Positive effect of shredders on microbial biomass and decomposition in stream microcosms

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SUMMARY

1. Animals play a major role in nutrient cycling via excretory processes. Although the positive indirect effects of grazers on periphytic algae are well understood, little is known about top-down effects on decomposers of shredders living on leaf litter.
2. Nutrient cycling by shredders in oligotrophic forest streams may be important for the microbial-detritus compartment at very small spatial scales (i.e. within the leaf packs in which shredders feed). We hypothesised that insect excretion may cause local nutrient enrichment, so that microorganism growth on leaves is stimulated.
3. We first tested the effect of increasing concentration of ammonium (+10, +20 and +40 μg NH_4^+ L^{-1}) on fungal and bacterial biomass on leaf litter in a laboratory experiment. Then we performed two experiments to test the effect of the presence and feeding activity of shredder larvae. We used two species belonging to the trichopteran family Sericostomatidae: the Palaearctic *Sericostoma vittatum* and the Neotropical *Myothrichia murina*, to test the effect of these shredders on fungal and bacterial biomass and decomposition on leaves of *Quercus robur* and *Nothofagus pumilio*, respectively. All experiments were run in water with low ammonium concentrations (2.4 ± 0.34 to 14.47 ± 0.95 μg NH_4^+ L^{-1}).
4. After 5 days of incubation, NH_4 concentrations were reduced to near-ambient streamwater concentrations in all treatments with leaves. Fungal biomass was positively affected by increased ammonium concentration. On the other hand, bacteria abundance was similar in all treatments, both in terms of abundance (bacteria cells mg^{-1} leaf DW) and biomass. However, there was a tendency towards larger mean cell size in treatments with 20 μg NH_4 L^{-1}.
5. In the experiment with *S. vittatum*, fungal biomass in the treatment with insects was more than twice that in the control after 15 days. Bacteria were not detected in treatments with insects, where hyphae were abundant, but they were abundant in treatments without larvae. In the decomposition experiment run with *M. murina*, leaf-mass loss was significantly higher in treatments with larvae than in controls.
6. Our hypothesis of a positive effect of shredders on fungal biomass and decomposition was demonstrated. Insect excretion caused ammonium concentration to increase in the microcosms, contributing to microbial N uptake in leaf substrata, which resulted in structural and functional changes in community attributes. The positive effect of detritivores on microbes has been mostly neglected in stream nutrient-cycling models; our findings suggest that this phenomenon may be of greater importance than expected in stream nutrient budgets.

Keywords: ammonium, bacteria, fungi, nutrient cycling, streams

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Introduction

Nutrient cycling involves multiple ecosystem processes such as the chemical transformation of one substance to another, and the flux of these compounds between organisms, habitats and ecosystems (Lavelle *et al*., 2005). Although microbes (bacteria and fungi) are key agents of this process in terrestrial, marine and freshwater ecosystems (Coleman, Reid & Cole, 1983; Fenchel, 1988; Tank *et al*., 2000), animals can also be important in cycling nutrients (Grimm, 1988; McNaughton, Banyikwa & McNaughton, 1997; Vanni, 2002). Via excretory processes, animals supply nitrogen and phosphorus at rates comparable to those released from major nutrient sources (Vanni, 2002; McManamay *et al*., 2011), although excretion is just a fraction of the whole cycling process. In terrestrial systems, those elements are mostly taken up by decomposers close to where they are released, while in aquatic systems, once the elements are released, they are dissolved in water and transported downstream (depending on the tightness of nutrient spiralling in the system, Newbold *et al*., 1983). In spite of this downstream loading, some studies have shown that nutrients released by grazers may have positive indirect effects on periphytic algae *in situ* (Rosemond, Mulholland & Elwood, 1993; Steinman, 1996; Liess & Hillebrand, 2004). In contrast, the effects of detritivore excretion on decomposers remain poorly understood, although some evidence indicates that detritivorous fish may exert a positive bottom-up effect on decomposers by enhancing nutrient loading in the water column (Hargrave, Hamontree & Gary, 2010). We argue that, similarly, nutrients released by detritivores feeding within detritus should favour decomposers (fungi and bacteria) colonising that litter.

Four ecological interactions can be established between detritivores and decomposers: (i) predator–prey interaction, where the former consume the latter; (ii) competition for dead organic matter; (iii) commensalism, because microbes soften leaf tissues and microbial enzymes may be used inside insect guts and (iv) mutualism, because the partial digestion by microbes benefits insects, and insects damage leaf epidermis, enabling fungal invasion to the inner layers of the leaf tissue (modified from Hildrew, 1996). Perhaps the least explored among the ecological trade-offs between aquatic detritivores and fungi is commensalism in which microorganisms take up nutrients released by detritivores (Canhoto & Graça, 2008). So, while bottom-up control of consumer populations is evident, the impact of detritivores on microbes, other than predation, remains little explored (Canhoto & Graça, 2008). Nutrient cycling by shredders may be especially important for the microbial-detritus compartment in oligotrophic forest streams at very small spatial scales (i.e. within the leaf packs in which they feed; Balseiro & Albariño, 2006).

