Fungal alteration of the elemental composition of leaf litter affects shredder feeding activity

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SUMMARY

1. Leaf litter from riparian vegetation provides the main source of matter and energy for food webs of small forest streams. Shredding macroinvertebrates mostly feed on this litter when it has been colonised and conditioned by microorganisms, especially by aquatic hyphomycetes. Since shredders feed selectively, they must make foraging decisions based on the physical and chemical characteristics of the food resource, which can change depending on the identity of fungal species.

2. Here, we addressed the effect of changes in fungal assemblage structure on the elemental composition of oak (Quercus robur) leaf litter and how variation in litter quality affects the feeding of a stream shredder. Leaf discs were incubated in microcosms for 2 weeks, inoculated with various fungal assemblages comprised of three species each, and offered to a shredder (Schizopelex festiva, Trichoptera: Sericostomatidae) as food.

3. This shredder ate more leaves with a high mycelial biomass, which depended on fungal assemblage composition. Leaf litter conditioned by different fungal assemblages resulted in different litter N and P concentrations. Mycelial biomass was positively related to litter P concentration, with the lowest and highest P concentrations differing by 40% at most, but not to litter N concentration, even though the lowest and highest N concentrations differed by as much as 35%. The caddisfly larvae ate more leaves with a low C/P ratio.

4. These findings suggest a key role of litter P concentration in eliciting fungal conditioning effects on shredder-mediated litter decomposition.

Keywords: ecosystem functioning, fungal community composition, leaf litter stoichiometry, Schizopelex festiva, species identity

Introduction

One challenge that all organisms face is to acquire sufficient energy and elements to maintain bioenergetic processes (Frost et al., 2005). A growing body of evidence indicates that organisms meet an imbalanced mixture of elements from their resources (Sterner & Elser, 2002; Frost et al., 2005). Elemental constraints can therefore strongly affect consumer growth and reproduction (Bruning, 1991; Sterner & Schulz, 1998; Aerts & Chapin, 2000; Smith, 2002; Frost et al., 2005), with important consequences for the dynamics of populations (Elser et al., 1998; Chen et al., 2004), interspecific interactions (DeMott & Gulati, 1999; Denno & Fagan, 2003) and key ecosystem processes (Sterner et al., 1997; Cebrian, 1999; Frost et al., 2005).

In small woodland streams, the primary source of carbon (C) and energy for aquatic food webs is terrestrially
derived organic matter supplied by the riparian vegetation, whose shade also limits instream primary production (Vannote et al., 1980). Leaf litter decomposition in these streams is therefore an important ecosystem process, which depends on the activity of shredding invertebrates and microorganisms (Hieber & Gessner, 2002; Pascoal & Cásio, 2004). The latter, especially aquatic hyphomycetes, convert leaf C into biomass and immobilise nutrients, which increases the palatability of leaf detritus for shredding invertebrates (Gessner, Dobson & Chauvet, 1999; Graça, 2001). Therefore, the microbially conditioned leaf litter often allows a higher survival, growth rate and/or reproductive output and thus enhances shredder fitness (Graça, 2001).

Early studies showed that aquatic hyphomycete species differ in degradative capabilities when they are inoculated individually onto leaf litter (Suberkropp, Arsuffi & Anderson, 1983; Suberkropp & Arsuffi, 1984; Butler & Suberkropp, 1986). This suggests that communities dominated by distinct fungal species would decompose leaf litter at different rates and therefore differentially improve the food quality of leaf detritus. Beyond interspecific variations due to those qualitative differences in enzymatic capabilities, Danger & Chauvet (2013) recently reported a strong plasticity in the elemental composition of several aquatic hyphomycete species.

Overall, these points could have important consequences for consumer–resource interactions, the supply of C and nutrients up the food web, and nutrient recycling. Detritivores are able to discriminate among leaves colonised by different fungal species (Arsuffi & Suberkropp, 1984; Gonçalves et al., 2014). Detritivores and primary consumers, in general, are among the organisms most affected by variation in resource quality. Detritivores face a world where the resources (mainly leaf litter) they consume are often of poor quality. Factors such as leaf toughness, structural constituents or secondary leaf compounds, and low nitrogen (N) concentration can affect detritivore performance (Schlie & Mutz, 2006; Danger et al., 2012). Several mechanisms for overcoming low nutritional quality have been suggested (Sterner & Hessen, 1994). Consumers could actively select resources with higher concentrations of the limiting mineral nutrient (Kimmerer & Potter, 1987; Butler, Suttle & Neill, 1989) or could increase their uptake of food with low nutritional quality to access sufficient amounts of the limiting nutrient (Fink & von Elert, 2006). However, Frost & Elser (2002) suggested that such compensatory mechanisms probably cause additional energetic costs, which reduce their adaptive value. To our knowledge, the mechanisms underlying feeding preferences of detritivores have been poorly explored.

Here, we assessed whether changes in the composition of aquatic hyphomycete assemblages would influence the nutrient concentration of decomposing oak leaf litter, and whether these variations in litter quality would affect the feeding activity of a stream detritivore. We hypothesised that variation in leaf litter resource quality, defined here as leaf litter elemental composition, would change consumer feeding performance, with consequences for the rate of leaf litter breakdown as a major ecological process in detritus-based headwater streams. We tested this hypothesis by conducting an experimental laboratory study, in which the structure of litter-associated fungal assemblages was manipulated in microcosms simulating a stream environment. The conditioned litter was then fed to a stream detritivore, the larva of the shredder Schizopelex festiva (Trichoptera: Sericostomatidae). We predicted that fungal assemblages comprising aquatic hyphomycete species that exhibit high degradative capabilities (Zemek et al., 1985) would also better enhance leaf litter nutritional value, resulting in an increased shredder feeding activity and litter decomposition rates.

**Methods**

**Microcosm set-up and fungal assemblages**

Leaf litter, fungal assemblages and microcosms. Oak leaves were collected at abscission in autumn 2008 using a suspended net in the riparian zone of the Alzeau river, Montagne Noire (south-western France; 02°13′23″ E, 43°25′51″ N; altitude 743 m a.s.l) and dried at room temperature. Before use, leaves were soaked in distilled water for 3 h and ~8000 leaf discs were cut from them with a 12-mm Ø cork borer, avoiding primary and secondary veins. Once cut, leaf discs were oven-dried (65 °C, >24 h), weighed individually (±0.01 mg) and distributed among 63 mass classes of 0.1 mg range (from 2.10 to 8.20 mg). Eight mass classes, among which two thickness levels were distinguished, were selected: 3.30–3.40, 3.40–3.50 mg [Thin, Early assemblage treatment]; 3.50–3.60, 3.60–3.70 mg [Thin, Late assemblage]; 3.90–4.00, 4.00–4.10 mg [Thick, Early assemblage]; and 4.10–4.20, 4.20–4.30 mg [Thick, Late assemblage].

