy for aquatic food webs is terrestrially derived organic matter supplied by the riparian vegetation, whose shade also limits primary production (Vannote *et al*., 1980). Decomposition of this organic matter is carried out mainly by aquatic hyphomycetes and shredding invertebrates (Hieber & Gessner, 2002; Pascoal & Cássio, 2004), through mineralization, incorporation into biomass and conversion into fine particulate organic matter (González & Graça, 2003; Gulis & Suberkropp, 2003b, c; Pascoal & Cássio, 2004). Litter decomposition, being primarily a biological process, is expected to be affected by increased water temperature. Correlative studies have demonstrated a positive relationship between water temperature and decomposition rates of litter incubated along altitudinal or latitudinal gradients (Irons *et al*., 1994; Fabre & Chauvet,

1998), probably mediated by temperature effects on the biota (Fabre & Chauvet, 1998). Simulations also predict higher decomposition rates with increased water temperature, through enhancement of invertebrate and microbial activity (Buzby & Perry, 2000). Higher water temperatures have been shown to affect litter decomposition in laboratory experiments, both directly, by promoting leaching of soluble compounds (Chergui & Pattee, 1990), and indirectly, by stimulating fragmentation and consumption by selected invertebrate shredders (González & Graça, 2003; Azevedo-Pereira *et al*., 2006) and by enhancing microbial activity (Carpenter & Adams, 1979). High temperatures also stimulate production of fungal assemblages associated with leaves (Suberkropp & Weyers, 1996), and growth and sporulation by some species of aquatic hyphomycetes cultivated individually (Koske & Duncan, 1974; Graça & Ferreira, 1995; Chauvet & Suberkropp, 1998; Rajashekar & Kaveriappa, 2000; Dang *et al*., 2009), which can result in accelerated decomposition rates. However, species have a temperature tolerance range below and above which their activity is reduced or suppressed. In face of the predicted increases in water temperature many species of invertebrates (Quinn *et al*., 1994) and fungi (Koske & Duncan, 1974; Rajashekar & Kaveriappa, 2000) may have their activity inhibited, making it difficult to predict the response of the decomposition process in streams under a global warming scenario. In addition, the influence of modified interspecific interactions is largely unknown (Webster *et al*., 1976).

Additionally, there are several factors that are associated with increased temperature, which might modulate its effects on stream biota and processes. One such factor is increased evapotranspiration, and consequently increased pollutant and nutrient concentrations in water bodies (Murdoch *et al*., 2000). This, associated with increasing needs for water and production of wastewater by a growing human population, will result in decreased ecological status of freshwaters, i.e. decreased ability to provide ecosystem services as water purification. The effects of dissolved nutrients on the decomposition of submerged litter and associated biological activity have been well studied. Nutrient enrichment generally stimulates litter decomposition and associated biota (Elwood *et al*., 1981; Suberkropp & Chauvet, 1995; Rosemond *et al*., 2002; Gulis & Suberkropp, 2003a; Niyogi *et al*., 2003; Pascoal *et al*., 2003; Gulis *et al*., 2004, 2006; Ferreira *et al*., 2006b). In streams where a given inorganic nutrient (i.e. N or P) is not limiting, further increases in its concentration in water may however not enhance litter decomposition or activity of associated microbes (Grattan & Suberkropp, 2001; Abelho & Graça, 2006; Baldy *et al*., 2007). Also, when increases in dissolved nutrients occur simultaneously with variation in other factors (e.g. increases in sedimentation, decreases in dissolved O_2) their stimulatory effect might be offset (Pascoal & Cássio, 2004). Inhibition of decomposition rates might occur when increases in dissolved nutrients reach toxic levels (Lecerf *et al*., 2006).

The effect of interactions between increased water temperature and nutrient concentrations on litter decomposition and associated biological activity remains to be assessed. Nonetheless, evidence from other systems suggests that the effects of factors associated with global changes acting in combination might not be predictable from the effects of factors considered individually (Rozema *et al*., 1997; Hoffman *et al*., 2003; Przeslawski *et al*., 2005). In this study, we addressed the effects of water temperature (three levels) and nutrient concentrations (two levels) on the decomposition of alder leaves, and associated aquatic hyphomycete biomass, activity and assemblage structure in simulated stream microcosms, in a complete factorial design. As the rate of biological processes is dependent on temperature, since they are basically enzyme driven (Brown *et al*., 2004), and fungi can retrieve nutrients from both the substrate and the water (Suberkropp, 1998), we predict that fungal biomass and activity, and consequently decomposition rates, will increase with temperature and nutrient concentrations. Given that aquatic hyphomycetes exhibit temperature and nutrients optima (Gulis & Suberkropp, 2003a; Pascoal *et al*., 2005a; Ferreira *et al*., 2006b; Artigas *et al*., 2008), changes in the structure of the assemblages are anticipated.

Materials and methods

Fungal species assemblage

An assemblage of six species of aquatic hyphomycetes was used, as representative of fungal diversity found on a single leaf decomposing in natural streams (Bärlocher, 1992). Strains were isolated from single conidia trapped in naturally occurring foam, or released from leaf accumulations, collected from a lowland stream in central Portugal [Ribeira do Botão; 40°18′22″N, 8°23′37″W; *Articulospora tetracladia* Ingold (ARTE)], a Mediterranean stream in the French Pyrenees [Maureillas; 42°28′18″N, 2°47′57″E; *Clavariopsis aquatica* de Wildeman (CLAQ), *Flagellospora curvula* Ingold (FLCU) and *Tetracladium marchalianum* de Wildeman (TEMA)] and a temperate mountain stream in the Massif Central, SW France [Oreval; 43°26′19″N, 2°5′41″E; *Heliscus lugdunensis* Saccardo & Thérry (HELU) and *Tumularia aquatica* (Ingold) Descals & Marvanová (TUAQ)]. Growing colonies were kept at 15 °C, in 9 cm diameter Petri dishes with ~10 mL of growth medium (10 g malt and 20 g agar per liter of sterile distilled water), until they were used to induce conidial production. Conidial inoculations (<1-day-old) were produced at 15 °C, by incubation of agar plugs taken from the leading edge of 7- to 14-day-old colonies (either grown from an individual agar plug or from conidia spread over a Petri dish) in 25 mL of nutrient solution

(75.5 mg $CaCl_2$, 10 mg $MgSO_4 \cdot 7H_2O$, 0.5 g 3-morpholinopropanesulfonic acid (MOPS), 0.55 mg K_2HPO_4 and 100 mg KNO_3 per liter of sterile distilled water), on an orbital shaker (100 rpm). An aliquot of each specific conidial suspension, based on conidial numbers, was used to make a combined conidial suspension to inoculate each microcosm.

Microcosms, medium and experimental setup

Alder [*Alnus glutinosa* (L.) Gaertner] leaf discs were incubated in laboratory microcosms designed to simulate stream conditions (Suberkropp, 1991). Each microcosm consisted of a 50 mL glass chamber aerated from the bottom by a continuous air flow (80–100 mL min^{-1}), which creates turbulence and keeps the leaf discs in permanent agitation. A tap at the bottom allowed for the aseptic drainage of the chamber and recovery of the conidial suspension. Fresh medium (40 mL) was added to microcosms through the open top which was otherwise closed with a glass cap. Microcosms were incubated in the dark at three temperatures (5, 10 and 15 °C), for the duration of the experiment.

Half of the microcosms at each temperature were filled with a low nutrient concentrations solution (low NP level treatment) and half with a high nutrient concentrations solution (high NP level treatment). The low nutrient solution was composed of 75.5 mg $CaCl_2$, 10 mg $MgSO_4 \cdot 7H_2O$, 0.5 g MOPS, 0.055 mg K_2HPO_4 and 10 mg KNO_3 per liter of sterile distilled water (= 0.01 mg PO_4-P L^{-1} and 1.39 mg NO_3-N L^{-1}), while the high nutrient solution was amended with 0.55 mg K_2HPO_4 and 100 mg KNO_3 (= 0.10 mg PO_4–P L^{-1} and 13.86 mg NO_3–N L^{-1}) (Dang *et al*., 2005). The phosphorus limitation in the solutions (N : P = 141) was intended to mimic the trophic conditions of most streams (Grattan & Suberkropp, 2001), although there are streams in which nitrogen naturally is the limiting nutrient (Ferreira *et al*., 2006b). There were 12 replicate microcosms for each of the six temperature–nutrient treatments.

Alder leaves collected just after abscission on December 30, 2006 at Gibel, Midi-Pyrenees, France (43°17′35″N, 1°40′51″E), and dried at room temperature, were moistened with distilled water and left to rehydrate overnight. Leaf discs were cut with a 12 mm diameter cork borer and oven dried (65 °C) during 3 days. Batches of 20 leaf discs were frozen overnight at −20 °C, lyophilized (20 h), weighed (± 0.1 mg) to determine initial dry mass (DM), placed inside glass tubes with an aliquot of distilled water and autoclaved (20 min at 121 °C). Seven batches of 20 leaf discs were given the same treatment and were used to create a correction factor for mass loss due to leaching during sterilization. Sterilized microcosm (30 min at 121 °C) were filled with 40 mL of the low or high nutrient solutions, received the corresponding leaf discs, were distributed by three incubator chambers (5, 10 and 15 °C), and aerated for 24 h. Nutrient solutions were changed, the microcosms inoculated with a total of 4800 conidia equally partitioned among all species of the aquatic hyphomycete assemblage, aerated for 10 min, left to rest for ca. 2 h, and aerated again. The nutrient solutions were replaced after 24 h and then every 3 days for the duration of the experiment (43 days). All manipulations of microcosms took place in a flow cabinet.

Fungal sporulation

Each of the 14 times the nutrient solutions were changed, the conidial suspensions from three microcosms (the same over time) from each treatment were stored into 50 mL centrifuge tubes, sample volume was adjusted to 42 mL with distilled water and preserved with 3 mL of 35% formalin. Additionally, after 10, 16, 28 and 43 days, three microcosms from each treatment were sacrificed, and the conidial suspensions preserved as above. When preparing filters for conidial counting and identification, 150 μL of polyethylene glycol *p*-(1,1,3,3-tetramethylbutyl)-phenyl ether (Triton X-100, 0.5%) were added to the suspension, mixed with a magnetic stirring bar, to ensure a uniform distribution of conidia, and an aliquot of the suspension was filtered (Millipore SMWP, pore size 5 μm). Filters were stained with 0.08% trypan blue in 60% lactic acid, and spores were identified and counted under a compound microscope at × 320 (Graça *et al*., 2005). For sacrificed microcosms, sporulation rates were expressed as number of conidia mg^{-1} DM day^{-1}. For microcosms repeatedly sampled throughout time, results were expressed as number of conidia $microcosm^{-1}$ and as mg conidia $microcosm^{-1}$. The total conidial mass at each sampling date was calculated by multiplying the number of conidia from each species by the average mass of individual conidia obtained from the literature (Chauvet & Suberkropp, 1998) or calculated from biovolume data (Bärlocher & Schweizer, 1983) assuming a 70% water content. Cumulative conidial mass production over time was calculated by summing up the total conidial mass at the preceding sampling dates.

Oxygen consumption

A subset of five leaf discs from each sacrificed microcosm was used to determine fungal oxygen consumption rates using a closed six-channel dissolved oxygen measuring system (Strathkelvin 928 System, North Lanarkshire, Scotland) connected to a computer. The oxygen electrodes were calibrated against a 4% sodium sulfite solution prepared immediately before use (0% O_2), and a 100% O_2 saturated low or high nutrient solution at the target temperature. Leaf discs were incubated in 3 mL volume chambers filled with 100% O_2 saturated low or high nutrient solution, homogenized with a magnetic stirring bar, kept at the target temperature of 5, 10 or 15 °C by circulation of water originating from a temperature-controlled water bath. Additional chambers without leaf discs were used as controls. After 1 h trial, leaf discs were enclosed in small sterile zip lock bags and promptly frozen at −20 °C for later DM determination and ergosterol extraction. Oxygen consumption rates were determined by the difference in the oxygen concentration in the sample and the control over a 20 min interval and corrected for the chamber's volume, time and discs mass. Results were expressed as mg O_2 g^{-1} DM h^{-1}.

Mass loss and mycelial biomass

The remaining 15 leaf discs from each microcosm were enclosed in small sterile zip lock bags and promptly frozen at −20 °C. All 20 leaf discs from each microcosm were combined, lyophilized,

promptly weighed (± 0.1 mg) to determine DM remaining, and used for ergosterol extraction as a surrogate for mycelial biomass (Gessner & Chauvet, 1993; Graça *et al.*, 2005). Lipid extraction and saponification were carried out in 5 mL KOH/methanol (8 g L^{-1}) at 80 °C for 30 min. The extract was then purified by solid phase extraction (Waters Oasis® HLB 3cc cartridges, Waters Corp., Milford, MA, USA; Graça *et al.*, 2005). Ergosterol was quantified by HPLC (HPLC pump 422, HPLC detector 432, HPLC autosampler 360; Kontron Inst., Neufahru, Germany) by measuring absorbance at 282 nm. The HPLC detector was equipped with a FLT 0.5 µm A-316 precolumn (Upchurch Sci., Oak Harbour, WA, USA) and a LispRP 18-5 250 × 4.6 mm column (Thermo-Hypersil Keystone, Bellefonte, PA, USA) maintained at 33 °C. The mobile phase was 100% methanol and the flow rate was set to 1.4 mL min^{-1}. Ergosterol was converted into mycelial biomass using specific conversion factors for ARTE, CLAQ, FLCU and TEMA, and 5.5 µg ergosterol mg^{-1} mycelial biomass for HELU and TUAQ (Gessner & Chauvet, 1993), applied to the relative contribution of each species to total ergosterol mass based on specific contribution to total conidial production. Results were expressed as mg mycelial biomass g^{-1} DM.