Decomposer microorganisms usually use organic matter as a source of carbon and nutrients. However, detritus has very high C : N and C : P ratios compared with bacterial and fungal biomass (Cross *et al*., 2005), making microorganisms additionally dependent on dissolved inorganic nutrients (Stelzer, Heffernan & Likens, 2003). This is supported by evidence that experimental nutrient enrichment may increase fungal biomass (Gulis & Suberkropp, 2003; Ferreira, Gulis & Graça, 2006; Chung & Suberkropp, 2008) and decomposition rates (Robinson & Gessner, 2000; Benstead *et al*., 2009), enhancing litter quality for invertebrates (Greenwood *et al*., 2007). If invertebrates inhabiting leaf packs enhance fungal biomass, then fungal biomass per unit remaining leaf-litter mass would be expected to be higher in patches or leaves thoroughly colonised by detritivores than in those that are sparsely colonised. This effect could be particularly apparent in leaf litter conditioned in coarse-mesh bags, compared with fine-mesh bags where invertebrates are excluded. However, results on the effect of shredders on fungi have been contradictory. While shredders may have indirect negative effects on fungal species richness (Bärlocher, 1980) and sporulation rates (Bärlocher, 1982), conidial production may be higher in coarse-mesh bags, which may indicate that insects stimulate fungal reproduction (Ferreira & Graça, 2006). And although Chung & Suberkropp (2008) found no significant differences in fungal biomass between the two types of bags, recent evidence indicates that degradative enzymes may show higher activity in leaf litter conditioned in more coarsely meshed bags (J. Morales, pers. comm.).

In this study, we assessed the importance of the presence of shredders for fungal and bacterial biomass and leaf-litter decomposition. We hypothesised that insect excretion may cause local nutrient enrichment, so that microbial growth on the leaves and decomposition are stimulated. Hence, we carried out two laboratory experiments to test microbial growth and decomposition rates in the presence and absence of shredders.

Methods

Our hypothesis was based on the premise that elevated ammonium concentration has positive effects on fungal and bacterial biomass. We tested this premise by a first experiment in which we incubated leaf litter under different ammonium concentrations. Subsequently, we

performed two experiments to test the effect of the presence and feeding activity of shredder larvae on microbial biomass and decomposition (leaf-mass loss). All experiments were run in water with low nitrogen concentrations (see details later), using one of two trichopteran species belonging to the family Sericostomatidae: the Palaearctic caddisfly *Sericostoma vittatum* Rambur and the Neotropical species *Myothrichia murina* Schmid. The family Sericostomatidae is globally distributed and is represented by species whose larval stages feed on leaf litter, functioning as shredders. *Sericostoma vittatum* is a common endemic species of the Iberian Peninsula (Feio & Graça, 2000) and is abundant in some low-order streams of central Portugal, where it can reach an annual mean density and biomass of 115 individuals m^{-2} and 83 mg m^{-2}, respectively (González & Graça, 2003). *Myothrichia murina* is a common species of Patagonian low-order streams (Buria *et al*., 2007; Brand & Miserendino, 2011a). This caddisfly may reach locally the highest abundance and secondary production among trichopterans (mean annual density and annual production in a second-order stream: 334 indiv. m^{-2} and 310 mg m^{-2} per year, Brand & Miserendino, 2011b).

The indirect effects of the larvae on microbial biomass and decomposition were measured on nutrient-poor leaves chosen as leaf substrata. The effect of the Patagonian species *M. murina* was tested on litter from *Nothofagus pumilio* (Poepp and Endl.) Krasser (lenga), with a C : N ratio of 91 (Satti *et al*., 2003). Lenga is the native mountain beech tree that dominates Patagonian-Andean forests up to the timberline, spanning 35–56°S latitude (Hildebrand-Vogel, Godoy & Vogel, 1990). The effect of the Iberian shredder *S. vittatum* was tested on leaf litter from *Quercus robur* L. (oak), attaining a C : N ratio of 47 (Díaz Villanueva, Albariño & Canhoto, 2011). Oak is a common native tree in Europe and frequent in riparian areas of low-order streams of central and northern Portugal (Euforgen, 2009). In both experiments, and to stimulate ammonium production, shredders were fed with *Alnus glutinosa* (L.) Gaertn (alder) leaf litter, with a C : N ratio of 27 (Díaz Villanueva *et al*., 2011), and a high-quality food for several shredders (Iversen, 1974).

Effect of ammonium on fungal and bacterial biomass

In the autumn before the experiments, undamaged lenga leaves were collected from the forest floor immediately after senescence, air-dried in the dark and stored until needed. Just before the experiments, leaves were preconditioned for 15 days in the laboratory (15 °C, 12-h light:12-h dark photoperiod) in aerated stream water. To test the effect of increasing ammonium concentration on fungal and bacterial biomass, leaf discs of lenga were exposed to four treatments: filtered stream water, +10, +20 and +40 μg L^{-1} NH_4^+. Enrichment was carried out by adding NH_4Cl to 250 mL pre-filtered (GF/F) stream water (2.4 ± 0.3 μg NH_4 L^{-1}, 1.85 ± 0.14 μg P L^{-1}; 4.52 μg NO_3 L^{-1}) in sterile plastic containers (three replicates per treatment) at 15 °C. Ten pairs of leaf discs of 10 mm diameter were symmetrically punched out of the leaves; a set of ten was introduced to the containers, and the corresponding discs of each pair were used to obtain initial leaf dry mass (DM_i). Every 5th day, the water was renewed and the NH_4 concentration was measured. Controls without leaves were established for all ammonium concentrations, to detect any change in dissolved NH_4 because of chemical conditions or any possible bacterial contamination. After 15 days, the leaf discs were removed. In this way, the effect of NH_4 enrichment on microbial decomposers was cumulative throughout the experiment. Ammonium was measured by the indophenol blue method (APHA, 1989).

Eight discs from each replicate were freeze-dried for later estimates of fungal biomass by ergosterol analysis. Extraction was performed with hot KOH/methanol and purified by solid-phase filtration (Gessner, 2005). Ergosterol was quantified by an HPLC (high-performance liquid chromatography) Dionex DX-120 and a reverse-phase C_{18} column (Brownlee SPHERI-5 RP-18; Applied Biosystems, Foster city, CA, U.S.A). A conversion factor of 5.5 mg of ergosterol g^{-1} fungal biomass was used for fungal biomass estimation (Gessner, 2005). Fungal carbon biomass was estimated as 43% carbon content of fungi (Baldy & Gessner, 1997).