Leaf discs were conditioned in laboratory microcosms designed to simulate the stream environment. Each microcosm consisted of a 1-L glass flask with a sealed cap through which passed three tubes: one tube was connected to an air pump and allowed continuous aeration.
Conidial inoculations (<1 day old) were produced at 15 °C, under light conditions, by agitation of agar plugs taken from the leading edge of 14- to 30-day-old colonies in 25 mL of nutrient solution (prepared as described previously). An aliquot of each specific conidial suspension, based on conidial density, was used to inoculate the microcosms. Eight microcosms were inoculated with an assemblage composed of aquatic hyphomycete species tending to be early colonisers (Early assemblage: Anguillospora, Tetrachaetum and Tricladium), and eight microcosms were inoculated with an assemblage composed of aquatic hyphomycete species tending to be late colonisers (Late assemblage: Articulospora, Clavariopsis and Tetracladium) (Gessner et al., 1993; Nikolcheva, Cockshutt & Bärlocher, 2003). The use of only three species allowed us to assess the importance of the dominant species identity on the decomposition process (Ferreira & Chauvet, 2012) and, more specifically, the extent to which this affected the food quality of leaf detritus. For each assemblage type (Early and Late), there were four dominance treatments: an even treatment, in which microcosms were inoculated with even proportions of conidia from each species (i.e. 33.3%), and three uneven treatments in which microcosms were inoculated with species 1, species 2 or species 3 as dominant (i.e. 60% and 20% for each of the two other species, cf. Fig. 2; Ferreira & Chauvet, 2012).

Conditioning. Five groups of 155 discs were set apart from the selected mass classes, weighed (±0.01 mg), placed inside each microcosm with 150 mL of distilled water and autoclaved (20 min at 121 °C). In parallel, three groups of five leaf discs of the selected mass classes were weighed (±0.01 mg), placed inside glass tubes with 10 mL of distilled water, given the same treatment and used to create a correction factor for mass loss due to leaching during sterilisation. After cooling to room temperature, the water from the microcosms (n = 20) was replaced with 500 mL of a sterile nutrient solution (described above) added to microcosms through the tubing system, which was otherwise closed with a clamp, and aerated for 24 h to allow further leaching. Nutrient solution was changed and the microcosms inoculated with conidial suspensions. Eight microcosms were inoculated with an early assemblage, and eight were inoculated with a late assemblage; a total of ~155 000 conidia were inoculated into each microcosm (Fig. 2). For the first 6 h, the microcosms were aerated for periods of 20 min interspaced by periods of 20-min quiescence; after this, microcosms were aerated continuously until the end of the experiment. The nutrient solution was

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Fig. 1 Laboratory microcosm designed to simulate stream conditions and used to inoculate and grow aquatic fungi axenically on leaf discs. Each microcosm consisted of a 1-L glass flask equipped with three sealed tubing systems running through the cap: one tube was connected to an air pump and allowed continuous aeration that created turbulence and kept the leaf discs in permanent agitation (air pump connection), the second tube allowed the aseptic filling of the microcosm (aseptic inlet), and the third tube allowed the aseptic drainage of medium and recovery of conidial suspensions (aseptic outlet).
replaced after 24 h and then every 2 days for the duration of the experiment (14 days). Microcosms were incubated at 15 °C under a 12-h light:12-h dark photoperiod. All manipulations of microcosms took place under aseptic conditions in a sterile laminar flow cabinet.

After 2 weeks, the 155 leaf discs from each microcosm were sampled. Ninety discs were promptly used in the invertebrate experiment. Three sets of five leaf discs were used for respirometry trials and then frozen at −20 °C, lyophilised, weighed (±0.01 mg) and used for ergosterol extraction. Three sets of 10 leaf discs were

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used for determination of DM, ground and used for determinations of phosphorus (P), N and C concentrations. Four microcosms (i.e. two microcosms per thickness level) were kept sterile and were used as controls for abiotic decomposition.

**Mass loss and leaf physical–chemical characteristics.** Specific leaf disc area (SLA) was determined for 96 discs as the area-to-mass ratio, and results were expressed as mm² mg⁻¹. Toughness was determined for the same leaf discs with a penetrometer after discs had been soaked in distilled water for 1 h, with results expressed as the mass (g) required to force an iron rod (1.89 mm² ∅) through the discs (Graça, Bärlocher & Gessner, 2005).

To characterise initial litter chemical composition before and after autoclaving, three sets of 10 air-dried and autoclaved litter discs were oven-dried (105 °C, 24 h) to allow estimation of initial DM and ground with a mortar and pestle (0.5 mm powder size) and subsamples weighed (±0.01 mg) to be analysed for C, N [IRMS Thermo Delta V advantage with a Flash EA (1112 series)], and P concentrations (APHA, 1998). Results were expressed as percentage of dry mass (% DM). After the end of the experiment, three sets of 10 leaf discs from each microcosm were oven-dried (105 °C, 24 h), weighed (±0.01 mg) to determine remaining dry mass (DMr) calculated as the difference between initial mass and final mass, and used to estimate leaf litter elemental quality after conditioning.

**Fungal sporulation.** Each of the seven times the nutrient solutions were changed, 40 mL of the suspension from each microcosm was sampled, stored in 50 mL Falcon tubes and preserved with 2 mL 37% formalin until processed. When preparing filters for conidial counting and identification, 150 μL of polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether (Triton X-100, 0.5%) was added to the suspension and mixed with a magnetic stirring bar to ensure a uniform distribution of conidia, and an aliquot of the suspension was filtered (25 mm ∅, pore size 5 μm, Millipore SMWP; Millipore Corp., Bedford, MA, U.S.A.). Filters were stained with 0.05% trypan blue in 60% lactic acid, and spores were identified and counted under a compound microscope at 320 × (Leitz Diaplan, Wetzlar, Germany) according to Graça et al. (2005). Cumulative conidial production over time was calculated by summing the numbers of conidia produced at the current and preceding sampling dates. The total conidial mass was calculated by multiplying the number of conidia from each species by the average mass of individual conidia obtained from the literature (Chauvet & Suberkropp, 1998) or calculated from biovolume data (Bärlocher & Schweizer, 1983). Results were expressed as μg of conidia released mg⁻¹ initial leaf DM.

