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Biodiversity and ecosystem functioning in stressed environments: primary producers and consumers at the basis of marine food webs

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**Biodiversity and ecosystem functioning in
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Biodiversity and ecosystem functioning in stressed environments: primary producers and consumers at the basis of marine food webs

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1 **Summary**

2 Biodiversity is increasingly altered by human activities, and there is a growing concern to what
3 extent biodiversity loss will affect ecological processes that are essential to ecosystem functioning
4 and ultimately to human well-being. These concerns have made the relationship between
5 **biodiversity and ecosystem functioning** (diversity-functioning hereafter) a core research area
6 in ecology, and two decades of intensive research have provided compelling evidence for a link
7 between biodiversity and ecosystem functioning.

8 **Anthropogenic stressors** such as an increasing number of chemical contaminants are the driving
9 force behind the ongoing biodiversity loss. Whilst diversity-functioning research is often framed
10 within the context of anthropogenic change, anthropogenic stressors are however rarely included
11 into the design of diversity-functioning studies. Similarly, the majority of diversity-functioning
12 experiments have focussed on single trophic levels, whereas the impact of anthropogenic
13 stressors across trophic levels has remained underrepresented in diversity-functioning research.

14 This PhD used marine primary producers (**benthic diatoms**) and consumers (**harpacticoid**
15 **copepods**) and two chemical stressors (atrazine and copper) to explore four central questions
16 which are still largely debated or unresolved in current diversity-functioning research: (i) Does
17 the classic design in diversity-functioning research, which consists of random species loss, predict
18 the diversity gradients and diversity-functioning relations induced by anthropogenic stress? (ii)
19 Can biodiversity loss under stress, and its impact on ecosystem functioning, be predicted from the
20 species' traits? (iii) Is the biodiversity effect on functioning in stressed communities determined
21 by the dominant species (dominance effect) or a better performance of species in community
22 (complementarity effect), and which are the mechanisms driving both effects? (iv) Are stress
23 effects on the energy content of primary producers (diet quality) transmitted to the consumer
24 level? The main findings regarding these questions are presented in Chapters 2 to 5, whilst
25 Chapter 6 proposes an integrative synthesis of the three key effects through which anthropogenic
26 stress drives the diversity-functioning relation.

27 The majority of diversity-functioning experiments to date used broad gradients of species
28 richness, and tested the effect of biodiversity loss on ecosystem functioning by removing species
29 in a random way. It remains unsure however whether this classic protocol is representative of the
30 biodiversity loss and diversity-functioning relations as induced by anthropogenic stress. The first
31 objective of this work was therefore to contrast the biodiversity gradients and diversity-
32 functioning relations in a benthic diatom community as obtained with the classic random design
33 with those induced by atrazine (**Chapter 2**). Biodiversity was quantified as species richness and
34 evenness, and functioning as the diatom's contribution to primary production, biomass

35 production, energy content and sediment stabilization. Biodiversity gradients induced by atrazine
36 exposure were narrower than predicted by the classic random approach, since the herbicide
37 altered evenness but did not cause any species loss. Diversity- functioning relations for sediment
38 stabilization and energy content were steeper in stressed than in randomly assembled
39 communities. This disproportionately large decrease of functioning with stressor-induced
40 biodiversity loss was related to selective atrazine effects on the species contributing most to
41 energy content and sediment stabilization (*Nitzschia* sp.), which was also most sensitive to stress.
42 The common approach in diversity-functioning research thus overestimated biodiversity loss
43 induced by chemical stressors, but underestimated the associated loss of function due to selective
44 stress effects targeting the species contributing most to functioning.

45 Given the plethora of anthropogenic stressors, it is logistically impossible to predict selective
46 stress effects by experimentally testing the tolerance of species to each of these stressors. **Chapter**
47 **3** therefore tested if response traits and effect traits could respectively predict the abundance
48 (numerical response) and functioning (functional response; potential contribution to primary
49 production, sediment stabilization and energy content) of 18 marine benthic diatom strains to
50 copper and atrazine. Furthermore, it was tested whether the species' numerical stress response
51 was related to their functional contribution and to their functional stress response, i.e. if atrazine
52 and copper targeted species of little or high importance for ecosystem functioning, and if tolerant
53 species could maintain their functional contribution under stress. The numerical and functional
54 response of diatoms to copper was predicted by the same set of intercorrelated morphological
55 traits (cell size, surface to volume ratio, cell length), with large cells being more resistant to the
56 metal. Under copper, the diatoms' numerical response stress response was positively related to
57 their functional contribution and functional stress response, meaning that copper-tolerant
58 species contributed most to functioning and performed best at maintaining their functioning.
59 Under atrazine, the capacity for mixotrophic growth predicted the numerical but not the
60 functional stress response of diatoms, and the diatoms' numerical response to atrazine was not
61 related to their functional contribution and functional stress response. Diatoms capable of
62 growing on organic carbonic sources could thus maintain their cell numbers, but contributed little
63 to functioning under herbicide stress. Overall, Chapter 3 indicated that if numerical and functional
64 stress response are predicted by the same set of response and effect traits, numerically tolerant
65 species might be able to maintain ecosystem functioning under stress. If response and effect traits
66 do not correspond, stress might cause a disproportionate loss of functioning.

67 Dominance (dominance by species with high or low functional contribution) and
68 complementarity (species contribute more to functioning in community than in monoculture) are
69 the two key effects driving the biodiversity effect on ecosystem functioning, but there is little

70 evidence on the effects of anthropogenic stress on dominance and complementarity.
71 Complementarity effects arise from ecological mechanisms such as niche partitioning or
72 facilitative interactions between species. Direct functional and ecophysiological tests of these
73 mechanisms are however scarce and there is no evidence attributing changes of complementarity
74 under stress to changing facilitative mechanisms. **Chapter 4** first examined whether the
75 biodiversity effect on diatom biomass production under atrazine and copper stress was driven by
76 dominance or complementarity effects. Next, it was tested if the release of extracellular polymers
77 as a facilitative mechanism could explain changes in complementarity under stress. Last, it was
78 analysed if complementarity was two-way or one-way, i.e. if complementarity was equally
79 distributed among species, or favoured species depending on their properties. The biodiversity
80 effect on diatom biomass production depended on two-way complementarity, which increased
81 under stress. Under atrazine and copper stress, diatom communities increased their production
82 of extracellular polymers which, in part, predicted increases in complementarity. Diatom species
83 benefited from complementarity depending on their properties, with complementarity under
84 atrazine and copper respectively favouring the growth of mixotrophic (*C. fusiformis*, *Nitzschia* sp.,
85 *N. acicularis*) and copper-sensitive species (*A. lineolata*, *N. digitoradiata*, *Gyrosigma* sp.). These
86 copper-sensitive species were however characterised by a low biomass production, causing a
87 negative one-way complementarity, which largely offset positive two-way complementarity
88 effects and limited the biodiversity effect on diatom biomass production under metal stress.
89 Chapter 4 provided first ecophysiological evidence for facilitation as driver of complementarity
90 under stress, by identifying extracellular polymers as a 'sleeping' facilitative mechanism, which
91 when stimulated under stress predicted the diversity effect on biomass production. This
92 facilitative mechanism was however not necessarily beneficial for ecosystem functioning when it
93 caused a negative one-way complementarity which benefited species with a low functional
94 contribution.

95 There is little evidence whether stressors drive functioning through selective effects (changes in
96 community structure) or context-dependent effects (changes in the species' functional
97 contribution), and if both types of stress knock on across trophic levels. Moreover, trophic
98 approaches to date have essentially focussed on diet quantity, i.e. primary producer biomass,
99 whilst diet quality (producer energy content) has not been considered when examining food web
100 functioning under stress. **Chapter 5** measured if atrazine and copper affected functioning in a
101 diatom community (diet quantity, sediment stabilization, diet quality) through selective stress
102 effects (by selectively targeting the species contributing most to functioning) or context-
103 dependent effects (by changing the species' functional contribution). Concomitant knock-on
104 effects of selective and context-dependent stress effects on the next trophic level were quantified
105 by testing the response of the diatoms' main grazer (the harpacticoid copepod *M. littorale*) to

106 changes in diet quality. Diatom diet quantity was reduced by copper stress but not by low atrazine
107 levels due to the presence of an atrazine-tolerant, mixotrophic species (*C. closterium*). The
108 diatoms' contribution to sediment stabilization was stimulated by context-dependent effects of
109 both stressors. At low atrazine levels, selective changes in community structure involving
110 dominance by the atrazine-tolerant but energy-poor species *C. closterium*, reduced diet quality by
111 more than half. Context-dependent stress effects only reduced diet quality at high atrazine and
112 copper levels. Furthermore, selective and context-dependent stress effects on diet quality affected
113 the energy transfer to the next trophic level, with *M. littorale* losing half of its energy content when
114 feeding on diatoms grown under atrazine and high copper stress. Chapter 5 identified selective
115 stress effects, causing shifts in community structure towards dominance by species with low
116 functional contribution, as a more potent threat for ecosystem functioning than any direct stress
117 effects on the species' functional contribution. The energy content of copepods depended on that
118 of their diatom diet, highlighting the relevance of diet quality as a key driver of energy transfer at
119 the primary producer-consumer interface.

120 **Chapter 6** integrates the three main effects influencing ecosystem functioning under stress: the
121 functional impact of selective stress, targeting the functionally most important species, increases
122 with differences in the species' functional contribution, and can be predicted from the species'
123 response and effect traits. Physiological stress, by directly reducing the species' functional
124 contribution, drives ecosystem functioning through context-dependent effects at high stress
125 levels. Increases in complementarity, driven by the activation of facilitative mechanisms under
126 stress, can enable primary producers to maintain their functional contribution and limit losses of
127 ecosystem functioning. By highlighting the three key effects driving the diversity-functioning
128 relation under stress, this work proposes an conclusive framework for a better integration of
129 anthropogenic stressors within diversity-functioning research.

130

131 **Samenvatting**

132 Biodiversiteit wordt in toenemende mate veranderd door menselijke activiteiten, en er is een
133 groeiende bezorgdheid in hoeverre het verlies aan biodiversiteit invloed zal hebben op
134 ecologische processen die essentieel zijn voor het functioneren van ecosystemen en uiteindelijk
135 voor het menselijk welzijn. Deze bekommerning zorgde ervoor dat de relatie tussen biodiversiteit
136 en het functioneren van ecosystemen (diversiteit - ecosysteem functionering) een
137 kernonderzoeksgebied in de ecologie werd, en twee decennia van intensief onderzoek hebben
138 overtuigend bewijs geleverd voor een link tussen biodiversiteit en het functioneren van
139 ecosystemen.

140 Antropogene stressoren zoals een toenemend aantal chemische verontreinigingen zijn de
141 drijvende kracht achter het voortdurende verlies aan biodiversiteit. Terwijl onderzoek naar
142 diversiteit - ecosysteem functionering vaak gekaderd wordt in de context van antropogene
143 veranderingen, worden de antropogene stressoren waardoor het voortdurende verlies aan
144 biodiversiteit echter zelden opgenomen in het ontwerp van diversiteit-functioneringsstudies. De
145 meeste diversiteit-functioneringsexperimenten richten zich op één trofische niveau, terwijl de
146 invloed van antropogene druk over trofische niveaus heen ondervertegenwoordigd is in
147 diversiteit-functioneringsonderzoek.

148 Dit doctoraat gebruikt mariene primaire producenten (benthische diatomeeën) en grazers
149 (harpacticoide copepoden) en twee chemische stressoren (atrazine en koper) om vier centrale
150 vragen die nog steeds grotendeels worden betwist of onopgelost zijn in het huidige onderzoek te
151 toetsen: (i) voorspelt het klassieke ontwerp in diversiteit-functioneringsonderzoek, dat bestaat
152 uit een willekeurig soortenverlies, de diversiteitsgradiënten en diversiteit-functionering relaties
153 veroorzaakt door antropogene stress? (ii) kan verlies van biodiversiteit onder stress en zijn
154 impact ervan op het functioneren van ecosystemen, worden voorspeld op basis van kenmerken
155 van de soort? (iii) is het biodiversiteitseffect op het functioneren van gemeenschappen onder
156 stress bepaald door de dominante soort (dominantie-effect) of een betere prestatie van soorten
157 in de gemeenschap (complementariteitseffect), en welke zijn de mechanismen achter beide
158 effecten? (iv) worden stresseffecten op de energie-inhoud van primaire producenten
159 (dieetkwaliteit) doorgegeven naar het niveau van de consument? De belangrijkste bevindingen
160 ten aanzien van deze vragen worden gepresenteerd in de hoofdstukken 2 tot 5, terwijl hoofdstuk
161 6 een integratieve synthese geeft van de drie belangrijkste effecten waardoor antropogene stress
162 de diversiteit-functioneringsrelatie drijft.

163 Het merendeel van de diversiteit-functioneringsexperimenten gebruikte tot op heden een ruime
164 gradiënt van de soortenrijkdom, en testte het effect van het verlies aan biodiversiteit op het

165 functioneren van ecosystemen door het verwijderen van soorten op een willekeurige manier. Het
166 blijft echter onzeker of dit klassieke protocol representatief is voor het verlies aan biodiversiteit
167 en de diversiteit-functioning relaties veroorzaakt door antropogene stress. De eerste doelstelling
168 van dit onderzoek was dan ook om de biodiversiteitsgradiënten en diversiteit-
169 functioneringsrelaties te contrasteren in een benthische diatomeeëngemeenschap zoals
170 verkregen met de klassieke willekeurig ontwerp versus een gemeenschap geïnduceerd door
171 atrazine (**Hoofdstuk 2**). Biodiversiteit werd gekwantificeerd als soortenrijkdom en 'evenness'
172 (spreiding van de individuen over de soorten), en functionering in termen van de bijdrage van de
173 diatomeeën aan de primaire productie, de productie van biomassa, energie-inhoud en
174 sedimentstabilisatie. Biodiversiteitsgradiënten die veroorzaakt werden door
175 atrazineblootstelling waren kleiner dan voorspeld door de klassieke willekeurige aanpak, omdat
176 het herbicide de spreiding van de individuen over de soorten ('evenness') wijzigde maar geen
177 verlies aan soorten veroorzaakte. Diversiteit-functioneringsrelaties voor sedimentstabilisatie en
178 energie-inhoud waren steiler in gestresseerde dan in willekeurig samengestelde
179 gemeenschappen. Deze onevenredig grote vermindering van functioneren met stress-
180 geïnduceerd verlies van biodiversiteit was gelinkt aan selectieve atrazine effecten op de soort die
181 het meest bijdroeg tot de energie-inhoud en sedimentstabilisatie (*Nitzschia* sp.). Dit was tevens
182 de meest stressgevoelige soort. De algemene aanpak in diversiteit-functioneringsonderzoek
183 overschat dus het verlies aan biodiversiteit veroorzaakt door chemische stressoren, maar
184 onderschat het daarmee gepaard gaande verlies van functie als gevolg van selectieve
185 stress-effecten gericht op de soorten die het meest bijdragen tot het functioneren.

186 Gezien de overvloed van antropogene stressoren is het logistiek onmogelijk om selectieve
187 stress-effecten te voorspellen door de tolerantie van soorten experimenteel te testen voor elk van
188 deze stressoren. **Hoofdstuk 3** testte daarom of de response-eigenschappen en effecteigenschappen
189 respectievelijk de abundantie (numerieke respons) en het functioneren (functionele respons;
190 potentiële bijdrage aan de primaire productie, sedimentstabilisatie en energie-inhoud) kunnen
191 voorspellen van 18 mariene benthische diatomeeënstammen tegenover koper en atrazine. Verder
192 werd onderzocht of numerieke stressreactie van soorten gerelateerd was aan hun functionele
193 bijdrage en hun functionele stressrespons. De numerieke en functionele respons van diatomeeën
194 ten opzichte van koper werd voorspeld door dezelfde set gecorreleerde morfologische
195 kenmerken (celgrootte, oppervlakte-volumeverhouding, cellengte), met grote cellen die beter
196 bestand zijn tegen koper. In de koperbehandelingen was de numerieke stressrespons van de
197 diatomeeën positief gerelateerd aan hun functionele bijdrage en functionele stressrespons, wat
198 betekent dat de koper-tolerante soorten het meest bijdroegen tot het functioneren en het best
199 presteerden bij het handhaven van hun functie. Bij de atrazinebehandelingen voorspelde de
200 mixotrofe groei de numerieke maar niet de functionele stressreactie van diatomeeën en de

201 numerieke respons van de diatomeeën ten opzichte van atrazine was niet gerelateerd met hun
202 functionele bijdrage en functionele stressreactie. Diatomeeën die kunnen groeien op organische
203 koolzuurbronnen kunnen dus celdensiteiten behouden, maar dragen weinig bij tot het
204 functioneren onder herbicidestress. Over het algemeen, onderstreept Hoofdstuk 3 dat als
205 numerieke en functionele reactie op stress worden voorspeld door dezelfde set van de respons-
206 en effecteigenschappen, numeriek tolerante soorten mogelijks het ecosysteem functioneren
207 kunnen behouden onder stress. Als respons- en effecteigenschappen niet overeenstemmen dan
208 kan stress een onevenredig verlies van functioneren veroorzaken.

209 Dominantie (dominantie van soorten met een hoge of lage functionele bijdrage) en
210 complementariteit (soorten die meer bijdragen aan het functioneren in de gemeenschap dan in
211 monocultuur) zijn de twee belangrijkste effecten die het effect van biodiversiteit op
212 ecosysteemfunctionering sturen maar er is weinig bewijs voor de effecten van antropogene druk
213 op dominantie en complementariteit. Complementariteitseffecten vloeien voort uit de ecologische
214 mechanismen zoals nichepartitionering of faciliterende interacties tussen soorten. Directe
215 functionele en ecofysiologische testen van deze mechanismen zijn echter schaars en er is geen
216 bewijs die wijzigingen in complementariteit onder stress kan toewijzen aan veranderende
217 faciliterende mechanismen. **Hoofdstuk 4** onderzocht eerst of het biodiversiteitseffect op de
218 productie van biomassa door diatomeeën onder atrazine- en koperstress werd gedreven door
219 dominantie of complementariteitseffecten. Vervolgens werd onderzocht of de afgifte van
220 extracellulaire polymeren als faciliterende mechanisme een verklaring kan zijn voor
221 veranderingen in complementariteit onder stress. Tenslotte werd geanalyseerd of de
222 complementariteit in twee richtingen of één richting voorkwam, met andere woorden of de
223 complementariteit evenredig verdeeld werd tussen soorten, of of soorten bevoordeeld werden op
224 basis van hun eigenschappen. Het biodiversiteitseffect op de productie van biomassa door
225 diatomeeën was afhankelijk van twee-richtingen complementariteit, die toenam onder stress.
226 Onder atrazine en koperstress verhoogden de diatomeeëngemeenschappen hun productie van
227 extracellulaire polymeren wat deels de toename in complementariteit voorspelde.
228 Diatomeeënsoorten profiteerden van complementariteit afhankelijk van hun eigenschappen,
229 waarbij complementariteit bij behandeling met atrazine en koper de groei van respectievelijk
230 mixotrofe (*C. fusiformis*, *Nitzschia* sp., *N. acicularis*) en kopergevoelige soorten (*A. lineolata*, *N.*
231 *digitoradiata*, *Gyrosigma* sp.) promootte. Deze kopergevoelige soorten werden echter gekenmerkt
232 door lage biomassa-opbrengsten, waardoor een negatieve éénrichtingscomplementariteit, die
233 grotendeels positieve tweerichtingscomplementariteit effecten teniet deed en beperkte het
234 biodiversiteitseffect op de productie van diatomeeënbiomassa bij stress door metalen. Hoofdstuk
235 4 leverde het eerste ecofysiologisch bewijs voor facilitatie als drijfveer van complementariteit
236 onder stress, door het aanduiden van extracellulaire polymeren als een 'slapend' faciliterend

237 mechanisme, dat wanneer gestimuleerd onder stress het diversiteitseffect op de
238 biomassaproductie voorspelde. Deze faciliterende mechanisme was echter niet per se gunstig
239 voor het functioneren van het ecosysteem indien het een negatieve
240 éénrichtingscomplementariteit veroorzaakte die soorten bevoordeligde met een lage functionele
241 bijdrage.

242 Er is weinig bewijs dat stressoren functionering sturen door middel van selectieve effecten
243 (veranderingen in de gemeenschapsstructuur) of context-afhankelijke effecten (veranderingen in
244 functionele bijdrage van de soorten), en of beide types van stress een impact hebben over
245 trofische niveaus heen. Bovendien hebben trofische benaderingen tot op heden zich voornamelijk
246 gericht op dieethoeveelheid, dat wil zeggen de biomassa van de primaire producent, terwijl de
247 kwaliteit van het dieet (energie-inhoud van de producent) niet beschouwd werd bij het onderzoek
248 van het functioneren van een voedselweb onder stress. In **Hoofdstuk 5** werd onderzocht of
249 atrazine en koper een effect hadden op het functioneren van een diatomeeëngemeenschap
250 (dieethoeveelheid, sedimentstabilisatie, dieetkwaliteit) door middel van selectieve stresseffecten
251 (door selectieve impact op de soorten die het meest bijdragen tot het functioneren) of context-
252 afhankelijke effecten (door het veranderen van de functionele bijdrage van de soorten).
253 Gelijktijdig knock-on effecten van selectieve en context-afhankelijke stress effecten op de
254 volgende trofische niveau werden gekwantificeerd door het testen van de respons van de
255 belangrijkste grazers op de diatomeeën (de harpacticoïde copepode *M. littorale*) ten opzichte van
256 veranderingen in de dieetkwaliteit. De hoeveelheid van het diatomeeëndieet werd verlaagd door
257 koperstress maar niet door lage atrazineniveaus door de aanwezigheid van een atrazine-
258 tolerante, mixotrofe soort (*C. closterium*). De bijdrage van diatomeeën aan sedimentstabilisatie
259 werd gestimuleerd door context-afhankelijke effecten van beide stressoren. Bij lage
260 atrazineniveaus, verminderden selectieve veranderingen in de gemeenschapsstructuur met
261 dominantie van de atrazine-tolerante maar energie-arme soort *C. closterium*, de dieetkwaliteit
262 met meer dan de helft. Context-afhankelijke stresseffecten alleen beïnvloedden het functioneren
263 van diatomeeën bij hoge atrazine- en koperniveaus. Selectieve en context-afhankelijke
264 stresseffecten op dieetkwaliteit beïnvloedden de energietransfer naar het volgende trofische
265 niveau, met *M. littorale* die de helft van zijn energie-inhoud verliest wanneer gevoed met
266 diatomeeën opgegroeid onder atrazine- en hoge koperstress. Hoofdstuk 5 duidt selectieve
267 stresseffecten aan, die verschuivingen in de gemeenschapsstructuur veroorzaken met een
268 dominantie van soorten met een lage functionele bijdrage, als een meer potente bedreiging voor
269 het functioneren van ecosystemen dan directe stresseffecten op de functionele bijdrage van de
270 soort. De energie-inhoud van de copepoden hing af van die van hun diatomeeënvoedsel, dit
271 onderlijnt de relevantie van de kwaliteit van het dieet als een belangrijke motor van de energie-
272 overdracht op de primaire producent en consument interactie.

273 **Hoofdstuk 6** integreert de drie hoofdeffecten die ecosysteemfunctionering beïnvloeden onder
274 stress: de functionele impact van selectieve stress, gericht op de functioneel belangrijke soorten,
275 verhoogt met de verschillen in de functionele bijdrage van de soort, en kan worden voorspeld op
276 basis van de respons van de soort en de effectkenmerken. Fysiologische stress, door rechtstreeks
277 verminderen van de functionele bijdrage van de soort, drijft ecosysteemfunctionering door
278 middel van context-afhankelijke effecten bij hoge stressniveaus. Verhogingen van
279 complementariteit, gedreven door de activering van faciliterende mechanismen onder stress,
280 kunnen primaire producenten in staat stellen hun functionele bijdrage te behouden en beperken
281 het verlies van het functioneren van ecosystemen. Door te wijzen op de drie belangrijkste effecten
282 die de diversiteit-functioneringsrelatie onder stress sturen, stelt dit werk een integrale
283 alomvattend kader voor om een betere integratie van antropogene stressoren binnen-diversiteit
284 functionerende onderzoek te garanderen.

285

286 **Chapter 1: General Introduction**

287 **1.1. Biodiversity and ecosystem functioning**

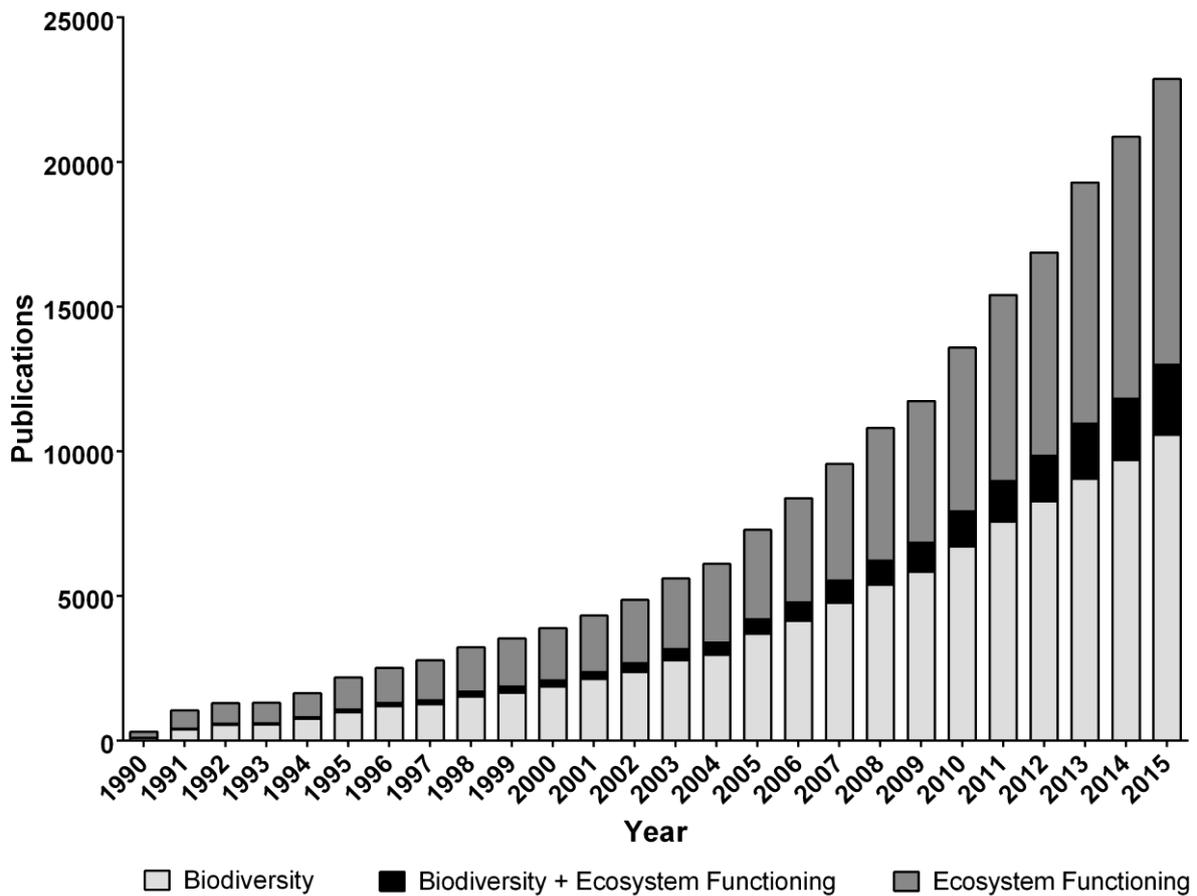
288 Over the past century, Earth has experienced an unprecedented and widespread biodiversity
289 turnover, and current predictions indicate that the rates of biodiversity change will continue to
290 rise in the near future (Sala et al. 2000, Pereira et al. 2010, Barnosky et al. 2011, Dornelas et al.
291 2014, Ceballos et al. 2015, Gonzalez et al. 2016). Human activities have been the main reason for
292 the ongoing extinctions and compositional turnover and will continue to extensively alter
293 biodiversity worldwide (Pimm et al. 1995, Sala et al. 2000, Chapin et al. 2000, Bellard et al. 2012,
294 McGill et al. 2015). Biodiversity has long been treated as a matter of ‘pure ecology’, which sees
295 biodiversity as a culmination of population and community ecological processes, and changes in
296 biodiversity have sparked mainly aesthetic and ethical concerns (Yarmin 1995, Martín-López et
297 al. 2007, Naeem et al. 2009). This conventional perspective of biodiversity has been
298 revolutionized by a question that had rarely been asked by ecology texts: What is the significance
299 of biodiversity to human wellbeing (Naeem et al. 2009)? This question broadened the
300 conventional perspective of biodiversity as a pure result of ecological processes towards the idea
301 of biodiversity as driver of ecosystem processes, which underpin the ecosystem goods and
302 services that ultimately determine human well-being (Loreau et al. 2001b, Hooper et al. 2005,
303 Srivastava and Vellend 2005, Cardinale et al. 2012, Naeem et al. 2012).

304 **1.1.1. From community and ecosystem ecology to biodiversity-ecosystem functioning** 305 **research**

306 Until the end of the 20th century, the inclusion of biodiversity into ecosystem science has not been
307 a focus in ecological research, which has traditionally been divided into the separate disciplines
308 of community ecology and ecosystem ecology (Loreau 2000, Loreau et al. 2001a). The former
309 focused on how biodiversity and community composition are governed by biotic interactions (e.g.
310 competition, predation, mutualism) and abiotic processes such as disturbance and climate. The
311 latter centred on the properties within ecosystems, such as the rates, dynamics or stability of
312 energy flow or nutrient cycling (Loreau et al. 2001a). In the early 1990s however, the separation
313 of community and ecosystem ecology was overcome when researchers started investigating the
314 impact of biota on ecosystem properties (Lubchenco et al. 1991, Chapin et al. 1992, Hobbs 1992).

315 Vitousek and Hooper (1993) formally merged community and ecosystem research and proposed
316 a common positive relationship between biodiversity and ecosystem functioning (hereafter BEF).
317 BEF research rapidly received widespread attention, and the first BEF experiments investigating
318 the effects of biodiversity on plant productivity in the mid-1990s (Naeem et al. 1994, Tilman and

319 Downing 1994, Tilman et al. 1996) were followed by in a dramatic increase of empirical and
 320 theoretical studies (Fig. 1.1).



321
 322 **Fig. 1.1:** The emerging synthesis of biodiversity and ecosystem functioning research. Results represent
 323 publications that included either biodiversity (B), ecosystem functioning (EF) or both (BEF, black fill in the
 324 centre of bars) in their titles, keywords or abstracts. Note the steep increase in biodiversity research, and
 325 the increasing proportion of studies linking biodiversity with ecosystem functioning. Data were obtained
 326 from the *ISI Web of Knowledge* using the 'advanced search' tool and the *Science Citation Index Expanded*
 327 database on 29th July 2016. The general search terms were TS=('biodiversity' OR 'species diversity' OR
 328 'species richness' OR 'evenness*') for publications on biodiversity, TS=('ecosystem function' OR 'ecosystem
 329 functioning' OR 'ecosystem process' OR 'ecosystem') for publications on ecosystem functioning, and
 330 TS=('biodiversity' OR 'species diversity' OR 'species richness' OR evenness) AND TS=('ecosystem function'
 331 OR 'ecosystem functioning' OR 'ecosystem process' OR 'ecosystem') for publications including both terms.
 332 The search included the keywords, titles and abstracts of all articles in all languages between 1990 and
 333 2015.

334
 335 Over the last decade, BEF research has provided an extensive amount of hypotheses and case
 336 studies, unraveled the core mechanisms (see 1.6) driving the BEF relation, and allowed for an
 337 unambiguous interpretation of the biodiversity effect on ecosystem functioning (Schläpfer and
 338 Schmid 1999, Loreau and Hector 2001, Loreau et al. 2001b, 2002, Fox 2005, Hooper et al. 2005,
 339 2012, Cardinale et al. 2011, Tilman et al. 2014). At present, the empirical evidence from more than

340 a thousand BEF studies indicates a positive effect of biodiversity on ecosystem processes such as
341 biomass production, nutrient flux and decomposition (Worm et al. 2006, Balvanera et al. 2006,
342 Cardinale et al. 2006, 2007, 2012, Stachowicz et al. 2007, Finkel et al. 2010, Quijas et al. 2010,
343 Hooper et al. 2012, Tilman et al. 2014, Gamfeldt et al. 2015, Hautier et al. 2015). Although
344 biodiversity effects were by no means universal and the slope of the BEF relation has shown
345 considerable variation, there appears to be a striking level in consistency of biodiversity effects
346 across various organism groups such as microbes, plants and predators, among trophic levels and
347 across the different types ecosystems that have been studied (Balvanera et al. 2006, Cardinale et
348 al. 2006, 2011, Gamfeldt et al. 2008, 2015, Wagg et al. 2014).

349 **1.1.2. Defining biodiversity, ecosystem functioning and ecosystem processes**

350 The term biodiversity encompasses a broad spectrum of biotic scales, which include genetic
351 variation within species, the numbers and relative abundances at the species level, and the
352 diversity of ecosystems on the planet (Purvis and Hector 2000, Mooney 2002, Hooper et al. 2005).
353 This broad scale is reflected in the biodiversity synthesis of the Millennium Ecosystem
354 Assessment (2005), which defines biodiversity as ‘the variability among living organisms from all
355 sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological
356 complexes of which they are part; this includes diversity within species, between species and of
357 ecosystems.’ BEF research commonly focuses on biodiversity at the species level (Loreau et al.
358 2002, Stachowicz et al. 2007), and this thesis consequently describes diversity in terms of both
359 the number of species (species richness) and their relative abundance (species evenness).

360 Although the terms ecosystem process and ecosystem function are often used interchangeably,
361 the term *ecosystem process* refers to the mechanistic processes which are performed by biota (De
362 Groot et al. 2002; in this work e.g. the production of extracellular polymers or fatty acids). These
363 processes regulate *ecosystem functions*, which can be defined as ‘the capacity of natural processes
364 and components to provide goods and services that satisfy human needs, directly or indirectly’
365 (De Groot et al. 2002; in this work e.g. sediment stabilization or trophic energy transfer). This
366 thesis refers to ecosystem processes when discussing the specific processes carried out by a
367 species or community, and to ecosystem functioning when discussing general concepts of BEF
368 research. Italicised terms are defined in a box after the paragraph where they were first
369 mentioned.

Ecosystem process	Processes performed by biota which regulate ecosystem functioning
Ecosystem functioning	The result of ecosystem processes, providing goods and services that satisfy human needs, directly or indirectly

370

371 **1.1.3. Evenness and Multifunctionality**

372 Most BEF research to date focused on individual ecosystem properties in isolation, with the
373 biomass production of primary producers being the most common functional endpoint
374 (Balvanera et al. 2006, Gamfeldt et al. 2013). Whilst biomass production is vital for delivering a
375 large range of ecosystem services (Imhoff et al. 2004), the contribution of biological communities
376 to decomposition, nutrient cycling, habitat structuring or trophic energy flow are also of key
377 importance for ecosystem functioning (Müller-Navarra et al. 2000, Gamfeldt et al. 2005, Rabaut et
378 al. 2007, Manzoni et al. 2008, Godbold et al. 2009). If diversity effects are not quantified for a larger
379 set of ecosystem functions, BEF research will only partially capture an ecosystem's response to
380 biodiversity loss, since functions other than primary production respond differently to
381 biodiversity change and diversity effects on ecosystem multifunctionality are larger than diversity
382 effects on single functions (Hector and Bagchi 2007, Jiang et al. 2008, Gamfeldt et al. 2013,
383 Lefcheck et al. 2015).

384 Moreover, most BEF studies have chosen *species richness* as measure of diversity, and have held
385 the relative abundance of species constant across richness treatments (Wilsey and Polley 2004,
386 Hillebrand et al. 2008, Isbell et al. 2009). Until the end of the last decade, 90% of BEF experiments
387 were based on manipulations of species richness, whereas less than 2.5% examined changes in
388 relative species abundances (i.e. *species evenness*, Balvanera et al. 2006, Hillebrand and
389 Matthiessen 2009). However, the impact of a species on ecosystem functioning depends not only
390 on whether it is present in an ecosystem, but also on how abundant it is (Díaz et al. 2003). If the
391 dominant species contributes more to ecosystem functioning than the mean of the community,
392 functioning will increase, and vice versa if the dominant species performs below average
393 (Hillebrand et al. 2008). Human activities do not only alter the number, but also the relative
394 abundance of species (Hillebrand et al. 2008). More importantly, evenness commonly responds
395 more sensitively to environmental change than species richness, and changes in evenness can
396 affect ecosystem functioning long before species are actually threatened by extinction (Chapin et
397 al. 2000, Hillebrand et al. 2008). Thus, the distribution and relative abundance of species within a
398 community might be more important for its contribution to ecosystem functioning than the
399 number of species (Norberg 2004, Hillebrand et al. 2008).

Species richness	The number of species in a community or ecosystem
Species evenness	The relative abundance of species in a community or ecosystem

400

401 **1.2. Biodiversity and ecosystem functioning in marine systems**

402 Marine ecosystems are of particular interest to conservation science due to their tremendous
403 biodiversity, with a significant number of marine species not yet described, and due to their global
404 significance for the storage and cycling of materials, nutrients, and energy flow (Snelgrove 1999,
405 Covich et al. 2004). Coastal, estuarine and freshwater ecosystems are among the most impaired
406 parts of the biosphere, experiencing some of the highest rates of species loss, but host a
407 disproportionately large fraction of productivity, providing essential services for human well-
408 being (Malmqvist and Rundle 2002, Covich et al. 2004, Gamfeldt et al. 2015, Turner and Schaafsma
409 2015). However, most of the early research on the effects of biodiversity on ecosystem functioning
410 has been focussing on terrestrial ecosystems, particularly grasslands, with producer biomass as
411 the predominant functional endpoint (Balvanera et al. 2006, Jiang et al. 2008). Marine and
412 freshwater systems on the other hand have remained a 'niche product' in early BEF research, and
413 conservationists stressed the need to test if conclusions derived from terrestrial experiments also
414 applied to aquatic ecosystems (Covich et al. 2004, Giller et al. 2004). During the last decade, a
415 number of groundbreaking studies responded to these needs and investigated the effects of
416 biodiversity loss on the functioning of marine and particularly coastal systems (e.g. Emmerson et
417 al. 2001, Bolam et al. 2002, Solan et al. 2004, Bracken et al. 2008, Godbold et al. 2009, Vanellander
418 et al. 2009, Davies et al. 2011, Gustafsson and Boström 2011, Baert et al. 2016). While the majority
419 of BEF research is still based on studies in terrestrial systems (60 % of all BEF contributions as
420 identified by Solan et al. 2009), marine systems are increasingly well represented (11%, the
421 remaining contributions being 13% freshwater studies and 17% generic reviews). By now, the
422 empirical evidence obtained in recent marine studies suggests that biodiversity tends to enhance
423 ecosystem functioning not only in terrestrial, but also in marine habitats (Gamfeldt et al. 2015).

424 **1.3. Anthropogenic stress**

425 In physiological and medical research, the term stress is most generally defined as a condition that
426 causes an organism to shift away from its homeostatic balance (Cannon 1926, Goldstein and Kopin
427 2007, Neumann-Lee 2016). In classic plant ecology, stress has been defined as the reduction in
428 growth or biomass production caused by an external constrain (Grime 1977, Maestre et al. 2009).
429 In an ecosystem functioning context, environmental stress is commonly defined as an
430 environmental condition that impacts ecosystem processes such as biomass production (Mullan
431 Crain and Bertness 2006, Gessner and Hines 2012, Steudel et al. 2012), although this terminology
432 depends on the organism and the range of environments considered (Körner 2003, Mullan Crain
433 and Bertness 2006). A change in environmental conditions which is caused by human activities
434 and affects ecosystem processes can be termed *anthropogenic stress* (Carlisle and Clements 2005,

435 Halpern et al. 2007, Johnston and Roberts 2009, McMahon et al. 2012, Malaj et al. 2014, Neumann-
436 Lee 2016).

437 Anthropogenic stress is among the driving forces behind the ongoing biodiversity losses (Lotze et
438 al. 2006, Halpern et al. 2008, Geiger et al. 2010). Among the many anthropogenic impacts on
439 natural ecosystems, chemical pollution is regarded as one of the most potent threats to aquatic
440 biodiversity (Wilcove and Master 2005). In the EU alone there are more than 100 000 registered
441 chemicals (EU 2001), many of which have not been tested for adverse biological effects. Global
442 chemical production is projected to double over the next quarter-century, rapidly outpacing the
443 rate of population growth, and many of these chemicals ultimately enter the environment
444 (Schwarzman and Wilson 2009). The list of chemicals eventually found in coastal ecosystems
445 includes industrial, domestic and agricultural nutrients, pesticides, road run-off, personal care
446 products, disinfectants and pharmaceuticals (European Marine Board 2013), amongst which
447 pesticides and their residues are reported to be among the most devastating agents for aquatic
448 ecosystems, affecting all levels of the food chain from the lowest to the top level (Islam and Tanaka
449 2004). Chemical pollution is jeopardizing biodiversity on a global scale (Wilcove and Master 2005,
450 Backhaus et al. 2012, Malaj et al. 2014), but the percentages of ecological studies addressing
451 pollution do not correspond at all to its ranked importance as a driver for biodiversity loss (Lawler
452 et al. 2006). This underrepresentation of anthropogenic stress in studies testing the effects of
453 biodiversity loss on ecosystem functioning has raised critical questions whether the patterns and
454 diversity-functioning relations obtained in classic BEF experiments reflect those induced by
455 anthropogenic activities (Naeem 2008, Fugère et al. 2012, O'Connor et al. 2015).