For bacteria counts, the other two discs were preserved in 4% filtered formalin, in the dark and at 5 °C, for further inspection under the microscope. Microorganisms were removed by adding 2 mL pyrophosphate buffer 0.02 M (final concentration 2 mM) and sonicating for 2 min in a Selecta sonication bath (J.P. Selecta S.A., Abrera, Spain) operating at 40 W and 40 kHz (Romaní *et al*., 2009). Slides for bacteria counting were prepared by filtering 1 mL of the suspension after it was stained for 1 min with 2% (V/V) DAPI (4,6-diamidino-2-phenylindole), through black polycarbonate filters (Poretics, 0.2 mm pore size; Porter & Feig, 1980). Counting was carried out at 1000× magnification with an Olympus BX50 epifluorescence microscope. Ten images for each sample were processed with an image analysis system (Image ProPlus; Media Cybernetics, Silver Spring, MD, USA), recording length and width to calculate bacterial biovolume by approximating shapes to an ellipsoid: $V = \frac{\Pi}{4} x D^2 x (L - \frac{D}{3})$(Bratbak, 1993). Carbon con-

tent was estimated with a conversion factor of 308 fg C mm^{-3} (Fry, 1990).

To estimate a mean uptake rate for each concentration, the difference between absolute values of ammonium in treatments and controls (calculated for 15 days) was divided by the absolute biomass of microorganisms on leaves after 15 days of colonisation.

Effects of shredders on fungal and bacteria biomass

To explore the effect of nutrient recycling by shredders on the microbial community, we measured fungal and bacterial biomass on leaves incubated in the presence and absence of *S. vittatum* larvae. A total of eight Erlenmeyer flasks filled with 150 mL artificial pond water ($CaCl_2{\cdot}2H_2O$ 294 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ 123.25 mg L^{-1}, $NaHCO_3$ 64.75 mg L^{-1}, KCl 5.75 mg L^{-1}, KH_2PO_4 410 mg L^{-1}, Suberkropp, Arsuffi & Anderson, 1983; without nitrogen source) and fine inorganic sediment (Ø < 2 mm) were sterilised (autoclaved). Each microcosm was provided with 7 oak-leaf discs (10 mm diameter) in fine-mesh bags (0.5 mm), to exclude larvae. Twelve larvae of similar size (1.68 ± 0.03 mg DM; mean indiv. mass ± SE) were used in each replicate and fed with conditioned alder leaves (15 days in the laboratory in aerated stream water, 15 °C, photoperiod 12 : 12). In all treatments and controls, 20 leaf discs (10 mm diameter) were used, and renewed every 3 days, to ensure similar quality and *ad libitum* food. The Erlenmeyer flasks were continuously aerated and kept at 15 °C under a photoperiod of 12 h light:12 h dark. Every 5th day, 1 mL was extracted from each microcosm to measure ammonium, and the water and leaf discs were renewed. The experiment stopped after 15 days. Oak-leaf discs were removed; five discs were used to estimate fungal biomass and two were preserved in formalin for bacteria counting (as explained previously).

Excretion rate

Larvae of *M. murina* were collected from a low-order forest stream in the northern Patagonian Andes, acclimated in the laboratory, and fed on lenga leaves for 2 days, at 10 °C and a photoperiod of 14 h light:10 h dark. Then, four larvae were transferred into each of 5 Erlenmeyer flasks with 100 mL filtered river water (polycarbonate 0.2 μm pore diameter, Poretic) and incubated for 3.5 h at 10 °C in the dark. Afterwards, the insects were removed, oven-dried (105 °C, 24 h) and weighed (±0.1 mg); water was filtered again through 0.2-μm-pore diameter polycarbonate filters for ammonium measurements. Ammonium from treatments and from filtered stream water was measured by the indophenol blue method (APHA (American Public Health Association), 1989). Excretion rates were expressed as the difference in NH_4 concentration in treatments and stream water, per mg larval DM per hour.

Decomposition experiment

The effect of the presence of the shredder on *N. pumilio* leaf decomposition was evaluated through a laboratory experiment. Leaf-mass remaining was estimated by incubating the leaf material in the presence and absence of larvae of the shredder *M. murina*. Fifteen larvae (0.88 ± 0.02 mg DM; mean ± SE) were placed in each container (n = 10) with 250 mL of filtered (GF/F filters) stream water (14.47 ± 0.95 μg NH_4 L^{-1}; 12.40 ± 0.86 μg P L^{-1}; 23.00 μg NO_3 L^{-1}), fine inorganic combusted sediments (Ø < 1–2 mm) and pre-conditioned (15 days in aerated stream water) alder leaves as food. Containers were aerated and kept at 10 °C, with a 12 h light:12 h dark photoperiod. Three groups of four pre-conditioned lenga leaf discs (10 mm diameter) were enclosed in fine-mesh bags (0.5 mm) and assigned to each container. One bag from each container was removed after 2 weeks (t_1), 4 weeks (t_2) and 8 weeks (t_3). Discs from the same leaf, obtained symmetrically in relation to the main vein, were exposed to a treatment and a control, so that each mesh bag incorporated into a treatment unit had its paired mesh bag in one control, run without larvae but with sediments and leaves (n = 10). Mass remaining (expressed as percentage) was calculated for both controls and treatments, at each time point, against mean initial dry mass; this value was estimated from a set of leaf discs punched from the same leaves used in the experiment. The intrinsic variance between paired leaf-disc groups, that is, the groups of four discs belonging to the same lenga leaves, was calculated from ten replicates of leaf discs at t_0. The difference in dry mass between the paired groups at t_0 was <4%.

Water, sediment and food were renewed every 7th day. Beforehand, an 11 mL of water sample was filtered (0.2-μm pore size) to measure the ammonium concentration at that time. In addition, the ammonium concentration in stream water to be added to the containers at each renewal date was measured by the indophenol blue method, to ensure that there were no differences at t_0. Phosphorus concentration (soluble reactive phosphorus) was measured in stream water (t_0) and at t_3 only, by the ascorbic acid method (APHA, 1989).

