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LETTER

Biodiversity effects on ecosystem functioning change along environmental stress gradients

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Abstract

Positive relationship between biodiversity and ecosystem functioning has been observed in many studies, but how this relationship is affected by environmental stress is largely unknown. To explore this influence, we measured the biomass of microalgae grown in microcosms along two stress gradients, heat and salinity, and compared our results with 13 published case studies that measured biodiversity–ecosystem functioning relationships under varying environmental conditions. We found that positive effects of biodiversity on ecosystem functioning decreased with increasing stress intensity in absolute terms. However, in relative terms, increasing stress had a stronger negative effect on low-diversity communities. This shows that more diverse biotic communities are functionally less susceptible to environmental stress, emphasises the need to maintain high levels of biodiversity as an insurance against impacts of changing environmental conditions and sets the stage for exploring the mechanisms underlying biodiversity effects in stressed ecosystems.

Keywords

Algae, biodiversity function, complementarity effect, environmental fluctuation, productivity, selection effect, species richness, stress intensity.

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INTRODUCTION

Understanding the influence of biodiversity for ecosystem functioning is crucial as we face extinction rates several orders of magnitude higher than those inferred for the last tens of millions of years, and expected to rise further as a result of climate change, landscape conversion, and other anthropogenic changes (Thomas *et al.* 2004; Loreau 2010). A large body of empirical studies conducted under more or less constant environmental conditions has documented in most cases positive effects of biodiversity on ecosystem functioning (Cardinale *et al.* 2011, 2012; Wagg *et al.* 2011; Naeem *et al.* 2012). Ecological theory predicts that biodiversity stabilizes and enhances ecosystem functioning, although ideas about this topic have changed over time (Loreau 2010). Following new experimental results, the current view again is that biodiversity generally buffers ecosystems against stress (Duffy 2009; Loreau 2010; Cardinale *et al.* 2012).

Ecosystems are subject to natural temporal and spatial variation of environmental conditions such as temperature, precipitation and nutrient availability, as well as to influences determined by other species (e.g. predators, competitors, invaders) and human activities (Tilman *et al.* 1997; Chapin *et al.* 2000; Bellemare *et al.* 2002). These fluctuations vary in their frequencies and intensities, ranging from limited, regularly recurring variations to which organisms living in a given environment are more or less adapted, to episodic, catastrophic disturbances that lead to extensive mortality and local extinction (Cooper-Ellis *et al.* 1999; Lugo 2008). If these fluctuations are detrimental to a species or ecosystem function, they are often called stress (Box 1). One of the major challenges in exploring the impact of stress intensity on biodiversity–ecosystem functioning relationships is that the term stress is a meta-concept that is difficult to define in a general way because a set of conditions that is detrimental (stressful) to one species may be beneficial for another (Loreau 2010). To circumvent this problem in this study, we use a scheme in which we define the terms stress, stress-response intensity, biodiversity effect and stress-response buffering effect, and we propose an approach to quantify these variables (Box 1).

Empirical studies of biodiversity–ecosystem functioning relationships under stressful conditions are rare and have resulted in contrasting results, ranging from clearly positive (e.g. Mulder *et al.* 2001; Goodsell & Underwood 2008; Steudel *et al.* 2011) to no or in some circumstances even negative effects (e.g. Bell 1990; Pfisterer & Schmid 2002; Caldeira *et al.* 2005; Downing & Leibold 2010). This currently unexplained variability of results led Loreau (2010) to conclude 'that current theory (on biodiversity–ecosystem functioning relationships) may still be missing some significant elements'. One such previously largely ignored element, both from an empirical and theoretical point of view, is the intensity of stress.

Microalgae have been shown in previous experiments to partly show a positive biodiversity effect on ecosystem functioning (Bell 1990; Behl *et al.* 2011). Importantly, these effects were dependent on the environmental conditions prevailing during the experiment and included a negative effect of biodiversity on algal biomass (Bell 1990). This suggests that microalgae are suitable organisms to explore the relationship between biodiversity effects and

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Box 1 Definition and Quantifications

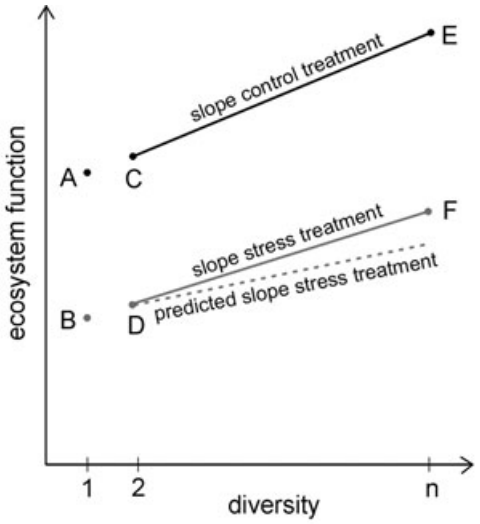

Illustration of the calculation of different parameters used in this study

stress response intensity (SRI) = $1 - \frac{B}{A}$

absolute biodiversity effect of control treatment (aBEc) = E – C

absolute biodiversity effect of stress treatment (aBEs) = F – D

absolute stress-response buffering effect (aSRBE) = $\frac{aBEc}{aBEs}$

relative biodiversity effect of control treatment (rBEc) = $\frac{E}{C}$

relative biodiversity effect of stress treatment (rBEs) = $\frac{F}{D}$

predicted rBEs (prBEs) = rBEc × SI

relative stress-response buffering effect (rSRBE) = rBEs - prBEs

Figure in Box illustrates the calculations used in the present study to quantify *stress response intensity* (SRI), *biodiversity effect* (BE), and *stress-response buffering effect* (SRBE). A and B are measured mean ecosystem responses (e.g., biomass production) of monocultures in control (A) and stress (B) treatments. C and E are mean ecosystem responses of mixed assemblages at low (C) and high (E) diversity levels in the control treatment, and D and F are the responses in the corresponding stress treatment. Variables used in analyses of the present study are marked in bold.

