Global Change Biology

Global Change Biology (2010) **16**, 3284–3296, doi: 10.1111/j.1365-2486.2009.02153.x

Effect of increased atmospheric CO_2 on the performance of an aquatic detritivore through changes in water temperature and litter quality

VERÓNICA FERREIRA*, ANA LÚCIA GONÇALVES*, DOUGLAS L. GODBOLD† and CRISTINA CANHOTO*

IMAR-CMA & Department Life Sciences, University of Coimbra, PO Box 3046, 3001-401 Largo Marquês de Pombal, Coimbra, Portugal, †Environment Centre Wales, Bangor University, Bangor LL57 2UW, UK

Abstract

Cold water woodland streams, where terrestrially derived organic matter fuels aquatic food webs, can be affected by increases in atmospheric CO_2 concentrations, as these are predicted to lead to increases in water temperature and decreases in organic matter quality. In fact, elevated CO_2 (580 ppm) decreased the initial phosphorus concentration of birch litter by 30% compared with litter grown under ambient conditions (380 ppm). Here, we first assessed the effect of differences in litter quality on mass loss, microbial colonization and conditioned litter quality after submersion in a mountain stream for 2 weeks. Leaching did not change the relative differences between litter types, while fungal biomass was two fold higher in elevated litter. We then offered this litter (conditioned ambient and elevated) to a stream detritivore that was kept at 10 and 15 °C to assess the individual and interactive effects of increased temperature and decreased litter quality on invertebrate performance. When given a choice, the detritivore preferred elevated litter, but only at 10 °C. When fed litter types singularly, there was no effect of litter quality on consumption rates; however, the effect of temperature depended on individual size and time of collection. Growth rates were higher in individuals fed ambient litter at 10 °C when compared with individuals fed elevated litter at 15 °C. Mortality did not differ between litter types, but was higher at 15 °C than at 10 °C. Increases in temperature led to alterations in the individual body elemental composition and interacted with litter type. The performance of the detritivore was therefore more affected by increases in temperature than by small decreases in litter quality. However, it seems conceivable that in a future global warming scenario the simultaneous increases in water temperature and decreases in litter quality might affect detritivores performance more than predicted from the effects of both factors considered individually.

Keywords: aquatic detritivore, global change, litter quality, *Sericostoma vittatum*, water temperature

Received 10 August 2009 and accepted 24 November 2009

Introduction

Atmospheric CO_2 concentrations are predicted to double by the end of the century [Intergovernmental Panel on Climate Change (IPCC) 2007], potentially affecting autotrophic and heterotrophic pathways in both terrestrial and aquatic systems (Bezemer & Jones, 1998; Norby *et al*., 2000; Rier *et al*., 2002; Cotrufo *et al*., 2005; Kominoski *et al*., 2007). The effects of increased atmospheric CO_2 on aquatic systems are, however, not well studied, and even less are the effects of multiple factors associated with global change. Small woodland, light-limited streams, where allochthonous organic matter constitutes the primary source of energy and carbon for aquatic food webs (Vannote *et al*., 1980), could be particularly affected (Tuchman *et al*., 2002; Frost & Tuchman, 2005) as increased atmospheric CO_2 concentrations might change the phenology of litter input as well as litter quantity and quality (Tricker *et al*., 2004; Cotrufo *et al*., 2005; Stiling & Cornelissen, 2007; Taylor *et al*., 2008).

Most studies of the effect of elevated CO_2 on tree leaf chemistry report a decrease in nitrogen concentration and an increase in lignin, starch and phenolic concentrations, resulting in impoverished nutritional quality (high C/N) of these leaves relative to leaves grown under ambient CO_2 concentrations (reviewed by Lindroth, 1996; Cotrufo *et al*., 1998a; Norby *et al*., 2001; Stiling & Cornelissen, 2007). Given that increased atmospheric CO_2 does not seem to affect readsorption efficiency of nutrients during senescence, lower nutritional quality of leaves grown under elevated CO_2 should be carried over to leaf litter (reviewed by Norby *et al*., 2000,

Correspondence: Verónica Ferreira, tel. + 351 239 855 760, fax + 351 239 855 789, e-mail: veronica@ci.uc.pt

2001; Cotrufo *et al*., 2005). This implies that in the future litter entering streams will be of poorer nutritional quality than at present.

A large body of evidence demonstrates that microbial and invertebrate performance is depressed in low- (high C/N) than in high-quality (low C/N) leaf litter (Gessner & Chauvet, 1994; Canhoto & Graça, 1995; Ferreira *et al*., 2006a), which results in slower decomposition of poor quality litter (Gessner & Chauvet, 1994; Ostrofsky, 1997; Richardson *et al*., 2004; Ferreira *et al*., 2006a). Therefore, CO_2-induced reductions in litter quality are likely to affect how microbes and detritivores use litter, and consequently alter litter decomposition and energy flow through the aquatic food web. However, evidence from terrestrial systems show that CO_2-induced decreases in leaf quality not always strongly affect invertebrate performance when there are postingestive compensatory mechanisms (Williams *et al*., 1994, 2000, 2003) or food choice (Williams *et al*., 1997; Peters *et al*., 2000). The effects of decreased leaf quality on invertebrates might also depend on developmental stage, with stronger effects on early instar larvae (Fajer, 1989; Bezemer & Jones, 1998; Williams *et al*., 1998; Asshoff & Hättenschwiler 2005; but see Williams *et al*., 1997). However, only a few studies have so far addressed the effect of CO_2-induced decreases in litter quality in aquatic systems (Rier, *et al*., 2002, 2005; Tuchman *et al*., 2002, 2003a, b; Kominoski *et al*., 2007).

The predicted increase in atmospheric CO_2 concentration will also result in a global 1.1–6.4 °C increase in atmospheric temperature (IPCC 2007). Stream water temperature should closely mirror this increase (Eaton & Scheller, 1996), potentially affecting stream biota since biological processes are temperature dependent (Brown *et al*., 2004). For invertebrates, an increase in water temperature, within nonstressful values, is expected to result in faster initial growth rates, shorter developmental time and smaller size at maturity (reviewed by Atkinson, 1995; Atkinson & Sibly, 1997). Effects of increased water temperature are, however, predicted to be stronger for invertebrates inhabiting cold waters when compared with those inhabiting warmer waters (e.g. highlands vs. lowlands, northward vs. southward, winter vs. summer) (Braune *et al*., 2008), because in cold water environments biological activities are more temperature limited (Brown *et al*., 2004). These alterations on detritivore life history will potentially affect instream litter processing and aquatic food web dynamics.

As the decrease in litter quality and the increase in water temperature might potentially have opposing effects on invertebrate performance, it is difficult to predict the combined effect of these two global change factors on detritivore ecology and consequently litter processing in woodland streams. Some of the studies that assessed the combined effect of different litter species (food quality) and water temperature on detritivore performance showed interactions among these factors (Sweeney & Vannote, 1984; Sweeney *et al*., 1986; González & Graça, 2003; Azevedo-Pereira *et al*., 2006). Accordingly, the simultaneous evaluation of CO_2-induced decreased litter quality and increased water temperature on aquatic detritivore performance will give a more realistic picture of the effects that elevated atmospheric CO_2 will have on these communities, compared with each factor individually (Williams *et al*., 2000, 2003).

Our goal was to assess the performance of a stream detritivore in a climate change scenario, when changes in both litter quality and water temperature are expected, because this might strongly affect litter processing in freshwaters. Specifically, we addressed the individual and combined effects of decreased birch litter quality (due to elevated atmospheric CO_2 levels) and increased water temperature (due to CO_2-induced increased atmospheric temperature) on food preferences, consumption rates, growth, survival and elemental composition of the caddisfly detritivore *Sericostoma vittatum* Rambur (Trichoptera: Sericostomatidae). Given the effects of increased temperature and decreased litter quality previously observed independently on invertebrates, we predict (i) higher consumption, growth and survival of individuals fed birch litter grown under ambient than elevated atmospheric CO_2, (ii) higher consumption and initial growth rates, but lower survival, of individuals at higher water temperature, (iii) higher difference in consumption rates between litter types for individuals at earlier than at later developmental stages, (iv) higher difference in consumption rates between temperatures for individuals collected in winter than for those collected in spring and (v) higher difference in consumption, growth and survival of individuals fed both litter types at the higher water temperature.