In this experiment, the mean NH_4 uptake rate (UpR) was estimated as μg NH_4 fixed by decomposers per day

for both control and treatment containers, by means of the following equation:

$$UpR = \frac{B + \mathrm{Ex} - M}{t}$$

where B is the basal concentration in stream water, Ex is the estimated total amount of ammonium excreted by *M. murina* larvae (see excretion experiment earlier) accumulated in 7 days (in controls without larvae Ex was zero), *M* is the measured ammonium concentration in each microcosm and *t* is the time interval (in this case, 7 days).

Data analysis

In the enrichment experiment, comparisons of final versus initial ammonium concentration in treatments (with leaves) and control (without leaves) were performed with one-way ANOVA, and *a posteriori* test for multiple comparisons between final (treatments and control) versus initial concentrations was run with Holm–Sidak method. Also bacteria abundance and biomass in the different treatments were compared with one-way ANOVA. A linear regression between the fungal biomass and the initial ammonium concentration was performed.

The effect of *S. vittatum* on fungal and bacterial biomass between treatments (with larvae) and controls (without larvae) was compared using *t*-test. In the decomposition experiment, differences in ammonium concentration and % leaf-mass remaining between controls (without larvae) and treatments (with *M. murina* larvae) on each sampling date were evaluated with a one-way repeated-measures ANOVA (RM-ANOVA) (shredder presence as factor, t_0 was excluded from the analysis) using SPSS. When differences were significant ($P < 0.05$), *a posteriori* tests for all pairwise multiple comparisons (Holm–Sidak method) were performed. Each analysis was performed after assumptions of normality and homoscedasticity were verified.

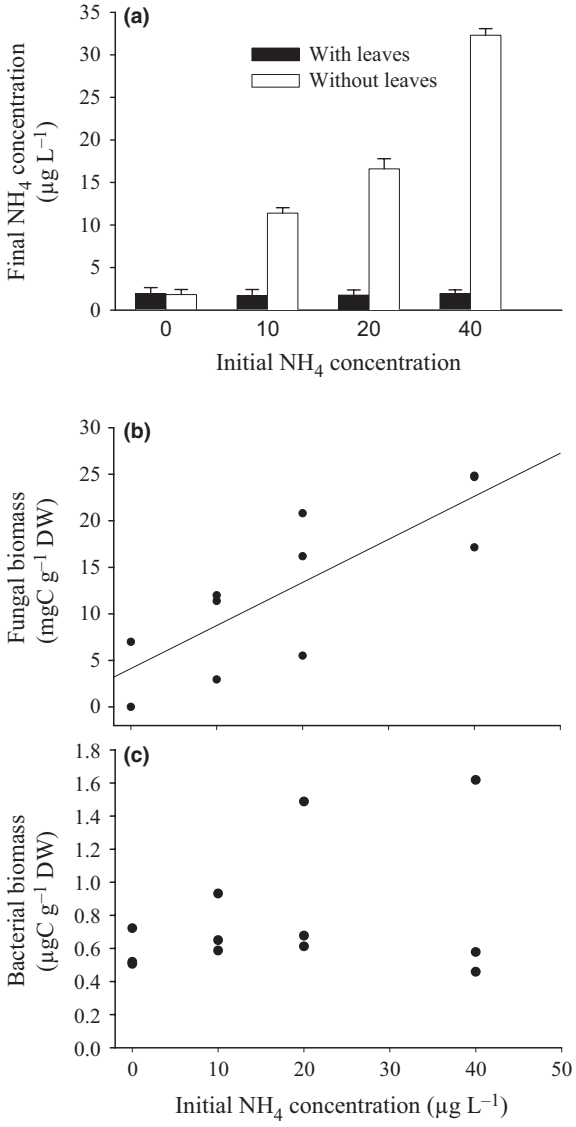

Fig. 1 (a) Final ammonium concentration (after 5 days incubation) in treatments with leaf discs and in controls. (b) Fungal biomass related to increasing ammonium concentration. Regression: fungal biomass = 4.117 + 0.463 × [NH_4] initial and (c) Effect of increasing ammonium concentration on bacterial biomass. The treatment with 'zero concentration' received no ammonium amendment and had the natural ammonium concentration in the stream. Bars in a) are means ± SE, and dots in b and c) are values for each replicate.

Results

Effect of ammonium on fungal and bacterial biomass

After 5 days of incubation, the NH_4 concentration was reduced to near-ambient streamwater concentrations in all treatments with leaves (one-way ANOVA, $F_{4,11} = 303.147$, $P < 0.001$), while no changes were observed in controls (*a posteriori* test, $P = 0.104$, Fig. 1a). Fungal biomass was positively affected by increased ammonium concentration ($R^2 = 0.671$, $P = 0.002$, Fig. 1b), converting leaf detritus into 0.463 mg C of fungal biomass per µg L^{-1} NH_4. Bacteria abundance was similar in all treatments, both in terms of cell abundance (one-way ANOVA, $F_{3,8} = 0.067$, $P = 0.976$, Table 1) and biomass (one-way ANOVA, $F_{3,8} = 0.431$, $P = 0.736$, Fig. 1c). The two replicates with the lowest bacterial biomass corresponded to the replicates with the highest fungal biomass (Fig. 2).

Estimated microbial uptake rate of ammonium was similar in all concentration treatments (one-way ANOVA, $P = 0.871$, 2.63 ± 1.21, 1.92 ± 0.93 and 2.07 ± 0.24 µg

Table 1: Bacterial abundance and cell size in the four ammonium concentration treatments. The treatment with 'zero concentration' received no ammonium amendment and had the ambient ammonium level of the source stream

Ammonium concentration (μg L^{-1})	0	10	20	40
Bacterial abundance (cells × 104 mg^{-1} DW)	3.93 ± 0.36	3.80 ± 0.16	3.19 ± 1.00	4.05 ± 1.41
Mean cell biovolume (10^2 μm^3)	4.91 ± 0.76	6.31 ± 1.23	11.19 ± 3.30	6.80 ± 0.62

Values are means ± SE; $n = 3$.