**Fungal respiration.** At the end of the experiment, three subsets of five discs from each microcosm were used to determine fungal oxygen consumption rates using a closed six-channel dissolved oxygen measuring system (Strathkelvin 929 System, North Lanarkshire, U.K.) connected to a computer. The oxygen electrodes were calibrated against a saturated solution of sodium sulphite in 0.01 m sodium borate (0% O₂) and a 100% O₂-saturated nutrient solution at 15 °C. Leaf discs were incubated in 3-mL chambers filled with a sterile 100% O₂-saturated nutrient solution, stirred with a magnetic bar, and kept at 15 °C by circulation of water originating from a temperature-controlled water bath.

Additional chambers without leaf discs were used as controls. After a 1-h trial, leaf discs were enclosed in small sterile zip lock bags and promptly frozen at −20 °C for later DM determination and ergosterol extraction. Oxygen consumption rates were determined by the difference in the O₂ concentration in the sample and the control (chamber with no leaf discs) over a 20-min interval during which O₂ consumption over time was linear, corrected for chamber volume, time and disc mass. Results were expressed as mg O₂ g⁻¹ leaf DM h⁻¹.

**Mycelial biomass.** The three subsets of five leaf discs from each microcosm, frozen after the respirometry trial, were lyophilised, promptly weighed (±0.01 mg) to determine DM, and used for ergosterol extraction as a surrogate for mycelial biomass (Gessner & Chauvet, 1993; Graça et al., 2005). Lipid extraction and saponification were carried out in 10 mL KOH/methanol (8 g L⁻¹) at 80 °C for 30 min. The extract was then purified by solid phase extraction (Waters Oasis HLB 3-cc cartridges; Waters Corp., Milford, MA, U.S.A.; Graça et al., 2005) and quantified with high-performance liquid chromatography (HPLC; Dionex DX-120, Sunnyvale, CA, U.S.A.) by measuring absorbance at 282 nm. The HPLC system was equipped with a LiChroCART 250-4 LiChrospher 100 RP-18 (5 μm) column (Merck, Darmstadt, Germany) maintained at 33 °C. The mobile phase was 100% methanol, and the flow rate was set to 1.4 mL min⁻¹. Fungal biomass was estimated from ergosterol using either specific ergosterol–fungal DM conversion factors (Articulospora, 4.2 mg ergosterol g⁻¹ fungal DM; Anguillospora, 10.9; Clavariopsis, 4.6; Tetracladium, 4.6; Tetracladium, 4.7) or a general 5.5 mg ergosterol g⁻¹ fungal DM.
conversion factor (Tricladium; no specific conversion factors were available) (Gessner & Chauvet, 1993; Suberkropp, Gessner & Chauvet, 1993), applied to the relative contribution of each species to total ergosterol mass based on specific relative contribution to total reproductive output. Results were expressed as mg mycelial biomass g⁻¹ leaf DM.

Consumption test. Here, we tested whether changes in the composition of aquatic hyphomycete assemblages, identity of the dominant species and leaf thickness would influence the nutrient concentration of decomposing leaf litter. We also evaluated whether this variation in nutrient concentrations affected the feeding activity of shredders by offering leaf discs inoculated in the 20 microcosms considered above (two thickness levels × two assemblage types × four dominance types + four sterile controls) to the detritivore Schizopelex festiva.

Similar-sized, early-instar larvae were collected from depositional areas in Ribeira de Múceres, Serra do Caraça (central Portugal; 40°32'01" N, 08°09'15" W; altitude 210 m a.s.l), during winter 2010, and transported to the laboratory in plastic containers with stream water and sand. Individuals were kept in plastic containers with stream sand and aerated stream water, fed ad libitum chestnut litter conditioned in the stream for 2 weeks and acclimated at 15 °C under a 12-h light:12-h dark photoperiod for 2 weeks before being used. The diameter of the case opening (CO, mm) of each individual was measured under a stereoscopic microscope at 16 × (Wild M38, Heerbrugg, Switzerland), at the beginning of the experiment, and individual DM (g) was estimated by the application of the regression model DM = 0.0002 × CO².068 (r² = 0.92, P < 10⁻³, n = 30). For the feeding experiment, the invertebrates were allocated to plastic containers (7 cm diameter × 8.5 cm high), with a standard amount (two tea spoons; ~19 g) of ashed stream sediment (<0.5 mm; 550 °C, 8 h) covering the bottom, and 200 mL of filtered stream water (47 mm Ø, pore size 0.7 μm, Millipore APFF04700; Millipore Corp.), aerated for the duration of the experiment (Graca et al., 2005). One pre-measured S. festiva larva was allocated to each of 300 plastic containers (20 treatments × 15 replicates) and allowed access to three conditioned leaf discs, while three other leaf discs were enclosed in a fine mesh bag that hung from the plastic containers’ edge and served as a control for mass loss due to leaching and microbial activity. After 48 h, when remaining litter in most containers was <50% of initial disc area, the remaining litter mass was recovered, oven-dried (105 °C; 24 h) and weighed (±0.01 mg). The litter DM eaten by the larva during the elapsed time (Lₑ, g) was calculated as Cₑ–Eₑ, where Cₑ is the final DM (g) of control litter discs and Eₑ is the final DM (g) of litter exposed to the larva (this assumes that control and exposed litter had the same initial mass after the conditioning step). Relative consumption rates (g leaf DM g⁻¹ individual DM h⁻¹) were calculated as Lₑ/(DMₑ × t), where DMₑ is the invertebrate initial DM (g) and t is the time elapsed (h).