Methods

Birch leaves, detritivores and laboratory incubation conditions

Birch (*Betula pendula* Roth.) leaves grew under ambient or elevated atmospheric CO_2 at the BangorFACE facility (Bangor, UK; http://www.senr.bangor.ac.uk/research/themes/fem/climate_change.php). The experiment was established in March 2004, and CO_2 enrichment carried out in 2005–2008. The climate is Hyperoceanic, with annual rainfall of about 1000 mm. In total, four ambient and four Free Air Carbon dioxide Enrichment (FACE) plots were randomly located within the plantation forming a complete replicated block design. Carbon enrichment started in April 2005 and was achieved by injecting pure CO_2 through laser-driller holes in tubing mounted on eight masts per plot. The elevated CO_2 concentrations,

measured at 1-min intervals, were within 30% deviation from the preset target concentration of 580 ppm CO_2 (approximately double of preindustrial values; IPCC 2007) for 75–79% of the time during the photosynthetically active period (2005–2008). The CO_2 used for enrichment originated from natural gas. Litter from ambient (380 ppm) or elevated atmospheric CO_2 plots (580 ppm) were collected after abscission in autumn 2007, air dried at room temperature and stored in paper bags until shipped to Coimbra, Portugal, for experimental use. *B. pendula* can be found naturally in riparian forests (Siefert & Mutz, 2001; Pascoal *et al*., 2005), and can contribute with significant amounts of leaf litter to streams (Lecerf, *et al*., 2005).

Each litter type was assembled into groups of ~3 g, enclosed in 0.5-mm-mesh bags (15 × 20 cm) and conditioned for 2 weeks (autumn 2008) in riffle habitats at Ribeira de S. João, a low-order stream in Central Portugal (Lousã mountain; 40°05′59″N, 8°14′02″W; for more information see Ferreira *et al*., 2006b). After retrieval, litterbags were transported to the laboratory on ice and the litter gently rinsed with distilled water. Litter disks were cut out individually with a cork borer (12 mm diameter), avoiding the central vein, placed on paper to air dry and then oven dried (105 °C) until needed. Just before use, litter disks were weighed (±0.1 mg) and rehydrated with distilled water. Conditioned litter was used in these experiments as previous studies have demonstrated that conditioning (microbial colonization) increases litter palatability to detritivores, in part due to the chemical changes they induce in the litter (Graça *et al*., 2001). Also, in stream conditions, invertebrates tend to colonize submerged litter after this had been leached and conditioned (Hieber & Gessner, 2002).

Experiments were performed with similar size, early-stage larvae of the caddisfly *S. vittatum*, a stream detritivore endemic to the Iberian Peninsula and common in Central Portugal. Individuals were collected from depositional areas in Ribeira de S. João in autumn and winter 2008, and transported to the laboratory in plastic containers with stream water and sand. In the laboratory, individuals were randomly split into two groups, allocated into plastic containers with stream sand at the bottom and aerated stream water, acclimatized at 10 and 15 °C, under a 12 h light : 12 h dark photoperiod, and fed conditioned alder litter *ad libitum* for at least 1 week before being used in the experiments. Just before being used, and when necessary during the experiments, the diameter of the case opening of each individual was measured under a stereoscopic microscope at ×16, and individual dry mass estimated by the application of the regression model DM = 0.0136 × CO–0.0162 ($R^2 = 0.83$, $P < 0.001$, $n = 35$), where DM is dry mass (g) and CO is case opening (mm).

Experiments took place at 10 °C to simulate actual water temperature in temperate mountain streams in autumn, and at 15 °C to simulate water temperature under a climate-warming scenario. This 5 °C increase in water temperature is within that predicted for streams in the United States (Eaton & Scheller, 1996). Air temperature in Portugal is expected to increase by up to 7 °C (Miranda *et al*., 2002) and so a 5 °C increase in water temperature is also reasonable. In all experiments, the invertebrates were allocated to plastic containers (7 cm diameter × 8.5 cm high), with ashed stream sediment (<0.5 mm; 550 °C, 8 h) covering the bottom, and 250 mL of filtered stream water (Millipore APFF), aerated for the duration of the experiment (Graça *et al*., 2005).

Initial and conditioned litter chemical composition and microbial colonization

In order to characterize initial litter chemical composition, air-dried litter samples were oven dried (105 °C, 24 h), milled (0.5 mm powder size) and subsamples weighed (±0.1 mg) to be analysed for carbon (C) and nitrogen (N) [IRMS Thermo Delta V advantage with a Flash EA (1112 series)], phosphorus (P; Graça *et al*., 2005), lignin (Goering & van Soest, 1970) and total phenolic (Graça *et al*., 2005) concentrations. Results were expressed as percentage of dry mass (%DM).

In order to characterise conditioned litter chemical composition and microbial colonization, four 0.5-mm-mesh bags (15 × 20 cm) were prepared with 3 g (±0.1 mg) of air dry mass of each litter type, and conditioned for 2 weeks in riffle habitats at Ribeira de S. João, simultaneously to litter incubation for the experiments with invertebrates. After retrieval (day 14), each litterbag was enclosed in an individual zip lock bag and transported to the laboratory on ice. Litter was gently rinsed with distilled water, and three sets of five litter disks were cut out with a cork borer (12 mm diameter), avoiding the central vein, for microbial determinations. (i) Sporulation by aquatic hyphomycetes was induced by incubation of litter disks in 100 mL Erlenmeyer flasks (Schott Duran, Mainz, Germany) with 25 mL of filtered stream water, on an orbital shaker at 100 rpm and 15 °C, for 48 h. Conidial suspensions were then saved into 50 mL centrifuge tubes and preserved with 2 mL of 37% formalin until filters were prepared for observation under a microscope and conidia were counted (Graça *et al*., 2005). (ii) Microbial oxygen consumption was determined using a flow-through system where litter disks were kept inside 8 mL glass chambers supplied with 100% oxygenated filtered stream water (Graça *et al*., 2005). (iii) Ergosterol was extracted from freeze-dried litter disks by microwave, separated by pentane (MAE; Young, 1995) and quantified by high-performance liquid chromatography (HPLC) by measuring absorbance at 282 nm. The HPLC system (Dionex, Sunnyvale, CA, USA) was equipped with the LiChroCART 250-4 LiChrospher 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany), maintained at 30 °C. The mobile phase was 100% methanol and the flow rate was set to 1.4 mL min^{-1}. Ergosterol was converted into fungal biomass using a conversion factor of 5.5 μg ergosterol mg^{-1} fungal dry mass (Gessner & Chauvet, 1993). Litter disks were weighed (±0.1 mg) after [in case of (i) and (ii)] or before [in case of (iii)] microbial determinations and their mass summed to the bulk remaining mass.

The bulk remaining material was oven-dried (105 °C, 24 h) and weighed (±0.1 mg) to determine dry mass remaining. The initial air dry mass to the initial oven dry mass conversion factor was obtained through short-term incubation (30 min) of four litterbags of each litter type in the stream at day 0; these bags were returned to the laboratory and dry mass was determined as above. Dry mass remaining was calculated as the difference between initial mass, corrected for the conver-

sion factor, and final mass. The remaining material was milled, and subsamples were analysed for C, N, P, lignin and total phenolic concentrations, as above, to characterize litter chemical composition after conditioning.

Feeding preferences

One premeasured early instar invertebrate was allocated to each of 40 containers; half of which were incubated at 10 °C and half at 15 °C. In each cup, one preweighed litter disk from each litter type (ambient and elevated), marked with coloured pins, was accessible to the invertebrate. Controls for invertebrate feeding were made of one litter disk from each litter type, enclosed in a small fine mesh (0.5 mm) bag attached to the cup's edge with a clip. The remaining litter disks and invertebrate from each container were sampled after 4 days when one of the litter disks was ~50% eaten. Disks were oven dried (105 °C, 24 h), and weighed (±0.1 mg), while the case opening of the invertebrate was measured to determine invertebrate mass. Dry mass of each litter disk eaten by the invertebrate (L_e, g) was calculated as $(I_i - I_f) - (I_i \times ((C_i - C_f)/C_i))$, where I_i and I_f are initial and final dry mass (DM, g) of litter disk exposed to the invertebrate, and C_i and C_f are the initial and final dry mass (g) of control litter disks (Graça *et al*., 2005). Individual consumption was calculated as $L_e/I \times t$, where I is the invertebrate dry mass (g) at time t, and results expressed as g disks DM g^{-1} individual DM day^{-1} (Graça *et al*., 2005).