Anthropogenic stress A change in environmental conditions which is caused by human
activities and affects ecosystem processes

456

457 **1.4. Incorporating anthropogenic stress into BEF research**

458 The overwhelming majority of BEF experiments to date have manipulated biodiversity in subsets
459 of species under controlled conditions, which enabled scientists to eliminate functional effects by
460 confounding factors other than biodiversity, and thus allowed an unambiguous interpretation of
461 the BEF relation (Loreau et al. 2001b, Naeem and Wright 2003, Díaz et al. 2003, Srivastava et al.
462 2004, Hooper et al. 2005, Cardinale et al. 2006, 2012, Tilman et al. 2014). The high degree of
463 control required in these experiments however implied that the environmental complexity and
464 the anthropogenic drivers that shape BEF relations under global change were largely neglected
465 (Zavaleta and Hulvey 2004, Srivastava and Vellend 2005, Schläpfer et al. 2005, Bracken et al. 2008,

466 Reich et al. 2012, De Laender et al. 2016). The plethora of anthropogenic drivers causing the
467 ongoing biodiversity changes in nature however made it unclear whether the observed BEF
468 patterns will hold for realistic extinction scenarios as induced by anthropogenic change, over
469 multiple trophic levels, and across different types of ecosystems (Giller et al. 2004, Srivastava and
470 Vellend 2005, Raffaelli 2006, Duffy et al. 2007, Hillebrand and Matthiessen 2009, Gamfeldt et al.
471 2013). After the first phase in BEF research had provided the empirical evidence on the direction
472 and the effects underpinning the diversity-functioning relation, the incorporation of realistic
473 biodiversity loss and trophic complexity were thus identified as key challenges for the next
474 generation of BEF research (Hooper et al. 2005, Srivastava and Vellend 2005, Cardinale et al.
475 2012).

476 **1.4.1. The classic BEF design**

477 After early BEF experiments (Naeem et al. 1994, Tilman and Downing 1994) were criticized for
478 confounding species number with species composition, BEF research soon adopted the principle
479 of using random species assemblages to evaluate the functional consequences of changing
480 biodiversity (McGrady-Steed et al. 1997, Hector et al. 1999, Naeem 1999, Tilman et al. 2001,
481 Downing and Leibold 2002). To date, most BEF research has involved experiments in which
482 diversity is varied through random draws from a species pool (Srivastava and Vellend 2005,
483 Cardinale et al. 2012, Eisenhauer et al. 2016, Wardle 2016). Random designs have considerably
484 advanced BEF research, by allowing to unequivocally attribute changes in functioning to species
485 richness, and by providing insights into the key mechanisms by which more diverse communities
486 can result in enhanced functioning (Loreau and Hector 2001, Cardinale et al. 2006, Eisenhauer et
487 al. 2016). However, the classic design of random species loss involves three implicit limitations,
488 which are addressed in the following three sections.

489 **1.4.2. Selective stress**

490 First, random species loss implies that the likelihood to be removed from the system is the same
491 for all species, regardless of their extinction-proneness in nature, an assumption which soon drew
492 criticism (Ives and Cardinale 2004, Giller et al. 2004). While it is impractical for experimental
493 studies to mimic all the factors driving species loss in nature, it is clearly an oversimplification to
494 accept random extinction as a representative scenario of species loss (Giller et al. 2004).
495 Anthropogenic stress is likely to cause non-random sequences of biodiversity loss, depending on
496 differences in the species' physiology, morphology, trophic position or habitat specialization,
497 which determine their sensitivity to different stressors (Jonsson et al. 2002, Duffy 2003, Ives and
498 Cardinale 2004, O'Connor and Crowe 2005, Bracken et al. 2008). Stress has therefore been termed
499 *selective stress*, if it disfavours some species more than others (Wittebolle et al. 2009). This biased

500 diversity loss has raised questions about how useful inferences from randomly assembled
501 communities will be for conservation efforts, and has led to calls for experimental designs that
502 remove the most vulnerable species first (Duffy 2003, Raffaelli 2004, Ball et al. 2008, Naeem 2008,
503 Tilman et al. 2014, Wardle 2016).

504 **1.4.3. Physiological stress**

505 Second, the same stressors that indirectly alter ecosystem functioning through selective changes
506 in biodiversity can also directly affect the physiology of the surviving species, and thus their
507 contribution to ecosystem functioning. Direct exposure to anthropogenic stressors is however
508 rarely included into the design of BEF studies (McMahon et al. 2012, but see Reusch et al. 2005,
509 Wittebolle et al. 2009, McMahon et al. 2012, Roger et al. 2012, Baert et al. 2016, Radchuk et al.
510 2016). This means that even when diversity gradients are established according to the species'
511 vulnerability in nature, the functional rates of the surviving 'tolerant' species are commonly
512 assumed to remain unaffected. However, stress tolerance may come at a physiological cost, e.g.
513 acclimation by allocating resources from growth to survival pathways (Schimel et al. 2007), and
514 this '*physiological stress*' can cause species to contribute differently to a given ecosystem processes
515 than in unstressed conditions (Relyea and Hoverman 2006, Schimel et al. 2007, De Laender et al.
516 2010). This is at least the case for chemical stress, which commonly causes sublethal adverse
517 effects on the surviving species (McWilliam and Baird 2002, Fleeger et al. 2003, Rohr et al. 2006,
518 Liebig et al. 2008). Even when explicitly including exposure to anthropogenic stressors, common
519 BEF designs cannot separate the direct functional impact of a stressor via effects on organismal
520 physiology from its indirect effects that are mediated by changes in biodiversity (Relyea and
521 Hoverman 2006, Radchuk et al. 2016). Disentangling direct stress effects at the physiological level
522 and from indirect effects at the community level thus represents a major challenge to determine
523 functioning in stressed communities (Relyea and Hoverman 2006, Schimel et al. 2007).

524 **1.4.4. Functional contribution**

525 Third, random species loss holds the implicit assumption that all species contribute in a more or
526 less similar way to ecosystem functioning, and that what matters is mainly the number, rather
527 than the characteristics, of species added or lost from a system (Díaz et al. 2003). Most
528 experimental BEF studies have thus documented the 'mean' effect of adding or removing species,
529 regardless of their individual contribution to ecosystem functioning (Lewis 2009). There is
530 mounting evidence, however, that often one or a few particular species have a disproportionate
531 influence on ecosystem properties (Power et al. 1996, Grime 1998, Emmerson et al. 2001, Bolam
532 et al. 2002, Waldbusser et al. 2004, Solan et al. 2004, Bruno et al. 2006, Cardinale et al. 2006). The
533 few studies that investigated selective biodiversity loss caused by anthropogenic disturbance

534 found that anthropogenic stressors can on the one hand have limited effects on functioning
535 despite causing significant diversity loss, as long as the most abundant species are not affected
536 (Smith and Knapp 2003, Solan et al. 2004, Radchuk et al. 2016). On the other hand, BEF studies
537 found a disproportionate loss of functioning when selective stress targeted the species that
538 contributed most to ecosystem functioning (Larsen et al. 2005, McIntyre et al. 2007). Integrating
539 information on the *functional contribution* of a species within scenarios of selective biodiversity
540 loss is thus necessary to successfully predict BEF relationships under stress (Hillebrand and
541 Matthiessen 2009).

542 These three limitations do not devalue the findings obtained in classic BEF studies which were
543 based on random manipulations of richness under constant experimental conditions. Far from
544 that, in the absence of detailed predictions, these early studies provided a general expectation on
545 how diversity affects ecosystem functioning and unravelled the fundamental mechanisms driving
546 the BEF relations (Hector et al. 2001, Loreau and Hector 2001, Srivastava and Vellend 2005).
547 However, after the incorporation of selective, non-random biodiversity loss was identified as a
548 key challenge for future BEF research, scientists were facing the delicate task to connect the
549 extinction vulnerability of individual species with their contribution to ecosystem functioning
550 (Hooper et al. 2005, Cardinale et al. 2012). The results of random-loss BEF experiments could
551 most easily be used to predict the effects of biodiversity loss when this loss naturally occurs
552 independently of species traits (Srivastava 2002, Lepš 2004, Srivastava and Vellend 2005).
553 However, as discussed above, anthropogenic stress is causing a plethora of non-random scenarios
554 of biodiversity loss, where species are lost according to their sensitivity to a given stressor
555 (McKinney 1997, Srivastava and Vellend 2005, Larsen et al. 2005, Rubach et al. 2012, Malaj et al.
556 2014). This species-specific sensitivity is correlated with the species' biological traits, which can
557 cause organisms to be either sensitive or tolerant to different types of stress (Lavorel and Garnier
558 2002, Solan et al. 2004, Buchwalter et al. 2008, Van den Brink et al. 2011). In their comprehensive
559 review on the future challenges of BEF research, Hooper et al. (2005) thus described 'the
560 covariance between traits affecting extinction and those affecting ecosystem processes' as 'the
561 first critical issue for predicting the consequences of non-random biodiversity loss'.

Functional contribution	The contribution of a species to a given ecosystem process
Physiological stress	A change in environmental conditions which affects an ecosystem process by altering the species' functional contribution
Selective stress	A change in environmental conditions which affects an ecosystem process by selectively targeting species with a high or low functional contribution

563 1.5. Response and effect traits

564 The concepts and definitions of 'traits' have varied over time and ecological disciplines (Violle et
565 al. 2007), but BEF research has mainly considered traits as functional traits, which are defined as
566 'morphological, physiological or phenological characteristics of an organism affecting its
567 individual performance' (Violle et al. 2007, Hillebrand and Matthiessen 2009). As such, functional
568 traits are the pillar for the concept of 'functional diversity'. Functional diversity can be described
569 as the diversity of distinct functional groups, in which species are classified according to their
570 traits, or as continuous gradients of different traits, which both allow to predict the changes in
571 ecosystem processes based on changes in community and thus trait composition (Diaz and Cabido
572 2001, Petchey and Gaston 2006, Griffin et al. 2009, Hillebrand and Matthiessen 2009). As such,
573 functional diversity, together with the above-mentioned species richness and evenness, rapidly
574 emerged as third main biodiversity endpoint in BEF research (Balvanera et al. 2006).

575 Suding et al. (2008) and Hillebrand and Matthiessen (2009) introduced a new trait concept into
576 BEF research, which connects *response traits* (traits that predict species abundance in responses
577 to environmental change) and *effect traits* (traits that reflect the effects of a species on ecosystem
578 processes). This concept corresponded to the need formulated by Hooper et al. (2005), to predict
579 the consequences of selective biodiversity loss by correlating traits predicting extinction with
580 those affecting ecosystem processes. If response traits and effect traits are correlated, i.e. the
581 species contributing most to functioning are also the ones most likely to be lost in response to
582 anthropogenic disturbance, selective biodiversity loss will lead to a disproportionate decline in
583 functioning relative to random biodiversity loss. If response and effect traits are uncorrelated, the
584 community might be able to compensate for the loss of effect traits and maintain its contribution
585 to functioning (Hillebrand and Matthiessen 2009). The presence of toxic chemicals alters not only
586 the abundance of species, but also their functional contribution (McWilliam and Baird 2002,
587 Fleeger et al. 2003, Rohr et al. 2006, but see 1.4.3). For the case of chemical pollution, response
588 traits should thus predict the numerical stress response of species (i.e. changes in abundance,
589 hereafter numerical stress response), and effect traits should furthermore capture the functional
590 stress response (i.e. if species can maintain their functional contribution, hereafter functional
591 stress response). When the numerical and functional stress response are positively related to the
592 same set of response and effect traits, a community decimated by species loss could still be
593 expected to retain most of its functioning. Alternatively, stress exposure could lead to a
594 disproportionate reduction in functioning if numerically tolerant species, while still being able to
595 grow, would lose most of their functional contribution.

596 The use of response traits as predictors of sensitivity to toxic substances has been increasingly
 597 advocated in ecotoxicology, as a mechanistic alternative to the otherwise empirical approach of
 598 estimating sensitivities using species sensitivity distributions (Baird and Van den Brink 2007, Van
 599 den Brink et al. 2011). For example, Baird and Van den Brink (2007) showed that only four
 600 response traits could predict the sensitivity of 12 species to a large array of chemical pollutants.
 601 Subsequently, several pioneering works have shown the capacity of traits to predict species
 602 sensitivity to chemical pollutants, linking traits to specific toxic mechanisms, and thus enabling a
 603 more deterministic description of the structure of biological communities under chemical stress
 604 (Buchwalter et al. 2008, Guénard et al. 2011, Larras et al. 2012, Rubach et al. 2012, Fischer et al.
 605 2013, Pomati and Nizzetto 2013). Whilst the use of response traits is well established, the
 606 identification of effect traits and the explicit linkage between both types of traits is still rare in BEF
 607 research (but see Pakeman 2011, Heuner et al. 2015, Zwart et al. 2015, Lennon and Lehmkuhl
 608 2016), and mostly limited to single species and not in the context of chemical stress. An increased
 609 focus on correlating response and effect traits should thus enable scientists to predict how
 610 selective changes in diversity caused by environmental change will affect the contribution of
 611 biological communities to ecosystem functioning.

Response trait	A trait predicting the abundance of a species or organism group in response to a change in environmental conditions
Effect trait	A trait reflecting the effect of a species or organism group on ecosystem processes

612

613 **1.6. Complementarity and dominance**

614 The identification of complementarity and selection effects have been among the most crucial
 615 findings of early BEF research (Loreau and Hector 2001). After more than two decades of BEF
 616 research, scientists now widely agree that complementarity and selection are the two key effects
 617 which cause diverse communities to perform differently than expected from their monocultures
 618 (Hector et al. 2009, Hodapp et al. 2016). The selection effect drives BEF relations through selective
 619 processes such as interspecific competition, which can cause dominance (high relative
 620 abundance) of species with particular traits. The selection effect can positively or negatively affect
 621 a community's contribution to ecosystem functioning, depending on whether the species that
 622 ultimately dominate the community have a relatively high or low functional contribution,
 623 respectively (Loreau and Hector 2001). In contrast to the competition-driven selection effect,
 624 complementarity refers to a class of mechanisms that result in a higher performance of a

625 community than would be expected from the separate performances of each component species
626 (Loreau and Hector 2001). These mechanisms are commonly thought to be facilitation, e.g. if
627 species modify the environment in a way that benefits co-occurring species, and niche
628 partitioning, e.g. different resource requirements among species causing interspecific competition
629 in diverse communities to be lower than intraspecific competition in monocultures (Tilman et al.
630 1997, Loreau and Hector 2001, Duffy et al. 2007, Vanellander et al. 2009).

631 One limitation of Loreau and Hector's (2001) bipartite partition of the biodiversity effect into
632 selection and complementarity is that it assumes that complementarity is distributed equally
633 across species, i.e. that species mutually benefit each other to the same degree (Hector et al. 2009).
634 However, species with particular traits can perform better than expected in communities either
635 due to competitive replacement of other species, or not at the expense of other species, e.g. by
636 benefitting from complementarity mechanisms without contributing to these mechanisms (the
637 so-called trait-dependent or 'one-way' complementarity, Fox 2005; Hector et al. 2009). The
638 bipartite partition assigns this one-way complementarity to the selection effect, and may
639 therefore over- or underestimate total complementarity, depending in whether species with
640 particular traits (e.g. high or low monoculture biomass, hereafter yield) benefit more or less from
641 complementarity (Fox 2005; Hector et al. 2009). Therefore, Fox (2005) proposed a tripartite
642 model, that partitions the biodiversity effect on ecosystem functioning into *dominance*, *one-way*
643 *complementarity* and *two-way complementarity* effects. The dominance effect occurs when species
644 with particular traits dominate communities at the expense of other species. Depending on
645 whether communities are dominated by high- or low-yield species, the dominance effect will be
646 positive or negative. Trait-dependent or one-way complementarity occurs when species with
647 particular traits perform better in community than in monoculture, but not at the expense of other
648 species. Trait-independent or two-way complementarity occurs when species perform better in
649 community, independently of their traits and not at the expense of other species. Two-way
650 complementarity is thus analogous to complementarity as defined by Loreau and Hector (2001).
651 To date, there have been few explicit comparisons of the bipartite and tripartite partitioning
652 methods (Hector et al. 2009), but where tested, the contribution of one-way complementarity to
653 the total biodiversity effect was minor (Hector et al. 2009, Karlson et al. 2010, Schmidtke et al.
654 2010, Fernandes et al. 2011, Long et al. 2013, Stachova et al. 2013, Siebenkäs et al. 2016). Thus,
655 using the bi- or tripartite partition should produce similar results on the relative importance of
656 dominance / selection and complementarity effects.

657 More importantly, experimental BEF studies keep producing contrasting results on the relative
658 contributions of dominance and complementarity effects, leading to an active debate on which
659 principal effect drives the diversity-functioning relation (Huston et al. 2000, Cardinale et al. 2006,

660 Fargione et al. 2007, Wang et al. 2013, Hodapp et al. 2016). The bottleneck which is limiting the
661 experimental quantification of complementarity and dominance effects, is that in order to
662 unequivocally determine the contribution of either effect to observed BEF relationships,
663 information is required on the yield of every species when grown in community and in
664 monoculture. This information is however largely unavailable (Hector et al. 2009), and as a result
665 complementarity and dominance underpinning observed BEF relationships are largely
666 interpreted indirectly from existing patterns, rather than from direct experimental tests
667 (Cardinale et al. 2006, Hector et al. 2009).

668 The contribution of dominance and complementarity effects to BEF relations under stress is even
669 less clear. Whilst anthropogenic stressors are increasingly incorporated into the design of BEF
670 experiments, these experiments found contrasting biodiversity effects on functioning in stressed
671 communities (clearly positive effects in Mulder et al. 2001, Solan et al. 2004, Zavaleta and Hulvey
672 2004, Larsen et al. 2005, Ives and Carpenter 2007, McMahon et al. 2012, Steudel et al. 2012, Wang
673 et al. 2013, Baert et al. 2016, neutral or even negative diversity effects in (Jonsson et al. 2002,
674 Petchey et al. 2002, Smith and Knapp 2003, Jiang 2007, Fernandes et al. 2011, Roger et al. 2012,
675 Fugère et al. 2012). However, few of these discrepant diversity-functioning relations could be
676 explicitly traced to changes in dominance and complementarity effects (Fernandes et al. 2011,
677 Fugère et al. 2012, Wang et al. 2013, Baert et al. 2016, Hodapp et al. 2016), since quantifying both
678 effects under stress requires data on the yield of every species in community and monoculture at
679 every stress level. Under stressful conditions, species will become dominant by being inherently
680 tolerant and thus replacing other more sensitive species. This is the selective stress described
681 before (see 1.4.2) and although dominance effects could not be explicitly quantified, the functional
682 contribution of these dominant, stress-tolerant species has been identified as key driver of BEF
683 relations under stress (Petchey et al. 2002, Solan et al. 2004, Zavaleta and Hulvey 2004, Larsen et
684 al. 2005, Radchuk et al. 2016). Complementarity effects in stressed communities depend on
685 potential changes in species interactions along stress gradients. Stressful conditions leading to an
686 increase in interspecific competition can cause negative complementarity effects (Fernandes et
687 al. 2011). The complementarity effect increases if facilitation or niche partitioning are enhanced
688 under stress (Fugère et al. 2012; Wang et al. 2013). Direct ecophysiological evidence for
689 interspecific facilitation as driver of complementarity effects is however still limited (Cardinale et
690 al. 2002, Temperton et al. 2007, Vanellander et al. 2009), and is lacking so far in stressed
691 communities. Determining the relative importance of dominance and complementarity effects as
692 drivers of BEF relations under stress, and connecting both effects to stress tolerance and
693 interactions at the species level, thus remains a largely unresolved task for explaining diversity-
694 functioning relations in stressed communities.

Dominance effect	Species with particular traits dominate communities at the expense of others.
Two-way complementarity	‘Trait-independent’ complementarity: growth in community rather than monoculture increases the functioning of species, independent of their traits and not at the expense of other species.
One-way complementarity	‘Trait-dependent’ complementarity: growth in community rather than monoculture increases the functioning of species with particular traits, but not at the expense of other species.

695

696 **1.7. Trophic complexity**

697 **1.7.1. Horizontal and vertical biodiversity effects**

698 BEF research has been dominated by experiments focussing on a single trophic level, mostly
699 primary producers in terrestrial grasslands (Petchey et al. 2004, Srivastava et al. 2004, O’Connor
700 and Crowe 2005, Raffaelli 2006, Scherber et al. 2010). Biodiversity loss can act horizontally, i.e.
701 affect the abundance, biomass or resource use at the same trophic level where biodiversity is lost
702 (Cardinale et al. 2006, Duffy et al. 2007, Scherber et al. 2010). However, horizontal species loss
703 may also have vertical consequences, by affecting other trophic levels, organism groups and
704 processes (Cardinale et al. 2006; Duffy et al. 2007; Scherber et al. 2010). Besides the incorporation
705 of realistic non-random species loss, the addition of a vertical dimension of biodiversity was thus
706 soon identified as a major task for BEF research (Hooper et al. 2005, Srivastava and Vellend 2005,
707 Duffy et al. 2007, Reiss et al. 2009, Scherber et al. 2010, McMahon et al. 2012).

708 Although the single trophic level approach has advanced BEF research considerably, it has a
709 number of limitations (Raffaelli 2006, Scherber et al. 2010). For instance, complementarity and
710 dominance effects have been identified as drivers of BEF relations within trophic levels, but these
711 approaches cannot be used to test biodiversity effects across multiple trophic levels, and it is
712 unclear if the same effects occur in multitrophic experiments (Fox 2006, Raffaelli 2006). Another
713 main reason to adopt a second, vertical perspective in BEF research is the need to recognise that
714 biodiversity loss due to anthropogenic activities usually occurs at all trophic levels, and taxa will
715 differ in their sensitivity to particular loss scenarios (Solan et al. 2004, Raffaelli 2006). Thus,
716 anthropogenic stressors can cause differential biodiversity loss and disparate consequences for
717 functioning across trophic levels (Raffaelli 2006, McMahon et al. 2012). There are likely to be
718 significant feedbacks between the trophic level targeted by a stressor and the higher and lower
719 trophic levels (Raffaelli 2006). If these feedbacks are not recognised, the outcomes of one-

720 dimensional, single trophic experiments will only be of limited value for policy makers interested
721 in the impacts of biodiversity loss (Raffaelli et al. 2002, Raffaelli 2006).

722 In principle, adopting a two-dimensional approach links the fields of BEF research (focusing on
723 horizontal diversity and ecosystem functioning) and predator-prey and food web ecology
724 (focusing of vertical diversity), which are thematically linked but have remained largely separate
725 research fields (Duffy et al. 2007). A large number of trophic ecology studies has analysed the
726 mechanisms and importance of trophic interactions (e.g. Pace et al. 1999, Chase et al. 2002, Borer
727 et al. 2005, De Troch et al. 2005, 2012b). Most of this research however has yet to be integrated
728 within BEF research and its (so far horizontal) focus on ecosystem processes (Duffy et al. 2007).
729 After the first generation of BEF research had established the generality of the diversity-
730 functioning relation, theoretical and experimental works began to merge the functional effects of
731 diversity and of trophic interactions (e.g. Fox 2004, Petchey et al. 2004, Thebaut and Loreau 2005,
732 Casula et al. 2006). Similar to traditional horizontal BEF approaches, vertical BEF research started
733 in terrestrial environments (Koricheva et al. 2000, Balvanera et al. 2006, Cardinale et al. 2006,
734 Haddad et al. 2009), with vertical BEF efforts in marine systems starting in the second half of the
735 last decade (Gamfeldt et al. 2005, Bruno et al. 2008, Scherber et al. 2010). Recent works found
736 strong correlations between primary producer diversity and ecosystem processes at higher
737 trophic levels, thus confirming the importance of biodiversity in a vertical dimension (Scherber et
738 al. 2010, Lefcheck et al. 2015). These findings also apply to chemical pollutants, which by causing
739 losses of producer diversity, can significantly impact processes at the associated consumer level
740 (Relyea and Hoverman 2006, McMahon et al. 2012). Whilst some of the strongest impacts of
741 biodiversity loss have been found at the plant-animal interface (Scherber et al. 2010, McMahon et
742 al. 2012, Lefcheck et al. 2015), these multitrophic diversity-functioning relationships were less
743 predictable than those found for single trophic levels (Duffy et al. 2007, Reiss et al. 2009, McMahon
744 et al. 2012).

745 **1.7.2. Diet quality**

746 The overwhelming majority of both BEF and trophic ecology experiments to date have focused on
747 *diet quantity* (i.e. the abundance and availability of food, mostly quantified as producer biomass),
748 which indeed is a critical determinant for food web functioning (Arts and Wainmann 1999,
749 Balvanera et al. 2006, Österblom et al. 2008, Scherber et al. 2010, Guo et al. 2016). However, the
750 recent integration of diet biochemistry with traditional studies of diet quantity has indicated that
751 not only abundance and availability of food items, but also *diet quality* can be of striking
752 importance for the reproduction and growth dynamics of many aquatic animals (Arts and
753 Wainmann 1999, Österblom et al. 2008).

754 The differences in the biochemical composition of organisms are most pronounced at the plant-
755 animal interface, which features the largest, but also the most variable energy transfer efficiencies
756 in the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, De Troch et al. 2012b,
757 Guo et al. 2016). For aquatic primary consumers, algae represent a high quality diet (compared to
758 e.g. terrestrial organic matter), due to their high *polyunsaturated fatty-acid (PUFA)* content
759 (Taipale et al. 2013, Guo et al. 2016). Fatty acids represent the densest form of energy in aquatic
760 ecosystems (Parrish 2009), and are therefore crucial for the energy flux from primary producers
761 to higher levels of the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Kainz
762 et al. 2004, Gladyshev et al. 2011). PUFAs are defined as fatty acids with more than one double
763 bond, and are almost exclusively generated by plants (Brett and Müller-Navarra 1997) from de
764 novo synthesis of palmitic acid and further enzymatic elongase and desaturation reactions
765 (Harwood and Guschina 2009, Cagliari et al. 2011, Taipale et al. 2013).

766 Amongst all algal classes, Cryptophyceae and Bacillariophyceae (diatoms) are considered as diets
767 of excellent quality for aquatic primary consumers, due to their high content of two specific PUFA
768 components, the so-called *essential fatty acids (EFAs)* eicosapentaenoic acid (EPA) and
769 docosahexaenoic acid (DHA, Dunstan et al. 1993, Brett et al. 2006, Taipale et al. 2013). Conversely,
770 primary producers with a low concentration of EFAs (e.g. cyanobacteria) are of very poor diet
771 quality for aquatic consumers (Brett et al. 2006, Burns et al. 2011, Taipale et al. 2013). Animals
772 (e.g. crustaceans and fish as well as humans) cannot synthesize these EFAs de novo, and therefore
773 need to obtain these molecules from their diet (Brett and Müller-Navarra 1997, Müller-Navarra
774 et al. 2000, Litzow et al. 2006, Parrish 2009). Diet quality in terms of EFA content is a critical
775 determinant of the growth rate and reproductive success of primary consumers (Müller-Navarra
776 1995, Müller-Navarra et al. 2000, von Elert 2002, Arendt et al. 2005). Moreover, primary
777 consumers such as copepods cannot maintain their energy content when feeding on EFA-poor
778 algae species (Koski et al. 1998, Werbrouck et al. 2016). Algal diet quality can even be a stronger
779 driver of primary consumer biomass than diet quantity (Gladyshev et al. 2011), and a lack of algal
780 EFA production can reduce food web functioning by causing trophic decoupling, i.e. high primary
781 productivity with little corresponding increases in consumer biomass (Guo et al. 2016). Algal EFA
782 production has thus been described as a crucial determinant of diet quality and energy transfer
783 across the food web (Brett and Müller-Navarra 1997, Müller-Navarra et al. 2000, Litzow et al.
784 2006, Parrish 2009, Taipale et al. 2013). The large variation in EFA content among and within
785 algal classes thus makes the diversity and composition of aquatic primary producer communities
786 a key factor for food web functioning (Dunstan et al. 1993, Wyckmans et al. 2007, Taipale et al.
787 2013, Guo et al. 2016).

788 Algal diet quality is strongly influenced by changes in abiotic conditions, and can be altered in
789 response to light intensity (Wainman et al. 1999, Guschina and Harwood 2006), nutrient regimes
790 (Guschina and Harwood 2009, Sanpera-Calbet et al. 2016) and temperature (Morgan-Kiss et al.
791 2006, Piepho et al. 2012, Werbrouck et al. 2016). Far less attention has been devoted to the effect
792 of anthropogenic stress on algal diet quality. Due to the large variation in the EFA content of
793 microalgae (Dunstan et al. 1993, Taipale et al. 2013, Guo et al. 2016), selective changes in algal
794 community composition could potentially change the energy supply for higher trophic levels.
795 Moreover, there is limited but compelling evidence that anthropogenic stressors affect algal EFA
796 production through direct physiological effects. For instance, metal pollution can cause an
797 increasing degree of algal fatty acid saturation (thus lowering the proportion of high-energy EFAs)
798 and a general decrease of EFA production (Chia et al. 2013a, 2013b). Similarly, pesticide stress
799 can cause a general decrease of fatty acid synthesis (Weisshaar et al. 1988, El-Sheekh et al. 1994),
800 or reduce the proportion of EFAs within the total fatty acid pool by inhibiting enzymes involved
801 in fatty acid elongation and thus EFA synthesis (Böger et al. 2000). Nonetheless, diet quality has
802 so far not been integrated in trophic diversity-functioning research. As it has become increasingly
803 clear that diet quality, and not quantity, is the more important factor regulating the efficiency of
804 energy flow through aquatic food webs (Müller-Navarra et al. 2000, Torres-Ruiz et al. 2007, Lau
805 et al. 2009, Gladyshev et al. 2011), the biochemical composition of primary producers should thus
806 be considered when analysing multitrophic BEF relations under anthropogenic stress.

Diet quantity	The abundance and availability of a food source, in this thesis quantified as biomass
Diet quality	The quality of a food source, in this thesis quantified as energy content
Polyunsaturated fatty acids (PUFAs)	Fatty acid molecules with more than one unsaturated carbon bond (double bond)
Essential fatty acids (EFAs)	PUFAs which are essential to organismal physiology, which animals cannot synthesize and thus have to take up from their diet

807

808 **1.8. Study system and thesis outline**

809 **1.8.1. Study system**

810 This research was conducted in microcosm experiments using benthic diatoms
811 (Heterokontophyta, Bacillariophyceae) as primary producers and harpacticoid copepods
812 (Crustacea, Copepoda, Harpacticoida) as their main consumers, and atrazine and copper as

813 anthropogenic stressors. Diatoms and copepods were collected at the Paulina intertidal area, a
814 muddy intertidal habitat in the Westerschelde estuary (SW Netherlands).

815 Within these muddy intertidal systems, microalgal assemblages (microphytobenthos) represent
816 the main primary producers (Underwood and Kromkamp 1999, Forster et al. 2006, Tang and
817 Kristensen 2007). Benthic diatoms are the dominant component of the microphytobenthos, and
818 play an important role for a number of ecosystem processes (Underwood and Kromkamp 1999,
819 Forster et al. 2006, Hicks et al. 2011). Diatoms constitute the main food source for several primary
820 consumers, which makes diatom biomass production essential for the functioning of intertidal
821 food webs (Moens and Vincx 1997, Buffan-Dubau and Carman 2000, De Troch et al. 2012a, Moens
822 et al. 2014, Cnudde et al. 2015). Due to their high essential fatty acid content (EFAs), diatoms are
823 among the most critical components for the energy transfer through marine food webs (Müller-
824 Navarra et al. 2000; Taipale et al. 2013; Guo et al. 2016, but see 1.7.2.). Moreover, benthic diatoms
825 excrete large amounts of extracellular polymeric substances (EPS), a mix of high-molecular-weight
826 and carbon-rich molecules (De Brouwer et al. 2000, Smith and Underwood 2000, Underwood and
827 Paterson 2003). EPS play a beneficial role for the diatoms themselves, as a protective measure
828 against various abiotic disturbances and by enabling diatom locomotion and vertical migration
829 (Pistocchi et al. 1997, Smith and Underwood 2000, Staats et al. 2000, Gerbersdorf et al. 2009b).
830 Moreover, EPS promote ecosystem functioning in marine coastal systems by increasing sediment
831 stability (Decho 1990, 2000, Smith and Underwood 2000, Gerbersdorf et al. 2009a).

832 Meiofauna (organism size between 38 μm and 1 mm) represent an important link between
833 microalgal primary production and higher trophic levels (Montagna et al. 1995, Buffan-Dubau and
834 Carman 2000, Moens et al. 2002, De Troch et al. 2005). Harpacticoid copepods represent an
835 important part of the meiofauna, and are among the main consumers (grazers) of benthic diatoms
836 (Decho and Fleeger 1988, De Troch et al. 2005, 2012a). Their high content of EFAs, which are
837 incorporated directly from their diatom diet, makes harpacticoids an energy-rich food source for
838 small and juvenile fish (Nanton and Castell 1998, Wyckmans et al. 2007). As such, harpacticoid
839 copepods represent a pivotal link for the energy transfer from primary producers to higher trophic
840 levels (e.g. Alheit and Scheibel 1982, De Troch et al. 1998, Buffan-Dubau and Carman 2000,
841 Andersen et al. 2005).

842

843 Atrazine is a herbicide which binds to the plastoquinone binding protein of photosystem II,
844 causing the disruption of photosynthetic electron flow (Dorigo and Le Boulanger 2001, Legrand et
845 al. 2006, Knauert 2008). When atrazine enters freshwater and estuarine environments through
846 flooding events and river runoff, it can have deleterious effects on the growth and photosynthesis
847 of aquatic primary producers, such as microalgae (Peterson et al. 1994, Bester et al. 1995,

848 DeLorenzo et al. 2001, Pennington et al. 2001, Larras et al. 2016). Atrazine is commonly not
849 acutely toxic to aquatic consumers, but has adverse chronic effects on consumers due to food
850 limitation, hormonal disruption and reduced reproduction, although these reproductive and
851 hormonal effects are not consistently observed (Jüttner et al. 1995, Bejarano and Chandler 2003,
852 Bejarano et al. 2005, Forget-Leray et al. 2005, Solomon et al. 2008, Hayes et al. 2011). Despite its
853 Europe-wide ban in 2004, atrazine is still a common pollutant European estuaries and remains
854 one of the most-used pesticide worldwide (Graymore et al. 2001, Carafa et al. 2007, Noppe et al.
855 2007, Benbrook 2016).

856 In contrast to organic pesticides, heavy metals occur naturally in the environment, and several of
857 them are essential for organism physiology (Rengel 1999, Hänsch and Mendel 2009). This is the
858 case for copper which is involved in several metabolic pathways in microalgae, as an essential
859 micronutrient and component of proteins and enzymes (Morelli and Scarano 2004, Hänsch and
860 Mendel 2009). However, copper concentrations above the required levels are toxic to marine
861 organisms at all trophic levels (Welsh et al. 1996, Millward and Grant 2000, Murray-Gulde et al.
862 2002, Real et al. 2003, Manimaran et al. 2012). Copper enters coastal environments through river
863 run-off, industrial and domestic activities, copper mine drainages and agricultural practices,
864 notably its usage in herbicides and antifouling paints (Kennish 1996, Stauber and Davies 2000,
865 Murray-Gulde et al. 2002). Copper affects both primary producers and consumers through the
866 formation of reactive oxygen species (ROS) which can lead to cell death by damaging cell
867 membranes and nucleic acids (Rijstenbil et al. 1994, Knauert and Knauer 2008, Rhee et al. 2013).
868 Copper also affects marine primary consumers by inhibiting membrane transport proteins
869 (Bianchini et al. 2004) and by limiting the quantity of their algal diet (Pinho et al. 2007). Further
870 copper effects on microalgae include the inhibition of electron transport in photosystem II and I
871 (for detailed mechanisms see Miao et al. 2005, Spijkerman et al. 2007), by limiting the reduction
872 of nitrate to nitrite (Manimaran et al. 2012) and reducing the uptake of silicon (in diatoms, Martin-
873 Jézéquel et al. 2000).

874

875 **1.8.2. Thesis outline**

876 The main challenges for future BEF research comprise:

877 (i) contrasting BEF relations obtained with the classic design of random species loss with those
878 induced by anthropogenic stress (addressed in Chapter 2)

879 (ii) identifying response and effect traits which predict the functional consequences of
880 biodiversity loss under anthropogenic stress (addressed in Chapter 3)

881 (iii) determining the relative importance of the two main biodiversity effects (dominance and
882 complementarity) for functioning in stressed communities, and identifying mechanisms which
883 drive both effects under stress (addressed in Chapter 4)

884 (iv) analysing the effects of anthropogenic stress on diet quality and food web functioning
885 (addressed in Chapter 5)

886

887 Here, in the general introduction (**Chapter 1**), the scientific setting of this thesis was outlined,
888 with a focus on the recent progress and future research scopes in BEF science. The inclusion of
889 anthropogenic stress into BEF research was identified as first main objective, since it is unclear
890 whether the biodiversity loss and the BEF relations obtained with the classic random BEF design
891 correspond to those induced by anthropogenic stress. Hence, **Chapter 2** contrasts diversity
892 gradients in diatom communities created by the classic design of random community assembly
893 with diversity gradients induced by applying the pesticide atrazine. Next, Chapter 2 tests if BEF
894 relations in both designs are comparable, i.e. if diversity-functioning relationships inferred from
895 classic random-assembly approaches predict pesticide-driven diversity-functioning
896 relationships. Functioning is measured as the diatoms' contribution to four processes:
897 photosynthetic efficiency and biomass production (as proxy for primary production), fatty acid
898 production (as proxy for energy content) and EPS production (as proxy for sediment
899 stabilization). Lastly, Chapter 2 tests if differences between the two types of diversity-functioning
900 relationships could be explained by selective atrazine effects, i.e. to what extent the species'
901 contribution to functioning and their stress tolerance combine in shaping BEF relations under
902 stress.

903 The lacking information on the species' stress tolerance is a main reason why BEF relations are
904 commonly tested with random species loss. **Chapter 3** therefore identifies a set of response and
905 effect traits, which predict the abundance (numerical stress response) and functional contribution
906 (functional stress response) of diatoms under copper and atrazine. By quantifying the effect of

907 atrazine and copper on the abundance (cell densities) and functional contribution (photosynthetic
908 efficiency, EPS and fatty acid production) of 17 diatom species, Chapter 3 represents a
909 comprehensive dataset on the effects of two common chemical stressors on benthic diatoms.
910 Moreover, by linking response and effect traits, Chapter 3 combines ecotoxicology and theoretical
911 ecology, and provides new insight into the extent to which metal- and herbicide-tolerant species
912 can maintain their contribution to ecosystem functioning under stress.

913 Dominance (dominance of communities by high- or low-yield species) and complementarity
914 (better performances of species in community due to mechanisms such as resource partitioning
915 or facilitation) are the two main effects which cause communities to perform differently than
916 expected from their component monocultures (see 1.6). However, both effects have rarely been
917 quantified under stress. Moreover, there is little ecophysiological evidence for facilitative
918 mechanisms as driver of complementarity in stressed communities, and BEF models commonly
919 assume complementarity to be mutual, with species reciprocally benefitting each other. **Chapter**
920 **4** tests whether the biodiversity effect on diatom biomass production under copper and atrazine
921 stress depends on dominance or complementarity effects. Moreover, Chapter 4 unravels the
922 fundamental mechanisms driving the diversity effect on functioning, by testing if
923 complementarity under stress is driven by the activation of facilitative mechanisms in stressed
924 diatom communities, and by linking the degree to which species benefit from complementarity to
925 their stress tolerance and trophic mode.

926 **Chapter 5** relates chemical stress effects on primary producers (diatoms) to the consumer level
927 (harpacticoid copepods). First, Chapter 5 measures the effects of atrazine and copper on the
928 contribution of a diatom community to diet quantity (biomass production), diet quality (essential
929 fatty acids production) and sediment stabilization (EPS production). Next, Chapter 5 tests if
930 diatom functioning under stress is driven by changes in community structure or by stress effects
931 on the species' functional contribution. Last, grazer experiments are used to test if atrazine and
932 copper effects on diatom diet quality affect the dominant grazer at the study site, the harpacticoid
933 copepod *Microarthridion littorale*. Thereby, Chapter 5 addresses a main bottleneck issue in
934 current BEF research, which rarely investigates stressor effects across trophic levels and nearly
935 essentially analyzes food web functioning in terms of diet quantity but not diet quality.

936 The main conclusions of this work are summarized and discussed together with future
937 perspectives in **Chapter 6**.

938 The **Addenda II to IV** comprise supplementary material to the chapters 2 to 5, respectively.

939 **Addendum V** reports the results of an additional experiment which quantified the tolerance of
940 five harpacticoid species collected at the Paulina intertidal area to direct copper exposure.