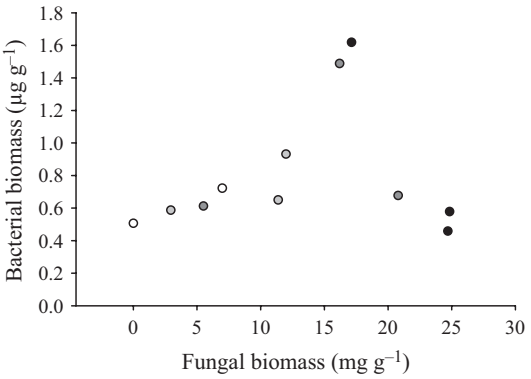

Fig. 2 Relation between fungal and bacterial biomass in leaf discs of treatments with different ammonium concentrations. Dots are single replicates (white dots correspond to: treatment 0 μg H_4 L^{-1}, grey dots: 10 μg NH_4 L^{-1}, dark grey dots: 20 μg NH_4 L^{-1} and black dots: 40 μg NH_4 L^{-1}).

NH_4 mg^{-1} C day^{-1} in 10, 20 and 40 μg L^{-1} treatments, respectively) and averaged 2.21 ± 0.46 μg NH_4 mg C^{-1} day^{-1}. Considering the fungal biomass in 1 g DM leaf, this rate could be expressed as 33.23 ± 4.50 μg NH_4 g^{-1} leaf DM day^{-1}.

Effects of shredders on fungal and bacterial biomass

In the experiment with *S. vittatum*, the fungal biomass in the treatment with insects was 36.81 ± 6.33 mg C g^{-1} leaf discs DM, more than threefold the fungal biomass in the control (10.05 ± 4.17 mg C g^{-1}) after 15 days of experiment (*t*-test, $P = 0.025$). In contrast, bacteria were an order of magnitude less in the treatment with insects (4.90 ± 0.66 × 10^5 cells g^{-1} DM) than in the control (3.90 ± 0.50 × 10^6 cells g^{-1} DM). This difference was clearly observed in microscopic images (Fig. 3).

Leaf-mass remaining

Ammonium concentration at each renewal (of water, sediment and food resource) date differed between control (without larvae) and treatment (with *M. murina* larvae) (one-way RM-ANOVA, $F_{1,26} = 33.381$, $P < 0.001$, Fig. 4a). After 2 and 4 weeks, ammonium in treatments was higher than in controls (post hoc comparisons, Holm–Sidak method, $P < 0.05$). However, after 8 weeks, ammonium concentration values were as low as in controls ($P > 0.05$). Similarly, after 8 weeks, phosphate concentration had decreased from 12.40 ± 0.86 μg P L^{-1} at t_0 to 4.96 ± 0.59 μg P L^{-1} in treatments (*t*-test, $P < 0.001$), while in controls, it increased to 27.57 ± 1.93 μg P L^{-1} (*t*-test, $P < 0.001$). Leaf-mass remaining was significantly lower in treatments than in controls (one-way RM-ANOVA, $F_{1,29} = 26.717$, $P < 0.001$, Fig. 4b).

Based on the measured *M. murina* excretion rates (0.59 ± 0.04 μg NH_4 mg^{-1} day^{-1}), the estimated accumulation of ammonium released by the 15 larvae (13.23 ± 0.24 mg total DM in each container) after 7 days was 219.18 ± 16.47 μg L^{-1}. Considering that the ammonium concentration in the stream water was 14.47 ± 0.95 μg L^{-1}, the estimated microbial uptake rate in containers with shredding activity was 8.02 ± 0.08 μg NH_4 day^{-1}, while in controls it was 0.44 ± 0.02 μg NH_4 day^{-1}.

Discussion

Our hypothesis of a positive effect of shredders on decomposer microbes was supported by data from direct (fungal biomass) and indirect (organic matter degradation by microbes) measurements, using two different aquatic shredders and leaves from Northern and Southern Hemisphere temperate forests. Insect excretion caused ammonium concentration to increase in the microcosms, contributing to microbial N uptake in leaf substrata, which resulted in structural (the proportion of fungi and bacteria) and functional (decomposition) changes. Our findings showed that detritus dynamics are complex, and the contributions of decomposers and detritivores to mass loss act not simply additively. Biotic forces that directly exploit the detritus pool act synergistically in transforming carbon and nutrients.

Accumulations of detritus are habitats where contrasting biotic interactions occur. On one hand, detritivores feed on dead organic matter, simultaneously preying on microbes (Graça, Maltby & Calow, 1993). On the other hand, we demonstrated that nutrient cycling by animals simultaneously enhances fungal