Consumption, growth and survival

One premeasured *S. vittatum* individual was allocated to each of 80 containers: half were incubated at 10 °C and half at 15 °C. At each temperature 20 individuals were fed ambient litter disks and 20 fed elevated litter disks. Each individual was provided *ad libitum* oven-died (105 °C) litter disks, which were changed bi-weekly together with the water and sediment. At that time, the case opening of the individuals was measured to determine their dry mass. Relative growth rates (RGR; mg DM g^{-1} DM day^{-1}) were calculated as $DM_g/(DM_f \times t)$, where DM_g is the dry mass gained during the elapsed time (t, 28 days) given by the difference between final (day 28) and initial dry mass (mg) and DM_f is the final dry mass (g) (Tuchman *et al*., 2002). Percentage mass gained relatively to initial values was also calculated. Survivorship was also registered bi-weekly for the duration of the experiment (112 days).

Consumption was determined for (a) small size individuals (14.6 ± 0.3 mg, average ± SE; 14 days) collected from the stream in winter (February 2009), (b) medium size individuals (23.8 ± 0.3 mg; 6 days) collected from the stream in winter (January 2009), and (c) medium size individuals (22.1 ± 0.3 mg; 6 days) collected from the stream in spring (May 2009), to evaluate the effect of larval developmental stage (a vs. b) and ambient acclimatization (b vs. c) to variation in litter quality and water temperature. For this, litter disks were weighed (±0.1 mg) before and after being exposed to the invertebrates. Controls (six containers per litter type per temperature) were run with litter material but without larvae. The dry mass of litter disks eaten by the invertebrate and the individual consumption were calculated as above. Relative consumption rates (RCR; g leaf DM g^{-1} individual DM day^{-1}) were calculated as $L_e/(DM_f \times t)$, where L_e is the litter dry mass eaten during the elapsed time (t, 14 and 6 days for small and medium size individuals) and DM_f is the final dry mass of individuals (g) (Tuchman *et al*., 2002).

Oxygen consumption and body elemental composition

Groups of seven medium size individuals of *S. vittatum* were allocated into 250 mL Erlenmeyer flasks filled with 100 mL of filtered and aerated stream water, and ashed sand. Ten flasks were incubated at 10 and 15 °C for 1 week, and half provided with *ad libitum* ambient or elevated litter; water, sand and litter were changed after 4 days. After the feeding phase, invertebrates' oxygen consumption rates were determined using a flow trough system (the seven individuals comprising a replicate were kept together; Graça *et al*., 2005), and results expressed as mg O_2 g^{-1} individual DM h^{-1}. Individuals were then sacrificed (105 °C), removed from their cases, oven dried (105 °C, 24 h), weighed (±0.1 mg), ground and analysed for C, N and P, as above.

Data analysis

Litter chemical composition was compared among litter types (ambient vs. elevated) and states (initial vs. conditioned) by two-way analysis of variances (ANOVAs); *t*-tests were used when only conditioned litter was considered. Elemental ratios on conditioned litter were compared among litter types by *t*-tests. Mass loss, microbial oxygen consumption, fungal biomass and sporulation rates after 2 weeks incubation in the stream were compared between litter types by *t*-tests.

Consumption rates of ambient and elevated litter types for the feeding preference experiment were compared within each temperature by paired *t*-test. Relative consumption rates, RGRs, oxygen consumption rates and body elemental composition were compared among treatments by two-way ANOVAs (temperature and litter type as categorical factors) (Zar, 1999). Median time to death (TTD) at each treatment was calculated by the Kaplan–Meier product-limit method, and survivorship of individuals was compared between paired treatments by log-rank tests.

Data were transformed [log, log(x + 1) or arcsine square root transformation] to achieve normality whenever necessary (Zar, 1999). Tukey's test was applied for post-hoc multicomparisons. Analyses were performed with STATISTICA 6 software (StatSoft, Tulsa, OK, USA).

Results

Initial and conditioned litter chemical composition and microbial colonization

Experimental increase in atmospheric CO_2 concentration significantly affected phosphorus concentration in

litter (two-way ANOVA, $F_{18,1} = 21.8$, $P < 0.001$), with birch litter grown under elevated CO_2 atmosphere having 30% lower phosphorus concentration than litter grown under ambient CO_2 atmosphere (Tukey's test, $P = 0.020$) (Table 1). This difference between litter types persisted even after litter was incubated in a stream for 2 weeks (Tukey's test, $P = 0.037$), although leaching resulted in a 50% decrease in phosphorus concentration for both litter types (two-way ANOVA, $F_{18,1} = 92.3$, $P < 0.001$) (Table 1). Nitrogen, carbon, phenols and lignin concentrations in litter were not affected by changes in atmospheric CO_2 concentration (two-way ANOVAs, $P > 0.278$), and incubation in the stream did not change this pattern; however, incubation of litter significantly reduced phenols (two-way ANOVA, $F_{16,1} = 8.3$, $P = 0.011$) and increased lignin (two-way ANOVA, $F_{15,1} = 13.7$, $P = 0.002$) concentrations (Table 1). After incubation, litter types also differed in elemental ratios with elevated litter presenting lower C/N and higher C/P and N/P ratios than ambient litter (*t*-tests, $P = 0.031$, 0.014 and < 0.001, respectively) (Table 1).

Incubation of litter in the stream resulted in microbial colonization and 8–10% initial litter mass loss (Table 2). However, from all biological determinations performed on conditioned litter, only fungal biomass was significantly different between litter types (*t*-test, $P < 0.001$), but contrary to expected fungal biomass was twofold higher for elevated than ambient litter (Table 2).

Feeding preferences

When conditioned ambient and elevated litter disks were offered simultaneously to the aquatic detritivore *S. vittatum*, feeding preferences were affected by water temperature. At 10 °C elevated litter was preferred over ambient litter (paired *t*-test, $P = 0.044$), while at 15 °C there was no significant difference in consumption between litter types ($P = 0.570$) (Fig. 1).

Consumption, growth and survival

Patterns in relative consumption rate among treatments varied between small and medium size individuals and between winter and spring individuals (Fig. 2). Small winter individuals had higher consumption rate at 10 than at 15 °C (two-way ANOVA, $F_{76,1} = 6.2$, $P = 0.015$; Fig. 2a) while medium winter individuals had higher consumption rate at 15 than at 10 °C (two-way ANOVA, $F_{74,1} = 4.2$, $P = 0.044$; Fig. 2b). On the other hand, medium spring individuals had a tendency to feed at the same rate at both temperatures (two-way ANOVA, $F_{76,1} = 3.6$, $P = 0.061$; Fig. 2c). In any case, there was not a significant difference in consumption rate between litter types (two-way ANOVAs, $P > 0.154$).

Table 1 Chemical composition (average ± SE; $n = 4$–12) of birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm), before (initial) and after (conditioned) incubation in the stream for 2 weeks

		Chemical composition					Elemental ratios*		
Litter state	Litter type	P (%DM)	N (%DM)	C (%DM)	Phenols (%DM)	Lignin (%DM)	C/N	C/P	N/P
Initial	Ambient	0.20 ± 0.03^{a}	1.46 ± 0.21^{a}	48.29 ± 5.40^{a}	4.42 ± 2.31^{a}	47.59 ± 2.20^{a}	33.04	247.73	7.38
	Elevated	0.14 ± 0.01^{b}	1.40 ± 0.17^{a}	49.62 ± 1.47^{a}	5.28 ± 1.40^{a}	47.28 ± 0.91^{a}	35.36	347.43	9.82
Conditioned	Ambient	0.10 ± 0.01^{c}	1.21 ± 0.13^{a}	52.41 ± 0.84^{a}	2.10 ± 0.08^{b}	52.78 ± 0.75^{ab}	44.69 ± 3.36^{a}	541.79^{a} ± 34.63	12.48 ± 2.42^{a}
	Elevated	0.07 ± 0.01^{d}	1.60 ± 0.15^{a}	50.56 ± 0.59^{a}	1.73 ± 0.08^{b}	50.52 ± 0.59^{b}	32.95 ± 3.13^{b}	749.09^{b} ± 54.41	23.11 ± 4.24^{b}

Different letters indicate significant differences among treatments (two-way analysis of variances for chemical composition and *t*-tests for elemental ratios, $P < 0.05$).
*Elemental ratios of initial litter were calculated from average values of phosphorus (P), nitrogen (N) and carbon (C), while those of conditioned litter were calculated by averaging the elemental ratios of each replicate.