Data analysis. Phosphorus and N concentrations of initial and autoclaved oak leaf discs, and initial SLA, were compared between thin and thick discs by t-tests. A three-way factorial ANOVA was used to assess differences in mycelial biomass, respiration rate and percentage of leaf mass loss at the end of the conditioning period and relative consumption rate of S. festiva larvae among treatments [leaf thickness (i.e. Thin or Thick), assemblage type (i.e. Early or Late) and dominance treatment as the main effects] (Zar, 1996). When significant differences were detected among treatments, Tukey’s HSD tests were then carried out for post hoc pairwise comparisons. No statistical analysis was performed on total conidial production and conidial biomass production because there was only one value per microcosm. The relationships between the specific relative conidial production and conidial biomass production after 2 weeks of incubation and the specific relative conidial abundance in the inocula were assessed across treatments within each assemblage type by linear regression. The relationship between mycelial biomass (expressed as log-transformed data) across dominance treatments and relative consumption rate of S. festiva larvae was assessed using simple linear regression analysis. Similarly, relationships between mycelial biomass across dominance treatments and relative consumption rate of S. festiva larvae and elemental composition ratios of leaf litter were examined with simple linear regression analyses. Data were log-transformed to improve homoscedasticity when necessary. All analyses were performed with STATISTICA 6 software (StatSoft Inc., 2001). For all analyses, a significance threshold of P = 0.05 was used.

Results

Initial litter chemical composition, toughness and specific leaf area

The thinnest leaf discs (mass classes 3.30–3.40, 3.40–3.50; and 3.50–3.60, 3.60–3.70 mg) had on average 10.6%
higher initial P concentration than leaf discs exhibiting the highest thickness (mass classes 3.90–4.00, 4.00–4.10 mg; and 4.10–4.20, 4.20–4.30 mg), although the difference was not significant (Table 1; \( P = 0.23 \)). Leaching by autoclaving affected thick and thin leaf discs differently, with greater losses in P concentration observed for the thinnest leaf discs (52.2%) compared with the thickest ones (29.6%; Table 1). Although leaching increased the relative difference in P concentration between the two sets of leaf discs to 24.9%, this still remained non-significant (Table 1, \( P = 0.11 \)). Leaf disc thickness did not significantly affect initial N concentration (\( P = 0.08 \)) in spite of a relative difference of 11.2% between thickness levels (Table 1). However, leaching by autoclaving significantly increased the relative difference in N concentration between the two sets of leaf discs to 19.7% (Table 1; \( P = 0.01 \)). Carbon concentrations before and after autoclaving were similar between thin and thick leaves (Table 1, <0.3% difference; \( P = 0.38 \) and 0.55, respectively). Based on a set of leaf discs randomly chosen, disc toughness was negatively related to SLA (linear regression, \( P < 0.001, r^2 = 0.27, n = 96 \), leaf disc mass from 2.2 to 6.7 mg or leaf disc SLA from 51.88 to 16.96 mm² mg⁻¹). However, the relationship remained weak in spite of a large difference between the extreme leaf disc mass or SLA values.

**Aquatic hyphomycete assemblages.** Overall, conidial biomass production from early assemblages was consistently higher than that from late assemblages, whatever the disc thickness (i.e. 1.4 and 1.5 times higher in the thin and thick discs, respectively; Table 2). Manipulation of the specific number of conidia inoculated into the microcosms allowed us to anticipate the identity of the dominant species in the *Anguillospora* (Early assemblage), *Clavariopsis* (Late assemblage) and *Tetracladium*

### Table 1 Phosphorus (P), nitrogen (N) and carbon (C) concentrations (mean ± 1 SE) of initial and autoclaved thin and thick oak leaf discs. Grey-shaded text shows early assemblages and unshaded late assemblages.

<table>
<thead>
<tr>
<th>Thickness</th>
<th>Litter state</th>
<th>P (% DM)</th>
<th>N (% DM)</th>
<th>C (% DM)</th>
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<tbody>
<tr>
<td>Thin</td>
<td>Initial non autoclaved</td>
<td>0.027 ± 0.000</td>
<td>0.86 ± 0.01</td>
<td>46.72 ± 0.09</td>
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<td>Initial autoclaved</td>
<td>0.011 ± 0.002</td>
<td>0.87 ± 0.07</td>
<td>47.44 ± 0.15</td>
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<td>0.93 ± 0.06</td>
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<tr>
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<table>
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<tr>
<th>Thickness</th>
<th>Total conidial production (µg mg⁻¹ DMi)</th>
<th>Anguillospora (%)</th>
<th>Tetracladium (%)</th>
<th>Tricladium (%)</th>
<th>Articulospora (%)</th>
<th>Clavariopsis (%)</th>
<th>Tetracladium (%)</th>
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<tr>
<td>Thin</td>
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</table>

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(Late assemblage) treatments for both thickness levels. Early assemblages were dominated by Anguillospora (Table 2), even when this species was represented by only 20% conidia in the initial inocula (see Methods). Conidial biomass production by Tetrachaetum across treatments was also not related to its abundance in the inocula ($r^2 = 0.37; P = 0.11$), and Tetrachaetum generally codominated with Anguillospora (Table 2). Tricladium conidial production across treatments was related to its initial abundance ($r^2 = 0.93; P < 0.001$), but was the species contributing the least to the total conidial pool within each treatment. Late assemblages were dominated by Tetrachaetum, except for Clavariopsis treatments where Clavariopsis dominated. Conidial biomass production by Tetrachaetum across treatments was related to its abundance in the inocula ($r^2 = 0.81; P < 0.01$). Articulospora was consistently outcompeted by the other two species, even in the Articulospora treatments. Nevertheless, each of the three species tended to produce relatively more conidial biomass in the microcosms where they were inoculated with the highest number of conidia (Table 2).

**Litter mass loss.** Mass loss of oak leaf discs after incubation in microcosms for 2 weeks varied between 13.2% (Thin, Early assemblage, Even) and 49.7% (Thin, Late assemblage, Tetrachaetum). Interestingly, leaf decomposition by late assemblages was much faster than that promoted by early assemblages, in both leaf thickness levels ($P < 0.001$; Tables 3 & 4). Decomposition rate was affected by species dominance identity within thickness level (i.e. Thin and Thick; $P < 0.006$) but also by dominant species identity within assemblage type (i.e. Early versus Late; $P < 0.001$; Tables 3 & 4). Mass loss was negatively affected by litter thickness ($P < 0.001$, Tables 3 & 4).