We define *stress response* as a negative impact of an environmental condition on an ecosystem function such as biomass production. A condition induces a stronger stress response in a given study system (the stress treatment) than another (the control) when it results in a relatively lower level of ecosystem function. The logical starting point, especially if the aim of the study is to assess effects of species interactions, is to estimate stress intensity in monocultures. Accordingly, we quantify the *intensity* of *stress response*, i.e., the stress response intensity (SRI), as reduction in the mean level of a given ecosystem function measured in monocultures in control versus stressed systems (1 − B/A).

BE is defined here as the increase in an ecosystem function with increasing biodiversity and is measured as the slope of ecosystem function versus diversity. Biodiversity effects can be measured in absolute terms as the difference in an ecosystem function achieved by high and low diversity assemblages, or in relative terms as the ratio of ecosystem functioning achieved by high and low diversity assemblages.

SRI can be related either to an absolute biodiversity effect (E − F for control treatment [aBEc] and F − D for stress treatment [aBEs]), respectively) or to a relative biodiversity effect (E/F for control treatment [rBEc]) and F/D for stress treatment [rBEs], respectively). We use the term SRBE to describe this relationship. A SRBE is high when BE of the stress treatment is higher than that of the control. If we apply this approach to absolute biodiversity effects, we divide the biodiversity effect of the stress treatment by that of the control (aBEs/aBEc). However, because by definition absolute values of ecosystem function are lower in stress treatments, this would often result in a

lower slope indicating a reduced SRBE with increasing stress intensity. To account for this bias, we also define the rSRBE as the difference between the predicted slope (prBEs = rBEc × SI) of the stressed system if its slope decreases proportionally to stress response intensity (SRI), and the observed slope of the stressed system, i.e. the rBEs (rBEs − prBEs). Although both SRI and the SRBE are derived from the same measure of ecosystem function, the two variables are independent in this approach, because (1) SRI is derived from monoculture data and thus is independent of any biodiversity effect and (2) the SRBE compares the slopes of two treatments (control and stress).

environmental stress. Microalgae show fewer differences in morphological traits than vascular plants, but the diversity of functional or physiological traits as well as of resource utilisation (e.g. light use efficiency) may be similar to those of higher plants (Striebel *et al.* 2009).

In this study, we set out to explore effects of stress by using both an experimental approach in which we varied stress intensity, and a comparative analysis of published studies assessing the effect of biodiversity–ecosystem functioning relationships under conditions of environmental stress. Our basic hypothesis was that stress intensity affects the strength of biodiversity–ecosystem functioning relationships, although there were no a priory reasons to decide whether a positive or negative net effect of stress intensity on the relationship was to be expected.

METHODS

Algal strains

Green microalgae were grown in microcosms under controlled environmental conditions to examine the effect of stress intensity on total algal biomass. We used 64 algal strains from the Culture Collection of Algae at Göttingen University (SAG), Germany. The strains were selected among about 400 cultures of monococcal aeroterrestrial and freshwater green algae suitable for our experiment. All strains were known to be easily cultivated and to grow quickly under the conditions of our control treatment (Table S1). We use the term species as a synonym of strain because assignment of the strains to species is currently fluent. For example, five of the strains we used were recently placed into two species. We used up to 64 species to cover a broad range of richness levels, similar to that of natural algal communities. Including algae from different habitats increased the likelihood that assemblages comprised species adapted to different environmental conditions. We made no effort to assemble natural communities.

Experimental design

All 64 strains were cultivated in microcosms as source cultures of inocula. They were inoculated every 2 weeks under the same conditions as the control treatments used in the experiment. Therefore, the algae did not require acclimatisation before or during the experiment. Two identical sets of algae microcosms were inoculated following the experimental design described below. One of these sets was treated as control, whereas the other was subjected to one of two stress treatments (NaCl and elevated temperature). In all cases, a control treatment was paired with a stress treatment. We repeated this approach 12 times, with six levels of stress intensity for both heat and salinity, resulting in a total of 12 control runs in parallel with 12 stress runs. The order of these runs was chosen randomly to avoid any temporal influence by directional changes of abundances or cell sizes of the source strains.

Microcosms were inoculated with microalgae at one of seven levels of species richness (1, 2, 4, 8, 16, 32 and 64) and incubated for a week prior to estimating total algal biomass. The selected algal strains were randomly numbered from 1 to 64. Thirty-two assemblages containing two species were created by combining species 1 and 2, 3 and 4, 5 and 6, etc. (Table S2). Similarly, the 16 four-species assemblages were combinations of 2 two-species assemblages (species 1–4, 5–8, 9–12, etc.). This procedure was repeated to produce eight-species assemblages, etc. To increase the number of replicates at the 16-species level and 32-species level to eight assemblages each, we created additional assemblages as shown in Table S2. Finally, at the 64-species level, we used eight replicates with identical species composition (Table S2). Although the cell densities of the algal suspensions were not initially determined, we were able to calculate biodiversity effects using the monoculture data as relative reference. All species combinations were assembled in duplicate, resulting in 288 microcosms for each run. In the entire experiment, we used 3456 microcosms.

