Trophic complexity enhances ecosystem functioning in an aquatic detritus-based model system

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Summary

1. Understanding the functional significance of species interactions in ecosystems has become a major challenge as biodiversity declines rapidly worldwide. Ecosystem consequences arising from the loss of diversity either within trophic levels (horizontal diversity) or across trophic levels (vertical diversity) are well documented. However, simultaneous losses of species at different trophic levels may also result in interactive effects, with potentially complex outcomes for ecosystem functioning.

2. Because of logistical constraints, the outcomes of such interactions have been difficult to assess in experiments involving large metazoan species. Here, we take advantage of a detritus–based model system to experimentally assess the consequences of biodiversity change within both horizontal and vertical food-web components on leaf-litter decomposition, a fundamental process in a wide range of ecosystems.

3. Our concurrent manipulation of fungal decomposer diversity (0, 1 or 5 species), detritivore diversity (0, 1 or 3 species), and the presence of predatory fish scent showed that trophic complexity is key to eliciting diversity effects on ecosystem functioning. Specifically, although fungi and detritivores tended to promote decomposition individually, rates were highest in the most complete community where all trophic levels were represented at the highest possible species richness. In part, the effects were trait-mediated, reflected in the contrasting foraging responses of the detritivore species to predator scent.

4. Our results thus highlight the importance of interactive effects of simultaneous species loss within multiple trophic levels on ecosystem functioning. If a common phenomenon, this outcome suggests that functional ecosystem impairment resulting from widespread biodiversity loss could be more severe than inferred from previous experiments confined to varying diversity within single trophic levels.

Key-words: aquatic hyphomycetes, biodiversity and ecosystem functioning, detritivores, fungi, litter decomposition, shredders, stream, trophic cascade

Introduction

What 'species do in ecosystems' (Lawton 1994) has become a leading theme of ecological inquiry. Central to this development have been investigations into ecosystem consequences arising from species interactions within trophic levels (Hooper *et al.* 2012). This research is generally conducted under the label of biodiversity effects on ecosystem functioning (Hillebrand & Matthiessen 2009; Reiss *et al.* 2009; Loreau 2010; Cardinale *et al.* 2012), motivated in part by the current rapid species loss worldwide (Dudgeon *et al.* 2006; Naeem, Duffy & Zavaleta 2012) and its repercussions for the human benefits afforded by

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ecosystems with diverse communities (Cardinale *et al.* 2012). Although initially focused on terrestrial plant diversity and primary productivity (e.g. Isbell *et al.* 2011), such relationships have now been documented for a wide range of ecosystem properties and community types (Stachowicz, Bruno & Duffy 2007; Scherber *et al.* 2010; Cardinale *et al.* 2011), including aquatic and terrestrial detritus-based systems in which leaf litter decomposition is a central process (Srivastava & Bell 2009; Gessner *et al.* 2010; Cardinale *et al.* 2012).

Similar attention has been given to the concept of trophic cascades (Baum & Worm 2009; Terborgh & Estes 2010) and their repercussions for ecosystem functioning. Trophic cascades refer to the phenomenon that top-predators suppress the biomass of intermediate consumers, which in turn releases consumers at the next lower trophic level from predation pressure, with this alternate suppression-and-release effect propagating down to the producer level. As originally formulated, trophic cascades operate over entire trophic levels (Hairston, Smith & Slobodkin 1960), but this stringent condition was later relaxed as it became clear that the concept is also useful to describe trophic relationships between individuals or clusters of species interacting across trophic levels in a cascading fashion (Knight *et al.* 2005; Mooney *et al.* 2010; Terborgh & Estes 2010). In addition, the concept has been expanded to encompass behaviourally mediated cascades (Schmitz 2008) and ecosystem-level effects other than biomass suppression at alternate trophic levels (Croll *et al.* 2005; Rasher & Hay 2010). This has led to a rich conceptual framework (Kéfi *et al.* 2012), and compelling evidence for the existence of trophic cascades in a wide variety of biological communities and ecosystems (Sommer 2008; Baum & Worm 2009; Terborgh & Estes 2010).

Investigations into trophic cascades and biodiversity effects on ecosystem functioning share the goal of gaining insight into the significance of species interactions for ecosystem properties. This notwithstanding, the two lines of research have proceeded largely independently, although there have been repeated calls to incorporate the vertical component of biodiversity across trophic levels into assessments of effects on ecosystem functioning arising from horizontal, or within-trophic level, biodiversity change (Petchey *et al.* 2004; Duffy *et al.* 2007; Hillebrand & Matthiessen 2009; Reiss *et al.* 2009; Gessner *et al.* 2010; Cardinale *et al.* 2012).

When trophic complexity has been addressed in previous biodiversity-ecosystem functioning experiments involving metazoans, it has been limited to either concomitant variation of both species richness within a single trophic level and food chain length (e.g. Wojdak 2005), or to species richness across two trophic levels (e.g. Gamfeldt, Hillebrand & Jonsson 2005). Results of the few published experiments show that resource diversity, as well as consumer presence and diversity, can change species interactions within-trophic levels and thereby modify

biodiversity effects on ecosystem processes, depending on consumer food selectivity or responses to predation (Duffy *et al.* 2007). However, consequences of biodiversity loss in more complex food webs involving metazoans have been poorly explored so far, owing to the logistic constraints associated with manipulating the large number of required experimental units.