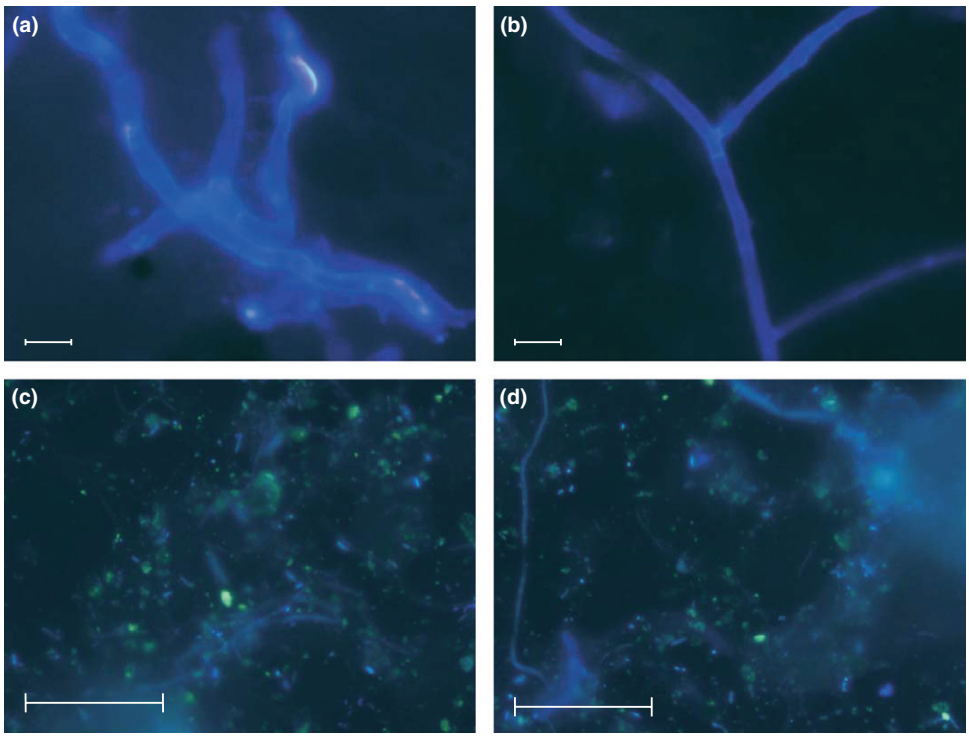

Fig. 3 Epifluorescence microscope images (1250×) corresponding to treatment and control of the feedback experiment: a–b) treatment with insects, where hyphae are abundant; and c–d) control without insects, where bacteria are abundant. Samples were stained with DAPI. Scale bar: 20 μm.

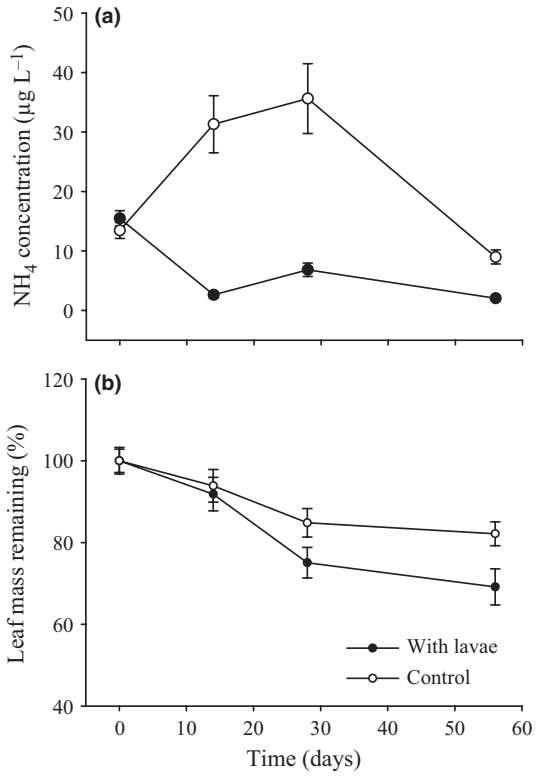

Fig. 4 (a) Ammonium concentration dynamics in treatments with insects and controls. (b) Leaf-mass remaining (%) dynamics in treatments with insects and controls. Values are means ± SE.

biomass and degradation activity within the microhabitat of leaf packs in which they feed/near where predation on fungi by shredders occurs. Because shredder nutrition is highly dependent on leaf-litter microorganisms (Graça, 2001), a positive effect of nutrient excretion on fungi and bacteria would imply a positive feedback on shredders.

Tank *et al.* (2000) demonstrated that ammonium demand was higher in microbes colonising leaf detritus, compared with epilithon, fine particulate organic matter or bryophytes. The authors reported an NH_4 uptake rate of 3.51 mg m^{-2} day^{-1}. The results of our first experiment, in which ammonium concentration was lowered to the streamwater concentration level in all treatments, would indicate that for a leaf-mass standing stock of 30 g DM m^{-2} found in a Patagonian second-order stream in winter (Díaz Villanueva, Buria & Albariño, 2010), the estimated uptake rate would be 1.00 mg N m^{-2} day^{-1}. The positive relationship between ammonium concentration and fungal biomass in our first experiment would indicate that N was a limiting nutrient for the fungal community inhabiting high C : N leaves. Therefore, nutrients excreted by animals that feed in (and on) detritus accumulations are probably rapidly immobilised by microbes growing on and decomposing the same

resource. Because water exchange within leaf packs is probably slow, nutrients produced by detritivore feeding activity may be expected to be retained and taken up by microbes within these accumulations. If this is true, detritus dynamics acting at the scale of a leaf pack will also depend on detritivore activity and not only on streamwater nutrient concentration, at least in oligotrophic environments. It seems plausible that where detritivore invertebrates are able to exert control on leaf-litter breakdown (e.g. the direct reduction of organic mass), they are also capable of indirectly increasing decomposition rate by stimulating decomposer growth.

It is well demonstrated that artificial nutrient enrichment may increase decomposition rates (Robinson & Gessner, 2000; Benstead *et al.*, 2009) and fungal biomass (Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006; Chung & Suberkropp, 2008) when dissolved nutrient concentrations in streams are low. However, in most of these studies, the enrichment consisted of nitrate addition (Sridhar & Bärlocher, 2000; Stelzer *et al.*, 2003; Ferreira *et al.*, 2006) or led to ammonium concentrations of more than 100 $\mu g\ L^{-1}$ (Gulis & Suberkropp, 2003; Chung & Suberkropp, 2008). Our enrichment experiment was run at low ammonium concentrations (up to 40 $\mu g\ L^{-1}$), in ranges that can be found in low-order streams, and demonstrated the potential effect of microenrichment by shredder excretion.