Inoculation of microcosms and growth conditions

Glass Erlenmeyer flasks (100 mL) were used as microcosms. They were filled with 30 mL of sterile culture medium following ISO 8692:2004 guidelines, closed with a cellulose plug and covered by aluminium foil. The flasks were inoculated with a total of 6.4 mL of algal suspension using sterile technique and ensuring they received equal volumes of all species. We used a relatively large total inoculation volume to ensure enough intact cells were added to microcosms even when assemblages contained 64 species (i.e. a minimum of 0.1 mL per species). Tips of the 1-mL Eppendorf micropipette we used were wide enough to avoid damage of the algae during inoculation. As the algal suspensions were cultivated under the same conditions as the control treatments, cell densities were relatively low compared with inocula obtained from solid or liquid media used in other experiments with microalgae (e.g. Bell 1990; Jiménez-Pérez *et al.* 2004).

Microcosms were prepared under sterile conditions one day before inoculation to ensure proper buffering of the culture medium as a result of CO_2 diffusion through the cellulose plugs. This buffering process followed the ISO 8692:2004 guidelines. After inoculation, the algae were grown for 24 h at 24 °C in a Percival Scientific I-36 LLVL incubator. Light intensity was about 70 μmol photons m^{-2} s^{-1} with a day–night cycle of 14:10 h. After the first 24 h, one set of the microcosms was exposed to stressful conditions, whereas the set of control microcosms was further cultivated as described above. Once a day, microcosms were manually shaken and randomly rearranged in the incubator to ensure similar average growth conditions and effective gas exchange.

Stress treatments

Two different stress treatments were used, elevated temperature and salinity. Stress intensities were increased in six steps for both stress types. For the salinity stress gradient, sterile NaCl solution in culture medium was added to microcosms to yield salinities of 0.125, 0.25, 0.5, 1, 1.5 and 2.5%. Control microcosms received the same volume of sterile culture medium without NaCl. For the temperature stress treatments, which had to be conducted in a separate incubator, the microcosms were placed in a New Brunswick Scientific Innova 4340 incubator (New Brunswick, Edison, NJ, USA) at temperatures of 27.5, 30, 32.5, 35, 37.5 or 40 °C. Light intensity was about 50 μmol photons m^{-2} s^{-1}, which is low but proved sufficient to ensure substantial growth during our 1-week incubations. The day–night regime was set to 14:10 h. The difference in light intensity of this (but not the salinity) stress treatment to the control might conceivably influence our results. However, our aim was not to study heat stress as such, but any type of environmental situation that would result in stress to the algae. In this case, our treatment involved mainly warming and slightly reduced light intensity. Furthermore, our conclusions are mainly based on the comparisons of treatments of different SI within a stress treatment, which were all cultivated under identical conditions, and are therefore unaffected by differences between the cultivation conditions of the control and heat stress treatments. One experimental run lasted for 1 week to obtain a measure of algal population growth, rather than yield, similar to the approach taken in grassland experiments when harvesting biomass as a measure of annual production (Duffy 2009; Hector *et al.* 2009). To avoid temporal effects of differences in inoculum abundances, we not only cultivated the source strains over the whole experimental time as described but adjusted the inoculum of the source strains optically for similar algae concentrations.

Absorption measurements

Absorption of the algal suspensions at 595 nm (Abs_{595}) was used as a measure of algal biomass. Absorption was measured in a Sunrise multiplate reader (Tecan, Männedorf, Switzerland) 7 days after inoculation using 200 μL of the suspensions. Measuring absorption is a common approach to estimating microbial biomass in liquid cultures (Sorokin 1973). That the absorption of two simultaneously cultivated control treatments comprising 53 species (monocultures and combinations) were tightly correlated ($r = 0.99$, $P < 0.001$, $n = 108$; Fig. S1) indicated high repeatability of Abs_{595} measurements and reproducibility of algal growth in identical environmental conditions (intercept = 0.0036, slope = 0.983, linear model, $P > 0.001$, $n = 108$). To assess whether differences in cell size influenced the absorption measurements, we first determined the area of 100 individual cells each of 16 of our study species using an Olympus© BX 60 microscope and imaging software (Olympus Cell*, Soft Imaging System GmbH, Münster, Germany) at 400× and 200× magnification. The mean surface area of the algal species ranged from 8.23 to 176.8 μm^2, while volumes ranged from 17.1 to 1743 μm^3. We then counted the density of individuals for each species in the algae suspension with a haemocytometer. By multiplying the mean surface area or volume, respectively, of each cell by the number of cells, we obtained a measure of the total surface area or biovolume of algae in 1 mL of the suspension. To vary the concentrations of the algae, we used dilution sequences. This resulted in 61 values of total algal surface area or biovolume, respectively, in relation to absorption values. For surface area, this resulted in a linear model with $r = 0.98$, $P < 0.001$, $F = 1204$, DF = 59 without species interactions, and $r = 0.99$, $P < 0.001$, $F = 77.3$, DF = 27 with species interactions (Fig. S2a). The corresponding analysis for biovolume yielded a linear model with $r = 0.68$, $P < 0.001$, $F = 50.9$, DF = 59 without species interactions and with $r = 0.998$, $P < 0.001$, $F = 273$, DF = 27 with species interactions (Fig. S2b). These results indicate that absorption was a suitable measure of algal biomass in the suspensions.

