Stressor-induced biodiversity gradients: revisiting biodiversity–ecosystem functioning relationships

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Biodiversity–ecosystem functioning experiments typically inspect functioning in randomly composed communities, representing broad gradients of taxonomic richness. We tested if the resulting evenness gradients and evenness–functioning relationships reflect those found in communities facing evenness loss caused by anthropogenic stressors. To this end, we exposed marine benthic diatom communities to a series of treatments with the herbicide atrazine, and analysed the relationship between the resulting gradients of evenness and ecosystem functioning (primary production, energy content and sediment stabilization). Atrazine exposure resulted in narrower evenness gradients and steeper evenness–functioning relations than produced by the design of random community assembly. The disproportionately large decrease in functioning following atrazine treatment was related to selective atrazine effects on the species that contributed most to the ecosystem functions considered. Our findings demonstrate that the sensitivity to stress and the contribution to ecosystem functioning at the species level should be both considered to understand biodiversity and ecosystem functioning under anthropogenic stress.

Synthesis

Biodiversity loss affects ecosystem functioning, yet biodiversity-ecosystem functioning relations have mainly been investigated using communities with random species loss. In nature however, species are lost according to their sensitivity to environmental stress. In the present study, biodiversity loss and biodiversity–ecosystem functioning relations in randomly composed diatom communities were compared to those induced by the pesticide atrazine. Stress exposure resulted in smaller biodiversity loss but steeper decrease in functioning than in randomly composed communities, due to selective atrazine effects on the best performing species. Therefore, species-specific sensitivity and contribution to ecosystem functioning need to be considered to predict biodiversity and ecosystem functioning under anthropogenic stress.

Biodiversity loss due to human activities has led scientists to enquire how the diversity of communities may regulate ecosystem functioning (Cardinale et al. 2012). During the last two decades, numerous studies have shown that biodiversity favours ecosystem functioning, by positively affecting processes such as productivity or decomposition (Loreau 2000, Hooper et al. 2012). Toxic chemicals are potential drivers of biodiversity decrease (De Laender et al. 2014) but remain understudied in biodiversity–ecosystem functioning research and conservation (Lawler et al. 2006). In diversity–functioning research, ecosystem functioning is commonly measured across broad gradients of species richness in randomly assembled communities (Deutschman 2001, Díaz et al. 2003). In the case of toxic chemicals, this makes the implicit assumption that pollution would eliminate a large number of species, and remove individuals in a random way. However, environmentally realistic levels of stressors such as chemical pollution or physical disturbance reduce the abundance of sensitive species (Davies et al. 2011), but without necessarily removing them from the ecosystem, resulting in gradients of evenness rather than species richness (Johnston and Roberts 2009). Whether the evenness gradients created by random manipulations of species richness are representative for those caused by environmental stress such as chemical toxicity remains elusive. Moreover, it is unclear how ecosystem functioning of randomly assembled communities compares to that of communities composed by environmental filtering through chemical toxicity. Chemicals can impact ecosystem functioning directly, by affecting the physiology of the concerned species (De Laender et al. 2010, Hooper et al. 2012), and indirectly through selective biodiversity loss, depending on

the species' sensitivity to the chemical (Vinebrooke et al. 2004). When species differ in their contribution to ecosystem functioning, consequences of biodiversity decline for a particular process can depend on the order in which the species are lost (Solan et al. 2004). If the most sensitive species also contributes most to a given ecosystem process, a chemical-induced reduction in density of this species, and therefore evenness reduction, can lead to more pronounced functional consequences than predicted from random community assembly design.

We compare biodiversity gradients and diversity–functioning relations obtained using the design of random community assembly with those generated by filtering through environmental stress. First, we contrast evenness gradients created by randomly assembling four benthic diatom species from an intertidal mudflat with those induced by applying the herbicide atrazine, one of the most frequently used herbicides worldwide (Graymore et al. 2001). Despite its EU-wide ban, environmentally critical concentrations of atrazine are still monitored in European intertidal mudflats, which are wetlands of high economic and ecological value (Graymore et al. 2001, Niquil et al. 2006). Next, we test if changes in ecosystem functioning in both sets of experiments are comparable: do evenness-functioning relationships inferred from the random assembly approach predict pesticide-driven non-random evenness–functioning relations? We measured three ecosystem processes and attributes that are particularly relevant in mudflat ecosystems: primary production, sediment stabilization and energy content of diatoms. Lastly, we tested if differences between the two types of diversity–functioning relationships could be explained by selective atrazine effects, measuring to what extent species-specific contributions to ecosystem functioning and species-specific sensitivity to chemicals combine in shaping the diversity–functioning relation.