Here we capitalize on a tractable, well characterized detritus based food web from forest streams to assess the extent to which interactions between horizontal and vertical components of biodiversity affect leaf litter decomposition. Detritus-based food webs in streams are amenable to diversity manipulations at all trophic levels, including basal resources (leaf litter), microbial decomposers (primarily aquatic hyphomycete fungi), detritivorous primary consumers (litter consuming arthropods) and predators. Using a realistic food-web configuration (Fig. 1a), in which species richness decreases with increasing trophic level (Petchey *et al.* 2004), we simultaneously manipulated
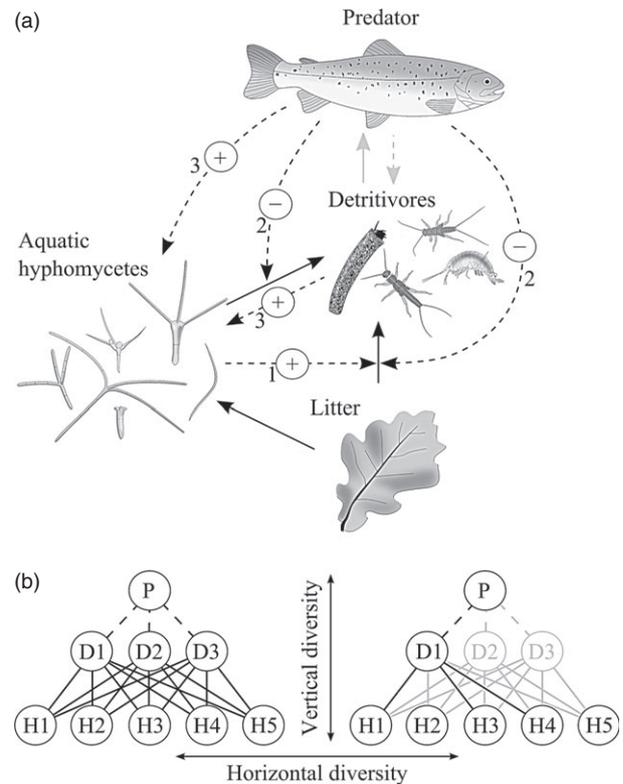

Fig. 1. Schematic of trophic (solid lines) and non trophic (dashed lines) interactions in detritus-based stream food webs. (a) Relationships between leaf litter, fungal decomposers, detritivores and predators as reflected in the experimental design of this study. Indirect interactions include (1) fungal facilitation of detritivores through leaf conditioning, (2) trait mediated reduction of detritivore feeding by predators, and (3) fungal stimulation through nutrient excretion by vertebrate and invertebrate consumers. Direct and indirect predation effects on detritivores (grey lines) are not addressed in this study. (b) Two of 234 food web configurations realized in this study, including the most diverse community (left), and a reduced food web with a single detritivore and three trophic links to fungal species (right). H1–H5: hyphomycete species, D1–D3: detritivore species, P: predator.

the species richness of both fungal decomposers and invertebrate detritivores, together with the presence or absence of predatory fish scent. We then used a statistical model to partition the effects arising from the presence of each trophic level, from richness within two of the three levels, and from the interaction of these factors. Our central hypothesis was that biodiversity effects on ecosystem functioning emerge, or grow stronger, when communities comprise multiple species at multiple trophic levels, because increasingly complex food webs increase the scope for species interactions and hence biodiversity effects to occur. Specifically, we hypothesized that (i) horizontal diversity effects depend on trophic structure, including the presence and/or diversity of higher and/or lower trophic levels, and (ii) these relationships are driven by the performance of detritivores, which can reach very high densities on decomposing leaf litter, and can greatly contribute to litter decomposition in streams (e.g. Hieber & Gessner 2002). We expected that detrivore performance will be affected by both the presence of predators (reflecting reduced feeding activity to minimize the risk of detection; Malmqvist 1993) and the presence and diversity of aquatic hyphomycete fungi (resulting in complementary selective feeding on a mosaic of patches on leaves colonized by distinct fungal species; Suberkropp 1992).

Materials and methods

We conducted this multitrophic biodiversity–decomposition experiment at the Moulis Experimental Ecological Station located in the Pyrenees, southwestern France. We simultaneously manipulated the species richness and composition of both fungal decomposers (all species of a pool of six in isolation, plus all six possible five-species combinations) and invertebrate detritivores (all of four species in isolation, plus all possible three-species combinations) (Fig. 1b). In addition, we crossed all fungal and detritivore combinations with a predator treatment where consumption was precluded (presence or absence of fish scent). Control treatments without fungi, detritivores or fish were also included. There were three replicates for each treatment combination, resulting in a total of 702 experimental units. We used aquatic microcosms consisting of plastic containers (11 × 8 × 4 cm) filled with 160 mL of decanted ($\geq$24 h), constantly aerated water from a nearby forest stream (Rémillassé stream). The microcosms were placed in a temperature-controlled room at 10 °C. Three pebbles of similar size (1.40 ± 0.43 cm^3 SD) were added to each microcosm to allow detritivores to seek shelter from predation risk.

Fungi and detritivores used in the experiment were collected from local populations. Recent single-spore isolates of six com-

mon species of aquatic hyphomycete (*Articulospora tetracladia* Ingold, *Clavariopsis aquatica* de Wildeman, *Flagellospora curvula* Ingold, *Heliscus lugdunensis* Saccardo et Thérry, *Tetrachaetum elegans* Ingold, and *Tetracladium marchalianum* de Wildeman), which co-occur on decomposing leaves in the region (Gessner et al. 1993), were obtained from freshly formed foam in stream riffles and maintained on 1% malt agar. Spores of these species were generated by submerging agar plugs from the leading edge of colonies in 40 mL of constantly aerated water. The resulting spore suspension was used to inoculate autoclaved oak (*Quercus robur* L.) leaf discs (10 mm diameter) submerged in 800 mL of constantly aerated and periodically renewed mineral nutrient solution (per liter 0.01 g KNO$_3$, 0.55 mg K$_2$HPO$_4$, 0.1 g CaCl$_2$, 0.01 g MgSO$_4$. 7H$_2$O, pH adjusted to 7). A total of approximately 90 000 fungal spores was added, corresponding to $\approx$18 000 spores of each of the five species in mixed communities or $\approx$90 000 spores of a single fungal species. Fungi were allowed to colonize the leaf disks for 35 days at 15 °C before batches of 6 discs from each fungal treatment were distributed to the microcosms. Spores of aquatic hyphomycetes produced during the colonization period were counted on 3 occasions, including after 35 days, to verify that all species developed in the experimental communities and no cross-contamination occurred. Forty mL of the spore suspensions were sampled for each species combination. Aliquots were filtered on membrane filters (SMWP, 5 μm pore size; Millipore, Bedford, MA, USA) and stained with 0.05% Trypan blue. The spores trapped on the filters were identified and counted under the microscope at × 200 (Gessner, Bärlocher & Chauvet 2003). All fungal treatments were successful, as indicated by sporulation of all inoculated species in all treatments and a complete lack of cross-contamination (Table S1).

Specimens of four species of detritivores (*Sericostoma personatum* Kirby and Spence, *Nemoura flexuosa–marginata* group Zwick, *Protonemura meyeri* Pictet and *Gammarus fossarum* Koch) were collected from three streams near the Ecological Station, kept in aquaria at 10 °C, and fed on naturally decomposing leaf litter collected in one of the above streams (Rémillassé). We placed six individuals of a single species (for each of the four detritivore species in single-species treatments) or two individuals each of three different species (for each of four detritivore species mixtures) in the microcosms. Throughout the experiment, any of the few dead or emerged individuals were replaced daily with similar-sized conspecifics.

Brown trout (*Salmo trutta fario* L.) of a local population were obtained from a nearby fish hatchery. Three individuals, one 2-yr-old and two yearlings, were placed in each of three 40-L aquaria (one per experimental block). Water in the aquaria was renewed daily to ensure similar water quality throughout the experiment (i.e. similar kairomone and nutrient concentrations; Table 1). Decanted stream water from the aquaria with fish was added to half of the microcosms at the beginning of the experiment to simulate the presence of predators while preventing

Table 1. Ion concentrations (mg L^{-1}) in water from aquaria with or without fish. Water was analysed by ion chromatography (Dionex DX–120; Dionex Corp., Sunnyvale, CA, USA) after 5 fold dilution (see Materials and methods) and after 24 h of fish exposure. Concentrations of PO$_4^{3-}$ and NO$_2^-$ were below 10 and 5 μg L^{-1}, respectively. Mean $\pm$ SD, N = 6 to 8

Treatment	N–NO$_3^-$	N–NH$_4^+$	S–SO$_4^{2-}$	Cl$^-$	Ca^{2+}	Mg^{2+}	K$^+$	Na$^+$
Fish	0.57 ± 0.004	0.56 ± 0.27	0.87 ± 0.03	1.85 ± 0.19	3.99 ± 0.21	1.54 ± 0.03	0.86 ± 0.16	3.10 ± 0.23
Control	0.58 ± 0.04	0.03 ± 0.02	0.86 ± 0.10	1.79 ± 0.22	4.06 ± 0.07	1.51 ± 0.03	0.69 ± 0.10	2.91 ± 0.07

actual predation on the detritivores. During the experiment, half of the water volume of the microcosms was renewed daily using water with or without fish scent in the fish presence and absence treatments, respectively. Water exposed to fish was diluted five-fold for this purpose. To avoid strong fluctuations in the composition of fish kairomones, which can vary with diet (e.g. Crowl & Covich 1990; Chivers, Wisenden & Smith 1996), fish were starved both during and 48 h before the experiment (i.e. for 178 h in total).

The experiment was stopped after 130 h when leaf mass loss approached 100% in the treatment experiencing the most rapid decomposition. Leaf discs and detritivores were collected, dried, and weighed to the nearest 0·01 mg. Leaf mass loss was determined as the difference between the average initial dry mass of batches of six leaf discs (29·7 ± 2·3 mg, mean ± SD; N = 30) and the final dry mass at the end of the experiment. Detritivore biomass was determined for each species in each microcosm, with average individual biomass subsequently calculated by dividing the total biomass of each species by the number of individuals for that species in each microcosm.

To assess variation in the responses of the detritivore species relative to their metabolic potential, we also calculated an index of detritivore performance that standardizes for differences in body mass (McKie et al. 2008). The index relates the observed leaf mass loss attributed to detritivores to their estimated metabolic capacity. It is based on the observation that large animals consume more food per capita than small ones but that the relationship between feeding rate and body size is not directly proportional. Metabolic capacity of the detritivores was calculated by assuming a power relationship between the body mass and feeding rate of the detritivores according to the metabolic theory of ecology (Brown et al. 2004). Thus, for a given microcosm, the metabolic capacity of the detritivores was determined as the sum, across all individuals and species, of the average individual body mass raised to the three-quarter power (Brown et al. 2004). Mortality during the experiment was accounted for by using the mean individual biomass, weighted according to the length of time an individual was most likely to have laid dead before replacement (i.e. 12 h). Finally, we calculated the ratio of the leaf mass loss attributed to detritivores to the metabolic capacity in each microcosm, where the leaf mass loss attributed to detritivores was calculated as the difference between the final leaf dry mass in microcosms with and without detritivores (for the same combination of fungal communities and predator presence or absence). The resulting index of detritivore performance is a standardized measure of whether and to what extent detritivore feeding was stimulated or inhibited (rates higher or lower than expected from the detritivore biomass and corresponding metabolic capacity) in microcosms differing in fungal diversity and predator presence or absence.

We performed an analysis of variance (ANOVA) on leaf mass loss with predator presence (P_P), detritivore community composition (D_C) and aquatic hyphomycete community composition (H_C) as factors, as well as species richness terms. For the latter, a priori contrasts were defined to distinguish between the effects of aquatic hyphomycete presence (H_P) and detritivore presence (D_P; by comparing the 0 vs. 1 species treatments), and aquatic hyphomycete species richness (H_R; comparing the 1 vs. 5 species treatments) and detritivore species richness (D_R; comparing the 1 vs. 3 species treatments). All richness effects were tested relative to variation among species combinations, which represent the

true replicates for the richness treatments, rather than among microcosms, by nesting the aquatic hyphomycete and detritivore species composition terms within fungal and detritivore richness, respectively (Schmid et al. 2002).

Our detritivore performance index was also analysed using a series of ANOVAs, to assess overall responses across detritivore treatments, and the responses of each detritivore single species treatment separately. As a first step, the effects of detritivore and aquatic hyphomycete richness, predator presence, and their interactions, were tested using the same ANOVA models as previously: Predator presence (P_P) was crossed with aquatic hyphomycete presence (H_P) as well as aquatic hyphomycete and detritivore richness (H_R and D_R), with aquatic hyphomycete composition (H_C) and detritivore composition (D_C) nested in the respective richness terms.

Additionally, four further analyses were performed focusing specifically on the detritivore single-species treatments, to evaluate the responses of each individual detritivore species (taken separately) to aquatic hyphomycete diversity and predator presence. This latter analysis thus included predator presence (P_P) crossed with aquatic hyphomycete presence (H_P) and richness (H_R), and aquatic hyphomycete composition (H_C) nested in richness. The presence/absence contrast in detritivore combinations (D_P) was not included in the latter analyses. We used Type I sums of squares in all ANOVAs, with response variables square root- or log-transformed to satisfy assumptions of normality and homoscedasticity. All statistics were performed using R version 2.12 (R Development Core Team 2011).

Results

LEAF MASS LOSS

Average leaf mass loss ranged from 0·71% to 93·6% with a mean of 29·9% ± 0·71 SE (Fig. 2). Community composition of detritivores and aquatic hyphomycetes ($P < 0.001$), but not species richness ($P = 0.74$ and 0·11,
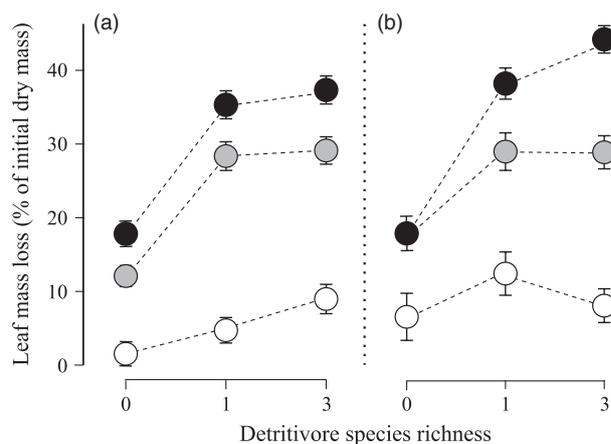
Fig. 2. Leaf mass loss as a function of aquatic hyphomycete and detritivore species richness. Mean percentage (± SE) of the initial dry mass in microcosms without aquatic hyphomycetes (○), with 1 aquatic hyphomycete species (◐) or 5 aquatic hyphomycete species (●) present, and with fish absent (a) or present (b).

Table 2. ANOVA results of square-root transformed data on leaf mass loss, testing for the effects of community composition and species richness at each of three trophic levels. Each richness (treatment) term is tested against the following composition (error) term, and error terms are tested against the residuals. E/F column indicates whether the factor is included as a factor (F) or error term (E)

Source of variation	E/F	d.f.	SS	P
Aquatic hyphomycete presence (H_P)	F	1	466·6	0·007
Aquatic hyphomycete species richness (H_R)	F	1	129·8	0·106
Aquatic hyphomycete community composition (H_C)	E	10	410·5	<0·001
Detritivore presence (D_P)	F	1	185·0	0·099
Detritivore species richness (D_R)	F	1	6·0	0·737
Detritivore community composition (D_C)	E	6	292·6	<0·001
$H_P \times D_P$	F	1	2·3	0·216
$H_P \times D_R$	F	1	<0·1	0·909
$H_R \times D_P$	F	1	5·8	0·052
$H_R \times D_R$	F	1	3·6	0·124
$H_C \times D_C$	E	92	136·8	0·002
Predator presence (P_P)	F	1	7·5	0·004
$H_P \times P_P$	F	1	3·9	0·038
$H_R \times P_P$	F	1	4·4	0·027
$D_P \times P_P$	F	1	0·6	0·399
$D_R \times P_P$	F	1	<0·1	0·946
$H_P \times D_P \times P_P$	F	1	0·6	0·428
$H_P \times D_R \times P_P$	F	1	9·0	0·002
$H_R \times D_P \times P_P$	F	1	6·3	0·009
$H_R \times D_R \times P_P$	F	1	1·6	0·181
$H_C \times D_C \times P_P$	E	108	94·7	0·690
Residuals	E	468	444·9	

respectively), explained a considerable portion of the overall variability (SS of main effects = 13·3 and 18·6%, respectively; Table 2), suggesting that decomposition rates were more strongly influenced by consumer community composition than by species richness. Among all main effects relating to presence–absence or species diversity, only aquatic hyphomycete ($P = 0.007$) and predator presence ($P = 0.004$) were significant, with higher leaf mass loss occurring when either group was present (+23·6% and +2·6%, respectively; Fig. 2). Although detritivore presence accounted for an average of 16·4% of total leaf mass loss, the effect was not significant ($P = 0.099$).

Multiple interactions involving both presence–absence and richness within trophic levels were apparent. In particular, there were several significant second-order interactions involving predator presence and both the presence and richness of aquatic hyphomycetes (Table 2). Further significant third-order interactions emerged (i) between aquatic hyphomycete diversity and the presence of predators and detritivores ($P = 0.009$), and (ii) between detritivore diversity and predator and aquatic hyphomycete presence ($P = 0.002$; Table 2). All these interactions increased decomposition rate with an increasing number of trophic levels (i.e. the presence–absence terms) and/or

within-trophic level species richness. Most strikingly, the highest decomposition rate (average mass loss of 43·9%) was observed when all trophic levels were present at the highest possible richness levels (Fig. 2).

DETRITIVORE PERFORMANCE

Detritivore performance (i.e. detritivore-mediated leaf decomposition in relation to the estimated metabolic capacity of the detritivore community [MC]; see Materials and methods) varied between 0 and 2·54 mg MC^{-1} (mean $\pm$ SE = 0·39 $\pm$ 0·01 mg MC^{-1}; Fig. 3). Species composition of detritivores and aquatic hyphomycetes explained a significant part of the observed variability ($P < 0.001$; SS of main effects = 1·9 and 17·6%, respectively; Table 3), as did the interaction between aquatic hyphomycete and detritivore species composition ($P = 0.005$, 7·9% of the total SS). Furthermore, the interaction between aquatic hyphomycete diversity and predator presence was highly significant ($P < 0.001$; Table 3), suggesting that detritivore performance was contingent on both resource diversity and the presence of predators. The latter interaction resulted in a negative effect of fish presence (decrease of 0·13 mg MC^{-1}) on detritivore performance for the single-species aquatic hyphomycete treatments, while it became positive (increase of 0·10 mg MC^{-1}) for diverse aquatic hyphomycete communities (Fig. 3).

Among the single-species detritivore treatments, the performance of *Gammarus* was lower (0·29 $\pm$ 0·03 mg MC^{-1}) than that of the three insect species (0·44 $\pm$ 0·04, 0·45 $\pm$ 0·06 and 0·42 $\pm$ 0·03 mg MC^{-1} for *Nemoura*, *Protonemura* and *Sericostoma*, respectively). Detritivore responses to predator presence and aquatic hyphomycete communities varied among species (Table 4; Fig. 4), with
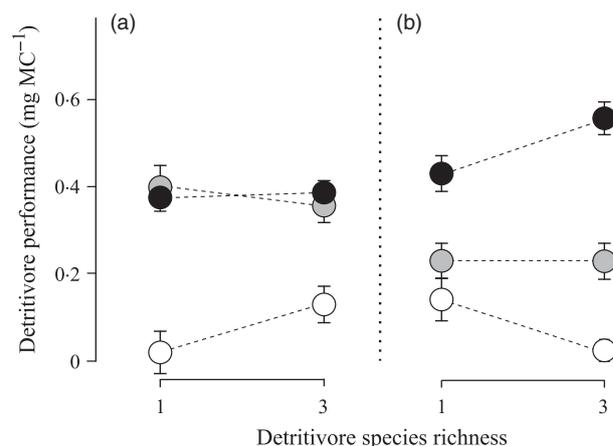

Fig. 3. Detritivore performance as a function of detritivore and aquatic hyphomycete species richness. Mean ($\pm$ SE; see Materials and methods for calculation of detritivore performance) in microcosms without aquatic hyphomycetes (○), with 1 aquatic hyphomycete species (◓) or 5 aquatic hyphomycete species (●) present, and with fish absent (a) or present (b).

Table 3. ANOVA results of log-transformed data on detritivore performance (see Materials and methods for calculation), testing for the effects of community composition and species richness at two trophic levels as well as predator presence. Each richness (treatment) term is tested against the following composition (error) term, and error terms are tested against the residuals. E/F column indicates whether the factor is included as a factor (F) or error term (E)

Source of variation	E/F	d.f.	SS	P
Aquatic hyphomycete presence (H_P)	F	1	1·92	0·114
Aquatic hyphomycete species richness (H_R)	F	1	1·71	0·135
Aquatic hyphomycete community composition (H_C)	E	10	6·44	<0·001
Detritivore species richness (D_R)	F	1	0·07	0·501
Detritivore community composition (D_C)	E	6	0·79	<0·001
$H_P \times D_R$	F	1	<0·01	0·736
$H_R \times D_R$	F	1	0·11	0·116
$H_C \times D_C$	E	82	3·63	0·005
Predator presence (P_P)	E	1	0·04	0·241
$H_P \times P_P$	F	1	<0·01	0·785
$H_R \times P_P$	F	1	1·10	<0·001
$D_R \times P_P$	F	1	0·04	0·317
$H_P \times D_R \times P_P$	F	1	0·15	0·047
$H_R \times D_R \times P_P$	F	1	0·03	0·410
$H_C \times D_C \times P_P$	E	98	3·76	0·039
Residuals	E	416	12·22	

interactions apparent for *Protonemura* ($H_R \times P_P$: $P = 0\cdot011$; Table 4), *Sericostoma* ($H_R \times P_P$: $P = 0\cdot015$; Table 4) and *Nemoura* ($H_R \times P_P$: $P = 0\cdot045$; Table 4). Specifically, *Protonemura* and *Nemoura* performance decreased in the presence of predators in the single-species treatments but not when five aquatic hyphomycete-species were present. In contrast, *Sericostoma* performance was unaffected by predator presence in the single-species aquatic hyphomycete treatments, and

increased when five aquatic hyphomycete species were present (Fig. 4). The performance of all detritivore species also varied with aquatic hyphomycete community composition (Table 4).

Discussion

The chief discovery emerging from our manipulation of a model detritus-based system is that the ecosystem process we targeted – litter decomposition – was most efficient when both horizontal (within-trophic level) and vertical (across trophic-level) diversity components were present. Diversity effects on decomposition rate did not primarily arise because of species interactions *within* a given trophic level, but reflected complex interactions between the presence and species richness of microbial decomposers, detritivorous invertebrates, and the non-trophic influence of a vertebrate predator. This experimental result supports outcomes of theoretical models suggesting that multitrophic interactions complicate assessments of the functional consequences of biodiversity change (Thébault & Loreau 2003, 2006), demonstrating that even relatively modest effects of change within single trophic levels can ramify and amplify through ecological networks to influence ecosystem functioning.

Unsurprisingly, the sheer presence of fungal decomposers strongly increased ecosystem process rates, reflecting their key role in leaf litter decomposition in streams and other ecosystems (Gessner *et al.* 2010). However, further increases in decomposition rate with increased fungal richness, although consistent (Fig. 2), were too small to be statistically significant unless predatory fish were present. This finding is in line with outcomes of previous biodiversity–decomposition experiments in the absence of detritivores where fungal diversity effects were weak or non-existent (Dang, Chauvet & Gessner 2005; Duarte *et al.* 2006). Increased detritivore richness

Table 4. ANOVA results of log-transformed data on detritivore performance (see Materials and methods for calculation), testing for the effects of aquatic hyphomycete community composition and diversity as well as predator presence in single-species detritivore treatments (*Gammarus*, *Nemoura*, *Protonemura* and *Sericostoma*). Each richness (treatment) term is tested against the following composition (error) term, and error terms are tested against the residuals. E/F column indicates whether the factor is included as a factor (F) or error term (E)

| Source of variation	E/F	d.f.	*Gammarus* SS	*Gammarus* P	*Nemoura* SS	*Nemoura* P	*Protonemura* SS	*Protonemura* P	*Sericostoma* SS	*Sericostoma* P
Aquatic hyphomycete presence (H_P)	F	1	0·11	0·228	0·40	0·184	0·24	0·238	0·17	0·232
Aquatic hyphomycete species richness (H_R)	F	1	0·04	0·451	0·35	0·206	<0·01	0·936	0·30	0·121
Aquatic hyphomycete community composition (H_C)	E	10	0·66	0·005	1·95	<0·001	1·55	0·031	1·03	<0·001
Predator presence (P_P)	F	1	0·02	0·331	0·21	0·011	0·02	0·555	0·04	0·026
$H_P \times P_P$	F	1	0·01	0·498	0·06	0·197	0·09	0·252	<0·01	0·823
$H_R \times P_P$	F	1	<0·01	0·731	0·15	0·045	0·46	0·011	0·05	0·015
$H_C \times P_P$	E	10	0·23	0·439	0·70	0·024	0·56	0·629	0·12	0·134
Residuals	E	52	1·17		1·58		3·64		0·39	
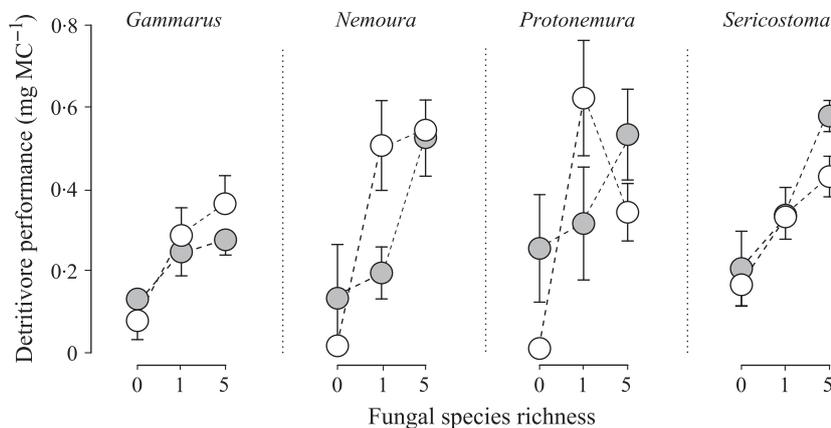
Fig. 4. Detritivore performance for each single-species treatment as a function of aquatic hyphomycete species richness. Mean (± SE; see Materials and methods for calculation of detritivore performance) in microcosm with fish present (●) or absent (○).

has more often yielded positive effects on decomposition in previous studies (McKie *et al.* 2008; Srivastava *et al.* 2009), though neutral or negative detritivore diversity effects have also been observed (Gessner *et al.* 2010). Differences in microbial diversity, which previous experiments with detritivores did not control for, could be an important factor accounting for the variable outcomes of other studies, given that detritivore diversity effects in our experiment were only apparent when microbial diversity was high (and fish were present). Two previous experiments have addressed the effects of varying fungal diversity in the presence and absence of detritivores, but neither was designed to test for interactive effects of fungal and detritivore diversity (Lecerf *et al.* 2005; Reiss *et al.* 2010). Nevertheless, in line with our results, both found generally stronger diversity effects (either positive or negative) when both trophic levels were present. This reinforces the conclusion suggested by our multitrophic experiment that reducing community complexity curtails biodiversity effects on ecosystem processes.

Detritivores feeding on leaf litter are key intermediate consumers linking microbial decomposers and top predators in detritus-based systems (Srivastava *et al.* 2009; Gessner *et al.* 2010). Accordingly, detritivore feeding could be influenced by both bottom-up (i.e. presence and diversity of microbial decomposers) and top-down forces (i.e. presence of predators). The bottom-up effect, termed conditioning, relates to the enhancement of leaf palatability, particularly by fungal biomass accumulation and changes in the properties of leaves resulting from partial enzymatic degradation (Suberkropp 1992). As palatability of different fungal species varies among detritivores (Suberkropp 1992), there is scope for complementary resource use when multiple detritivore species encounter diverse fungal communities. Positive fungal diversity × detritivore diversity effects on decomposition can thus arise, driven by distinct feeding preferences. This hypothesis is supported by our finding that diverse aquatic hyphomycete communities enhanced consumer performance in the multi-species detritivore treatment (Fig. 3). However, this effect was only significant when fish were present, revealing that bottom-up and top-down forces

acted together to produce a detritivore diversity effect on decomposition. This again highlights the significance of complex interactions in communities composed of multiple species within the same and at different trophic levels.

The observed cascading top-down effect from predators to detritivores and decomposition was clearly due to changes in detritivore feeding rates arising from non-trophic mechanisms, as fish scent rather than live trout was varied in our experiment. This finding demonstrates that non-consumptive (i.e. trait-mediated) responses (Kéfi *et al.* 2012) of primary consumers can elicit biodiversity effects on ecosystem processes in complex communities, similar to effects propagating in trophic cascades (Mooney *et al.* 2010; Strong & Frank 2010; Terborgh & Estes 2010). Clearly, trait-mediated species interactions not only can change trophic structure, but also rates of key ecosystem processes.

Fish can affect detritivore behaviour and consequent effects on ecosystems in at least two opposing ways. First, positive responses to simulated fish presence could arise through cascading bottom-up effects elicited by the release of nutrients from fish waste products. Nutrients released by fish (e.g. McIntyre *et al.* 2007), including NH_4^+ (Table 1), can stimulate microbial litter decomposers (Gulis & Suberkropp 2003), detritivores (Robinson & Gessner 2000), or detritivore–microbe interactions, and thus enhance decomposition rates. However, it is not clear whether a fish-mediated nutrient effect was important in the present study, because concentrations of dissolved P, the limiting nutrient in microcosms as inferred from high N : P ratios, were not measurably influenced by the presence of fish.

Alternatively, fish release specific chemical cues (kairomones) that restrain the activity of prey who seek to limit their vulnerability to predators (Chivers & Smith 1998; Brönmark & Hansson 2012). In accordance with this second mechanism, the two stonefly species in our experiment reduced feeding in the presence of fish when fungal diversity was low. However, at the high fungal diversity level, the stoneflies maintained their feeding rates even when fish were present (Fig. 4). One possible explanation is that higher fungal diversity is associated with

greater small-scale resource heterogeneity, which would allow stoneflies to maintain their feeding activity in their immediate surroundings, even if the presence of fish constrains active foraging over a larger area. In contrast to the stoneflies, *Sericostoma personatum*, a large caddisfly larva armoured by a case of coarse sand grains, increased its feeding rate in the presence of fish (Fig. 4). Protection by a sturdy case reduces vulnerability to predation, consistent with the observation that various cased caddisfly taxa, including *Sericostoma*, are insensitive to fish chemical cues (Pestana *et al*. 2009). Thus, our data on detritivore feeding performance indicate that their lower vulnerability could allow cased caddisflies to take advantage of reduced competition from other detritivore species that reduce feeding activity in the presence of vertebrate predators.

Biodiversity experiments assessing the consequences of species loss on ecosystem processes have been criticized for their simplicity (Duffy 2008), which limits extrapolation of results from such experiments to natural ecosystems. The study presented here overcomes one of the major limitations of those previous studies by explicitly testing for effects on litter decomposition that result from species interactions occurring simultaneously within and across trophic levels. Although still reflecting a simplified experimental setting, this design improves realism of the experimental food-web configurations. Densities of the detritivores in our experiment were high relative to the amount of food available. However, densities were not completely unrealistic (cf. Malmqvist, Nilsson & Svensson 1978; Hieber & Gessner 2002), especially in view of the fact that leaf-shredding detritivores show strong aggregation behaviour on leaf litter patches in streams (Presa Abós *et al*. 2006) and that such patches become scarce following resource depletion as a result of litter decomposition or downstream transport during floods (Argerich *et al*. 2008). Therefore, our results are most relevant for field situations in winter when litter resources have become scarce and consumer biomass is still high, although discrepancies between our experimental and natural field densities were within limits suggesting that our results could apply for situations even during leaf fall in autumn.

One could also argue that our experimental set-up might have exaggerated the effects of fish presence on detritivore performance, because even though addition of three pebbles provided some refuge in our microcosms, the caged detritivores were unable to move away from the fish scent imposed on them. However, such is the situation even in natural streams, where it is impossible for consumers to evade fish scent, which is ubiquitous along stream reaches with healthy fish populations and induces changes in the activity of stream consumers via trait-mediated mechanisms, as has been demonstrated in a field experiment (Peckarsky *et al*. 2002). Consequently, it is unlikely that fish scent in our microcosms triggered unnatural detritivore behaviour.

Ecosystems worldwide are suffering unprecedented rates of biodiversity loss at present (Dudgeon *et al*. 2006; Butchart *et al*. 2010), with top predators often most vulnerable to extinction (Duffy 2003). It has long been understood that such extinctions can affect multiple ecosystem properties through trophic cascades (Terborgh & Estes 2010). Our results from a detritus-based model food web involving invertebrates and vertebrate consumers indicate that the situation is yet more complex in that top predator extinction is likely to interact with biodiversity declines at lower trophic levels to alter ecosystem functioning. Indeed, according to our results, it is the coupling between top predator loss and diversity declines at either the microbial or detritivore level that reduces decomposition rates most strongly.

In natural systems, predators alter food-web interactions not only through behavioural (i.e. trait-mediated) mechanisms (Werner & Peacor 2003; Mooney *et al*. 2010), as examined here, but also through direct consumption, and they can exert strong control on ecosystem processes, especially when primary consumers face multiple predator species (Zhang, Richardson & Negishi 2004). Thus, the range of possible interactions between vertical and horizontal diversity with repercussions on ecosystem process rates is likely to be even greater in nature than observed in our experiment. The implication of the results presented here is that mimicking scenarios of trophic complexity in experiments as realistically as possible is likely to strengthen rather than weaken evidence for the importance of biodiversity effects on the functioning of ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Contribution of each of 6 aquatic hyphomycete species used to assemble 12 experimental communities.