Table 2 Mass remaining and associated microbial variables (average ± SE; $n = 8$) after incubation of litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm) in the stream for 2 weeks

Litter type	Mass remaining (%DM)	Oxygen consumption ($mg\,O_2\,g^{-1}\,DM\ h^{-1}$)	Fungal biomass ($mg\,g^{-1}\,DM$)	AH sporulation (conidia $mg^{-1}\,DM\,day^{-1}$)
Ambient	92.59 ± 1.08[a]	0.43 ± 0.04[a]	8.28 ± 0.97[a]	22.86 ± 5.13[a]
Elevated	90.00 ± 0.86[a]	0.44 ± 0.07[a]	17.60 ± 0.87[b]	29.72 ± 13.61[a]

Different letters indicate significant differences between treatments (*t*-tests, $P < 0.05$).
AH, aquatic hyphomycetes; DM, dry matter.

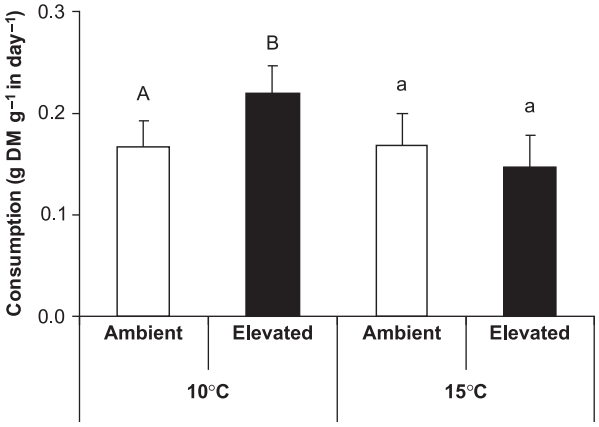

Fig. 1 Feeding preferences (average ± SE; $n = 20$) of *Sericostoma vittatum* kept at 10 and 15 °C, and provided with birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm) for 4 days. Different letters indicate significant differences between litter types, within the same temperature (*t*-test, $P < 0.05$).

Small size individuals (14.6 ± 0.3 mg, average ± SE) kept at 10 and 15 °C and fed ambient and elevated litter increased their body mass over 28 days, after which growth stopped and mortality started. However, this increase was higher for individuals at 10 °C (27–30% of initial mass) than for individuals at 15 °C (17–23%), which translated into significantly faster RGRs at lower temperature (14–38% faster; two-way ANOVA, $F_{76,1} = 20.4$, $P < 0.001$) (Fig. 3). The tendency for faster growth rates for individuals fed ambient litter than for those fed elevated litter was not significant (Tukey's test, $P > 0.136$). However, as we predicted a simultaneous increase in temperature and decrease in litter quality, the most relevant comparison is between growth rates of individuals kept at 10 °C and fed ambient litter and those kept at 15 °C and fed elevated litter, and in this case the difference was significant (Tukey's test, $P < 0.001$), with a 38% reduction in RGR and a 13% reduction in body mass gained under future climate change scenario.

Mortality of individuals was high at all treatments, and by day 112 <4% of the total number of individuals remained (Fig. 4). Median TTD was 56 days for individuals at 15 °C, 84 for individuals fed ambient litter at 10 °C and 98 for individuals fed elevated litter at 10 °C. Litter type did not affect survival (log-rank, test statistic = 1.471, $P = 0.141$ at 10 °C, and test statistic = −0.785, $P = 0.433$ at 15 °C); however, higher water temperature accelerated the mortality (log-rank, test statistic = −3.927, $P < 0.001$ for ambient litter, and test statistic = −4.631, $P < 0.001$ for elevated litter), with the percentage of survival at 15 °C lagging ~40 days behind that at 10 °C, after day 28 (Fig. 4).

Oxygen consumption and body elemental composition

Oxygen consumption by invertebrates was stimulated (11–38%) by an increase in water temperature (two-way ANOVA, $F_{16,1} = 5.8$, $P = 0.028$), but did not significantly differ between litter types (two-way ANOVA, $F_{16,1} = 0.1$, $P = 0.740$) (Fig. 5). Invertebrates kept at 15 °C for 1 week had higher nitrogen concentration than those kept at 10 °C (two-way ANOVA, $F_{16,1} = 10.8$, $P = 0.005$), but only when fed ambient litter (Tukey's test, $P = 0.030$), or when the increase in temperature occurred simultaneously to a decrease in litter quality (Tukey's test, $P = 0.024$) (Table 3). Even though litter types differed in phosphorus concentration, no difference was found in body phosphorus concentration between litter types (two-way ANOVA, $F_{16,1} = 0.5$, $P = 0.475$) or temperatures (two-way ANOVA, $F_{16,1} = 0.7$, $P = 0.431$). Body carbon concentration decreased with increasing temperature for individuals fed elevated litter (two-way ANOVA, $F_{16,1} = 6.2$, $P = 0.024$) (Table 3). This was translated into a significant decrease in body C/N ratio for individuals fed elevated litter at 15 °C when compared with individuals at 10 °C (Tukey's test, $P < 0.006$); no significant differences in C/P and N/P ratios were found among treatments (two-way ANOVAs, $P > 0.093$) (Table 3).

Discussion

Increased atmospheric CO_2 concentrations are expected to affect aquatic ecosystems through multiple pathways, including elevated water temperature (as a direct

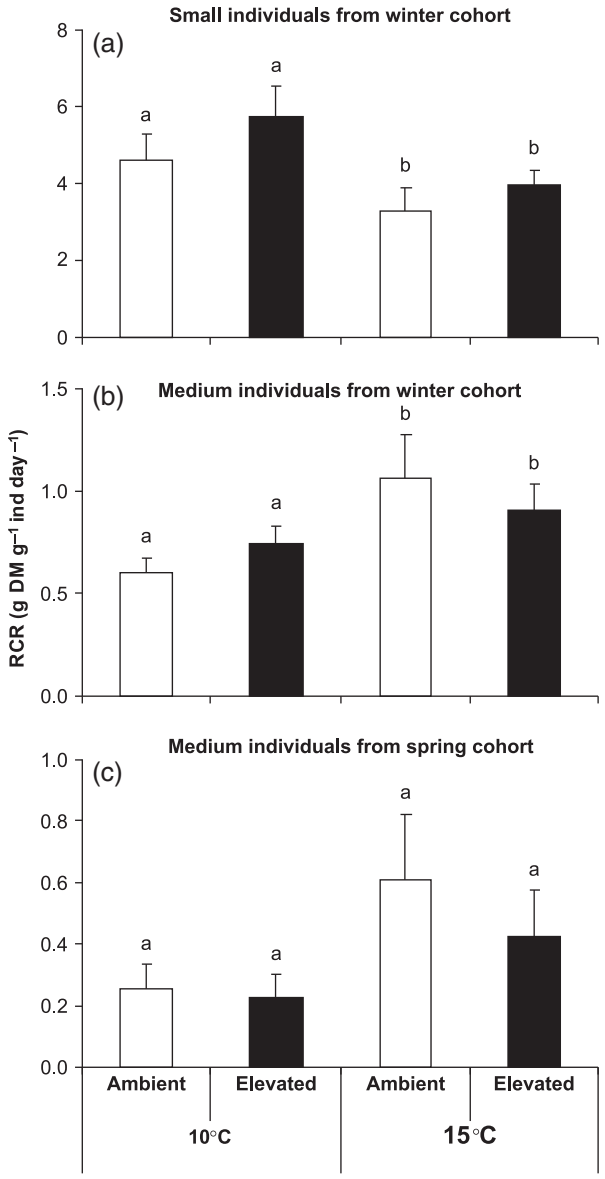

Fig. 2 Relative consumption rate (RCR; average ± SE; $n = 20$) by *Sericostoma vittatum* individuals kept at 10 and 15 °C, and provided with birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm). (a) Small individuals from winter cohort, (b) medium individuals from winter cohort and (c) medium individuals from spring cohort. Different letters indicate significant differences among treatments (two-way analysis of variance, $P < 0.05$).