941 **Chapter 2: Stressor-induced biodiversity gradients: revisiting**
942 **biodiversity – ecosystem functioning relationships**

943

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948

949 **Abstract**

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

964

965 **2.1. Introduction**

966 Biodiversity loss due to human activities has led scientists to enquire how the diversity of
967 communities may regulate ecosystem functioning (Cardinale et al. 2012). During the last two
968 decades, numerous studies have shown that biodiversity favours ecosystem functioning, by
969 positively affecting processes such as productivity or decomposition (Loreau 2000, Hooper et al.
970 2012). Toxic chemicals are potential drivers of biodiversity decrease (De Laender et al. 2014) but
971 remain understudied in biodiversity-ecosystem functioning research and conservation (Lawler et
972 al. 2006). In diversity-functioning research, ecosystem functioning is commonly measured across
973 broad gradients of species richness in randomly assembled communities (Deutschman 2001, Díaz

974 et al. 2003). In the case of toxic chemicals, this makes the implicit assumption that pollution would
975 eliminate a large number of species, and remove individuals in a random way. However,
976 environmentally realistic levels of stressors such as chemical pollution or physical disturbance
977 reduce the abundance of sensitive species (Davies et al. 2011), but without necessarily removing
978 them from the ecosystem, resulting in gradients of evenness rather than species richness
979 (Johnston and Roberts 2009). Whether the evenness gradients created by random manipulations
980 of species richness are representative for those caused by environmental stress such as chemical
981 toxicity remains elusive. Moreover, it is unclear how ecosystem functioning of randomly
982 assembled communities compares to that of communities composed by environmental filtering
983 through chemical toxicity. Chemicals can impact ecosystem functioning directly, by affecting the
984 physiology of the concerned species (De Laender et al. 2010), and indirectly through selective
985 biodiversity loss, depending on the species' sensitivity to the chemical (Vinebrooke et al. 2004).
986 When species differ in their contribution to ecosystem functioning, consequences of biodiversity
987 decline for a particular process can depend on the order in which the species are lost (Solan et al.
988 2004). If the most sensitive species also contributes most to a given ecosystem process, a
989 chemical-induced reduction in density of this species, and therefore evenness reduction, can lead
990 to more pronounced functional consequences than predicted from a random community
991 assembly design.

992 We compare biodiversity gradients and diversity-functioning relations obtained using the design
993 of random community assembly with those generated by filtering through environmental stress.
994 First, we contrast evenness gradients created by randomly assembling 4 benthic diatom species
995 from an intertidal mudflat with those induced by applying the herbicide atrazine, one of the most
996 frequently used herbicides worldwide (Graymore et al. 2001). Despite its EU-wide ban,
997 environmentally critical concentrations of atrazine are still monitored in European intertidal
998 mudflats, which are wetlands of high economic and ecological value (Graymore et al. 2001, Niquil
999 et al. 2006). Next, we test if changes in ecosystem functioning in both sets of experiments are
1000 comparable: Do evenness-functioning relationships inferred from the random assembly approach
1001 predict pesticide-driven non-random evenness-functioning relations? We measured three
1002 ecosystem processes and attributes that are particularly relevant in mudflat ecosystems: primary
1003 production, sediment stabilization and energy content of diatoms. Lastly, we tested if differences
1004 between the two types of diversity-functioning relationships could be explained by selective
1005 atrazine effects, by measuring to what extent species-specific contributions to ecosystem
1006 functioning and species-specific sensitivity to chemicals combine in shaping the diversity-
1007 functioning relation.

1008 Diversity-functioning research commonly focuses on the relation between species richness and
1009 primary production (Hillebrand and Matthiessen 2009). Yet, evenness responds faster to
1010 environmental stress and can be a stronger predictor for ecosystem functioning than species
1011 richness *per se* (Chapin et al. 2000, Kirwan et al. 2007). Also, ecosystem processes other than
1012 primary production can respond differently to diversity loss (Hector and Bagchi 2007). We
1013 therefore quantified biodiversity as evenness, and analyzed its relation to three ecosystem
1014 processes and attributes. Biovolume, the most frequently used proxy for phytoplankton biomass
1015 (Hillebrand et al. 1999), and maximum quantum yield of photosynthesis, a proxy for
1016 photosynthetic efficiency (Hartig et al. 1998, Kromkamp et al. 1998), were used to quantify
1017 primary production. Sediment stabilization was quantified as extracellular polymeric substances
1018 (EPS) production. These biopolymers secreted by microphytobenthos and other microorganisms
1019 enhance ecosystem functioning through biogenic sediment stabilization (Gerbersdorf et al.
1020 2009a). The diatom energy content, an attribute that serves as proxy for energy transfer to higher
1021 trophic levels, was estimated by the production of polyunsaturated fatty acids (PUFAs), an
1022 essential diet component for primary consumers (De Troch et al. 2012a). PUFAs are therefore
1023 commonly used as marker for food quality and energy transfer efficiency (Brett and Müller-
1024 Navarra 1997, De Troch et al. 2012a).

1025

1026 **2.2. Methods**

1027 **Experimental organisms and culture conditions.** Experimental communities were composed
1028 of 4 benthic epipelagic diatom species. *Navicula arenaria*, *Nitzschia* sp. and *Entomoneis paludosa*
1029 were sampled from intertidal mudflats in the Westerschelde estuary (SW Netherlands, 51°21'N,
1030 3°43'E). *Seminavis robusta* was obtained from the culture collection of the Protistology & Aquatic
1031 Ecology Research Group (UGent). Cultures of the species were maintained in a climate room at
1032 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons m⁻²s⁻¹, in culture
1033 medium consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2
1034 nutrients (Guillard 1975).

1035 **Atrazine and random assembly design.** In the first experiment, diatom communities were
1036 constructed by random community assembly, the common approach in diversity-functioning
1037 research. All possible 15 species combinations were assembled, thereby simulating all
1038 possibilities of random species loss. In the second experiment (atrazine design), diatom
1039 communities were composed selectively through atrazine, by exposing the full species community
1040 to concentrations of 0 (control treatment), 100, 500 and 1000 µg/l atrazine respectively.
1041 Technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 99.8% pure) was used in

1042 all treatments. Atrazine was dissolved in the culture medium by means of a 30 min ultrasonic bath.
1043 The resulting atrazine concentrations determined by GC-MS analysis were 91, 465 and 980 µg/l
1044 in the atrazine treatments and <1 µg/l (below detection limit) atrazine in the control treatment.
1045 Treatments in the random assembly design were run twice in polystyrene 6-well-plates,
1046 treatments in the atrazine design in 3 polystyrene 6-well-plates: an equivalent of 12 and 18
1047 replicated microcosms per treatment. Each microcosm was inoculated with a total cell density of
1048 10 000 cells/ml (100 000 cells in 10 ml of culture medium), with equal cell densities per species.
1049 Treatments were inoculated with diatom cells from exponentially growing cultures, and
1050 incubated in a climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons
1051 m⁻²s⁻¹. All experiments were terminated after 18 days.

1052 **Biodiversity and ecosystem functioning measurements.** Evenness was calculated every
1053 second day from cell densities per species. Cell densities were obtained by magnifying and
1054 photographing (x100) an area of 0.33 mm² per microcosm, using an inverted Axiovert 135 Zeiss
1055 microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11
1056 digital camera). Cell numbers per species were subsequently determined by identifying and
1057 counting cells on the computer (ImageJ cell counting software) and converted into cell densities
1058 in cells/ml. Evenness for every treatment and replicate was calculated as follows:

1059
$$J = \frac{H'}{H'_{\max}}$$

1060
$$H' = - \sum_{i=1}^s \frac{n_i}{N} \times \log \frac{n_i}{N}$$

1061 Where J is evenness, H' is the Shannon index, n_i is cell density of the *i*th species. N is total cell density
1062 of the microcosm and H'_{max} is log₁₀ of species richness (log₁₀(4) in every treatment). Time-
1063 averaged evenness was used as biodiversity parameter in every treatment.

1064 Biovolume was calculated every second day from cell densities and mean cell biovolume per
1065 species according to (Hillebrand et al. 1999), using measured linear dimensions and formulas
1066 representing the closest approximation of geometric shape for each genus (Addendum I Table S1).
1067 Time-averaged biovolume was calculated as proxy for primary production for every treatment.

1068 Maximum quantum yield of photosynthetic activity was measured at 48-hour-intervals by pulse-
1069 amplitude modulated (PAM) fluorometry. Maximum quantum yield is determined as the ratio of
1070 variable and maximum fluorescence (F_v/F_m). Maximum fluorescence F_m is the maximum
1071 fluorescence emission level after a dark adaptation of 20 minutes, measured with a saturating
1072 pulse of light (emission peak at 450 nm, 2700 photons m⁻²s⁻¹, 800 ms). Variable fluorescence F_v is

1073 calculated from the difference between initial fluorescence (F_0) and maximum fluorescence ($F_v =$
1074 $F_m - F_0$).

1075 Extracellular polymeric substances were measured by spectrophotometry at the end of the
1076 experiments after 18 days, with 3 replicates of 10 ml suspended diatom culture per treatment.
1077 The suspended diatom cultures were centrifuged for 15 min at 15 °C and 3500*g*. The supernatant
1078 yielded the soluble EPS fraction, which was left to precipitate overnight at -20°C in 30 ml cold
1079 ethanol (98%), and subsequently centrifuged for 15 min at 15 °C and 3500*g*. The pellet was dried
1080 under a flow of nitrogen and resuspended in 2 ml of 1.5% NaCl. Samples of 200 µl of this
1081 suspension per replicate were used for the EPS analysis. EPS was measured according to a
1082 modified version of the phenol/H₂SO₄ assay by Dubois et al. (1956). In 24-well-plates, 1 ml
1083 concentrated H₂SO₄ and 200 µl phenol (5%, w / v in distilled water) were added to 200 µl sample.
1084 The mixture was shaken, incubated for 20 min and the absorbance measured at 488 nm using a
1085 Victor³ multilabel reader (Perkin-Elmer). EPS concentrations were quantified with a function
1086 obtained from a linear regression of known concentrations of glucose standards (ranging from 0.1
1087 to 100 µg/ml).

1088 Fatty acid profiles in 2 replicates of 37 ml suspended diatom culture per treatment were analyzed
1089 after 18 days. The suspension was centrifuged for 20 minutes at 4 °C and 2700*g*. The supernatant
1090 was removed except 5 ml, the pellet was resuspended, transferred into a 10 ml glass tube and
1091 centrifuged for 15 minutes at 4 °C and 2700*g*. The supernatant was removed and the pellet was
1092 stored at -80 °C until fatty acid extraction. Hydrolysis of total lipid extracts of duplicate diatom
1093 cell suspensions per treatment and methylation to FA methyl esters (FAME) was achieved by a
1094 modified one-step derivatisation method (Abdulkadir and Tsuchiya 2008, De Troch et al. 2012a).
1095 The boron trifluoride-methanol reagent was replaced by 3.5 ml of a 2.5% H₂SO₄-methanol
1096 solution per sample since BF₃-methanol can cause artefacts or loss of PUFA (Eder 1995). The fatty
1097 acid Methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the
1098 quantification (see further). Samples were centrifuged and vacuum dried. The FAME thus
1099 obtained were analysed using a gaschromatograph (HP 6890N) with a mass spectrometer (HP
1100 5973). The samples were run in splitless mode, with a 1 µL injection per run, at an injector
1101 temperature of 250°C using a HP88 column (Agilent J&W; Agilent Co., USA). The oven temperature
1102 was programmed at 50°C for 2 min, followed by a ramp at 25°C min⁻¹ to 175°C and then a final
1103 ramp at 2°C min⁻¹ to 230°C with a 4 min hold. The FAME were identified by comparison with the
1104 retention times and mass spectra of authentic standards and mass spectral libraries (WILEY,
1105 NITS05), and analysed with the software MSD ChemStation (Agilent Technologies). Quantification
1106 of individual FAME was accomplished by the use of external standards (Supelco # 47885, Sigma-
1107 Aldrich Inc., USA). The quantification function of each individual FAME was obtained by linear

1108 regression of the chromatographic peak areas and corresponding known concentrations of the
1109 standards (ranging from 5 to 250 ng/ml).

1110 **Data analysis.** Biodiversity-ecosystem functioning relationships in randomly assembled and
1111 stressed communities were analyzed with a generalized least squares model (Model 1), with the
1112 respective ecosystem process or attribute as response variable and biodiversity as predictor
1113 ($E[EF|a, b] = a + b \cdot B$). B represents biodiversity, EF ecosystem functioning, and a and b the
1114 intercept and the slope (i.e. the effect of B on EF). In cases when biodiversity significantly (α
1115 = 0.05) predicted ecosystem functioning for both random and stressed treatments, we compared
1116 the slopes (b) and their confidence intervals between the two model fits. The absence or presence
1117 of overlap of the 95% pointwise confidence intervals for b in both models was used as a criterion
1118 for the similarity or difference of diversity-functioning relations between the atrazine-induced
1119 and random biodiversity loss. Normality and homogeneity of model residuals across the evenness
1120 range were inspected using quantile-quantile plots and by plotting residuals versus explanatory
1121 variables, respectively. If homogeneity was violated, the model was refitted using an exponential
1122 variance structure allowing residuals to change with the predictor X ($\text{var}(\varepsilon) = \sigma^2 \cdot e^{2 \cdot \delta \cdot X}$), and
1123 homogeneity was evaluated again (Model 2). By means of likelihood ratio testing, the significance
1124 of these structures was tested ($\alpha = 0.05$).

1125 Effects of species identity were assessed by fitting a generalized least squares model to the
1126 respective ecosystem process or attribute as response variable and the cell density of each of the
1127 4 species as predictors ($E[EF|a, b] = a + \sum_{i=1}^4 b_i \cdot S_i$). EF represents ecosystem functioning, a is the
1128 intercept, S is the cell density of *Navicula arenaria*, *Entomoneis paludosa*, *Seminavis robusta* or
1129 *Nitzschia sp.* respectively, and b represents the slope, i.e. the effect of species identity on EF.
1130 Normality and homogeneity of model residuals was tested as for the diversity-functioning models,
1131 i.e. by plotting residuals vs. all predictors. All calculations were performed in R 2.10.1, using
1132 RStudio (R development Core Team 2015) and the package nlme (Pinheiro et al. 2015).

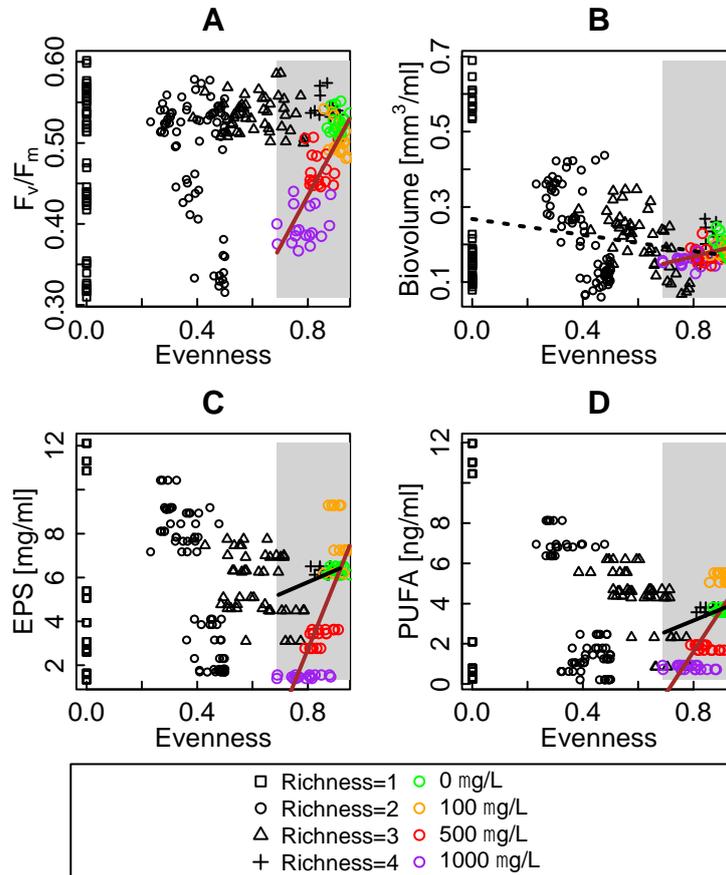
1133

1134 **2.3. Results**

1135 Atrazine reduced diatom evenness, but to a lesser extent than random species removal. Evenness
1136 gradients obtained in the atrazine design (0.69 to 0.98) were narrower than those established in
1137 the random assembly design (0 to 0.96, Fig. 2.1). Evenness-functioning relationships differed in
1138 randomly assembled and atrazine-exposed communities. Evenness was positively related to all 4
1139 ecosystem functioning proxies (all $p < 0.0001$) in the atrazine design (Fig. 2.1, Table 2.1). In the
1140 random assembly design, evenness was significantly related to biovolume only ($p = 0.0006$, Fig.
1141 2.1, Table 2.1). Ecosystem functioning was also increasingly variable with decreasing evenness in

1142 the random assembly design (Fig. 2.1). This was confirmed by likelihood ratio testing, which
 1143 showed that diversity-functioning relations in these communities were more accurately
 1144 described by models including a variance structure allowing the variance of ecosystem
 1145 functioning to change with evenness ($p < 0.0001$; Table 2.1).

1146



1147

1148 **Fig. 2.1:** Proxies of ecosystem functioning as a function of evenness in randomly assembled and atrazine-
 1149 exposed diatom communities. Ecosystem functioning is quantified as primary production (photosynthetic
 1150 efficiency and biovolume, panels A and B), sediment stabilization (EPS, panel C) and energy content (PUFA,
 1151 panel D). Black dots represent diatom communities from the random assembly design, coloured dots denote
 1152 diatom communities from the atrazine design. The area marked in grey denotes the evenness gradient
 1153 induced by atrazine. Regression lines show significant relations between evenness and functioning. Brown
 1154 lines are linear models based on the atrazine design. Dashed and solid black lines are linear models based
 1155 on the random assembly design for the whole evenness range or for the evenness range induced by atrazine
 1156 (grey area), respectively.

1157

1158 To enable contrasts of evenness-functioning relations induced by the random assembly approach
 1159 with those found in stressed communities within the same evenness gradient, we repeated our
 1160 analyses, only considering the overlap of both evenness ranges (0.69-0.98) (Fig. 2.1, marked in
 1161 grey). This was because the evenness gradient resulting from atrazine exposure was narrower
 1162 than that in the random assembly design. These analyses revealed that, in the randomly composed

1163 communities, evenness within a range of 0.69 to 0.98 was positively related to EPS ($p=0.02$) and
 1164 PUFA ($p=0.02$) production (Fig. 2.1, Table 2.1). The slopes of these evenness-functioning
 1165 relationships however were 3x (EPS) and 6x (PUFA) lower than those found in the atrazine design
 1166 (Fig. 2.1, Table 2.1), i.e. slopes were less steep at random community composition. Diversity-
 1167 functioning relationships were thus different when created by random community assembly than
 1168 by exposure to atrazine.

1169

1170

Ecosystem Function	Data	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Primary Production: (Photosynth. Efficiency)	R	1	0,08	0,02	4,05	0,0001	-416	211	No		
		2	0,09	0,01	4,90	<0,0001	-441	224	No	N.A.	N.A.
	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	N.A.	N.A.
Primary Production: Biovolume [mm ³ /ml]	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 [mg/ml]	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	N.A.	N.A.
	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]	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

1171

1172 **Table 2.1:** Relationship between evenness and ecosystem functioning in randomly composed and atrazine-
 1173 exposed diatom communities. Results of generalized least squared models predicting ecosystem
 1174 functioning from evenness, without (model 1) or with (model 2) a variance structure. 'Data' denotes the
 1175 data on which the models are based: the classical experiment with random community assembly (R), the
 1176 atrazine experiment (A), the classical experiment for evenness values overlapping with those from the
 1177 atrazine experiment (R'); 's.e.' is the standard error on the estimated slopes; T and P denote the t- and p-
 1178 values, bold values are statistically significant. AIC is the Akaike information criterion; 'Validity' denotes if

1179 residuals were homogeneous and normally distributed ('yes') or not ('no'); 'LR' is the likelihood ratio of
 1180 model 1 vs. model 2, P LR the corresponding p-value.

1181

Ecosystem Function	Data	Predictor	Slope	s.e.	T	P
Sediment stabilization: EPS [mg/ml]	A	<i>Navicula</i>	-1,31E-01	4,66E-02	-2,8243	0,0062
		<i>Entomoneis</i>	5,08E-03	2,08E-02	0,2445	0,8076
		<i>Seminavis</i>	9,85E-03	4,29E-02	0,2296	0,8191
		<i>Nitzschia</i>	2,57E-01	3,74E-02	6,8742	<0.0001
	R'	<i>Navicula</i>	1,94E-02	2,55E-02	0,7579	0,4578
		<i>Entomoneis</i>	-1,01E-02	8,76E-03	-1,1494	0,2647
		<i>Seminavis</i>	-4,93E-03	1,31E-03	-3,7631	0,0013
		<i>Nitzschia</i>	1,83E-02	2,27E-03	8,0803	<.00001
Energy content: PUFA [ng/ml]	A	<i>Navicula</i>	-1,60E+00	1,06E+00	-1,5046	0,1371
		<i>Entomoneis</i>	1,02E-01	4,69E-01	0,2175	0,8285
		<i>Seminavis</i>	-1,08E+00	9,70E-01	-1,1102	0,2709
		<i>Nitzschia</i>	6,12E+00	8,50E-01	7,1985	<0.0001
	R'	<i>Navicula</i>	2,93E-01	5,41E-01	0,5429	0,5935
		<i>Entomoneis</i>	-2,95E-01	1,77E-01	-1,6690	0,1115
		<i>Seminavis</i>	-2,12E-01	2,64E-02	-8,0345	<0.0001
		<i>Nitzschia</i>	4,89E-01	7,29E-02	6,7162	<0.0001
Primary Production: Biovolume [mm ³ /ml]	A	<i>Navicula</i>	1,73E-01	1,28E-02	13,5532	<0.0001
		<i>Entomoneis</i>	6,97E-02	6,03E-03	11,5579	<0.0001
		<i>Seminavis</i>	-8,01E-03	1,24E-02	-0,6463	0,5203
		<i>Nitzschia</i>	9,59E-02	1,05E-02	9,1230	<0.0001
	R'	<i>Navicula</i>	1,13E-01	1,99E-02	5,6587	<0.0001
		<i>Entomoneis</i>	1,46E-01	1,56E-02	9,3537	<0.0001
		<i>Seminavis</i>	3,01E-03	2,75E-03	1,0917	0,2886
		<i>Nitzschia</i>	2,08E-02	1,33E-03	15,6292	<0.0001
Primary Production: (Photosynth. Efficiency)	A	<i>Navicula</i>	-3,41E-02	3,78E-02	-0,9010	0,3708
		<i>Entomoneis</i>	2,34E-02	1,96E-02	1,1984	0,2350
		<i>Seminavis</i>	8,30E-02	4,18E-02	1,9843	0,0513
		<i>Nitzschia</i>	1,23E-01	3,09E-02	3,9923	<0.0001
	R'	<i>Navicula</i>	-2,82E-02	3,85E-02	-0,7319	0,4732
		<i>Entomoneis</i>	-1,48E-04	1,89E-02	-0,0078	0,9938
		<i>Seminavis</i>	-4,12E-03	2,87E-03	-1,4325	0,1683
		<i>Nitzschia</i>	6,53E-03	2,14E-03	3,0548	0,0065

1182

1183 **Table 2.2:** Relationship between ecosystem functioning and diatom cell density per species in randomly
 1184 composed and atrazine-exposed communities. Results of generalized least squared models predicting
 1185 ecosystem functioning from cell density per species. 'Data' denotes the data on which the models are based:
 1186 the atrazine experiment (A), or the classical experiment (random community assembly) for evenness values
 1187 overlapping with those from the atrazine experiment (R'); 's.e.' is the standard error on the estimated
 1188 slopes; bold values are statistically significant.

1189

1190 As we considered community performance as more relevant for ecosystem functioning than
1191 performance per cell, we initially analyzed diversity-functioning relationships in terms of absolute
1192 values. In order to know if PUFA and EPS production were also related to diversity at the cellular
1193 level, we included supplemental analyses between evenness and PUFA and EPS concentrations
1194 normalized to cell density. Normalized PUFA and evenness were not correlated in the random
1195 assembly design, but positively related in the atrazine design ($p < 0.0001$, Addendum I Fig. S1,
1196 Table S2). EPS production per cell was positively correlated with evenness, both in atrazine-
1197 exposed communities and over the whole random diversity gradient (both $p < 0.0001$). Similar to
1198 non-normalized values, the diversity-functioning relation was steeper when evenness gradients
1199 were induced by atrazine. In randomly assembled communities of similar evenness as induced by
1200 atrazine, the relation between evenness and normalized EPS was negative ($p = 0.02$, Addendum I
1201 Fig. S1, Table S2).

1202 In the atrazine design, diversity-functioning relations were steeper, although diversity loss was
1203 less severe than predicted by the random assembly approach. In the random assembly design,
1204 *Nitzschia sp.*, if present, always became the dominant species. Exposure to atrazine did not lead to
1205 species removal, but to a change in dominance: instead of *Nitzschia sp.*, *Navicula arenaria*
1206 dominated communities exposed to 500 and 1000 $\mu\text{g/l}$ atrazine, reaching its highest cell density
1207 at the two highest atrazine concentrations. Detailed cell densities per species for every treatment
1208 are indicated in Addendum I Fig. S2 for the random assembly design and Addendum I Fig. S3 for
1209 the atrazine design. Moreover, *Nitzschia sp.* cell density was positively correlated with all proxies
1210 of ecosystem functioning, in both atrazine-exposed and randomly composed communities (Table
1211 2.2). PUFA and EPS production, the processes that were related to evenness in both the atrazine
1212 and the random assembly design, thus also correlated with the abundance of *Nitzschia sp.*
1213 Evenness gradients found in atrazine and random assembly treatments hence coincided with a
1214 change in the identity of the dominant species (dominance of *Navicula arenaria* and *Nitzschia sp.*
1215 respectively). At high atrazine concentrations the growth of *Nitzschia sp.*, the species contributing
1216 most to all analyzed functions, was suppressed. Instead, communities were dominated by
1217 *Navicula*, which was negatively correlated with EPS production in the atrazine-exposed
1218 communities (Table 2.2).

1219 The observed diversity-functioning relationships were thus related to the identity of the most
1220 productive species, and a selective suppression of this species by atrazine can explain the steeper
1221 decline of functioning at stressor-induced than at random biodiversity loss.

1222

1223 **2.4. Discussion**

1224 In biodiversity-ecosystem functioning research, one typically aims at experimental biodiversity
1225 gradients that are as broad as possible, to test the effects of widespread diversity loss on
1226 ecosystem functioning (Naeem 2008). Indeed, narrow, evenness-based biodiversity gradients are
1227 uncommon in experimental diversity-functioning research (Hillebrand et al. 2008). Instead,
1228 biodiversity loss by anthropogenic activity is typically mimicked with broad diversity gradients,
1229 covering the entire range from minimal to maximal species richness of the considered ecosystem
1230 (Hillebrand et al. 2008), corresponding to evenness ranges from 0 to 1. In the case of toxic
1231 chemicals however, such extensive biodiversity loss is unlikely to happen. Realistic biodiversity
1232 loss most often only affects a fraction of the existing species pool, resulting in alterations of
1233 evenness rather than richness (Pennington et al. 2001, Hillebrand and Matthiessen 2009), even at
1234 chemical concentrations well above environmental concentrations, as represented by the highest
1235 atrazine concentrations in our study (Pennington et al. 2001). Evenness alterations induced by
1236 atrazine were less severe than those in the richness-based random assembly approach, which in
1237 this case overestimated the biodiversity loss induced by chemical stress. Yet in the random
1238 assembly design, we recorded positive effects of diversity on energy content and sediment
1239 stabilization within a narrow range of high evenness, but not over the whole broad evenness
1240 gradient. Rather than being a tradeoff to realism, we thus suggest narrow biodiversity gradients,
1241 with evenness instead of richness as diversity proxy, as a more realistic simulation of pollution-
1242 induced biodiversity loss that can facilitate the detection of biodiversity effects on ecosystem
1243 functioning.

1244 Diversity-functioning relations were steeper when diversity gradients were chemically-induced
1245 rather than randomly assembled, denoting a disproportionately large decrease of functioning in
1246 diatom communities under stress conditions. Contrasting the design of random community
1247 assembly with scenarios of selective biodiversity loss due to environmental filtering is among the
1248 main challenges for future diversity-functioning research (Srivastava 2002, Giller et al. 2004), but
1249 has rarely been tested experimentally (Symstad and Tilman 2001, Smith and Knapp 2003, Solan
1250 et al. 2004, Larsen et al. 2005). Chemical stress moreover has not been studied as driver for
1251 realistic biodiversity loss and ecosystem functioning, although toxic chemicals emitted by
1252 industry, agriculture and households are among the most potent threats to aquatic species (Geiger
1253 et al. 2010). We found a larger discrepancy in random and stressor-induced diversity-functioning
1254 relations (3 to 6 time steeper relations in stressed communities in terms of EPS and PUFA
1255 production) than in other cases where the random assembly design could be contrasted with
1256 realistic diversity loss (Smith and Knapp 2003, Solan et al. 2004, Schlöpfer et al. 2005, Larsen et
1257 al. 2005). This can be due to the organisms and ecosystem processes studied, and to selective
1258 atrazine effects on the physiological rates and composition of the diatom communities.

1259 In our diatom communities that were randomly assembled, primary production, the most
1260 common measure of ecosystem functioning (Hooper et al. 2005), was not related to diversity. In
1261 contrast, sediment stabilization and energy content showed a steeper relation to diversity than
1262 primary production in both the atrazine and the random assembly design, which facilitated the
1263 contrast of the two experimental approaches. The observed functional consequences of stressor-
1264 induced diversity loss thus depended on the measured ecosystem function, which underlines the
1265 value of analyzing multiple ecosystem processes (Hector and Bagchi 2007, Hiddink et al. 2009).
1266 Also, the pool of 4 species could have contributed to the steep diversity-functioning relations. In
1267 our experimental communities, the functionally most important species was most affected by
1268 atrazine (but see below). In a larger species pool, this selection effect could be compensated by
1269 functionally redundant, but less sensitive species. Moreover, as randomized and stress-induced
1270 biodiversity and ecosystem functioning have so far not been assessed in marine microalgae, we
1271 cannot relate our findings to other studies using similar organisms. The disproportionate loss of
1272 functioning following selective loss of diatom biodiversity however corresponds to existing
1273 knowledge on non-random diversity loss in grassland primary producers (Symstad and Tilman
1274 2001, Zaveleta and Hulvey 2004). Yet, the steep diversity-functioning relationships in our stressed
1275 communities can not only be explained by the number and type of different organisms and
1276 ecosystem processes. Loss of functioning in stressed communities can be related to selective
1277 alterations of biodiversity by the stressor, as well as direct stressor effects (Schimel et al. 2007).
1278 In our case, atrazine could affect functioning indirectly through selective changes of community
1279 composition and directly by reducing physiological rates of the species. Atrazine treatments in
1280 which functioning was reduced with regard to the control were essentially dominated by *Navicula*
1281 *arenaria*, the only species which was able to grow at the highest atrazine concentration. To inspect
1282 potential direct physiological effects, we compared PUFA and EPS production per cell in these *N.*
1283 *arenaria*-dominated stressed communities with those of unexposed *N. arenaria* monocultures.
1284 Cells in the *N. arenaria*-dominated communities produced 1.8 and 3.5 times less EPS, but 2 and 3
1285 times more PUFA than in the monocultures of this species (Addendum I Fig. S4). The reduction in
1286 EPS in stressed communities could thus be due to physiological effects of atrazine on *N. arenaria*.
1287 The reduction in PUFA however does not seem to be caused by physiological effects, as *N. arenaria*
1288 actually seems to increase its PUFA production when exposed to atrazine. To understand the steep
1289 loss of functioning in our stress-exposed communities, we must therefore, depending on the
1290 ecosystem process, look beyond direct stressor effects and consider selective atrazine effects on
1291 the community composition.

1292 In diversity-functioning research, consequences of biodiversity loss are commonly predicted
1293 using randomly assembled communities, whereas environmental filtering due to stress results in
1294 distinct non-random community compositions. Furthermore, evenness is often held constantly

1295 high across species richness treatments (Wilsey and Polley 2004), thus minimizing effects of
1296 relative abundances (i.e. species identity) on ecosystem functioning. Due to the increasing
1297 recognition that biodiversity and species identity combine in shaping ecosystem functioning
1298 (Bruno et al. 2006), research on realistic biodiversity loss has started by removing rare and
1299 uncommon species from the community (Smith and Knapp 2003). Despite making the step from
1300 random to a more realistic, abundance-based biodiversity loss, this approach still assumes that
1301 the naturally abundant species are also most resistant to environmental stressors and less likely
1302 to be lost. In our diatom communities, *Nitzschia sp.* was the most abundant species in the natural
1303 samples as well as in the experimental communities, contributing most to the measured
1304 ecosystem processes. However, when exposed to stress, *Nitzschia sp.* was the species whose cell
1305 density was most reduced by atrazine. This shows that both in stressed and randomly assembled
1306 communities, community composition tends to be uneven, and that the most abundant species is
1307 not necessarily the most stress-resistant. Biodiversity loss generally reduces ecosystem
1308 functioning, but general physiological and selective stressor effects on the most abundant and
1309 functionally most efficient species lead to a disproportionate loss of ecosystem functioning in
1310 stressed communities compared the random assembly design.

1311 Benthic diatoms are the dominant primary producers and food source in many intertidal, soft-
1312 sediment environments as well as on rocky shores (Tang and Kristensen 2007, Speybroeck et al.
1313 2008). Diatom communities play a pivotal role in controlling energy flow, nutrient fluxes and
1314 productivity in these systems (Underwood and Kromkamp 1999, De Troch et al. 2012a). Diatom
1315 biodiversity loss resulting in a two-fold reduction in diatom biomass, three-fold reduction of
1316 unsaturated fatty acids and six-fold decrease of EPS production would strongly impact the
1317 ecosystem under concern. The standing stock and energy flux to higher trophic levels would be
1318 affected (Decho 1990, Müller-Navarra et al. 2000), zoobenthos and as a consequence fish biomass
1319 production limited (De Troch et al. 2012a), and sediment erosion promoted. Concomitantly,
1320 knock-on effects on the variety of processes undertaken by sediment-associated organisms
1321 collectively contributing to ecosystem functioning can be expected (Tolhurst et al. 2003).
1322 Stressor-induced biodiversity loss at the base of the marine food web could thus affect ecosystem
1323 functioning more seriously than predicted by the random assembly design in diversity-
1324 functioning research. In our study, the random assembly approach overestimated biodiversity
1325 loss, but underestimated the associated loss of ecosystem functioning. These contrasting results
1326 strengthen the need to more realistically predict biodiversity gradients and diversity-functioning
1327 relations caused by anthropogenic stress, by considering the prevailing stressors, and the
1328 sensitivity and contribution to ecosystem functioning of the concerned species.

1329 In conclusion, we infer three general statements from our results. First, existing diversity-
1330 functioning research largely overestimates the biodiversity gradients induced by common
1331 environmental stressors such as herbicides. Second, diversity-functioning relations differ
1332 depending on whether the diversity gradient is generated by random community assembly, or by
1333 stressor-induced non-random assembly. Third, physiological stressor effects can affect ecosystem
1334 functioning, and the slope of the diversity-functioning relation depends on stressor-induced
1335 dominance shifts.

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1344

1345 **Chapter 3: Different response-effect trait relationships underlie**
1346 **contrasting numerical and functional responses to two chemical**
1347 **stressors**

1348

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1353

1354 **Abstract**

1355 Environmental stress can impact ecosystem functioning through the loss of species, but also by
1356 altering their densities and functional contribution. Our understanding of the capacity of species
1357 to withstand stress effects on density ('numerical response') and on functional contribution
1358 ('functional response'), and how these are related to one another, is limited, which hampers
1359 efforts to predict ecosystem functioning under stress.

1360 We first identified the response traits and effect traits, driving the numerical and functional
1361 response respectively (potential contribution to primary production, sediment stabilization and
1362 energy content) of 18 marine benthic diatom strains to two different chemical stressors (a
1363 pesticide and a metal). We then examined if the functional contribution of diatoms in unstressed
1364 conditions is related to their numerical and functional stress response. Finally, we investigated
1365 the relationship between numerical and functional response, i.e. whether numerically tolerant
1366 species could maintain their contribution to ecosystem functioning under stress.

1367 The response-effect trait relationship was different depending on stressor type: diatom numerical
1368 and functional response (for all three functions) to the metal were predicted by the same set of
1369 intercorrelated morphological traits, with large cells being more stress-resistant. Numerical and
1370 functional response to the metal were positively related, with species whose density was least
1371 affected also maintaining most of their functional contribution. These species also contributed
1372 most to primary production and sediment stabilization in unstressed conditions.

1373 In contrast, the capacity for mixotrophic growth predicted the numerical, but not the functional
1374 response to the pesticide. The relation between numerical and functional response to the
1375 pesticide was positive for primary production, but negative for energy content and sediment
1376 stabilization, with numerically tolerant species being most affected in their functional
1377 contribution.

1378 *Synthesis.* Ecosystem functioning loss following exposure to stress can be predicted from response
1379 and effect traits, which drive the species' numerical and functional stress response. The extent of
1380 functional loss will depend on the relation of both types of traits, with a disproportionate loss of
1381 functioning if traits predicting numerical response are not related to those driving functional
1382 response.

1383

1384 **3.1. Introduction**

1385 Understanding and predicting the consequences of anthropogenic stress on the functioning of
1386 ecosystems is becoming increasingly critical in view of the profound pressure of human activities
1387 on natural ecosystems and the goods and services they deliver (Balvanera et al. 2006, Halpern et
1388 al. 2008). Environmental stress can affect ecosystem functioning by causing species loss, but also
1389 by altering their densities and contributions to functioning (Fox 2006). While the effects of species
1390 loss on ecosystem functioning have been extensively documented (Naeem et al. 2012), realistic
1391 scenarios which also incorporate changes in species densities and functional contributions are
1392 still rare, which hampers realistically predicting the overall effect of environmental stress on
1393 ecosystem functioning (Duffy et al. 2007, Hooper et al. 2012).

1394 Community structure along environmental gradients has been related to the morphological,
1395 biochemical and physiological traits of the species present (Litchman et al. 2007, Violle et al.
1396 2007). Consequently, trait-based approaches are promising to predict species sensitivities to
1397 different types of stress (Baird and Van den Brink 2007, Larras et al. 2012). Suding et al. (2008),
1398 Hillebrand and Matthiessen (2009) and Litchman et al. (2015) proposed a trait-based framework
1399 to evaluate ecosystem functioning under global change which incorporates the relationship
1400 between traits predicting species densities under stress (response traits, which affect the
1401 numerical stress response of species) to traits affecting ecosystem functioning (effect traits, which
1402 affect the functional stress response of species). This is essential as correlations between response
1403 and effect traits can strongly modify the impact of stress on ecosystem functioning. If the
1404 numerical and functional stress response are positively related to the same set of response and
1405 effect traits, a community decimated by species loss could still be expected to retain most of its
1406 functioning. Alternatively, stress exposure could lead to a disproportionate reduction in
1407 functioning if stress-resistant species, while still being able to grow, would lose most of their
1408 functional contribution. Stressor effects on ecosystem functioning would then be more important
1409 than merely estimated from species densities. Experimental approaches addressing this
1410 relationship are still rare (Hillebrand and Matthiessen 2009, but see Heuner et al. 2015), and
1411 identifying traits which generally predict stressor effects on a taxon's numerical and functional
1412 stress response would be a key step towards predicting community composition and functioning

1413 under different types of stress. Toxic chemicals are among the most common abiotic stressors.
1414 They cause widespread loss of biodiversity and ecosystem functioning (Malaj et al. 2014) but
1415 remain understudied in conservation science (Lawler et al. 2006). The numerical and functional
1416 response to chemical stress has only been quantified for a few species and toxicants, because
1417 generating these data is time- and labour-intensive (Larras et al. 2014). As a result, in
1418 ecotoxicological and also ecological research in general, species loss is often assumed to be
1419 random (O'Connor and Crowe 2005, Baird and Van den Brink 2007, Mensens et al. 2015), and
1420 contributions of species to ecosystem functioning are considered to be equivalent (Díaz et al.
1421 2003). However, species tend to differ in their numerical and functional stress response, as toxic
1422 substances do not only alter species densities, but may also impose a physiological cost, as such
1423 affecting their contribution to ecosystem functioning (Schimel et al. 2007, De Laender et al. 2010).

1424 In the present paper, we relate the traits driving the numerical and functional response of 18
1425 benthic diatom strains to metal (copper) and pesticide (atrazine) stress. Benthic intertidal
1426 diatoms play a pivotal role in the functioning of estuarine ecosystems, where they can provide up
1427 to 50% of primary production (Underwood and Kromkamp 1999), enhance sediment stabilization
1428 through the secretion of extracellular polymers (Decho 2000), and are of essential dietary
1429 importance for higher trophic levels (Dunstan et al. 1993). Copper is one of the most common
1430 pollutants in coastal ecosystems, due to input through urban and industrial runoff and its use in
1431 herbicides and antifouling paints (Kennish 1996; Murray-Gulde et al. 2002). Atrazine is one of the
1432 most frequently used herbicides worldwide (Graymore et al. 2001) and represents a common
1433 stressor in intertidal mudflats, which are wetlands of high economic and ecological value
1434 (Graymore et al. 2001; Niquil et al. 2006). Atrazine and copper are both reported to affect diatom
1435 cell densities and functioning by different modes of action: atrazine blocks the electron transport
1436 chain of Photosystem II (PSII), resulting in the excitation energy generated in PSII being lost as
1437 heat or fluorescent light instead of being used for CO₂ assimilation in the Calvin cycle (Dorigo and
1438 Le Boulanger 2001). An excess of copper decreases the uptake of silicon, inhibits the reduction of
1439 nitrate to nitrite and leads to the production of reactive oxygen species that damage cell
1440 membranes, nucleic acids and chloroplasts (Knauert and Knauer 2008; Masmoudi et al. 2013).