Fungal biomass, respiration and leaf litter quality for consumers. After 2-week conditioning, fungal biomass on leaf discs varied between 83 (Thin, Early assemblage, Anguillospora) and 389 mg g$^{-1}$ leaf DM (Thin, Late assemblage, Articulospora) (Table 3). Higher fungal biomass accrual (~three times) was obtained in microcosms where leaf discs were inoculated with late assemblages ($P < 0.001$; Tables 3 & 4), with the difference being more pronounced between assemblage types in the thin than in the thick discs ($P < 0.001$; Tables 3 & 4). Interestingly, the increase in disc thickness led to a significant increase in fungal biomass in microcosms with early assemblages, whereas it resulted in a decrease in fungal biomass with late assemblages ($P < 0.001$; Table 4). Mycelial biomass accrual was affected by changes in identity of the dominant species: thin discs in microcosms inoculated with Articulospora as the dominant species had higher values than thin discs in microcosms inoculated with Anguillospora or Tetrachaetum as the dominant species (Table 3).

Table 3 Mycelial biomass, fungal activity, dry mass remaining and elemental composition (mean ± 1 SE) of oak leaf discs at two leaf thickness levels and two assemblage types for the four dominance types over 2 weeks. Grey-shaded text shows early assemblages and unshaded late assemblages. Treatments with the same letter are not significantly different (Tukey’s HSD, $P > 0.05$). Fungal species: Anguillospora filiformis (Anguillospora), Articulospora tetractia (Articulospora), Clavariopsis aquatica (Clavariopsis), Tetrachaetum elegans (Tetrachaetum), Tetrachaetum marchalium (Tetrachaetum) and Tricladium chaetocladium (Tricladium).

<table>
<thead>
<tr>
<th>Thickness type</th>
<th>Mycelial biomass (mg g$^{-1}$ leaf DM)</th>
<th>Fungal activity (mg O$_2$ g$^{-1}$ DM h$^{-1}$)</th>
<th>DMr (%)</th>
<th>P (% DM)</th>
<th>N (% DM)</th>
<th>C (% DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin Even</td>
<td>101 ± 9a</td>
<td>0.71 ± 0.04a</td>
<td>86.76 ± 0.56e</td>
<td>0.106 ± 0.006abc</td>
<td>1.68 ± 0.02ab</td>
<td>48.40 ± 0.09a</td>
</tr>
<tr>
<td>Articulospora</td>
<td>83 ± 9a</td>
<td>0.82 ± 0.07ab</td>
<td>81.89 ± 1.02de</td>
<td>0.097 ± 0.007ab</td>
<td>1.84 ± 0.06ab</td>
<td>47.99 ± 0.39a</td>
</tr>
<tr>
<td>Tetrachaetum</td>
<td>86 ± 3a</td>
<td>0.86 ± 0.02abc</td>
<td>80.95 ± 1.69de</td>
<td>0.093 ± 0.000a</td>
<td>1.72 ± 0.11ab</td>
<td>46.86 ± 0.95a</td>
</tr>
<tr>
<td>Tricladium</td>
<td>104 ± 9a</td>
<td>1.09 ± 0.10abcd</td>
<td>80.54 ± 1.71d</td>
<td>0.138 ± 0.007efg</td>
<td>1.89 ± 0.06ab</td>
<td>50.30 ± 2.52a</td>
</tr>
<tr>
<td>Even</td>
<td>296 ± 5def</td>
<td>1.36 ± 0.07cdef</td>
<td>50.95 ± 0.38a</td>
<td>0.135 ± 0.004efg</td>
<td>1.95 ± 0.07ab</td>
<td>47.64 ± 0.13a</td>
</tr>
<tr>
<td>Articulospora</td>
<td>389 ± 21g</td>
<td>1.82 ± 0.16f</td>
<td>53.43 ± 2.10ab</td>
<td>0.155 ± 0.002g</td>
<td>1.87 ± 0.03ab</td>
<td>48.23 ± 0.09a</td>
</tr>
<tr>
<td>Clavariopsis</td>
<td>346 ± 15fg</td>
<td>1.51 ± 0.13ef</td>
<td>52.39 ± 0.83a</td>
<td>0.139 ± 0.003efg</td>
<td>1.46 ± 0.32a</td>
<td>40.42 ± 5.99a</td>
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<tr>
<td>Tetrachaetum</td>
<td>318 ± 10ef</td>
<td>1.41 ± 0.11def</td>
<td>50.31 ± 0.78a</td>
<td>0.132 ± 0.010def</td>
<td>1.93 ± 0.12ab</td>
<td>48.30 ± 0.46a</td>
</tr>
<tr>
<td>Thick Even</td>
<td>88 ± 15a</td>
<td>1.01 ± 0.07abcde</td>
<td>77.81 ± 0.36d</td>
<td>0.108 ± 0.002abcd</td>
<td>1.47 ± 0.19a</td>
<td>47.68 ± 0.72a</td>
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<tr>
<td>Articulospora</td>
<td>114 ± 9a</td>
<td>1.01 ± 0.02abcde</td>
<td>76.82 ± 0.34d</td>
<td>0.106 ± 0.003abc</td>
<td>1.80 ± 0.12ab</td>
<td>48.28 ± 0.37a</td>
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<tr>
<td>Tetrachaetum</td>
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<td>0.94 ± 0.04abcde</td>
<td>80.91 ± 0.68de</td>
<td>0.095 ± 0.004ab</td>
<td>1.50 ± 0.23a</td>
<td>44.51 ± 3.95a</td>
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<td>Tricladium</td>
<td>134 ± 4a</td>
<td>1.14 ± 0.09abcde</td>
<td>81.50 ± 2.08de</td>
<td>0.124 ± 0.004def</td>
<td>1.93 ± 0.06ab</td>
<td>48.15 ± 0.30a</td>
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<td>Even</td>
<td>284 ± 7cde</td>
<td>1.23 ± 0.03bcde</td>
<td>59.07 ± 0.27bc</td>
<td>0.138 ± 0.005efg</td>
<td>1.78 ± 0.17ab</td>
<td>48.03 ± 0.24a</td>
</tr>
<tr>
<td>Articulospora</td>
<td>238 ± 5bc</td>
<td>1.82 ± 0.21f</td>
<td>65.12 ± 0.40c</td>
<td>0.145 ± 0.005fg</td>
<td>2.24 ± 0.19b</td>
<td>50.45 ± 2.84a</td>
</tr>
<tr>
<td>Clavariopsis</td>
<td>261 ± 7bce</td>
<td>1.13 ± 0.09abcde</td>
<td>63.79 ± 1.69c</td>
<td>0.119 ± 0.005abcde</td>
<td>2.07 ± 0.11ab</td>
<td>47.51 ± 0.88a</td>
</tr>
<tr>
<td>Tetrachaetum</td>
<td>228 ± 18bc</td>
<td>1.22 ± 0.10abcde</td>
<td>52.31 ± 0.93a</td>
<td>0.120 ± 0.002bdcde</td>
<td>1.83 ± 0.02ab</td>
<td>47.94 ± 0.24a</td>
</tr>
</tbody>
</table>
Fungal respiration after incubation for 2 weeks was strongly affected by changes in identity of the dominant species in the inoculum \( (P = 0.001; \text{Table 4}) \) and varied between 0.71 mg O\(_2\) g\(^{-1}\) C \( \text{DM h}^{-1}\) (Thin, Early assemblage, Even) and 1.82 mg O\(_2\) g\(^{-1}\) C \( \text{DM h}^{-1}\) (Thin, Late assemblage, Articulospora) and Thin, Late assemblage, Articulospora. Overall, leaf litter thickness did not affect respiration rate \( (P = 0.84; \text{Tables 3 & 4}) \), and higher values for fungal respiration were consistently obtained in microcosms inoculated with late assemblages \( (P < 0.001; \text{Table 3}) \). As previously shown for fungal biomass, the increase of leaf litter thickness led to a significantly higher fungal respiration in microcosms with early assemblages, conversely to late assemblages \( (P = 0.002; \text{Table 4}) \). Fungal respiration showed a significant positive correlation with mycelial biomass \( (r^2 = 0.66; \ P < 0.001) \).

