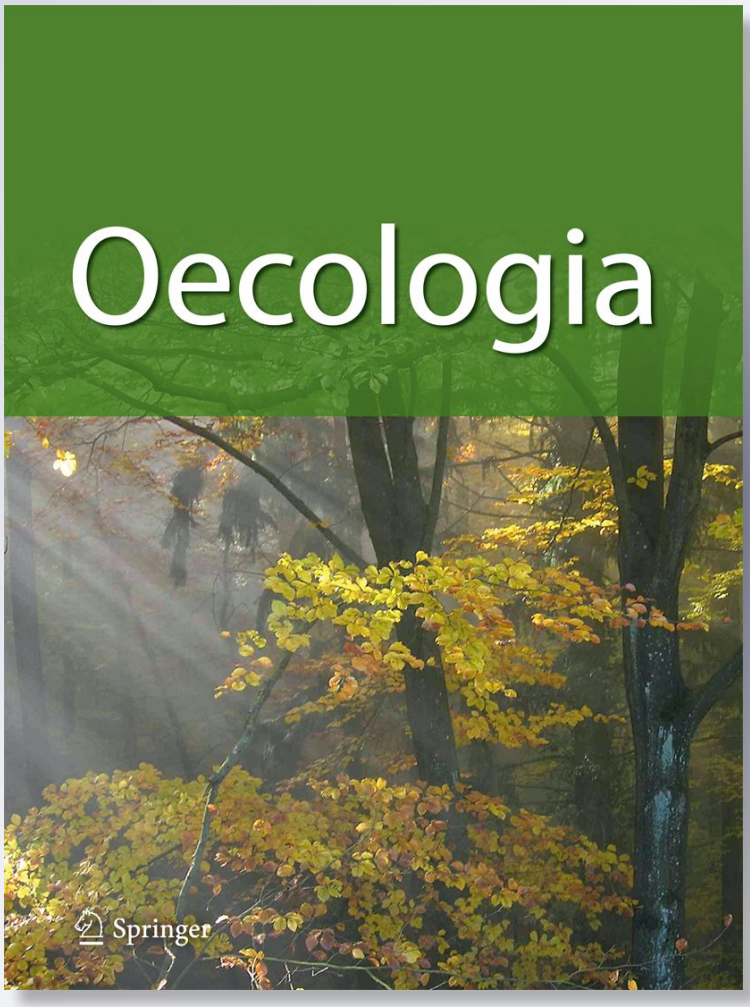
Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams

Verónica Ferreira & Eric Chauvet

Oecologia

ISSN 0029-8549
Volume 167
Number 1

Oecologia (2011) 167:279-291
DOI 10.1007/s00442-011-1976-2



Springer

Oecologia (2011) 167:279–291
DOI 10.1007/s00442-011-1976-2

GLOBAL CHANGE ECOLOGY - ORIGINAL PAPER

Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams

Verónica Ferreira · Eric Chauvet

Received: 23 July 2010 / Accepted: 18 March 2011 / Published online: 3 April 2011

Abstract The predicted increase in atmospheric CO_2 concentration for this century is expected to lead to increases in temperature and changes in litter quality that can affect small woodland streams, where water temperature is usually low and allochthonous organic matter constitutes the basis of the food web. We have assessed the individual and interactive effect of water temperature (5 and 10°C) and alder litter quality produced under ambient CO_2 levels (ambient litter) or under CO_2 concentrations predicted for 2050 (elevated litter) on litter decomposition and on fungal activity and assemblage structure. Litter decomposition rates and fungal respiration rates were significantly faster at 10 than at 5°C, but they were not affected by litter quality. Litter quality affected mycelial biomass accrual at 5 but not at 10°C, while increases in temperature stimulated biomass accrual on ambient but not on elevated litter. A similar pattern was observed for conidial production. All variables were stimulated on elevated litter at 10°C (future scenario) compared with ambient litter at 5°C (present scenario), but interactions between temperature and litter quality were additive. Temperature was the factor that most strongly affected the structure of aquatic hyphomycete assemblages. Our results indicate that if future increases in atmospheric CO_2 lead to only slight modifications in litter quality, the litter decomposition and fungal activities and community structure will be strongly controlled by increased water temperature. This may have serious consequences for aquatic systems as faster litter decomposition may lead to food depletion for higher trophic levels.

Keywords Aquatic hyphomycetes · Atmospheric CO_2 · Ecosystem functioning · Global change · Interactive effects

Communicated by Elena Litchman.

V. Ferreira (✉)
IMAR-CMA, Department of Life Sciences, University of Coimbra, P.O. Box 3046-401, Coimbra, Portugal
e-mail: veronica@ci.uc.pt

E. Chauvet
UPS, INPT; EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), Université de Toulouse 3–Université Paul Sabatier, Bâtiment 4R1, 31062 Toulouse, France

E. Chauvet
CNRS, EcoLab, 31062 Toulouse, France

Introduction

In temperate regions, small woodland streams constitute more than 80% of the length of hydrological networks (Alan and Castillo 2007). These are usually cold water, light-limited streams in which allochthonous organic matter constitutes the major source of energy and carbon (C) (Vannote et al. 1980). As a consequence, aquatic communities in these streams are highly sensitive to changes in water temperature (Poff et al. 2002), litter seasonality, and the quantity and quality of litter inputs, which constitute the basal resource of aquatic food webs (Cummins and Klug 1979).

The predicted doubling of atmospheric carbon dioxide (CO_2) concentration up to the end of this century will result in a 1.1–6.4°C increase in global air temperature (Solomon et al. 2007). This increase will be paralleled by similar increases in stream water temperature (Eaton and Scheller 1996), with consequences on biological processes and ecosystem functions (Brown et al. 2004). Litter decomposition may be particularly affected, since increased water temperature stimulates litter mass loss directly by promoting

leaching (Chergui and Pattee 1990) and indirectly by enhancing litter consumption by shredding invertebrates (González and Graça 2003). Higher temperatures stimulate fungal production (mycelia and conidia) by some species of aquatic hyphomycetes cultivated individually (Chauvet and Suberkropp 1998; Rajashekar and Kaveriappa 2000; Dang et al. 2009), which can also enhance decomposition (Ferreira and Chauvet 2011). However, since temperature tolerance ranges are species specific (Rajashekar and Kaveriappa 2000), and the effect of water temperature changes on interspecific interactions is mostly unknown (Webster et al. 1976), it is difficult to anticipate the impact of global warming scenarios on decomposition processes.

To complicate the matter further, increased atmospheric CO_2 concentration might also lead to delayed litter fall, increased litter production, and decreased litter quality (King et al. 2001; Stiling and Cornelissen 2007; Taylor et al. 2008). Most studies examining the effect of elevated CO_2 concentrations on tree leaf chemistry report a decrease in nutrient concentration [mostly nitrogen (N)] and an increase in structural and secondary compounds, resulting in an altered nutritional quality (higher C/N) of these leaves compared to leaves grown under ambient CO_2 concentration (Cotrufo et al. 1998; Norby et al. 2001; Stiling and Cornelissen 2007). The lower nutritional quality of leaves grown under elevated CO_2 atmosphere should be reflected in leaf litter, since increased atmospheric CO_2 does not seem to affect the readsorption efficiency of nutrients during senescence (Norby et al. 2000, 2001; King et al. 2001). These results imply that, under a climate change scenario, higher amounts of lower quality litter might enter streams later in the year.

Decreased leaf litter quality might negatively affect aquatic communities, as these are known to colonize high-quality litter faster than low-quality litter, and to be more active on the former (Gessner and Chauvet 1994). Although most comparisons have been done among litter species differing in nutritional quality, this should also hold true between genotypes within the same species (LeRoy et al. 2007) or trees from the same species grown under different environmental conditions (Cotrufo et al. 1995; Lecerf and Chauvet 2008). A CO_2-induced decrease in litter quality might therefore affect its use by aquatic decomposers and, thereby affect litter decomposition. However, to date, only a few studies have addressed this question (Tuchman et al. 2002, 2003b; Rier et al. 2002, 2005; Kelly et al. 2010), and in all of these studies CO_2-induced changes in litter quality were considered irrespective of variations in temperature, despite the fact these two factors vary simultaneously.