Statistical analyses

Stress-response intensity (SRI), *absolute stress-response buffering effect* (aSRBE) and *relative stress-response buffering effect* (rSRBE) were quantified as detailed in Box 1.

To compare total algal biomass produced under different environmental conditions (Fig. 1, Table 1), we used a linear mixed model with square-root transformed absorption as response variable and species richness as explanatory variable. Random variables were (1) the individual run, (2) the species combination of the assemblages and (3) the pair of control and stress treatment corresponding to each other.

We also compared the coefficient of variation of biomass produced under different stress levels across the diversity gradient using linear models. Models including an interaction of the slopes (resulting in non-parallel regression lines) were significantly better than those without interaction (ANOVA $P < 0.001$). Statistical analyses were conducted with R 2.13.1 (R Development Core Team 2011).

Analysis of literature data

For the analysis of literature data, we used all published experimental studies that (1) included at least two levels of diversity, (2) involved variation in environmental conditions leading to a decrease of the measured ecosystem function, that is, stress as defined here, and (3) measured at least one response variable suitable as proxy for ecosystem functioning. To identify these studies, we scanned recent literature and searched the Thomson Reuters Web of Knowledge (www.webofknowledge.com) and Google scholar (www.scholar.google.ch) using the search terms 'stress', 'ecosystem functioning', 'biodiversity effect', 'environmental fluctuation', 'biomass production', 'productivity' and related terms, in various combinations. Additionally, we checked the literature cited by the identified publications for additional original studies.

Where regression slopes of the ecosystem functions were provided in the publications, we used them for our analyses. In other cases, we calculated slopes based on the lowest and highest diversity levels studied. Where possible, we calculated slopes with monoculture data excluded, to obtain independent measures of SRI and SRBE (Box 1). However, this was only possible for six case studies. Therefore, we present results both for these six studies with monoculture data excluded, and for all 13 studies with monoculture data included. We analysed the data by applying linear mixed models with the relative biodiversity effect as response variable and SRI as explanatory variable (Box 1). Case study was treated as random variable. The specific data sources we used for all analyses are given in Table S3.