1441 We first test if the numerical and functional response to copper and atrazine in diatoms can be
1442 predicted from a set of morphological, physiological and biochemical traits.

1443 Next, we analyze the relation between the functional contribution in unstressed conditions and
1444 the numerical and functional response to atrazine and copper, for instance if the species with the
1445 highest functioning (e.g. contributing most to primary production or energy flow in intertidal
1446 systems) tend to be particularly tolerant or sensitive to either of the stressors.

1447 Last, we test if and how the numerical and functional response are related, for example if species
1448 that are tolerant in terms of density (i.e. their growth is unaffected) are also able to maintain their
1449 functional contribution under chemical stress.

1450 The functional contribution of each diatom strain was quantified as the contribution per cell to
1451 three processes that are crucial for ecosystem functioning in intertidal systems. The potential
1452 contribution to sediment stabilization was estimated as extracellular polymeric substances (EPS)
1453 production. These biopolymers secreted by microphytobenthos enhance ecosystem functioning
1454 through biogenic sediment stabilization (Gerbersdorf et al. 2009a). Maximum light use efficiency
1455 in photosystem II (hereafter photosynthetic efficiency), an essential component in primary
1456 production, was measured as maximum quantum yield of photosynthesis in photosystem II
1457 (Cleveland et al. 1989, Consalvey et al. 2005, Barnett et al. 2015). The diatom energy content for
1458 higher trophic levels was quantified as the production of polyunsaturated fatty acids, an essential
1459 diet component for primary consumers and a marker for energy transfer efficiency (Brett and
1460 Müller-Navarra 1997; De Troch et al. 2012a).

1461 To predict numerical and functional stress response, we selected morphological and biochemical
1462 traits (biovolume, surface-to-volume ratio, cell length, total organic carbon, total nitrogen and
1463 chlorophyll a content), which have been linked to the functional properties and response to
1464 environmental change in microalgae (Finkel et al. 2010, Kruk et al. 2010). Furthermore we tested
1465 if the capacity for mixotrophic growth, which explained diatom sensitivity to herbicides in recent
1466 studies (Debenest et al. 2009, Larras et al. 2014), could explain the numerical and functional
1467 response of diatoms to chemical stress.

1468

1469 **3.2. Methods**

1470 **Experimental organisms and culture conditions.** In total 18 diatom strains (belonging to 17
1471 species, Addendum II Table S1) were isolated from intertidal mudflats in the Westerschelde
1472 estuary (The Netherlands, 51°21'N, 3°43'E) and the Veerse meer (The Netherlands, 51°32'N,
1473 3°47'E), where diatoms represent the dominant component of the microphytobenthos (Forster et
1474 al. 2006). The strains were deposited in the diatom culture collection (BCCM/DCG) of the Belgian
1475 Coordinated Collection of Micro-organisms (<http://bccm.belspo.be>); accession numbers are
1476 indicated in Addendum II Table S1. Diatoms were maintained in a climate room at 15±1 °C, a
1477 light/dark cycle of 12h / 12h and an illumination of 90 µmol photons m⁻²s⁻¹, in culture medium
1478 consisting of filtered and autoclaved natural seawater (salinity 32±1) enriched with f/2 nutrients
1479 (Guillard 1975).

1480 **Atrazine and copper experiments.** Diatoms were exposed to concentrations of 0, 50, 100, 200,
1481 500 and 1000 µg/l atrazine and 0, 10, 30, 90, 270 and 810 µg/l copper respectively.
1482 Concentrations were based on trial tests (data not shown) as well as published sensitivity data for
1483 marine benthic diatoms (Pistocchi et al. 1997, Levy et al. 2007, Wood et al. 2014) and were
1484 prepared from atrazine and copper stock solutions. The atrazine stock solution was prepared by
1485 dissolving 50 mg technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 99.8%
1486 pure, Sigma-Aldrich Chemie GmbH, Munich, Germany) in 10 ml acetone as a carrier to increase the
1487 solubility of atrazine, with a maximum final volume of 0.025% acetone in the treatments. An
1488 acetone control treatment of 0.025% acetone was included and compared to an acetone-free
1489 control to test for carrier effects. All atrazine treatments were compared to the acetone control.
1490 Copper (as a Cu[II]Cl₂ solution, analytical grade; VWR International) was spiked directly into the
1491 culture medium before exposure of the diatoms. F/2 culture medium for the copper experiments
1492 was prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The
1493 resulting atrazine and copper concentrations are indicated in Addendum II Table S2. All
1494 experiments were run in polystyrene 6-well-plates, an equivalent of 6 replicated microcosms per
1495 treatment. Each microcosm was inoculated with a total cell density of 5000 diatom cells/ml (50
1496 000 cells in 10 ml of culture medium) from exponentially growing cultures, and incubated in a
1497 climate room at 15±1 °C, under a light / dark cycle of 12h / 12h at 90 µmol photons m⁻²s⁻¹. Culture
1498 medium spiked with atrazine and copper respectively was refreshed after 12 days, and all
1499 experiments were terminated after 25 days.

1500 **Cell density, traits and functional contribution.** Cell densities were obtained on days 0, 2, 5, 10,
1501 15, 20 and 25 by magnifying and photographing (x100) an area of 0.33 mm² per microcosm, using
1502 an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and a connected digital
1503 camera (Canon PowerShot G11 digital camera). Cell numbers (cell densities in cells/ml) were
1504 subsequently determined by digitally counting the cells by means of ImageJ cell counting software
1505 (Schneider et al. 2012).

1506 Morphological traits (biovolume, length, surface to volume ratio) were obtained by
1507 photographing diatom cells in every replicate, using an inverted Axiovert 135 Zeiss microscope
1508 (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11 digital camera).
1509 The linear dimensions were digitally measured using ImageJ software calibrated to a stage
1510 micrometer and a standardized set of equations representing the closest approximation of
1511 geometric shape for each genus (Hillebrand et al. 1999).

1512 Chlorophyll a content was measured *in vivo* on days 0, 2, 5, 10, 15, 20 and 25 of the experiments
1513 by PAM fluorometry (MAXI Imaging PAM fluorometer, Walz, Germany) as the minimum
1514 chlorophyll fluorescence signal (F₀) after 15 minutes of dark-adaptation.

1515 Total organic carbon (TOC) and total nitrogen (TN) were measured in a separate experiment,
1516 under the same conditions as the atrazine and copper experiments described above. After 25 days
1517 two replicates of 10 ml suspended diatom culture per treatment were harvested and centrifuged
1518 10 minutes at 4 °C and 1000*g*. The pellets were freeze-dried, homogenized and acidified with 1%
1519 HCl and TOC and TN content were measured using a Flash EA 1112+ MAs 200 elemental analyser
1520 (Thermo Interscience).

1521 Mixotrophy was measured in a separate experiment as the capacity to compensate growth
1522 inhibition by atrazine in the presence of organic carbon sources. Each diatom strain was grown in
1523 4 treatments: (a) f/2 culture medium, (b) f/2 medium + atrazine (at EC50 concentration), (c) f/2
1524 medium + atrazine + 0.1 mM Glucose (d) f/2 medium + atrazine + 0.6 mM Glucose. Mixotrophy
1525 was assessed as the ratio of the maximum cell density (carrying capacity *K*) that each strain
1526 reached under atrazine (treatment b) and atrazine + glucose (treatments c and d): $K(c)/K(b)$ and
1527 $K(d)/K(b)$. The methodology and glucose concentrations of this experiment were set as described
1528 in Lewin and Hellebust (1975). Each microcosm was inoculated with a total cell density of 5000
1529 diatom cells/ml, cell densities were measured on days 0, 5, 10, 15, 20, and 25, and the experiment
1530 was performed under the same conditions as the atrazine and copper experiments described
1531 above.

1532 Photosynthetic efficiency was measured as maximum quantum yield of photosynthetic activity on
1533 days 0, 2, 5, 10, 15, 20 and 25 by pulse-amplitude modulated (PAM) fluorometry (MAXI Imaging
1534 PAM fluorometer, Walz, Germany). Maximum quantum yield is determined as the ratio of variable
1535 and maximum fluorescence (F_v/F_m). Maximum fluorescence F_m is the maximum fluorescence
1536 emission level after a dark adaptation of 20 minutes, measured with a saturating pulse of light
1537 (emission peak at 450 nm, 2700 photons $m^{-2}s^{-1}$, 800 ms). Variable fluorescence F_v is calculated
1538 from the difference between initial fluorescence (F_0) and maximum fluorescence ($F_v = F_m - F_0$).

1539 EPS were measured by spectrophotometry at the end of the experiments after 25 days. Three
1540 replicates of 10 ml suspended diatom culture per treatment were centrifuged for 15 min at 15 °C
1541 and 3500*xg*. The supernatant yielded the soluble EPS fraction, which was left to precipitate
1542 overnight in 30 ml cold ethanol (98%), and subsequently centrifuged for 15 min at 15 °C and
1543 3500*g*. The pellet was dried under a flow of nitrogen and resuspended in 2 ml of 1.5% NaCl.
1544 Samples of 200 μ l of this suspension per replicate were used for the EPS analysis. EPS was
1545 measured according to a modified version of the phenol/ H_2SO_4 assay (Dubois et al. 1956). In 24-
1546 well-plates, 1 ml concentrated H_2SO_4 and 200 μ l phenol (5%, w / v in distilled water) were added
1547 to 200 μ l sample. The mixture was shaken, incubated for 20 min and the absorbance measured at
1548 488 nm using a Victor³ multilabel reader (Perkin-Elmer). EPS concentrations were quantified with

1549 a function obtained from a linear regression of known concentrations of glucose standards
1550 (ranging from 5 to 400 µg/ml).

1551 Fatty acid profiles in 2 replicates per treatment were analyzed after 25 days. A suspension of 10
1552 ml suspended diatom culture was centrifuged for 20 minutes at 4 °C and 2700*g*. The supernatant
1553 was removed and the pellet was stored at -80 °C until fatty acid extraction. Hydrolysis of total lipid
1554 extracts of duplicate diatom cell suspensions per treatment and methylation to fatty acid methyl
1555 esters (FAME) was achieved by a modified one-step derivatisation method (Abdulkadir and
1556 Tsuchiya 2008; De Troch et al. 2012a). The boron trifluoride-methanol reagent was replaced by
1557 3.5 ml of a 2.5% H₂SO₄-methanol solution per sample since BF₃-methanol can cause artefacts or
1558 loss of PUFA (Eder 1995). The fatty acid methylnonadecanoate C19:0 (Fluka 74208) was added
1559 as an internal standard for the quantification (see further). Samples were centrifuged and vacuum
1560 dried. The FAME thus obtained were analysed using a gas chromatograph (HP 6890N) coupled to
1561 a mass spectrometer (HP 5973). The samples were run in splitless mode, with a 1 µL injection per
1562 run, at an injector temperature of 250°C using a HP88 column (Agilent J&W; Agilent Co., USA). The
1563 oven temperature was programmed at 50°C for 2 min, followed by a ramp at 25°C min⁻¹ to 175°C
1564 and then a final ramp at 2°C min⁻¹ to 230°C with a 4 min hold. The FAME were identified by
1565 comparison with the retention times and mass spectra of authentic standards and mass spectral
1566 libraries, and analysed with the software MSD ChemStation (Agilent Technologies). The
1567 quantification function of each individual FAME was obtained by linear regression of the
1568 chromatographic peak areas and corresponding known concentrations of the external standards
1569 (Supelco # 47885, Sigma-Aldrich Inc., USA) ranging from 5 to 250 ng/ml).

1570 All traits and functional contribution measurements were normalized to cell density and are
1571 indicated in Addendum II Table S1.

1572 **Data analysis.** The numerical stress response was quantified as EC₅₀, i.e. the effective copper and
1573 atrazine concentration inducing a 50% loss of each species' maximum cell density (carrying
1574 capacity). The functional stress response was quantified as the EC₅₀ of photosynthetic efficiency,
1575 EPS and fatty acids production, representing the effective concentration of copper and atrazine
1576 inducing a 50% reduction in these processes. EC₅₀ values as a sensitivity endpoint represent the
1577 baseline for pesticide risk assessment in Europe (Van den Brink et al. 2011) and were calculated
1578 using a three-parameter log-logistic model (Equation 1) and a three-parameter Weibull model in
1579 two different parameterisations (Equations 2 and 3) as described in Ritz and Streibig (2005).

$$1580 \quad Y = d / (1 + \exp\{b[\log x - \log e]\}) \quad [1]$$

$$1581 \quad Y = d(1 - \exp\{-\exp[b(\log x - \log e)]\}) \quad [2]$$

1582 $Y = d(\exp\{-\exp[b(\log x - e)]\})$ [3]

1583 Y represents the response variable (cell density, photosynthetic efficiency, EPS or fatty acids per
 1584 cell), b the relative slope of the curve, d the upper limit, e the inflection point, and x the
 1585 concentration of atrazine or copper.

1586 To account for the hormetic growth and EPS production of several species under stress, dose-
 1587 response models that can describe hormesis were fitted as described by Brain and Cousens (1989,
 1588 Equation 4) and Cedergreen et al. (2005, Equation 5).

1589 $Y = c + (d - c + fx)/(1 + \exp\{b[\log x - \log e]\})$ [4]

1590 $Y = (d + f \exp(-1 / x^\alpha)) / (1 + \exp(b(\log x - \log e)))$ [5]

1591 c is the lower and b the upper horizontal asymptote and f is the hormesis effect. Model 5 was fitted
 1592 with $\alpha = 1, 0.5$ and 0.25 , respectively. Model fit for every dose-response model was evaluated
 1593 visually as well as by likelihood ratio testing, lack-of-fit test and Akaike information criterion
 1594 (AIC), and the best-fitting model was used to calculate EC50 values.

1595 Trait-sensitivity relations were analyzed with generalized least squares models (Equation 6), with
 1596 numerical and functional response to copper and atrazine as response variable and traits as
 1597 predictors (biovolume, surface to volume ratio, length, chlorophyll a content, mixotrophy at 0.1
 1598 and 0.6 mM Glucose, TN and TOC).

1599 $S = a + \sum(b_i \cdot T_i)$ [6]

1600 T_i represents the traits, S the numerical and functional response (EC50) in terms of respectively
 1601 cell density, and photosynthetic efficiency, EPS and fatty acid production, a the intercept and b_i
 1602 the slope (i.e. the effect of trait i on stress response). Generalized least squares models were fitted
 1603 to all uncorrelated traits (correlation factor < 0.5) as predictors, correlated traits were separately
 1604 included as predictors in additional model fits. In all models, predictors were removed in a
 1605 stepwise manner, model fit was assessed at every step (AIC and likelihood ratio testing), and the
 1606 best-fitting model was selected for the trait-sensitivity analyses. When a trait significantly
 1607 predicted numerical or functional stress response, we tested in additional analyses if the
 1608 respective trait also predicted stress response when species with outlier trait values were
 1609 omitted.

1610 The relation between the functional contribution in unstressed conditions and the numerical as
 1611 well as functional stress response was analyzed by fitting generalized least squares models to

1612 functional contribution per cell as response variable and numerical as well as functional stress
1613 response as predictors (Equation 7).

$$1614 \quad F = a + b \cdot S_D + c \cdot S_F \quad [7]$$

1615 F is the functional contribution (photosynthetic efficiency, fatty acid and EPS production per cell
1616 under control conditions), S_D is the numerical and S_F the functional stress response, a is the
1617 intercept, b and c represent the slopes, i.e. the relation of functional contribution to numerical and
1618 functional stress response. If S_D and S_F were correlated (correlation factor > 0.5), models were
1619 fitted separately for both predictors.

1620 The relation of numerical and functional stress response was assessed by fitting generalized least
1621 squares models to numerical response as predictor and the response of the respective function as
1622 response variable (Equation 8).

$$1623 \quad S_F = a + b \cdot S_D \quad [8]$$

1624 S_F is the functional stress response in terms of photosynthetic efficiency, EPS and fatty acid
1625 production respectively, a is the intercept, S_D the numerical stress response in terms of cell
1626 density, b represents the slope, i.e. the relation of numerical and functional stress response.

1627 For all generalized least squares models, normality and homogeneity of model residuals were
1628 evaluated using quantile-quantile plots and by plotting residuals versus explanatory variables,
1629 respectively. If homogeneity was violated, the model was refitted using an exponential variance
1630 structure allowing residuals to change separately with each predictor X ($\text{var}(\varepsilon) = \sigma^2 \cdot e^{2 \cdot \delta \cdot X}$), the
1631 best-fitting model (lowest AIC) was selected, and homogeneity was tested again. By means of
1632 likelihood ratio testing, the significance of this structure was tested ($\alpha = 0.05$). All calculations
1633 were performed in R 2.10.1, using RStudio and the packages drc and nlme (Ritz and Streibig 2005,
1634 Pinheiro et al. 2015, R Development Core Team 2016).

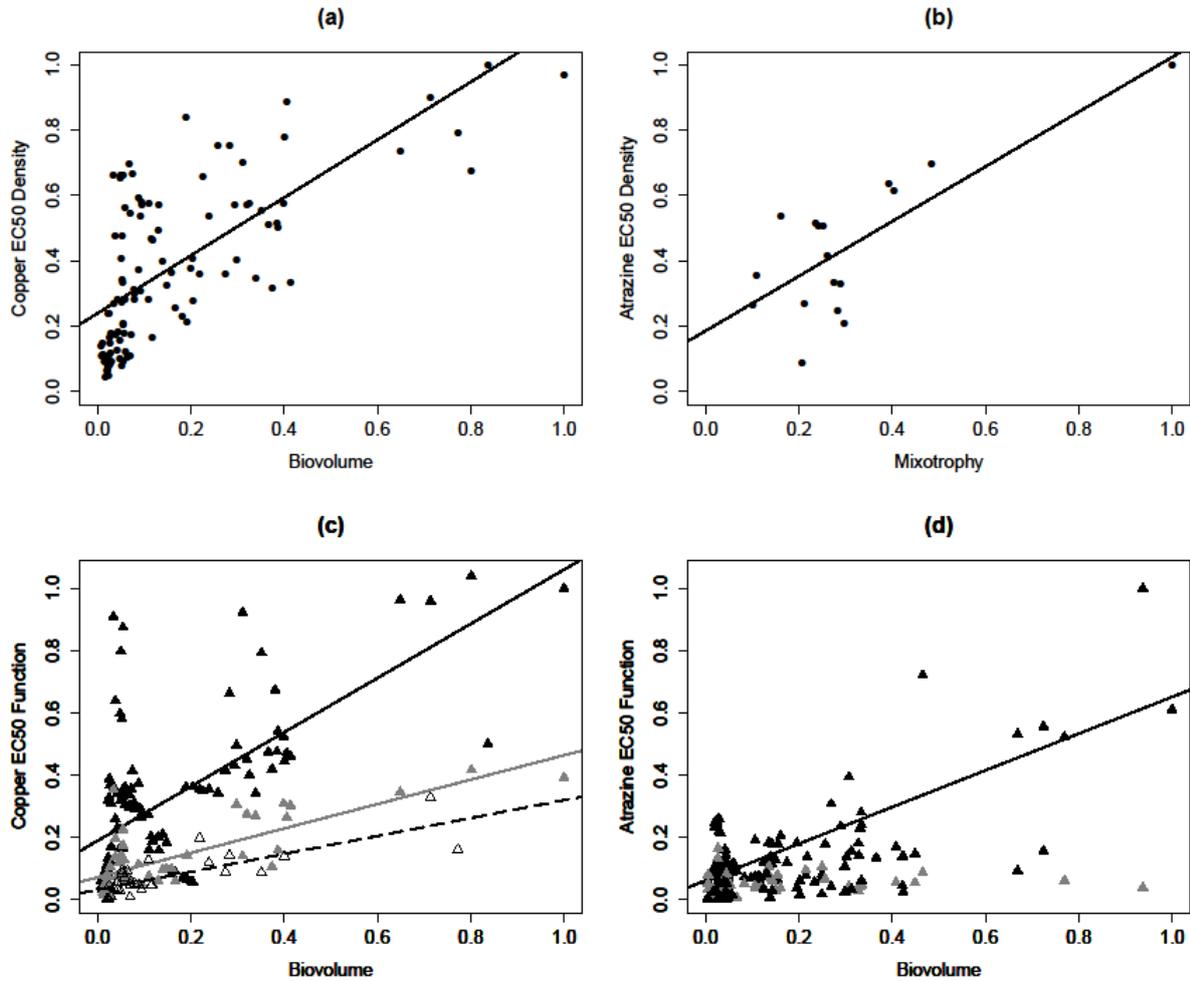
1635

1636 **3.3. Results**

1637 The numerical response of diatoms to copper was strongly related to biovolume, with larger
1638 species being more tolerant to copper ($p < 0.0001$, Fig. 3.1a, Addendum II Table S3). Other traits
1639 that were correlated with biovolume (surface to volume ratio, length, chlorophyll a content) also
1640 predicted the numerical response to copper in separate model fits (Addendum II Table S4). In
1641 contrast, the numerical response to atrazine was not related to any morphological trait, but to the
1642 capacity for mixotrophic growth ($p = 0.0003$, Fig. 3.1b, Addendum II Table S3). Diatom species
1643 which could increase their density in response to glucose turned out to be least affected by

1644 atrazine (see mixotrophic growth response and atrazine sensitivity per species in Addendum II
1645 Tables S1 and S5). Biovolume and mixotrophy still predicted the numerical response to copper
1646 and atrazine in additional analyses where the largest respectively most mixotrophic species
1647 (*Entomoneis* sp. and *Cylindrotheca closterium*, data points in upper right quadrant of Fig. 3.1a and
1648 3.1b) were omitted (Addendum II Table S4).

1649 The functional response to copper was predicted by biovolume for all three ecosystem processes
1650 measured, with larger species being least affected in their photosynthetic efficiency, fatty acid
1651 content and EPS production (all $p < 0.0001$, Fig. 3.1c, Addendum II Table S3). The functional
1652 response to atrazine in terms of photosynthetic efficiency was also positively related to biovolume
1653 ($p < 0.0001$, Fig. 3.1d, Addendum II Table S3). The same outcome was obtained when the largest
1654 species (*Entomoneis* sp.) was omitted from the analysis (Addendum II Table S4). In separate
1655 model fits, other morphological traits that were correlated with biovolume (surface to volume
1656 ratio, length) also predicted the response of photosynthetic efficiency, EPS and fatty production
1657 to copper as well as the response of photosynthetic efficiency to atrazine (Addendum II Table S4).
1658 None of the measured traits predicted the functional response to atrazine in terms fatty acid and
1659 EPS production (Fig. 3.1d, Addendum II Table S3), if outlier data were omitted from the analysis
1660 (Addendum II Table S4). EC50s for all diatom strains are indicated in Addendum II Table S5, and
1661 the growth curves for every strain and stressor are shown in Addendum II Fig. S2. The diatoms'
1662 morphological traits were not altered during exposure to atrazine and copper, with the exception
1663 of *Astartiella bahusiensis*, which increased its cell size when exposed to copper (Addendum II
1664 Table S8 and Fig. S1).



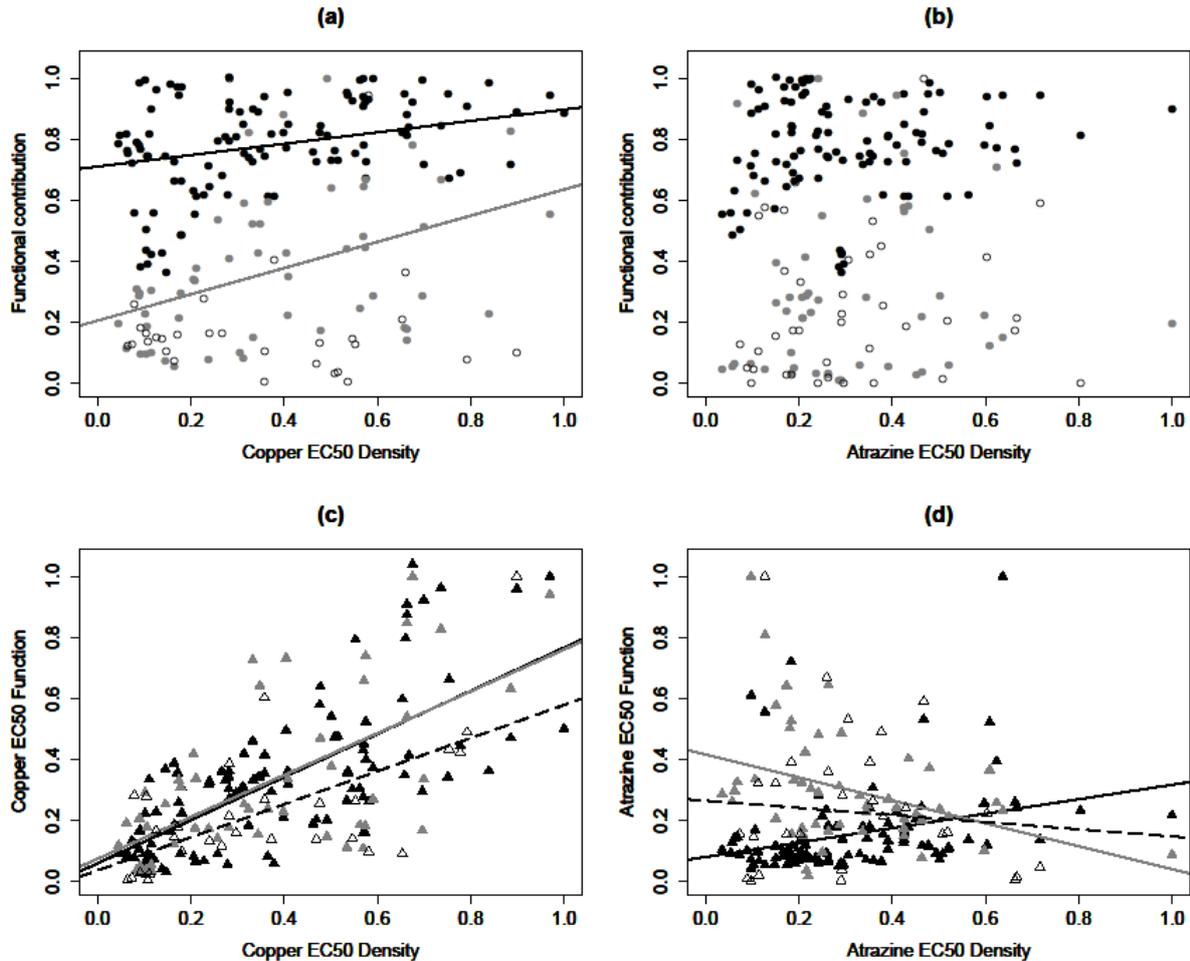
1665

1666 **Fig. 3.1:** Diatom numerical and functional response to copper and atrazine as a function of biovolume and
 1667 mixotrophy. Panel a) shows numerical response to copper (EC50 cell density) as a function of biovolume.
 1668 Panel b) shows numerical response to atrazine as a function of mixotrophy. Regression lines show
 1669 significant relations between biovolume or mixotrophy and numerical response to copper and atrazine.
 1670 Panels c) and d) show functional response (EC50 of photosynthetic efficiency, fatty acids and EPS
 1671 production) to copper and atrazine as a function of biovolume. Regression lines show significant relations
 1672 between biovolume and functional response: photosynthetic efficiency (black triangles, black lines), fatty
 1673 acids (white triangles, dashed line), and EPS production (grey triangles, grey line). Biovolume, mixotrophy
 1674 and EC50 values are indicated normalized to the highest respective value recorded.

1675

1676 The functional contributions of diatoms in unstressed conditions were not correlated to their
 1677 numerical or functional response to atrazine for any of the three processes (Fig. 3.2b, Addendum
 1678 II Table S6). Diatom numerical and functional response to copper was positively related to
 1679 photosynthetic efficiency ($p=0.0008$ for numerical and $p=0.0006$ for functional response) as well
 1680 as EPS production ($p=0.001$ for numerical and $p=0.009$ for functional response), with productive
 1681 species being more copper-resistant, but not fatty acid content (Fig. 3.2a, Addendum II Table S6).

1682



1683

1684 **Fig. 3.2:** Diatom functional contribution and functional stress response as a function of numerical response
 1685 to copper and atrazine. Panels a) and b) show functional contribution in unstressed conditions as a function
 1686 of numerical response (EC50 cell density) to copper and atrazine. Significant relations are indicated by
 1687 regression lines. Functional contributions are photosynthetic efficiency (black dots, black line), fatty acids
 1688 (white dots) and EPS production (grey dots, grey line). Panels c) and d) show the relation between
 1689 numerical response and functional response (EC50 of photosynthetic efficiency, fatty acids and EPS
 1690 production) to copper and atrazine. Significant relations are indicated by regression lines. Functional
 1691 response is indicated in terms of photosynthetic efficiency (black triangles, black line), fatty acids (white
 1692 triangles, dashed line) and EPS production (grey triangles, grey line). All functional contribution and EC50
 1693 values are indicated normalized to the highest respective value recorded.

1694

1695 The numerical and functional stress response were related, this relationship however differed
 1696 between copper and atrazine. For copper, species which were least affected in their density were
 1697 also the most tolerant in their functional contribution. Numerical and functional response to
 1698 copper were positively related for all three processes ($p < 0.0001$ for photosynthetic efficiency and
 1699 EPS production, $p = 0.0015$ for fatty acid content, Fig. 3.2c, Addendum II Table S7). A similar
 1700 positive relation was observed between the numerical response and the response of
 1701 photosynthetic efficiency to atrazine ($p = 0.003$, Fig. 3.2d, Addendum II Table S7). Conversely, the
 1702 numerical and functional response to atrazine in terms of EPS and fatty acid production were

1703 negatively related ($p=0.0015$ and $p=0.03$, Fig. 3.2d, Addendum II Table S7). These negative
1704 relations between the numerical and functional response to atrazine were largely driven by
1705 species whose density was least affected by atrazine (e.g. *Cylindrotheca closterium*, *Gyrosigma* sp.
1706 2, *Cylindrotheca fusiformis*, *Biremis ambigua*), but which were amongst the most atrazine-sensitive
1707 ones concerning their functional contribution (Addendum II Table S5). The functional response
1708 to copper and atrazine stress was thus not only predicted by traits, but was also related to the
1709 species' capacity to maintain their density under both types of stress: the numerical and functional
1710 response to atrazine were not driven by the same set of traits, and atrazine-resistant species in
1711 terms of density were amongst the most sensitive in their EPS and fatty acid output. The numerical
1712 and functional response to copper were driven by the same morphological traits, and numerically
1713 resistant species were least affected in their functional contribution.

1714

1715 **3.4. Discussion**

1716 The outcome of our experiments demonstrates that the numerical and functional response to two
1717 common chemical stressors can be predicted from traits, but also that these stressors are likely to
1718 have a differential impact on ecosystem functioning as the relationship between response and
1719 effect traits is different depending on stressor and functional process. When exposed to copper,
1720 response and effect traits were correlated and the numerically tolerant species were least affected
1721 in their functioning, suggesting that under metal stress the first species to be numerically
1722 decimated will probably have little effect on overall ecosystem functioning. In contrast,
1723 mixotrophic growth can help to maintain the density of certain species in response to
1724 photoinhibiting stressors such as atrazine, but not their contribution to particular functional
1725 processes, which would cause the functional consequences of stress exposure to be more
1726 important than estimated from mere species densities.

1727 Trait-based approaches have made a considerable breakthrough in explaining community
1728 structure along gradients of environmental stress (Baird and Van den Brink 2007, Litchman et al.
1729 2007, Fontana et al. 2014). Yet, effect traits leading to increased or decreased ecosystem
1730 functioning are rarely linked the response traits needed to maintain density under stress
1731 (Hillebrand and Matthiessen 2009, but see e.g. Pomati and Nizzetto 2013, Heuner et al. 2015). This
1732 represents a major shortcoming for predicting ecosystem functioning in stressed environments,
1733 given that stress can affect ecosystem functioning by altering both the density and the functional
1734 output of the concerned species (Fox 2006). For this challenge, morphological traits are expected
1735 to be suitable tools, since they are relatively simple to measure, capture most of the functional
1736 properties of microalgae, and can play an important role in algae-pollutant interactions (Kruk et
1737 al. 2010).

1738 In the case of copper, the expectations of morphological traits as predictors of any type of stress
1739 response were fulfilled as biovolume, surface to volume ratio and cell length all predicted the
1740 response of diatom density, photosynthetic efficiency, fatty acid and EPS production to copper.
1741 The case of atrazine however demonstrated that morphological traits alone can not generally
1742 predict numerical and functional response. As common chemical stressors in coastal and
1743 estuarine environments, the effects of atrazine and other photoinhibiting herbicides on primary
1744 producers in these ecosystems have been extensively analysed, but mainly on the density and not
1745 on the functions sustained by the species concerned, and in relation to taxonomy rather than
1746 species traits. Mixotrophy is common in benthic diatoms (Tuchman et al. 2006), and the few
1747 approaches which related the effects of photoinhibiting herbicides to mixotrophy suggest the
1748 numerical response of diatoms to these stressors can be modulated by their trophic mode
1749 (Debenest et al. 2009; Larras et al. 2014). Here, mixotrophy predicted the numerical response of
1750 diatoms, but not their contribution to any of the three functional processes under atrazine.
1751 Estimating the effects of photoinhibiting stress on ecosystem functions sustained by primary
1752 producers is therefore less straightforward than under metal stress, since the numerical and
1753 functional response to photoinhibiting stress are not driven by the same traits and mixotrophy
1754 cannot be quantified as easily as morphological traits. Nevertheless, the capacity for mixotrophic
1755 growth can be obtained from the literature for many taxa (Hellebust and Lewin 1977, Tuchman
1756 et al. 2006). Our study indeed corroborates literature reports for *Cylindrotheca closterium*
1757 (Vanellander et al. 2009), which was also the most mixotrophic species in our experiments.

1758 Relating traits to stress response should be done by linking traits to groups of stressors with
1759 similar modes of action, to generalize the trait-sensitivity relation for distinct types of stressors
1760 (Baird and Van den Brink 2007). Like copper, the toxicity of other heavy metals to primary
1761 producers lies in the formation of reactive oxygen species (ROS), which can damage cell
1762 membranes, plastids and other intracellular structures (Masmoudi et al. 2013). Metal uptake is
1763 commonly related to cell size, with smaller species having a larger surface to volume ratio and
1764 thus being more prone to be adversely affected by metal pollution (Miao et al. 2005, Quigg et al.
1765 2006). Not only morphological traits, but also protective mechanisms such as the production of
1766 metallothioneins equally play an important role in mediating metal toxicity in various organisms
1767 (Cobbett and Goldsbrough 2002). Nevertheless, morphological traits appear as a promising,
1768 general tool to estimate both the numerical and functional response of microalgae to metal
1769 pollution.

1770 In contrast to metals, triazine herbicides are photoinhibiting stressors, which disrupt
1771 photosynthesis rather than causing physical damage to cell structures. In this respect, the effects
1772 of triazine herbicides resemble those of other types of photoinhibiting stress in benthic intertidal

1773 habitats, caused by extreme light conditions due to tidal emersion in combination with
1774 unpredictably changing weather conditions (Barnett et al. 2015). Within growth forms
1775 constraints (motile vs non-motile), diatoms can adapt to the excess light stress by behavioural
1776 (e.g. vertical migration in motile species) and/or physiological photoprotective mechanisms such
1777 as non-photochemical quenching (McQuoid and Hobson 1996, McLachlan et al. 2009, Barnett et
1778 al. 2015). However, in contrast with mixotrophic capacity, none of these adaptations would
1779 probably be efficient against herbicide-induced blocking of PS II. Phylogenetic indices integrate
1780 over other traits that can potentially mediate algal resistance to PS II inhibitors (e.g. absence or
1781 presence of a raphe structure, Larras et al. 2014) and are therefore, alongside the capacity for
1782 mixotrophic growth, suggested as promising predictors of microalgae response to herbicide
1783 stress (Larras et al. 2014).

1784 The variability of traits and the multitude of potential sensitivity endpoints should be considered
1785 when using trait-based approaches to predict stress response (Albert et al. 2010, Rubach et al.
1786 2012). We observed little changes in morphological traits under stress exposure, but our
1787 experiments lasted only a limited number of vegetative growth cycles and hence negligible size
1788 reduction (inherent to the diatom life cycle, cf. Chepurnov et al. 2008). Diatoms can shift their
1789 trophic mode with almost immediate effect (Lewin and Hellebust 1975), whereas significant
1790 changes in morphology occur over longer time spans which makes trait variability more likely in
1791 long-term experiments and in scenarios of constant stress exposure. Also, in toxicology, sensitivity
1792 is defined primarily as the median of a tolerance distribution (the 'EC50') along an environmental
1793 gradient (Baird et al. 1996). Conversely, in ecology, sensitivity is often defined as the variance of
1794 the tolerance distribution, irrespective of its median, with respect to an environmental gradient
1795 (Baird et al. 1996). While less straightforwardly linked to non-morphological traits than, for
1796 example, toxicokinetic measurements (e.g. uptake or elimination rate constants, Rubach et al.
1797 2012), EC50 values were selected as sensitivity endpoints because of their widespread use in
1798 trait-sensitivity research (Baird and Van den Brink 2007; Van den Brink et al. 2011; Larras et al.
1799 2014). However, our analysis could also be performed based on other sensitivity endpoints that
1800 better capture the variance of the tolerance distribution and relate to a wider variety of traits
1801 (Rubach et al. 2012).

1802 To evaluate the effects of different stressor types on ecosystem functioning, we need to contrast
1803 and disentangle the response traits driving the species densities and the effect traits driving the
1804 contribution to ecosystem functioning under stress. Mixotrophy as a response trait predicted
1805 diatom densities, but not functioning under atrazine exposure, and those species which could best
1806 maintain their cell densities and photosynthetic efficiency under atrazine were most sensitive in
1807 their fatty acid content and EPS production. These contrasting effects of atrazine on density and

1808 functioning imply that at herbicide stress levels where species densities are not yet impacted,
1809 sediment stabilization and energy flux to higher trophic levels would already be affected, with
1810 concomitant knock-on effects on other sediment-associated organisms and the food web in
1811 intertidal systems (Müller-Navarra et al. 2000, Tolhurst et al. 2003, De Troch et al. 2012a).

1812 For metal pollution, the functional consequences seem at a first sight smaller: response and effect
1813 traits were positively correlated, meaning that species that were little affected in their density also
1814 performed best at maintaining their contribution to the functional processes. Furthermore,
1815 copper mostly reduced the densities of those species that contributed least to EPS and fatty acids
1816 production. Since the same traits that predicted the numerical and functional response to copper
1817 in this study also explain tolerance to pollution by other metals (Miao et al. 2005, Lahive et al.
1818 2011), large, metal-resistant species could potentially sustain ecosystem functioning under metal
1819 pollution. However, the real threat for species loss and ecosystem functioning is from the presence
1820 of multiple stressors that primary producers are subjected to in intertidal environments
1821 (Underwood et al. 1998, Hillebrand et al. 2000, McLachlan et al. 2009, Souffreau et al. 2010). Cell
1822 size is a master trait that shapes the performance of microalgae along environmental gradients
1823 (Litchman and Klausmeier 2008, Pomati and Nizzetto 2013, Pomati et al. 2013), but large size is
1824 correlated with other extinction-prone traits such as lower nutrient acquisition, slower growth
1825 and longer generation times (Litchman and Klausmeier 2008, Pomati et al. 2013, Litchman et al.
1826 2015). If simultaneously with metal pollution diatoms are subjected to other types of stress that
1827 favour traits such as small cell size or short growth cycles, the competitive advantage and the
1828 capacity of large-bodied species to sustain ecosystem functioning will be limited.

1829 Lastly, we should consider the relation between numerical and functional stress response not only
1830 from an ecosystem, but also from a diatom perspective. The processes performed by diatom
1831 biofilms are essential contributions to ecosystem functioning (sediment stabilization, primary
1832 production, energy transfer to higher trophic levels). But for the diatoms themselves, a high
1833 photosynthetic efficiency will be beneficial under most types of growth-limiting stress, whereas
1834 for example producing large amounts of fatty acids will not be advantageous in the case of
1835 herbicide stress. Oxidative stress resulting from metal exposure on the other hand results in the
1836 damage of fatty acid rich structures such as cell membranes due to lipid peroxidation (Li et al.
1837 2006), and a high fatty acid production could help diatoms to better tolerate the damage to cellular
1838 structures caused by heavy metal stress. Similarly, producing EPS can also protect diatoms from
1839 metal stress, through the establishment of an organic carbon matrix that protects biofilms by
1840 chelating metal ions (Pistocchi et al. 1997). Generally, stressed organisms will allocate most
1841 resources to those processes that favour survival and growth under stress and we should consider

1842 the interconnection of numerical and functional stress response, when interpreting their
1843 divergent relation under different types of stress.