Since a CO_2-induced increase in water temperature and decrease in litter quality might have contradictory effects on decomposers and litter decomposition, the extent to which they interact with each other is unknown. This lack of knowledge makes responses of ecosystem processes rates under future climate change scenarios difficult to predict unless the combined effects of global change factors on communities and processes are examined. In the study reported here, we assessed the individual and interactive effect of CO_2-induced changes in water temperature (two levels) and alder litter quality (two types) on litter decomposition and the associated aquatic hyphomycetes activity and assemblage structure in microcosms. Based on the effects of each factor individually, we predicted (1) lower fungal performance on, and slower decomposition of alder litter produced under elevated CO_2 than under ambient atmosphere because of the predicted decrease in litter quality under elevated CO_2, and (2) higher fungal performance and faster decomposition at higher water temperature due to the predicted increase in metabolic activities with increased temperature. Also, (3) if quality markedly differs between the two litter types (ambient vs. elevated), then higher differences in fungal performance and litter decomposition would be expected between litter types at higher versus lower temperature, since metabolic activity might become nutrient limited at higher temperature (Ferreira and Chauvet 2011). Finally, (4) the extent to which the stimulatory effect of increased temperature on fungal performance and litter decomposition would be compensated by decreases in litter quality depends on the differences between litters and on the interaction between litter quality and temperature.

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): *Articulospora tetracladia* Ingold, *Clavariopsis aquatica* de Wildeman, *Heliscus lugdunensis* Saccardo and Thérry, *Lemonniera terrestris* Tubaki, *Tetracladium marchalianum* Ingold, and *Tetrachaetum elegans* Ingold. Strains were isolated from single conidia trapped in naturally occurring foam collected from streams in southern France. Growing colonies were kept at room temperature in 9-cm-diameter petri dishes containing approximately 10 mL of growth medium (2% malt and 2% agar) until they were used to induce conidial production. Conidial inoculations (<1 day old) were produced at 15°C by agitation of agar plugs taken from the leading edge of growing colonies, in 25 mL of distilled water or nutrient solution [100 mg $CaCl_2{\cdot}2H_2O$, 10 mg $MgSO_4{\cdot}7H_2O$, 0.5 g 3-morpholinopropanesulfonic acid (MOPS), 5.5 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 experimental microcosm.

Leaf litter, microcosms, and experimental set-up

Alder (*Alnus glutinosa* (L.) Gaertner) litter produced under ambient and elevated CO_2 atmosphere were provided by the Bangor FACE project (Bangor, UK). In this project, trees were planted in March 2004 and grown under either ambient atmosphere (present CO_2 level: 380 ppm; hereafter called "ambient" litter) or elevated CO_2 atmosphere (predicted CO_2 level by 2050: 580 ppm; hereafter called "elevated" litter), from April 2005 until August 2008; within each CO_2 level, trees were planted in four different areas to account for location variability. Both litters were collected after abscission in autumn 2007, air dried at room temperature, and stored in paper bags until experimental use.

Leaf litter was moistened with distilled water and left to rehydrate for 0.5–1 h. Litter discs were obtained using a 12-mm-diameter cork borer and oven dried (>3 days at 105°C). Sterile litter discs were used in this experiment to prevent microbial contamination (as litter is expected to carry its own biological community) from confounding the possible effects of the factors under study. However, any sterilization method (e.g., autoclave, UV-C radiation) can potentially alter the litter quality, which would be of minor importance if litter quality was not one of the factors of interest. Thus, several tests were performed, followed by determination of the phosphorus (P), N, and C concentrations in litter discs, to select the best sterilization method, so that the relative difference in chemical quality would be maintained between litters. Twelve batches of 20 oven-dry litter discs from each area ($n = 4$) within each CO_2 level were split into groups of three and (1) used to determine the initial chemical quality of the litter discs, (2) incubated in glass tubes with 10 mL of distilled water for 1 day at 10°C, (3) placed in glass tubes with 10 mL of distilled water and autoclaved (20 min at 121°C), and (4) irradiated with UV-C light in a sterile laminar flow cabinet for 1 day (being turned over several times so that both sides of the litter discs would be irradiated), and then incubated in glass tubes with 10 mL of distilled water for 3 h at 10°C. After treatments (1–4), litter discs were oven-dried (24 h at 105°C), grinded using a mortar and pestle, and the powder weighed (±0.1 mg) and analyzed for P (APHA 1998), N and C (NA 2100 Protein, CE Instruments; ThermoQuest, Milan, Italy). The results were expressed as % litter dry mass (DM).

Specific leaf area (SLA), as a measure of thickness given by the area-to-mass ratio, was determined on batches of 20 individual, oven-dried litter discs (diameter 12 mm). Determinations were performed for each area within each CO_2 level, and the results were expressed as millimeter squared per milligram.

Forty-eight batches of 20 oven-dry litter discs were weighed (±0.1 mg) to determine the initial DM, placed inside glass tubes with 10 mL distilled water, and autoclaved (20 min at 121°C; see above). Twenty-four batches of 20 litter discs were subjected to the same treatment and were used to calculate the correction factor for mass loss due to leaching during sterilization.

Alder litter 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 created turbulence and kept the litter discs in permanent agitation. A tap at the bottom allowed for the aseptic drainage of the chamber and recovery of the conidial suspension without removing litter discs. Fresh nutrient solution was added to microcosms through the open top, which was otherwise covered with a glass cap.

Incubations took place at 5°C to simulate the actual water temperature in temperate mountain streams in winter, and at 10°C to simulate predicted stream water temperature under a future climate warming scenario. The 5°C increase in water temperature is within that predicted for streams in the USA under a warming scenario (Eaton and Scheller 1996). In some areas of the world, air temperature is predicted to increase up to 7°C (e.g., Portugal; Miranda et al. 2002); consequently a 5°C increase in water temperature in temperate streams is realistic. Forty-eight sterilized (30 min at 121°C) microcosms were distributed into two temperature-controlled chambers, the corresponding sterile litter discs were added, and the microcosms were then filled with 40 mL of a nutrient solution (100 mg $CaCl_2{\cdot}2H_2O$, 10 mg $MgSO_4{\cdot}7H_2O$, 0.5 g MOPS, 0.055 mg K_2HPO_4 and 1 mg KNO_3 per liter of sterile distilled water) and aerated. After 24 h, the solution was changed and the microcosms inoculated with a total of 5,000 conidia equally partitioned among all species of the aquatic hyphomycete assemblage. For the first 2 h, the microcosms were aerated for periods of 20 min interspaced with periods of 20 min in which the conidia were allowed to settle. The microcosms were then aerated continuously until the end of the experiment. The nutrient solution was replaced after 24 h and then every 3 days for the duration of the experiment (73 days). All manipulations of microcosms took place in a sterile laminar flow cabinet.

Fungal sporulation

Four microcosms (one for each area of litter origin) from each temperature × litter quality treatment were sacrificed

after 19, 43, and 73 days. The conidial suspensions were poured into 50-mL Falcon tubes; the volume was adjusted to 42 mL with distilled water and 3 mL of 37% formalin was added as a preservative. In preparation for conidia counting and identification, Triton X-100 (0.5%) was added to the suspensions, followed by mixing to ensure a uniform distribution of conidia; an aliquot of the suspensions was then filtered (SMWP, pore size 5 μm; Millipore, Bedford, MA). Filters were stained with 0.05% trypan blue in 60% lactic acid, and spores were identified and counted under a compound microscope at a magnification of 320× (Bärlocher 2005). Sporulation rates were expressed as milligram conidial C per gram litter C per day. Conidial DM was calculated by multiplying the number of conidia from each species by the average mass of individual conidia (based on data reported in the literature; Chauvet and Suberkropp 1998) or calculated from biovolume data assuming 70% water content (Bärlocher and Schweizer 1983). Conidial and litter C mass was calculated assuming a 50% C content of conidia and litter DM, respectively (Gulis and Suberkropp 2003). In addition, for the 16 microcosms that were sacrificed by day 73, the conidial suspensions were sampled each time the nutrient solution was changed (every 3 days), collected into 1-L jars, and preserved with 37% formalin. Preparation for conidia counting and identification were as described above, and the results were expressed as the total number of conidia produced per microcosm.