Leaf litter conditioned by different fungal assemblages resulted in different N and P concentrations on leaves, with a tendency for higher concentrations when leaf discs were inoculated with the late assemblage \( (P < 0.001 \text{ for P, } P = 0.03 \text{ for N}) \).

Consumption of leaf discs by a stream detritivore

Larval mortality during the short-term feeding assay was very low (1.0%). Larval consumption rate averaged 0.071 g leaf DM g\(^{-1}\) ind DM h\(^{-1}\) across treatments, but there were clear differences between disc thickness levels, fungal assemblages and dominance.

Fig. 3 Relative consumption rate (RCR) of Schizopelex festiva caddisfly larvae for thin and thick oak leaf discs, for each fungal assemblage type and species dominance treatment. Grey bars represent early assemblages, and open bars represent late assemblages. Treatments within each thickness level were compared with three-way ANOVA, followed by Tukey’s HSD; different letters indicate significant differences among treatments \( (P < 0.05) \). Fungal species: Anguillospora filiformis (Anguillospora), Articulospora tetracladia (Articulospora), Clavariopsis aquatica (Clavariopsis), Tetrachaetum elegans (Tetrachaetum), Tetracladium marchalianum (Tetracladium) and Tricladium chaetocladium (Tricladium).
types (Table 4). The relative consumption rate of caddisfly larvae was two-three times higher on late than on early assemblages for both thickness levels. Interestingly, for both thin and thick discs, species dominance identity was the prime factor affecting larval consumption within early but not within late assemblages (Fig. 3). In parallel, fungal biomass ($r^2 = 0.71; \ P < 0.001$) and fungal activity measured as oxygen consumption ($r^2 = 0.62; \ P < 0.001$) consistently explained the relative consumption rate of caddisfly larvae (Figs 3 & 4; Table 3).

**Relationship between leaf litter quality and invertebrate consumption rate**

There was a strong and positive relationship between relative consumption rate of *S. festiva* larvae and mycelial biomass across all treatments ($r^2 = 0.90, \ P < 0.001$; Fig. 4). Caddisfly larvae consumption rate and mycelial biomass were also related to litter P concentration ($r^2 = 0.63, \ P < 0.001$ and $r^2 = 0.63, \ P < 0.001$, respectively), with P concentration differing between thickness and fungal assemblage treatments by 40% at most. In contrast, caddisfly larvae consumption rate and mycelial biomass were not significantly related to litter N concentration ($r^2 = 0.23, \ P = 0.06$ and $r^2 = 0.07, \ P = 0.31$, respectively), even though N concentration differed between thickness and fungal assemblage treatments by as much as 35%. Caddisfly larvae consumption rate and mycelial biomass were strongly related to C/P leaf litter ratio ($r^2 = 0.71, \ P < 0.001$ and $r^2 = 0.72, \ P < 0.001$, respectively; Figs 5a, 6a). In contrast, although being significantly related to caddisfly larvae consumption rate ($r^2 = 0.39, \ P = 0.01$; Fig. 5b), C/N leaf litter ratio was not related to mycelial biomass ($r^2 = 0.22, \ P = 0.07$; Fig. 6b). Caddisfly larvae consumption rate and mycelial biomass were weakly related to N/P leaf litter ratio ($r^2 = 0.26, \ P = 0.04$ and $r^2 = 0.38, \ P < 0.01$, respectively; Figs 5c, 6c).

**Discussion**

Changes in community structure as an early outcome of anthropogenic induced stresses are expected to precede a reduction in species richness (Ellison *et al.*, 2005; Hillebrand, Bennett & Cadotte, 2008). Recent laboratory experiments and field studies have shown that changes in dissolved nutrient concentration and temperature induce shifts in fungal species abundance (Dang *et al.*, 2005; Artigas, Romaní & Sabater, 2008; Ferreira & Chauvet, 2011). While these studies have suggested that changes in abundance of particular species could strongly alter nutrient transfer efficiency in food webs, the relationship between leaf litter elemental composition and changes in associated fungal community structure has not been investigated. Thus, it is relevant to assess whether these changes have the potential to affect ecosystem processes. Here, we show how changes in decomposing litter quality, due solely to differences in the species composition of the fungal assemblage, can affect the feeding activity of a stream detritivore, which might ultimately affect its growth and survival (Arsuffi & Suberkropp, 1986; Chung & Suberkropp, 2009). Our data suggest that changes in assemblage structure have a strong influence on the feeding activity of *S. festiva* caddisfly larvae through changes in fungal biomass accumulation and on the elemental composition of its resource.