1844 In conclusion we infer two general statements from our results. First, the numerical and functional
1845 response of primary producers to two of the most common chemical stressors in coastal
1846 environments, herbicide and metal pollution, is predictable based on the species' morphological
1847 traits and trophic mode. Second, the impacts of stress exposure on ecosystem functioning are
1848 underpinned by the relation of the numerical and functional stress response and the functional
1849 importance of those species whose density is reduced first. Linking response and effect traits
1850 driving the numerical and functional stress response will reveal the capacity of tolerant species to
1851 maintain their contribution to ecosystem functioning, and should be considered when estimating
1852 ecosystem functioning under global change.

1853

1854 **Chapter 4: Facilitation and tolerance explain the diversity effect on**
1855 **functioning in stressed diatom communities**

1856

1857 **Abstract**

1858 The relation between biodiversity and ecosystem functioning depends on complementarity and
1859 dominance effects, but the ecological processes driving complementarity and dominance under
1860 anthropogenic stress remain largely unknown.

1861 We exposed two benthic diatom communities to metal and pesticide stress (copper and atrazine),
1862 and measured complementarity and dominance effects on biomass yield under stressed and
1863 unstressed conditions. Next, we analysed if complementarity under stress was ‘two-way’
1864 (affecting species independent of their biomass yield) or ‘one-way’ (differentially affecting high-
1865 and low-yield species). Last, we tested if the production of extracellular polymers as a facilitative
1866 mechanism as well as the species’ stress tolerance and capacity for mixotrophic growth were
1867 ecological processes that explained complementarity and dominance under stress.

1868 The net biodiversity effect on diatom biomass yield was consistently positive, and was mainly
1869 caused by two-way complementarity, which was higher in diatom communities stressed by
1870 copper (both communities) and atrazine (one community) than in unstressed communities.
1871 Stimulation by stress of the diatom’s extracellular polymer production predicted, in part, these
1872 increases of complementarity under stress.

1873 Complementarity in communities stressed by the atrazine, a photosynthesis inhibitor, was driven
1874 by the presence of mixotrophic species, which can grow on organic substances. Under copper
1875 stress, complementarity in one community was driven by the species which were least tolerant to
1876 the metal in monoculture. These species were characterised by low biomass yields, resulting in a
1877 negative one-way complementarity, reducing the net biodiversity effect under metal stress.

1878 *Synthesis.* Biodiversity effects on diatom biomass were caused by two-way complementarity
1879 effects, which increased under stress and were driven by extracellular polymer production, a
1880 ‘sleeping’ facilitative mechanism stimulated by stress. The degree to which diatom species
1881 benefited from complementarity depended on their trophic mode and stress tolerance. When
1882 complementarity favoured the growth of sensitive but low-yield species, the net biodiversity
1883 effect on diatom biomass yield decreased under stress.

1884

1885

1886 **4.1. Introduction**

1887 Understanding the influence of biodiversity on ecosystem functioning is a key challenge, as global
1888 change is causing unprecedented rates of biodiversity change (Pereira et al. 2010, Dornelas et al.
1889 2014, Gonzalez et al. 2016). A large amount of empirical research has documented in most cases
1890 positive effects of biodiversity on ecosystem processes (most commonly biomass yield, Balvanera
1891 et al. 2006, Cardinale et al. 2011, Naeem et al. 2012), with results being largely consistent across
1892 trophic levels and ecosystem types (Cardinale et al. 2006, Griffin et al. 2013).

1893 Anthropogenic stressors are a main driver of biodiversity change (Wilcove and Master 2005, Malaj
1894 et al. 2014), and can substantially affect biodiversity-ecosystem functioning relationships (Steudel
1895 et al. 2012, Baert et al. 2016). An increasing number of studies have tested the biodiversity effect
1896 on ecosystem functioning under stressful conditions, but these have yielded contrasting results
1897 (clearly positive effects in Mulder et al. 2001, Ives and Cardinale 2004, Solan et al. 2004, Zavaleta
1898 and Hulvey 2004, Larsen et al. 2005, McMahon et al. 2012, Steudel et al. 2012, Mensens et al. 2015,
1899 Baert et al. 2016 and neutral or even negative diversity effects in Jonsson et al. 2002, Petchey et
1900 al. 2002, Smith and Knapp 2003, Jiang 2007, Jiang et al. 2008, Roger et al. 2012). This variability
1901 of results calls for more mechanistic studies on how stress affects diversity effects on functioning,
1902 relating ecological processes to stress-induced alterations of biodiversity effects on function.
1903 (Naeem 2008, Solan et al. 2009, O'Connor et al. 2015, Baert et al. 2016).

1904 Dominance (dominance of communities by high- or low-yield species) and complementarity
1905 (better performances of species in community due to mechanisms such as resource partitioning
1906 or facilitation) are the main diversity effects which cause communities to perform differently than
1907 expected from monocultures of the constituent species (Loreau and Hector 2001, Fox 2005).
1908 Dominance and complementarity mostly operate simultaneously (Loreau et al. 2001).
1909 Consequently, which effect drives the diversity-functioning relation and how both effects are
1910 affected by anthropogenic stress is actively debated (Cardinale et al. 2007, Fargione et al. 2007,
1911 Jiang et al. 2008, Loreau 2010, Wang et al. 2013, Baert et al. 2016, Hodapp et al. 2016).
1912 Complementarity and dominance effects can drive ecosystem functioning under stressful
1913 conditions through stress-induced increases of facilitation (Wang et al. 2013) and when
1914 communities are dominated by stress-tolerant species with above-average contribution to
1915 functioning (Baert et al. 2016), respectively. Even though still scarce at the moment, stressor-
1916 induced changes in dominance and complementarity are increasingly tested for (Wang et al. 2013,
1917 Baert et al. 2016), using analytical methods based on the covariance between species performance
1918 in a community and in monoculture (Loreau and Hector 2001, Fox 2005).

1919 On itself, quantifying complementarity and dominance does not offer direct mechanistic insight
1920 into the ecological processes driving stress effects on biodiversity-ecosystem functioning
1921 relationships. That is because complementarity and dominance (*sensu* Fox 2005 or Loreau and
1922 Hector 2001, who refer to complementarity and selection) are statistical effects that can result
1923 from multiple ecological processes (Cardinale et al. 2011, Tilman et al. 2014). One such process
1924 is facilitation, which can drive complementarity in legumes, insects and microalgae (Cardinale et
1925 al. 2002, Temperton et al. 2007, Vanellander et al. 2009). Further tests of facilitative mechanisms
1926 as drivers of complementarity are however lacking and there is no direct evidence attributing
1927 changes of complementarity effects under stress to changing facilitative mechanisms.

1928 Here, we quantify complementarity and dominance effects on the biomass yield of two benthic
1929 diatom communities under chemical stress, and compare these with effects determined in
1930 unstressed controls. We use benthic diatom communities as an experimental model because they
1931 are the main primary producers in many intertidal, soft-sediment habitats, and diatom biomass
1932 production is crucial as it represents the basis of the food web in these systems (Montagna et al.
1933 1995, Underwood and Kromkamp 1999, Tang and Kristensen 2007). We use copper and the
1934 herbicide atrazine as model stressors, the former being a common pollutant in coastal ecosystems
1935 due to urban and industrial runoff (Kennish 1996, Murray-Gulde et al. 2002), and the latter
1936 representing one of the most-used agricultural herbicides worldwide (Graymore et al. 2001,
1937 Chirnside et al. 2007).

1938 We then test if complementarity under stress was 'two-way' (affecting diatom species
1939 comparably) or 'one-way' (differentially affecting species with high or low biomass yields).
1940 Benthic diatoms produce large amounts of extracellular polymeric substances (EPS, Decho 1990,
1941 Underwood and Paterson 2003). These organic exudates have been linked to metal tolerance in
1942 diatom monocultures (Pistocchi et al. 1997, Levy et al. 2007) and can cause facilitation in diatom
1943 communities containing mixotrophic species (Vanellander et al. 2009). We therefore analyse if
1944 the release of EPS could predict complementarity in stressed diatom communities, and test if
1945 stressor-induced changes in complementarity were related to the species' stress tolerance in
1946 monoculture and the presence of mixotrophic species.

1947

1948 **4.2. Methods**

1949 **Experimental organisms and culture conditions.** The two diatom communities (hereafter
1950 community A and B) were each composed of six benthic diatom species (Addendum III Table S1)
1951 collected in the Westerschelde estuary (The Netherlands, 51°21'N, 3°43'E) and the Veerse meer
1952 (The Netherlands, 51°32'N, 3°47'E), where they represent the dominant component of the

1953 microphytobenthos (Forster et al. 2006). Monoclonal cultures of each strain were obtained and
1954 preserved in the culture collection of the Protistology & Aquatic Ecology Research Group (UGent,
1955 <http://bccm.belspo.be/>). Diatoms were maintained in a climate room at 15 ± 1 °C, a light/dark
1956 cycle of 12h / 12h and an illumination of $90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, in culture medium consisting of
1957 filtered and autoclaved natural seawater (salinity 32 ± 1) enriched with f/2 nutrients (Guillard
1958 1975).

1959 **Atrazine and Copper experiments.** Diatoms were exposed to 0 (control), 200 and 500 $\mu\text{g/l}$
1960 (hereafter 'low' and 'high') atrazine and copper respectively. In a previous experiment, these
1961 concentrations resulted on average in a 50% and 90% reduction of diatom monoculture yield
1962 (Chapter 3). Atrazine treatments were prepared from a stock solution obtained by dissolving 50
1963 mg technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 99.8% pure, Sigma-
1964 Aldrich Chemie GmbH, Munich, Germany) in 10 ml acetone as a carrier to increase the solubility
1965 of atrazine, with a maximum final volume of 0.01% acetone in the treatments. An acetone control
1966 treatment of 0.01% acetone was included and compared to an acetone-free control to test for
1967 carrier effects. All atrazine treatments were compared to the acetone control. Copper (as a
1968 Cu[II]Cl_2 solution, analytical grade; VWR International) was spiked directly into the culture
1969 medium before exposure of the diatoms. F/2 culture medium for the copper experiments was
1970 prepared without EDTA, to avoid complexation of free copper ions (Pistocchi et al. 1997). The
1971 resulting atrazine and copper concentrations were determined by respectively GC-MS and
1972 graphite furnace AAS analyses (Addendum III Table S2). All experiments were run in tissue
1973 culture flasks (Greiner BioOne, CELLSTAR® TC, 175 cm^2 growth surface), with nine replicates per
1974 treatment (three replicates in the monoculture treatments). Each microcosm was inoculated with
1975 a total cell density of 5000 diatom cells/ml (5000 cells/ml for each species in monoculture,
1976 between 800-850 cells/ml per species in communities). Microcosms were inoculated with cells
1977 from exponentially growing cultures and were incubated in a climate room at 15 ± 1 °C, under a
1978 light / dark cycle of 12h / 12h at $90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. Culture medium of all treatments was
1979 refreshed after eight and 15 days, and experiments were terminated after 25 days.

1980 **Diatom biomass.** Diatom biomass was quantified as biovolume, and was calculated from cell
1981 densities and mean cell biovolume per species, using measured linear dimensions and formulas
1982 representing the closest approximation of geometric shape for each genus Hillebrand *et al.* (1999).
1983 Cell densities were obtained at day 0, 2, 5, 10, 15, 20 and 25 of the experiment by magnifying and
1984 photographing (x100) an area of 0.66 mm^2 per microcosm, using an inverted Axiovert 135 Zeiss
1985 microscope (Carl Zeiss, Jena, Germany) and a connected digital camera (Canon PowerShot G11
1986 digital camera). Cell numbers per species were subsequently determined by identifying and
1987 counting cells on the computer (ImageJ cell counting software, Schneider et al. 2012) and

1988 converted into cell densities in cells/ml. All further analyses use the biomass on day 15 (late
1989 exponential growth phase).

1990 **EPS production.** Diatom biofilms are characterized by a high production of EPS (Underwood and
1991 Paterson 2003), which represent a mix of organic compounds consisting mostly of
1992 polysaccharides, but also proteins and nucleic acids (Baines and Pace 1991). These organic
1993 compounds play an important role for sediment stabilization in muddy intertidal areas (Decho
1994 2000, Underwood and Paterson 2003). The release of organic compounds into the medium has
1995 also been identified as driver of complementarity effects in benthic diatoms (Vanelslander et al.
1996 2009). Furthermore, benthic diatoms can modulate their EPS production in response to abiotic
1997 stress, notably copper (Pistocchi et al. 1997, Levy et al. 2007). We therefore tested whether the
1998 EPS released by diatoms into the medium could predict potential changes of complementarity
1999 under chemical stress. EPS were measured by spectrophotometry after 15 days, in three
2000 replicates of 10 ml suspended diatom culture per treatment. The suspended diatom cultures were
2001 centrifuged for 15 min at 15 °C and 3500xg. The supernatant yielded the soluble EPS fraction,
2002 which was left to precipitate overnight in 30 ml cold ethanol (98%), and subsequently centrifuged
2003 for 15 min at 15 °C and 3500xg. The pellet was dried under a flow of nitrogen and resuspended in
2004 1 ml of 1.5% NaCl. Samples of 200 µl of this suspension per replicate were used for the EPS
2005 analysis. EPS were measured according to a modified version of the phenol/H₂SO₄ assay by Dubois
2006 et al. (1956). In 24-well-plates, 200 µl phenol (5%, w / v in distilled water) and 1 ml concentrated
2007 H₂SO₄ were added to 200 µl sample. The mixture was shaken, incubated for 20 min and the
2008 absorbance measured at 488 nm using a Victor³ multilabel reader (Perkin-Elmer). EPS
2009 concentrations were quantified with a function obtained from a linear regression of known
2010 concentrations of glucose standards (ranging from 5 to 400 µg/ml).

2011 **Data analysis.** For every treatment, we partitioned the net biodiversity effect on diatom biomass
2012 into dominance, two-way complementarity and one-way complementarity, using the method
2013 developed by Loreau and Hector (2001) and later extended by Fox (2005, Equation 1).

$$\begin{aligned} \Delta Y = & N \times \text{cov} \left(M_i, \frac{RY_i}{RYT} - RY_{Ei} \right) \\ & + N \times (\overline{\Delta RY}) \times (\bar{M}) \\ & + N \times \text{cov} \left(M_i, RY_i - \frac{RY_i}{RYT} \right) \end{aligned} \quad [1]$$

2014 ΔY is the net biodiversity effect on diatom biomass yield, i.e. the difference between the observed
2015 community yield and the community yield that is expected from the monoculture yields of the
2016 component species. M_i is the mean monoculture yield of species i . RY_i is the observed relative

2017 yield of species i with $RY_i = \frac{O_i}{M_i}$, where O_i is the observed biomass of species i in the community.
2018 RYT is the relative yield total with $RYT = \sum_{i=1}^N RY_i$ where N is the total number of species in the
2019 community (six). $\frac{RY_i}{RYT}$ is the observed frequency of species i in the community, i.e. its share of the
2020 relative yield total. \bar{M} is the average monoculture yield of all species. $RY_{Ei} = \frac{1}{N}$ is the expected
2021 relative yield of species i in the community, with $\sum_{i=1}^N RY_{Ei} = 1$. The mean deviation in relative
2022 yield $\overline{\Delta RY}$ was calculated as the mean difference in observed relative yield and expected relative
2023 yield $\overline{\Delta RY} = \frac{1}{N} \times \sum_{i=1}^N (RY_i - RY_{Ei})$.

2024 The first term on the right-hand side of equation 1 is the ‘dominance effect’ (Fox 2005), which is
2025 the covariance between the species’ monoculture yields and the difference between their
2026 observed and expected frequencies in community. The dominance effect is positive when species
2027 with high monoculture yields also reach high yields in community at the expense of low-yield
2028 species. It is negative if communities are dominated by low-yield species at the expense of high-
2029 yield species. Mechanisms for this effect include interspecific competition for shared resources.

2030 The second term is the ‘two-way’ or ‘trait-independent’ complementarity (TIC) effect (Loreau and
2031 Hector 2001, Fox 2005), which quantifies the extent to which the species’ observed yields in
2032 community deviate from the expected yield, but in a way that is independent of the individual
2033 species’ monoculture yields. Two-way complementarity is positive when growth in community
2034 rather than monoculture increases the yield of species, independent of their monoculture yield
2035 and not at the expense of other species. Mechanisms underlying this effect can be niche
2036 partitioning or facilitation.

2037 The third term is the ‘one-way’ or ‘trait-dependent’ complementarity (TDC) effect (Fox 2005),
2038 which is the covariance between the species’ monoculture yield and the deviation of their
2039 observed relative yield from their observed frequency in community. One-way complementarity
2040 effects are observed when growth in community rather than monoculture increases the
2041 functioning of species with high or low monoculture yields, but not at the expense of other species.
2042 One-way complementarity can be positive if, for example, species with high monoculture yield are
2043 facilitated by species with low monoculture yield, but not vice-versa. One-way complementarity
2044 effects can become negative if low-yield species but not high-yield species benefit from facilitation
2045 when being grown in community.

2046 To analyse if the net biodiversity effect, DOM, TIC and TDC changed under atrazine and copper
2047 stress, we used generalised least squares models with the biodiversity effects as response
2048 variables and the treatment type as categorical predictor (Equations 2-5).

2049 $\Delta Y = a + b \times T$ [2]

2050 $DOM = a + b \times T$ [3]

2051 $TIC = a + b \times T$ [4]

2052 $TDC = a + b \times T$ [5]

2053 ΔY is the net biodiversity effect on diatom biomass yield, DOM is the dominance effect, TIC and
 2054 TDC are two-way and one-way complementarity, a is the intercept, T is the treatment type
 2055 (control, low and high atrazine and copper) with the control set as reference level, b is the slope,
 2056 i.e. the effect of the treatment type on each biodiversity effect.

2057 Next, we tested the role of EPS as a potential driver of complementarity in diatom communities
 2058 by fitting generalized least squares models to two-way complementarity as response variable and
 2059 diatom EPS production as predictor (Equation 6).

2060 $TIC = a + b \times EPS$ [6]

2061 TIC is two-way complementarity, a is the intercept, EPS the amount of EPS produced per unit
 2062 diatom biomass and b is the slope, i.e. the effect of EPS production on two-way complementarity.
 2063 Models were fitted separately for each diatom community and stressor.

2064 In a last step, we tested if changes in one-way complementarity were driven by species with a low
 2065 or high monoculture yield and/or stress tolerance benefiting most from being grown in
 2066 community. We fitted generalized least squares models to the monoculture yield and stress
 2067 tolerance of the diatom species as predictors, and the deviation of their observed relative yield
 2068 from their observed frequency in community as response variable (Equation 7)

$$RY_{i,j} - \frac{RY_{i,j}}{RYT_j} = a + b \times M_{i,j} + c \times \frac{M_{i,j}}{M_{i,j=0}} \quad [7]$$

2069 The fraction on the left-hand side of Equation 7 is the deviation of the observed relative yield from
 2070 the observed frequency of species i when grown in community at stress level j . This term
 2071 (hereafter relative performance) quantifies the extent to which each species was effected by one-
 2072 way complementarity (see Equation 1). $M_{i,j}$ is the monoculture yield of species i at the stress level
 2073 j , $\frac{M_{i,j}}{M_{i,j=0}}$ is the tolerance of species i , quantified is the ratio of the monoculture yield at stress level
 2074 j and the monoculture yield in the control ($j=0$), a is the intercept, b and c are the slopes, i.e. the

2075 relation between the species' yield and tolerance in monoculture and the deviation of their
2076 relative performance in community. If the monoculture yield and tolerance were correlated
2077 (correlation factor > 0.5), models were fitted separately for both predictors. Models were fitted
2078 separately for each diatom community and stressor.

2079 For all least squares model fits, normality and homogeneity of model residuals were inspected by
2080 evaluation of quantile-quantile plots and Shapiro-Wilk's test, and by Levene's test and plotting
2081 residuals versus explanatory variables respectively. If normality was violated (for model fits in
2082 equation 7 only), data were log-transformed. If homogeneity was violated, the model was refitted
2083 using a variance structure allowing different variances per treatment T ('weights =
2084 varIdent(form ~ 1 | T' in the R package nlme (Pinheiro et al. 2015) and homogeneity was
2085 evaluated again. By means of likelihood ratio testing, the significance of these structures was
2086 tested (alpha = 0.05). All calculations were performed in R 3.0.1. using RStudio (R Development
2087 Core Team 2016).

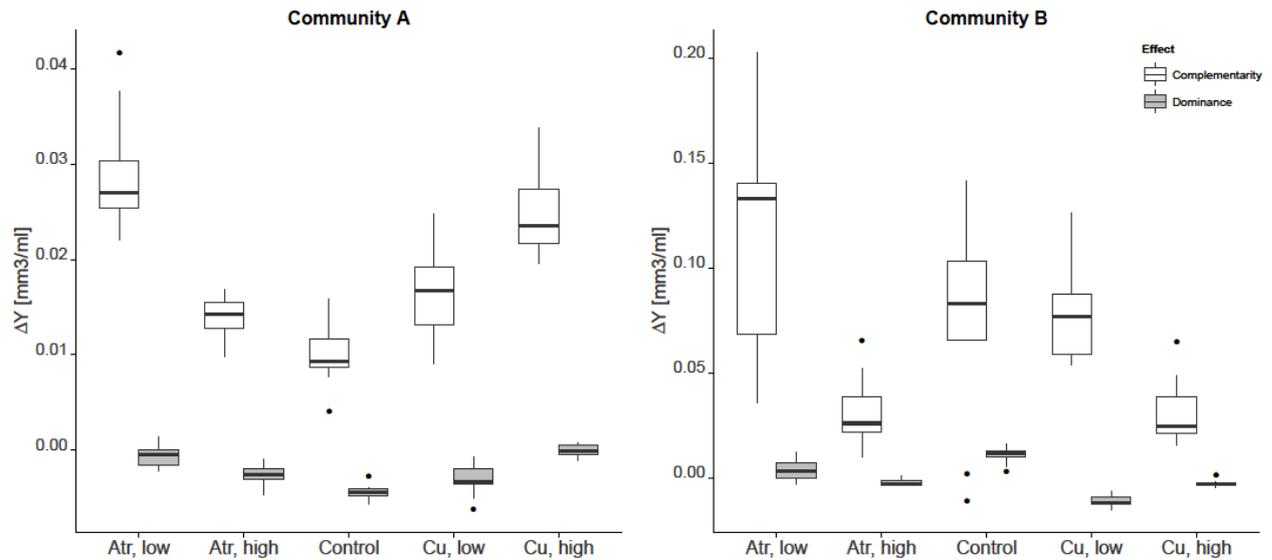
2088

2089 **4.3. Results**

2090 **Effects of stress on dominance and complementarity.** The net biodiversity effect was positive
2091 in all treatments of both diatom communities, i.e. the biomass yield of diatoms in community was
2092 higher than that expected from the component species' monocultures (Addendum III Fig. S1). In
2093 both communities and across all treatments, complementarity was the main effect causing the
2094 yield of diatom communities to deviate from that expected from their monocultures (Fig. 4.1).

2095 In community A, all tested levels of atrazine and copper increased the two biodiversity effects (Fig.
2096 4.1A). This increase drove the small and negative dominance effect closer to zero and made the
2097 large and positive complementarity effect more positive (Fig. 4.1A, Addendum III Tables S3 and
2098 S4).

2099 In community B, both stressors also changed complementarity and dominance (Fig. 4.1B).
2100 However, in contrast to community A, these changes were negative at high stress levels. The small
2101 and positive dominance effect was reduced by all stress levels, driving it closer to zero (Fig. 4.1B,
2102 Addendum III Table S3). The large and positive complementarity effect was reduced by high levels
2103 of both stressors, but was still well above zero (Fig. 4.1B, Addendum III Table S4).

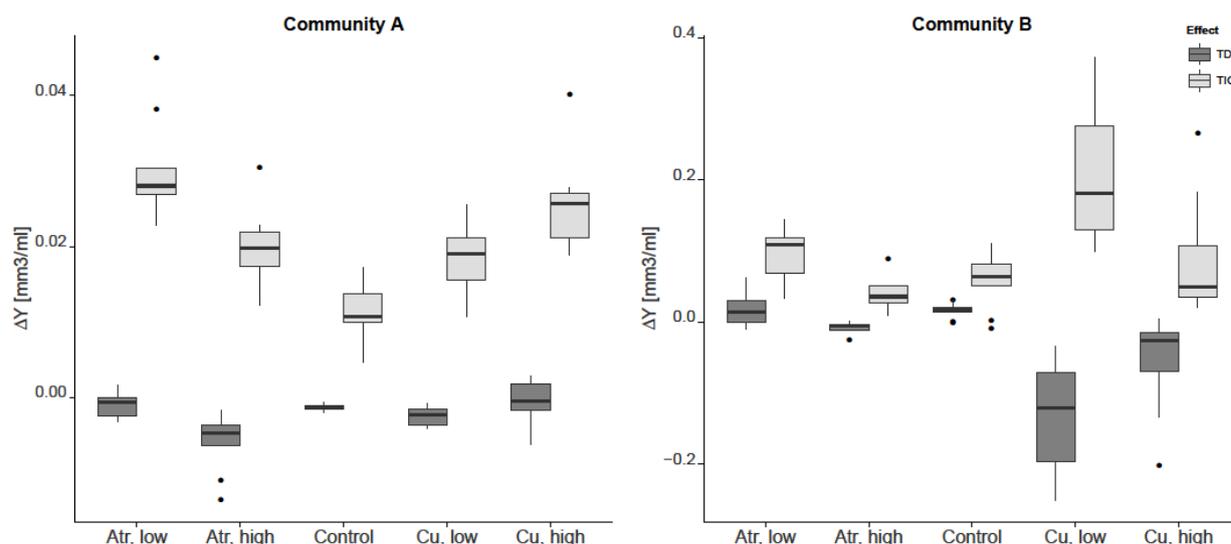


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Fig. 4.1: Dominance and complementarity effects in two benthic diatom communities exposed to low and high (200 and 500 $\mu\text{g/L}$) concentrations of copper (Cu) and atrazine (Atr). ΔY is the deviation in the observed from the expected yield of diatom communities that could be attributed to either dominance and complementarity effects. Complementarity is indicated as the sum of two-way and one-way complementarity effects.

2110

2111 Complementarity effects in community A were essentially composed of two-way
2112 complementarity (TIC, Fig. 4.2A), which was consequently always positive and positively affected
2113 by all stress levels (Fig. 4.2A, Addendum III Table S5). The contribution of two-way and one-way
2114 complementarity (TDC) were more comparable in community B than in community A.
2115 Complementarity effects in community B were mostly composed of TIC (control and atrazine
2116 treatments), and by both TDC and TIC (copper treatments, Fig. 4.2B). Interestingly, low stress had
2117 no (atrazine) or negative (copper) effects on TDC and positive (copper) or no (atrazine) effects on
2118 TIC, while high levels of both stressors had negative effects on TDC (Fig. 4.2B, Addendum III Table
2119 S5). Thus, negative effects on TDC were partly offset by positive effects on TIC when stress was
2120 low, but not when stress was high.



2121 **Fig. 4.2:** Two-way complementarity effects (TIC) and one-way complementarity effects (TDC) in two
 2122 benthic diatom communities (A and B) exposed to low and high (200 and 500 $\mu\text{g/L}$) concentrations of
 2123 copper and atrazine. ΔY is the deviation in the observed from the expected yield of diatom communities that
 2124 could be attributed to either TIC or TDC.
 2125

2126

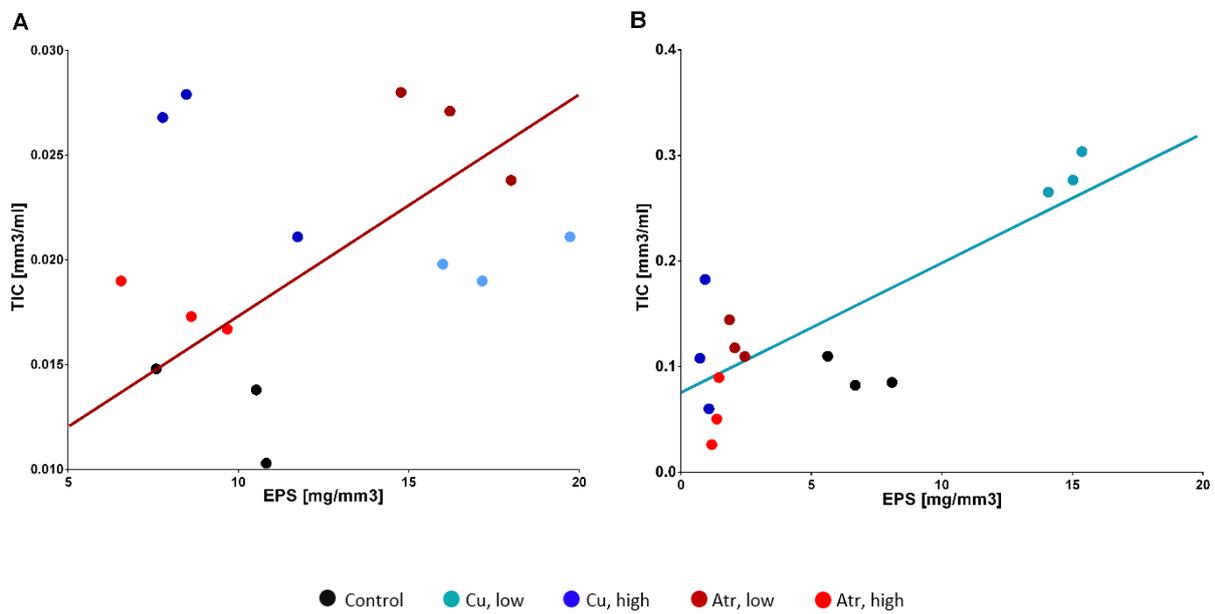
2127 **Linking stress effects on two-way complementarity to EPS production.** The release of EPS
 2128 predicted the increase of TIC in community A under atrazine and in community B under copper
 2129 (Fig. 4.3). In community A, diatoms increased their EPS production at low atrazine and copper
 2130 stress. EPS secretion and TIC were positively related when diatoms were exposed to atrazine (Fig.
 2131 4.3A, Addendum III Table S6), but not to copper. In community B, diatoms increased their EPS
 2132 production at low copper, but not atrazine stress. EPS and TIC were not related under atrazine
 2133 stress, but the amount of EPS produced by the diatoms positively affected TIC in communities
 2134 exposed to copper (Fig. 4.3B, Addendum III Table S6).

2135 **Linking stress effects on one-way complementarity to stress tolerance and monoculture**
 2136 **yield.** In community A, the relative performance of diatoms (the deviation of the observed relative
 2137 yield from the observed frequency in community, the main component of TDC) was not related to
 2138 stress tolerance or monoculture yield (Fig. 4.4 A1-A2, Addendum III Table S7). Diatoms in this
 2139 community differed in both their stress tolerance and monoculture yield (see x-axes in Fig. 4.4 A1-
 2140 A2), but growth in community did not benefit species with either above- or below-average stress
 2141 tolerance or monoculture yields.

2142 In community B, atrazine tolerance and monoculture yield also did not predict the relative
 2143 performance of diatoms in communities exposed to atrazine (Fig. 4.4 B1-B2, Addendum III Table
 2144 S7). Under copper stress, the relative performance of diatoms was negatively related to both their
 2145 copper tolerance and monoculture yield (Fig. 4.4 B1-B2, Addendum III Table S7). These negative

2146 correlations were caused by *Amphora lineolata*, *Gyrosigma* sp. 1 and *Navicula digitoradiata*, which
 2147 were the least copper-tolerant and lowest-yield-species within community B (Fig. 4.4 B1-B2).
 2148 Despite their low monoculture yields and stress tolerance, *N. digitoradiata*, *Gyrosigma* sp. 1, and
 2149 *A. lineolata* however showed the highest relative performance when grown in community (Fig. 4.4
 2150 B1-B2). In community B one-way complementarity under copper stress thus benefited the species
 2151 which were most sensitive to the metal, but also had the lowest biomass yields, which led to a
 2152 decline of total complementarity and ultimately caused a decrease of the net biodiversity effect
 2153 on diatom biomass under copper stress.

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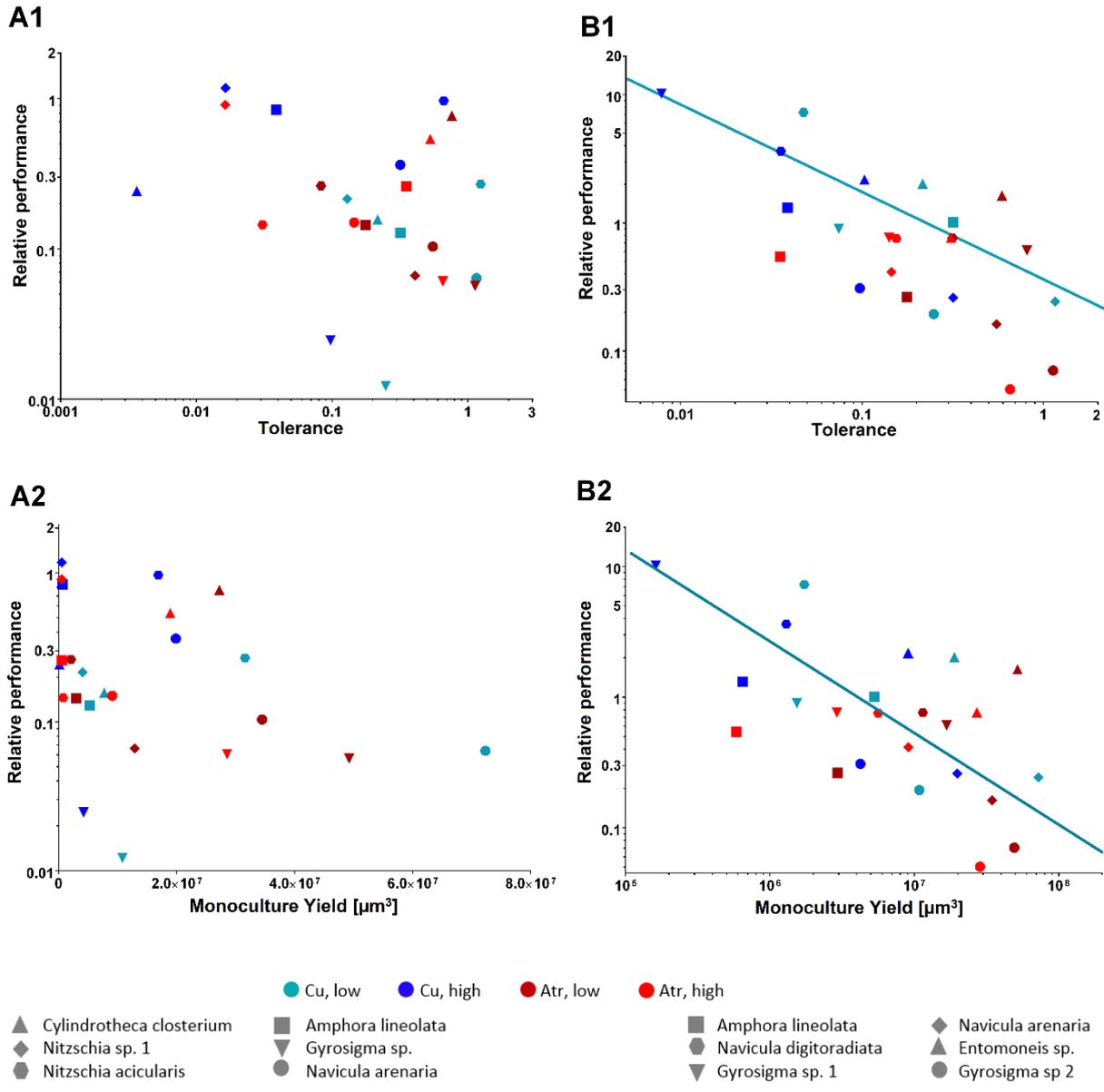


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2156

2157 **Fig. 4.3:** Two-way complementarity (TIC) as a function of the release of extracellular polymeric substances
 2158 (EPS) by two benthic diatom communities (panels A and B) exposed to low and high (200 and 500 µg/L)
 2159 concentrations of copper (Cu) and atrazine (Atr). Regression lines show significant relations between EPS
 2160 and TIC in diatom communities when exposed to copper (blue line) and atrazine (red line). Regressions
 2161 were fitted over three treatments (control, 200 and 500 µg/L) for each stressor.

2162



2163

2164

2165 **Fig. 4.4:** Relative performance of diatom species in community as a function of their stress tolerance and
 2166 biomass yield in monoculture. The panels A1 and B1 show the relative performance of diatom species
 2167 (calculated as the deviation of observed relative yield (RY) from the relative frequency (RY/RYT) as a
 2168 function of stress tolerance (calculated as the ratio of the monoculture yield at each stress level and in the
 2169 control) in community A (panel A1) and B (panel B1). Panels A2 and B2 show the relative performance as a
 2170 function of monoculture yield for diatoms in community A (panel A2) and B (panel B2). Stress levels are
 2171 low and high (200 and 500 $\mu\text{g/L}$) atrazine (Atr) and copper (Cu). Regression lines show significant relations
 2172 between the deviation of the species' relative performance in community and their stress tolerance and
 2173 biomass yield in monoculture. Data are shown on a log scale, as models were fitted to log-transformed data.

2174

2175 4.4. Discussion

2176 The outcome of our experiment shows a consistently positive diversity effect on diatom biomass
 2177 production which was mainly caused by complementarity rather than dominance effects. The
 2178 diatoms' production of extracellular polymers predicted, in part, increases in two-way

2179 complementarity. These increases could however be offset if complementarity was one-way:
2180 when low-yield species were least stress-tolerant in monoculture but benefited most from
2181 complementarity, the diversity effect on the biomass yield of stressed diatom communities was
2182 reduced.

2183 Diatom biomass production is crucial for the functioning of muddy intertidal systems, since
2184 benthic diatoms represent the main primary producers and thus the basis of the food web in these
2185 areas (Underwood and Kromkamp 1999, Tang and Kristensen 2007). Our findings of
2186 complementarity as the main driver of diatom biomass corresponds to previous works which also
2187 found complementarity rather than dominance to cause positive diversity effects on microalgae
2188 biomass yield (Vanelslander et al. 2009, Cardinale 2011, Stockenreiter et al. 2012, Schabhüttl et
2189 al. 2013). More importantly, these complementarity effects increased when diatoms were
2190 exposed to pesticide and metal stress, highlighting the value of producer diversity for maintaining
2191 biomass production at the basis of intertidal food webs under stressful conditions. Changes in
2192 environmental conditions can increase complementarity effects on primary producer biomass
2193 yield if facilitation, one of the mechanisms potentially leading to complementarity, becomes more
2194 important under harsh environmental conditions (Wang et al. 2013, Hodapp et al. 2016).

2195 The increase of two-way complementarity was, in part, predicted by a stimulation of diatom EPS
2196 production under stress. The release of these extracellular polymers is a common stress response
2197 in diatom biofilms: diatoms can increase their EPS production in response to nitrogen depletion,
2198 low temperatures or other unfavourable abiotic conditions (Staats et al. 2000a, Wolfstein and Stal
2199 2002). EPS production has furthermore been linked to protection from desiccation, physical
2200 disturbance and resistance to toxins (Decho 1990, Gerbersdorf et al. 2009b). Here, diatom
2201 communities increased their EPS production at low stress levels, thus confirming the stimulation
2202 of EPS as a response to low stress, which can however not be maintained under highly stressful
2203 conditions. This corroborates previous findings, which identified the release of organic exudates
2204 as a protective mechanism against chemical stress (Pistocchi et al. 1997), which can also lead to
2205 facilitation and thus increased complementarity in diatom biofilms (Vanelslander et al. 2009).

2206 Diatoms in community B did not increase their EPS production under atrazine stress, and there
2207 was no relation between EPS production and complementarity, which did not change under
2208 atrazine. In community A however, a stimulated EPS production predicted the increase of
2209 complementarity under atrazine. Atrazine blocks the electron transport chain of Photosystem II
2210 (PSII, Dorigo and Le Boulanger 2001). Some diatom species can however reduce their dependency
2211 on photosynthesis and thus their sensitivity to PSII inhibitors by mixotrophic growth, i.e. the
2212 uptake of organic substances, if such substances are present in sufficient amounts in the medium

2213 (Debenest et al. 2009, Larras et al. 2014). Amongst the diatom species in community A,
2214 *Cylindrotheca closterium* has been shown to increase its growth in response to organic exudates
2215 by other diatom species (Vanellander et al. 2009), and *C. closterium*, *Nitzschia* sp. 1 and *Nitzschia*
2216 *acicularis* can grow in the presence of atrazine when the growth medium contains organic carbon
2217 sources (Chapter 3). When community A was exposed to atrazine, these three species showed the
2218 highest relative performance (deviation of observed relative yield from observed frequency, see
2219 y-axis Fig. 4.4 A1). Although our findings show that the mixotrophic species in community A
2220 performed best under atrazine, one-way complementarity stayed close to zero. This was due to
2221 the – compared to the other species in community A – average monoculture yields of these best-
2222 performing mixotrophic species, which resulted in neither positive nor negative covariances
2223 between the species' relative performance and monoculture yields, and thus only minor one-way
2224 complementarity effects. The increase of diatom EPS production under atrazine stress thus served
2225 as a potential facilitative mechanism, which drove complementarity effects through the presence
2226 of mixotrophic species capable of utilizing these organic exudates. Since these mixotrophic species
2227 were not characterised by any above- or below average monoculture yields, the stimulation of EPS
2228 production led to an increase of two-way rather than one-way complementarity.