Fungal respiration

A subset of five litter discs from each sacrificed microcosm was used to determine fungal oxygen consumption rates using a closed 6-channel dissolved oxygen measuring system (Strathkelvin 928 System; Strathkelvin Instruments, North Lanarkshire, Scotland) connected to a computer. The oxygen electrodes were calibrated against a fresh 4% sodium sulfite solution [0% oxygen (O_2)], and a 100% O_2-saturated nutrient solution at the target temperature. Litter discs were incubated in 3-mL chambers containing a 100% O_2-saturated nutrient solution, with constant stirring, and kept at the target temperature of 5 or 10°C by the circulation of water originating from a temperature-controlled water bath. O_2 consumption rates were determined by the difference in the oxygen concentration in the sample and the control over a 20-min interval during which O_2 consumption over time was linear, corrected for the chamber's volume, time, and disc mass. After a 1-h incubation, the litter discs were enclosed in small sterile zip lock bags and promptly frozen at −20°C for storage until remaining DM determination and ergosterol extraction (see below). O_2 consumption rates were converted into C production rates considering a 50% C content of litter DM and a respiratory quotient of 1 (Gulis and Suberkropp 2003; Ferreira and Chauvet 2011). The results were expressed as milligram C per gram litter C per hour.

Litter mass loss and mycelial biomass

The remaining 15 litter discs from each microcosm were enclosed in small sterile zip lock bags and promptly frozen at −20°C. All 20 litter discs from each microcosm were combined, lyophilized, and weighed (±0.1 mg) to determine remaining DM and subsequently used for ergosterol extraction as a surrogate for mycelial biomass (Gessner and Chauvet 1993; Gessner 2005). Ergosterol was extracted in 5 mL of KOH/methanol (8 g L^{-1}) for 30 min at 80°C. The extract was then purified by solid phase extraction (Waters Oasis HLB 3 cc cartridges; Waters Corp, Milford, MA; Gessner 2005) and quantified with high-performance liquid chromatography (HPLC pump 422, HPLC detector 432, HPLC autosampler 360; Kontron Instruments, Neufahru, Germany) by measuring absorbance at 282 nm. The HPLC detector was equipped with a FLT 0.5-μm A-316 precolumn (Upchurch Scientific, Oak Harbour, WA) and a LispRP 18–5 250 × 4.6-mm column (Thermo-Hypersil Keystone, Bellefonte, PA) maintained at 33°C. The mobile phase was 100% methanol, and the flow rate was set to 1.4 mL min^{-1}. The relative contribution of each species to total ergosterol mass was estimated based on the specific contribution to total conidial mass production. Ergosterol mass was converted into mycelial biomass using either specific conversion factors for *A. tetracladia* (5.0 μg ergosterol mg^{-1} mycelial biomass), *C. aquatica* (8.0), *L. terrestris* (5.3), *T. elegans* (2.9), and *T. marchalianum* (2.6), or the overall conversion factor for *H. lugdunensis* (5.5 μg ergosterol mg^{-1} mycelial biomass; no specific conversion factor available) (Gessner and Chauvet 1993). The results were expressed as milligram mycelial C per gram litter C, considering a 50% C content of mycelial and litter DM.

Fungal C budget

Fungal C budgets were calculated assuming a 50% C content of litter, conidial and mycelial DM and a respiratory coefficient of 1 (Gulis and Suberkropp 2003; Ferreira and Chauvet 2011). The amount of initial litter C converted into conidia C by the end of the experiment was estimated by multiplying the specific number of conidia accumulated each 3 days over the experiment by the average mass of individual conidia obtained from the literature (Chauvet and Suberkropp 1998) or calculated from biovolume data (Bärlocher and Schweizer 1983). The amount of initial litter C converted into mycelial C was estimated for the date when maximum biomass was

recorded (day 73). The amount of initial litter C converted into CO_2-C was estimated by summing up values of daily CO_2-C, assuming linearity between sampling dates. Fractions of initial litter C converted into conidia, mycelia or mineralized where calculated as C converted by a given fungal activity × 100/total litter C loss. The difference between total litter C loss and the C loss due to overall fungal activities was attributed to the release of fine particulate organic matter (FPOM) and dissolved organic matter (DOM).

Data analysis

Phosphorus, N, and C concentrations of initial, leached, autoclaved, and irradiated alder discs, and the initial SLA were compared between ambient and elevated litter by *t* tests. The relationship between SLA and litter P, N, and C was assessed by linear regression.

Exponential decomposition rates (k) of alder discs were calculated by linear regression of ln-transformed fraction of remaining C mass over time, with the intercept fixed at 0. Slopes were compared among treatments by analysis of covariance (ANCOVA), with time as the continuous variable and temperature and litter quality as categorical variables (Zar 1999).

Fungal respiration rate, biomass, and sporulation rate from sacrificed microcosms were compared among treatments by three-way analyses of variance (ANOVAs); time, temperature, and litter quality as categorical variables). Total and specific cumulated number of conidia per microcosm, and litter C fractions converted into mycelial C, conidial C, and CO_2-C were compared among treatments by two-way ANOVAs (temperature and litter quality as categorical variables).

Aquatic hyphomycete assemblages associated with alder discs were compared among treatments by principal response curve (PRC) analysis, which is a constrained form of principal component analysis (ter Braak and Smilauer 1998). The analysis output is a graph with time on the x axis and the principal responses, i.e., compositional differences of treatments (5°C, elevated; 10°C, ambient; 10°C, elevated) against the control (5°C, ambient), displayed on the y axis. Species scores reflect the contribution of each species to the overall community response as described by the PRCs (CANOCO for Windows 4.5; Microcomputer Power, Ithaca, NY; ter Braak and Smilauer 1998).

Data were log $(x + 1)$ transformed when necessary to achieved normality and homoscedasticity. Tukey's test was used for post hoc comparisons. All analyses were performed with STATISTICA v7 software (StatSoft, Tulsa, OK), unless otherwise indicated.

Results

Litter chemical quality and specific leaf area

Litter produced under elevated CO_2 atmosphere had 41% lower initial P concentration than litter produced under ambient atmosphere (*t* test, $P = 0.001$). Growth conditions did not affect litter initial N (*t* test, $P = 0.922$) or C concentration ($P = 0.114$; Table 1).

Litters lost 46–63% of their initial P concentration after leaching for 1 day or autoclaving; however, the relative difference in P concentration between litters was maintained (36 and 31% for leaching and autoclaving, respectively; *t* test, $P < 0.005$; Table 1). Leaching led to a 40% decrease in initial N concentrations, while autoclaving resulted in a 6–8% increase; this did not affect the initial differences between litters (*t* test, $P > 0.672$; Table 1). Leaching and autoclaving increased the initial C concentration by 0.7–2.8%, and differences between litters became significant (*t* test, $P < 0.022$; Table 1).

Irradiating litter with UV-C light led to smaller decreases in initial P concentration compared with leaching or autoclaving, while the differences between litters remained significant (*t* test, $P < 0.004$; Table 1). However, the relative difference in N (*t* test, $P = 0.018$) and C concentration (*t* test, $P = 0.020$; Table 1) was altered between litters.

Summarizing, autoclaving the litter led to changes in chemical composition that mimicked those due to leaching and maintained the relative differences in P and N between litters, while irradiation changed the relative differences in chemical composition between litters. Autoclaving was therefore chosen as the sterilization method.

Contrary to expected, SLA was higher for elevated than for ambient litter (14.1 vs. 17.3 $mm^2\ mg^{-1}$; *t* test, $P < 0.001$). SLA and P concentration were negatively related (linear regression, $P = 0.016$, $R^2 = 0.65$, $n = 8$).

Litter decomposition

Alder litter discs lost between 26 and 44% of their initial C mass after being incubated in microcosms for 73 days (Fig. 1a), which resulted in decomposition rates between 0.0043 and 0.0075 day^{-1} (Table 2). There was a tendency, although non-significant (Tukey's test, $P > 0.459$), for elevated litter to decompose faster than ambient litter at both temperatures. The increase in water temperature by 5°C significantly stimulated decomposition of both litters ($P < 0.009$). Elevated litter incubated at 10°C (future global change scenario) also decomposed faster than ambient litter incubated at 5°C (present scenario) (Tukey's test, $P < 0.001$; Table 2).