Manipulation of the relative abundances of fungal species inoculated did not determine the identity of the dominant species in all fungal assemblages, but only in six of 16. Nevertheless, we obtained sixteen different fungal assemblages that differed in the relative proportion of each species. The strong discrepancies between the specific relative conidial production over the incubation time and that initially present in the inoculum might be attributed to differences in conidial traits (e.g. shape and size) likely to influence the probability of their settlement and germination (Dang, Gessner &
Moreover, aquatic hyphomycete assemblage structure in our study was based on specific reproductive output, which might not necessarily reflect specific mycelial biomass in the

Fig. 5 Relative consumption rate (RCR) of *Schizopelex festiva* cadisfly larvae as a function of leaf litter (a) C/P, (b) C/N and (c) N/P molar ratios. Grey symbols represent early assemblages, and open circles represent late assemblages. Lines and corresponding $r^2$- and $P$-values are shown for simple linear regression.

Fig. 6 Leaf litter (a) C/P, (b) C/N and (c) N/P molar ratios as a function of mycelial biomass. Grey symbols represent early assemblages, and open circles represent late assemblages. Lines and corresponding $r^2$- and $P$-values are shown for simple linear regression.
leaf litter. A recurrent and unique problem with microbial systems is the difficulty of keeping track of individual species. Recent advances in molecular approaches, although prone to uncertainty, can circumvent such obstacles by allowing the quantification of species contribution to total fungal biomass (Bärlocher, 2007, 2010; Krauss et al., 2011).

Our study contrasts with other studies suggesting functional redundancy among aquatic hyphomycete assemblages (Bärlocher & Graça, 2002; Pascoal, Cássio & Marvanová, 2005; Ferreira, Gulis & Graça, 2006b; Ferreira & Chauvet, 2012). Conversely, they agree with studies that have demonstrated the important role played by the identity of dominant species in the relationship between community evenness and ecosystem processes (McKie et al., 2008; Gonçalves & Canhoto, 2009; Swan, Gluth & Horne, 2009).

Thereby, our results demonstrated that experimental changes in fungal community structure [i.e. assemblage type (Early versus Late), species identity and evenness] resulted in an increased nutritional quality of leaf litter which, in turn, translated into an increased proportion of leaf litter consumed by a detritivore. Changes in fungal community structure associated with leaf litter can alter the nutritional quality of leaf detritus via a number of mechanisms that are not mutually exclusive and that may act synergistically.

First, changes in fungal species composition can translate into an increased biomass and activity of fungi associated with leaf detritus, which are often differentially ingested by invertebrate detritivores (Suberkropp, 1992) or assimilated at a higher efficiency than non-living detritus (Graça, Maltby & Calow, 1993a). It is widely accepted that the degree of conditioning (usually defined by measures of microbial biomass or activity) is often the main factor affecting resource quality and constitutes the standard by which all types of shredders discriminate among leaves. The diversity of feeding patterns exhibited by shredders reported by numerous studies (e.g. Suberkropp et al., 1983; Suberkropp & Arsuffi, 1984; Butler & Suberkropp, 1986; Arsuffi & Suberkropp, 1989; Friberg & Jacobsen, 1994), with a few others reporting ambiguous or less well-defined patterns, suggests that this factor alone does not determine foraging behaviour. For example, there was no correlation between palatability and protein increase or mass loss (as indicator of overall enzymatic activity; Bärlocher & Kendrick, 1973). Graça, Maltby & Calow (1993b, 1994) reported that no leaf parameter was correlated with resource preference of Gammarus pulex in experiments where leaf discs were conditioned by different pure cultures of individual fungal species. Similarly, consumption by Gammarus tigrinus of leaf discs conditioned by six pure cultures of individual aquatic hyphomycete species was not correlated with leaf protein, phenols, lipids or ergosterol concentrations (Rong, Sridhar & Bärlocher, 1995).

Second, heterotrophic microorganisms contain high concentrations of nutrients (Sterner & Elser, 2002; Cross et al., 2005), which may directly affect the growth and production of consumers (Iversen, 1974; Urabe, Clasen & Sterner, 1997). Leaf litter nutrient concentration has been shown to increase shredder consumption rates in headwater streams (Iversen, 1974; Irons, Oswood & Bryant, 1988).

Regression analysis in our study indicated that leaf litter quality was affected by the amount of fungal biomass associated with the leaf discs. There was quite strong variation in fungal biomass, depending on different fungal assemblages (e.g. 4.7 times between the two extreme values). In line with previous studies, fungal colonisation led to nutrient immobilisation (Gulis & Suberkropp, 2003), which in our case translated into lower C/P ratios, without significantly affecting C/N ratios.

Interestingly, several studies that documented foraging preferences of caddisfly larvae (i.e. three families, five genera; Suberkropp et al., 1983; Arsuffi & Suberkropp, 1984; Suberkropp & Arsuffi, 1984; Suberkropp & Arsuffi, 1984; Arsuffi & Suberkropp, 1986) reported that all caddisfly species examined had similar preferences when offered leaf litter colonised by the same aquatic hyphomycete species. This similarity among trichopterans contrasts strongly with different ranked responses of different groups of detritivores (i.e. Amphipoda, Plecoptera, Diptera and Gastropoda) to leaves colonised by various fungi (Arsuffi & Suberkropp, 1989), and our findings suggest that stoichiometric imbalances between consumers and resources might affect consumer feeding activity.

Heterotrophic microorganisms also contain biochemicals, such as polyunsaturated fatty acids (PUFA) (Brett & Mueller-Navarra, 1997; Arce Funck et al., 2015), which are almost exclusively synthesised by autotrophic organisms. Animals can convert from one form of PUFA to another through elongation and desaturation, but very few can synthesise PUFA de novo. Accordingly, most consumers depend on PUFA supplied through their dietary intake to meet their physiological requirements (Arts, Brett & Kainz, 2009). Polyunsaturated fatty acids are essential for invertebrates owing to their involvement in a wide range of physiological processes, such as regulation of cell membrane properties or as precursors to many hormones (Brett & Mueller-Navarra, 1997).
Despite the pivotal role played by aquatic hyphomycetes as the main basal food sources in stream food webs, data on their lipid composition are extremely scarce (but see Cargill et al., 1985 and Arce Funck et al., 2015). Accordingly, further investigation in this direction might deepen our understanding of the role of aquatic hyphomycetes in forested headwater stream ecosystems.