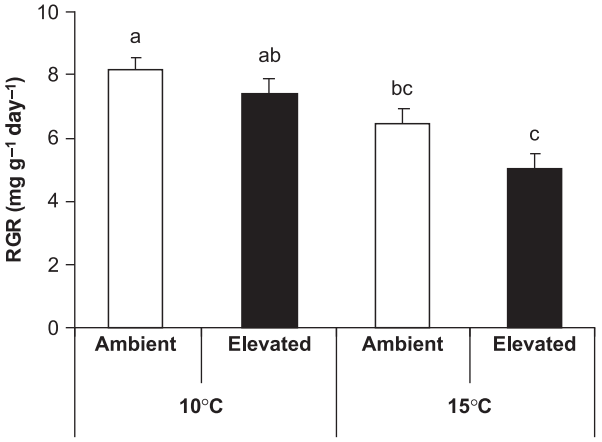

Fig. 3 Relative growth rate (RGR; average ± SE; $n = 20$) of *Sericostoma vittatum* individuals kept at 10 and 15 °C, and provided with birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm) for 28 days. Different letters indicate significant differences among treatments (two-way analysis of variance, $P < 0.05$).

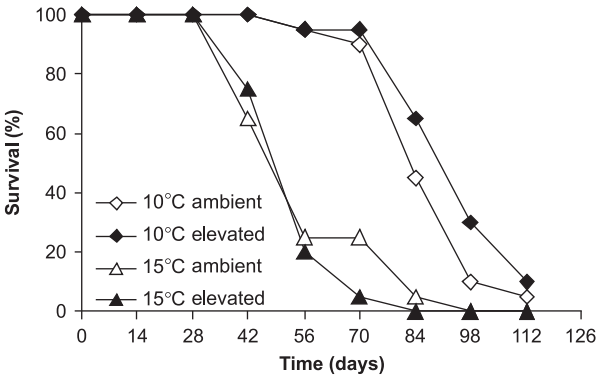

Fig. 4 Survival (%) of *Sericostoma vittatum* individuals ($n = 20$) kept at 10 and 15 °C, and provided with birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm) for a maximum period of 112 days.

reflex of CO_2-induced increases in atmospheric temperature; Eaton & Scheller, 1996; IPCC 2007) and decreased litter quality (as a result of CO_2-induced decreases in nutrient concentration and increases in structural and secondary compounds concentrations on leaves; Lindroth, 1996; Cotrufo *et al*., 1998a; Norby *et al*., 2001; Stiling & Cornelissen, 2007). However, the evaluation of how these two combined factors will affect aquatic detritivores performance is still lacking. Because detritivores are key components of aquatic food webs in woodland streams, where they are major players in the decomposition of litter provided by the riparian vegetation (Vannote *et al*., 1980; Cummins *et al*., 1989), this assessment is urgent. The way detritivores are affected by these interacting factors is therefore expected to be important for ecosystem functioning.

We carried out laboratory experiments to assess the performance of the freshwater detritivore *S. vittatum* under a future climate scenario, whereby temperate streams in autumn are expected to be ~5 °C warmer and provided with litter of poorer nutritional quality

than at present (Eaton & Scheller, 1996; Norby *et al*., 2000, 2001; Cotrufo *et al*., 2005).

The birch (*B. pendula*) litter we used in these experiments was by itself of poor nutrient quality (initial ambient litter: C/N = 33, C/P = 248). Growing leaves under elevated CO_2 atmosphere resulted in a decrease in litter quality; however, the difference between ambient and elevated litter was limited to a reduction of 30% in phosphorus concentration. This could have resulted from a differential allocation of phosphorus within the plant; under elevated CO_2 atmosphere birch trees could have invested more carbon into fine roots than leaves (Rey & Jarvis, 1997), which would have required higher concentration of phosphorus and could have diverted it from leaves. Decreases in litter phosphorus concentration under growth in an elevated CO_2 atmosphere have been observed previously (Cotrufo *et al*., 1999). Because we limited the assessment of structural and secondary compounds to lignin and phenolic concentrations, respectively, we cannot assure absence of wider differences between litter types. Previous studies on the effects of elevated CO_2 concentrations on birch (*B. pendula*) leaf quality reported stronger effects of increased atmospheric CO_2 than those found here, although these could in part result from the higher CO_2 concentrations tested and different methods used [600–730 ppm in solardomes (Cotrufo & Ineson, 1996) and open top chambers (Oksanen *et al*., 2005)]. Experiments using FACE technology (our case) usually report less strong effects of increased CO_2 concentration on plant responses, including leaf chemical composition, than those using enclosures which emphasises the potential of artefacts associated with enclosures (Norby *et al*., 2001; Long *et al*., 2004; Ainsworth & Long, 2005). Although changes in nitrogen and structural and secondary compounds concentrations are most frequently observed (Lindroth, 1996; Stiling & Cornelissen, 2007), effects of elevated CO_2 atmosphere on leaf quality are species specific (Hättenschwiller & Schafellner, 2004; Rier *et al*., 2005) and dependent on other environmental factors (e.g. nutrient availability, light limitation; Cotrufo & Ineson, 1996; Tricker *et al*., 2004).

Contrary to terrestrial systems where soil detritivores are promptly exposed to initial litter quality, in aquatic

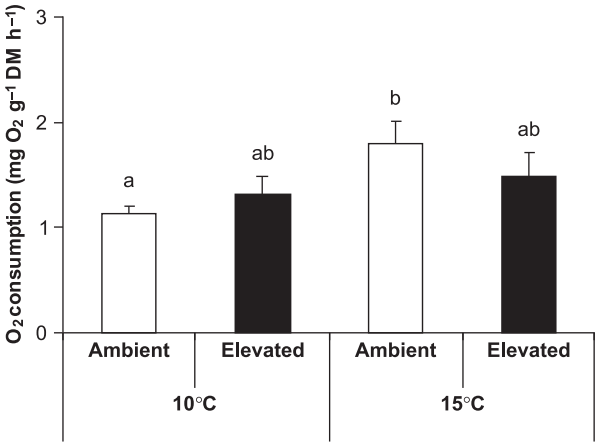

Fig. 5 Oxygen consumption (average ± SE; $n = 5$) by *Sericostoma vittatum* individuals kept at 10 and 15 °C and fed *ad libitum* birch litter grown under ambient and elevated CO_2 atmosphere (380 vs. 580 ppm) for 1week. Different letters indicate significant differences among treatments (two-way analysis of variance, $P < 0.05$).

Table 3 Elemental composition and ratios (average ± SE) of birch litter grown under ambient and elevated CO_2 atmospheres (380 vs. 580 ppm), after incubation in the stream for 2 weeks ($n = 3$) and of detritivores ($n = 5$) kept for 1 week at 10 and 15 °C and fed *ad libitum* conditioned birch litter

Temperature (°C)	Litter type	Elemental composition*			Elemental ratios			Elemental imbalance		
		N (%DM)	P (%DM)	C (%DM)	C/N	C/P	N/P	C/N	C/P	N/P
Conditioned litter										
	Ambient	1.04 ± 0.01[a]	0.10 ± 0.004[a]	51.30 ± 1.03[a]	49.08 ± 0.52[a]	504.90 ± 18.53[a]	10.29 ± 0.40[a]			
	Elevated	1.65 ± 0.24[a]	0.07 ± 0.003[b]	49.31 ± 0.24[a]	31.11 ± 4.23[b]	708.60 ± 30.25[b]	23.43 ± 2.40[b]			
Detritivore										
10 °C	Ambient	9.33 ± 0.17[a]	0.76 ± 0.06[a]	48.94 ± 0.79[ab]	5.25 ± 0.03[a]	63.32 ± 5.17[a]	12.05 ± 0.93[a]	9.35	7.97	0.85
	Elevated	9.72 ± 0.18[ab]	0.85 ± 0.01[a]	50.60 ± 0.59[b]	5.21 ± 0.10[a]	59.69 ± 0.68[a]	11.47 ± 0.24[a]	5.97	11.87	2.04
15 °C	Ambient	10.04 ± 0.14[b]	0.74 ± 0.04[a]	49.70 ± 0.77[ab]	5.96 ± 0.13[ab]	67.52 ± 4.29[a]	13.58 ± 0.60[a]	9.90	7.48	1.32
	Elevated	10.07 ± 0.16[b]	0.69 ± 0.12[a]	47.55 ± 0.88[a]	4.72 ± 0.06[b]	64.23 ± 8.36[a]	13.59 ± 1.70[a]	6.59	11.03	1.72