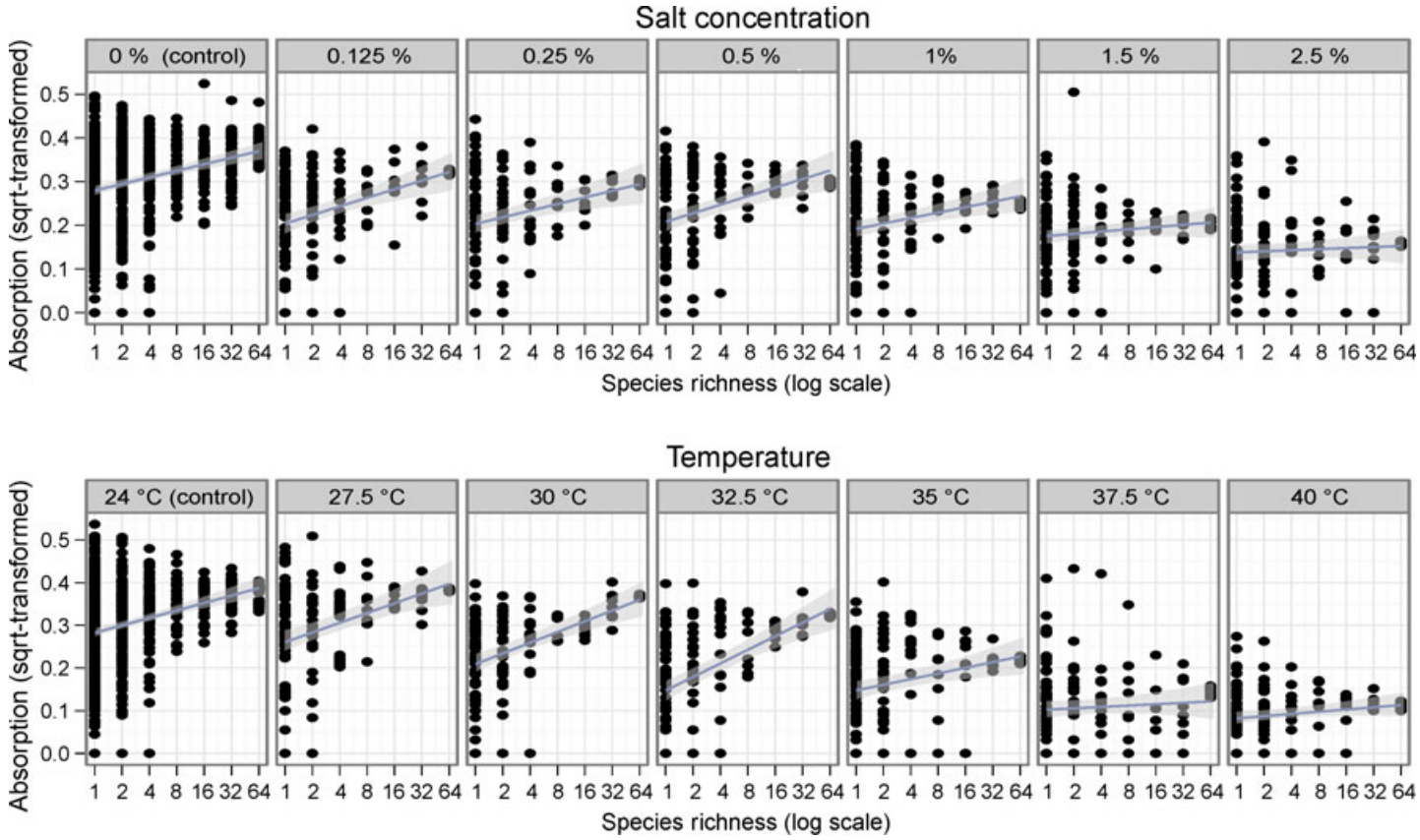

Figure 1 Relationships between algal species richness and biomass in microcosms exposed to increasingly stressful salinity and temperature conditions. Lines and shaded grey areas are linear regression slopes with 95% confidence limits of the absorption of algal suspensions (square-root transformed) as a proxy of algal biomass (Fig. S2). Data from all control microcosms (i.e. one for each environmental stress treatment) for both salinity and heat stress are combined in the panels furthest to the left, resulting in 864 data points in the control and 144 in each stress treatment.

Table 1 Effects of salinity and temperature stress intensities on algal biomass. Intercepts and slopes predicted by mixed linear models of the absorption of algal suspension (square-root transformed) as a proxy of biomass (Fig. S2) against the species richness (log-transformed) using experimental run, species composition and the individual pairs (control and stress treatment) as random factors. Results are shown as mean and 95% CI

Salinity (%)	Intercept	Slope	Temperature (°C)	Intercept	Slope
0*	0.280 (0.019)	0.0217 (0.0052)	24*	0.282 (0.016)	0.0252 (0.0054)
0.125	0.216 (0.032)	0.0317 (0.0132)	27.5	0.259 (0.034)	0.0310 (0.0148)
0.25	0.214 (0.032)	0.0242 (0.0132)	30	0.188 (0.034)	0.0387 (0.0148)
0.5	0.209 (0.032)	0.0188 (0.0132)	32.5	0.178 (0.034)	0.0407 (0.0148)
1	0.157 (0.032)	0.0169 (0.0132)	35	0.161 (0.034)	0.0232 (0.0148)
1.5	0.163 (0.032)	0.0108 (0.0132)	37.5	0.092 (0.034)	0.0066 (0.0148)
2.5	0.161 (0.032)	0.0050 (0.0132)	40	0.070 (0.034)	0.0060 (0.0148)

*Control

RESULTS

Biodiversity effects of microalgae

In control microcosms (0% NaCl and 24 °C), we found a significant positive relationship between biodiversity and average algal biomass (Fig. 2), although species mixtures did not generally produce more biomass than the fastest growing monocultures.

Increases in salinity or temperature reduced total biomass of the algae in our microcosms (Fig. 1, Table 1) and thus reflected stress as defined by us. In the monocultures, there were gradual decreases of biomass with increasing environmental stress intensity (ESI) in both the salinity and temperature treatment (Fig. S3). In the temperature treatment, significantly more species went extinct in the monocultures when stress intensity increased, but the same effect was not observed along the salinity gradient (Fig. S4). In the mixed assemblages, at low ESI, the reduction of biomass was often relatively more pronounced in the species-poor assemblages than in the species-rich ones, resulting in lower intercepts and thus steeper slopes of the algal richness–biomass relationship (Fig. 1). With further increases in ESI, the slope of the relationship (aSRBE) decreased to become indistinguishable from zero at the highest ESI of both stress types (Fig. 1).

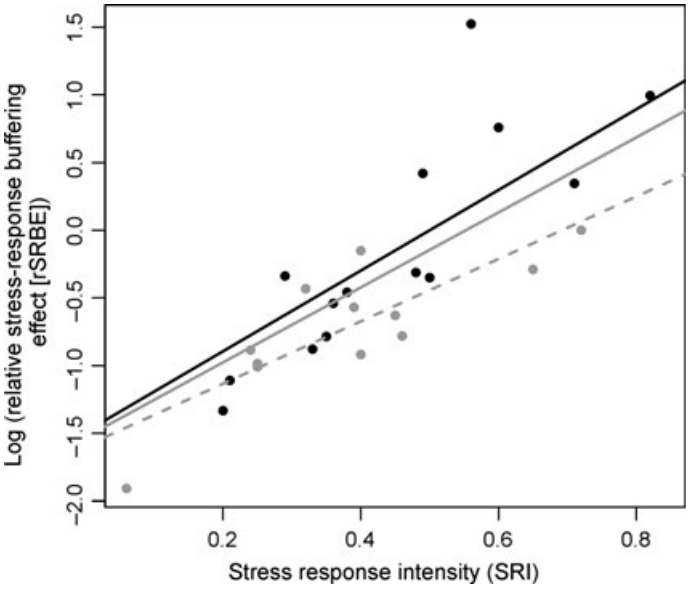

Figure 2 Relationships between relative stress-response buffering effect (rSRBE) calculated without monoculture data and stress-response intensity (SRI) derived from monoculture data. Black dots represent literature data, grey dots are for algae in the present experiment. The black line is the regression line of a linear mixed model including published data only, with 'study' treated as random factor (intercept = −1.49, SE = 0.48, t = −3.1, slope = 2.98, SE = 1.03, t = 2.9, P = 0.023, n = 6). The grey line is the regression line of a linear mixed model including published data and those of the present experiment, with 'study' treated as random factor (intercept = −1.53, SE = 0.29, t = −5.3, slope = 2.77, SE = 0.61, t = 4.6, P < 0.001, n = 7). The dashed grey line represents a regression line predicted by a linear model for the data of the present study only (intercept = −1.59, SE = 0.21, t = −7.7, slope = 2.30, SE = 0.49, t = 4.7, P < 0.001, n = 12).