Table 1 Phosphorus, nitrogen, and carbon concentrations of initial, leached, autoclaved and UV-C irradiated alder discs produced under ambient or elevated carbon dioxide concentrations

Litter state	Litter quality[a]	P (% DM)	N (% DM)	C (% DM)
Initial	Ambient	0.25 ± 0.02 a	3.37 ± 0.06 a	49.9 ± 0.1 a
	Elevated	0.15 ± 0.02 b	3.35 ± 0.11 a	49.5 ± 0.2 a
Leached	Ambient	0.12 ± 0.01 a	2.03 ± 0.10 a	51.0 ± 0.2 a
	Elevated	0.08 ± 0.01 b	2.02 ± 0.19 a	49.9 ± 0.2 b
Autoclaved[b]	Ambient	0.09 ± 0.01 a	3.58 ± 0.08 a	51.3 ± 0.1 a
	Elevated	0.07 ± 0.01 b	3.63 ± 0.11 a	50.7 ± 0.2 b
UV-C irradiated	Ambient	0.23 ± 0.01 a	3.54 ± 0.09 a	50.6 ± 0.1 a
	Elevated	0.16 ± 0.02 b	3.21 ± 0.10 b	49.9 ± 0.3 b

Values are given as the mean concentration ± 1 standard error (SE). Values within the same treatments (column) and litter state (row) followed with the same letter are not significantly different (*t* test, $P > 0.05$)

P phosphorus, *N* nitrogen, *C* carbon, CO_2, carbon dioxide, *DM* dry mass

[a] Ambient, ambient atmosphere (present CO_2 level: 380 ppm); elevated, elevated CO_2 atmosphere (predicted CO_2 level by 2050: 580 ppm)

[b] Chosen sterilization method

Fungal respiration, biomass, and sporulation

Maximum values of fungal respiration (0.029–0.030 mg C g^{-1} C h^{-1}; Fig. 1b), mycelial biomass (72–82 mg C g^{-1} C; Fig. 1c) and sporulation rates (0.08–0.11 mg C g^{-1} C day^{-1}; Fig. 1d) were attained at 10°C. Litter quality did not affect respiration rates at any temperature (Tukey's test, $P > 0.299$), while it affected mycelial biomass accrual and sporulation rates at 5°C only ($P = 0.044$ and $P = 0.023$, respectively; Table 3). The increase in water temperature by 5°C stimulated fungal respiration and sporulation rates on both litters (Tukey's test, $P < 0.037$) and increased biomass accrual on ambient litter only ($P < 0.001$; Table 3). Mycelial biomass and sporulation rates were also higher on elevated litter at 10°C (future global change scenario) than on ambient litter at 5°C (present scenario) (Tukey's test, $P < 0.001$; Table 3).

Fungal C budget

Litter C loss due to conidial production, although low (<1.2%), was higher at 10°C than at 5°C on both litters (Tukey's test, $P < 0.009$) and higher on elevated litter at 10°C than on ambient litter at 5°C ($P = 0.034$; Fig. 2; Table 4). C loss due to mycelial production was similar among treatments (15–22%; 2-way ANOVA, $P > 0.050$), while C loss due to mineralization varied between 15 (5°C, elevated) and 29% (10°C, ambient) and was higher on ambient than on elevated litter at both temperatures (Tukey's test, $P < 0.041$) and higher on elevated litter at 10°C than at 5°C ($P = 0.004$; Fig. 2; Table 4). C loss explained by overall fungal activities varied between 31 (5°C, elevated) and 52% (10°C, ambient) of total C loss and tended to be higher at higher temperature and for ambient litter, although significant differences were found only between elevated and ambient litter at 5°C ($P = 0.043$; Fig. 2; Table 4). C loss as fine particulate organic matter (FPOM) and dissolved organic matter (DOM) varied between 48 (10°C, ambient) and 69% (5°C, elevated).

Aquatic hyphomycete assemblages

Aquatic hyphomycete assemblages on both ambient and elevated litter at 10°C were most dissimilar from those on ambient litter at 5°C (control; Fig. 3a), indicating that temperature was more important than litter quality in structuring fungal assemblages. Temperature and litter quality treatments explained 49.3%, while time explained 22.3% of the variability among samples (Monte Carlo permutation, $P = 0.002$). *C. aquatica* was the species that was most related to the pattern shown by the PRCs, followed by *T. elegans* and *L. terrestris*, suggesting that these species were the ones most stimulated by increases in temperature (Fig. 3b, c).

The total number of conidia produced per microcosm during the incubation period varied between 91,200 (5°C, elevated) and 1,018,726 (10°C, ambient; Table 5). There was no effect of litter quality on total conidial production at either temperature (Tukey's test, $P > 0.276$). Contrary, the number of conidia was six and ninefold higher at 10 than at 5°C on ambient and elevated litter, respectively (Tukey's test, $P < 0.002$). There were species specific differences in conidial production among treatments. *C. aquatica* (101 fold; Tukey's test, $P = 0.008$), *T. elegans* (45; $P = 0.008$), *T. marchalianum* (22; $P = 0.003$) and *L. terrestris* (5; $P = 0.011$) had higher conidial production on elevated litter at 10 than at 5°C; the same discrepancies, although with different proportions, were also observed on ambient

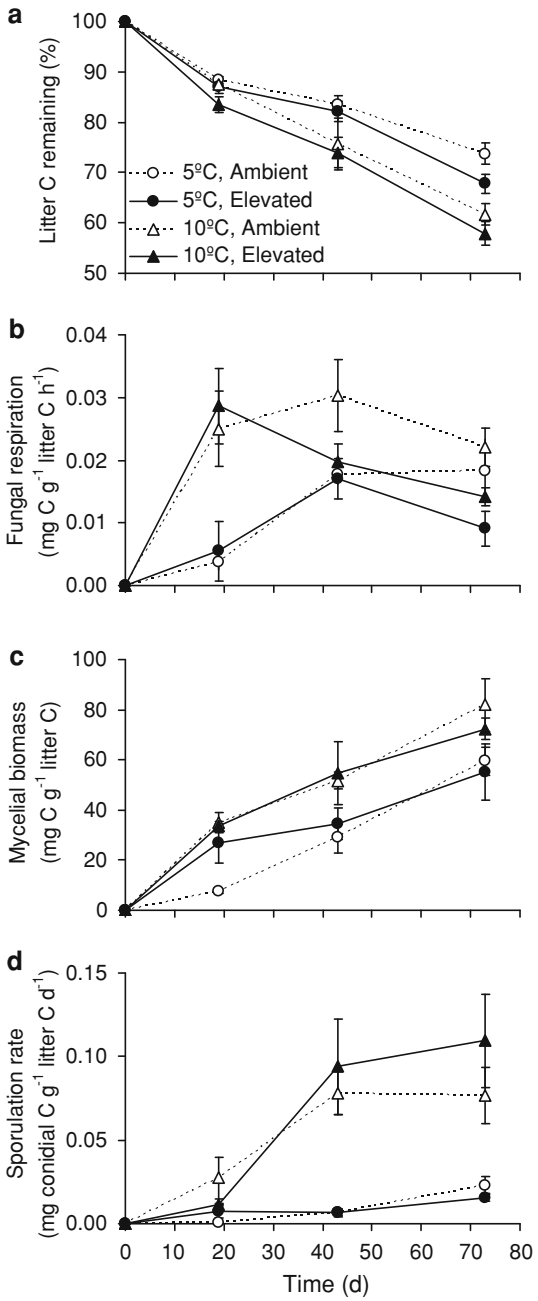


Fig. 1 Remaining alder leaf litter mass (**a**), fungal respiration rate (**b**), mycelial biomass (**c**), sporulation rate (**d**) associated with alder discs produced under ambient (present CO_2 level: 380 ppm) or elevated (predicted CO_2 level by 2050: 580 ppm) CO_2 concentrations and incubated in microcosms at 5 and 10°C for 73 days. Data are presented as the mean ± 1 standard error (SE)

litter between 10 and 5°C (Table 5). Conversely, differences between species contribution among litter quality treatments were generally weak. These changes translated into shifts in the assemblages' structure with *A. tetracladia* and *L. terrestris* co-dominating at 5°C and *C. aquatica* and *L. terrestris* (together with *T. marchalianum* on ambient litter) dominating at 10°C (Fig. 3c).