2229 Both diatom communities also increased their EPS production when exposed to copper.
2230 Microalgae are usually among the first organisms to be affected by metal pollution due to their
2231 large surface to volume ratio (Miao et al. 2005, Quigg et al. 2006, Manimaran et al. 2012). The
2232 toxicity of heavy metals to microalgae primarily lies in the formation of reactive oxygen species,
2233 which can damage cell membranes, plastids and other intracellular structures (Masmoudi *et al.*
2234 2013). However, microalgae can increase their metal tolerance through the excretion of
2235 extracellular polymers (Hall et al. 1979, Fisher and Froud 1980, Pistocchi et al. 1997). The
2236 excretion of EPS allows microalgae to chelate metal ions in the medium, thus reducing metal
2237 accumulation within the algal cells (Pistocchi et al. 1997). Compared to other microalgal species,
2238 diatoms are characterised by a high EPS production (De Brouwer et al. 2000). This capacity to
2239 excrete EPS under metal stress can enable diatoms to grow at copper concentrations which are
2240 up to one magnitude higher than those limiting the growth of other microalgae classes (Pistocchi
2241 et al. 1997).

2242 In community A, most species performed better when exposed to copper in community rather
2243 than in monoculture, but this complementarity was evenly distributed amongst species, and not
2244 predicted by their EPS production. The positive diversity effect on the biomass yield of this diatom
2245 community under copper was thus likely caused by other mechanisms than the excretion of
2246 extracellular polymers. Such mechanisms can consist of an intracellular accumulation of metal
2247 ions in vacuoles or metal immobilization in the cell wall, selective copper exclusion from algal

2248 cells, and an increased production of amino acids protecting cell membranes from copper damage
2249 (Fernandes and Henriques 1991, Lage et al. 1994, Wu et al. 1995).

2250 The diatoms' EPS production predicted complementarity in community B under copper stress.
2251 However, this complementarity was not equally distributed amongst species, and essentially
2252 favoured *A. lineolata*, *Gyrosigma* sp. 1, *N. digitoradiata*. These species had the lowest biomass
2253 yields and were least copper-tolerant in monoculture, but showed the highest relative
2254 performance when exposed to the metal in community. These high relative performances did not
2255 come at the expense of other species (neutral dominance effect), but were due to a one-way
2256 complementarity which only benefited sensitive species with low monoculture yields, causing the
2257 increases in two-way complementarity to be mirrored and offset by a negative one-way
2258 complementarity. Diversity thus increased the biomass yield of diatom species under copper
2259 stress, but since sensitive and low-yield species benefited most from being grown in community,
2260 the net diversity effect on diatom biomass yield was not altered or even reduced at low and high
2261 copper stress respectively.

2262 Complementarity effects tend to increase over time (Fargione et al. 2007). Also, complementarity
2263 is strongest along gradients of broad environmental niche space, which allow for resource
2264 partitioning between species under natural conditions (Špačková and Lepš 2001, Hodapp et al.
2265 2016). Our experiments were performed over a relatively short time (one diatom growth cycle)
2266 in an artificial experimental system which limited environmental fluctuations and spatial
2267 heterogeneity, which can lead to an underestimation of complementarity effects. Also, to confirm
2268 EPS excretion as a facilitative mechanism under stress, it is recommended to quantify the extent
2269 to which extent the production of EPS reduced metal exposure of diatom cells, as well as the
2270 uptake of these organic exudates by mixotrophic species. The former could be achieved by
2271 measuring the intracellular copper concentrations in the diatoms (e.g. see Pistocchi *et al.* 1997),
2272 the latter by a colorimetric quantification of the metabolization of organic substances by diatoms
2273 (e.g. see Tuchman *et al.* 2006). Moreover, EPS production was a 'sleeping' facilitative mechanism,
2274 which was stimulated when diatom communities were exposed to chemical stress, and only
2275 predicted complementarity in diatom communities containing either stress-sensitive species, or
2276 species capable of heterotrophic growth.

2277 We infer four conclusions from our results: first, a positive biodiversity effect causes diatom
2278 communities to produce more biomass than expected from their monoculture. Second, this
2279 positive biodiversity effect is largely underpinned by complementarity effects. Third,
2280 complementarity in stressed diatom communities can be driven by the release of extracellular
2281 polymers as a facilitative mechanism, leading to a better performance of species characterised by

2282 a mixotrophic growth mode or a low stress tolerance in monoculture. Fourth, a negative one-way
2283 complementarity can cause the net diversity effect to decrease under stress, if low-yield species
2284 perform better when grown with different species.

2285

2286 **Chapter 5: Selective and context-dependent effects drive chemical**
2287 **stressor impacts across trophic levels at the basis of marine food webs**

2288

2289 **Abstract**

2290 Human activities increasingly impact the functioning of marine food webs, but anthropogenic
2291 stressors are seldom included into ecological study designs and diet quality, as distinct from just
2292 diet quantity, has rarely been highlighted in food web studies in a stress context.

2293 We first measured the effects of metal and pesticide stress (copper and atrazine) on the
2294 contribution of a benthic intertidal diatom community to three processes that are key to the
2295 functioning of intertidal systems: biomass production (diet quantity), energy content (diet
2296 quality) and extracellular polymer production (sediment stabilization). We then examined if
2297 stressors affected diatom functioning by selectively targeting the species contributing most to
2298 functioning (selective stress effects) or by changing the species' functional contribution (context-
2299 dependent effects). Finally, we tested the response of the diatoms' main grazers (harpacticoid
2300 copepods) to changes in diet quality.

2301 Diatom diet quantity was reduced by metal stress but not by low pesticide levels due to the
2302 presence of an atrazine-tolerant, mixotrophic species. Context-dependent effects of both stressors
2303 increased the diatoms' contribution to sediment stabilization by stimulating the release of
2304 extracellular polymers.

2305 Selective effects of the pesticide reduced diatom diet quality by 60% and 75% at low and high
2306 pesticide levels respectively, by shifting diatom community structure from dominance by lipid-
2307 rich species towards dominance by atrazine-tolerant, but lipid-poor species. Context-dependent
2308 effects did not affect individual diatom lipid content at low levels of both stressors, but caused
2309 diatoms to lose 40% of their lipids at high copper stress.

2310 Copepod energy content was related to the quality of their diatom diet, with copepods losing half
2311 of their energy content (in terms of lipids) when feeding on diatoms grown under low and high
2312 pesticide and high metal stress. Selective atrazine effects on diatom community structure affected
2313 the energy flow to their grazers at stress levels where no context-dependent effects of both
2314 stressors on diatom diet quality were detected.

2315 *Synthesis.* Chemical stress differentially affected the contribution of marine primary producers to
2316 diet quantity, sediment stabilization and diet quality. Selective community shifts were a more
2317 potent threat for diet quality than context-dependent stress effects on primary producers. Diet

2318 quality predicted the energy flow from marine producers to their consumers and should be
2319 considered when measuring food web functioning under anthropogenic change.

2320

2321 **5.1. Introduction**

2322 The impact of human activities on biological communities and their contribution to ecosystem
2323 functioning has become a central topic in ecological research (Halpern et al. 2008, Cardinale et al.
2324 2012, Gamfeldt et al. 2015). Although conservation research is framed within the context of
2325 anthropogenic change, exposure to anthropogenic stressors is rarely included into ecological
2326 study designs (McMahon et al. 2012, Steudel et al. 2012).

2327 Stress can affect ecosystem functioning by causing biodiversity loss in terms of species richness,
2328 as well as through changes in community structure, without necessarily causing species to go
2329 extinct (Hillebrand et al. 2008, Wittebolle et al. 2009, Mensens et al. 2015). Selective stress effects
2330 on community structure (hereafter 'selective stress effects', Wittebolle et al. 2009) can reduce
2331 ecosystem functioning if stressed communities are dominated by tolerant species with a low
2332 functional contribution (Larsen et al. 2005, Mensens et al. 2015). If the functionally most
2333 important species are also most stress-tolerant, loss of functioning under stress will be limited
2334 (Radchuk et al. 2016). Moreover, functioning in stressed communities can be driven by 'context-
2335 dependent effects', i.e. changes in the species' functional contribution (Fox 2006, Fox and Harpole
2336 2008, Tylianakis et al. 2008, Hiddink et al. 2009). Context-dependent effects can arise from direct
2337 effects of environmental drivers on the species' functional contribution (Fox and Harpole 2008,
2338 e.g. physiological stress, Schimel et al. 2007), as well as from differential species interactions
2339 under stress (e.g. changes in complementarity, Fox 2006, Fox and Harpole 2008).

2340 The majority of experiments designed to address ecosystem functioning under anthropogenic
2341 change have focused on single trophic levels, usually primary producers (Raffaelli 2006, Cardinale
2342 et al. 2011). Stressors that alter functioning at the producer level can however have concomitant
2343 impacts on their consumers (Rohr and Crumrine 2005, McMahon et al. 2012). The trophic impacts
2344 of anthropogenic stressors are commonly examined with regard to diet quantity, which has been
2345 linked to the abundance and biomass of consumers (e.g. Kasai and Hanazato 1995, Wendt-Rasch
2346 et al. 2004, Rohr and Crumrine 2005). Far less attention has been devoted to diet quality, which
2347 considers a diet's biochemical composition and is commonly expressed as lipid content (Jodice et
2348 al. 2006, Guo et al. 2016). Nevertheless, the growth, reproduction and energy profile of consumers
2349 is strongly linked to the quality of their diet (Giles et al. 2002, Österblom et al. 2008, Taipale et al.
2350 2013). The biochemical composition of primary producers is increasingly affected by human
2351 disturbance (Vitousek et al. 1997, Wang and Frei 2011, Guo et al. 2016, Sanpera-Calbet et al.

2352 2016), which has made the integration of food biochemistry with traditional studies of diet
2353 quantity a key challenge for estimating food web functioning under stress (Arts and Wainmann
2354 1999, Guo et al. 2016).

2355 Here, we first measure the effects of metal and pesticide stress (copper and atrazine) on the
2356 structure of a benthic diatom community and its contribution to three ecosystem processes:
2357 biomass production (diet quantity), energy content (diet quality) and extracellular polymer
2358 production (sediment stabilization). Benthic diatoms are the main primary producers in many
2359 soft-sediment intertidal habitats, enhancing sediment stabilization in these systems through the
2360 production of extracellular polymeric substances (EPS, Underwood and Kromkamp 1999, Decho
2361 2000). Diatom diet quality in terms of essential fatty acid (EFA) content plays a crucial role for
2362 trophic energy transfer (Arts et al. 2001, Taipale et al. 2013). EFAs cannot be synthesized by
2363 animals but are key determinants of the growth and energy content of aquatic consumers (Brett
2364 and Müller-Navarra 1997, von Elert 2002, Arendt et al. 2005, Litzow et al. 2006). Harpacticoid
2365 copepods are among the main consumers (grazers) of benthic diatoms and incorporate large
2366 amounts of EFAs from their algal diet, making harpacticoids pivotal for the energy transfer from
2367 primary producers to higher trophic levels (Alheit and Scheibel 1982, De Troch et al. 1998, Nanton
2368 and Castell 1998, Buffan-Dubau and Carman 2000, Andersen et al. 2005).

2369 Next, we test if stressor-induced changes in diatom functioning (EFA and EPS production) were
2370 caused by selective or context-dependent stress effects. Context-dependent effects are measured
2371 by comparing functioning in experimental diatom communities with that in synthetic
2372 communities. Synthetic communities are computed from the species' EFA and EPS production in
2373 unstressed monocultures and reflect the same community structure observed at each stress level,
2374 thus allowing to exclude any confounding effects of community structure on diatom functioning.
2375 Selective stress effects are quantified by contrasting synthetic communities which reflect the
2376 community structure under stress and control conditions, thus excluding any confounding
2377 context-dependent effects on diatom functioning.

2378 Last, we test if potential changes in the survival and energy content of the harpacticoid copepod
2379 *Microarthridion littorale*, being the dominant grazer at the intertidal study site, are related to
2380 atrazine and copper effects on the quality of its diatom diet. For instance, whether potential
2381 changes in grazer energy content are linked to context-dependent stress effects on diatom energy
2382 content, or to changes in community structure leading to dominance of diatoms with a low or high
2383 contribution to energy flow in intertidal systems.

2384 **5.2. Methods**

2385 **Experimental organisms & culture conditions.** The harpacticoid copepod *Microarthridion*
2386 *littorale* (family Tachidiidae) was collected from intertidal mud at the Paulina intertidal flat (SW
2387 Netherlands, 51° 21'N, 3°43'E), where it represented the dominant grazer (~ 90% of all
2388 harpacticoid individuals). *M. littorale* specimens were extracted alive from the sediment using a
2389 mixed technique of sediment decantation and extraction via white light attraction. Adult
2390 specimens were randomly collected with a glass pasteur pipette using a Wild M5 binocular.
2391 Copepods were washed 3 times over a 38 µm sieve and placed in glass jars with filtered and
2392 autoclaved natural seawater overnight in order to empty their intestines prior to the start of the
2393 experiment.

2394 The diatom community was composed of six species representing the most abundant genera
2395 observed at the sampling site (Addendum IV Table S1). For this experiment, all species were
2396 obtained from the culture collection of the Protistology and Aquatic Ecology Research Group
2397 (UGent) (<http://bccm.belspo.be>). Prior to the set-up of the experiments, the diatoms were grown
2398 in tissue bottles (Greiner BioOne, CELLSTAR® TC, 175 cm² growth surface) during 10 days in a
2399 climate room at 15±1 °C, a light/dark cycle of 12h / 12h and an illumination of 90 µmol photons
2400 m⁻²s⁻¹, in culture medium consisting of filtered and autoclaved natural seawater (salinity 32±1)
2401 enriched with f/2 nutrients (Guillard 1975).

2402 **Diatom experiments.** The experimental diatom communities were exposed in five treatments to
2403 0, 200 (hereafter 'low') and 500 (hereafter 'high') µg/l atrazine and copper respectively,
2404 representing levels of atrazine and copper stress resulting on average in a 50% and 90%
2405 reduction in growth of the diatom species (Chapter 3). Atrazine treatments were prepared from a
2406 stock solution obtained by dissolving 50 mg technical atrazine (2-chloro-4-ethylamino-6-
2407 isopropylamino-*s*-triazine, 99.8% pure, Sigma-Aldrich Chemie GmbH, Munich, Germany) in 10 ml
2408 acetone as a carrier to increase the solubility of atrazine, with a maximum final volume of 0.01%
2409 acetone in the treatments. An acetone control treatment of 0.01% acetone was included and
2410 compared to an acetone-free control to test for carrier effects. All atrazine treatments were
2411 compared to the acetone control. Copper (as a Cu[II]Cl₂ solution, analytical grade; VWR
2412 International) was spiked directly into the culture medium before exposure of the diatoms. F/2
2413 culture medium for the copper experiments was prepared without EDTA, to avoid complexation
2414 of free copper ions (Pistocchi et al. 1997). The obtained atrazine and copper concentrations that
2415 were finally applied in the experiment are listed in Addendum IV Table S2. Additionally, the six
2416 diatom species were assembled in monoculture under control conditions, to quantify each
2417 species' biomass, EFA and EPS production in the absence of the stressors (Addendum IV Table
2418 S1). All treatments were run in tissue culture flasks (Greiner BioOne, CELLSTAR® TC, 175 cm²

2419 growth surface), with nine replicates per treatment (three replicates in the monoculture
2420 treatments). Each microcosm was inoculated with a total cell density of approximately 5000
2421 diatom cells/ml (500000 cells in 100 ml medium per culture flask, between 800-850 cells/ml per
2422 species in the diatom communities) from exponentially growing cultures, and incubated in a
2423 climate room at 15 ± 1 °C, under a light / dark cycle of 12h / 12h at $90 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. Culture
2424 medium was refreshed after eight and 15 days, diatom biofilms as food for the copepod
2425 experiment as well as for the EPS and EFA analyses (see further) were harvested after 15 days
2426 (late exponential growth phase) and experiments were terminated after 25 days.

2427 Diatom biofilms in three replicates of 100 ml cell suspension per treatment were harvested as
2428 food for the corresponding treatments in the copepod experiment, and purified from copper and
2429 atrazine. In the purification process, the diatom cells were resuspended and placed in 50 ml
2430 centrifuge tubes (2 tubes per replicate). All samples were then centrifuged at a speed of $50g$ for
2431 10 minutes. Supernatant fluid was removed and replaced with f/2 culture medium (25 ml total
2432 volume) and centrifuged again for 10 minutes. After the second centrifuge process, supernatant
2433 fluid was removed again, and 5 ml of concentrated diatom pellet per replicate were transferred to
2434 nine Eppendorf microtubes (0.56 ml pellet per microtube), freeze-dried and preserved at -80 °C.
2435 The individual microtubes contained the food aliquot for each day of the respective treatments in
2436 the copepod experiment (see further).

2437 **Copepod experiment.** We tested the effect of diatom diet quality by offering *M. littorale* diatom
2438 diets of equal biomass under unstressed conditions. The copepod experiment consisted of five
2439 treatments, each with three replicates of 100 *M. littorale* copepods (a natural mix of adult males
2440 and (gravid) females), that were fed for 10 d an equal biomass of diatoms grown under unstressed
2441 conditions and low and high atrazine and copper stress, respectively. The experiment was
2442 conducted in glass jars containing 100 ml of filtered and autoclaved seawater in a climate room at
2443 15 ± 1 °C with a 12:12 h light:dark cycle and 40 to $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. To ensure a constant
2444 food supply, each treatment was inoculated with a concentrated diatom pellet of 0.23 mm^3
2445 biovolume every day, and unconsumed diatoms were removed from the bottom of the jars. Over
2446 the whole duration of the experiment, a total diatom biovolume of 2.05 mm^3 was applied per
2447 experimental unit, corresponding to 3 to 5×10^6 diatom cells per treatment. At the end of each day,
2448 there was no food depletion in any of the treatments. Based on our previous experiments (De
2449 Troch et al. 2005, 2007), the provided quantity of diatoms can be considered as above the feeding
2450 saturation level. Copepod mortality was determined at the end of the experiment, and surviving
2451 (85-100%) individuals from each experimental unit were washed in natural seawater to remove
2452 food particles, left 12h to empty their gut, and stored at -80 °C for further fatty acid analysis.

2453 **Biomass, EPS and EFA analyses.** Diatom biomass was quantified as biovolume after 0, 2, 5, 10,
2454 15, 20 and 25 days of incubation. Biovolume was calculated from cell densities, linear dimensions
2455 (measured digitally using ImageJ, Schneider et al. 2012) and formulas representing the closest
2456 approximation of geometric shape for each genus (Hillebrand et al. 1999, Addendum IV Table S1).
2457 Cell densities (in cells/ml) were determined by digitally counting the cells (ImageJ cell counting
2458 software) in photographs obtained by magnifying and photographing (x100) an area of 0.66 mm²
2459 per microcosm, using an inverted Axiovert 135 Zeiss microscope (Carl Zeiss, Jena, Germany) and
2460 a connected digital camera (Canon PowerShot G11). All further analyses use the biomass on day
2461 15.

2462 Extracellular polymeric substances (EPS) secreted by the diatom communities were measured in
2463 three replicates of 10 ml diatom suspension which were collected after 15 days of incubation. EPS
2464 concentrations were analysed by spectrophotometry according to a modified version of the
2465 phenol/H₂SO₄ assay (Dubois et al. 1956) as described in Mensens *et al.* (2015).

2466 Essential fatty acids (EFAs) as marker of energy content were quantified as the content of
2467 eicosapentaenoic acid (20:5 ω 3, EPA) and docosahexaenoic acid (22:6 ω 3, DHA). Three replicates
2468 of 8 ml suspended diatom culture per treatment of the diatom communities and monocultures
2469 were collected after 15 days. The samples were centrifuged for 10 minutes at 10 °C and a speed
2470 of 50g. After undergoing the same purification process as the diatom food samples for the grazer
2471 experiment, pellets were resuspended and 1.5 ml of concentrated diatom suspension per replicate
2472 were placed in a glass vial and stored at -80 °C for fatty acid analysis.

2473 Copepod and diatom EFA content was measured through hydrolysis of total lipid extracts and
2474 methylation to FA methyl esters (FAME), followed by the analysis of the obtained FAME using a
2475 gaschromatograph (HP 6890N) coupled to a mass spectrometer (HP 5973) according to the
2476 protocol described in De Troch *et al.* (2012) for copepods and Mensens *et al.* (2015) for diatoms.
2477 The quantification function of each individual FAME was obtained by linear regression of the
2478 chromatographic peak areas and corresponding known concentrations of the standards (ranging
2479 from 5 to 250 ng/ml). All EPS and EFA concentrations were standardized to diatom biomass or
2480 the number of copepod individuals for diatoms and copepods, respectively.

2481 **Data analysis.** An analysis of the diatom community structure among the treatments (control,
2482 low and high atrazine and copper) was conducted with a non-metric multidimensional scaling
2483 method based on Bray-Curtis similarity. A one-way analysis of similarity (ANOSIM) was used to
2484 test for significant biomass differences between the treatments. Subsequently, percentages of
2485 similarity (SIMPER) were calculated to determine the main species contributing to any differences
2486 in community structure.

2487 Differences in diatom biomass, EFA and EPS production among the treatments were tested with
2488 a generalized least squares model, with biomass, EFA and EPS as response variables and
2489 treatment type as categorical predictor (Equation 1)

$$2490 \quad Y \sim \beta_T \cdot T \quad [1]$$

2491 where Y is the response variable (biomass, EFA or EPS), T is the treatment type (control, low and
2492 high atrazine and copper), and the beta coefficient β_T is the slope measuring the effect of the
2493 treatment type on biomass, EFA or EPS production. Biomass, EFA and EPS production among
2494 treatments were compared with pairwise Tukey's tests correcting p-values for multiple
2495 comparisons by the single-step method.

2496 Next, we tested if potential changes in diatom EFA and EPS production were due to selective or
2497 context-dependent effects of atrazine and copper. Selective and context-dependent effects were
2498 quantified by comparing the EFA and EPS production in experimental and synthetic diatom
2499 communities. The synthetic communities have the same community structure as observed in the
2500 corresponding treatments of the experimental communities, but are computed from the species'
2501 EFA and EPS production in unstressed monocultures (Equation 2). The synthetic communities
2502 thus reflect the EFA and EPS production expected at the same community structure as induced by
2503 copper and atrazine, however without any stress exposure or species interactions.

$$2504 \quad Y_{SYN,j} = \frac{\sum_{i=1}^N M_{i,j=0} \times B_{i,j}}{B_{T,j}} \quad [2]$$

2504 $Y_{SYN,j}$ is the EFA or EPS production in synthetic communities of the same structure as the
2505 experimental communities at the atrazine or copper concentration j . $M_{i,j=0}$ is the mean EFA or EPS
2506 production per unit biomass of species i in monoculture under unstressed conditions ($j=0$). $B_{i,j}$ is
2507 the biomass of species i observed in the experimental community at the stress level j . $B_{T,j}$ is the
2508 total biomass observed in the experimental community at the stress level j .

2509 Selective and context-dependent effects of both stressors were analysed with a generalized least
2510 squares model (Equation 3) and pairwise comparisons of the EFA and EPS production in
2511 experimental and synthetic diatom communities.

$$2512 \quad Y \sim \beta_T \cdot T \quad [3]$$

2513 where Y is the diatom EFA or EPS production per unit biomass, T is the treatment type (control,
2514 low and high atrazine and copper in the experimental and synthetic diatom communities), and
2515 the beta coefficient β_T is the slope measuring the effect of the treatment type on EFA or EPS

2516 production. Pairwise comparisons were performed with a Tukey's test correcting p-values for
2517 multiple comparisons by the single-step method.

2518 Context-dependent effects occur when synthetic and experimental communities within the same
2519 treatment differ in their EFA or EPS production. Since both community types have the same
2520 structure, any differences in EFA or EPS between the two community types result from direct
2521 stress effects or species interactions in the experimental community. Consequently, any
2522 differences between experimental and synthetic communities of the same treatment point at
2523 context-dependent effects.

2524 Selective stress effects occur when synthetic communities reflecting the control community
2525 structure differ in their EFA or EPS production from synthetic communities reflecting the
2526 community structure under stress. Since synthetic communities are computed from unstressed
2527 monocultures, any differences in EFA or EPS between synthetic communities are related to
2528 differences in community structure rather than direct stress effects or species interactions.
2529 Consequently, any differences among synthetic communities are linked to selective rather than
2530 context-dependent effects. Addendum IV Fig. S1 provides a scheme visualizing the quantification
2531 of context-dependent and selective stress effects.

2532 The response of copepod energy content to stressor-induced alterations in diet quality was
2533 analysed with generalized least squares models, with copepod energy content as response
2534 variable and diatom diet quality in terms of energy content and community structure as predictors
2535 (Equation 4). Diatom energy content was quantified as EFA production, diatom community
2536 structure as the Bray-Curtis percent dissimilarity from the average community structure in
2537 controls (Equation 5). Models were fitted separately for copepods feeding on atrazine- and
2538 copper-exposed diatoms respectively, to test if the effects of either stressor on copepod energy
2539 content can be predicted from changes in diatom energy content or community structure.

$$2540 \quad E_C \sim a + b \cdot E_D + c \cdot C_D \quad [4]$$

2541 E_C is the copepod energy content (EFA content per copepod individual), E_D is diatom energy
2542 content (EFA production per unit biomass), C_D is diatom community structure, a is the intercept,
2543 b and c represent the slopes, i.e. the relation of copepod energy content to diatom energy content
2544 and diatom community structure. If E_D and C_D were correlated (correlation factor > 0.5), models
2545 were fitted separately for both predictors.

$$2546 \quad C_D = 100 \cdot \{1 - \Sigma |B_{ij} - \mu B_{ij=0}| / \Sigma(B_{ij} + \mu B_{ij=0})\} \quad [5]$$

2547 B_{ij} is the biomass of species i at the atrazine or copper concentration j and $\mu_{B_{ij=0}}$ is the mean
2548 biomass of species i in the control ($j=0$).

2549 For all least squares model fits, normality and homogeneity of model residuals were inspected by
2550 evaluation of quantile-quantile plots and Shapiro-Wilk's test, and by Levene's test and plotting
2551 residuals versus explanatory variables respectively. Untransformed data did not violate normality
2552 (Shapiro-Wilk's test, $\alpha > 0.1$). If indications of deviations from normality were detected ($0.1 < \alpha <$
2553 0.15), an optimal Box-Cox transformation (Box and Cox 1964, Venables and Ripley 2002) was
2554 applied to maximize normality of model residuals. If homogeneity was violated, the model was
2555 refitted using an exponential variance structure allowing residuals to change with the continuous
2556 predictor X (weights = varExp(form ~ 1 | X) or allowing different variances according to the
2557 categorical predictor P (weights = varIdent(form ~ 1 | P). By means of likelihood ratio testing, the
2558 significance of these structures was tested ($\alpha = 0.05$).

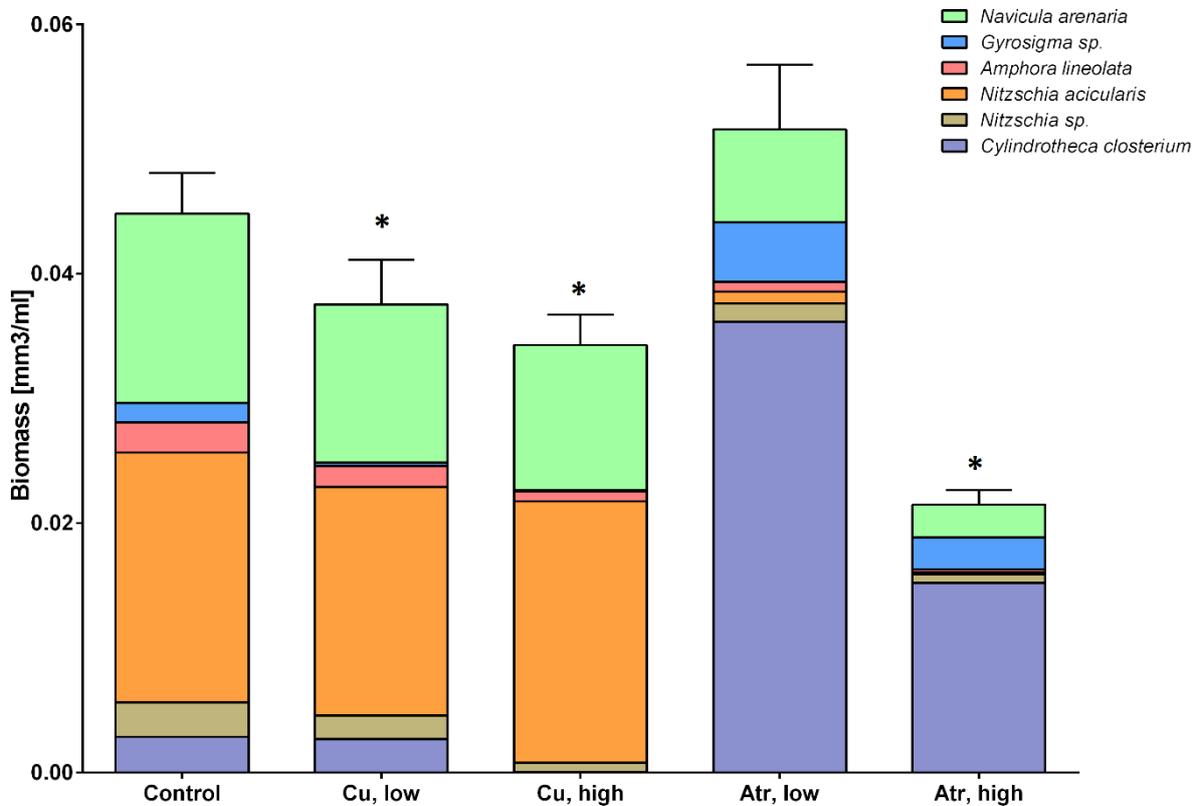
2559 Multivariate, ANOSIM and SIMPER analyses of diatom community structure were performed
2560 using Primer 6 software (Clarke and Gorley 2006). All other calculations were done in R 3.0.1.
2561 using RStudio (R Development Core Team 2016). The package nlme (Pinheiro et al. 2015) was
2562 used for the fitting of generalized least squares models and optional variance structures. Optimal
2563 Box-Cox transformations were performed using MASS (Venables and Ripley 2002). Pairwise
2564 Tukey's tests on the fitted models were performed with the package multcomp (Hothorn et al.
2565 2008), using the general linear hypothesis test (glht) function, correcting p-values for multiple
2566 comparisons by the single-step method (default procedure in multcomp).

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2568 **5.3. Results**

2569 **Diatom community structure.** The structure of diatom communities under atrazine differed
2570 from the structure of communities grown under control conditions and copper exposure (see non-
2571 metric multidimensional scaling in Addendum IV Fig. S2). The ANOSIM confirmed the significance
2572 of treatment type for community structure (global $R = 0.833$, $p=0.001$). The community structure
2573 at both copper levels resembled the community structure observed under control conditions
2574 (14% and 18% dissimilarity respectively), with *N. acicularis* and *N. arenaria* contributing the most
2575 biomass in both types of communities (Fig. 5.1, Addendum IV Table S3). In contrast, both atrazine
2576 levels induced a change in community structure (70% and 76% dissimilarity from the control)
2577 due to an increase in biomass of *C. closterium*, which compared to the control showed a 6- and 12-
2578 fold increase in biomass in the high and low atrazine treatments, respectively. This resulted in a
2579 dominance by *C. closterium* in the atrazine-exposed communities, as it contributed more than 70%
2580 of the total biomass at both atrazine levels (Fig. 5.1, Addendum IV Table S3). Within the control,

2581 copper and atrazine treatments, the community structure of diatom communities showed little
 2582 variance (within-treatment similarities between 84% and 92%, Addendum IV Table S4).



2583 **Fig. 5.1:** Total biomass production per treatment and biomass of the component species for diatom
 2584 communities grown in control, low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')
 2585 treatments. Significant differences in biomass production from the control are indicated with asterisks (*).
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 2587

2588 **Diatom biomass, EPS and EFA production.** Diatom biomass, EPS and EFA production changed
 2589 depending on treatment type (all $p < 0.0001$, Fig. 5.1, Fig. 5.2). The post-hoc analyses showed that
 2590 diatom biomass was reduced at both low and high copper as well as at high but not at low atrazine
 2591 concentrations (Fig. 5.1, Table 5.1). Diatom EPS production increased at low atrazine and copper
 2592 stress, with diatom communities producing twice as much EPS than under control conditions, but
 2593 was not affected at high levels of both stressors (Fig. 5.2A grey bars, Table 5.1). Diatom EFA
 2594 production was reduced at both levels of atrazine, but only at high copper stress (Fig. 5.2B grey
 2595 bars, Table 5.1). High copper stress reduced the EFA production of diatom communities by 40%,
 2596 low and high atrazine stress by 60% and 75%, respectively (Fig. 5.2B grey bars).

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Process	Treatment	Treatment	Est	lwr	upr	Z	P
Biomass	Control	Atr, low	-6.756	-9.18	-4.33	2.78	0.059
		Atr, high	23.327	20.90	25.75	9.61	<0.001
		Cu, low	7.273	4.85	9.70	-3.00	0.036
		Cu, high	10.533	8.11	12.96	4.34	<0.001
	Atr, low	Atr, high	30.083	27.66	32.51	12.39	<0.001
	Cu, low	Cu, high	3.260	0.83	5.69	1.34	0.666
EPS	Control	Atr, low	-0.251	-0.44	-0.06	-4.03	<0.001
		Atr, high	0.066	-0.13	0.26	1.06	0.984
		Cu, low	-0.290	-0.48	-0.10	-4.66	<0.001
		Cu, high	0.016	-0.18	0.21	0.25	1.000
	Atr, low	Atr, high	0.317	0.13	0.51	5.09	<0.001
	Cu, low	Cu, high	0.305	0.11	0.50	4.91	<0.001
EFA	Control	Atr, low	2.143	1.30	2.99	8.00	<0.001
		Atr, high	3.191	2.34	4.04	12.08	<0.001
		Cu, low	-0.046	-0.89	0.80	-0.17	1.000
		Cu, high	1.372	0.52	2.22	5.12	<0.001
	Atr, low	Atr, high	0.317	0.13	0.51	5.72	<0.001
	Cu, low	Cu, high	0.305	0.11	0.50	4.91	<0.001

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Table 5.1: Pairwise comparisons of biomass, EFA and EPS production in diatom communities as estimated by generalised least squares model fits. 'Process' indicates to which response variable models were fitted (biomass, EFA or EPS production). 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicates which treatments are compared. 'Est' indicates the difference in biomass (in 10³ mm³/ml), EFA (in µg/mm³) and EPS (in mg/mm³) production between the compared treatments as estimated by generalized least squares models fitted to untransformed biomass and Box-Cox transformed EFA and EPS data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

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Selective and context-dependent stress effects on diatom EPS and EFA production. The EPS and EFA production in the experimental and synthetic diatom communities changed depending on treatment type (both $p < 0.0001$). Pairwise comparisons of the EPS production in the experimental and synthetic communities showed no difference among the two community types in the control and the high copper and atrazine treatments (Fig. 5.2A, Table 5.2). At low levels of both stressors however, the EPS production in experimental communities was double of that expected in unstressed synthetic communities of the same structure (Fig. 5.2A, Table 5.2). Within the synthetic communities there was no difference in EPS among any of the treatments (Fig. 5.2A, Table 5.2).

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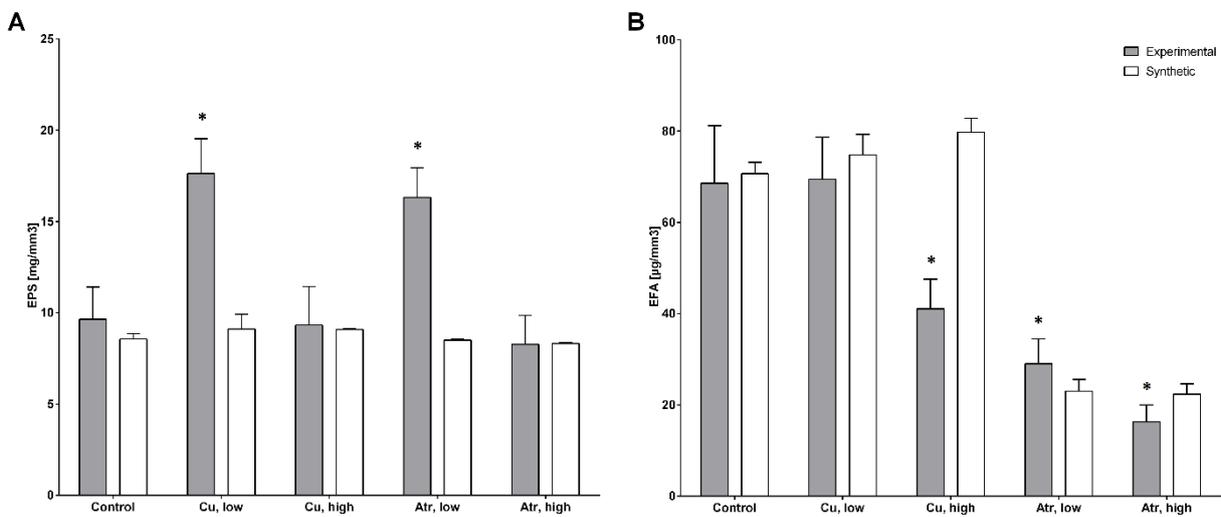
2623

Pairwise comparisons of the EFA production in experimental and synthetic communities did not show differences among the two community types in the control and low copper treatments (Fig. 5.2B, Table 5.2). At high copper stress, the EFA production in the experimental community was lower than in the corresponding synthetic community (Fig. 5.2B, Table 5.2). The EFA loss induced by high copper stress in experimental communities was thus not reflected in unstressed synthetic

2624 communities of the same structure, whose EFA production did not differ from the control (Fig.
2625 5.2B, Table 5.2).

2626 In the atrazine treatments, both the experimental and synthetic communities had a lower EFA
2627 production than the control (Fig. 5.2B, Table 5.2). Within each atrazine treatment, the EFA
2628 production of experimental and synthetic communities did not differ (Fig. 5.2B, Table 5.2). The
2629 EFA loss induced by atrazine in the experimental communities was thus reflected by the synthetic
2630 communities, which mimicked the community structure under atrazine without actual exposure
2631 to the herbicide (Fig. 5.2B, Table 5.2).

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2634 **Fig. 5.2:** Atrazine and copper effects on diatom sediment stabilization (EPS production, panel A) and energy
2635 content (EFA production, panel B). Experimental communities were grown in control, low (200 µg/L) and
2636 high (500 µg/L) atrazine ('Atr') and copper ('Cu') treatments. Synthetic communities were computed from
2637 unstressed monocultures in the same community structure observed at each stress level. Significant
2638 differences in the EFA or EPS production of experimental communities from the control are indicated with
2639 asterisks (*).

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Process	Effect	Com	Treatment	Com	Treatment	Est	lwr	upr	Z	P
EPS	SE	Syn	Control	Syn	Atr, low	0.002	-0.05	0.05	0.15	1.000
					Atr, high	0.012	-0.04	0.05	0.78	0.998
					Cu, low	-0.026	-0.07	0.02	-1.68	0.765
					Cu, high	-0.026	-0.07	0.02	-1.65	0.779
	CD	Exp	Control	Syn	Control	0.047	-0.09	0.18	-1.05	0.984
					Atr, low	0.301	-0.16	0.44	6.64	<0.001
					Atr, high	-0.006	-0.15	0.13	-0.13	1.000
					Cu, low	0.311	0.17	0.45	6.86	<0.001
					Cu, high	0.006	-0.13	0.15	0.14	1.000
EFA	SE	Syn	Control	Syn	Atr, low	2.695	1.85	3.54	10.06	<0.001
					Atr, high	2.746	1.90	3.60	10.25	<0.001
					Cu, low	-0.173	-1.02	0.67	-0.65	0.999
					Cu, high	-0.381	-1.23	0.47	-1.42	0.921
	CD	Exp	Control	Syn	Control	-0.110	-0.96	0.74	0.41	1.000
					Atr, low	0.442	-0.41	1.29	1.65	0.823
					Atr, high	-0.554	-1.40	0.29	-2.07	0.550
					Cu, low	-0.237	-1.08	0.61	0.89	0.997
					Cu, high	-1.863	-2.71	-1.02	-6.95	<0.001

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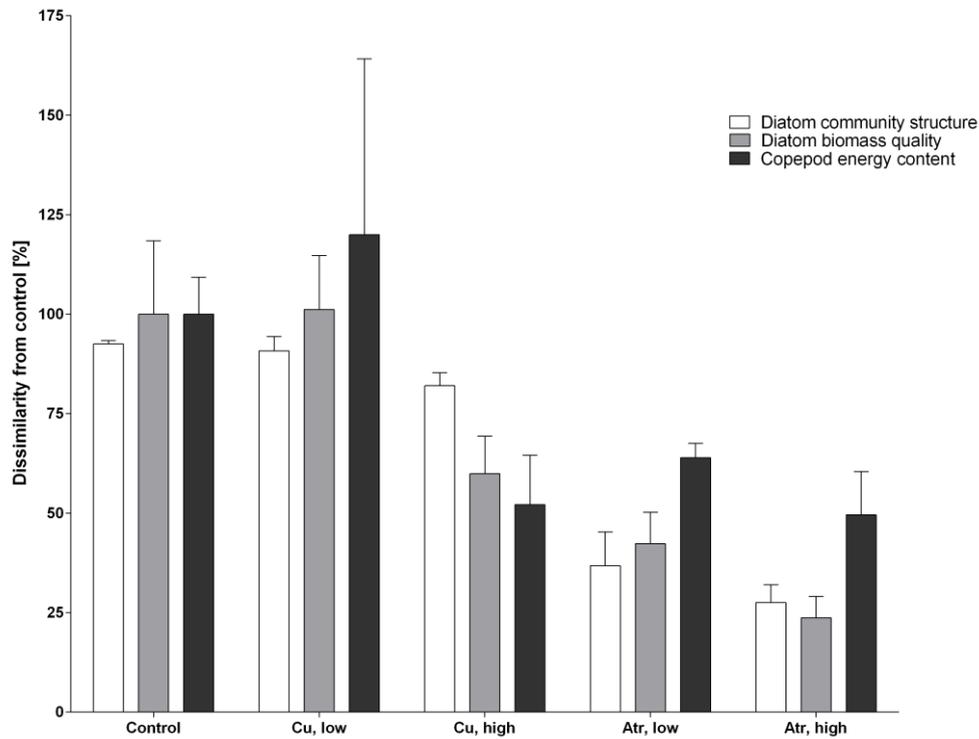
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Table 5.2: Pairwise comparisons of EFA and EPS production in treatments of experimental and synthetic diatom communities as estimated by generalised least squares model fits. 'Process' indicates to which response variable models were fitted (EFA or EPS production). 'Effect' indicates which type of stress is analyzed: Selective stress effects ('SE': comparison of synthetic communities reflecting the community structure under control and stress conditions), Context-dependent stress effects ('CD': comparison of stressed and synthetic communities of the same community structure). 'Com' ('Exp': Experimental and 'Syn': Synthetic) and 'Treatment' (low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu')) indicate which communities and treatments are compared. 'Est' indicates the difference in EFA and EPS production between the compared treatments as estimated by generalized least squares models fitted to Box-Cox transformed EFA and EPS data, 'lwr' and 'upr' indicate the lower and upper confidence intervals of the estimated difference. 'Z' and 'P' denote the z- and p-values corrected for multiple comparisons by the single-step method, bold values are statistically significant.

Diet quality effect on copepods. The energy content of *M. littorale* was related to the stressor-induced changes in diatom energy content (both stressors) and diatom community structure (atrazine only, Fig. 5.3, Table 5.3). Copepods maintained their control EFA content when feeding on diatoms from the low copper treatment, but lost half of their EFAs when feeding on diatoms grown under high copper stress (Fig. 5.3). This resulted in a positive correlation of copepod EFA content and diatom EFA content (Fig. 5.3, Table 5.3). When offered diatoms from the low and high atrazine treatments, copepods also lost half of their EFA content, which was predicted not only by the diatoms' EFA content, but also by the changes in diatom community structure (Fig. 5.3, Table 5.3). Addendum IV Fig. S3 shows the EFA content per diatom biomass and per copepod individual, as well as the relative proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In diatoms, the essential fatty acids consisted mainly of EPA, whereas DHA was the main component in copepods (EPA:DHA ratio 4 to 6 in diatoms, 0.3 to 0.6 in copepods, Addendum IV Fig. S3). The copepod survival rate in the experimental units was between 85-100%. The EFA

2670 content of copepods feeding on diatoms from control conditions (65.3 ± 6.1 ng copepod⁻¹) did not
 2671 differ significantly from copepods at the start of the experiment (i.e. animals collected in the field,
 2672 68.8 ± 11.8 ng copepod⁻¹, t-test, $p=0.99$, Addendum IV Fig. S3).

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2675 **Fig. 5.3:** Community structure and energy content (EFA content) of diatoms exposed to stress, and energy
 2676 content (EFA content) of *Microarthridion littorale* copepods after 10d of feeding on the respective diets,
 2677 visualised as percent dissimilarity from the corresponding control. The dissimilarity of diatom community
 2678 structure is calculated as the Bray-Curtis similarity to the control mean (see methods section). The
 2679 dissimilarity of diatom and copepod EFA content is calculated as fraction percentage of the EFA
 2680 concentration in treatment i and the mean (μ) EFA concentration in corresponding control c :
 2681 $(EFA_i/\mu EFA_c) \times 100$. Diatom communities were exposed to low (200 $\mu\text{g/L}$) and high (500 $\mu\text{g/L}$) atrazine
 2682 ('Atr') and copper ('Cu') concentrations, copepods were not exposed to any of the stressors.

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Stressor	Quality	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Atrazine	EFA	1	0.059	0.008	7.38	0.0002	28.46	-11.23	Yes	0.69	0.41
		2	0.060	0.007	9.06	<0.0001	29.77	-10.89	Yes		
	Comp	1	0.045	0.008	5.88	0.0006	31.69	-12.85	Yes	0.07	0.79
		2	0.045	0.008	5.64	0.0008	33.62	-12.81	Yes		
Copper	EFA	1	0.137	0.021	6.57	0.0003	35.21	-14.61	Yes	3.64	0.06
		2	0.133	0.014	9.17	<0.0001	33.57	-12.79	Yes		
	Comp	1	0.204	0.126	1.62	0.1486	44.82	-19.41	No	0.75	0.39
		2	0.071	0.150	0.48	0.6494	46.07	-19.03	Yes		

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Table 5.3: Results of generalized least squared models predicting copepod energy content (EFA content) from diatom community structure and energy content (EFA production). 'Stressor' denotes which diet the copepods were offered: diatom communities exposed to copper or atrazine. 'Quality' indicates the predictor: diet quality in terms of diatom community composition or EFA production. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) variance structure. 'Slope' indicates the relation between predictors and copepod energy content, i.e. the effect of diatom energy content and community structure on copepod energy content. 's.e.' 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, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change with the predictor. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

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2700 5.4. Discussion

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The outcome of our experiments demonstrates that chemical stress can stimulate the contribution of marine primary producers to sediment stabilization but reduces diet quantity and diet quality. Selective and context-dependent stress effects on diet quality were furthermore found to lead to concomitant knock-on effects at the consumer level.

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Biomass production is the most widespread functional endpoint in research investigating ecosystem functioning under anthropogenic change, and producer biomass (diet quantity) has been the emphasis of most trophic experiments (Arts and Wainmann 1999, Balvanera et al. 2006, Cardinale et al. 2011). The loss of diatom biomass induced by copper corresponds to previous findings on the toxicity of copper to marine diatoms (Stauber and Florence 1989, Cid et al. 1995, Pistocchi et al. 1997, Masmoudi et al. 2013). Surprisingly, diatom biomass was less affected by atrazine, although atrazine has been shown to reduce diatom biomass at concentrations lower than those used in our study (Bester et al. 1995, DeLorenzo et al. 1999, 2001, Magnusson et al. 2008). The capacity of diatom communities to maintain their biomass under atrazine exposure was related to a change in community composition, with *C. closterium* increasing its biomass in

2715 the presence of atrazine, and dominating all communities in the atrazine treatments. Atrazine
2716 blocks the electron transport chain of Photosystem II (PSII, Dorigo and Le Boulanger 2001). Some
2717 diatom species can however reduce their dependency on photosynthesis and thus their sensitivity
2718 to PSII inhibitors by mixotrophic growth (i.e. the uptake of organic substrates, Debenest et al.
2719 2009; Larras et al. 2014). *C. closterium* is capable of mixotrophic growth, which reduces its
2720 sensitivity to herbicide stress (Vanellander et al. 2009, Chapter 3). The presence of a mixotrophic,
2721 atrazine-tolerant species thus caused the changes in community composition and underpinned
2722 the limited loss of biomass under atrazine.

2723 Neither atrazine nor copper reduced the diatoms' contribution to sediment stabilization. On the
2724 contrary, EPS production doubled at low levels of both stressors. In our low atrazine and copper
2725 treatments, diatom EPS production was also double than expected in synthetic communities of
2726 the same community structure. The increase in EPS production thus resulted from context-
2727 dependent effects rather than from changes in community structure. The secretion of EPS by
2728 microphytobenthos does not only play a vital role for ecosystem functioning through the
2729 stabilization of sediment surfaces, but also represents a physiological response of benthic diatoms
2730 to various abiotic stressors (Pistocchi et al. 1997, Decho 2000, Staats et al. 2000a, Underwood and
2731 Paterson 2003, Levy et al. 2007). At sublethal stress levels, context-dependent stress effects are
2732 thus unlikely to cause a reduction in the diatom's contribution to sediment stabilization. Selective
2733 stress can reduce diatom EPS production, by causing dominance by tolerant species producing
2734 little amounts of EPS (Mensens et al. 2015). The dominant species in our atrazine, copper and
2735 control communities however showed little difference in their EPS output, which resulted in an
2736 absence of selective stress effects on EPS production.

2737 Selective atrazine stress determined the energy content of diatom communities, which lost 60%
2738 and 75% of their essential fatty acids at low and high levels of the pesticide. The same extent of
2739 energy loss was observed in synthetic communities which reflected the community structure
2740 under atrazine without actual exposure to the pesticide. Conversely, the energy content in
2741 atrazine-exposed experimental communities and unexposed synthetic communities did not differ.
2742 The energy loss under atrazine was thus caused by selective changes in community structure
2743 rather than by context-dependent atrazine effects on diatom energy content. This selective
2744 atrazine stress was underpinned by the dominant species *C. closterium*, which produced eight and
2745 three times less EFAs than the species contributing the most biomass under control conditions (*N.*
2746 *acicularis* and *N. arenaria* respectively, see fatty acids per species in Addendum IV Table S1). In
2747 the presence of copper, communities were dominated by the same lipid-rich species as in the
2748 control, and no such selective effects on energy content were observed. The loss of diatom energy
2749 content at high copper levels was caused by context-dependent rather than selective copper

2750 effects, which was reflected in the lower energy content in experimental compared to synthetic
2751 communities. Context-dependent effects also likely caused the further loss of EFAs at high
2752 compared to low atrazine stress, since community structure at the two herbicide levels did not
2753 differ. This loss of energy content at high levels of both stressors could be due to physiological
2754 stress effects such as an alteration of photosynthesis and thus of the carbon supply for fatty acid
2755 synthesis, an inhibition of the enzymes involved in lipid biosynthesis or an increase in the degree
2756 of fatty acid saturation, which are all reported to reduce the EFA production of microalgae under
2757 metal and pesticide stress (Böger et al. 2000, Guschina and Harwood 2006, Chia et al. 2013a).

2758 Our results however highlight selective stress as the main driver of primary producer energy
2759 content. Selective atrazine stress caused a more important loss of diatom energy content than the
2760 context-dependent effects of both stressors, at chemical concentrations where no context-
2761 dependent effects on individual fatty acid content were recorded. The high and low copper and
2762 atrazine concentrations used in our experiments are respectively far above field concentrations,
2763 or have only been recorded in extreme pollution events or at chronically contaminated sites
2764 (Millward and Grant 2000, Graymore et al. 2001, Pennington et al. 2001, Lockert et al. 2006).
2765 Apart from scenarios of severe chemical pollution, pesticide and metal stress are thus unlikely to
2766 reduce the energy flow in intertidal systems by inhibiting algal EFA synthesis. Conversely,
2767 chemical pollution can cause shifts in algae community structure at stress levels lower than those
2768 used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010). The impact of such
2769 selective chemical stress on ecosystem functioning will largely depend on the functional
2770 importance of the most stress-tolerant species (Larsen et al. 2005, Mensens et al. 2015, Radchuk
2771 et al. 2016). Not only pollution, but also long-term changes in environmental variables are causing
2772 considerable shifts in microalgae community structure (Pomati et al. 2012, Litchman et al. 2015).
2773 Due to the pronounced differences in lipid profiles within and among algal classes (Dunstan et al.
2774 1993, Taipale et al. 2013, Guo et al. 2016), these changes in community structure rather than
2775 context-dependent changes in algal energy content represent the potentially stronger driver of
2776 trophic energy flow under anthropogenic change.

2777 The energy content of the copepod *M. littorale* closely tracked that of its diets. Selective and
2778 context-dependent stress effects on diatom energy content resulted in a concomitant loss in the
2779 energy content of their main copepod grazer, confirming algal EFAs as a key component of diet
2780 quality which is directly linked to trophic energy transfer. For diatoms exposed to atrazine, the
2781 response of *M. littorale* was predicted by both its diet's energy content and community structure.
2782 Energy content in terms of fatty acids is the most common measure of diet quality (Jodice et al.
2783 2006, Österblom et al. 2008, Taipale et al. 2013, Guo et al. 2016), but requires extensive analyses
2784 of a diet's biochemical composition. For stressors causing selective shifts in community structure,

2785 effects on trophic energy transfer can thus more straightforwardly be estimated from the species
2786 dominating under stress, given that the nutritional value of these species is known.

2787 However, our results have to be treated carefully due to the limited number of stress levels and
2788 replicates. To obtain a more robust correlation of the consumer response to stressor-induced
2789 changes in diet quality, models should be fitted to gradients containing more than three
2790 concentrations per stressor and more than three replicates per treatment. Also, offering *M.*
2791 *littorale* preserved rather than live diatom food might have influenced food uptake. Freeze-drying
2792 does not alter the biochemical composition within diatoms cells, but modifies the exterior of
2793 diatom cells through the loss of exudates (e.g. EPS) or bacteria associated to the diatom frustule,
2794 which can affect the ingestion of diatoms by harpacticoid copepods (Cnudde et al. 2011). Feeding
2795 *M. littorale* live diatom cultures under unstressed conditions would however have resulted in a
2796 dissimilar diatom community structure than that induced by the stressors: atrazine and copper
2797 did not eliminate any of the diatom species, but caused alterations of community evenness, which
2798 could not be maintained when diatoms were grown in the absence of the stressors.

2799 The DHA:EPA ratio of *M. littorale* was higher than in the diatom communities, which corresponds
2800 to previous findings on the relative concentrations of both essential fatty acids in copepods and
2801 their algal diets (De Troch et al. 2012a, Arndt and Sommer 2014). Diatoms are characterised by a
2802 high EPA content (Taipale et al. 2013, Guo et al. 2016), but DHA appears to be the most important
2803 fatty acid for copepods (Taipale et al. 2013). Planktonic primary consumers such as cladocerans
2804 or calanoid copepods directly depend on the DHA taken up from their diet (Bell et al. 2007, Bell
2805 and Tocher 2009, De Troch et al. 2012a), but several harpacticoid copepod species are able to
2806 bioconvert EPA to the longer chain DHA, a capacity which has notably been demonstrated in *M.*
2807 *littorale* (De Troch et al. 2012a). While the total EFA content of *M. littorale* reflected that of its
2808 different diatom diets, this capacity to convert EPA to DHA likely enabled *M. littorale* to maintain
2809 high relative levels of DHA.

2810 Since their EFA content ranks among the highest of all algae classes, diatoms are regarded as high-
2811 quality food source and crucial link for the energy flow at the basis of aquatic food webs (Brett
2812 and Müller-Navarra 1997, Taipale et al. 2013, Guo et al. 2016). Here, diatom diet quality
2813 responded more sensitively to chemical stress than the diatom's contribution to habitat structure
2814 (sediment stabilization) and diet quantity. The selective and context-dependent stress effects on
2815 diatom diet quality were caused by the large interspecific differences in energy content and the
2816 loss of diatom energy content under stress. Indeed, the energy content of benthic diatoms shows
2817 more interspecific variation and is more affected by chemical pollutants than their contribution
2818 to diet quantity or sediment stabilization (Mensens et al. 2015, Chapter 3).

2819 Losses in diet quality occurred at copper concentrations which were close to the LC50 value of *M.*
2820 *littorale* (Addendum V). Next to indirect effects on copepod energy content through changes in
2821 diet quality, high metal stress can thus be expected to reduce copepod abundance through direct
2822 toxic effects. Conversely, atrazine changes microalgal community structure at concentrations
2823 lower than the 200 µg/L used in our study (Bérard and Benninghoff 2001, Debenest et al. 2010),
2824 whereas direct acute effects of atrazine on copepods and other marine invertebrates mostly occur
2825 at >1 mg/L atrazine (Hall et al. 1995, Bejarano and Chandler 2003). Herbicides can thus first affect
2826 marine primary consumers indirectly through selective changes in the structure of their diet
2827 rather than through direct effects on the consumers themselves. The loss of diet quality did not
2828 result in increased harpacticoid mortality. Low diet quality rarely causes acute copepod mortality,
2829 but reduces copepod energy content and, on a longer term, growth rate and reproduction (Müller-
2830 Navarra 1995, Müller-Navarra et al. 2000, Arendt et al. 2005, Gonçalves et al. 2011). The energy
2831 content of copepods is crucial for their main consumers, especially larval fish whose development
2832 can depend on the EFAs taken up from their copepod prey (Sargent et al. 1995, Payne et al. 1998).
2833 Rather than acutely eliminating consumers, the main risk of losses in producer diet quality thus
2834 consists in reducing the energy transfer at the plant-animal interface, which is a key limiting factor
2835 for the functioning of marine ecosystems (Brett and Müller-Navarra 1997, De Troch et al. 2012b).
2836 Nonetheless, the importance of algal diet quality, as distinct from just diet quantity, is rarely
2837 highlighted in research on food web functioning (Guo et al. 2016). Due to its sensitive response to
2838 selective stress, algal diet quality in terms of EFA production and community structure provides
2839 a powerful approach to integrate our understanding of coastal ecosystem functioning under
2840 anthropogenic change.

2841 In conclusion we infer three statements from our results. First, chemical stress differentially
2842 affects the contribution of marine primary producers to diet quantity, sediment stabilization and
2843 diet quality, with diet quality being most sensitive. Second, the loss of diet quality is underpinned
2844 by selective and context-dependent stress effects. Selective stress causes a more important loss of
2845 diet quality at lower stress levels than context-dependent stress effects, and thus represents the
2846 main risk for energy flow at the basis of marine food webs. Third, changes in producer diet quality
2847 closely predict consumer energy content. The integration of diet quality within traditional studies
2848 of diet quantity is recommended to assess energy flow in marine food webs under anthropogenic
2849 change.

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2851 **6. General discussion**

2852 Since the first BEF experiments were conducted in the 1990s (Naeem et al. 1994, Tilman and
2853 Downing 1994, Tilman et al. 1996, Hector et al. 1999), nearly a thousand studies have examined
2854 the functional role of biodiversity in terrestrial, freshwater and marine ecosystems (Covich et al.
2855 2004, Cardinale 2011, Cardinale et al. 2012, Gamfeldt et al. 2015). After initially controversial
2856 discussions (van der Heijden et al. 1999, Wardle 1999, Hector et al. 2000, Huston et al. 2000),
2857 consensus is now emerging that declines in biodiversity have negative consequences for
2858 ecosystem functioning (for quantitative data syntheses see Worm et al. 2006, Balvanera et al.
2859 2006, Cardinale et al. 2006, 2011, 2012, Stachowicz et al. 2007, Finkel et al. 2010, Quijas et al.
2860 2010, Hooper et al. 2012, Tilman et al. 2014, Gamfeldt et al. 2015, Hautier et al. 2015). In addition,
2861 BEF research has generated new ecological and mathematical theory (Tilman et al. 1997, Loreau
2862 and Hector 2001, Pacala and Kinzig 2002, Fox 2005), provided decision support tools for
2863 policymakers (Naidoo and Ricketts 2006, Kareiva et al. 2011, InVEST 2016), and led to the
2864 establishment of intergovernmental initiatives to preserve global biodiversity (CBD 2010,
2865 Larigauderie and Mooney 2010, IPBES 2016).

2866 Despite this progress, early (Hooper et al. 2005) and recent (Cardinale et al. 2012, Tilman et al.
2867 2014, De Laender et al. 2016) BEF consensus papers continue to highlight the need to include the
2868 environmental drivers of biodiversity loss into the next generation of BEF experiments.
2869 Contrasting BEF relations under stress with the classic BEF protocol of random species loss, and
2870 the identification of response and effect traits capable of predicting biodiversity and ecosystem
2871 functioning under stress, have been identified among the most urgent need to explore the
2872 functional consequences of realistic scenarios of biodiversity change (Suding et al. 2008, Naeem
2873 2008, Cardinale et al. 2012, Tilman et al. 2014, Wardle 2016).

2874 This dissertation, together with other recent efforts to include stressors into BEF theory (see
2875 further), has shown that stress can profoundly alter the biodiversity effect on ecosystem
2876 functioning. Diversity loss under stress is not **random**, but **selective** according to the species'
2877 stress tolerance, and the functional contribution of the most tolerant species plays a key role for
2878 functioning under stress. BEF relations in diatom communities under atrazine were steeper than
2879 predicted by the classic random design, since the herbicide inhibited the growth of the species
2880 (*Nitzschia* sp.) contributing most to energy content and sediment stabilization, and communities
2881 were dominated by a tolerant species (*N. arenaria*) contributing little to both processes (Chapter
2882 2). Theory predicts that under stress, the abundance and functional contribution of species can be
2883 predicted from their biological traits. The numerical and functional response of diatoms to copper
2884 stress was related to the same set of **response and effect traits** (cell size and surface-to-volume

2885 ratio), whilst the diatoms' capacity for mixotrophic growth predicted the numerical but not the
2886 functional stress response to atrazine (Chapter 3). **Complementarity**, the main statistical effect
2887 used to explain the better performance of species in community, has rarely been quantified under
2888 stress and it is unclear what **mechanisms** underpin potential changes in complementarity.
2889 Complementarity in diatom communities increased under atrazine and copper stress, driven by
2890 **facilitation** (an increased release of extracellular polymers). Complementarity benefited the
2891 growth of mixotrophic species (e.g. *C. closterium*) under atrazine, and of copper-sensitive species
2892 with low biomass yields (e.g. *A. lineolata*) under metal stress, reducing the net diversity effect on
2893 biomass yield in the latter case (Chapter 4). There is little evidence whether stressors drive
2894 functioning through **selective effects** (changes in community structure) or **context-dependent**
2895 **effects** (changes in the species' functional contribution), and if both types of stress knock on
2896 across **trophic levels**. Selective atrazine effects, leading to dominance by a mixotrophic but lipid-
2897 poor species (*C. closterium*), caused a 60% loss of diatom energy content at low atrazine levels.
2898 Context-dependent stress effects only affected diatom functioning at high atrazine and copper
2899 levels. Both types of stress affected the energy transfer to the next trophic level, causing a
2900 concomitant loss in the energy content of their main copepod consumer (*M. littorale*, Chapter 5).

2901 Based on these findings, three main effects that potentially drive functioning in stressed
2902 communities can be put forward. This discussion will first focus on **selective stress** effects, i.e.
2903 shifts in community structure causing stressed communities to be dominated by species with an
2904 above- or below-average functional contribution. Subsequently, context-dependent stress effects
2905 on the species' functional contribution will be addressed, which can be mediated by **physiological**
2906 **stress** effects and changes in **complementarity**, i.e. differential interspecific interactions in
2907 stressed communities. These three effects are not inherently exclusive, could not always be
2908 separated, and will in most cases simultaneously determine the impact of stressor-induced
2909 biodiversity changes on ecosystem functioning. Nevertheless, the most important stressor-
2910 induced changes in the contribution of diatom communities to ecosystem functioning, including
2911 concomitant effects at the consumer level, can be interpreted with regard to these three effects.
2912 Each effect is first discussed (sections 6.1 to 6.3) and then examined with regard to its impact on
2913 three ecosystem processes (biomass production, sediment stabilization and energy content¹,
2914 section 6.4), before presenting future challenges for BEF research which arise from the outcome
2915 of this work (section 6.5).

¹ Energy content was quantified as polyunsaturated fatty acids (PUFAs, fatty acid molecules with more than one double bond) in Chapters 2 and 3, and as essential fatty acids (EFAs, the two most important PUFAs for animal physiology: EPA and DHA) in Chapter 5. For the sake of simplicity, this chapter only uses the terms 'energy content' and 'fatty acids'.

2916 **6.1. Selective stress**

2917 Selective stress (Fig. 6.1.1) can be defined as a change in environmental conditions which affects
2918 ecosystem functioning by selectively targeting species with a high or low functional contribution
2919 (Chapter 1.4.2). Random species loss is the classic protocol in BEF research (Naeem 2008,
2920 Cardinale et al. 2012, Wardle 2016). Theory and simulations however predict stress to cause non-
2921 random scenarios of biodiversity loss, where species are targeted selectively according to their
2922 stress tolerance, and the functional contribution of the most tolerant species plays a key role for
2923 the overall functioning in stressed communities (Solan et al. 2004, Bunker et al. 2005, Cardinale
2924 et al. 2012).

2925 Here, stressor-induced changes in diatom community structure had little effect on the biomass
2926 production of stressed diatom communities, which depended on complementarity (see 6.3) rather
2927 than on the biomass production of the dominant species (Chapter 4). Selective atrazine effects
2928 reduced the contribution of stressed diatom communities to sediment stabilization, by causing
2929 dominance of an atrazine-tolerant species (*N. arenaria*) which however produced little EPS
2930 (Chapter 2). When stress-sensitive and stress-tolerant species did not differ in their functional
2931 contribution, selective changes in community structure however had no effect on the contribution
2932 of stressed diatom communities to sediment stabilization (Chapter 5). The strongest selective
2933 stress effects were recorded in terms of diatom energy content. Selective atrazine effects inhibited
2934 the growth of a lipid-rich species (*Nitzschia* sp.), causing a disproportionate reduction in the
2935 energy content of diatom communities exposed to the herbicide, which was not predicted by the
2936 classic design of random species removal (Chapter 2). Similarly, atrazine induced a shift in diatom
2937 community structure from dominance by a lipid-rich species (*N. acicularis*) under control
2938 conditions towards dominance by a highly mixotrophic, but lipid-poor species (*C. closterium*)
2939 under atrazine (Chapter 5). This selective shift in community structure caused a 60% loss in the
2940 energy content of diatom communities under atrazine, which furthermore knocked on to the next
2941 trophic level and reduced copepod energy content by half, despite invariant diatom biomass and
2942 in the absence of any context-dependent stress effects. Selective atrazine stress thus represented
2943 the main risk for energy flow at the producer-consumer interface. Conversely, no selective copper
2944 effects on diatom energy content were detected, since exposure to the metal did not change
2945 diatom community structure (Chapter 5).

2946 Moreover, BEF research has been sparked by concerns about the loss of species and, with few
2947 exceptions (e.g. Balvanera et al. 2005, Wittebolle et al. 2009), the majority of BEF experiments
2948 have manipulated diversity in terms of species richness, holding evenness constant across
2949 richness treatments (Hillebrand et al. 2008, Hillebrand and Matthiessen 2009, Zhang et al. 2012).
2950 However, critics often assert that anthropogenic stress is more likely to alter evenness than

2951 richness, with changes in evenness potentially affecting ecosystem functioning before species are
2952 driven to extinction (Chapin et al. 2000, Balvanera et al. 2005, Hillebrand et al. 2008). Here,
2953 atrazine and copper consistently altered evenness, but did not cause any species loss, highlighting
2954 the need to investigate diversity loss not only in terms of species extinctions, but also altered
2955 community structure. More importantly, when changes in evenness involved the dominance of
2956 species with low functional contribution, loss of function was more important than could be
2957 predicted from randomly composed communities (Chapter 2).

2958 Selective biodiversity loss can in theory cause more rapid or slower declines in ecosystem
2959 functioning compared to random loss scenarios (Ives and Cardinale 2004, Solan et al. 2004,
2960 Bunker et al. 2005, Gross and Cardinale 2005), and experimental evidence suggests the functional
2961 impact of selective stress depends on the stress tolerance of those species contributing most to
2962 functioning (Larsen et al. 2005, McIntyre et al. 2007, Ball et al. 2008, Bracken et al. 2008, Davies
2963 et al. 2011, Radchuk et al. 2016). For instance, diversity loss caused by accelerated wave exposure
2964 and overfishing reduced nutrient cycling in macroalgae and fish communities respectively to a
2965 larger extent than expected from random diversity loss (McIntyre et al. 2007, Bracken et al. 2008).
2966 In both cases, wave stress and fishermen targeted the species contributing most to nutrient
2967 cycling. In the terrestrial environment, habitat loss caused a disproportionate loss of pollination,
2968 because large-bodied insects contributing most to pollination were most sensitive to this type of
2969 stress (Larsen et al. 2005). Conversely, insecticide stress had no effect on ecosystem processes in
2970 an aquatic food web, since the insecticide only targeted species contributing little to functioning,
2971 and the functionally most important species could maintain their functional contribution under
2972 stress (Radchuk et al. 2016).

2973 Suding *et al.* (2008) and Hillebrand and Matthiessen (2009) highlighted the need for BEF research
2974 to consider the functional consequences of selective biodiversity loss, by proposing a trait-based
2975 framework which connects species traits that influence the stress tolerance ('response traits') to
2976 species traits that influence ecosystem functioning ('effect traits'). Although experimental tests of
2977 this framework are still rare (but see Heuner et al. 2015, Lennon and Lehmkuhl 2016), relating
2978 response and effect traits has been identified as a key issue to estimate ecosystem functioning
2979 under stress (Cardinale et al. 2012, Wardle 2016).

2980 Here, the response-effect trait relationship was different depending on stressor type. The capacity
2981 of diatom species to withstand copper effects on density ('numerical response') and on functional
2982 contribution ('functional response', in terms of photosynthetic efficiency, energy content and
2983 sediment stabilization) was predicted by the same set of morphological traits (cell size and surface
2984 to volume ratio). Species which were numerically tolerant to copper contributed most to

2985 functioning under unstressed conditions, and performed best at maintaining functioning when
2986 exposed to the metal, indicating that tolerant species might be able to sustain functioning under
2987 copper stress. A physiological trait (mixotrophy) predicted the diatoms' numerical response to
2988 atrazine, but not their functional response. Numerically tolerant diatoms did not contribute most
2989 to functioning, and the numerical and functional response to atrazine were not correlated,
2990 indicating a limited capacity of tolerant species to maintain functioning under atrazine (Chapter
2991 3).

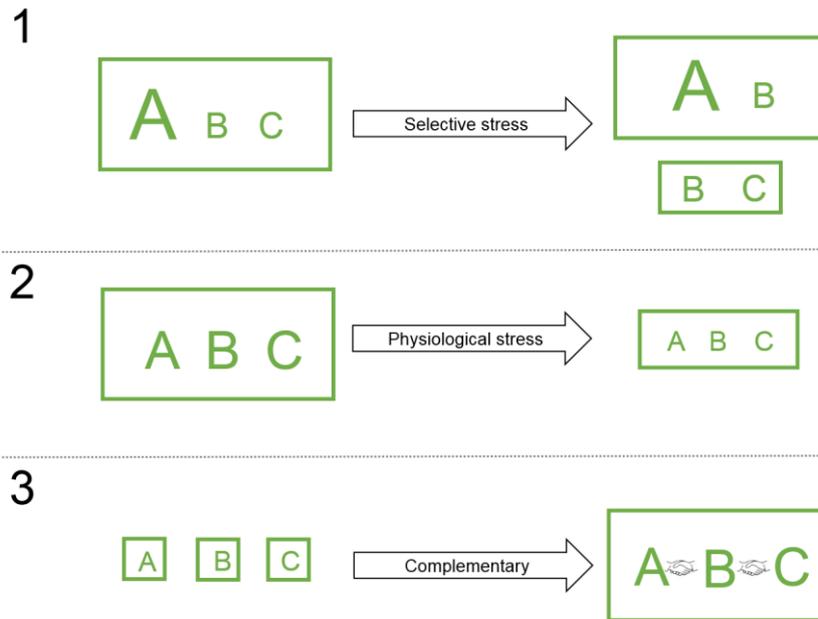
2992 The diatoms' numerical stress response to atrazine and copper corresponded to the community
2993 structure observed under both stressors: copper-exposed diatom communities were dominated
2994 by *Entomoneis* sp. and *N. acicularis* (Chapters 4 & 5) which were among the numerically and
2995 functionally most tolerant species to the metal. Consequently, the functioning of diatom
2996 communities was only affected at high (500 µg/L) copper concentrations. Atrazine-exposed
2997 diatom communities were dominated by two species which were numerically tolerant to the
2998 herbicide (*N. arenaria* and *C. closterium*). These species contributed seven times less to sediment
2999 stabilization and respectively fifteen and forty times less to energy content than the atrazine-
3000 sensitive species which had dominated communities under unstressed conditions (*Nitzschia* sp.
3001 and *N. acicularis*, Chapter 2 and 5), causing the negative selective atrazine effects on diatom
3002 functioning (see above). *Entomoneis* sp. and *C. closterium* were the most copper- and atrazine-
3003 tolerant species among a set of 17 diatom species, and were characterized by the largest cell size
3004 and lowest surface-to-volume-ratio, and by the highest capacity for mixotrophic growth
3005 respectively (Chapter 3).

3006 Whilst the above examples confirm the potential of traits to predict changes in community
3007 structure and their effect on functioning under stress (Hillebrand and Matthiessen 2009), this
3008 trait-based approach could not consistently predict community structure under stress: For
3009 instance, *N. arenaria* reached high relative abundances in atrazine-exposed communities (Chapter
3010 2), despite having no capacity for mixotrophic growth (Chapter 3). Similarly, the good
3011 performance of *N. acicularis* in copper-exposed communities (Chapter 5) was not predictable
3012 from its traits, since this species was neither characterized by a large cell size nor by a low surface
3013 to volume ratio (Chapter 3). Selective stress effects on diatom community structure and
3014 functioning were thus consistently predicted by the species' tolerance (EC50 values), but not
3015 always by the species' traits, arguably because this work did not include other traits relevant to
3016 microalgal stress tolerance. Such traits involve metal-binding metallothioneins and
3017 phytochelatin, or the structure of the quinone binding site in photosystem II, which determine
3018 algal tolerance to copper and atrazine respectively (Galloway and Mets 1984, Gekeler et al. 1988,
3019 Robinson 1989, Fernandes and Henriques 1991, Galgani et al. 1999). Furthermore, stress

3020 tolerance in monoculture, which was predicted by response traits, could be decoupled from stress
3021 tolerance in community when species benefited differentially from complementarity. For
3022 example, in the diatom communities in Chapter 4, *A. lineolata* was the least copper-tolerant
3023 species in monoculture, which could be expected based on its response traits (smallest cell size,
3024 largest surface to volume ratio of all component species). *A. lineolata* however benefited most
3025 from an increase in complementarity and thus performed well in communities exposed to the
3026 metal (but see 6.3), thus limiting the capacity of response traits to predict community structure
3027 under stress.

3028 Nevertheless, the identification of key morphological and physiological traits determining, in part,
3029 the structure and functioning of primary producer communities under stress, highlighted the
3030 value of trait-based approaches to predict the consequences of selective stress for ecosystem
3031 functioning. In ecotoxicology, approaches relating traits to stress tolerance have been tested
3032 (Baird and Van den Brink 2007, Rubach et al. 2012, Larras et al. 2014) and trait-based bioindicator
3033 tools are being used to link the pesticide load in European waters to the structure of aquatic
3034 communities (Liess and Von Der Ohe 2005, Beketov et al. 2009). In BEF research, the theoretical
3035 framework for the incorporation of trait-based approaches has been set (Suding et al. 2008,
3036 Hillebrand and Matthiessen 2009, Litchman et al. 2015). Methodological advances now allow for
3037 time-efficient quantifications of traits at the individual level (Fontana et al. 2014), and recent
3038 experimental approaches have used response and effect traits to understand the structure and
3039 functioning of communities under stress (Koide et al. 2014, Heuner et al. 2015, Zwart et al. 2015,
3040 Lennon and Lehmkuhl 2016). These theoretical and methodological advances should allow to
3041 progressively complement the classic design of random species loss with trait-based approaches
3042 that more realistically reflect community structure and ecosystem functioning under
3043 anthropogenic change.

3044



3045

3046 **Fig. 6.1:** Scheme indicating three effects which potentially drive functioning in stressed communities. For
 3047 the sake of simplicity, this scheme uses communities with only three species (A, B and C). The font size of A,
 3048 B and C indicates the species' functional contribution. Communities are marked by green boxes, with the
 3049 size of the box marking the community's functional contribution. **Panel 1** shows **selective stress** (for
 3050 details see Chapter 1.4.2), which occurs when the species contributing most to functioning are also least
 3051 stress-tolerant. The example community in Panel 1 includes a species with a high functional contribution
 3052 (species A) and two species with a low functional contribution (species B and C). Selective stress has limited
 3053 effect on community functioning, if the stressor does not remove species A, which contributes most to
 3054 functioning (upper community on the right-hand side of panel 1). Selective stress disproportionately
 3055 reduces community functioning, if the stressor removes species A (lower community on the right-hand side
 3056 of panel 1). **Panel 2** shows **physiological stress** (for details see Chapter 1.4.3), which affects ecosystem
 3057 functioning when stress alters the species' functional contribution. For the sake of simplicity, Panel 2
 3058 indicates a community in which the species, A, B and C contribute equally to functioning, with each species'
 3059 functional contribution altered to the same extent by stress. **Panel 3** shows **complementarity** (for details
 3060 see Chapter 1.6), which occurs when species contribute more to functioning when grown in community
 3061 than in monoculture. Facilitation and niche partitioning are the main mechanisms driving complementarity.
 3062 In contrast to selective and physiological stress, complementarity is not a 'stress effect', and can operate
 3063 under stressed and unstressed conditions. The example in Panel 3 shows three species A, B and C with a
 3064 low functional contribution when being exposed to stress in monoculture, and an increased functional
 3065 contribution when being exposed to stress in community due to facilitative interactions.

3066

3067 6.2. Physiological stress

3068 Physiological stress can be defined as stress which affects ecosystem functioning by directly
 3069 altering the species' functional contribution (Fig. 6.1.2). Direct stress exposure to anthropogenic
 3070 stressors is rarely included into the design of BEF experiments (McMahon et al. 2012, Steudel et
 3071 al. 2012), which remove species to mimic stressed ecosystems, but assume that the functional
 3072 contribution of the species in these low-diversity-communities remains unchanged. However,
 3073 stressors that cause species loss can impose a physiological cost on the surviving species, e.g.
 3074 acclimation by allocating resources from growth to survival pathways, which can affect the
 3075 contribution of stressed communities to ecosystem functioning (Schimel et al. 2007, Hawlena and

3076 Schmitz 2010). This has resulted in calls to consider sublethal physiological effects on the species'
3077 functional contribution, when estimating ecosystem functioning under stress (Relyea and
3078 Hoverman 2006).

3079 In monoculture, atrazine and copper reduced diatom growth and thus biomass production
3080 (Chapters 3 & 4). The main physiological mechanisms through which atrazine and copper affect
3081 diatom growth comprise an inhibition of electron transport in photosystem II (atrazine, Dorigo
3082 and Leboulanger 2001) and the damage of cell membranes, thylakoids and chloroplasts (copper,
3083 Knauert and Knauer 2008, for more details see Chapter 1.8.1). Conversely, most diatom species
3084 increased their contribution to sediment stabilization by producing more EPS at low atrazine and
3085 copper levels (Chapter 3). At high levels of both stressors diatoms could not maintain their EPS
3086 production (Chapter 3). As well as playing an important functional role in sediment stabilization
3087 (De Brouwer et al. 2000, Decho 2000), the excretion of EPS also represents a physiological stress
3088 response of benthic diatoms to metal, nutrient and salinity stress (Pistocchi et al. 1997, Liu and
3089 Buskey 2000, Staats et al. 2000a, Levy et al. 2007). However, diatoms cannot maintain their EPS
3090 production when photosynthesis is inhibited (e.g. by herbicides, Staats et al. 2000b), which can
3091 explain the loss of EPS at high stress levels. Diatom energy content was consistently reduced by
3092 both stressors, with most diatom species producing less fatty acids at stress levels where their
3093 growth was still unaffected (Chapter 3). Physiological pesticide and metal effects that can lead to
3094 such losses in algal energy content include a reduced carbon supply for fatty acid synthesis (when
3095 photosynthesis is inhibited), the conversion of unsaturated to saturated fatty acids, or the
3096 inhibition of enzymes involved in fatty acid synthesis (Böger et al. 2000, Guschina and Harwood
3097 2006, Chia et al. 2013a, 2013b).

3098 To what extent did these physiological effects impact the contribution of stressed diatom
3099 communities to ecosystem functioning? Linking physiological responses to environmental stress
3100 to processes at the ecosystem level has been a major theme in plant ecology (Grime 1977, Chapin
3101 et al. 2002). The few BEF experiments conducted under stressful conditions could not disentangle
3102 whether changes in ecosystem functioning were mediated by biodiversity loss, or by effects of
3103 environmental disturbance on the species' functional contribution (Steudel et al. 2012, Radchuk
3104 et al. 2016, but see Fox and Harpole 2008, Baert et al. 2016).