The relative rSRBE increased significantly with increasing SRI (Fig. S5a). The rSRBE calculated with monocultures included also significantly increased with increasing SRI, indicating that our conclusions are robust (Fig. S5b).

The variability of biomass at different stress levels, expressed as coefficient of variation, significantly declined with increasing species richness in both the heat and salinity treatments (Fig. S6; Table S4). Whether this decline was only due to increasing similarity of assemblages with increasing species richness (Morin & McGrady-Steed 2004) could not be assessed because our experiment did not involve replicate assemblages with identical species composition (except for the 64-species assemblage). The different control treatments cannot be treated as replicates because algal densities are unknown and may have varied among runs. Coefficients of variation increased with increasing ESI (Fig. S6; Table S4).

Analysis of published data

We found 13 published studies and a total of 31 individual observations that conformed to the requirements for inclusion in our comparative analysis (Table 2). These studies were very heterogeneous. Biodiversity was either measured as genetic diversity, species richness or functional group richness, ranging from 1 to 3 species in one study to 1 to 32 species in another. Six studies representing 14 observations provided the data necessary to calculate slopes of ecosystem functioning against biodiversity without inclusion of the monoculture data (Box 1). For the other seven studies with 17 observations, we were unable to calculate slopes without the monoculture data. Because the first approach is preferable in that it allowed us to calculate slopes independently from SI, we focus on the first, restricted data set, but also report on the analysis of all data combined.

The mixed-effects model analysis with study as a random grouping factor revealed a significant negative relationship between the aSRBE and SRI for the full data set (Fig. S7) but not for the data set excluding monocultures in the calculation of slopes (intercept = −0.22, SE = 0.74, t = −0.30, slope = 1.78, SE = 1.53, t = 1.16, P = 0.28, n = 6). When we added the data points from our own experiments, the slope of this relationship changed only marginally. When we analysed our data on algae only, aSRBE also decreased with increasing SRI, indicating that the literature data and our algal data follow the same fundamental pattern (Fig. S7) for the full data set. A similar pattern was observed when an outlier (27.5 °C in the temperature treatment) was removed from the mixed model (intercept = 1.14, SE = 0.36, t = 3.21, slope = −2.48, SE = 0.64, t = −3.90, P < 0.001, n = 14). For the data set excluding the monoculture data for the calculation of biodiversity effects, this pattern was not significant either (intercept = 0.41, SE = 0.53, t = 0.76, slope = −0.059, SE = 1.00, t = −0.059, P = 0.95, n = 7).

The same models focusing on the relative stress-response buffering effect (rSRBE) revealed a significant positive relationship between rSRBE and SRI (Fig. 2). When we added the data points from our own experiments, the slope of this relationship changed only marginally (Fig. 2). This was also the case when the outlier was removed (intercept = −1.44, SE = 0.31, t = −4.61, slope = 2.57, SE = 0.67, t = 3.85, P = 0.0013, n = 7).

Finally, our mixed-effects model analysis based on the calculation of rSRBE including all 13 literature studies revealed an almost identical pattern. We found a significant positive relationship between rSRBE and SRI for the literature data and also for the literature data with our experimental data on algae added (Fig. S8). As above, a similar result was obtained when we excluded the outlier (intercept = −1.70, SE = 0.26, t = −6.44, slope = 3.54, SE = 0.52, t = 6.84, P < 0.001, n = 14).

DISCUSSION

The main results of our study are that aSRBE decreased with ESI and SRI, respectively (Fig. 1, Fig. S7), whereas rSRBE increased (Fig. 2). This result emerged from both of our experiments as well as from our literature analysis. Both of these analytical approaches have their limitations and strengths. Our experiment with algal cultures was conducted in an artificial system but is the only study to date in which a quantified stress intensity gradient was applied. The published studies mostly involved single levels of stress intensity, but they cover a wide range of taxa and study systems and hence add generality to the observed patterns. A striking result of our study is that despite these differences, the patterns founds by both parts of our study are congruent (Fig. 2). This suggests that the gradient of stress intensity covered by our experimental study reached levels corresponding those of other, partly natural, systems. Accordingly, both of our approaches indicated that rSRBE was relatively more pronounced at high levels of ESI and SRI, respectively, a relationship that has commonly been assumed based on very limited empirical evidence so far.