Lastly, changes in dominance/evenness and/or identity of species among assemblages are likely to result in qualitative differences in enzymatic capabilities among species belonging to early- or late-successional stages and may alter leaf detritus degradation and, in turn, modulate the digestibility of detritus to consumers (Bärlocher, 1985; Sinsabaugh, Linkins & Benfield, 1985; Suberkropp, 1992). Based on a study by Güsewell & Gessner (2009), and given the N/P ratio of the nutrient solution adopted in our study (31.3 on a molar basis) and the overall nutrient supply used in our microcosms, we assume that the fungal assemblages were likely to be N-limited, constraining them to maintain N content in their own biomass at a basal metabolic level. This was apparent from the absence of a significant relationship between C/N leaf litter ratio and mycelial biomass. In contrast, P limitation for fungi is expected to be reached at higher N/P ratios than that used here (Güsewell & Gessner, 2009). Accordingly, the negative relationship between C/P leaf litter ratio and mycelial biomass among treatments was mainly due to P immobilisation by fungi, which might be related to an increase in fungal decomposition activity and biomass. In addition, fungi growing in P-unlimited conditions, as was the case here, might store P in excess in their own biomass, mainly in membranes or in vacuoles (Beever & Burns, 1980), thereby reducing C/P leaf litter ratios. It is important, however, to note that these results have been obtained with assemblages of heterotrophic decomposer assemblages solely composed of fungi. Hodge, Robinson & Fitter (2000) proposed that bacteria should dominate decomposition processes on substrates with low C/N ratios, while the contribution of fungi should prevail on substrates with high C/N ratios, due to the contrasting N requirements of these organisms (but see Hieber & Gessner, 2002). Similarly, fungi are known to have lower nutrient requirements and have lower metabolic activity than bacteria (Hieber & Gessner, 2002; Cross et al., 2005). Accordingly, the relative P requirements of fungi should be lower than those of bacteria (Smith, 2002), so that fungi are expected to become P-limited at higher N/P ratios than bacteria. Consequently, it is likely that the presence of bacteria in our experiment altered the observed patterns regarding the elemental composition of leaf detritus, potentially leading to changes in the feeding activity of shredder-mediated litter decomposition.

Relatively few studies have explored stoichiometric detritivore–resource relationships in running waters (Cross et al., 2003, 2005; Hladyz et al., 2009; Danger et al., 2012, 2013; Lauridsen et al., 2012, 2014). In this study, litter C/P was a better predictor of caddisfly larvae feeding performance than C/N or N/P, suggesting that P rather than N might have been limiting for these consumers. Accordingly, our results differ from those of Ferreira et al. (2010), which indicated that invertebrates prefer litter with higher N than P concentration, and also with the notion that litter N concentration generally determines invertebrate litter choice because it is often the limiting nutrient for primary consumers (Evans-White, Stelzer & Lamberti, 2005). In contrast, our results agree with the growth rate hypothesis, which proposes that consumer growth rather tends to be limited by the resource P concentration (Elser et al., 2003).

Organisms require a mixture of energy, vitamins, biochemicals and minerals to grow and reproduce. Imbalanced supply of one or more of these elements slows the growth of animals (Elser et al., 2000a, 2003; Frost & Elser, 2002) and alters their physiology, life history and behaviour (Sterner & Elser, 2002; Frost et al., 2005). While we did not assess the elemental body composition of our invertebrates, Cross et al. (2003) and Hladyz et al. (2009) found similar molar C/N ratios for shredders: 6.7 [mean for 11 shredder species including six genera: Leuctra and Tallaperla (Plecoptera), Tipula (Diptera), Lepidostoma and Pycnocnusche (Trichoptera), the mean also included multiple size classes for Tallaperla and Fattigia] and 6.7 [mean for four shredder species: Potonemura meyeri (Plecoptera), Gammarus duebeni (Amphipoda), Halesus radiatus (Trichoptera) and Potamophylax cingulatus (Trichoptera)] or 6.5 (mean for two Trichoptera species: Halesus radiatus and Potamophylax cingulatus), respectively, which suggests very narrow constraints on tissue N concentration across even distantly related and geographically separated taxa (Hladyz et al., 2009). Molar C/P ratios were also quite similar in both studies [498 and 487 (values from caddisfly larvae only) on average, respectively]. In accordance with the elemental body composition values of caddisfly shredders from the literature (Cross et al., 2003; Hladyz et al., 2009), our results show that C/N imbalances between S. festiva caddisfly larvae and oak leaf litter were relatively high (i.e. range from 4.0 to 5.7 times) and, therefore, consumers were particularly limited by N. In parallel, C/P imbalances between consumers and resources were relatively low when compared to C/N (i.e. range from 1.5
Meeting nutrient requirements is particularly challenging for leaf-shredding invertebrates because their food resources exhibit a dramatic imbalance from their body composition (Cross et al., 2003, 2005). According to the principles of ecological stoichiometry, the nutritional requirements of a given consumer are defined by its nutrient concentration and relative growth rate (Sterner & Elser, 2002; Cross et al., 2003). Consequently, consumers (or specific life stages) with high body N or P concentration and high growth rates require food that is high N or P, to maintain optimal growth (Cross et al., 2003). Here, we show that the higher sensitivity regarding food P rather than N content in S. festiva caddisfly larvae may be explained by its early life stage. High growth rates during early stages of development are correlated with a high content of (P-rich) ribosomal RNA and associated high body P demand (Elser et al., 2000b). These results are in accordance with predictions of ecological stoichiometry and have considerable empirical support, as underlined by Cross et al. (2003), who suggested that differences in ontogeny, life history strategy and relative allocation of structural biomolecules may all contribute to changes regarding the nutritional requirements of a given consumer.

In conclusion, we demonstrated that changes in fungal community structure affected the elemental composition of leaves, which significantly affected the feeding performance of S. festiva caddisfly larvae. Stimulated (Late assemblages) or reduced (Early assemblages) feeding activities of caddisfly larvae were related to changes in aquatic hyphomycete community structure. We showed that microbial identity can regulate leaf litter decomposition. Changes in consumption rates can potentially alter growth and mortality rates, and body elemental composition, and therefore influence shredder population dynamics, which will directly affect the processes in which these organisms are involved (e.g. litter decomposition, nutrient cycling). The relationship between fungi and invertebrates under changing environments remains to be explored in further studies to identify cascading effects on aquatic food-web structure and related ecosystem processes.

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Author contribution

J.C., V.F., A.L.G., E.C. and C.C. designed the experiments; J.C., V.F and A.L.G. carried out the experiments; and J.C., V.F., A.L.G., E.C. and C.C. wrote the manuscript.

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