Elemental imbalances were calculated as the ratio between elemental ratios of litter and its consumer. Different letters indicate significant differences among treatments (*t*-tests (conditioned litter) and two-way analysis of variances (detritivore), $P < 0.05$).
*Elemental composition of conditioned litter slightly differed from that in Table 1 because only a subset of the litter was used here.
P, phosphorus; N, nitrogen; C, carbon; DM, dry matter.

systems litter undergo loss of water soluble compounds before being colonized by aquatic biota (Gessner *et al*., 1999). This could mitigate the potentially negative effect of CO_2-induced decreases in litter quality on aquatic invertebrates and litter mass loss. Here, although leaching resulted in decreases of 50% in phosphorus and 53–67% in phenols concentrations, which was anticipated (Nykvist, 1959; Canhoto & Graça, 1996; Ferreira *et al*., 2006c), decreases were similar for both litter types. Still, the incubation also resulted in a tendency for a higher difference in nitrogen concentration between litter types, as nitrogen percentage (i) increased in elevated litter, perhaps due to fungal biomass accrual and (ii) decreased in ambient litter, probably due to leaching (Nykvist, 1959). Decreases in litter nitrogen concentration during the first stages of decomposition have been observed (Canhoto & Graça, 1996; Molinero *et al*., 1996; Robinson & Jolidon, 2005), however, this is uncommon given that fungal colonization generally leads to nitrogen immobilization (Gulis & Suberkropp, 2003). In our case, the lower fungal colonization of ambient litter might not have compensated for nitrogen leaching, as it might have happened in elevated litter, which translated into significantly lower C/N ratio for this litter type. In recent studies, leaching and initial microbial colonization have been observed not to affect (Tuchman *et al*., 2002, 2003a; Rier *et al*., 2005) or to eliminate (Rier *et al*., 2002, 2005) initial chemical differences between ambient and elevated litter. Litter mass loss over 14 days was similar between litter types (8–10%), which might be explained by the short incubation period, even though recent studies have shown that CO_2 effects on litter decomposition are detectable mostly on early decomposition stages (<30 days) – heavy colonization by microbes after the leaching period mitigates differences between litter types (Rier *et al*., 2002; Tuchman *et al*., 2003b). The twofold higher fungal biomass concentration on elevated litter, and the absence of significant differences in all other microbial variables between litter types, contradict recent results from stream incubation experiments performed by other authors where microbial respiration and bacterial biomass have been most depressed by CO_2-induced decreases in litter quality, while fungal biomass has been insensitive to these changes (Rier *et al*., 2002, 2005; Tuchman *et al*., 2002). In our case, the higher fungal biomass built up on elevated litter might have resulted from its slightly lower phenol concentration compared with ambient litter (Canhoto & Graça, 1999). Elevated litter could also have higher concentration of labile carbon compounds (e.g. sugars, not measured) (Stiling & Cornelissen, 2007), which might have enhanced fungal colonization. However, given the reduced number of studies addressing this question, differences on how the microbial community responds to CO_2-induced changes of litter quality might at this moment be considered litter species specific.

The use of air dried litter instead of freshly fallen litter may have affected litter nutrient dynamics and microbial colonization, as leaching of soluble compounds is usually more intense in dried litter (Gessner, 1991), which allows faster colonization of this litter by microbes (Bärlocher, 1991). However, because lateral litter inputs to streams might constitute up to 30% of total litter inputs (Molinero & Pozo, 2004), and it can be assumed that this litter can be air dried before being submerged, the use of air dried litter in this study remains ecologically sound.

Differences in chemical quality between ambient and elevated litter affected invertebrate feeding preferences at 10 °C, but not at 15 °C, which seems to indicate that water temperature affects invertebrate perception of litter quality. However, contrary to anticipated, invertebrates preferred elevated litter over ambient litter (Cotrufo *et al*., 1998b), which might indicate that invertebrates prefer litter with higher fungal biomass and lower C/N ratio than those with higher phosphorus concentration. Litter nitrogen concentration is usually the factor determining invertebrate litter choice because it is usually the limiting nutrient for primary consumers (Evans-White *et al*., 2005).

Consumption rates did not significantly differ between litter types, contrary to what was reported for the stream detritivore *Tipula abdominalis* who had a 50% slower relative consumption rate when fed elevated litter than when fed ambient litter (Tuchman *et al*., 2002). Also, larval stage did not influence the effect of litter quality (review by Bezemer & Jones, 1998), either because early instars are not more sensitive than later instars to impoverishment of litter quality or because the difference in quality between litter types was not large enough. However, water temperature differentially affected consumption rate by each animal group. Small individuals had higher consumption rate at lower temperature while medium individuals had higher consumption at higher temperature. This can partially be explained by a trade off between optimal temperature, which for winter organisms is ~10 °C, and higher energetic requirements at 15 °C, that affect larger individuals more than small ones. However, González & Graça, (2003) reported increased consumption rates for *S. vittatum* with increases in temperature, within the range used here, that were independent of animal size. Also, while medium size winter individuals had higher consumption rates at 15 °C, spring individuals tended to feed at similar rates at both temperatures, which partially agree with the prediction that increased temperature will strongly affect individuals from colder waters (Braune *et al*., 2008).

RGRs of invertebrates fed a given litter type were 14–38% faster at 10 than at 15 °C, which contradicts the general temperature–size rule, predicting faster initial growth rates at higher temperature (Atkinson, 1995; Atkinson & Sibly, 1997). However, in our case, individuals were feeding on an overall poor-quality litter that might not have allowed them to fulfil their nutritional requirements at 15 °C and support a faster initial growth rate there. The higher body mass at the lowest temperature was, however, predicted by the general temperature–size rule. Within a non stressful temperature range, aquatic invertebrates have been reported to have higher body mass when kept at lower than at higher temperature (Rempel & Carter 1987; Atkinson 1995; Hogg *et al*., 1995; Hogg & Williams 1996; Blanckenhorn 1997; Turner & Williams 2005). There was also a tendency for higher differences in growth rates between litter types at 15 °C (20%) than at 10 °C (10%). This was expected since at higher temperatures polymerase catalysis is stimulated and consequent increases in DNA synthesis require high amounts of phosphorus (Atkinson & Sibly 1997), which existed in lower concentration in elevated litter. However, the (nonsignificant) differences in growth rate between individuals fed elevated litter and those fed ambient litter, which were attributed to the lower phosphorus concentration on elevated litter that might have limited nucleic acid synthesis necessary for growth (Cross *et al*., 2005), fall behind those found by Tuchman *et al*. (2002) who reported a 12 times slower growth rate for a crane fly larvae and 25–78% slower developmental rates for mosquito larvae (Tuchman *et al*., 2003a) fed elevated than for those fed ambient aspen leaves. The differences in effect size between ours and other studies are most likely explained by the smaller differences in quality between litter types in our study. However, individuals submitted to the simultaneous increase in water temperature and altered in litter quality (future climate scenario) had a 38% reduction in growth rate and a 13% reduction in mass gained when compared with individuals at 10 °C fed ambient litter (present scenario), which is higher than the reduction predicted from both factors considered alone (22% and 10%, respectively), indicating synergistic effects between factors on invertebrates' performance. The effects of multiple global change factors on terrestrial, freshwater and intertidal invertebrates can not be predicted from their individual effects, i.e. they are not additive (Williams *et al*., 2003; Przeslawski *et al*., 2005; Kashian *et al*., 2007; David & Gillon 2009).

The high mortality, and the absence of differences in survival between both litter types, seems to support the conclusion that birch litter is by itself a poor resource. Also, *B. pendula* is quite rich in essential oils (Demirci *et al*., 2004), which are known to be toxic to invertebrates (Siramon *et al*., 2009). However, the earlier and initially faster mortality at 15 °C than at 10 °C seem to indicate that increases in temperature will reduce the ability of invertebrates to survive on poor nutrient diets (Hanson *et al*., 1983). If increased CO_2 concentrations results in a stronger decrease of litter quality, invertebrates under future climate scenarios will have lower survival (Tuchman *et al*., 2003a; Adams *et al*., 2005).