Table 2 Empirical studies used for analysing relationships between the relative stress-response buffering effect (rSRBE) and stress intensity (SRI)

Reference and treatment	log (aSRBE)	log (aSRBE) without monocultures	log (rSRBE)	log (rSRBE) without monocultures	Stress-response intensity (SRI)
Allison (2004) (Low1)	−0.416	NA	0.751	NA	0.73
Allison (2004) (Low2)	−1.05	NA	−0.361	NA	0.48
Caldeira *et al.* (2005)	−1.653	NA	2.091	NA	0.84
De Boeck *et al.* (2008) (A)	−0.916	NA	−1.216	NA	0.33
De Boeck *et al.* (2008) (B)	−1.05	NA	−1.848	NA	0.29
De Boeck *et al.* (2008) (C)	−0.223	NA	−0.266	NA	0.4
De Boeck *et al.* (2008) (D)	−0.844	NA	−2.367	NA	0.25
De Boeck *et al.* (2008) (E)	−0.619	NA	−2.141	NA	0.2
De Boeck *et al.* (2008) (F)	0.89	NA	−2.692	NA	0.17
Downing & Leibold (2010) (Macrophyte)	−0.811	NA	−0.949	NA	0.37
Downing & Leibold (2010) (Herbivore)	−0.172	NA	0.275	NA	0.45
Dukes (2002) (new)	0.513	1.792	0.3	−0.785	0.35
Dukes (2002) (established)	0.03	−0.42	−0.679	−0.878	0.33
Goodsell & Underwood 2008 (Bungan Head)	0.932	NA	−0.78	NA	0.21
Goodsell & Underwood (2008) (Narrabeen Head)	0.56	NA	−0.949	NA	0.24
Hughes & Stachowicz (2004)	1.887	−0.26	−0.375	−0.312	0.48
Ji *et al.* (2009) (Grasses 2004)	0.194	NA	−0.373	NA	0.34
Ji *et al.* (2009) (N fixers 2004)	−1.444	−1.641	−0.862	NA	0.5
Ji *et al.* (2009) (N fixers 2005)	−0.0073	0.755	1.042	−0.35	0.6
Ji *et al.* (2009) (Forbs 2005)	0.087	NA	−0.234	0.759	0.22
Joshi *et al.* (2000)	0.513	2.351	1.397	0.346	0.71
Liiri *et al.* (2002)	−0.083	NA	0.641	NA	0.47
Mulder *et al.* (2001)	2.14	NA	−0.057	NA	0.34
Nagase & Dunnett (2010) (root)	−0.827	1.674	0.167	1.524	0.56
Nagase & Dunnett (2010) (shoot)	0	−0.272	−1.215	−1.246	0.29
Steudel *et al.* (2011) (above-ground drought)	0.573	0.57	−0.639	−1.109	0.21
Steudel *et al.* (2011) (above-ground salt)	0.61	0.525	−0.63	−1.333	0.2
Steudel *et al.* (2011) (above-ground shade)	0.772	0.733	0.009	−0.458	0.38
Steudel *et al.* (2011) (below-ground drought)	0.578	0.788	0.357	0.42	0.49
Steudel *et al.* (2011) (below-ground salt)	0.501	0.564	−0.033	−0.54	0.36
Steudel *et al.* (2011) (below-ground shade)	−0.675	0.96	1.408	0.995	0.82

(A)-(F) Different runs that were conducted in this study.

Current ecological theory does not explicitly cover the relationship of biodiversity effects to stress intensity. However, the so-called insurance (Yachi & Loreau 1999) and portfolio effects (Doak *et al.* 1998; Tilman *et al.* 1998) predict that biodiversity buffers and enhances ecosystem functioning in the face of environmental fluctuations. While stress intensity is not explicitly addressed by these hypotheses, it might be implicit in that buffering and enhancing effects could be more pronounced as stress intensity increases. Our study is thus in accordance with previous ecological theory and adds the important component of relating responses of ecosystems to environmental fluctuations differing in strength.

There is currently no evident mechanistic explanation of the pattern we observed, partly because possible effects of varying stress intensity on biodiversity–ecosystem functioning relationships have not been theoretically examined. Furthermore, the standard mathematical methods to explore mechanisms behind biodiversity effects on ecosystem functioning could not be applied to our experimental data. This is because additive partitioning (Loreau & Hector 2001) and related methods to distinguish between the so-called complementarity and selection effects rely on quantifying the relative abundances of individual species at the time of sampling (Hector *et al.* 2009), but many of the microalgae used in our experiment cannot be reliably identified under a microscope. The increase in biomass observed in a previous experiment with green algae under benign culture conditions was due to spectral niche partitioning, which is a complementarity effect (Behl *et al.* 2011). It is not clear, however, whether the same mechanism is important also in stressed systems, because high stress intensities limit the scope for complementarity effects, making selection effects more likely. We therefore assessed the coefficient of variation of biomass at different levels of species richness in different stress treatments and found that variation increased at higher stress intensity (Fig. S6). While not conclusive evidence, this result provides some support for the notion that selection effects, by which different species dominate under different environmental conditions, become more prevalent at higher levels of environmental stress intensity. The relative contribution of complementarity and selection effects to biodiversity effects in ecosystems subject to environmental stress thus deserves closer examination.