Diversity–functioning research commonly focuses on the relation between species richness and primary production (Hillebrand and Matthiessen 2009). Yet, evenness responds faster to environmental stress and can be a stronger predictor for ecosystem functioning than species richness per se (Chapin et al. 2000, Kirwan et al. 2007). Also, ecosystem processes other than primary production can respond differently to diversity loss (Hector and Bagchi 2007). We therefore quantified biodiversity as evenness, and analyzed its relation to three ecosystem processes and attributes. Biovolume, the most frequently used proxy for phytoplankton biomass (Hillebrand et al. 1999), and maximum quantum yield of photosynthesis, a proxy for photosynthetic efficiency (Hartig et al. 1998, Kromkamp et al. 1998), were used to quantify primary production. Sediment stabilization was quantified as extracellular polymeric substances (EPS) production. These biopolymers secreted by microphytobenthos and other microorganisms enhance ecosystem functioning through biogenic sediment stabilization (Gerbersdorf et al. 2009). The diatom energy content, an attribute that serves as proxy for energy transfer to higher trophic levels, was estimated by the production of polyunsaturated fatty acids (PUFAs), an essential diet component for primary consumers (De Troch et al. 2012). PUFAs are therefore commonly used

as marker for food quality and energy transfer efficiency (Brett and Müller-Navarra 1997, De Troch et al. 2012).

Methods

Experimental organisms and culture conditions

Experimental communities were composed of four benthic epipelic diatom species. *Navicula arenaria, Nitzschia* sp. and *Entomoneis paludosa* were sampled from intertidal mudflats in the Westerschelde estuary (southwestern Netherlands, 51°21′N, 3°43′E). *Seminavis robusta* was obtained from the culture collection of the Protistology and Aquatic Ecology Research Group (UGent). Cultures of the species were maintained in a climate room at $15 \pm 1°C$, a light/dark cycle of 12 h / 12 h and an illumination of 90 μmol E m^{-2} s^{-1}, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32 ± 1) enriched with f/2 nutrients (Guillard 1975).

Atrazine and random assembly design

In the first experiment, diatom communities were constructed by random community assembly, the common approach in diversity–functioning research. All possible 15 species combinations were assembled, thereby simulating all possibilities of random species loss. In the second experiment (atrazine design), diatom communities were composed selectively through atrazine, by exposing the full species community to concentrations of 0 (control treatment), 100, 500 and 1000 μg l^{-1} atrazine respectively. Technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 99.8% pure) was used in all treatments. Atrazine was dissolved in the culture medium by means of a 30 min ultrasonic bath. The resulting atrazine concentrations determined by GC-MS analysis were 91, 465 and 980 μg l^{-1} in the atrazine treatments and < 1 μg l^{-1} (below detection limit) atrazine in the control treatment. Treatments in the random assembly design were run twice in polystyrene 6-well-plates, treatments in the atrazine design in three polysterene 6-well-plates: an equivalent of 12 and 18 replicated microcosms per treatment. Each microcosm was inoculated with a total cell density of 10 000 cells ml^{-1} (100 000 cells in 10 ml of culture medium), with equal cell densities per species. Treatments were inoculated with diatom cells from exponentially growing cultures, and incubated in a climate room at $15 \pm 1°C$, under a light/dark cycle of 12 h/12 h at 90 μmol E m^{-2} s^{-1}. All experiments were terminated after 18 days.

Biodiversity and ecosystem functioning measurements

Evenness was calculated every second day from cell densities per species. Cell densities were obtained by magnifying and photographing ($\times$ 100) an area of 0.33 mm^2 per microcosm, using an inverted microscope and a connected digital camera. Cell numbers per species were subsequently determined by identifying and counting cells on the computer (ImageJ cell counting software) and converted into cell densities in

cells ml^{-1}. Evenness for every treatment and replicate was calculated as follows:

$$J = \frac{H'}{H'_{max}}$$

$$H' = -\sum_{i=1}^{s} \frac{n_i}{N} \times \log \frac{n_i}{N}$$

Where J is eveness, H′ is the Shannon index, n_i is cell density of the *i*th species. N is total cell density of the microcosm and H′$_{max}$ is log10 of species richness (log10(4) in every treatment). Time-averaged evenness was used as biodiversity parameter in every treatment.