Interestingly, fungi and bacteria responded differently to ammonium enrichment. Bacterial reduced growth in the experiment with *S. vittatum*, with high fungal biomass (more than 80 $mg\ g^{-1}$ leaf DM), and bacterial slight decline in the enrichment experiment, with fungal biomass higher than 20 $mg\ g^{-1}$ leaf DM, suggest a negative effect of fungi on bacteria. Although our data are not conclusive (only two replicates), they support evidence from other experiments (Bergur & Frieberg, 2012) in which bacteria reduction increased fungal respiration. This contrasts with Romaní *et al.* (2006), who found that bacteria grew better together with fungi than alone, but at the same time bacteria suppressed fungal growth. However, Mille-Lindblom, Fischer & Tranvik (2006) showed that the antagonism between bacteria and fungi can be drastically altered if fungi are given an opportunity to establish before inoculation of bacteria, where established fungi can outcompete bacteria. In our experiments, fungi and bacteria were allowed to colonise leaf litter at the same time, but it seemed that fungi were more nutrient limited than bacteria because supplying ammonium stimulated fungal growth. Stelzer *et al.* (2003) also found that dissolved nutrients had a strong positive effect on fungal biomass but not on bacterial biomass. Perhaps the ability of fungi to respond to nutrient enrichment enabled them to outcompete bacteria from the leaf substrata.

Because fungi proved to rely partly on nutrients released by shredders, it would be expected that any change in excretion rates could alter fungal biomass and decomposition. Leaf species composition (Lecerf *et al.*, 2011), fungal and invertebrate assemblages (Gessner *et al.*, 2010) and abiotic conditions (such as stream nutrient concentration and temperature, Chung & Suberkropp, 2008; Díaz Villanueva *et al.*, 2011) are the major driving forces that interact to affect the outcomes of these relationships. In the global change scenarios (Intergovernmental Panel on Climate Change (IPCC), 2007) where riparian vegetation cover and composition are expected to change, and water temperature and nutrient status of the streams are expected to increase, further studies are needed to understand how the strength and direction of those interactions are to be adjusted within ecosystem functioning.

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References

APHA (American Public Health Association) (1989) *Standard Methods for the Examination of Water, Sewage, and Wastewater*. American Public Health Association, Washington D.C.

Baldy V. & Gessner M.O. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus de l'Académie des Sciences, Paris, Life Sciences*, **320**, 747–758.

Balseiro E.G. & Albariño R.J. (2006) C–N mismatch in the leaf litter–shredder relationship of an Andean Patagonian stream detritivore. *Journal of the North American Benthological Society*, **25**, 607–615.

Bärlocher F. (1980) Leaf-eating invertebrates as competitors of aquatic hyphomycetes. *Oecologia*, **47**, 303–306.

Bärlocher F. (1982) On the ecology of ingoldian fungi. *BioScience*, **32**, 581–586.

Benstead J.P., Rosemond A.D., Cross W.F., Wallace J.B., Eggert S.L., Suberkropp K. *et al.* (2009) Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology*, **90**, 2556–2566.

Bergur J. & Frieberg N. (2012) Trade-offs between fungal and bacterial respiration along gradients in temperature, nutrients and substrata: experiments with stream derived microbial communities. *Fungal Ecology*, **5**, 46–52.

Brand C. & Miserendino M.L. (2011a) Characterizing Trichoptera trophic structure in rivers under contrasting land use in Patagonia, Argentina. *Zoosymposia*, **5**, 29–40.

Brand C. & Miserendino M.L. (2011b) Life history strategies and production of caddisflies in a perennial headwater stream in Patagonia. *Hydrobiologia*, **673**, 137–151.

Bratbak G. (1993) Microscope methods for measuring bacterial biovolume: epifluorescence microscopy, scanning electron microscopy, and transmission electron microscopy. In: *Handbook of Methods in Aquatic Microbial Ecology* (Eds P.F. Kemp, B.F. Sherr, E.B. Sherr & J.J. Cole), pp. 309–317. Lewis Publishers, Boca Rataon, FL, U.S.A.

Buria L., Albariño R.J., Díaz Villanueva V., Modenutti B. & Balseiro E. (2007) Impact of exotic rainbow trout on the benthic macroinvertebrate community from Andean-Patagonian headwater streams. *Fundamental and Applied Limnology*, **168**, 145–154.

Canhoto C. & Graça M.A.S. (2008) Interactions between fungi and stream invertebrates: back to the future. In: *Novel Techniques and Ideas in Mycology* (Eds K.R. Sridhar, F. Bärlocher & K.D. Hyde). *Fungal Diversity Research Series*, **20**, 205–325.

Chung N. & Suberkropp K. (2008) Influence of shredder feeding and nutrients on fungal activity and community structure in headwater streams. *Fundamental and Applied Limnology*, **173**, 35–46.

Coleman D.C., Reid C.P.P. & Cole C.V. (1983) Biological strategies of nutrient cycling in soil systems. *Advances in Ecological Research*, **13**, 1–55.

Cross W.F., Benstead J.P., Frost P.C. & Thomas S.A. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**, 1895–1912.

Díaz Villanueva V., Albariño R.J. & Canhoto C. (2011) Detritivores feeding on poor quality food are more sensitive to increased temperatures. *Hydrobiologia*, **678**, 155–165.

Díaz Villanueva V., Buria L. & Albariño R.J. (2010) Primary consumers and resources: annual variation in two contrasting reaches of a Patagonian mountain stream. *International Journal of Limnology*, **46**, 21–28.

Euforgen 2009. *European Forest Genetic Resources Programme*. Available at: http://www.euforgen.org/distribution_maps.html

Feio M.J. & Graça M.A.S. (2000) Food consumption by the larvae of *Sericostoma vittatum* (Trichoptera), an endemic species from the Iberian Peninsula. *Hydrobiologia*, **439**, 7–11.

Fenchel T. (1988) Marine plankton food chains. *Annual Review of Ecology and Systematics*, **19**, 19–38.