One limitation of our experiment is that the density of algae in the microcosms was unknown at both the beginning and the end of the experimental runs. However, biomass in the species mixtures was calculated relative to that of the monocultures which were derived from the same suspensions. Therefore, differences in the cell density of inocula were accounted for. Furthermore, an independent growth experiment involving 53 species with different species mixtures

Table 3 Measurements of stress-response buffering effects (SRBE) and stress-response intensity (SRI) of the present study

Salinity or temperature level	log (aSRBE)	log (aSRBE) without monocultures	log (rSRBE)	log (rSRBE) without monocultures	Stress-response intensity (SRI)
0.125%	0.488	0.472	−0.488	−0.885	0.24
0.25%	0.155	0.258	−0.745	−0.985	0.25
0.5%	−0.18	−0.321	−0.851	−1.0095	0.25
1%	−0.248	−0.183	−0.517	−0.569	0.39
1.5%	−0.847	−1.264	−0.843	−0.918	0.4
2.5%	−1.724	−0.816	−0.932	−0.781	0.46
27.5 °C	0.151	0.211	−1.677	−1.908	0.06
30 °C	0.538	0.538	−0.17	−0.432	0.32
32.5 °C	0.311	0.705	0.306	−0.151	0.4
35 °C	0.032	−0.280	−0.176	−0.629	0.45
37.5 °C	−1.553	−1.333	−0.309	−0.289	0.65
40 °C	−1.305	−0.889	−0.008	0.001	0.72

(n = 108) and using similar inoculum densities showed that biomass continued to increase over twice the duration of our experiment, so that it is very unlikely that our microcosms reached stationary growth phase, which may influence the results since an assemblage growing quickly to stationary phase may be caught up by an assemblage growing slower so that differences arising during exponential growth may later be undetectable. Nevertheless, our experiment, like most previous studies, was conducted in an artificial experimental system over a relatively short time, requiring caution when extrapolating such experimental results to natural systems (Duffy 2009).

A second limitation of our experiment is that we had to use different incubators for the control and corresponding temperature stress treatments. This resulted in lower light intensity in the temperature stress treatments compared with the corresponding controls and may have slowed growth in the stressed treatments. However, as all temperature stress treatments were exposed to the same light intensity, there was no systematic bias along the temperature gradient. Moreover, the results we obtained along the temperature stress gradient are very similar to those of the salt stress treatment, in which light intensity of the stressed treatments and corresponding controls were identical because they were placed in the same incubator. Accordingly, while our temperature stress treatment should not be used to draw conclusions as to the specific effects of heat on algal growth (cf. Kessler 1977; Kessler & Huss 1992), it clearly represents a stress gradient affecting the relationship between algal richness and biomass production.

A further issue is to which degree our experimental results, along with others, can be applied to natural ecosystems and to fluctuations in environmental conditions. Because different experiments were conducted in very different study systems and considered different environmental factors, a formal quantitative comparison between effects of experimentally applied environmental stress intensity (i.e. variation of environmental conditions) and those of SRI, defined here in terms of ecosystem functioning (Box 1), is not possible. Furthermore, many studies, including our temperature stress experiment (see above), involved combinations of stress factors whose relative effects cannot be disentangled. Nevertheless, qualitative comparisons suggest that high levels of environmental stress also result in high levels of SRI. For example, a natural decline in annual precipitation (by 33%) combined with 49% more frost days compared with the 29-year average at the study site resulted in an SRI of 0.84 in a grassland; plant monocultures produced only 16% of the biomass than was observed the previous year experiencing normal rainfall (Caldeira *et al.* 2005). This is similar to the results of our experiment, the only study based on a quantitatively defined environmental stress gradient, where SRI increased with increasing salinity and heat (Fig. 1, Tables 1 and 3). Likewise, strong heating of macroalgae for 90 min, a perturbation considered similar in strength to natural temperature variations (Allison 2004), resulted in a SRI of 0.73. Invasion by a root hemiparasite yielded a SRI of 0.71 in host community biomass in another grassland study (Joshi *et al.* 2000). Conversely, most of the published studies for which we deduced low levels of SRI also showed relatively low environmental stress intensities. This includes a study in which temperature was increased by 3 °C relative to ambient conditions (De Boeck *et al.* 2008), resulting in SRIs ranging from 0.17 to 0.40. In a trampling experiment to stress macroalgae (Goodsell & Underwood 2008), SRIs were 0.21 and 0.24. These examples indicate that ESI and SRI tend to be correlated.

The levels of environmental stress intensity (ESI) we applied can also be compared with results from physiological experiments with selected species. Several species of *Ankistrodesmus, Chlorella* and *Scenedesmus*, all genera included in our experiments, tolerate maximum salinities between < 1% and 3–6% NaCl and maximum temperatures ranging from 28 to 42 °C (Kessler 1977; Kessler & Huss 1992). These upper limits are below or close to the highest salinity (2.5% NaCl) and temperature (40 °C) in our experiments, conditions that resulted in strong biomass declines of both the monocultures (Fig. S3) and mixed cultures (Fig. 1). Thus, the ESIs we applied reflect very harsh environmental conditions at the high stress intensities, corresponding to the physiological limits of the algal species.

In summary, the concordance of our study and of the analysis of previously published experiments strongly suggests that biodiversity enhances ecosystem functioning under stressful environmental conditions in relative, but not in absolute, terms. This relationship is commonly implicit in discussions of the major benefits of biodiversity, but is experimentally documented here for the first time. Our results thus add to mounting evidence that conserving biodiversity is a crucial precautionary measure to maintain ecosystem functioning at current levels (Cardinale *et al.* 2012; Naeem *et al.* 2012). Our study prepares the ground for further experimental studies and theoretical approaches to better understand the mechanisms driving biodiversity –ecosystem functioning relationships along stress gradients.

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AUTHORSHIP

B.S. and M.K. conceived the study. M.L. and T.F. chose the algal strains and provided cultivation knowledge. B.S., C.L. and M.W. conducted the experiments. B.S. and A.H. analysed the data. B.S., A.H. and M.K. led the writing with all other authors commenting on the results and on the manuscript.

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