Elemental imbalances between litter and the detritivore were high, and similar considering element concentrations and C/nutrient ratios (6–12 fold difference in both cases), and were within values from previous reports (Evans-White *et al*., 2005; Hladyz *et al*., 2009). Our data further support the notion of nutrient-poor quality of birch litter, and conditioning it for 2 weeks did not greatly enhance its nutritional quality (even though substantially decreasing phenols concentration, which is a requirement for fungal growth; Canhoto & Graça 1999). Invertebrates were homoeostatic regarding nutrient composition within a given temperature, which can be attributed to differential acquisition, incorporation and release of chemical elements (Frost *et al*., 2005). However, variation in temperature induced deviations from strict homoeostasis that were dependent on food quality as indicated by the increased nitrogen concentration for individuals fed ambient litter, and reduced carbon concentration and C/N ratio for individuals fed elevated litter when temperature was increased by 5 °C. Changes in nutritional quality of food resources have been previously reported to induce changes in body elemental composition and C/nutrient ratios of invertebrates (Frost & Elser 2002), while increases in water temperature by 4.5 °C above ambient did not result in changes in elemental composition of invertebrates in mesocosms mimicking shallow pond environments (Ventura *et al*., 2008). Nevertheless, when individuals were submitted to a simulation of future climate scenario (changes in temperature and litter quality) there was an 8% increase in body nitrogen concentration and a 10% decrease in body C/N ratio, which might lead to increases in instream nitrogen retention and body nutritional quality for predators (Vanni 2002). Potential alterations on stream nutrient dynamics caused by CO_2-induced changes on litter quality, through its effect on invertebrates, were already suggested (Frost & Tuchman 2005). It seems that higher fungal colonization of elevated litter and consequently a slight increase in nitrogen concentration offset the potential negative effect of its lower phosphorus concentration on animal body quality.

In conclusion, the performance of our stream detritivore was more affected by increases in water temperature than by decreases in litter phosphorus concentration. Higher microbial colonization (increased

fungal biomass and nitrogen concentration) of elevated litter was an important determinant of invertebrate preference for this litter as it offset its lower phosphorus concentration. However, in a future warming scenario the simultaneous increase in water temperature and decrease in litter quality might affect detritivores performance more than predicted from the effects of both factors considered individually. The CO_2-induced negative effects on invertebrate performance observed in this study might, however, be considered conservative given the quite small reduction in quality of elevated litter. Nevertheless, this study shows that even small reductions in litter quality induced by increases in atmospheric CO_2 concentration will exacerbate the negative effect of increased water temperature on invertebrate ecology, with consequences at the population level and ecosystem functioning. Changes in consumption rates, lower growth rates, higher mortality and changes in body composition under future climate scenario can result in a decrease in populations that will directly impact the processes in which these organisms are involved (e.g. litter decomposition, nutrient cycling).

As the increase in CO_2 concentration has climatic impacts that cannot be reduced without a concerted global action, something that is reasonably achievable is the protection of naturally diverse riparian forests and the recovery of degraded ones by reforestation with native diverse vegetation. This would ensure high diversity of leaf litter in the stream benthos, which would increase the probability of high-quality litter given that CO_2 effects on litter quality are species dependent, and would give detritivores feeding choices (Williams *et al*., 1997; Hättenschwiller *et al*., 1999; Peters *et al*., 2000).

Acknowledgements

Elsa Rodrigues and Cristina Docal are thanked for the HPLC and IRMS analysis, and for providing technical help during the growth experiment. The suggestions made by Professor Manuel A. S. Graça and by four anonymous reviewers to an earlier version of the ms were most appreciated. Financial support granted by the Portuguese Foundation for Science and Technology (FCT) to V. F. (reference POCTI2010/SFRH/BPD/34368/2006) and A. L. G. (reference SFRH/BD/47089/2008) and by the IMAR are gratefully acknowledged. This manuscript constitutes an output of the project PTDC/CLI/67180/2006, financed by the FCT. The development of the Bangor FACE site was funded by SRIF. We thank the Forestry Commission Wales and CIRRE for support for the running costs.

References

Adams JA, Tuchman NC, Moore PA (2005) Effects of CO_2-altered detritus on growth and chemically mediated decisions in crayfish (*Procambarus clarkii*). *Journal of the North American Benthological Society*, **24**, 330–345.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosysthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, **165**, 351–371.

Asshoff R, Hättenschwiler S (2005) Growth and reproduction of the alpine grasshopper *Miramella alpina* feeding on CO_2-enriched dwarf shrubs at treeline. *Oecologia*, **142**, 191–201.

Atkinson D (1995) Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. *Journal of Thermal Biology*, **20**, 61–74.

Atkinson D, Sibly RM (1997) Why are organisms usually bigger in colder environments? Making sense of a life puzzle. *Trends in Ecology and Evolution*, **12**, 235–239.

Azevedo-Pereira HVS, Graça MAS, González JM (2006) Life history of *Lepidostoma hirtum* in an Iberian stream and its role in organic matter processing. *Hydrobiologia*, **559**, 183–192.

Bärlocher F (1991) Fungal colonization of fresh and dried leaves in the River Teign (Devon, England). *Nova Hedwigia*, **52**, 349–357.

Bezemer TM, Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. *Oikos*, **82**, 212–222.

Blanckenhorn WU (1997) Altitudinal life history variation in the dung flies *Scathophaga stercoraria* and *Sepsis cynipsea*. *Oecologia*, **109**, 342–352.

Braune E, Richter O, Sondgerath D, Suhling F (2008) Voltinism flexibility of a riverine dragonfly along thermal gradients. *Global Change Biology*, **14**, 1–13.

Brown JH, Gilloly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Canhoto C, Graça MAS (1995) Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis*. *Freshwater Biology*, **34**, 209–214.

Canhoto C, Graça MAS (1996) Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. *Hydrobiologia*, **333**, 79–85.

Canhoto C, Graça MAS (1999) Leaf barriers to fungal colonization and shredder consumption of decomposing *Eucalyptus globulus*. *Microbial Ecology*, **37**, 163–172.

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

Cotrufo MF, De Angelis P, Polle A (2005) Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE). *Global Change Biology*, **11**, 971–982.

Cotrufo MF, Ineson P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. *Oecologia*, **106**, 525–530.

Cotrufo MF, Ineson P, Scoot A (1998a) Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology*, **4**, 43–54.

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. *Functional Ecology*, **13**, 343–351.

Cross WF, Benstead JP, Frost PC, Thomas SA (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**, 1895–1912.

Cummins KW, Wilzbach MA, Gates DM, Perry JB, Taliaferro WB (1989) Shredders and riparian vegetation. *BioScience*, **39**, 24–30.

David J-F, Gillon D (2009) Combined effects of elevated temperatures and reduced leaf litter quality on the life-history parameters of a saprophagous macroarthropod. *Global Change Biology*, **25**, 156–165.

Demirci B, Paper DH, Demirci F, Baser KHC, Franz G (2004) Essential oil of *Betula pendula* Roth. Buds. *Evidence-Based Complementary and Alternative Medicine*, **1**, 301–303.

Eaton JG, Scheller RM (1996) Effects of climate warming on fish thermal habitat in streams on the United States. *Limnology and Oceanography*, **41**, 1109–1115.

Evans-White MA, Stelzer RS, Lamberti GA (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biology*, **50**, 1786–1799.

Fajer ED (1989) The effects of enriched CO_2 atmospheres on plant-insect herbivore interactions: growth responses of larvae of the specialist butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae). *Oecologia*, **81**, 514–520.

Ferreira V, Elosegi A, Gulis V, Pozo J, Graça MAS (2006c) Eucalyptus plantations affect fungal communities associated with leaf litter decomposition in Iberian streams. *Archiv für Hydrobiologie*, **166**, 467–490.

Ferreira V, Graça MAS, de Lima JLMP, Gomes R (2006b) Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. *Archiv für Hydrobiologie*, **165**, 493–513.

Ferreira V, Gulis V, Graça MAS (2006a) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, **149**, 718–729.

Frost PC, Elser JJ (2002) Growth responses of littoral mayflies to the phosphorus content of their food. *Ecology Letters*, **5**, 232–240.

Frost PC, Evans-White MA, Finkel ZV, Jensen TC, Matzek V (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos*, **109**, 18–28.