Fungal carbon budgets

Fungal carbon (C) budgets were calculated assuming a 50% C content of leaf, conidia and mycelial DM and a respiratory quotient of 1 (Gulis & Suberkropp, 2003b). The cumulative fungal C production was calculated for day 43 by summing up the cumulative conidial C production and the mycelial C production, the sum of which was maximum at day 43. The fungal yield coefficient (%) was calculated as cumulative fungal C production (%)/leaf C mass loss (%) × 100. The fungal production efficiency (%) was calculated as cumulative fungal C production (mg g^{-1})/(cumulative fungal C production (mg g^{-1}) + cumulative C respired (mg g^{-1})) × 100. The explained leaf C mass loss by a given fungal activity (conidial C production, mycelial C production or mineralization; %) was calculated as C allocated to this fungal activity/leaf C mass loss × 100. The total explained leaf C mass loss (%) resulted from the sum of leaf C mass loss due to conidial C production, mycelial C production and mineralization (Baldy *et al.*, 1995; Gulis & Suberkropp, 2003b, c).

Data treatment

Decomposition rates of alder leaf discs were calculated assuming (a) an exponential decay, by linear regression of the Ln transformed negative exponential model $M_t = M_o \times e^{-kt}$ (where M_o is the initial mass, M_t is the remaining mass at time t and k is the decomposition rate) and (b) a linear decay, by regression of the linear model $M_t = M_o \times (1 - k \times t)$ (Pozo & Colino, 1992). In both cases, a fixed intercept was used at $M_o = 0$ or $M_o = 1$, for the exponential and linear model, respectively. Although the decomposition rates derived from the exponential model might be useful for comparisons with published literature, where this is the most commonly used model, further analysis were performed considering only a linear decay, since the Akaike Information Criterion (AIK) and the Bayesian Information Criterion (BIC) as tools for model selection gave lower values for the linear model than for the exponential one (AIK = −147 vs. −123 and BIC = −143 vs. −118), indicating that the linear model fits the data better. Slopes were compared among treatments by ANCOVA with time as continuous variable and temperature and nutrient level as categorical variables, followed by Tukey's Honest Significant Difference (HSD). The relationship between remaining mass (% initial mass) after 43 days and water temperature for the low and high nutrient levels was assessed by linear regression.

The sensitivities of decomposition rates to temperature were calculated as Q_{10-q} values: $Q_{10-q} = (t_c/t_w)^{(10/(T_c/T_w))}$, where t_c and t_w are the time (d) at the cooler and warmer temperature to decompose 20% DM, respectively, and T_w and T_c are the warmer and cooler temperature (°C), respectively (Conant *et al.*, 2008). These have the advantage over Q_{10} values of allowing determination of sensitivities of decomposition to temperature for temperature intervals different from 10 °C, and of keeping the substrate quality (q) consistent between temperatures and thus eliminating changes on litter quality as a source of variation on sensitivities to temperature (Conant *et al.*, 2008).

Fungal oxygen consumption, biomass and sporulation rates associated with alder discs from the sacrificed microcosms were compared among treatments by three-way ANOVAs (time, temperature and nutrient level as categorical variables), followed by Tukey's HSD. Total number of conidia, total mass of conidia and sporulating species richness per microcosm determined over the 14 sampling dates were compared among treatments by repeated measures ANOVAs, followed by Tukey's HSD. Cumulative conidial production (mass and number) was compared among treatments by ANCOVA, followed by Tukey's HSD. The cumulative number of conidia produced by each aquatic hyphomycete species by day 43 was compared among treatments by two-way ANOVAs (temperature and nutrient level as categorical variables), followed by Tukey's HSD. For the common sampling dates (day 10, 16, 28 and 43) there was no significant difference in conidial production between the three sacrificed microcosms and the three microcosms sampled though time, for any treatment (one-way ANOVA, $P > 0.08$).

Fungal assemblages over all sampling dates were analyzed by nonmetric multidimensional scaling ordination (NMDS) based on Bray Curtis similarity index of $\log(x + 1)$ transformed conidial abundance data (PRIMER v6; Clarke & Gorley, 2001). However, the effect of time in determining the structure of the assemblage (analysis of similarity, ANOSIM) hid the potential effects of temperature and nutrient level. To overcome this, fungal assemblages were analyzed by sampling date, and two-way ANOSIM performed with temperature and nutrient level as factors.

Leaf C mass loss due to conidial production, mycelial production, mineralization, total mass loss due to overall fungal activities, yield coefficient and production efficiency were compared among treatments by two-way ANOVAs (temperature and nutrient level as categorical variables), followed by Tukey's HSD. Mass loss due to overall fungal activities was arcsine-square root transformed.

Data were $\log(x + 1)$ transformed when necessary to achieve normality and homoscedasticity, unless otherwise indicated. All analyses were performed with STATISTICA 6 software unless otherwise indicated.

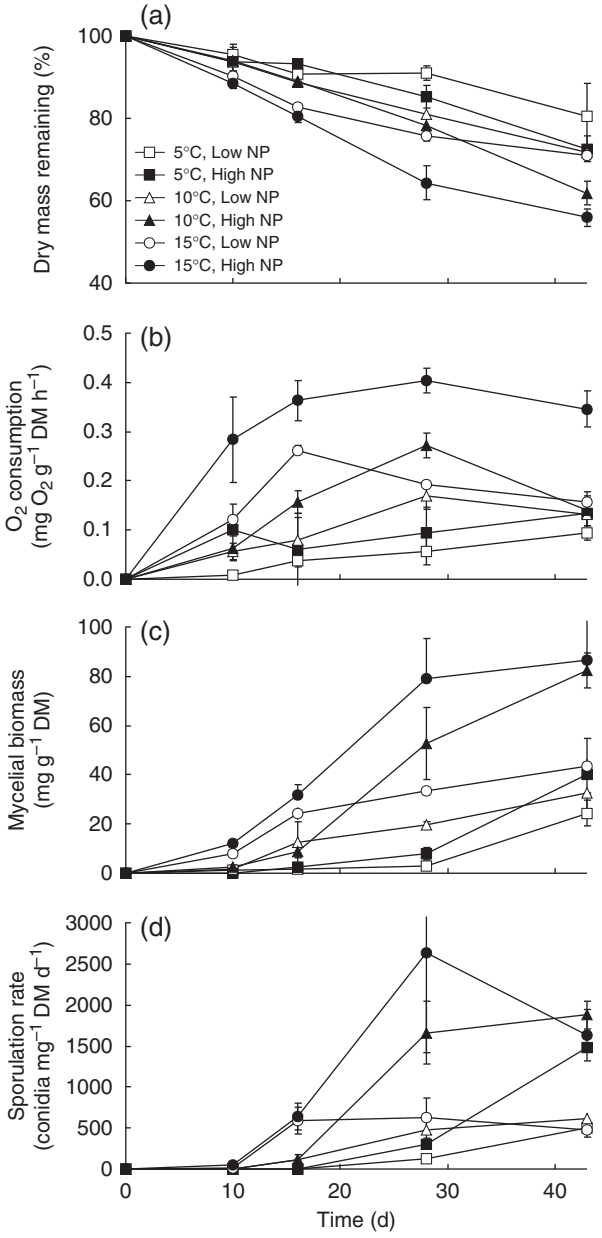

Fig. 1 Dry mass remaining of alder leaf discs (a), fungal oxygen consumption (b), mycelial biomass (c) and fungal sporulation rate (d) associated with alder leaf discs incubated in microcosms at three temperatures and two NP levels for 43 days. Data are from sacrificed microcosms and treatments are as in Table 1. Values are averages ± 1 SE.

Results

Mass loss

Mass loss of alder leaf discs after incubation in simulated stream microcosms for 43 days varied between 19% (at 5 °C and low NP level) and 44% (at 15 °C and high NP level) (Fig. 1a), which translated into decomposition rates between 0.0041 and 0.0100 day^{-1} (linear model; Table 1). Decomposition rates were stimulated by high NP level only at 15 °C (0.0055 day^{-1} at low NP vs. 0.0100 day^{-1} at high NP; Tukey's HSD, $P = 0.008$); for both low and high NP levels, decomposition rates were faster at 15 °C than at lower temperatures (15 > 5 °C at low NP; 15 > 5 °C and 15 > 10 °C at high NP; Tukey's HSD, $P < 0.002$; Table 1). Additionally, when temperature and NP level were increased simultaneously simulating a future scenario of both warming and eutrophication in winter (increase from 5 °C and low NP to 10 °C and high NP) or spring (increase from 10 °C and low NP to 15 °C and high NP) decomposition rates also increased (Tukey's HSD, $P < 0.001$; Table 1). This increase was however higher than that predicted from the sum of the effects of both factors considered individually (0.0097 day^{-1} observed vs. 0.0092 day^{-1} predicted for winter scenario, and 0.0100 day^{-1} observed vs. 0.0086 day^{-1} predicted for spring scenario). Leaf discs mass loss at high NP level after 43 days increased with increasing temperature (linear regression, $R^2 = 0.74$ and $P = 0.003$), while at low NP level no relationship was found (linear regression, $R^2 = 0.24$ and $P = 0.182$).

The sensitivity of litter decomposition to temperature, although overall low, depended on nutrient level and temperature interval. Decomposition rates at low NP level were more stimulated than decomposition rates at high NP level when temperature increased from 5 to 10 °C ($Q_{10-q} = 2.62$ vs. 2.10), while an increase in temperature from 10 to 15 °C almost did not had an effect on decomposition rates at high NP level ($Q_{10-q} = 1.06$) and inhibited those at low NP level ($Q_{10-q} = 0.70$). When temperature increased from 5 to 15 °C decomposition rates were slightly more stimulated at high than at low NP level ($Q_{10-q} = 1.49$ vs. 1.36).

Table 1 Decomposition rates (day^{-1}) of alder leaf discs incubated in microcosms at 5, 10 or 15 °C and low or high NP levels for 43 days, and coefficient of determination of the regression

Temperature (°C)	NP level	Exponential model		Linear model	
		k	R^2	k	R^2
5	Low NP	0.0048^{a}	0.38	0.0041^{a}	0.39
	High NP	0.0067^{ab}	0.79	0.0067^{ab}	0.83
10	Low NP	0.0076^{ab}	0.97	0.0066^{abc}	0.97
	High NP	0.0101^{b}	0.85	0.0097^{bc}	0.90
15	Low NP	0.0089^{b}	0.81	0.0055^{c}	0.88
	High NP	0.0141^{c}	0.89	0.0100^{d}	0.89

Treatments with the same letter are not significantly different (Tukey's HSD, $P > 0.05$).

Table 2 Summary table for three-way ANOVAs performed on fungal oxygen consumption, mycelial biomass [log($x+1$) transformed], and sporulation rates [log($x+1$) transformed], associated with alder discs incubated in microcosms at three temperatures and two NP levels for 43 days

		O_2 consumption		Mycelial biomass		Sporulation rate	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Three-way ANOVA							
Intercept	1	496.6	<0.001	3237.2	<0.001	3383.0	<0.001
Temperature	2	51.0	<0.001	128.3	<0.001	95.0	<0.001
NP level	1	24.2	<0.001	20.1	<0.001	35.3	<0.001
Time	3	7.5	<0.001	145.2	<0.001	255.9	<0.001
Temperature × NP level	2	10.9	<0.001	1.9	0.155	1.1	0.336
Temperature × time	6	2.6	0.033	7.6	<0.001	20.2	<0.001
NP level × time	3	2.3	0.095	4.5	0.007	0.7	0.580
Temperature × NP level × time	6	1.6	0.167	1.3	0.274	0.9	0.534
Error	43						
Tukey's HSD							
5 °C, low NP			a		a		a
5 °C, high NP			ab		a		ab
10 °C, low NP			abc		b		b
10 °C, high NP			bc		c		c
15 °C, low NP			c		c		c
15 °C, high NP			d		d		d

Tukey's HSD are presented, with treatments with the same letter not being significantly different ($P>0.05$). Data are from sacrificed microcosms and treatments are as in Table 1.

Oxygen consumption

Oxygen consumption by fungi generally increased until a peak was reached (days 10–28), except at 5 °C and low NP level where it increased over time (Fig. 1b). Similarly to decomposition rates, oxygen consumption rates were stimulated by high NP level only at 15 °C (peak values: 0.26 mg O_2 g^{-1} DM h^{-1} at low NP vs. 0.40 mg O_2 g^{-1} DM h^{-1} at high NP; Tukey's HSD, $P<0.001$). For both nutrient levels, oxygen consumption rates were also higher at 15 °C than at lower temperatures (15 > 5 °C at low NP level; 15 > 10 °C and 15 > 5 °C at high NP; Tukey's HSD, $P<0.002$; Table 2). When temperature and NP level were increased simultaneously as in a future scenario of both warming and eutrophication in winter (increase from 5 °C and low NP to 10 °C and high NP) or spring (increase from 10 °C and low NP to 15 °C and high NP) oxygen consumption rates also increased (Tukey's HSD, $P<0.015$; Table 2).

Mycelial biomass

Mycelial biomass increased over time for all treatments, attaining maximum values between 24 mg g^{-1} DM (at 5 °C and low NP) and 86 mg g^{-1} DM (at 15 °C and high NP; Fig. 1c). Mycelial biomass was stimulated by high NP level at 10 and 15 °C (maximum values: 32 mg g^{-1} DM vs. 82 mg g^{-1} DM at 10 °C for low and high NP, respectively; 44 mg g^{-1} DM vs. 86 mg g^{-1} DM at 15 °C; Tukey's HSD, $P<0.020$), but not at 5 °C. Mycelial biomass increased with increasing temperature for both NP levels (15 > 10 > 5 °C; Tukey's HSD, $P<0.001$; Table 2). Additionally, mycelial biomass at 10 °C and high NP level resembled that at 15 °C and low NP level (Tukey's HSD, $P=0.641$), and both were higher than that at 10 °C and low NP level (Tukey's HSD, $P<0.014$), which indicate similar effects of a 5 °C increase in water temperature or 10-fold increase in NP level for mycelial biomass in systems initially at 10 °C and low NP level (Table 2). Also, when temperature and NP level were increased simultaneously, in a scenario combining warming and eutrophication in winter or spring, mycelial biomass also increased (Tukey's HSD, $P<0.001$; Table 2).

Fungal sporulation

Fungal sporulation rates from sacrificed microcosms increased, reached a peak and then decreased (at 10 °C and high NP and at 15 °C and both NP) or increased continuously over time (at 5 °C and both NP and 10 °C and low NP; Fig. 1d). Maximum sporulation rates were attained at 15 °C and high NP level (2637 conidia mg^{-1} DM day^{-1}). Sporulation rates were stimulated by high NP level at 10 and 15 °C (peak values: 620 con-

idia mg^{-1} DM day^{-1} vs. 1877 conidia mg^{-1} DM day^{-1} at 10 °C for low and high NP, respectively; 587 conidia mg^{-1} DM day^{-1} vs. 2637 conidia mg^{-1} DM day^{-1} at 15 °C; Tukey's HSD, $P < 0.020$), but not at 5 °C. Sporulation rates increased with increasing temperature at both NP levels (15 > 10 > 5 °C; Tukey's HSD, $P < 0.001$; Table 2). Additionally, and in similitude to mycelial biomass, sporulation rates at 10 °C and high NP level resembled those at 15 °C and low NP level (Tukey's HSD, $P = 0.947$), and both were higher than those at 10 °C and low NP level (Tukey's HSD, $P < 0.001$), which indicate similar effects of a 5 °C increase in water temperature or 10-fold increase in NP level for sporulation rates in systems initially at 10 °C and low NP level. When both temperature and NP level were increased, in a scenario combining warming and eutrophication in winter or spring, sporulation rates also increased (Tukey's HSD, $P < 0.001$; Table 2).

The mass of conidia produced per microcosm tended to increase over time, although at higher temperatures (10 and 15 °C) and NP level a first peak was reached by day 25 and then a second one close to the end of the experiment. At 15 °C and low NP level a peak was reached by day 22 (Fig. 2a). Conidial mass production was stimulated at high NP level at 10 and 15 °C (Tukey's HSD, $P < 0.047$), but not at 5 °C; conidial production was higher at 10 and 15 °C than at 5 °C, but only at high NP level (Tukey's HSD, $P < 0.001$; Table 3). This translated into cumulative conidial mass production being higher at 10 and 15 °C and high NP level than at the other four treatments (Tukey's HSD, $P < 0.020$; Fig. 2b). The same patterns (data not shown) and statistical differences were found when considering the number of conidia per microcosm. When temperature and NP level were increased simultaneously, in a future scenario combining warming and eutrophication in winter, total conidial production was stimulated to a higher extent than that predicted from the sum of the effects of both factors considered individually (1.205 mg microcosms^{-1} observed vs. 0.644 predicted, and 2 655 703 conidia microcosms^{-1} observed vs. 1 328 238 predicted; Fig. 2b, Table 4). Sporulating species richness increased until the maximum value, i.e. six, was attained at day 13 at 15 °C, day 22 at 10 °C and day 25 at 5 °C, which translated into statistical differences among treatments (Fig. 2c, Table 3).

The assemblages of aquatic hyphomycetes associated with alder discs at each sampling date differed among treatments. However, the factor that most affected the structures of assemblages varied among sampling dates: temperature was the most important factor up to day 25, while nutrient level became more important afterwards (Table 5). The total number of conidia produced by each species over 43 days differed among treatments (except for ARTE; two-way ANOVA, $P > 0.243$;

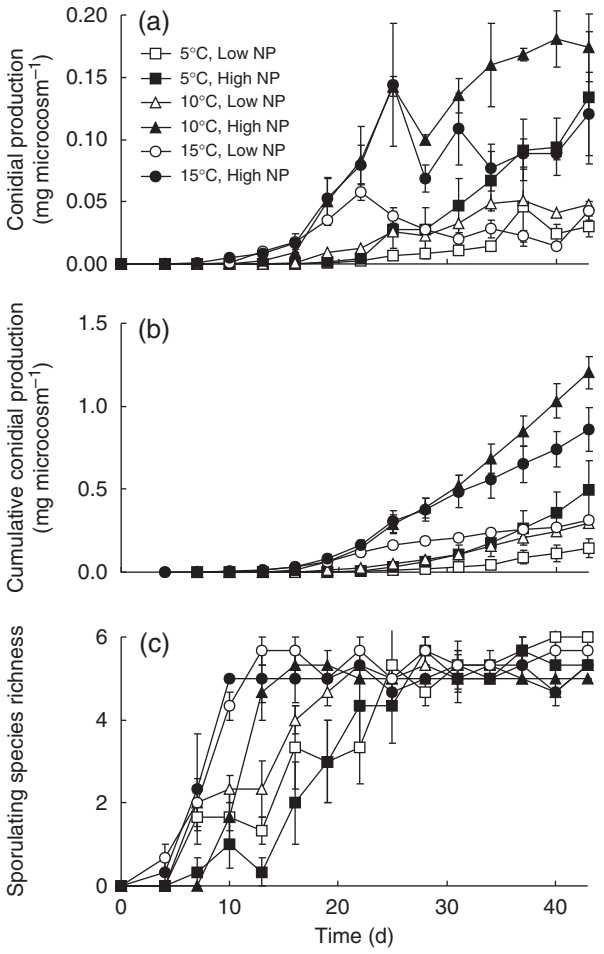

Fig. 2 Conidial production (a), cumulative conidial production (b) and sporulating species richness (c) associated with alder leaf discs incubated in microcosms at three temperatures and two NP levels for 43 days. Data are from microcosms sampled over time and treatments are as in Table 1. Values are averages ± 1 SE. The same patterns were found when conidial production and cumulative conidial production were based on the number of conidia per microcosm.

Table 4). On the one hand, individual conidial production at low NP level did not differ among temperatures, while it was generally higher at 10 and/or 15 °C than at 5 °C at high NP level. On the other hand, higher NP level stimulated specific conidial production at 5 °C (HELU and TUAQ), 10 °C (CLAQ, FLCU and HELU) and 15 °C (CLAQ, HELU and TEMA; Table 4). ARTE was the least sporulating species at all treatments, while FLCU was by far the dominant species, except at high NP level at 5 and 15 °C where it co-dominated with HELU and CLAQ, respectively. TEMA and TUAQ ranked third to fifth in dominance (based on conidial production), while HELU and CLAQ ranked first to

Table 3 Summary table for repeated measures ANOVAs performed on conidial production per microcosm [log(x + 1) transformed] and sporulating species richness, associated with alder discs incubated in microcosms at three temperatures and two NP levels for 43 days

		Conidial production		Species richness	
	Df	*F*	*P*	*F*	*P*
Repeated measures ANOVA					
Intercept	1	11 414.0	<0.001	4825.5	<0.001
Temperature	2	59.9	<0.001	25.9	<0.001
NP level	1	15.8	0.002	2.6	0.131
Temperature × NP level	2	4.5	0.035	1.3	0.301
Error	12				
Time	12	321.9	<0.001	52.3	<0.001
Time × temperature	24	28.4	<0.001	8.6	<0.001
Time × NP level	12	7.3	<0.001	1.0	0.472
Time × temperature*NP level	24	6.1	<0.001	2.1	0.004
Error	144				
Tukey's HSD					
5 °C, low NP			ab		ab
5 °C, high NP			a		a
10 °C, low NP			bc		abc
10 °C, high NP			de		bcd
15 °C, low NP			cd		d
15 °C, high NP			e		cd

Tukey's HSD are presented, with treatments with the same letter not being significantly different ($P > 0.05$). Data are from microcosms sampled over time and treatments are as in Table 1.

Table 4 Specific and total number of conidia produced at three temperatures and two NP levels over 43 days

	5 °C		10 °C		15 °C	
Aquatic hyphomycete species	Low NP	High NP	Low NP	High NP	Low NP	High NP
ARTE	5123^{a}	4197^{a}	571^{a}	872^{a}	2935^{a}	2644^{a}
CLAQ	20 540^{a}	168 914^{a}	145 470^{a}	570 277^{b}	192 317^{a}	642 742^{b}
FLCU	320 581^{a}	293 411^{a}	369 380^{a}	1 053 139^{b}	230 251^{a}	527 769^{a}
HELU	61 346^{a}	344 479^{bc}	64 714^{a}	312 743^{b}	100 665^{a}	452 333^{c}
TEMA	22 759^{a}	42 384^{a}	64 215^{ab}	316 422^{bc}	76 999^{ab}	402 410^{c}
TUAQ	40 787^{a}	210 371^{bc}	91 266^{abc}	402 250^{c}	93 038^{abc}	75 032^{ab}
Total number of conidia produced	471 136^{a}	1 063 757^{a}	735 617^{a}	2 655 703^{b}	696 206^{a}	2 102 930^{b}

Data are average values from replicate microcosms sampled over time, and treatments are as in Table 1. Treatments with the same letter are not significantly different (Tukey's HSD, $P > 0.05$).
ARTE, *Articulospora tetracladia*; CLAQ, *Clavariopsis aquatica*; FLCU, *Flagellospora curvula*; HELU, *Heliscus lugdunensis*; TEMA, *Tetracladium marchalianum*; TUAQ, *Tumularia aquatica*.

fifth being favored at 5 °C and at 10 and 15 °C, respectively (Fig. 3).

Fungal carbon budgets

Leaf C directed to fungal production ranged 6–22%, and although it was not stimulated by high NP level within any temperature, or by increasing temperature at any NP level (Tukey's HSD, $P > 0.050$), it was significantly higher at 10 and 15 °C and high NP level than at 5 °C and low NP level (20–22% vs. 6%; Tukey's HSD, $P < 0.023$; Table 6). Leaf C loss due to mineralization was stimulated at higher temperature for the high NP level only (37% at 15 °C vs. 19% at 5 °C; Tukey's HSD, $P = 0.039$). Fungal production efficiency was relatively high (31–50%), and seemed to be stimulated at high NP

Table 5 Summary table for two-way ANOSIM performed on aquatic hyphomycete assemblages associated with alder discs incubated in microcosms at three temperatures and two NP levels for 43 days

Sampling day	Temperature		NP level	
	Overall *R*	*P*	Overall *R*	*P*
7	0.46	0.008*	0.51	0.017*
10	0.34	0.019*	0.21	−0.110
13	0.55	0.001*	0.45	0.006*
16	0.54	0.001*	0.20	−0.096
19	0.60	0.001*	0.30	−0.088
22	0.49	0.003*	0.17	−0.182
25	0.33	0.002*	0.49	0.026*
28	0.45	0.001*	0.57	0.007*
31	0.29	0.004*	0.53	0.003*
34	0.33	0.005*	0.44	0.004*
37	0.35	0.011*	0.42	0.027*
40	0.34	0.001*	0.59	0.002*
43	0.15	−0.088	0.41	0.011*

Data are from microcosms sampled over time. Overall *R* gives an absolute measure of how separate the groups are (0, indistinguishable; 1, all similarities within groups are less than any similarity between groups; Clarke & Gorley, 2001).
*Denote significant effects.
ANOSIM, analysis of similarity.

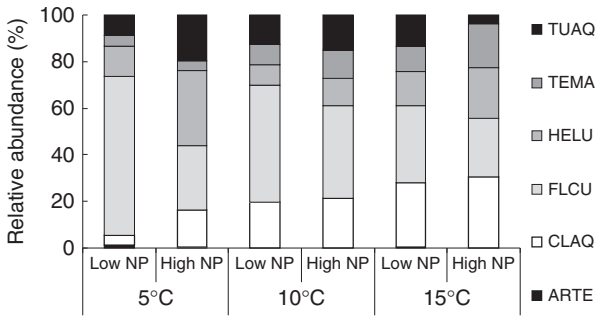

Fig. 3 Relative contribution of fungal species to the total conidial production at three temperatures and two NP levels over 43 days. Data are from microcosms sampled over time and treatments are as in Table 1.

level, although no significant differences were found among treatments (two-way ANOVA, $P > 0.130$). Mass loss attributed to fungi varied between 26% and 57%, and although there was a tendency to increase with increasing temperature and NP level, it was only significantly higher at 15 °C and high NP level than at 5 °C and low NP level (Tukey's HSD, $P = 0.027$). As a consequence, leaf C loss as fine particulate and dissolved organic matter (total leaf C loss minus leaf C loss due to fungi) varied between 43% and 74% in the opposite direction.

Discussion

The individual effects of water temperature and nutrient concentrations on decomposers and litter decomposition have been widely studied, with most studies reporting a stimulation of decomposers biomass and activity (litter decomposition included) with increasing temperature or nutrient concentrations (see Introduction). However, as far as we know, the combined effects of these two global change factors on litter decomposition in aquatic environments has not yet been addressed, even though they are predicted to increase simultaneously in the near future (Murdoch *et al*., 2000; MEA, 2005), which might have profound effects in the carbon cycle (Clark, 2004; Knorr *et al*., 2005; Cornelissen *et al*., 2007). Here we aimed at assessing the combined effect of increased water temperature, in an attempt to simulate future warming in winter (increase from 5 to 10 °C) and spring (increase from 10 to 15 °C) (Stefan & Sinokrot, 1993; Eaton & Scheller, 1996), and nutrients enrichment, in an attempt to simulate future eutrophication as expected from decreased stream discharges, resulting from increased evapotranspiration and water abstraction for human uses, and increased nutrients loads (Murdoch *et al*., 2000), on litter decomposition and associated fungal assemblages.

Overall, responses of fungal biomass, activity and consequently decomposition rates, to increased temperature and nutrient concentrations were consistent. On the one hand, the stimulation of all biological variables at high NP level only at high temperatures (10 and/or 15 °C) indicates that nutrient enrichment of stream water will have a higher stimulatory effect on fungal performance and decomposition rates under a warming scenario than at lower temperatures. On the other hand, the stimulation of fungal biomass and sporulation with increasing temperature at both NP levels suggests that the predicted increase in water temperature will enhance fungal growth and reproduction in both oligotrophic and eutrophic streams. The stimulation of fungal oxygen consumption and litter decomposition with increasing temperature at high NP level might indicate that a stimulation of carbon mineralization will probably occur at eutrophied streams, while oligotrophic conditions seem to require a 10 °C (from 5 to 15 °C) increase in water temperature to result in stimulated fungal respiration and litter decomposition. Since predictions indicate a maximum increase of 6.4 °C in atmospheric temperature for this century (Stefan & Sinokrot, 1993; Eaton & Scheller, 1996; IPCC, 2007), litter decomposition in oligotrophic streams

Table 6 Carbon budgets for the fungal assemblage associated with alder discs incubated in microcosms at three temperatures and two NP levels for 43 days

	5 °C		10 °C		15 °C	
	Low NP	High NP	Low NP	High NP	Low NP	High NP
Leaf C loss (%)	21.4 ± 8.0	30.3 ± 4.0	30.6 ± 0.7	43.3 ± 2.1	32.1 ± 1.0	48.9 ± 2.6
Total conidial production (mg C g^{-1} initial leaf C)	1.7 ± 0.6	5.2 ± 1.8	3.5 ± 0.3	13.0 ± 1.3	3.5 ± 0.2	9.9 ± 1.7
Leaf C loss due to conidial production (%)	0.9 ± 0.2[a]	1.6 ± 0.3[ab]	1.1 ± 0.1[ab]	3.0 ± 0.3[c]	1.1 ± 0.1[ab]	2.0 ± 0.3[bc]
Mycelial biomass (mg C g^{-1} initial leaf C)	16.0 ± 9.6	40.3 ± 14.6	32.5 ± 0.3	82.4 ± 7.1	43.7 ± 3.2	86.5 ± 17.4
Leaf C loss due to mycelial production (%)	5.5 ± 2.8[a]	12.6 ± 2.8[a]	10.6 ± 0.3[a]	19.3 ± 2.6[a]	13.7 ± 1.1[a]	17.5 ± 2.9[a]
Cumulative fungal production (mg C g^{-1} initial leaf C)*	17.7 ± 10.1	45.5 ± 16.3	35.9 ± 0.2	95.4 ± 7.1	47.2 ± 3.4	96.4 ± 18.9
Yield coefficient (%)†	6.4 ± 2.7[a]	14.2 ± 3.1[ab]	11.8 ± 0.3[ab]	22.3 ± 2.7[b]	14.8 ± 1.2[ab]	19.5 ± 3.1[b]
Respiration (mg C g^{-1} initial leaf C)‡	29.0	55.2	65.5	93.3	104.1	180.3
Leaf C loss due to mineralization (%)	19.4 ± 8.4[a]	18.8 ± 2.2[a]	21.4 ± 0.5[ab]	21.7 ± 1.1[ab]	32.5 ± 1.0[ab]	37.1 ± 1.9[b]
Production efficiency (%)§	31.4 ± 15.2[a]	42.6 ± 8.0[a]	35.4 ± 0.1[a]	50.4 ± 1.9[a]	31.1 ± 1.6[a]	34.2 ± 4.6[a]
Explained mass loss (%)¶	25.8 ± 5.7[a]	33.0 ± 1.0[ab]	33.2 ± 0.8[ab]	43.9 ± 3.7[ab]	47.3 ± 1.9[ab]	56.6 ± 2.6[b]

Values are averages ± 1 SE. Data are from sacrificed microcosms and treatments are as in Table 1. Treatments with the same letter are not significantly different (Tukey's HSD, $P > 0.05$).

*Total conidial production + mycelial production.

†Cumulative fungal production (%)/mass loss (%) × 100.

‡There is no SE present as values are based on cumulative values by the last sampling date for different microcosms sampled at four dates.

§Cumulative fungal production/(cumulative fungal production + respiration) × 100.

¶[Cumulative fungal production (%) + respiration (%)]/mass loss (%) × 100.

might be 'protected', at least to some extent, from global warming. This is further supported by the absence of a relationship between remaining mass after 43 days and water temperature at low NP level, while it was significant and negative at high NP level. These observations result from fungal performance being controlled by temperature, as all biological processes since increased temperature up to an optimum stimulates enzyme activity (Brown *et al*., 2004), and dissolved nutrients, as these are more readily accessible to microorganisms than those from litter substrates (Suberkropp, 1998), which means that if one of these factors is limiting an increase in the other may not result in a stimulation of fungal performance and litter decomposition. This allows us to speculate that if water quality of presently eutrophied streams is improved with a reduction in nutrient concentrations, the potential stimulatory effects of future increase in water temperature on aquatic biota and processes might be mitigated.

The sensitivities of decomposition rates to temperature (Q_{10-q}) were higher at lower temperature (5–10 °C) than at higher temperature (10–15 °C) which agrees with previous reports from terrestrial systems (Chen *et al*., 2000; Bekku *et al*., 2003), and are explained by a temperature limitation of enzymatic activity. In terrestrial systems, sensitivities of decomposition rates to temperature have been shown to be controlled by other factors as litter quality (Fierer *et al*., 2005; Conant *et al*., 2008) and soil nutrient content (Fierer *et al*., 2003). In our study, sensitivities to temperature differed between NP levels, indicating that dissolved nutrients can also modulate temperature sensitivities of organic matter processing in streams. The general pattern indicates (slightly) higher temperature sensitivity at high NP level (Q_{10-q} = 1.49 and 1.36 for high and low NP, respectively, from 5 to 15 °C), as expected when nutrients are not limiting. However, when only an increase in water temperature from 5 to 10 °C was considered, decomposition was stimulated to a higher extent at low NP level than at high NP level, which remains difficult to explain without considering consequences of modified interspecific interactions.

Another aspect to be noted is that for fungal biomass and sporulation rate at 10 °C and low NP level, a 10-fold increase in nutrient concentrations was equivalent to a 5 °C increase in water temperature, indicating that in some cases the effects of warming will resemble those of eutrophication, and some presently eutrophied streams might serve as models to predict the effects of future warming on similar noneutrophied streams. However, predictions are that water temperature and nutrient concentrations will increase simultaneously in many streams, unless efficient measures to reduce water abstraction from and nutrients loads to streams are

undertaken (Murdoch *et al*., 2000; MEA, 2005). In such a scenario, all biological variables assessed here will be stimulated, independently of the original water temperature, as a result of synergistic interactions between increased temperature and nutrient enrichment. This might have profound impacts on forest streams where decomposition of allochthonous organic matter is at the base of aquatic food webs. Changes in fungal growth and activity, including litter decomposition, might lead to altered energy and nutrient flow through the food chain (Greenwood *et al*., 2006), and might affect aquatic organisms that have their life cycles synchronized with the autumnal litter supply in temperate regions (Bärlocher, 2000; Gulis & Suberkropp, 2004). Faster decomposition of organic matter under warmer and nutrient rich conditions will lead to its earlier disappearance from the stream bed which can result in food depletion for higher trophic levels. However, this is still difficult to predict, with the increase in atmospheric CO_2 concentration, besides being responsible for an increase in temperature, also possibly leading to an increase in leaf production but of poorer quality (Norby *et al*., 2001; Stiling & Cornelissen, 2007), which might counteract the faster litter decomposition predicted here.

In addition to the described alterations on fungal biomass and activity, increased water temperature and nutrient levels also affected the structure of aquatic hyphomycete assemblages, but their relative importance varied over time. Water temperature was more important during the initial colonization of leaf discs by fungal species, leading to faster colonization of discs at higher temperature. However, this can not simply be attributed to differences in temperature tolerances of species since eventually all species sporulated at all temperatures and overall conidial production (number and mass) did not differ among temperatures at low NP level. Actually, the fact that all six species sporulated at all treatments indicates that conidia germinated and mycelia grew from all species within the 24 h period of leaf inoculation with the combined conidial suspension (Dang *et al*., 2007, 2009). The delay in sporulation at lower temperatures might be explained by a delay in biomass built-up; although sporulation rates usually peak before mycelial biomass (Suberkropp & Chauvet, 1995; Gulis *et al*., 2006; Lecerf & Chauvet, 2008), they can be delayed until enough biomass accumulates (Gonçalves *et al*., 2007). Also, none of the six species of aquatic hyphomycetes used here were markedly cold- or warm-water species, except TEMA which is known to grow and sporulate best at high temperatures (25 °C; Chauvet & Suberkropp, 1998), although here its overall sporulation did not differ among temperatures. However, temperature might have changed interspecific relationships (Webster *et al*., 1976), which translated into modifications in species rank in a dominance scale, with CLAQ acquiring competitive advantage and HELU and TUAQ loosing it with increasing temperature. Changes in communities dominance induced by temperature have been observed before (Bärlocher *et al*., 2008; Dang *et al*., 2009). After day 25, when all species were sporulating at all treatments, NP level became more important than temperature leading to overall higher conidial production at high NP level (Suberkropp & Chauvet, 1995; Gulis & Suberkropp, 2003a, b; Ferreira *et al*., 2006b). Surprisingly, ARTE conidial production did not differ between NP levels, although it was previously reported to be stimulated by increasing nutrient concentrations in microcosms (Gulis & Suberkropp, 2003b) and in a whole-stream nitrate addition experiment (Ferreira *et al*., 2006b). Nutrient enrichment has also been reported to induce shifts in dominance of fungal communities, maybe due to changes in species interactions (Gulis & Suberkropp, 2003a; Pascoal *et al*., 2005a; Ferreira *et al*., 2006b; Artigas *et al*., 2008). These shifts also occurred here within each treatment, with CLAQ being favored and FLCU unflavored by increased NP level at 5 and 15 °C. Changes in dominance rank of HELU, TEMA and TUAQ also occurred with increasing NP level, but the direction of these changes depended on water temperature. Although aquatic hyphomycete assemblages have been considered functionally redundant (Dang *et al*., 2005; Pascoal *et al*., 2005a, b; Ferreira *et al*., 2006a), shifts in assemblage composition and identity of the dominant species might have indirect effects on litter decomposition since aquatic detritivores derive a large portion of C from fungal mycelium (Chung & Suberkropp, 2009), and seem to have preferences for certain fungal species (Suberkropp *et al*., 1983). This might result in differential performances when invertebrates feed on different fungal species (Arsuffi & Suberkropp, 1986), which might lead to differential invertebrate induced decomposition rates of leaves with different dominant fungal species (Arsuffi & Suberkropp, 1984). Additionally, if changes in temperature and trophic state of freshwaters lead to decreases in fungal species richness, either because they reach their upper thermal tolerance limit or due to exclusion by competition, this might negatively affect invertebrate performance (Lecerf *et al*., 2005) with further consequences for ecosystem functioning.

Carbon budgets for fungi associated with decomposing alder leaf discs were similar to those reported in previous studies (Baldy *et al*., 1995; Baldy & Gessner, 1997; Gulis & Suberkropp, 2003b, c; Pascoal & Cássio, 2004; Pascoal *et al*., 2005b). Fungal production was responsible for 6–22% of leaf C mass loss; however, a larger portion of leaf C mass loss was due to fungal respiration (19–37%). Nevertheless, fungal production

efficiency was high (31–50%). Overall, fungi were responsible for 26–57% of the initial leaf C mass loss, which adds to the large body of evidence indicating aquatic hyphomycetes as important players in litter processing (Baldy *et al*., 1995; Hieber & Gessner, 2002; Gulis & Suberkropp, 2003c; Pascoal *et al*., 2005b), and their importance to leaf mass loss clearly increased with increasing temperature and nutrient concentrations (Gulis & Suberkropp, 2003b; this study). An important amount of leaf C mass was also lost from the system as fine particulate and dissolved organic matter (43–74% in this study; Gulis & Suberkropp, 2003b, c), and this pathway was more important at lower temperatures and nutrient concentrations. These modifications of C budgets under simultaneous increases in water temperature and nutrient concentrations might have serious consequences on the functioning of small heterotrophic streams and reaches downstream, as the portion of leaf C incorporated by fungi will locally increase resulting in higher retention of carbon and nutrients within the system. This, together with the increase in the portion of leaf C respired within the system and the decrease in its loss as fine particulate and dissolved organic matter will impact downstream reaches where collectors become the most abundant functional feeding group (Vannote *et al*., 1980) and thus may lack appropriate resources. Effects of global change factors on fungal activity and community structure might therefore cascade along the food web, with effects on detritivores and collectors (Greenwood *et al*., 2006). These might further affect higher trophic levels which depend on these organisms as a food resource.

In conclusion, simultaneous increases of water temperature and nutrient concentrations accelerated decomposition rates of alder leaf discs (up to 52%, and up to 24% above expected increases), stimulated fungal growth, reproduction and overall activity, changed the structure of assemblages and altered fungal carbon budgets. Some of these modifications were however attenuated at low nutrient concentrations, which suggest that the recovery of presently eutrophied streams might mitigate the effects of future climate warming on aquatic fungal communities and fungal mediated processes. This suggestion is further supported by previous studies which have demonstrated that water temperature and quality (e.g. nutrient status, acidification) interact to affect aquatic communities structure and processes (Marcarelli & Wurtsbaugh, 2006; Durance & Ormerod, 2007), and that improvement of water quality might have delayed changes due to global warming in macroinvertebrate community structure like in the French Rhône River (Daufresne *et al*., 2007).

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