Table 2 Exponential decomposition rates of alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C for 73 days, standard error and coefficient of determination of the regression

Temperature (°C)	Litter quality	k (day^{-1})	Standard error	R^2
5	Ambient	0.0043 a	0.0003	0.72
	Elevated	0.0052 a,b	0.0003	0.81
10	Ambient	0.0067 b,c	0.0005	0.76
	Elevated	0.0075 c	0.0004	0.84

Values (treatments) followed by the same letter are not significantly different (Tukey's test, $P > 0.05$)

k exponential decomposition rate

Discussion

The future increase in water temperature and alterations in litter quality that are anticipated due to the predicted increase in atmospheric CO_2 levels are expected to affect small woodland streams, where the water temperature is usually low and food webs strongly depend on the terrestrial litter input (Vannote et al. 1980). To the best of our knowledge, no study has yet assessed the combined effects of increased water temperature and changes in litter quality on aquatic microbial communities and processes. Here, we have attempted to fill this gap by assessing the performance of aquatic hyphomycetes, the most important group of microorganisms involved in litter decomposition in freshwaters (Weyers and Suberkropp 1996), when submitted to changes in both water temperature and litter quality, as those expected in a future climate change scenario. In such a scenario (10°C, elevated litter), decomposition rates and all fungal variables increased, although not more than expected from the individual effects of temperature and litter quality, indicating additive effects. Increased water temperature was, however, the most important factor driving responses of fungal activities and decomposition rates. The possibility that the effect of decreased litter quality can to some degree compensate for the effect of increased water temperature on litter mass loss probably depends on the litter species and on the extent to which litter chemistry and toughness are modified by increased CO_2. In this study, ambient and elevated litter apparently differed only in P concentration, which can be considered a small difference compared with the discrepancies in N and C often reported in the literature (Cotrufo et al. 1998; Norby et al. 2001; Stiling and Cornelissen 2007).

Growing alder leaves under elevated CO_2 atmosphere resulted in a 41% decrease in litter P concentration. This was a surprising result since trees grown under elevated CO_2 tend to have faster growth rates than those grown under ambient CO_2 (Curtis and Wang 1998) and should therefore have higher P demands and sequestration.

Table 3 Summary table for three-way ANOVAs performed on microbial variables associated with alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C for 73 days

Analysis	Fungal respiration rate			Mycelial biomass			Sporulation rate		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Three-way ANOVA									
Intercept	1	244.7	<0.001	1	5,492.0	<0.001	1	1,575.5	<0.001
Temperature	1	25.7	<0.001	1	34.7	<0.001	1	112.9	<0.001
Litter quality	1	2.9	0.095	1	2.8	0.103	1	4.1	0.051
Time	2	2.7	0.085	2	40.5	<0.001	2	39.2	<0.001
Temperature × litter quality	1	0.2	0.636	1	4.7	0.037	1	5.1	0.031
Temperature × time	2	5.6	0.008	2	3.0	0.061	2	3.0	0.064
Litter quality × time	2	2.1	0.132	2	3.3	0.047	2	3.9	0.029
Temperature × litter quality × time	2	0.7	0.495	2	2.8	0.076	2	10.9	<0.001
Error	34			35			36		
Tukey's test[a]									
5°C, ambient			a,b			a			a
5°C, elevated			a			b			b
10°C, ambient			c			b			c
10°C, elevated			b,c			b			c

Data are from sacrificed microcosms

ANOVA analysis of variance

[a] Tukey's test results are presented, with treatments with the same letter not being significantly different ($P > 0.05$)

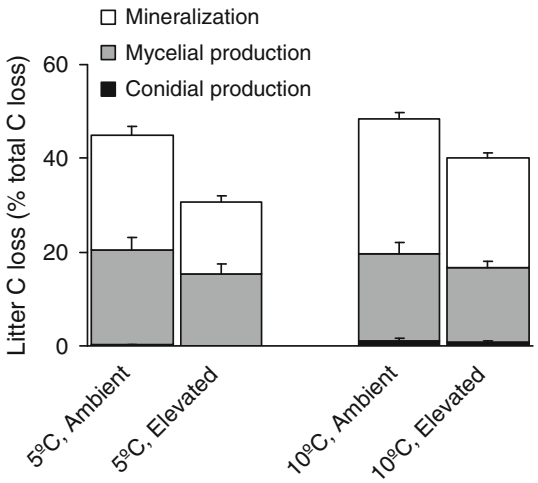


Fig. 2 Litter mass loss due to fungal activities of alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C over 73 days. Data are presented as the mean ± 1 standard error (SE)

However, alder trees might have invested more in the growth of fine roots than in that of leaves, diverting P from the latter to the former (Rey and Jarvis 1997). Similar decreases in P concentration in leaf litter grown under elevated CO_2 levels have been reported for *Quercus pubescens* (60%, Cotrufo et al. 1999) and *Betula pendula* (30%, Ferreira et al. 2010). Additionally, elevated CO_2 is most often reported to lead to decreases in N concentration in leaves (Cotrufo et al. 1998), although for N-fixers, the opposite trend might occur (Vogel et al. 1997). Neither of these patterns was observed in our study as both litters presented similar N concentrations, which might be attributed to alder being an N-fixing species (Cotrufo et al. 1998; Temperton et al. 2003). The small differences in litter quality between CO_2 levels in this study might also be partially explained by the conditions in which the alder trees grew. The elevated CO_2 concentration used in our study was intended to reproduce the concentration predicted by 2050, i.e., 580 ppm, which is lower than the concentrations that have been used in most studies performed to date (e.g., 600–1200 ppm; Cotrufo et al. 1998). Also, the free air CO_2 enrichment technology used in our study has been reported to produce less strong effects on plant responses than those observed when plants are grown in enclosures (e.g., open top chambers, solar domes), indicating the potential for artifacts associated with enclosures (Norby et al. 2001). Even though the differences in quality between ambient and elevated litter were not those that had been anticipated, elevated litter was of poorer nutrient quality, given that P is frequently the limiting nutrient for microbial growth (Francoeur 2001; Rosemond et al. 2002; Elser et al. 2007). Therefore, elevated litter was expected to have a lower fungal activity and decomposition rate than ambient litter (Gessner and Chauvet 1994). However, contrary to the findings reported for most studies, namely, an increased leaf thickness when

Table 4 Summary table for two-way ANOVAs performed on the fraction of litter C lost due to fungal activities on alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C for 73 days

Analysis	Litter C loss due to conidial production			Litter C loss due to mycelial production			Litter C loss due to mineralization			Litter C loss due to overall fungal activities		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Two-way ANOVA												
Intercept	1	72.6	<0.001	1	222.5	<0.001	1	1,335.2	<0.001	1	660.7	<0.001
Temperature	1	56.8	<0.001	1	0.2	0.681	1	23.9	<0.001	1	6.1	0.029
Litter quality	1	6.0	0.033	1	4.7	0.050	1	33.2	<0.001	1	15.5	0.002
Temperature × litter quality	1	1.4	0.259	1	0.0	0.881	1	2.0	0.187	1	0.2	0.705
Error	11			12			12			12		
Tukey's test[a]												
5°C, ambient			a			a			ab			a
5°C, elevated			a			a			c			b
10°C, ambient			b			a			b			a
10°C, elevated			b			a			a			ab

[a] Tukey's test results are presented, with treatments with the same letter not being significantly different ($P > 0.05$)

leaves are grown under elevated CO_2 (Stiling and Cornelissen 2007; but see Heagle et al. 2002), we found that the SLA was higher on elevated litter than on ambient litter. This result, associated with the fact that SLA was negatively related to litter P concentration, might to some extent compensate for the negative effects expected from poorer nutrient quality on fungal performance and litter decomposition (see below).

Aquatic organisms, however, are not usually exposed to the initial chemical composition of the litter since once in water litter starts to leach soluble compounds and its chemical composition changes (Gessner et al. 1999). Changes in quality after leaching for 1 day were also observed in our litter. Since litter discs had to be sterilized, autoclaving was the chosen method because it simulated the leaching process, which would be intensive during the first 24–48 h of litter submersion, and it maintained the relative difference in quality between litters. Incubation of ambient and elevated leaf litter in streams is associated with changes in litter quality, with some studies reporting that the relative difference in initial chemical composition between litters disappeared (Rier et al. 2002, 2005; Adams et al. 2003; Tuchman et al. 2003b), was maintained (Tuchman et al. 2002, 2003a; Adams et al. 2003; Ferreira et al. 2010), or was even amplified (Rier et al. 2005).

Litter decomposition rates increased by more than 30% with a 5°C increase in water temperature, but they were insensitive to litter quality. Faster litter decomposition rates at higher temperatures have been documented in aquatic and terrestrial systems (Irons et al. 1994; Aerts 2006; Dang et al. 2009; Ferreira and Chauvet 2011) and can be explained by stimulated biological activity (Fabre and Chauvet 1998; Dang et al. 2009; Ferreira and Chauvet 2011; see below). The anticipated lower decomposition rates of elevated litter due to its lower P concentration were not observed and might be attributed to a trade-off between lower litter P concentration, which would reduce the nutritional quality of the litter, and lower litter toughness, which would facilitate mycelial colonization. Litter toughness has been shown to determine litter decomposition rates, sometimes even overcoming the importance of litter chemistry (Quinn et al. 2000; Li et al. 2009). The few studies addressing the effects of changes on litter quality induced by increases in atmospheric CO_2, on aquatic microbial communities and litter decomposition have shown that these effects are stronger at early stages of litter decomposition than at later stages, when secondary compounds have been leached; however, overall they remain weak and species-dependent (Rier et al. 2002, 2005; Tuchman et al. 2003b; Kelly et al. 2010). Evidence from terrestrial systems also indicates that the effects of elevated CO_2 on litter quality are species specific and not always translated into differences in litter decomposition rates (Gorissen and Cotrufo 2000; King et al. 2001; Norby et al. 2001).

Fungal respiration rates, a measure of overall activity, were stimulated at 10°C, as expected, since increases in temperature within a non-stressful range stimulate enzymatic activities (Bekku et al. 2003). To the contrary, the similarity in respiration rates between litters might indicate that differences in litter quality were not strong enough or that litter quality was not the most important factor driving microbial activities. Microbial activity has been reported to be higher on ambient litter than on elevated litter when there is a difference in quality (Rier et al. 2002; Tuchman et al. 2003b). This indicates that microbial activity sensitivity to temperature is not always dependent on litter

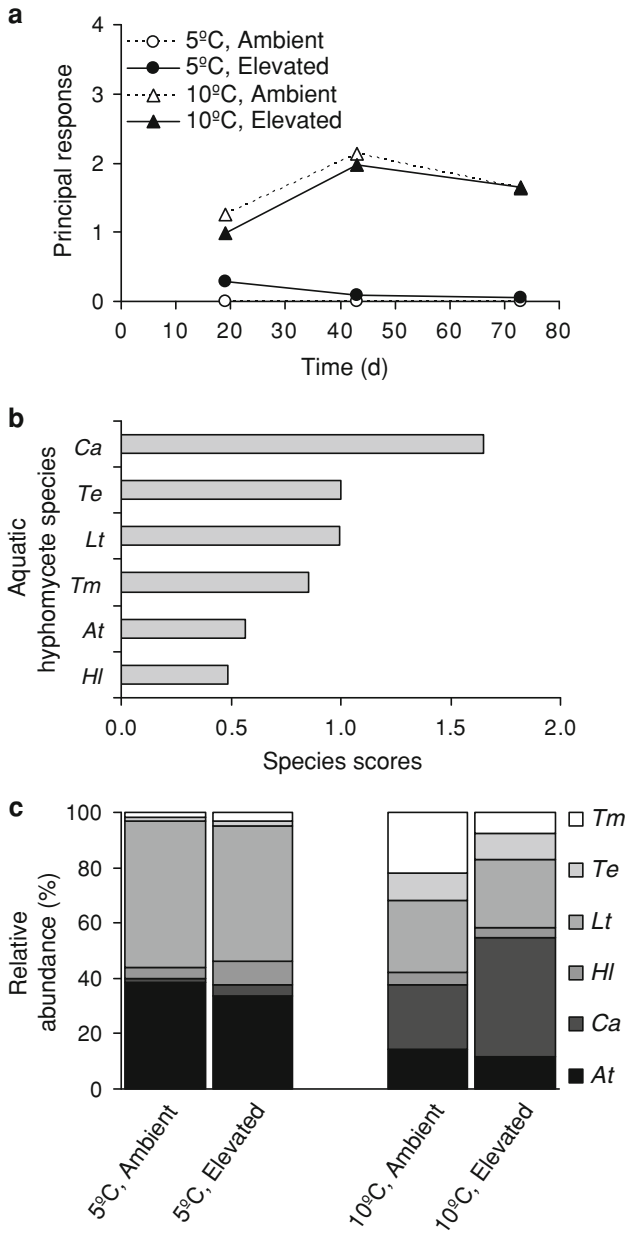


Fig. 3 **a** Principal response curves for aquatic hyphomycetes assemblages associated with alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C for 73 days, **b** species scores (the higher the score, the more the species was stimulated by the treatments), **c** relative contribution of each species to the total conidial production. *At Articulospora tetracladia* Ingold, *Ca Clavariopsis aquatica* de Wildeman, *Hl Heliscus lugdunensis* Saccardo and Thérry, *Lt Lemonniera terrestris* Tubaki, *Te Tetrachaetum elegans* Ingold, *Tm Tricladium marchalianum* Ingold

quality. This result is in contrast with reports from terrestrial systems where differences in litter quality are high (Conant et al. 2008a, b). The increase in CO_2 release from litter with increased temperature might have consequences on the C cycle, leading to a positive feedback on global warming (Knorr et al. 2005). The effect of elevated temperature on the decomposition of litter submerged in aquatic environments should therefore be taken into consideration when the effects of CO_2 release from decomposing litter are modeled on atmospheric CO_2 concentrations.

As expected, mycelial biomass accrual and fungal sporulation rates were stimulated by an increase in temperature (Chauvet and Suberkropp 1998; Rajashekar and Kaveriappa 2000; Dang et al. 2009; Ferreira and Chauvet 2011). At 5°C, fungal biomass accrual and sporulation were higher on ambient litter than on elevated litter, which might indicate that on elevated litter, both variables were more sensitive to decreased P concentration than to decreased toughness. This result is probably due to the increasing needs of P for nucleic acid production, which is necessary to sustain higher mycelial and conidial production. However, contrary to our expectations, differences in biomass accrual and sporulation were greater between litters at 5°C than between those at 10°C. Since higher water temperature resulted in stimulated fungal production, the P demand for nucleic acid production should have been higher to sustain higher mycelial and conidial production. Given that P concentration was lower on elevated litter, this should have translated into differences between litters at 10°C. At the lower water temperature, this P demand would be low on both litters because growth was lower, and elevated litter would therefore be able to support the P demand for fungal production. The fact that the difference between litters occurred only at 5°C might be attributed to the structure of the assemblages and the species-specific P needs at 5 and 10°C. Because a species such as *T. elegans*, which dominates assemblages at 5°C, but not 10°C, apparently behaves differently from other species in terms of N and P use and requirements, as judged from preliminary experiments (M Danger and E Chauvet, results not shown), this may have resulted in the observed contrast in response to litter quality at the two temperatures.

Fungal-induced mass loss was higher from ambient litter than from elevated litter, likely due to differences in overall fungal respiration, which was higher on the ambient litter. In contrast, mass loss through FPOM and DOM release was higher on elevated litter than on ambient litter, resulting in similar amounts of mass being lost from the system (mineralization, FPOM, and DOM) for both litters. In a stream situation, this would mean, on one hand, that the local invertebrate detritivores might not be strongly affected by small changes in litter quality as the ones reported here (but see Ferreira et al. 2010) while, on the other hand, downstream communities in which collectors become the dominant functional feeding group of invertebrates (Vannote et al. 1980) might be favored since the release of FPOM from elevated litter might increase. The higher levels of DOM leached from elevated litter might

Table 5 Specific and total number of conidia on alder discs produced under ambient or elevated CO_2 concentrations and incubated in microcosms at 5 and 10°C over 73 days

Temperature (°C)	5		10	
Litter quality	Ambient	Elevated	Ambient	Elevated
*Articulospora tetracladia**	66,170 ± 3,285 a,b	30,664 ± 7,546 a	147,305 ± 54,274 b	95,773 ± 24,728 a,b
*Clavariopsis aquatica**	2,098 ± 912 a,b	3,534 ± 1,926 a	235,469 ± 59,893 a,b	358,043 ± 103,673 b
*Heliscus lugdunensis**	6,766 ± 3,607 a	7,936 ± 1,798 a	48,748 ± 18,688 a	29,835 ± 2,414 a
*Lemonniera terrestris**	90,413 ± 27,789 a,b	44,525 ± 9,459 a	264,632 ± 84,971 b	204,407 ± 41,784 b
*Tetracladium elegans**	2,460 ± 655 a,b	1,700 ± 1,203 a	99,597 ± 20,068 b	76,103 ± 20,535 b
*Tetrachaetum marchalianum**	2,962 ± 719 a	2,841 ± 722 a	222,975 ± 95,324 b	63,133 ± 27,360 b
Total conidia microcosm^{-1}	170,870 ± 27,859 a	91,200 ± 16,792 a	1,018,726 ± 245,818 b	827,293 ± 105,806 b

Values are given as the mean number ± 1 SE

Values (treatments) followed by the same letter are not significantly different ($P > 0.05$)

also affect periphytic algal accrual and further affect grazing invertebrates (Kominoski et al. 2007).

Litter quality did not affect the absolute or relative specific conidial contribution to total conidial production, probably because the differences between litters were small. A recent study also found that fungal communities were not affected by litter quality changes induced by increased atmospheric CO_2 (Kelly et al. 2010). However, when differences in litter quality are greater, for example, between litter species, aquatic hyphomycete community structure can be affected (Gulis 2001; Ferreira et al. 2006). In contrast, increased water temperature affected the assemblage structure, as expected (Bärlocher et al. 2008; Dang et al. 2009; Ferreira and Chauvet 2011); this change was due to species having temperature preferences (e.g., *T. elegans* and *T. marchalianum* were stimulated at higher temperatures, Chauvet and Suberkropp 1998). These effects of water temperature on the structure of assemblages help in explaining the higher litter decomposition at 10°C since different species might differ in degradative capabilities (Suberkropp et al. 1983). In streams, temperature-induced changes in the structure of fungal assemblages might also indirectly affect litter decomposition through their effects on shredding invertebrates. Shredders exhibit preferences for certain species of aquatic hyphomycetes (Arsuffi and Suberkropp 1984) and derive a large amount of energy from fungal mycelium (Chung and Suberkropp 2009). Therefore, litter bearing preferred species in higher amounts will be more palatable, resulting in higher invertebrate-induced mass loss (Arsuffi and Suberkropp 1989).

In conclusion, our results indicate that if increased atmospheric CO_2 concentration affects litter quality only slightly, then litter decomposition and fungal activities and community structure will be strongly controlled by the future increase in water temperature. This may have serious consequences for aquatic systems, as faster litter decomposition will probably lead to food depletion at higher trophic levels (Poff et al. 2002). The fast disappearance of litter from the streambed can, however, be mitigated if increases in atmospheric CO_2 result in higher litter production (Finzi et al. 2001; Stiling and Cornelissen 2007).

More studies on the effects of elevated CO_2 on litter quality and the decomposition rates of different litter species are necessary to allow a better understanding of the effects of this global change factor on aquatic communities and ecosystem functioning. Since an increased atmospheric CO_2 concentration can also change plant phenology and litter production, as well as co-vary with other environmental factors, such as dissolved nutrients, multi-factor experiments will likely provide a better picture of ecosystem functioning under a future climate change scenario.

Acknowledgments Alder litter was kindly provided by Prof. Douglas Godbold (School of Agricultural and Forest Sciences, University of Wales, Bangor, UK) and was a product of the Bangor FACE project. We thank Franck Gilbert for laboratory equipment, Julien Cornut and Michael Danger for the isolation of aquatic hyphomycete species, and Marion Bottollier, Sylvain Lamothe, and Didier Lambrigot for providing technical help. The comments provided by Dr. Elena Litchman and two anonymous reviewers were most welcome. Financial support granted by the Portuguese Foundation for Science and Technology (FCT, Reference SFRH/BPD/34368/2006) to VF and by the National Center for Scientific Research (CNRS) to EC, as well as the Pessoa exchange program between Portugal and France (PHC 22808 TB) from the French Ministry of Foreign and European Affairs, are gratefully acknowledged.

References

Adams JA, Tuchman NC, Moore PA (2003) Atmospheric CO_2 enrichment alters leaf detritus: impacts on foraging decisions of crayfish (*Orconectes virilis*). J N Am Benthol Soc 22:410–422

Alan JD, Castillo MM (2007) Stream ecology. Structure and function of running waters, 2nd edn. Springer SBM, Dordrecht

Aerts R (2006) The freezer defrosting: global warming and litter decomposition rates in cold biomes. J Ecol 94:713–724

APHA (American Public Health Association) (1998) Standard methods for the examination of water and wastewater, 20th edn. APHA, Washington, DC

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

Arsuffi TL, Suberkropp K (1989) Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. Oecologia 79:30–37

Bärlocher F (ed) (1992) The ecology of aquatic hyphomycetes. Ecological Monographs, vol 94. Berlin Heidelberg New York, Springer

Bärlocher F (2005) Sporulation by aquatic hyphomycetes. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition. A practical guide. Springer SBM, Dordrecht, pp 185–187

Bärlocher F, Schweizer M (1983) Effects of leaf size and decay rate on colonization by aquatic hyphomycetes. Oikos 41:205–210

Bärlocher F, Seena S, Wilson KP, Williams DD (2008) Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. Freshw Biol 53:368–379. doi:10.1111/j.1365-2427.2007.01899.x

Bekku YS, Nakatsubo T, Kume A, Adachi M, Koizumi H (2003) Effect of warming on the temperature dependence of soil respiration rate in arctic, temperate and tropical soils. Appl Soil Ecol 22:205–210. doi:10.1016/S0929-1393(02)00158-0

Brown JH, Gilloly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789. doi:10.1890/03-9000

Chauvet E, Suberkropp K (1998) Temperature and sporulation of aquatic hyphomycetes. Appl Environ Microbiol 64:1522–1525

Chergui H, Pattee E (1990) The influence of season on the breakdown of submerged leaves. Arch Hydrobiol 120:1–12

Chung N, Suberkropp K (2009) Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichopetra: Limnephilidade). Freshw Biol 54:2212–2224. doi:10.1111/j.1365-2427.2009.02260.x

Conant RT, Drijber RA, Haddix ML, Parton WJ, Paul EA, Plante AF, Sixsand J, Steinweg JM (2008a) Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biol 14:868–877. doi:10.1111/j.1365-2486.2008.01541.x

Conant RT, Steinweg JM, Haddix ML, Paul EA, Plante AF, Sixs J (2008b) Experimental warming shows that decomposition temperature sensitivity increases with soil organic matter recalcitrance. Ecology 89:2384–2391. doi:10.1890/08-0137.1

Cotrufo MF, Briones MJI, Ineson P (1998) Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. Soil Biol Biochem 30:1565–1571

Cotrufo MF, Ineson P, Roberts JD (1995) Decomposition of birch leaf litter with varying C-to-N ratios. Soil Biol Biochem 27:1219–1221

Cotrufo MF, Raschi A, Lanini M, Ineson P (1999) Decomposition and nutrient dynamics of *Quercus pubescens* leaf litter in a naturally enriched CO_2 Mediterranean ecosystem. Funct Ecol 13:343–351

Cummins KW, Klug MJ (1979) Feeding ecology of stream invertebrates. Annu Rev Ecol Syst 10:147–172

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313

Dang CK, Schindler M, Chauvet E, Gessner MO (2009) Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90:122–131

Eaton JG, Scheller RM (1996) Effects of climate warming on fish thermal habitat in streams on the United States. Limnol Oceanogr 41:1109–1115

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Deabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Fabre E, Chauvet E (1998) Leaf breakdown along an altitudinal stream gradient. Arch Hydrobiol 141:167–179

Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biol 17:551–564. doi:10.1111/j1365-2486.2010.02185.x

Ferreira V, Gonçalves AL, Godbold DL, 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 Biol 16:3284–3296. doi:10.1111/j1365-2486.2009.02153.x

Ferreira V, Gulis V, Graça MAS (2006) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149:718–729. doi:10.1007/s00442-006-0478-0

Finzi AC, Allen AS, DeLucia EH, Ellsworth DS, Schlesinger WH (2001) Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology 82:470–484

Francoeur SN (2001) Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. J N Am Benthol Soc 20:358–368

Gessner MO (2005) Ergosterol as a measure of fungal biomass. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition. A practical guide. Springer SBM, Dordrecht, pp 189–195

Gessner MO, Chauvet E (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl Environ Microbiol 59:502–507

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

Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. Oikos 85:377–383

González JM, Graça MAS (2003) Conversion of leaf litter to secondary production by a shredding caddis-fly. Freshw Biol 48:1578–1592. doi:10.1046/j.1365-2427.2003.01110.x

Gorissen A, Cotrufo MF (2000) Decomposition of leaf and root tissue of three perennial grass species grown at two levels of atmospheric CO_2 and N supply. Plant Soil 224:75–84

Gulis V (2001) Are there any substrate preferences in aquatic hyphomycetes? Mycol Res 105:1088–1093

Gulis V, Suberkropp K (2003) Interactions between fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. Aquat Microb Ecol 30:147–157

Heagle AS, Burns JC, Fisher DS, Miller JE (2002) Effects of carbon dioxide enrichment on leaf chemistry and reproduction by twospotted spider mites (Acari: Tetranychidae) on white clover. Environ Entomol 31:594–601

Irons JG, Oswood MW, Stout RJ, Pringle CM (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshw Biol 32:401–411

Kelly JJ, Basal A, Winkelman J, Janus LR, Hell S, Wencel M, Belt P, Kuehn KA, Rier ST, Tuchman NC (2010) Growth of trees under elevated atmospheric CO_2 alters microbial communities colonizing leaf litter in a temperate woodland stream. Appl Environ Microbiol 76:4950–4959. doi:10.1128/AEM.00221-10

King JS, Pregitzer KS, Zak DR, Kubiske ME, Holmes WE (2001) Correlation of foliage and litter of sugar maple, *Acer saccharum*, as affected by elevated CO_2 and varying N availability, and

effects on decomposition. Oikos 94:403–416. doi:10.1034/j.1600-0706.2001.940303.x

Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil carbon turnover to warming. Science 433:298–301. doi:10.1038/nature03226

Kominoski JS, Moore PA, Wetzel RG, Tuchman NC (2007) Elevated CO_2 alters leaf-litter-derived dissolved organic carbon: effects on stream periphyton and crayfish feeding preferences. J N Am Benthol Soc 26:663–672. doi:10.1899/07-002.1

Lecerf A, Chauvet E (2008) Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic Appl Ecol 9:598–605. doi:10.1016/j.baae.2007.11.003

LeRoy CJ, Whitham TG, Wooley SC, Marks JC (2007) Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. J N Am Benthol Soc 26:426–438. doi:10.1899/06-113.1

Li AOY, Mg LCY, Dudgeon D (2009) Effects of leaf toughness and nitrogen content on litter breakdown and macroinvertebrates in a tropical stream. Aquat Sci 71:80–93. doi:10.1007/s00027-008-8117-y

Miranda P, Coelho FES, Tomé AR, Valente MA (2002) 20th century Portuguese climate and climate scenarios. In: Santos FD, Forbes K, Moita R (eds) Climate change in Portugal. Impacts and adaptation measures. SIAM Project, Scenarios. Gradiva Publications, Lda, pp 23–83

Norby RJ, Long TM, Hartz-Rubin JS, O'Neill EG (2000) Nitrogen resorption in senescing tree leaves in a warmer, CO_2-enriched atmosphere. Plant Soil 224:15–29

Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG (2001) Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia 127:153–165. doi:10.1007/s004420000615

Poff NL, Brinson MM, Day JW Jr (2002) Aquatic ecosystems and global climate change. Potential impacts on inland freshwater and coastal wetland ecosystems in the United States. Pew Center on Global Climate Change, Arlington

Quinn JM, Burrell GP, Parkin SM (2000) Influences of leaf toughness and nitrogen content on in-stream processing and nutrient uptake by litter in a Waikato, New Zealand, pasture stream and streamside channels. NZJ Mar Freshw Res 34:253–271

Rajashekar M, Kaveriappa KM (2000) Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. Hydrobiologia 441:149–153

Rey A, Jarvis PG (1997) Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO_2 exposure. Ann Bot 80:809–816. doi:10.1006/anbo.1997.0526

Rier ST, Tuchman NC, Wetzel RG, Teeri JA (2002) Elevated-CO_2-induced changes in the chemistry of quaking aspen (*Populus tremuloides* Michaux) leaf litter: subsequent mass loss and microbial responses in a stream ecosystem. J N Am Benthol Soc 21:16–27

Rier ST, Tuchman NC, Wetzel RG (2005) Chemical changes to leaf litter grown under elevated CO_2 and the implications for microbial utilization in a stream ecosystem. Can J Fish Aquat Sci 62:185–194. doi:10.1139/F04-148

Rosemond AD, Pringle CM, Ramírez A, Paul MJ, Meyer JL (2002) Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnol Oceanogr 47:278–289

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Stiling P, Cornelissen T (2007) How does elevated carbon dioxide (CO_2) affect plant-herbivore interaction? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. Global Change Biol 13:1823–1842. doi:10.1111/j.1365-2486.2007.01392.x

Suberkropp K (1991) Relationships between growth and sporulation of aquatic hyphomycetes on decomposing litter. Mycol Res 95:843–850

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

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Giolis B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, Kets K, Long SP, Karnosky DF (2008) Future atmospheric CO_2 leads to delayed autumnal senescence. Global Change Biol 14:264–275. doi:10.1111/j.1365-2486.2007.01473.x

Temperton VM, Garyston SJ, Jackson G, Barton CVM, Millard P, Jarvis PG (2003) Effects of elevated carbon dioxide concentration on growth and nitrogen fixation in *Alnus glutinosa* in a long-term field experiment. Tree Physiol 23:1051–1059. doi:10.1093/treephys/23.15.1051

ter Braak CJF, Smilauer P (1998) CANOCO reference manual and user's guide to CANOCO for windows: software for canonical community ordination, version 4. Microcomputer Power. Ithaca, NY

Tuchman NC, Wetzel RG, Rier ST, Wahtera KA, Teeri JA (2002) Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Global Change Biol 8:163–170. doi:10.1046/j.1365-2486.2002.00460.x

Tuchman NC, Wahtera KA, Wetzel RG, Russo NM, Kilbane GM, Sasso LM, Teeri JA (2003a) Nutritional quality of leaf detritus altered by elevated atmospheric CO_2: effects on development of mosquito larvae. Freshw Biol 48:1432–1439. doi:10.1046/j.1365-2427.2003.01102.x

Tuchman NC, Wahtera KA, Wetzel RG, Teeri JA (2003b) Elevated atmospheric CO_2 alters leaf litter quality for stream ecosystems: an in situ leaf decomposition study. Hydrobiologia 495:203–211. doi:10.1023/A:1025493018012

Vannote RL, Minshal GW, Cummins KW, Sedall JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137

Vogel CS, Curtis PS, Thomas RB (1997) Growth and nitrogen accretion of dinitrogen-fixing *Alnus glutinosa* (L.) Gaertn. under elevated carbon dioxide. Plant Ecol 130:63–70

Webster J, Moran ST, Davey RA (1976) Growth and sporulation of *Tricladium chaetocladium* and *Lunulospora curvula* in relation to temperature. Trans Br Mycol Soc 67:491–549

Weyers HS, Suberkropp K (1996) Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. J N AmBenthol Soc 15:408–420

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Englewood Cliffs