Frost PC, Tuchman NC (2005) Nutrient release rates and ratios by two stream detritivores fed leaf litter grown under elevated atmospheric CO_2. *Archiv für Hydrobiologie*, **163**, 463–477.

Gessner MO (1991) Differences in processing dynamics of fresh and dried leaf litter in a stream ecosystem. *Freshwater Biology*, **26**, 387–398.

Gessner MO, Chauvet E (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied Environmental Microbiology*, **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**, 377–384.

Goering HK, Van Soest PJ (1970) *Forage Fiber Analysis (Apparatus, Reagents, Procedures and some Applications). USDA Agricultural Handbook No. 379*, US Gov Office, Washington, DC, USA.

González JM, Graça MAS (2003) Conversion of leaf litter to secondary production by a shredding caddis-fly. *Freshwater Biology*, **48**, 1578–1592.

Graça MAS, Bärlocher F, Gessner MO (2005) *Methods to Study Litter Decomposition. A Practical Guide*. Springer, the Netherlands.

Graça MAS, Cressa C, Gessner MO, Feio MJ, Callies KA, Barrios C (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, **46**, 947–957.

Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123–134.

Hanson BJ, Cummins KW, Cargill AS, Lowry RR (1983) Dietary effects on lipid and fatty acid composition of *Clistoronia magnifica* (Trichoptera: Limnephilidae). *Freshwater Invertebrate Biology*, **2**, 2–15.

Hättenschwiller S, Bühler S, Körner C (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. *Oikos*, **85**, 271–281.

Hättenschwiller S, Schafellner C (2004) Gypsy moth feeding in the canopy of a CO_2-enriched mature forest. *Global Change Biology*, **10**, 1899–1908.

Hieber M, Gessner MO (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–1038.

Hladyz S, Gessner MO, Giller PS, Pozo J, Woodward G (2009) Resource quality and stoichiometric constrains on stream ecosystem functioning. *Freshwater Biology*, **54**, 957–970.

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

Hogg ID, Williams DD, Eadie JM, Butt AS (1995) The consequences of global warming for stream invertebrates: a field simulation. *Journal of Thermal Biology*, **20**, 199–206.

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate change 2007: the physical science basis. In: *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmantal Panel on Climate Change* (eds Solomon SQD, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL), pp. 749–845. Cambridge University Press, Cambridge, UK.

Kashian DR, Zuelling RE, Mitchell KA, Clements WH (2007) The cost of tolerance: sensitivity if stream benthic communities to UV-B and metals. *Ecological Applications*, **17**, 365–375.

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. *Journal of the North American Benthological Society*, **26**, 663–672.

Lecerf A, Dobson M, Dang CK, Chauvet E (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. *Oecologia*, **146**, 432–442.

Lindroth RL (1996) Consequences of elevated atmospheric CO_2 to forest insects. In: *Carbon Dioxide, Populations, and Communities* (eds Körner C, Bazzaz FA), pp. 347–361. Academic Press Inc., San Diego, CA, USA.

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plant FACE the future. *Annual Review of Plant Biology*, **55**, 591–628.

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

Molinero J, Pozo J (2004) Impacts of a eucalyptus (*Eucalyptus globulos* Labill.) plantation on the nutrient content and dynamics of coarse particulate organic matter (CPOM) in a small stream. *Hydrobiologia*, **528**, 143–165.

Molinero J, Pozo J, González E (1996) Litter breakdown in streams of the Agüera catchment: influence of dissolved nutrients and land use. *Freshwater Biology*, **36**, 745–756.

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.

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.

Nykvist N (1959) Leaching and decomposition of litter I. Experiments on leaf litter of *Fraxinus excelsior*. *Oikos*, **10**, 190–211.

Oksanen E, Riikonen J, Kaakinen S, Holopainen T, Vapaavuori E (2005) Structural characteristics and chemical composition of birch (*Betula pendula*) leaves are modified by increasing CO_2 and ozone. *Global Change Biology*, **11**, 732–748.

Ostrofsky ML (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society*, **16**, 750–759.

Pascoal C, Marvanová L, Cássio F (2005) Aquatic hyphomycete diversity in streams of Northwest Portugal. *Fungal Diversity*, **19**, 109–128.

Peters HA, Baur B, Bazzaz F, Körner C (2000) Consumption rates and food preferences of slugs in a calcareous grassland under current and future CO_2 conditions. *Oecologia*, **125**, 72–81.

Przeslawski R, Davis AR, Benkendorff K (2005) Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology*, **11**, 515–522.

Rempel RS, Carter JCH (1987) Temperature influences on adult size, development, and reproductive potential of aquatic diptera. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 1743–1752.

Rey A, Jarvis PG (1997) Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO2 exposure. *Annals of Botany*, **80**, 809–816.

Richardson JS, Shaugnhessy CR, Harrison PG (2004) Litter breakdown and invertebrate association with thee types of leaves in a temperate rainforest stream. *Archiv für Hydrobiologie*, **159**, 309–325.

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. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 185–194.

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. *Journal of the North American Benthological Society*, **21**, 16–27.

Robinson CT, Jolidon C (2005) Leaf breakdown and the ecosystem functioning of alpine streams. *Journal of the North American Benthological Society*, **24**, 495–507.

Siefert J, Mutz M (2001) Processing of leaf litter in acid waters of the post-mining landscape in Lusatia, Germany. *Ecological Engineering*, **17**, 297–306.

Siramon P, Ohtani Y, Ichiura H (2009) Biological performance of *Eucalyptus camaldulensis* leaf oils from Thailand against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of Wood Science*, **55**, 41–46.

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 Biology*, **13**, 1823–1842.

Sweeney BW, Vannote RL (1984) Influence of food quality and temperature on life history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. *Freshwater Biology*, **14**, 621–630.

Sweeney BW, Vannote RL, Dodds PJ (1986) The relative importance of temperature and diet to larval development and adult size of the winter stonefly, *Soyedina carolinensis* (Plecoptera: Nemouridae). *Freshwater Biology*, **16**, 39–48.

Taylor G, Tallis MJ, Giardina CP *et al*. (2008) Future atmospheric CO_2 leads to delayed autumnal senescence. *Global Change Biology*, **14**, 264–275.

Tricker PJ, Calfapietra C, Kuzminsky E *et al*. (2004) Long-term acclimation of leaf production, development, longevity and quality following 3 yr exposure to free-air CO_2 enrichment during canopy closure in *Populus*. *New Phytologist*, **162**, 413–426.

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. *Freshwater Biology*, **48**, 1432–1439.

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.

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 Biology*, **8**, 163–170.

Turner D, Williams DD (2005) Sexual dimorphism and the influence of artificial elevated temperatures on body size in the imago *Nemoura trispinosa* (Plecoptera: Nemouridae). *Aquatic Insects*, **27**, 243–252.

Vanni MJ (2002) Nutrients cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Ventura M, Liboriussen L, Lauridsen T, Sondergaard M, Sondergarrd M, Jeppesen E (2008) Effects of increased temperature and nutrient enrichment on the stoichiometry of primary producers and consumers in temperate shallow lakes. *Freshwater Biology*, **53**, 1434–1452.

Williams RS, Lincoln DE, Norby RJ (1998) Leaf age effects of elevated CO_2-grown white oak leaves on spring-feeding lepidopterans. *Global Change Biology*, **4**, 235–246.

Williams RS, Lincoln DE, Norby RJ (2003) Development of gypsy moth larvae feeding on red maple saplings at elevated CO_2 and temperature. *Oecologia*, **137**, 114–122.

Williams RS, Lincoln DE, Thomas RB (1994) Loblolly pine grown under elevated CO_2 affects early instar pine sawfly performance. *Oecologia*, **98**, 64–71.

Williams RS, Lincoln DE, Thomas RB (1997) Effects of elevated CO_2-grown loblolly pine needles on the growth, consumption, development, and pupal weight of red-headed pine sawfly larvae reared within open-topped chambers. *Global Change Biology*, **3**, 501–511.

Williams RS, Norby RJ, Lincoln DE (2000) Effects of elevated CO_2 and temperature-grown red and sugar maple on gypsy moth performance. *Global Change Biology*, **6**, 685–695.

Young JC (1995) Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids. *Journal of Agriculture and Food Chemistry*, **43**, 2904–2910.

Zar JH (1999) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ.