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Testing the stress-gradient hypothesis with aquatic detritivorous invertebrates: insights for biodiversity-ecosystem functioning research

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Summary

1. The stress-gradient hypothesis (SGH) states that environmental stress modulates species interactions, causing a shift from negative interactions to net positive interactions with increasing stress.

2. Potentially, this modulation of species interactions could in turn influence biodiversity-ecosystem function (B-EF) relationships along stress gradients. Although the SGH has been extensively discussed in plant community ecology in the past two decades, it has received little attention from animal ecologists.

3. To explore whether the SGH could be applied to animal communities, we conducted a litter decomposition experiment with aquatic detritivorous invertebrates in which we manipulated litter quality and measured species interactions along this resource quality gradient. Litter quality was manipulated by presenting detritivores with leaves of plant species varying in specific leaf area and decomposition rate in streams.

4. We found a switch from negative to neutral interactions with increasing resource quality stress, in line with the SGH. However, by re-examining other published results with aquatic detritivores from the perspective of the SGH, we found that a diversity of patterns seem to characterize detritivore interactions along stress gradients.

5. Although the basic pattern proposed by the SGH may not apply to animal systems in general, we show that aquatic detritivore interactions do change along stress gradients, which underlines the importance of incorporating environmental stressors more explicitly in B-EF research.

Key-words: aquatic invertebrates, competition, ecosystem function, environmental stress facilitation, litter decomposition, specific leaf area

Introduction

The stress-gradient hypothesis (SGH) in plant community ecology predicts that positive interspecific interactions increase in frequency with environmental stress (Bertness & Callaway 1994). When the SGH was originally discussed, it was proposed that a switch from negative to positive interactions should occur as the magnitude of environmental stress increases; in low stress environments, species from the same trophic level should mainly compete (leading to net negative interactions), whereas in high stress environments, if one species can somewhat lower the stress that another species experiences (e.g. by providing a refuge, by making a food item of poor quality more readily available, etc.), facilitative interactions would increase in frequency and offset competition, thus leading to net positive interactions (Callaway 2007). The original hypothesis has since been refined to incorporate various stressors (resource, non-resource, biotic) and response variables (measures of facilitation), leading to a diversity of possible stress-facilitation relationships while keeping the basic framework of the SGH (Maestre *et al.* 2009; Smit, Rietkerk & Wassen 2009; Malkinson & Tielbörger 2010).

The SGH has received much attention from plant ecologists in recent years (see for example Brooker & Callaway

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2009 and the other articles from the same issue for reviews of facilitation in plant communities), but despite its potential relevance for community ecology in general it has almost been ignored completely by animal ecologists. In fact, to our knowledge only one study has formally tested the SGH with animals (barnacles and mussels), using environmental, non-resource stressors only (physical disturbance and thermal stress; Kawai & Tokeshi 2007). Considering the SGH in animal ecology is both timely and relevant because of the strong current interest in biodiversity-ecosystem function (B-EF) relationships in both terrestrial and aquatic systems (Gessner *et al.* 2004, 2010; Hooper *et al.* 2005; Hector *et al.* 2007; Lecerf & Richardson 2009). Indeed, one of the proposed mechanisms through which biodiversity influences ecosystem functioning is interspecific facilitation (see for example Cardinale, Palmer & Collins 2002; Jonsson & Malmqvist 2003). If positive interactions vary along environmental gradients, as predicted by the SGH, then so should B-EF relationships (see also Cardinale, Nelson & Palmer 2000). For example, if positive interactions do gain importance with increasing environmental stress, then biodiversity loss in high-stress environments would be even more consequential for ecosystem functioning than low-stress environments. By focusing on the environmental modulation of positive interactions, the SGH could help provide a mechanistic explanation as to why B-EF relationships should change across environmental conditions.

To test whether the SGH could be applied to animal communities (besides the marine invertebrate example cited above), and to investigate the effect of a resource stressor on animal species interactions, we conducted a litter decomposition experiment in an Andean stream with macroinvertebrate shredders (aquatic invertebrates that feed on coarse detritus of terrestrial origin) and a variety of plant species. Differences in leaf traits among riparian plant species (nutrient content and presence of defensive and secondary compounds) greatly influence the rate at which leaves decompose in streams (Webster & Benfield 1986; Lecerf *et al.* 2007; Hladyz *et al.* 2009; Schindler & Gessner 2009), which should in turn affect the rate at which detrital carbon is converted into shredder biomass. SGH studies usually define stress (*sensu* Grime 1977) as conditions that limit the conversion of energy into biomass by producers (Maestre *et al.* 2009). Therefore, if litter quality influences shredder growth (conversion of leaf organic matter to animal biomass; Albariño & Balseiro 2001; Graça *et al.* 2001; Iversen 1974; Pritchard & Berté 1987), one can manipulate the quality of litter available to shredders, and create a resource quality gradient that approximately corresponds to the definition of stress used by plant ecologists in typical SGH studies.

We established a resource quality gradient by collecting leaves from five plant species from families known to differ in decomposition rate (Webster & Benfield 1986; Canhoto & Graça 1996). We confirmed that these species constituted a gradient of leaf (litter) quality using two approaches. First, we measured specific leaf area (SLA), the ratio of leaf area to leaf dry weight, which is a measure of leaf toughness that correlates well with leaf nutrient content and with leaf decomposition rate in terrestrial ecosystems, including neotropical ones (Cornelissen 1996; Santiago 2007; Poorter *et al.* 2009). Then, we verified that species with higher SLA decompose faster in streams by placing leaves in mesh bags in a stream, and monitoring mass loss over time (Bärlocher 2005). After confirming that our selection of plant species formed a resource quality gradient, we placed shredders in experimental enclosures containing one of either of the five plant species and measured whether interactions between the two shredder species varied with litter quality.

We used two shredder species for which we had a mechanistic hypothesis to predict interspecific facilitation at high stress: an amphipod (*Hyallela* sp.; Gammaridae) and a trichopteran (*Anomalocosmoecus* sp.; Limnephilidae). It was previously found that some gammarid shredders feed by grazing along the edge of leaf pieces whereas trichopteran shredders cut through the middle of larger pieces, thereby increasing the number of leaf pieces and the total leaf perimeter available to shredders feeding preferentially on edges (Jonsson *et al.* 2002). This difference in shredding behaviour has led some authors to suggest that trichopterans could facilitate the processing of low-quality litter by gammarids (Jonsson *et al.* 2002; see also Dangles *et al.* 2011). However, for high-quality litter that is easily broken down in smaller pieces by microbes and fungi, gammarids would not benefit from the presence of trichopterans, and competition for the litter resource would result instead of facilitation. We therefore hypothesized that gammarid–trichopteran interactions should follow the pattern predicted by the SGH, i.e. switch from negative (competitive) interactions in high-quality litter (low stress) to positive (facilitative) interactions in low-quality litter (high stress). Then, to see whether the SGH could apply to other systems besides the species-stressor combination used in our experiment, we reviewed other studies with aquatic detritivores that included some measure of species interactions in different environmental (stress) conditions. We examined whether their results (and ours) support the general applicability of the SGH to aquatic (freshwater) detritivore communities.

Materials and methods

FIELD SITE

Fieldwork was conducted between April and July 2010 in a section of the Antisana Ecological Reserve, Ecuador (0°30′36·44″S; 78°12′56·96″W; 4050 m asl). The reserve is a protected area of Andean páramo characterized by high-altitude tussock-like grasslands (predominantly of *Calamagrostis intermedia* Steud.) drained by small, low-diversity streams of glacial or groundwater origins, occasionally bordered by riparian shrubs. We performed all experiments in a 15 m-long, low-discharge pool of a first order groundwater-fed stream (conductivity = 140 μS cm^{-1}, pH = 7·0, diel temperature range = 7–11 °C). We used a groundwater-fed stream rather than a glacier-fed stream because the latter experience strong afternoon discharge because of glacial ablation, which could have caused much mechanical abrasion of the leaves in our mesh bags and experimental enclosures (see below).

SPECIFIC LEAF AREA AND DECOMPOSITION RATE OF PLANT SPECIES

We harvested fresh leaves from five species of plants common in the area: *Buddleja incana* Ruiz & Pav. (Scrophulariaceae), *Solanum* sp. (Solanaceae), *Brugmansia sanguinea* D. Don (Solanaceae), *Baccharis latifolia* Pers. (Asteraceae), and *Eucalyptus globulus* Labill. (Myrtaceae); the latter was introduced from Australia in the 19th century to help control soil erosion in the Andes (Sklenář *et al.* 2005). To measure the average SLA of those five plant species, 30 leaves from each species were scanned, oven-dried at 60 °C for 48 h, and weighed to the nearest mg. Leaf area was measured from the scans using IMAGEJ. SLA was then calculated by dividing leaf area by leaf dry mass. We compared average SLA (log-transformed) across plant species with a one-way ANOVA ($n = 30$ replicates).

We measured decomposition rates in stream using litter bags (Bärlocher 2005). We also used fresh leaves for the decomposition experiment: because there is no seasonal abscission of the leaves in this area, using fresh leaves was necessary to ensure that we would use leaves at equivalent stages of senescence. Indeed, leaves found on the ground could have fallen at very different times and show different extent of decomposition. Moreover, although fresh litter might normally be of limited use in benthic food webs, our aim was only to create a food quality gradient as a test system to study the SGH, and the differences in leaf traits and decomposition rate between fresh leaves of different plant species were sufficient for that purpose. Leaves from the five species were dried at 60 °C for 48 h before being placed in 15 × 10 cm litter bags. We used bags of two different mesh sizes: 0·3 and 5 mm. Fine mesh bags (0·3 mm) excluded stream macroinvertebrates and were used to measure leaf decomposition because of leaching and microbes (bacteria and fungi), whereas coarse mesh bags (5 mm) allowed macroinvertebrates in and were used to measure leaf decomposition because of consumption by stream shredders (in addition to microbes and leaching). We prepared five litter bags of each mesh size for each plant species (5 bags × 2 mesh sizes × 5 plant species = 50 litter bags). The content of each bag was weighed to get initial dry mass. The bags were then anchored to the stream bottom at random locations along the length of the experimental pool. The litter bags were retrieved from the stream 60 days later, after which their remaining litter content was rinsed in freshwater, dried at 60 °C for 48 h, and weighed. Invertebrates in the bags were discarded. We did not combust the leaf material to get ash-free dry mass because sediment accumulation on the leaves was negligible in the clear, groundwater experimental pool (especially after rinsing the bag content with freshwater).

We calculated % leaf mass remaining in fine mesh bags at the end of the experiment by dividing the final dry mass by the initial dry mass mass. We calculated the average ($n = 5$ bags) % mass remaining in fine mesh bags for each plant species to get a correction factor representing the expected mass loss because of leaching and microbes alone. We used these species-specific correction factors to adjust the initial dry weight of coarse mesh bags, to get an estimate of initial dry weight after leaching and microbial decomposition. We then calculated % leaf mass loss using the following equation: $100 - (\text{mass}_{\text{final}}/\text{mass}_{\text{initial, adjusted}}) \times 100$. This measure of leaf mass loss in coarse mesh bags after taking into account mass loss because of leaching and microbes represents the estimated % litter mass consumed by shredders over the 60 days of the experiment. As shredders in the stream had access to many different food types (the five plant species in litter bags in addition to the abundant detritus already present in the stream), we interpret a high % litter mass consumed as evidence for high palatability of the plant species to shredders (i.e. shredders chose to consume that litter). We compared average % mass consumed (arcsine-transformed) across plant species with a one-way ANOVA ($n = 5$ replicates). Then, we performed a linear regression between species average SLA (log-transformed) and species average % litter mass consumed (arcsine-transformed) to see whether there was a relationship between leaf SLA and leaf palatability to detritivores.

STRESS-GRADIENT HYPOTHESIS EXPERIMENT

Using a sharpened piece of metal tubing, we cut 1 cm diameter-disks in fresh leaves of the five plant species described above. Leaf disks were used to standardize the total perimeter and area of litter available to shredders, which was especially important given our hypothesized mechanism of interspecific facilitation (see Introduction). Oven-dried leaf disks were placed in 15 × 10 cm mesh enclosures (0·3 mm mesh size; the same fabric that we used for the fine mesh bags described above), with 10 disks of a single leaf species in each enclosure. We prepared 40 enclosures for each plant species (200 enclosures in total) and weighed the content of each enclosure to get initial dry mass. We then placed the enclosures in the experimental pool for 1 week to allow microbial and fungal colonization. We were constrained to only 1 week of leaf conditioning for logistical reasons; although this is a short time for microbial colonization, it was enough to provide a resource quality gradient to shredders (see results below). Although the gradient might therefore have arisen from differences in the speed at which the different leaf species were colonized by microbes in addition to chemical differences related to leaf palatability, for our experiment it only mattered that leaves differed in palatability, regardless of the cause of this difference.

After leaf conditioning, we retrieved the enclosures to add the invertebrate treatments. We collected *Hyallela* and *Anomalocosmoecus* downstream of the experimental pool using a Surber sampler. We sorted the invertebrates and chose individuals from both species that were approximately similar in size, to avoid problems resulting from the allometric relationship between the size and leaf processing efficiency (LPE) of shredder species (see McKie *et al.* 2008). We opened the enclosures and assigned them randomly one of the following four invertebrate treatments: *Hyallela* monoculture (four individuals), *Anomalocosmoecus* monoculture (four individuals), *Hyallela* and *Anomalocosmoecus* mixture (2 + 2 individuals), or control (no shredders), for a total of 10 replicates × 4 shredder treatments × 5 leaf species. We then closed the enclosures using twist ties and secured them to the bottom of the experimental pool. The only food source available to the invertebrates during this period was the litter content of the enclosure, such that they could not switch to alternative food sources when exposed to litter of low palatability. Two weeks later, we re-opened the enclosures and collected the invertebrates and remaining leaf material. For each enclosure, we verified that all individuals survived; in the case of mortality, we assumed that the individual lived for half of the experiment (as described in Jonsson *et al.* 2002). The invertebrates were preserved in 95% ethanol and weighed 24 h later. The remaining leaf material was rinsed, dried at 60 °C for 48 h, and then weighed to get the final dry weight.

We calculated the average leaf mass loss of the control enclosures ($\text{weight}_{\text{final}}/\text{weight}_{\text{initial}}$) for each of the five leaf species to get a correction factor representing mass loss because of leaching and microbial decomposition, as we did for litter bags. We then calculated leaf mass loss of the enclosures with shredder treatments by dividing $\text{weight}_{\text{final}}$ from the adjusted $\text{weight}_{\text{initial}}$, to get a measure of % mass loss because of invertebrate consumption alone. We divided this value by the total weight of invertebrates in the enclosure, to obtain a value of LPE (% leaf mass consumed per g of shredder; see McKie *et al.* 2008, 2009) for all enclosures. We assessed the effect of leaf species and invertebrate treatment (and the interaction between the two) on LPE with a two-way ANOVA.

To measure species interactions, we generated expected LPE values for the mixture enclosures (those that contained both *Hyallela* and *Anomalocosmoecus*) based on the assumption that if there is no species interactions (or if there is a net neutral interaction) then the LPE of the mixture for a given leaf species should be the average of the LPE of the two shredder monocultures for that leaf species. We calculated expected LPE values by averaging the LPE of one randomly chosen *Hyallela* monoculture and one randomly chosen *Anomalocosmoecus* monoculture; we repeated the process 10 times, until all monoculture enclosures were used, thus generating 10 expected LPE values per leaf species. Species interactions were then inferred when the observed LPE was lesser or greater than the expected LPE (showing net negative and net positive interactions respectively). Although plant community ecologists have developed a variety of indices of species interactions to test the SGH (Seifan *et al.* 2010), we could unfortunately not use those as we did not have independent measures of performance for the two shredder species in the mixture enclosures (we could only assess the total performance of the mixture and not of each species within the enclosure). We compared the observed vs. expected LPE of mixtures using Welch two-sample *t*-tests (two-sided).

To generate a stress-species interaction curve that could be used to graphically compare our results with existing SGH models (Kawai & Tokeshi 2007), we needed an index of stress that could be correlated with species interactions (defined as observed LPE − expected LPE of mixtures, as mentioned above). We calculated a value of relative resource quality stress for each leaf species with the following equation: expected LPE_{max} − expected $\text{LPE}(x_i)$ + expected LPE_{min}, where expected LPE_{max} is the average expected LPE of mixtures for the plant species of highest palatability, expected $\text{LPE}(x_i)$ is the average expected LPE of the mixtures for the plant species for which the stress value is being calculated, and expected LPE_{min} is the average expected LPE of mixtures for the plant species of lowest palatability. Subtracting the expected LPE of a plant species from the expected LPE of the species of highest palatability basically ‘reverses’ the resource quality gradient, and gives a high stress value to species of low palatability and a low stress value to species of high palatability. Then, we added the LPE of the plant species of lowest palatability to these stress values to ensure that all stress values were > 0, which was necessary to fit non-linear relationships between stress and species interactions. We used expected LPE as a measure of stress rather than decomposition rate in litter bags because the latter represents leaf palatability to the whole stream shredder community whereas expected LPE represents palatability to *Hyallela* and *Anomalocosmoecus* only, which is a more precise measure of stress in the experiment. We used least square regression to find the function that best described the relationship between this index of resource stress and species interactions. All statistical analyses described above were performed in R (version 2.14).

APPLICABILITY OF THE STRESS-GRADIENT HYPOTHESIS TO OTHER AQUATIC DETRITIVORE STUDIES

We searched the literature for controlled experiments performed with aquatic detritivores that included a measure of species interactions under different environmental (stress) conditions. Most of our examples were taken from studies examining species richness effects on ecosystem function (e.g. leaf decomposition rate). For these studies, as facilitation or competition was not directly measured, species interactions were inferred from the difference in performance of species mixtures from the expected performance calculated from monocultures, similar to what we calculated with our experimental data. We inferred stress levels by examining the performance of detritivore monocultures in the different environmental conditions; for example, if growth was lower in one environment than another environment, then the low-growth environment was classified as more stressful than the high-growth environment, even if the authors did not call these environmental differences a ‘stress gradient’. Because of differences in experimental methods, stress gradients and response variables, we could only obtain the direction of the change in species interactions along the gradient, and not the magnitude of this change. We only found a handful of studies that fulfilled the needs of this exercise. Therefore, rather than adopting a quantitative meta-analytical approach, we simply report how many studies showed a pattern compatible with the SGH, a pattern opposite to the SGH, or no pattern at all, and examine whether the evidence available to date suggests that species interactions among aquatic detritivores from various systems might follow the pattern described by the SGH.

Results

SPECIFIC LEAF AREA AND DECOMPOSITION RATE OF PLANT SPECIES

The five plant species differed greatly in both SLA ($F_{4,145}$ = 144·3, P < 0·0001; Fig. 1a) and decomposition rate in coarse-mesh bags, after accounting for mass loss because of leaching and microbes ($F_{4,20}$ = 7·84, P = 0·0006; Fig. 1b). There was a strong relationship between species average SLA and mass loss in-stream because of shredder consumption (r^2 = 0·9497, $t_{(3)}$ = 5·2519, P = 0·0134; Fig. 1c), suggesting that leaves of high SLA are indeed more palatable to detritivores.

STRESS-GRADIENT HYPOTHESIS EXPERIMENT

Mortality in the enclosures was generally low (mean ± standard deviation for all leaf species combined = 7·5 ± 6% for *Hyallela* and 6 ± 2·6% for *Anomalocosmoecus*). The five leaf species that we used were consumed to varying degrees in the enclosures (effect of leaf species on LPE: $F_{4,130}$ = 48·308, P < 0·0001; Fig. 2a); some leaf species were clearly more palatable than others to the two shredder species used in the experiment, similar to what we found with coarse mesh bags exposed to the entire stream shredder community. Shredder treatments also differed in their average LPE when pooling all leaf species (effect of shredder treatment on LPE: $F_{2,130}$ = 14·769, P < 0·0001), but this effect varied across leaf species (effect of the interaction between shredder treatment and leaf species on LPE: $F_{8,130}$ = 4·923, P < 0·0001; Fig. 2a). For the four leaf species of lesser quality, there was no significant difference between the observed and expected LPE of the mixture (*E. globulus*: $t_{13·221}$ = 1·1437, P = 0·2731; *B. incana*: $t_{13·533}$ = 0·0936, P = 0·9268; *Sola-*

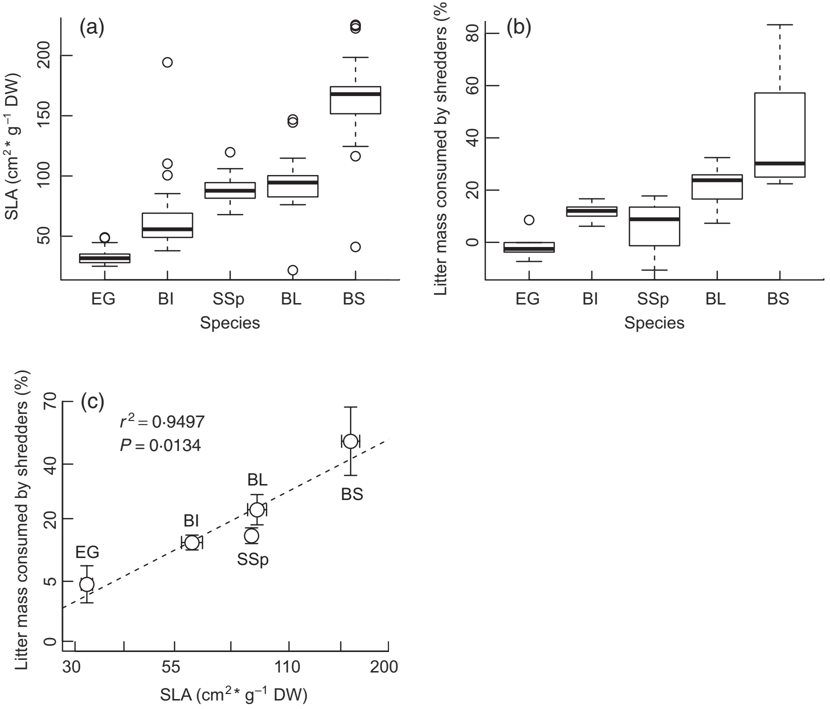

Fig. 1. (a, b) Boxplots showing the distribution of observations of SLA (a) and % litter mass loss in coarse-mesh bags because of shredder consumption (b), for all plant species. The bottom and top whiskers indicate the lowest and highest observations respectively, the bottom and top of the box represent the lower and upper quartiles, and the thick line denotes the median. Outliers are represented as open circles. (c) Relationship between average SLA and average shredder consumption in coarse-mesh bags for the five plant species. SLA was log-transformed and % mass consumed was arcsine-transformed for the linear regression. Axis labels were back-transformed into original units for illustrative purpose. Error bars = SEM. EG, *Eucalyptus globulus*; BI, *Buddleja incana*; SSp, *Solanum* sp.; BL, *Baccharis latifolia*; BS, *Brugmansia sanguinea*; SLA, specific leaf area; DW, dry weight.

num sp.: $t_{12{\cdot}523} = 1{\cdot}1224$, $P = 0{\cdot}2828$; *B. sanguinea*: $t_{7{\cdot}679} = 0{\cdot}8586$, $P = 0{\cdot}4166$). However, for the leaf species of highest palatability (*B. latifolia*), the mixture performed significantly worse than expected from the performance of the monocultures, suggesting negative interactions ($t_{17{\cdot}134} = 3{\cdot}1015$, $P = 0{\cdot}0064$). This net negative interaction only at low stress led to a positive relationship between resource quality stress and the interaction between *Hyallela* and *Anomalocosmoecus*, in line with the SGH (Fig. 2b; see Material and methods for a description of the stress index). The function that best described this relationship was a logarithmic function ($r^2 = 0{\cdot}9629$, $t_{(\text{slope})} = 8{\cdot}818$, $P = 0{\cdot}0031$). Contrary to what we hypothesized initially, however, we did not observe net positive interactions at high stress.

RESULTS FROM OTHER STUDIES

We found a few other experimental studies with macroinvertebrate detritivores in which species interactions in different environmental conditions could be examined from the perspective of the SGH, even though the experiments were performed for a different purpose (Table 1). Despite the small set of studies available, all three possible stress-species interactions relationships were found; three experiments resulted in the pattern predicted by the classical SGH (increasingly more positive net interactions with increasing stress), three found a pattern opposite to the SGH (increasingly more negative net interactions with increasing stress) and three others found complex patterns or no pattern in species interactions along the gradient. Importantly, regardless of the direction of change in species interactions following stress, all but one of the nine experiments listed in Table 1 found that species interactions did vary with environmental conditions.

Discussion

By looking at how the net interaction between two aquatic shredder species changed along a resource quality gradient, we explored whether the SGH in plant community ecology could be applied to aquatic detritivores and possibly to animal communities in general. We manipulated resource quality by forcing shredders to feed on litter from plant species varying in SLA and decomposition rate in streams (Fig. 1a,b). We found a positive logarithmic relationship between resource quality stress and the sign and strength of species interaction: interactions became more positive with increasing stress, but no net positive interactions were observed even at high stress (Fig. 2b). This relationship corresponds to what was previously described as a 'competition-only, monotonic increase' relationship (Kawai & Tokeshi 2007). It is possible that if we used an even broader stress gradient (using leaves of yet lower palatability) we would

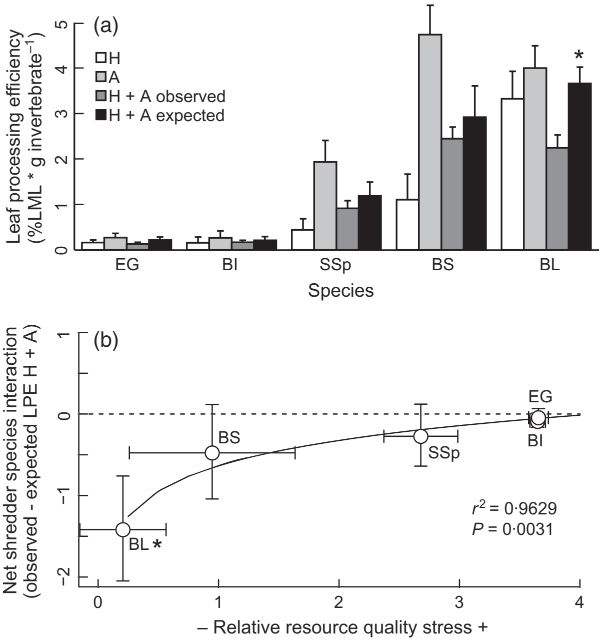

Fig. 2. (a) Average LPE of the three treatments and of the expected performance of the mixture for the five plant species. Error bars = SEM. (b) Relationship between resource quality stress and species interactions for the five plant species. The light grey dotted line indicates net neutral interactions. The dark line represents the best-fit relationship (a logarithmic function), for which the coefficient of determination and P value are given. Error bars = 95% confidence intervals. H, *Hyallela* monoculture; A, *Anomalocosmoecus* monoculture; H + A, *Hyallela* and *Anomalocosmoecus* mixture; LPE, leaf processing efficiency; LML, leaf mass loss. Plant species are abbreviated as in Fig. 1. *Significant difference between the observed and expected LPE of the mixture treatment ($P < 0{\cdot}01$).

have observed facilitation at very high stress. However, given that SLA (Fig. 1a), leaf decomposition in coarse-mesh bags (Fig. 1b), and leaf consumption in experimental enclosures (Fig. 2a) were all very low for our leaf species of lowest palatability, we are confident that our gradient included species of sufficiently low quality to observe any potential positive interactions. Other authors have shown that *E. globulus*, our leaf species of lowest quality, has a very low palatability to shredders and decompose slowly in streams (Canhoto & Graça 1995, 1996).

By measuring both the average SLA and decomposition rate of various plant species to establish a resource quality gradient, we observed a very strong relationship between SLA and leaf mass loss in coarse mesh bags after accounting for mass loss because of leaching and microbial decomposition (Fig. 1c). SLA is a measure of leaf toughness that has been shown to predict leaf decomposition rate in terrestrial systems (Cornelissen 1996; Cornelissen & Thompson 1997; Santiago 2007; Poorter *et al.* 2009). As SLA correlates well with leaf nutrient concentrations as well as leaf lignin content (Lambers & Poorter 1992; Santiago 2007; Poorter *et al.* 2009), both of which are well-known predictors of leaf decomposition rate in streams (Lecerf *et al.* 2007; Lecerf & Chauvet 2008; Hladyz *et al.* 2009; Schindler & Gessner 2009), it seems likely that SLA would also be a good predictor of decomposition rate in aquatic systems, as we found in this study. However, a recent study of decomposition of alder (*Alnus glutinosa*) in European streams found no relationship between SLA and decomposition rate (Lecerf & Chauvet 2008). The discrepancy between these results and ours might be explained by the fact that Lecerf & Chauvet (2008) measured SLA among different populations of the same species, whereas we compared distantly related species from different families. The range of SLA in the *A. glutinosa* study was only 117–155 cm^2 g^{-1} (compared to 35–180 cm^2 g^{-1} in our study), which might not have been sufficient to detect a relationship between SLA and decomposition rate.

Besides from SLA, we also used mass loss in litter bags as another proxy for leaf palatability to shredders. For both leaf disks in the SGH experiment and leaves in litter bags, we measured % mass loss after adjusting (reducing) initial dry mass to take into account the predicted mass loss because of leaching and microbial consumption. Any additional mass loss after adjusting the initial mass should represent consumption by detritivores (McKie *et al.* 2009), which has been shown to be a good indicator of resource palatability (quality) in other systems (Cornelissen *et al.* 1999; Hladyz *et al.* 2009). However, higher consumption might not necessarily represent higher palatability in situations where shredders have access to a single food type. Indeed, some studies report that shredders can increase their consumption of litter mass when the litter is of poor quality, perhaps to satisfy the same nutritional requirements for growth and metabolism, despite the low nutritional value of the food available (e.g. Friberg & Jacobsen 1999). This could clearly be an issue in our SGH (enclosure) experiment, as the only food source available to shredders was the litter that we placed in the enclosures. However, in the litter bag experiment with coarse mesh bags (Fig. 1b), differences between plant species in % mass consumed should reflect the palatability of the different leaves to shredders. Stream shredders had access to all five plant species as well as to the abundant benthic organic matter already present in the stream before the experiment. Shredders would have no reason to consume litter of low palatability in these conditions, which are somewhat similar to 'cafeteria' experiments in the laboratory in which resource palatability is estimated from consumption (e.g. Cornelissen *et al.* 1999). As the consumption gradients are almost identical in our litter bag and enclosure experiments (see ordering of plant species in Figs 1b and 2a), we believe that higher consumption in enclosures also reflected higher palatability. The only difference in the two gradients is for the species that was most consumed: *B. sanguinea* in coarse mesh bags and *B. latifolia* in the enclosures. This difference could be because of the relative abundance of shredder species in the experimental pool; *Anomalocosmoecus* was twice as abundant as *Hyallela* in the study stream (O. Dangles & D. Jacobsen, unpublished data) and *B. sanguinea* was the leaf of highest palatability to *Anomalocosmoecus* (as shown by the monoculture treatments in the SGH experiment; see Fig. 2a). The close correspondence between consumption gradients in our enclosures and coarse

Table 1. Examples of controlled experiments with aquatic invertebrates from which species interactions in different stress conditions can be inferred

Reference	Species	Stressor	Interaction measure	Result
Supports SGH				
Cardinale & Palmer (2002; Fig. 6)	Three trichopteran suspension feeders	One episode of random mortality in stream mesocosm or not	Rate of POM flux, monocultures vs. mixtures	Shift from neutral to positive interactions as stress increases
Jonsson (2006; Fig. 1)	Three plecopteran shredders	Resource availability diminishing over time	Detritivore LPE, monocultures vs. mixtures	Shift from neutral to positive interactions as stress increases
McKie *et al.* (2009; Fig. 2c)	Three plecopteran shredders	Liming (improvement of stream conditions)	Detritivore LPE, monocultures vs. mixtures	Shift from negative to neutral interactions as stress increases
Opposite of SGH				
Daugherty & Juliano (2002; Fig. 5)	Scirtid beatles (shredders) and mosquitoes (filter-feeders)	State of detrital resource (ease of resource acquisition)	Survivorship and biomass of facilitated species (mosquitoes)	Shift from positive to neutral interactions as stress increases
McKie *et al.* (2009; Fig. 4)	Three plecopteran shredders	Liming (improvement of stream conditions)	Detritivore relative growth rate, monocultures vs. mixtures	Shift from neutral to negative interactions as stress increases
McKie *et al.* (2009; Fig. 4)	Three plecopteran shredders	Nutrient enrichment (improvement of stream conditions)	Detritivore relative growth rate, monocultures vs. mixtures	Shift from neutral to negative interactions as stress increases
Complex pattern or absence of pattern				
Bastian, Pearson & Boyero 2008; Fig. 4	Three trichopteran shredders	Quality of detrital resource	Detritivore LPE, monocultures vs. mixtures	Some negative interactions but no pattern along stress gradient
Daugherty & Juliano (2002; Fig. 3)	Scirtid beatles (shredders) and mosquitoes (filter-feeders)	Quality of detrital resource	Development time of facilitated species (mosquitoes)	No interaction along entire gradient
Jonsson *et al.* (2002; Figs 2 and 3) and Dangles, Jonsson & Malmqvist (2002)	Three shredder species from different invertebrate taxa	Quality of detrital resource	Detritivore LPE, monocultures vs. mixtures	Shift from positive to neutral to positive interactions again as stress increases

LPE, leaf processing efficiency; SGH, stress-gradient hypothesis; POM, particulate organic matter.

mesh bags, as well as the strong correlation between SLA and consumption, make us believe that differences in consumption in the SGH experiment did indeed reflect a resource quality gradient, and therefore a stress gradient that should have influenced species interactions.

We did find that species interactions changed along the litter quality gradient in our experiment; however, we did not observe net positive interactions at high stress, as predicted by the SGH. The other experiments that we reviewed do not fully support the applicability of the SGH to aquatic detritivores either. Although an increase in stress led to more positive interactions in some cases, many other relationships between stress and net species interactions were possible (see Table 1). Plant ecologists have also observed deviations from the pattern predicted by the SGH in many systems, which helped extend the conceptual framework of the SGH (Maestre, Valladares & Reynolds 2005, 2006). The relationship (or absence of relationship) between stress and species interactions resulting from any given experiment will likely depend on the species tested, on the nature of the stressor and on the measure of species interaction that is used (Kawai & Tokeshi 2007; Maestre *et al.* 2009; Smit, Rietkerk & Wassen 2009; Seifan *et al.* 2010). For example, McKie *et al.* (2009) found that stream liming led to a general increase in LPE by shredders, but that this liming-induced reduction in stress (*sensu* Grime 1977) led to both a lower than expected performance of the detritivore species mixtures when LPE was considered and a higher than expected performance of the mixtures when the relative growth rate of detritivores was measured instead. In this case, the choice of performance measure would determine if one finds a positive or a negative stress-species interaction relationship, both resulting from the same stressor.

Although the direction of the shift in species interactions following stress seems hard to predict, one commonality is that aquatic detritivore interactions do appear to change along most stress gradients (Table 1). Many mechanisms could account for such changes, the specifics of which are likely to depend on the species pair and environmental context that are examined. For example, suspension-feeding caddis fly species may compete for limited habitat space in undisturbed streams with a high density of larvae, but in streams with occasional flooding that reduces larval density this competition would disappear (competition only at low stress, if stress is defined here as the frequency of flooding; Cardinale & Palmer 2002). Scirtid beetles (shredders) could benefit mosquito larvae (filter-feeders) co-occurring in water-filled tree holes by increasing the availability of fine particulate organic matter, but only when this resource is scarce (facilitation only at high resource stress; Daugherty & Juliano 2002).

Among the mechanisms that have been proposed to account for B-EF relationships, a 'release from intraspecific competition' can happen when a decrease in intraspecific density occurs alongside an increase in diversity (Jonsson & Malmqvist 2003; Lecerf & Richardson 2009). Intraspecific effects could indeed explain the difference between the expected and observed performance of the species mixtures in our experiment and in the other studies that we reviewed. In a substitutive experimental design (like ours) where total [invertebrate] density is held constant across treatments, monoculture and mixture treatments not only differ in species diversity (in our case, one vs. two species) but also in intraspecific density (four vs. two conspecifics; see also Jolliffe 2000). Therefore, a lower than expected performance of the mixture could be because of a loss of intraspecific facilitation (rather than interspecific competition) and a higher than expected performance of the mixture could be because of a release from intraspecific competition (rather than interspecific facilitation or complementarity; see McKie *et al.* 2008, 2009). Such intraspecific effects are also likely to vary along environmental gradients; for example, a recent experiment testing the SGH at the intraspecific level (with beech) found that the net interactions between conspecifics switched from negative to positive with increasing environmental stress (Fajardo & McIntire 2011). Importantly, regardless of whether the changes in net interactions that occur along environmental gradients are because of intraspecific or interspecific effects, the important point is that net interactions do fluctuate with environmental stress, and so should biodiversity effects on ecosystem functioning (but see Maestre *et al.* 2010).

Other authors have looked at the environmental modulation of B-EF relationships in animal communities (Cardinale & Palmer 2002; McKie *et al.* 2009). Indeed, it has been suggested previously that disparities in the B-EF literature might be because of the fact that similar experiments are performed under different environmental conditions (Cardinale, Nelson & Palmer 2000). What the SGH can add to such research, if not clear predictions as to what interaction *patterns* may be observed along environmental gradients, is a focus on the *mechanisms* that can account for the context-dependence of B-EF relationships, i.e., the environmental modulation of inter- and intraspecific interactions (because of, say, stress-related habitat amelioration or release from predation; see Maestre *et al.* 2009 for examples of mechanisms applying to plant communities). We believe that this is a promising avenue for future research that could lead to a better understanding of the proximate mechanisms underlying B-EF relationships. Also, we suggest that B-EF research should incorporate environmental stress more explicitly in richness-function experiments, if we are to ever understand how biodiversity loss will influence ecosystem functioning in different environments.

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