Biovolume was calculated every second day from cell densities and mean cell biovolume per species according to Hillebrand et al. 1999, using measured linear dimensions and formulas representing the closest approximation of geometric shape for each genus (Supplementary material Appendix 1 Table A1). Time-averaged biovolume was calculated as proxy for primary production for every treatment.

Maximum quantum yield of photosynthetic activity was measured at 48-hour-intervals by pulse–amplitude modulated (PAM) fluorometry. Maximum quantum yield is determined as the ratio of variable and maximum fluorescence (F_v/F_m). Maximum fluorescence F_m is the maximum fluorescence emission level after a dark adaptation of 20 min, measured with a saturating pulse of light (emission peak at 450 nm, 2700 photons m^{-2} s^{-1}, 800 ms). Variable fluorescence F_v is calculated from the difference between initial fluorescence (F_0) and maximum fluorescence ($F_v = F_m - F_0$).

Extracellular polymeric substances were measured by spectrophotometry at the end of the experiments after 18 days, with three replicates of 10 ml suspended diatom culture per treatment. The suspended diatom cultures were centrifuged for 15 min at 15°C and 3500 g. The supernatant yielded the soluble EPS fraction, which was left to precipitate overnight at –20°C in 30 ml cold ethanol (98%), and subsequently centrifuged for 15 min at 15°C and 3500 g. The pellet was dried under a flow of nitrogen and resuspended in 2 ml of 1.5% NaCl. Samples of 200 μl of this suspension per replicate were used for the EPS analysis. EPS was measured according to a modified version of the phenol/H$_2$SO$_4$ assay by (DuBois et al. 1956). In 24-well-plates, 1 ml concentrated H$_2$SO$_4$ and 200 μl phenol (5%, w/v in distilled water) were added to 200 μl sample. The mixture was shaken, incubated for 20 min and the absorbance measured at 488 nm using a Victor³ multilabel reader. EPS concentrations were quantified with a function obtained from a linear regression of known concentrations of glucose standards (ranging from 0.1 to 100 μg ml^{-1}).

Fatty acid profiles in two replicates of 37 ml suspended diatom culture per treatment were analyzed after 18 days. The suspension was centrifuged for 20 min at 4°C and 2700 g. The supernatant was removed except 5 ml, the pellet was resuspended, transferred into a 10 ml glass tube and centrifuged for 15 min at 4°C and 2700 g. The supernatant was removed and the pellet was stored at –80°C until fatty acid extraction. Hydrolysis of total lipid extracts of duplicate diatom cell suspensions per treatment and methylation to FA methyl esters (FAME) was achieved by a modified one-step derivatisation method (Abdulkadir and Tsuchiya 2008, De Troch et al. 2012). The boron trifluoride-methanol reagent was replaced by 3.5 ml of a 2.5% H$_2$SO$_4$-methanol solution per sample since BF3-methanol can cause artefacts or loss of PUFA (Eder 1995). The fatty acid methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification. Samples were centrifuged and vacuum dried. The FAME thus obtained were analysed using a gaschromatograph with a mass spectrometer. The samples were run in splitless mode, with a 1 μl injection per run, at an injector temperature of 250°C using a HP88 column. The oven temperature was programmed at 50°C for 2 min, followed by a ramp at 25°C min^{-1} to 175°C and then a final ramp at 2°C min^{-1} to 230°C with a 4 min hold. The FAME were identified by comparison with the retention times and mass spectra of authentic standards and mass spectral libraries, and analysed with the software MSD ChemStation. Quantification of individual FAME was accomplished by the use of external standards. The quantification function of each individual FAME was obtained by linear regression of the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 ng ml^{-1}).

Data analysis

Biodiversity–ecosystem functioning relationships in randomly assembled and stressed communities were analyzed with a generalized least squares model (model 1), with the respective ecosystem process or attribute as response variable and biodiversity as predictor (E[EFIa, b] = a + b·B]). B represents biodiversity, EF ecosystem functioning, and a and b the intercept and the slope (i.e. the effect of B on EF). In cases when biodiversity significantly (alpha = 0.05) predicted ecosystem functioning for both random and stressed treatments, we compared the slopes (b) and their confidence intervals between the two model fits. The absence or presence of overlap of the 95% pointwise confidence intervals for b in both models was used as a criterion for the similarity or difference of diversity–functioning relations between the atrazine-induced and random biodiversity loss. Normality and homogeneity of model residuals across the evenness range were inspected using quantile–quantile plots and by plotting residuals versus explanatory variables, respectively. If homogeneity was violated, the model was refitted using an exponential variance structure allowing residuals to change with the predictor X (var(ε) = $\sigma2 \times e^{2 \times \delta \times X}$), and homogeneity was evaluated again (model 2). By means of likelihood ratio testing, the significance of these structures was tested (alpha = 0.05).

Effects of species identity were assessed by fitting a generalized least square model to the respective ecosystem process or attribute as response variable and the cell density of each of the four species as predictors [E[EFIa, b] = a + $\Sigma_{i=1}^{4}$ b$_i$ × S$_i$]. EF represents ecosystem functioning, a is the intercept, S is the cell density of *Navicula arenaria, Entomoneis paludosa, Seminavis robusta* or *Nitzschia* sp. respectively, and b represents the slope, i.e. the effect of species identity on EF. Normality and homogeneity of model residuals was tested as for the diversity–functioning models, i.e. by plotting residuals versus all predictors. All calculations were
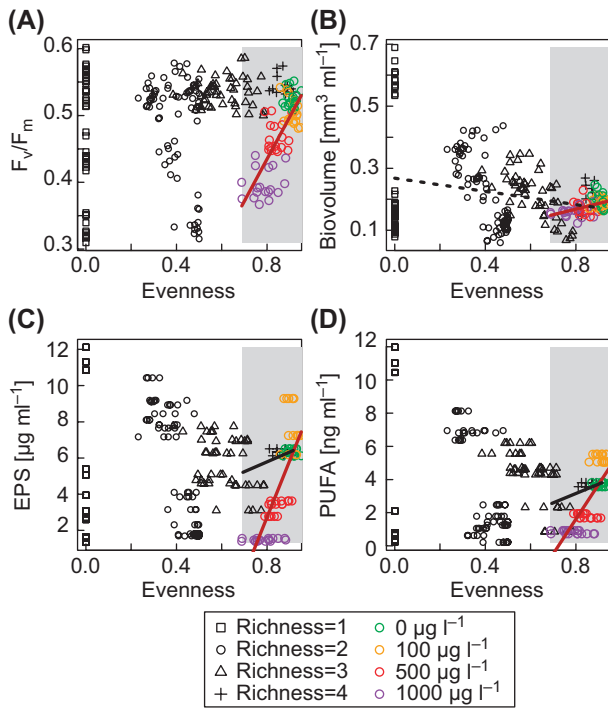
Figure 1. Proxies of ecosystem functioning as a function of evenness in randomly assembled and atrazine-exposed diatom communities. Ecosystem functioning is quantified as primary production (photosynthetic efficiency and biovolume, panels A and B), sediment stabilization (EPS, panel C) and energy content (PUFA, panel D). Black dots represent diatom communities from the random assembly design, coloured dots denote diatom communities from the atrazine design. The area marked in grey denotes the evenness gradient induced by atrazine. Regression lines show significant relations between evenness and function. Brown lines are linear models based on the atrazine design. Dashed and solid black lines are linear models based on the random assembly design for the whole evenness range or for the evenness range induced by atrazine (grey area), respectively.

performed in R ver. 2.10.1, using RStudio and the package nlme (Pinheiro et al. 2014).

Results

Atrazine reduced diatom evenness, but to a lesser extent than random species removal. Evenness gradients obtained in the atrazine design (0.69 to 0.98) were narrower than those established in the random assembly design (0 to 0.96) (Fig. 1). Evenness–functioning relationships differed in randomly assembled and atrazine-exposed communities. Evenness was positively related to all four ecosystem functioning proxies (all $p < 0.0001$) in the atrazine design (Fig. 1, Table 1). In the random assembly design, evenness was significantly related to biovolume only ($p = 0.0006$, Fig. 1, Table 1). Ecosystem functioning was also increasingly variable with decreasing evenness in the random assembly design (Fig. 1). This was confirmed by likelihood ratio testing, which showed that diversity–functioning relations in these communities were more accurately described by models including a variance structure allow-

ing the variance of ecosystem functioning to change with evenness ($p < 0.0001$; Table 1).

To enable contrasts of evenness–functioning relations induced by the random assembly approach with those found in stressed communities within the same evenness gradient, we repeated our analyses, only considering the overlap of both evenness ranges (0.69–0.98) (Fig. 1, marked in grey). This was because the evenness gradient resulting from atrazine exposure was narrower than that in the random assembly design. These analyses revealed that, in the randomly composed communities, evenness within a range of 0.69 to 0.98 was positively related to EPS ($p = 0.02$) and PUFA ($p = 0.02$) production (Fig. 1, Table 1). The slopes of these evenness–functioning relationships however were three times (EPS) and six times (PUFA) lower than those found in the atrazine design (Fig. 1, Table 1), i.e. slopes were less steep at random community composition. Diversity–functioning relationships were thus different when created by random community assembly than by exposure to atrazine.

As we considered community performance as more relevant for ecosystem functioning than performance per cell, we initially analyzed diversity–functioning relationships in terms of absolute values. In order to know if PUFA and EPS production were also related to diversity at the cellular level, we included supplemental analyses between evenness and PUFA and EPS concentrations normalized to cell density. Normalized PUFA and evenness were not correlated in the random assembly design, but positively related in the atrazine design ($p < 0.0001$, Supplementary material Appendix 2 Fig. A2, Table A2). EPS production per cell was positively correlated with evenness, both in atrazine-exposed communities and over the whole random diversity gradient (both $p < 0.0001$). Similar to non-normalized values, the diversity–functioning relation was steeper when evenness gradients were induced by atrazine. In randomly assembled communities of similar evenness as induced by atrazine, the relation between evenness and normalized EPS was negative ($p = 0.02$, Supplementary material Appendix 2 Fig. A2, Table A2).

In the atrazine design, diversity–functioning relations were steeper, although diversity loss was less severe than predicted by the random assembly approach. In the random assembly design, *Nitzschia* sp., if present, always became the dominant species. Exposure to atrazine did not lead to species removal, but to a change in dominance: instead of *Nitzschia* sp., *Navicula arenaria* dominated communities exposed to 500 and 1000 μg l⁻¹ atrazine, reaching its highest cell density at the two highest atrazine concentrations. Detailed cell densities per species for every treatment are indicated in the Supplementary material Appendix 3, Fig. A3 for the random assembly design and Appendix 4 Fig. A4 for the atrazine design. Moreover, *Nitzschia* sp. cell density was positively correlated with all proxies of ecosystem functioning, in both atrazine-exposed and randomly composed communities (Table 2). PUFA and EPS production, the processes that were related to evenness in both the atrazine and the random assembly design, thus also correlated with the abundance of *Nitzschia* sp. Evenness gradients found in atrazine and random assembly treatments hence coincided with a change in the identity of the dominant species (dominance of *Navicula arenaria* and *Nitzschia* sp.

Table 1. Relationship between evenness and ecosystem functioning in randomly composed and atrazine-exposed diatom communities. Results of generalized least squared models predicting ecosystem functioning from evenness, without (model 1) or with (model 2) a variance structure. 'Data' denotes the data on which the models are based: the random assembly design (R), the atrazine design (A), the random assembly design for evenness values overlapping with those induced by atrazine (R'); SE is the standard error on the estimated slopes; t and p denote the t- and p-values, bold values are statistically significant. AIC is the Akaike information criterion; 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'); 'LR' is the likelihood ratio of model 1 versus model 2, P LR the corresponding p-value.

Ecosystem function	Data	Model	Slope	SE	t	p	AIC	Log lik.	Validity	LR	P LR
Primary production: (photosynth. efficiency)	R	1	0.8	0.02	4.05	**0.0001**	−416	211	no		
		2	0.09	0.01	4.90	**<0.0001**	−441	224	no	na	na
	A	1	0.63	0.06	9.84	**<0.0001**	−264	135	yes		
		2	0.63	0.07	8.81	**<0.0001**	−265	137	yes	2.62	0.1057
	R'	1	0.03	0.06	0.44	0.6619	−98	52	yes		
		2	0.04	0.06	0.64	0.5274	−99	54	yes	na	na
Primary production: biovolume (mm^3 ml^{-1})	R	1	−0.11	0.03	−3.01	**0.0030**	−205	106	no		
		2	−0.11	0.03	−3.50	**0.0006**	−263	136	yes	59.82	**<0.0001**
	A	1	0.18	0.04	4.56	**<0.0001**	−333	170	yes		
		2	0.18	0.04	5.05	**<0.0001**	−332	170	yes	0.93	0.3340
	R'	1	0.33	0.17	1.99	0.0597	−55	30	yes		
		2	0.42	0.16	2.59	**0.0166**	−56	32	yes	3.05	0.0806
Sediment stabilization: EPS (μg ml^{-1})	R	1	−0.32	0.81	−0.40	0.6895	904	−449	no		
		2	0.11	0.74	0.15	0.8835	868	−430	yes	na	na
	A	1	31.12	2.91	10.69	**<0.0001**	273	−133	yes		
		2	29.32	2.63	11.14	**<0.0001**	274	−133	yes	1.07	0.3006
	R'	1	6.43	3.38	1.90	0.0706	81	−38	no		
		2	5.43	2.29	2.37	**0.0272**	62	−27	yes	21.60	**<0.0001**
Energy content: PUFA (ng ml^{-1})	R	1	−2.36	0.91	−2.60	**0.0101**	945	−470	no		
		2	−1.05	0.73	−1.44	0.1503	874	−433	yes	72.77	**<0.0001**
	A	1	20.83	2.09	9.94	**<0.0001**	224	−109	yes		
		2	18.56	1.80	10.30	**<0.0001**	225	−108	yes	1.80	0.1794
	R'	1	4.33	3.35	1.30	0.2086	77	−36	no		
		2	6.12	2.38	2.57	**0.0175**	62	−27	yes	17.30	**<0.0001**

respectively). At high atrazine concentrations the growth of *Nitzschia* sp., the species contributing most to all analyzed functions, was suppressed. Instead, communities were dominated by *Navicula*, which was negatively correlated with EPS production in the atrazine-exposed communities (Table 2).

The observed diversity–functioning relationships were thus related to the identity of the most productive species, and a selective suppression of this species by atrazine can explain the steeper decline of functioning at stressor-induced than at random biodiversity loss.

Discussion

In biodiversity–ecosystem functioning research, one typically aims at experimental biodiversity gradients that are as broad as possible, to test the effects of widespread diversity loss on ecosystem functioning (Naeem 2008). Indeed, narrow, evenness-based biodiversity gradients are uncommon in experimental diversity–functioning research (Hillebrand et al. 2008). Instead, biodiversity loss by anthropogenic activity is typically mimicked with broad diversity gradients, covering the entire range from minimal to maximal species richness of the considered ecosystem (Hillebrand et al. 2008), corresponding to evenness ranges from 0 to 1. In the case of toxic chemicals however, such extensive biodiversity loss is unlikely to happen. Realistic biodiversity loss most often only affects a fraction of the existing species pool, resulting in alterations of evenness rather than richness

(Pennington et al. 2001, Hillebrand and Matthiessen 2009), even at chemical concentrations well above environmental concentrations, as represented by the highest atrazine concentrations in our study (Pennington et al. 2001). Evenness alterations induced by atrazine were less severe than those in the richness-based random assembly approach, which in this case overestimated the biodiversity loss induced by chemical stress. Yet in the random assembly design, we recorded positive effects of diversity on energy content and sediment stabilization within a narrow range of high evenness, but not over the whole broad evenness gradient. Rather than being a tradeoff to realism, we thus suggest narrow biodiversity gradients, with evenness instead of richness as diversity proxy, as a more realistic simulation of pollution-induced biodiversity loss that can facilitate the detection of biodiversity effects on ecosystem functioning.

Diversity–functioning relations were steeper when diversity gradients were chemically-induced rather than randomly assembled, denoting a disproportionately large decrease of functioning in diatom communities under stress conditions. Contrasting the design of random community assembly with scenarios of selective biodiversity loss due to environmental filtering is among the main challenges for future diversity–functioning research (Srivastava 2002, Giller et al. 2004), but has rarely been tested experimentally (Symstad and Tilman 2001, Smith and Knapp 2003, Solan et al. 2004, Larsen et al. 2005, Davies et al. 2011). Chemical stress moreover has not been studied as driver for realistic biodiversity loss and ecosystem functioning, although toxic

Table 2. Relationship between ecosystem functioning and diatom cell density per species in randomly composed and atrazine-exposed communities. Results of generalized least squared models predicting ecosystem functioning from cell density per species. 'Data' denotes the data on which the models are based: the random assembly design (R), the atrazine design (A), the random assembly design for evenness values overlapping with those induced by atrazine (R'); SE is the standard error on the estimated slopes; bold values are statistically significant.

Ecosystem function	Data	Predictor	Slope	SE	t	p
Sediment stabilization: EPS ($\mu g\ ml^{-1}$)	A	Navicula	-1.31×10^{-1}	4.66×10^{-2}	-2.8243	**0.0062**
		Entomoneis	5.08×10^{-3}	2.08×10^{-2}	0.2445	0.8076
		Seminavis	9.85×10^{-3}	4.29×10^{-2}	0.2296	0.8191
		Nitzschia	2.57×10^{-1}	3.74×10^{-2}	6.8742	**<0.0001**
	R'	Navicula	1.94×10^{-2}	2.55×10^{-2}	0.7579	0.4578
		Entomoneis	-1.01×10^{-2}	8.76×10^{-3}	-1.1494	0.2647
		Seminavis	-4.93×10^{-3}	1.31×10^{-3}	-3.7631	**0.0013**
		Nitzschia	1.83×10^{-2}	2.27×10^{-3}	8.0803	**<0.00001**
Energy content: PUFA (ng ml^{-1})	A	Navicula	-1.60×10^{0}	1.06×10^{-0}	-1.5046	0.1371
		Entomoneis	1.02×10^{-1}	4.69×10^{-1}	0.2175	0.8285
		Seminavis	-1.08×10^{-0}	9.70×10^{-1}	-1.1102	0.2709
		Nitzschia	6.12×10^{-0}	8.50×10^{-1}	7.1985	**<0.0001**
	R'	Navicula	2.93×10^{-1}	5.41×10^{-1}	0.5429	0.5935
		Entomoneis	-2.95×10^{-1}	1.77×10^{-1}	-1.6690	0.1115
		Seminavis	-2.12×10^{-1}	2.64×10^{-2}	-8.0345	**<0.0001**
		Nitzschia	4.89×10^{-1}	7.29×10^{-2}	6.7162	**<0.0001**
Primary production: biovolume ($mm^3\ ml^{-1}$)	A	Navicula	1.73×10^{-1}	1.28×10^{-2}	13.5532	**<0.0001**
		Entomoneis	6.97×10^{-2}	$6.03E-03$	11.5579	**<0.0001**
		Seminavis	-8.01×10^{-3}	1.24×10^{-2}	-0.6463	0.5203
		Nitzschia	9.59×10^{-2}	1.05×10^{-2}	9.1230	**<0.0001**
	R'	Navicula	1.13×10^{-1}	1.99×10^{-2}	5.6587	**<0.0001**
		Entomoneis	1.46×10^{-1}	1.56×10^{-2}	9.3537	**<0.0001**
		Seminavis	3.01×10^{-3}	2.75×10^{-3}	1.0917	0.2886
		Nitzschia	2.08×10^{-2}	1.33×10^{-3}	15.6292	**<0.0001**
Primary production: (photosynth. efficiency)	A	Navicula	-3.41×10^{-2}	3.78×10^{-2}	-0.9010	0.3708
		Entomoneis	2.34×10^{-2}	1.96×10^{-2}	1.1984	0.2350
		Seminavis	8.30×10^{-2}	4.18×10^{-2}	1.9843	0.0513
		Nitzschia	1.23×10^{-1}	3.09×10^{-2}	3.9923	**<0.0001**
	R'	Navicula	-2.82×10^{-2}	3.85×10^{-2}	-0.7319	0.4732
		Entomoneis	-1.48×10^{-4}	1.89×10^{-2}	-0.0078	0.9938
		Seminavis	-4.12×10^{-3}	2.87×10^{-3}	-1.4325	0.1683
		Nitzschia	6.53×10^{-3}	2.14×10^{-2}	3.0548	**0.0065**

chemicals emitted by industry, agriculture and households are among the most potent threats to aquatic species (Geiger et al. 2010). We found a larger discrepancy in random and stressor-induced diversity–functioning relations (three to six time steeper relations in stressed communities in terms of EPS and PUFA production) than in other cases where the random assembly design could be contrasted with realistic diversity loss (Smith and Knapp 2003, Solan et al. 2004, Larsen et al. 2005, Schlapfer et al. 2005). This can be due to the organisms and ecosystem processes studied, and to selective atrazine effects on the physiological rates and composition of the diatom communities.

In our diatom communities that were randomly assembled, primary production, the most common measure of ecosystem functioning (Hooper et al. 2005), was not related to diversity. In contrast, sediment stabilization and energy content showed a steeper relation to diversity than primary production in both the atrazine and the random assembly design, which facilitated the contrast of the two experimental approaches. The observed functional consequences of stressor-induced diversity loss thus depended on the measured ecosystem function, which underlines the value of analyzing multiple ecosystem processes (Hector and Bagchi 2007, Hiddink et al. 2009). Also, the pool of four species

could have contributed to the steep diversity–functioning relations. In our experimental communities, the functionally most important species was most affected by atrazine. In a larger species pool, this selection effect could be compensated by functionally redundant, but less sensitive species. Moreover, as randomized and stress-induced biodiversity and ecosystem functioning have so far not been assessed in marine microalgae, we cannot relate our findings to other studies using similar organisms. The disproportionate loss of functioning following selective loss of diatom biodiversity however corresponds to existing knowledge on non-random diversity loss in grassland primary producers (Symstad and Tilman 2001, Zavaleta and Hulvey 2004). Yet, the steep diversity–functioning relationships in our stressed communities can not only be explained by the number and type of different organisms and ecosystem processes. Loss of functioning in stressed communities can be related to selective alterations of biodiversity by the stressor, as well as direct stressor effects (Hooper et al. 2012). In our case, atrazine could affect functioning indirectly through selective changes of community composition and directly by reducing physiological rates of the species. Atrazine treatments in which functioning was reduced with regard to the control were essentially dominated by *Navicula arenaria*, the only species

which was able to grow at the highest atrazine concentration. To inspect potential direct physiological effects, we compared PUFA and EPS production per cell in these *N. arenaria*-dominated stressed communities with those of unexposed *N. arenaria* monocultures. Cells in the *N. arenaria*-dominated communities produced 1.8 and 3.5 times less EPS, but 2 and 3 times more PUFA than in the monocultures of this species (Supplementary material Appendix 5 Fig. A5). The reduction in EPS in stressed communities could thus be due to physiological effects of atrazine on *N. arenaria*. The reduction in PUFA however does not seem to be caused by physiological effects, as *N. arenaria* actually seems to increase its PUFA production when exposed to atrazine. To understand the steep loss of functioning in our stress-exposed communities, we must therefore, depending on the ecosystem process, look beyond direct stressor effects and consider selective atrazine effects on the community composition.

In diversity–functioning research, consequences of biodiversity loss are commonly predicted using randomly assembled communities, whereas environmental filtering due to stress results in distinct non-random community compositions. Furthermore, evenness is often held constantly high across species richness treatments (Wilsey and Polley 2004), thus minimizing effects of relative abundances (i.e. species identity) on ecosystem functioning. Due to the increasing recognition that biodiversity and species identity combine in shaping ecosystem functioning (Bruno et al. 2006), research on realistic biodiversity loss has started by removing rare and uncommon species from the community (Smith and Knapp 2003). Despite making the step from random to a more realistic, abundance-based biodiversity loss, this approach still assumes that the naturally abundant species are also most resistant to environmental stressors and less likely to be lost. In our diatom communities, *Nitzschia* sp. was the most abundant species in the natural samples as well as in the experimental communities, contributing most to the measured ecosystem processes. However, when exposed to stress, *Nitzschia* sp. was the species whose cell density was most reduced by atrazine. This shows that both in stressed and randomly assembled communities, community composition tends to be uneven, and that the most abundant species is not necessarily the most stress-resistant. Biodiversity loss generally reduces ecosystem functioning, but general physiological and selective stressor effects on the most abundant and functionally most efficient species lead to a disproportionate loss of ecosystem functioning in stressed communities compared the random assembly design.

Benthic diatoms are the dominant primary producers and food source in many intertidal, soft-sediment environments as well as on rocky shores (Thompson et al. 2004, Tang and Kristensen 2007, Speybroeck et al. 2008). Diatom communities play a pivotal role in controlling energy flow, nutrient fluxes and productivity in these systems (Underwood and Kromkamp 1999, De Troch et al. 2012). Diatom biodiversity loss resulting in a two-fold reduction in diatom biomass, three-fold reduction of unsaturated fatty acids and six-fold decrease of EPS production would strongly impact the ecosystem under concern. The standing stock and energy flux to higher trophic levels would be affected (Decho 1990,

Müller-Navarra et al. 2000), zoobenthos and as a consequence fish biomass production limited (De Troch et al. 2012), and sediment erosion promoted. Concomitantly, knock-on effects on the variety of processes undertaken by sediment-associated organisms collectively contributing to ecosystem functioning can be expected (Tolhurst et al. 2003). Stressor-induced biodiversity loss at the base of the marine food web could thus affect ecosystem functioning more seriously than predicted by the random assembly design in diversity–functioning research. In our study, the random assembly approach overestimated biodiversity loss, but underestimated the associated loss of ecosystem functioning. These contrasting results strengthen the need to more realistically predict biodiversity gradients and diversity–functioning relations caused by anthropogenic stress, by considering the prevailing stressors, and the sensitivity and contribution to ecosystem functioning of the concerned species.

In conclusion, we infer three general statements from our results. First, existing diversity–functioning research largely overestimates the biodiversity gradients induced by common environmental stressors such as herbicides. Second, diversity–functioning relations differ depending on whether the diversity gradient is generated by random community assembly, or by stressor-induced non-random assembly. Third, physiological stressor effects can affect ecosystem functioning, and the slope of the diversity–functioning relation depends on stressor-induced dominance shifts.

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