Ferreira V. & Graça M.A.S. (2006) Do invertebrate activity and current velocity affect fungal assemblage structure in leaves? *International Review of Hydrobiology*, **91**, 1–14.

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

Fry J.C. (1990) Direct methods and biomass estimation. In: *Methods in Microbiology* Vol. 22 (Eds R. Grigorova & J.R. Norris), pp. 41–85. Academic Press, London.

Gessner M.O. (2005) Ergosterol as a measure of fungal biomass. In: *Methods to Study Litter Decomposition. A practical Guide* (Eds M.O. Graça, F. Bärlocher & M.O. Gessner), pp. 189–196. Springer, Dordrecht, the Netherlands.

Gessner M.O., Swan C.M., Dang C.K., McKie B.G., Bardgett R.D., Wall D.H. *et al.* (2010) Diversity meets decomposition. *Trends in Ecology and Evolution*, **25**, 372–380.

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

Graça M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams – a Review. *International Review of Hydrobiology*, **86**, 383–394.

Graça M.A.S., Maltby L. & Calow P. (1993) Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* I: feeding strategies. *Oecologia*, **93**, 139–144.

Greenwood J.L., Rosemond A.D., Wallace J.B., Cross W.F. & Weyers H.S. (2007) Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. *Oecologia*, **151**, 637–649.

Grimm N.B. (1988) Role of macroinvertebrates in nitrogen dynamics of a desert stream. *Ecology*, **69**, 1884–1893.

Gulis V. & Suberkropp K. (2003) Effect of inorganic nutrients on relative contributions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology*, **45**, 11–19.

Hargrave C., Hamontree S. & Gary K. (2010) Direct and indirect food web regulation of microbial decomposers in headwater streams. *Oikos*, **119**, 1785–1795.

Hildebrand-Vogel R., Godoy R. & Vogel A. (1990) Subantarctic-Andean *Nothofagus pumilio* forests. Distribution area and systematic overview, vegetation and soils as demonstrated by an example of a South Chilean stand. *Vegetation*, **89**, 55–68.

Hildrew A.G. (1996) Food webs and species interactions. In: *River Biota* (Eds G. Petts & P. Calow), pp. 123–144. Blackwell Science Ltd., Oxford.

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate change 2007: the physical science basis. In: IPCC Fourth Assessment Report (Eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller), pp. 339–378. IPCC, Geneva.

Iversen T.M. (1974) Ingestion and growth in *Sericostoma personatum* (Trichoptera) in relation to the nitrogen content of ingested leaves. *Oikos*, **25**, 278–282.

Lavelle P.R., Dugdale R., Scholes R., Asefaw A., Carpenter B.E., Codispoti L. *et al.* (2005) Nutrient cycling. In: *Ecosystems and Human Well-Being: Current State and Trends*. (Eds H. Etchevers & J. Tiessen), pp. 331–353. Millennium

Ecosystem Assessment. Available at: http://www.millenniumassessment.org/documents/document.281.aspx.pdf.

Lecerf A., Marie G., Kominoski J.S., LeRoy C.J., Bernadet C. & Swan C.M. (2011) Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. *Ecology*, **92**, 160–169.

Liess A. & Hillebrand H. (2004) Invited review: direct and indirect effects in herbivore periphyton interactions. *Fundamental and Applied Limnology*, **159**, 433–453.

McManamay R.A., Webster J.R., Valett H.M. & Dolloff C.A. (2011) Does diet influence consumer nutrient cycling? Macroinvertebrate and fish excretion in streams. *Journal of the North American Benthological Society*, **30**, 84–102.

McNaughton S.J., Banyikwa F.F. & McNaughton M.M. (1997) Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science*, **278**, 1798–1800.

Mille-Lindblom C., Fischer H. & Tranvik L.J. (2006) Antagonism between bacteria and fungi: substrate competition and a possible trade off between fungal growth and tolerance towards bacteria. *Oikos*, **113**, 233–242.

Newbold J.D., Elwood J.W., O'Neil R.V. & Sheldon A.L. (1983) Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. *Ecology* **64**, 1249–1265.

Porter K.G. & Feig Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnology and Oceanography*, **25**, 943–948.

Robinson C.T. & Gessner M.O. (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia*, **122**, 258–263.

Romaní A.M., Artigas J., Camacho A., Graça M.A.S. & Pascoal C. (2009) La biota de los ríos: los microorganismos heterotróficos. In: *Conceptos y técnicas en ecología fluvial* (Eds A. Elosegi & S. Sabater), pp. 169–218. Fundación BBVA, España.

Romaní A.M., Fischer H., Mille-Lindblom C. & Tranvik L. (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. *Ecology*, **87**, 2559–2569.

Rosemond A.D., Mulholland P.J. & Elwood J.W. (1993) Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. *Ecology*, **74**, 1264–1280.

Satti P., Mazzarino M.J., Gobbi M., Funes F., Roselli L. & Fernandez H. (2003) Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. *Journal of Ecology*, **91**, 173–181.

Sridhar K.R. & Bärlocher F. (2000) Initial colonization, nutrient supply and fungal activity on leaves decaying in streams. *Applied and Environmental Microbiology*, **67**, 5063–5068.

Steinman A.D. (1996) Effects of grazers on freshwater benthic algae. In: *Algal Ecology: Freshwater Benthic Ecosystems* (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe), pp. 341–374. Academic Press, San Diego, CA.

Stelzer R.S., Heffernan J. & Likens G.E. (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology*, **48**, 1925–1937.

Suberkropp K., Arsuffi T.L. & Anderson J.P. (1983) Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. *Applied and Environmental Microbiology*, **46**, 237–244.

Tank J.L., Meyer J.L., Sanzone D.M., Mulholland P.J., Webster J.R., Peterson B.J. *et al.* (2000) Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N-tracer addition. *Limnology and Oceanography*, **45**, 1013–1029.

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

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