3105 Stressors can affect ecosystem functioning through selective biodiversity loss (e.g. by targeting
3106 species with a high functional contribution, see 6.1), or through 'context-dependent effects', i.e.
3107 changes in the species' functional contribution (Fox 2006, Fox and Harpole 2008, Tylianakis et al.
3108 2008, Hiddink et al. 2009). These changes in functional contribution can arise from direct effects
3109 of environmental drivers (Fox and Harpole 2008), i.e. the here-described physiological effects, as

3110 well as from species interactions (e.g. a change in complementarity, Fox 2006, Fox and Harpole
3111 2008)

3112 Here, selective and context-dependent stress effects could be disentangled by contrasting
3113 functioning (energy content and sediment stabilization) in stressed diatom communities with that
3114 expected from unstressed monocultures (Chapters 2 & 5). The increased contribution to sediment
3115 stabilization (EPS production) of diatom communities under copper and atrazine was driven by
3116 positive context-dependent effects (Chapter 5). Since physiological effects of both stressors also
3117 increased the EPS production of most diatom species in monoculture (see above), it can be
3118 assumed that the increased EPS production of stressed diatom communities represented a direct
3119 physiological stress response. Nevertheless, the extent to which species interactions contributed
3120 to this increased EPS production under stress could not be quantified. No selective stress effects
3121 on EPS production were recorded when species did not differ in their functional contribution
3122 (Chapter 5). When species however differed in their functional contribution and selective stress
3123 caused dominance by an unproductive species (*N. arenaria*, Chapter 2), the resulting decline in
3124 EPS production could not be compensated by any context-dependent effects.

3125 Context-dependent stress effects generally reduced diatom energy content, with the fatty acid
3126 production of most species being reduced at stress levels which did not affect other processes. In
3127 Chapter 5, this loss of diatom energy content also knocked on to the consumer level and caused a
3128 concomitant loss in copepod energy content. However, compared to selective stress effects,
3129 context-dependent effects were of minor importance for diatom energy content. By promoting
3130 dominance of tolerant but lipid-poor species, selective atrazine effects caused a more important
3131 loss of diatom energy content than the context dependent effects of both stressors, at chemical
3132 concentrations where no context dependent effects on diatom energy content were recorded. At
3133 atrazine levels which, on average, reduced diatom fatty acid content by half (150 µg/L, see EC50
3134 values in Chapter 3), the herbicide caused a shift in diatom community structure involving
3135 dominance by tolerant species (*C. closterium* and *N. arenaria*) producing fifteen and forty times
3136 less fatty acids than those dominating under unstressed conditions (*Nitzschia* sp., *N. acicularis*).

3137 When species showed large differences in their functional contribution, selective stress effects via
3138 changes in community structure were thus a more potent threat to ecosystem functioning than
3139 stress effects on the species' functional contribution. Previous work also found that whilst
3140 physiological stress effects commonly affect ecosystem processes on the short term, selective
3141 changes in community structure can have strong long-term effects on functioning, which prevail
3142 after the stressful conditions (Schimel et al. 2007). Moreover, physiological stress effects on the

3143 functional contribution of diatoms in monoculture could, in part, be compensated by positive
3144 species interactions in stressed communities, as will be discussed in the next section.

3145 **6.3. Complementarity**

3146 Complementarity effects occur when species contribute more to functioning in community than
3147 in monoculture (Fig. 6.1.3), and result from ecological mechanisms such as niche partitioning or
3148 facilitative interactions between species (Loreau and Hector 2001, Fox 2005). Experimental
3149 evidence linking these mechanisms to complementarity effects is however still scarce, and the
3150 extent to which these mechanisms broadly contribute to ecosystem functioning has yet to be
3151 confirmed (Vanellander et al. 2009, Carroll et al. 2011, Cardinale et al. 2011, 2012). Two-way
3152 complementarity occurs when niche partitioning or facilitative interactions benefit all species,
3153 whilst one-way complementarity occurs if these mechanisms are not vice-versa and favour
3154 species with a high or low functional contribution (Fox 2005, Hector et al. 2009).

3155 As such, complementarity is not a 'stress effect', but operates under unstressed conditions, and
3156 has been in the focus of major debates in BEF research (Huston 1997, Huston et al. 2000, Cardinale
3157 et al. 2006, Fargione et al. 2007, Hodapp et al. 2016). Most of these debates have centred on
3158 whether biodiversity effects on ecosystem functioning are driven by complementarity effects, or
3159 by the competitive dominance of single, highly productive species (dominance effects, but see
3160 Chapter 1.6 and Loreau and Hector 2001, Fox 2005). Over the last decade, BEF research has shown
3161 that complementarity and dominance effects are not inherently exclusive and jointly control
3162 ecosystem functioning (Hector et al. 2009, Cardinale et al. 2012, Hodapp et al. 2016). On average
3163 over many ecosystems, each effect contributes roughly 50% of the biodiversity effect on
3164 functioning, whilst complementarity appears to be the main effect driving BEF relations in aquatic
3165 ecosystems (Cardinale et al. 2011, 2012).

3166 There is still little evidence on how complementarity is affected by anthropogenic stress (but see
3167 Wang et al. 2013, Baert et al. 2016). Potential changes of complementarity are however crucial for
3168 determining how stress will alter species' contribution to ecosystem functioning. If
3169 complementarity is not affected by stress, i.e. species interactions are the same under stressed
3170 and unstressed conditions, stress should affect the BEF relation only through selective
3171 biodiversity loss and physiological effects. The biodiversity effect on functioning would thus
3172 depend on the functional contribution of the most stress-tolerant (and thus dominant) species
3173 (Baert et al. 2016). If, however complementarity becomes more positive under stress, e.g. through
3174 an increase in facilitative interactions, adverse selective and physiological stress effects on
3175 functioning could be compensated. This potential for functional compensation among stressed
3176 communities has thus been a major subject of discussion BEF research (Fox 2006, Duffy et al.

3177 2007, Fox and Harpole 2008) and stress ecology (Bertness and Callaway 1994, Maestre et al. 2009,
3178 2010, He et al. 2013).

3179 Here, diatoms produced more biomass when grown in community than in monoculture. This
3180 positive biodiversity effect was caused by complementarity, which increased under stress
3181 (Chapter 4). Atrazine and copper reduced diatom biomass in monoculture (see 6.2), but this loss
3182 of biomass could partially be compensated by an increase in complementarity when diatoms were
3183 exposed to the same stress levels in community. Facilitative mechanisms can drive
3184 complementarity effects in unstressed conditions, for example in legumes and insects (Cardinale
3185 et al. 2002, Temperton et al. 2007). In microalgae, the release of organic exudates can act as a
3186 facilitative mechanism driving complementarity effects (Vanellander et al. 2009). At present,
3187 there is however no ecophysiological evidence linking changes in complementarity under stress
3188 to changing facilitative mechanisms. Under atrazine and copper stress, diatom communities
3189 increased their EPS production which, in part, predicted increases in complementarity under
3190 stress (Chapter 4). This role of EPS as a 'sleeping' facilitative mechanism, which when stimulated
3191 under stress predicted changes in complementarity highlights the two roles of microalgal EPS
3192 production: first, as an ecosystem process *per se*, promoting sediment stabilization (De Brouwer
3193 et al. 2000, Decho 2000, Gerbersdorf et al. 2009a). Second, as a physiological stress response
3194 which not only operates in monoculture (Pistocchi et al. 1997, Staats et al. 2000a), but also acts as
3195 driver of complementarity effects which helps to maintain biomass production under stress.

3196 Moreover, complementarity under stress was largely one-way, and benefited species depending
3197 on their properties: under atrazine and copper respectively, complementarity favoured the
3198 growth of mixotrophic (*C. closterium*, *N. acicularis*, *Nitzschia* sp.) and copper-sensitive species (*A.*
3199 *lineolata*, *N. digitoradiata*, *Gyrosigma* sp.). To date, the few BEF experiments which partitioned
3200 two-way and one-way complementarity found only minor contributions of one-way
3201 complementarity to the net biodiversity effect on ecosystem functioning (Hector et al. 2009,
3202 Fernandes et al. 2011, Long et al. 2013, Stachova et al. 2013, Siebenkäs et al. 2016). Here, copper-
3203 sensitive species were characterised by low biomass yields, causing a negative one-way
3204 complementarity under copper stress, which largely offset positive two-way complementarity
3205 effects and thus limited the biodiversity effect on diatom biomass production. The release of
3206 extracellular polymers as a facilitative mechanism thus predicted changes in complementarity in
3207 stressed diatom communities, but was not necessarily beneficial for ecosystem functioning under
3208 stress when complementarity benefited species with a low functional contribution. In total
3209 however, complementarity was consistently positive, increased under stress when one-way
3210 complementarity did not favour species with a low functional contribution, and thus represented
3211 the key driver of diatom biomass production under stress.

3212 **6.4. Multifunctionality**

3213 The large majority of BEF research has focused on one ecosystem process at a time, most
3214 commonly the biomass production of primary producers (Balvanera et al. 2006, Jiang et al. 2008,
3215 Cardinale et al. 2011). This focus on biomass is understandable, given the importance of biomass
3216 production for e.g. food and material production or carbon sequestration, which represent
3217 important ecosystem services that human society heavily relies on (Jiang et al. 2008, Cardinale et
3218 al. 2012). Here, however, different species contributed to different ecosystem processes (see 6.1-
3219 6.3). These results correspond to a growing body of BEF studies which found that sustaining
3220 multiple rather than single ecosystem processes requires a greater number of species, and
3221 suggested that more biodiversity is needed to maintain the 'multifunctionality' of ecosystems
3222 (Isbell et al. 2011, Maestre et al. 2012, Pasari et al. 2013, Lefcheck et al. 2015, Soliveres et al. 2016).

3223 If the covariation among diversity effects driving different ecosystem processes is strong, e.g. if
3224 multiple processes are regulated by either complementarity or the presence of one dominant
3225 species, a subset of diversity should be sufficient to sustain multifunctionality (Cardinale et al.
3226 2011). This principle also applies to the maintenance of multifunctionality under stress. If the
3227 diversity-multifunctionality relation is regulated by similar stress effects, e.g. by an increase in
3228 complementarity or by selective stress effects on the dominant species, focussing on one of these
3229 effects allows to predict multiple ecosystem processes under stress. If different ecosystem
3230 processes are driven by different stress effects, the maintenance of a particular ecosystem process
3231 under stress might reduce other processes, and multifunctionality imposes a tradeoff for the
3232 conservation of ecosystem functioning under stress. Here, for example, a facilitation-driven
3233 increase in complementarity stimulated the growth of a highly mixotrophic but lipid-poor species
3234 (*C. closterium*). This allowed to maintain diatom biomass under herbicide stress, but came at the
3235 cost of a negative selective stress effect on diatom energy content.

3236 The functional impact of selective biodiversity loss relative to changes in complementarity
3237 increases with differences in the species' functional contribution (Jiang 2007): when species
3238 contribute similarly to functioning, selective biodiversity loss can be compensated by an increase
3239 in complementarity effects, whilst this potential for compensation decreases when biodiversity
3240 loss selectively targets highly productive species. Complementarity commonly drives biomass
3241 production, but tends to have weaker effects on certain biochemical or trophic processes (Duffy
3242 2002, Jiang et al. 2008, Cardinale et al. 2011). Processes such as decomposition, trophic energy
3243 transfer, nutrient cycling, bioturbation or ocean carbon export can instead depend on the
3244 presence of organisms with a disproportionately high contribution to functioning (Emmerson et
3245 al. 2001, Hillebrand and Cardinale 2004, Waldbusser et al. 2004, Solan et al. 2004, Jiang 2007,

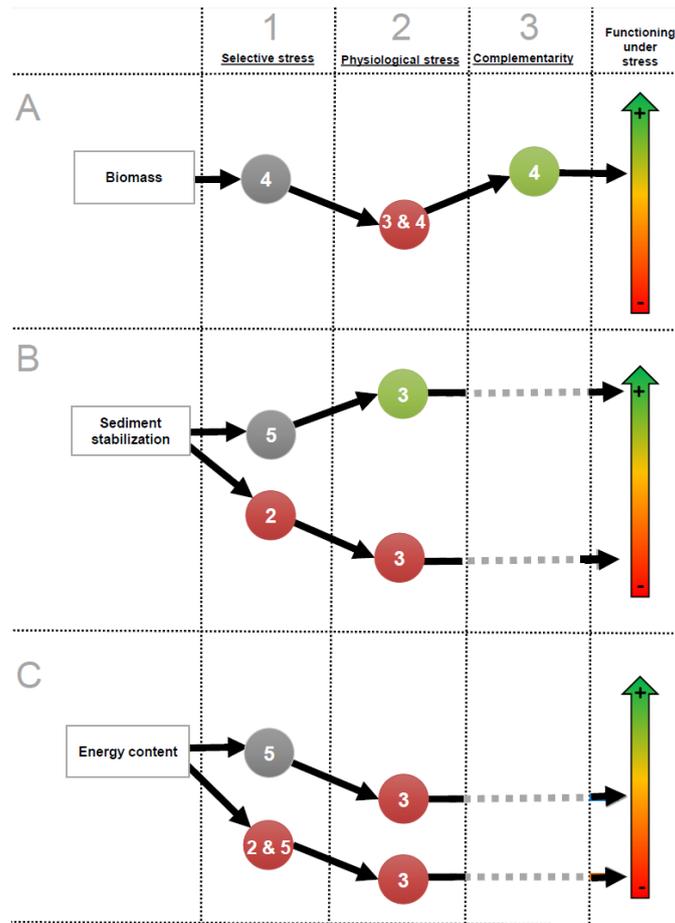
3246 Jiang et al. 2008, Cardinale et al. 2011, Bracken and Low 2012, Van Colen et al. 2012, Litchman et
3247 al. 2015).

3248 Here, differences in functional contribution amongst diatom species were limited in terms of
3249 biomass production, and most pronounced in terms of energy content. The monoculture biomass
3250 of the most productive species (*Nitzschia* sp. in Chapter 2, *N. arenaria* in Chapters 4 and 5,
3251 respectively) exceeded that of the least productive species (*S. robusta* and *A. lineolata*) by a factor
3252 three and four, respectively. The species contributing most to sediment stabilization (*Nitzschia* sp.
3253 and *A. lineolata* in Chapters 2 and 5, respectively) produced seven times more EPS than the least
3254 productive species (*S. robusta* and *N. arenaria*). The most lipid-rich species (two different
3255 *Nitzschia* sp.) however produced forty and fifteen times more fatty acids than the most lipid-poor
3256 species in the diatom communities of Chapters 2 and 5 (*N. arenaria* and *C. closterium*).

3257 In accordance with these differences in functional contribution, biomass production did not
3258 depend on the identity of the dominant species, and was reduced by physiological atrazine and
3259 copper effects, which could however be compensated by positive complementarity effects on
3260 stressed diatom communities (Fig. 6.2 A). The extent to which complementarity could compensate
3261 stress effects on diatom biomass production depended on the stress level, and whether
3262 complementarity favoured the growth of low- or high-yield species. For energy content and
3263 sediment stabilization, complementarity effects could not be quantified, since additive
3264 partitioning would require information on the fatty acid and EPS production of every species in
3265 monoculture and in community. Fig. 6.2 consequently does not make a statement on
3266 complementarity as driver of diatom energy content and sediment stabilization under stress.

3267 EPS was affected by selective stress, when stress-sensitive and tolerant species differed in their
3268 functional contribution. At low stress levels, diatoms increased their EPS production as a
3269 physiological stress response, whilst at high stress diatoms could not maintain their EPS
3270 production (Fig. 6.2 B). In a best-case scenario, stress thus did not target the functionally most
3271 important species, diatoms increased their EPS production at low stress, and stressed diatom
3272 communities produced more EPS than under control conditions. In a worst-case scenario, stress
3273 targeted the most productive species and diatoms could not maintain their EPS production under
3274 high stress, causing a disproportionate loss of EPS production in stressed diatom communities
3275 (Fig. 6.2 B).

3276



3277

3278 **Fig. 6.2:** Summary of three effects (Selective stress – Panel 1, Physiological stress – Panel 2,
 3279 Complementarity – Panel 3) determining the contribution of diatom communities to three ecosystem
 3280 processes under stress (Biomass production – Panel A, Sediment stabilization – Panel B, Energy content –
 3281 Panel C). Red, grey and green circles respectively represent negative, neutral and positive impacts of each
 3282 effect on ecosystem processes. The numbers within the circles indicate the chapters in which the respective
 3283 effects were analysed (Chapters 2 to 5). The coloured arrows on the right-hand side of panels A, B and C
 3284 represent an approximation to which extent stressed diatom communities could maintain their
 3285 contribution to ecosystem functioning (green: high functioning under stress; red: low functioning under
 3286 stress). For the sake of simplicity this figure does not present the extent of ecosystem functioning change in
 3287 the different treatments of Chapters 2 to 5. Instead, this figure has to be understood as a qualitative rather
 3288 than quantitative interpretation of the impact of selective stress, physiological stress and complementarity
 3289 on the contribution of stressed diatom communities to ecosystem functioning. Whilst the impact of selective
 3290 stress, physiological stress and complementarity on diatom biomass production were consistently neutral,
 3291 negative and positive respectively, changes in diatom energy content and sediment stabilization differed
 3292 depending on stress type (atrazine or copper) and stress level. For diatom energy content and sediment
 3293 stabilization, this figure thus indicates a best-case and worst-case scenario, resulting from the combination
 3294 of selective and physiological atrazine and copper effects on the functional contribution of diatom
 3295 communities.

3296

3297 Diatom energy content was most affected by selective atrazine stress, due to the large differences
 3298 in the species' fatty acid production (but see above). As predicted from the species' EC50 values,
 3299 the most copper-tolerant species also produced most fatty acids, and selective copper stress had
 3300 no effect on diatom energy content. Compared to biomass production and sediment stabilization,
 3301 diatom energy content was also most affected by physiological stress, with the fatty acid

3302 production of most species being reduced at stress levels which did not affect other processes.
3303 Context-dependent effects of both stressors thus also caused a loss of diatom energy content, even
3304 in the absence of selective stress effects (Fig. 6.2 C). In a best-case scenario, diatom energy content
3305 was not affected by selective copper stress, and only reduced by context-dependent effects at high
3306 copper levels. In a worst-case scenario, low atrazine levels reduced diatom energy content
3307 through selective changes in community structure, whilst high levels of the herbicide further
3308 reduced diatom energy content through context-dependent effects.

3309 These strong effects of chemical stress on diatom energy content which, partly despite invariant
3310 biomass, knocked on to the next trophic level highlight the relevance of diet quality in a trophic
3311 BEF context. Trophic BEF research has mostly focussed on diet quantity, i.e. the biomass
3312 production of primary producers (Balvanera et al. 2006, Duffy et al. 2007). Diet quality received
3313 broad attention in marine trophic ecology when population declines of some well-known marine
3314 top predators could be explained by changes in the quality of their diet. For example, reproductive
3315 failures in seabirds (Wanless et al. 2005, Kitaysky et al. 2006, Österblom et al. 2008) and declines
3316 in sea lion populations (Anderson and Piatt 1999, Rosen and Trites 2005, Trites et al. 2007) have
3317 been linked to losses in the energy content of their diet, and to shifts in prey composition from
3318 lipid-rich to lipid-poor prey species.

3319 However, diet quality in terms of energy content does not only play a crucial role at the top of the
3320 food web, but is the key driver of energy transfer at the primary producer-consumer interface. In
3321 aquatic ecosystems, algal diet quality is highly sensitive to changing environmental conditions,
3322 and energy content shows considerably variation among and within algal classes (Dunstan et al.
3323 1993, Taipale et al. 2013, Guo et al. 2016). Community shifts from high- to low-energy primary
3324 consumers can cause trophic decoupling, i.e. low energy transfer efficiencies to the consumer level
3325 despite high producer biomass, which reduces consumer energy content and ultimately
3326 reproduction (Müller-Navarra et al. 2000, Guo et al. 2016). The limited duration of the
3327 experiments in this work allowed to test for the former (energy transfer) but not the latter
3328 (consumer reproduction) consequence of altered diet quality. Nevertheless, anthropogenic
3329 change is increasingly altering the energy content of primary producers (Teoh et al. 2013, Guo et
3330 al. 2016) and is causing large-scale shifts in both local and global phytoplankton compositions
3331 (e.g. from energy-rich diatoms to energy-poor cyanobacteria, Bopp et al. 2005, Moran et al. 2010,
3332 Litchman et al. 2015). Evaluating the functional impact of these changes not only in terms of diet
3333 quantity, but also diet quality and trophic energy transfer will be major task for future BEF
3334 research.

3335 **6.5. Future perspectives**

3336 This doctoral thesis sets in many ways a baseline for the incorporation of anthropogenic stress
3337 into future research on biodiversity and ecosystem functioning research. The outcome of this
3338 work poses several challenges to further increase the level of realism in diversity-functioning
3339 research.

- 3340 • A first challenge is to test which level of diversity is most relevant for ecosystem
3341 functioning under stress. In this work, stress consistently altered evenness, which was a
3342 better predictor of functioning than richness (but see 6.1), whilst most BEF experiments
3343 to date have focused on the functional role of species richness (Wittebolle et al. 2009,
3344 Cardinale et al. 2011, Tilman et al. 2014). Describing diversity at the species level however
3345 only stands in the middle of a broad diversity spectrum, which includes genetic diversity
3346 (Hughes et al. 2008), functional trait diversity (Díaz et al. 2003, Cadotte et al. 2011) and
3347 the number of distinct communities within a system (Pasari et al. 2013).
- 3348 • To quantify which type of diversity is most relevant for ecosystem functioning
3349 under stress, future BEF research would need experiments which explicitly
3350 manipulate biodiversity at multiple levels. For example, is ecosystem functioning
3351 under stress driven by genetic diversity (e.g. through the presence of tolerant
3352 genotypes), trait diversity (e.g. the number of effect traits and their correlation
3353 with response traits), species diversity or community diversity (e.g. the number of
3354 distinct communities in a stressed system)?
- 3355 • A second challenge is to include multiple anthropogenic stressors into BEF experiments.
3356 The few BEF experiments which included explicit exposure to anthropogenic stressors
3357 examined the impact of one type of stress in isolation (McMahon et al. 2012, Steudel et al.
3358 2012, Radchuk et al. 2016, including this work). Ecosystems are however commonly
3359 exposed to multiple stressors (Lawler et al. 2006, Malaj et al. 2014).
- 3360 • Future BEF research will need approaches to predict community structure and
3361 ecosystem functioning in the presence of multiple anthropogenic stressors.
3362 Community ecotoxicology has developed advanced mechanistic and predictive
3363 approaches to predict biodiversity in the presence of multiple contaminants
3364 (Koelmans et al. 2001, Rohr et al. 2006, Clements and Rohr 2009, Halstead et al.
3365 2014). Explicitly linking these multistressor approaches with the species'
3366 functional contribution represents a promising basis for testing the functional
3367 impact of multiple stressors, to further increase realism in BEF designs.
- 3368 • A third challenge for future BEF research is to examine not only the functional
3369 consequences of species loss, but also of species gain. BEF research has been motivated by

3370 concerns about biodiversity loss, but global change, through species invasions and range
3371 expansions, is homogenizing the distribution of organisms worldwide (Cardinale et al.
3372 2012, Dornelas et al. 2014). While global biodiversity indeed seems to decrease,
3373 biodiversity at local scales has been observed to remain unaffected or even increase (Sax
3374 and Gaines 2003, Dornelas et al. 2014, McGill et al. 2015).

3375 • In order to keep pace with a changing world, BEF research will need to develop
3376 designs that predict ecosystem functioning under simultaneous biodiversity
3377 losses and gains. The marine environment is largely affected by the
3378 homogenization of Earth's biota (Molnar et al. 2008, Vansteenbergue et al. 2016),
3379 and coastal ecosystems are particularly vulnerable to invasive species (Carlton
3380 1998, Gamfeldt et al. 2015). Marine studies could thus lead the way in integrating
3381 simultaneous invasion and extinction into BEF frameworks.

3382 • Last, BEF research should further increase realism by including the spatial and temporal
3383 complexity driving the BEF relation in natural ecosystems. There still remains a large gap
3384 between the small spatial and temporal scales at which most BEF experiments are being
3385 performed, and those scales at which conservation efforts are taking place (Srivastava and
3386 Vellend 2005, Cardinale et al. 2011). Larger spatial and temporal scales allow for a greater
3387 niche partitioning, a key mechanism for complementarity (Cardinale et al. 2007,
3388 Stachowicz et al. 2008a, Venail et al. 2010). Facilitative interactions also grow stronger
3389 with time and can change with fluctuating abiotic conditions (Mulder et al. 2001, Cardinale
3390 et al. 2007, Stachowicz et al. 2008b).

3391 • This discrepancy of experimental and natural systems highlights the need to cross-
3392 check experimental results with observed patterns in natural ecosystems, and to
3393 transform the results of short-term laboratory or mesocosm BEF experiments into
3394 meaningful predictions at scales which matter to conservation. To extrapolate the
3395 results of controlled short-term experiments to more complex environments, BEF
3396 experiments should test biodiversity effects on functioning under constant
3397 conditions as well as different regimes of temporal and abiotic fluctuations. Once
3398 such an extrapolation degree has been established for various ecosystem
3399 processes, we should be able to transform the outcome of controlled small-scale
3400 BEF experiments to scales which are relevant for management and conservation
3401 efforts.

3402 In a world affected by human activities at an increasing scale and speed, BEF research has come a
3403 long but fast way, from being a 'niche research area' at the interface of community and ecosystem
3404 ecology only twenty years ago to having a broad impact on both ecological research and public
3405 awareness. BEF research went through a contentious confirmatory phase in the 1990s and an

3406 exploratory phase in the 2000s revealing the core drivers of the biodiversity effect in functioning.
3407 Now, BEF research faces a realism phase with the challenge to include the natural and
3408 anthropogenic drivers of diversity-functioning relations into its experimental design. By
3409 complementing current BEF designs with the inherent variability of natural systems and the
3410 anthropogenic drivers of the ongoing biodiversity change, we should be able to gain a more
3411 thorough understanding of ecosystem functioning.

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3413

3414 **Addendum I – Supporting Material Chapter 2**

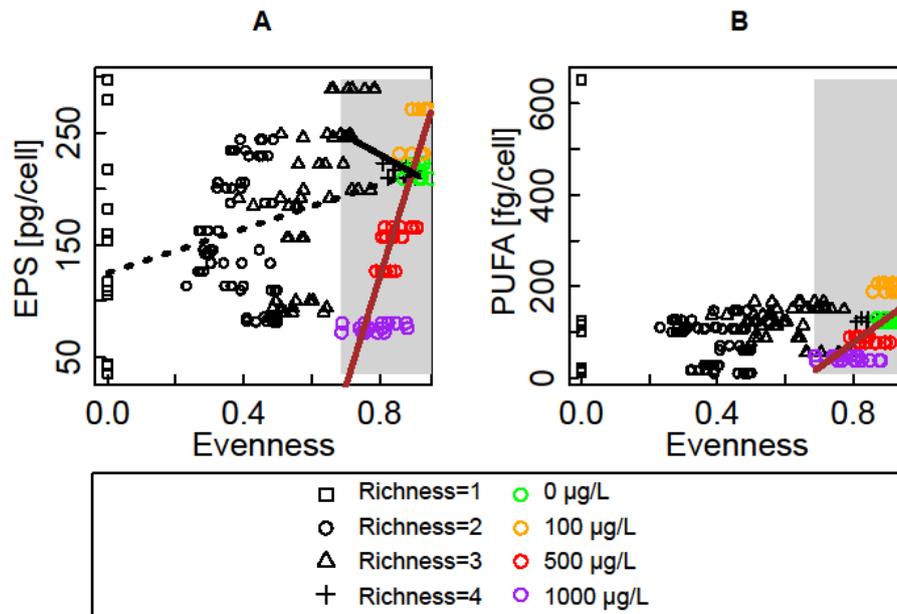
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Species	Shape	Biovolume equation	Length (a) [μm]	Width (b) [μm]	Height (c) [μm]	Biovolume [μm ³]
<i>Navicula arenaria</i>	Elliptic prism	$V = \frac{\pi}{4} \times a \times b \times c$	47	18	15	9896
<i>Entomoneis paludosa</i>	Elliptic prism	$V = \frac{\pi}{4} \times a \times b \times c$	40	5	50	7854
<i>Seminavis robusta</i>	cymbelloid	$V = \frac{1}{6} \pi \times (2b)^2 \times a \times \frac{\beta}{360};$ with $\sin \frac{\beta}{2} = \frac{c}{2 \times b}$	25	15	10	2813
<i>Nitzschia sp.</i>	Prism on parallelogram	$V = \frac{1}{2} \times a \times b \times c$	45	15	17	5738

3417

3418 **Table S1:** Cell biovolume per species. Biovolume was calculated based on the closest approximation of
 3419 geometric shape and the mean measured linear dimensions during the experiments.



3420

3421 **Fig. S1:** EPS (A) and PUFA (B) concentrations normalized to cell density as a function of evenness.
 3422 Regression lines show significant relations between evenness and function. Brown lines are linear models
 3423 based on the atrazine experiment. Dashed and solid black lines are linear models based on the random
 3424 assembly experiment for the whole evenness range or for the evenness range of the atrazine experiment
 3425 (grey area), respectively.

3426

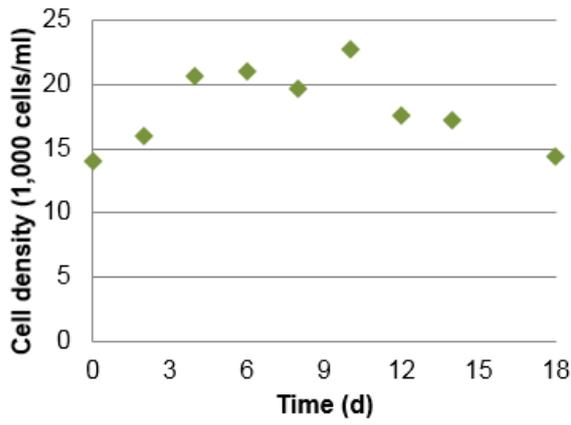
3427

Ecosystem Function	Data	Model	Slope	s.e.	T	P	AIC	Log lik.	Validity	LR	P LR
Sediment stabilization: EPS [pg/ml]	R	1	78,22	18,45	4,24	<0,0001	2029	-1012	Yes		
		2	98,30	18,30	5,37	<0,0001	2023	-1008	Yes	7,78	0,005
	A	1	971,96	98,94	9,82	<0,0001	781	-387	Yes		
		2	894,93	83,61	10,70	<0,0001	780	-386	Yes	2,88	0,0897
	R'	1	-188,23	81,60	-2,31	0,0309	234	-114	Yes		
		2	-166,25	64,94	-2,56	0,0179	221	-107	Yes	14,87	0,0001
Energy content: PUFA [fg/ml]	R	1	-25,99	31,09	-0,84	0,4043	2217	-1106	No		
		2	61,55	17,88	3,44	0,0007	2052	-1022	No	NA	NA
	A	1	663,37	74,62	8,89	<0,0001	740	-367	Yes		
		2	541,24	56,28	9,62	<0,0001	736	-364	Yes	5,80	0,0160
	R'	1	-37,56	96,31	-0,39	0,7003	242	-118	No		
		2	63,13	63,25	1,00	0,3291	222	-107	No	NA	NA

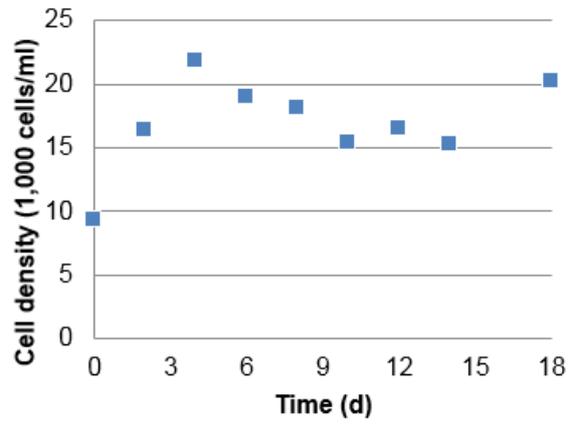
3428

3429 **Table S2:** Results of generalized least squared models predicting PUFA and EPS production (normalized to
 3430 cell density) from evenness, without (model 1) or with (model 2) a variance structure. 'Data' denotes the
 3431 data on which the models are based: the random assembly experiment (R), the atrazine experiment (A), the
 3432 random assembly experiment for evenness values overlapping with those from the atrazine experiment
 3433 (R'); 's.e.' is the standard error on the estimated slopes; T and P denote the t- and p-values, bold values are
 3434 statistically significant. AIC is the Akaike information criterion; 'Validity' denotes if residuals were
 3435 homogeneous and normally distributed ('yes') or not ('no'); 'LR' is the likelihood ratio of model 1 vs. model
 3436 2, P LR the corresponding p-value.

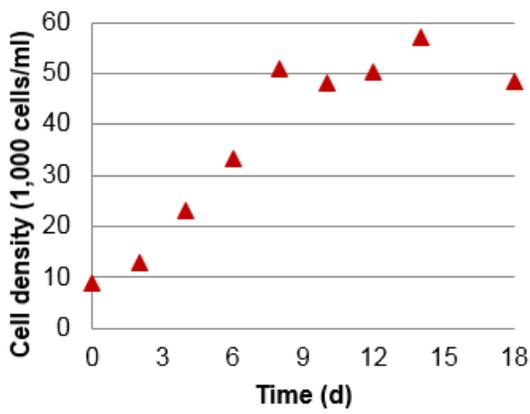
(1) *Navicula*



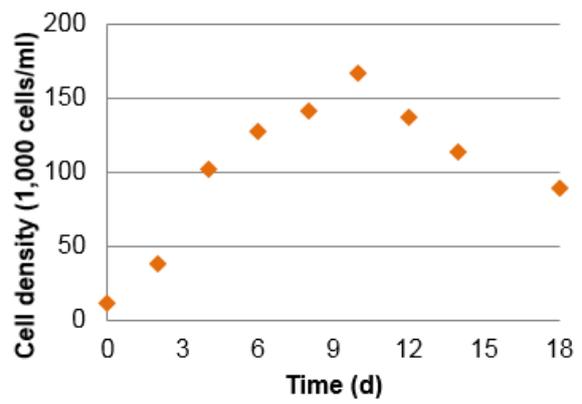
(2) *Entomoneis*



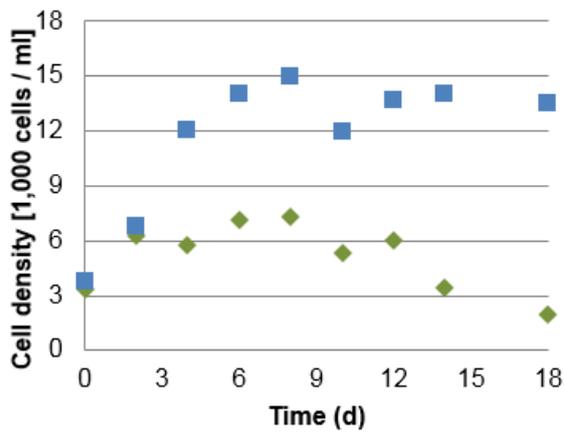
(3) *Seminavis*



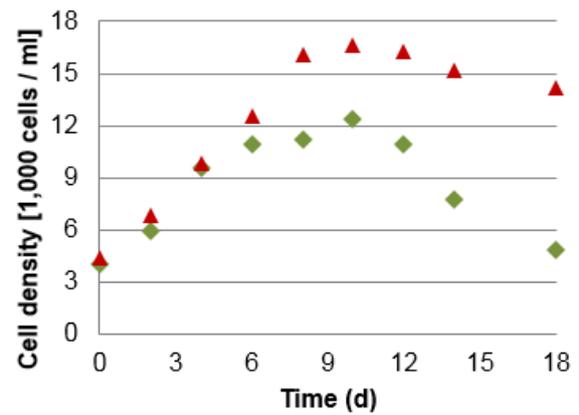
(4) *Nitzschia*



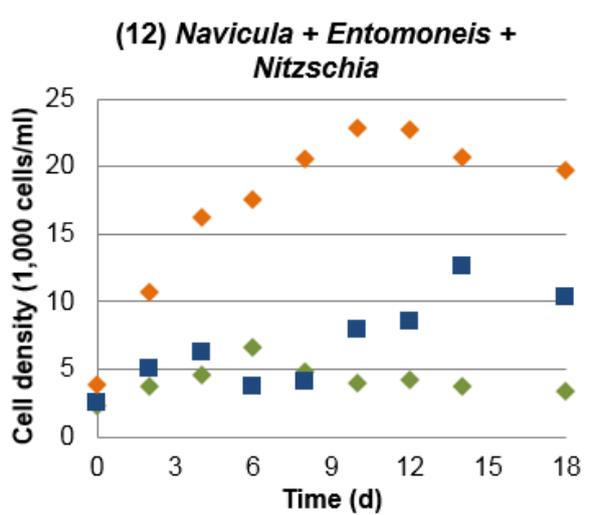
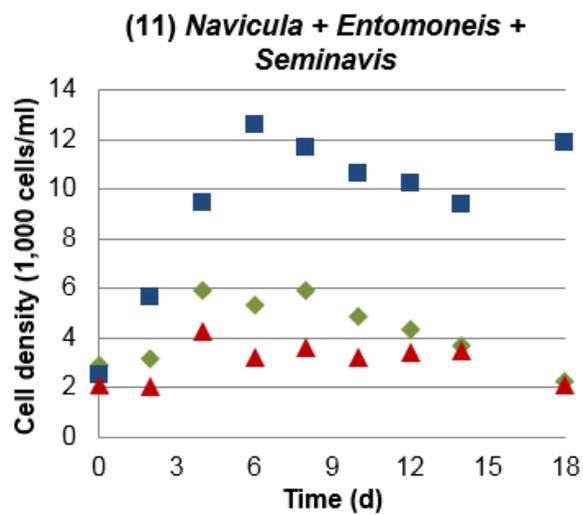
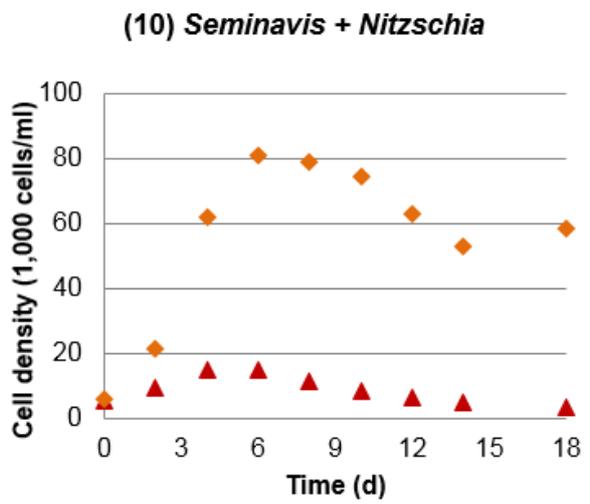
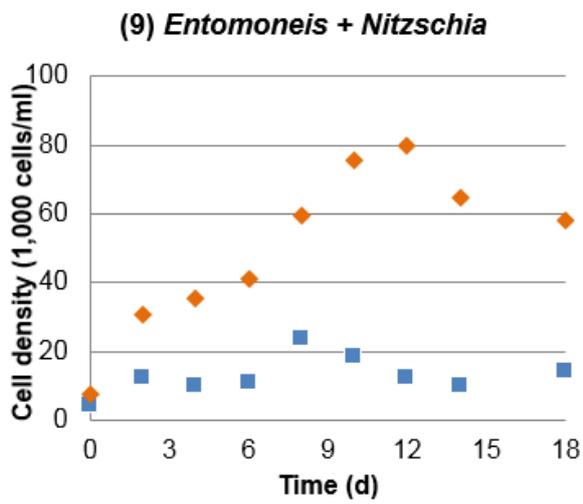
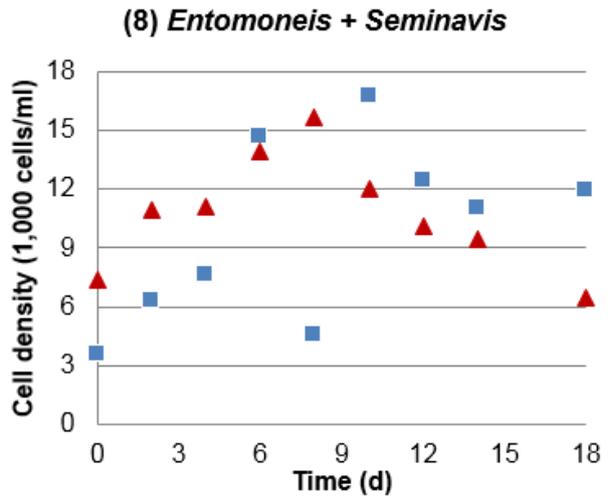
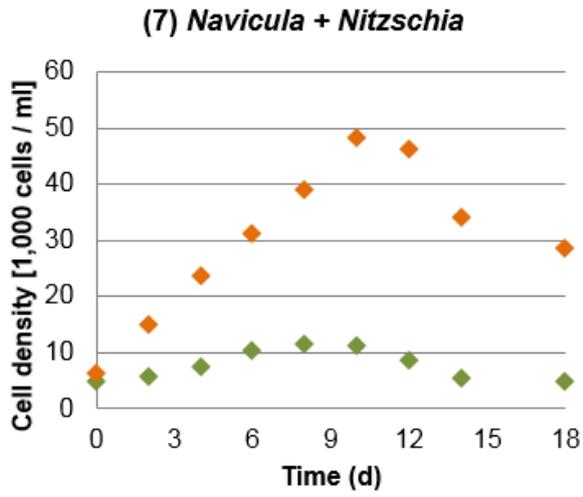
(5) *Navicula + Entomoneis*

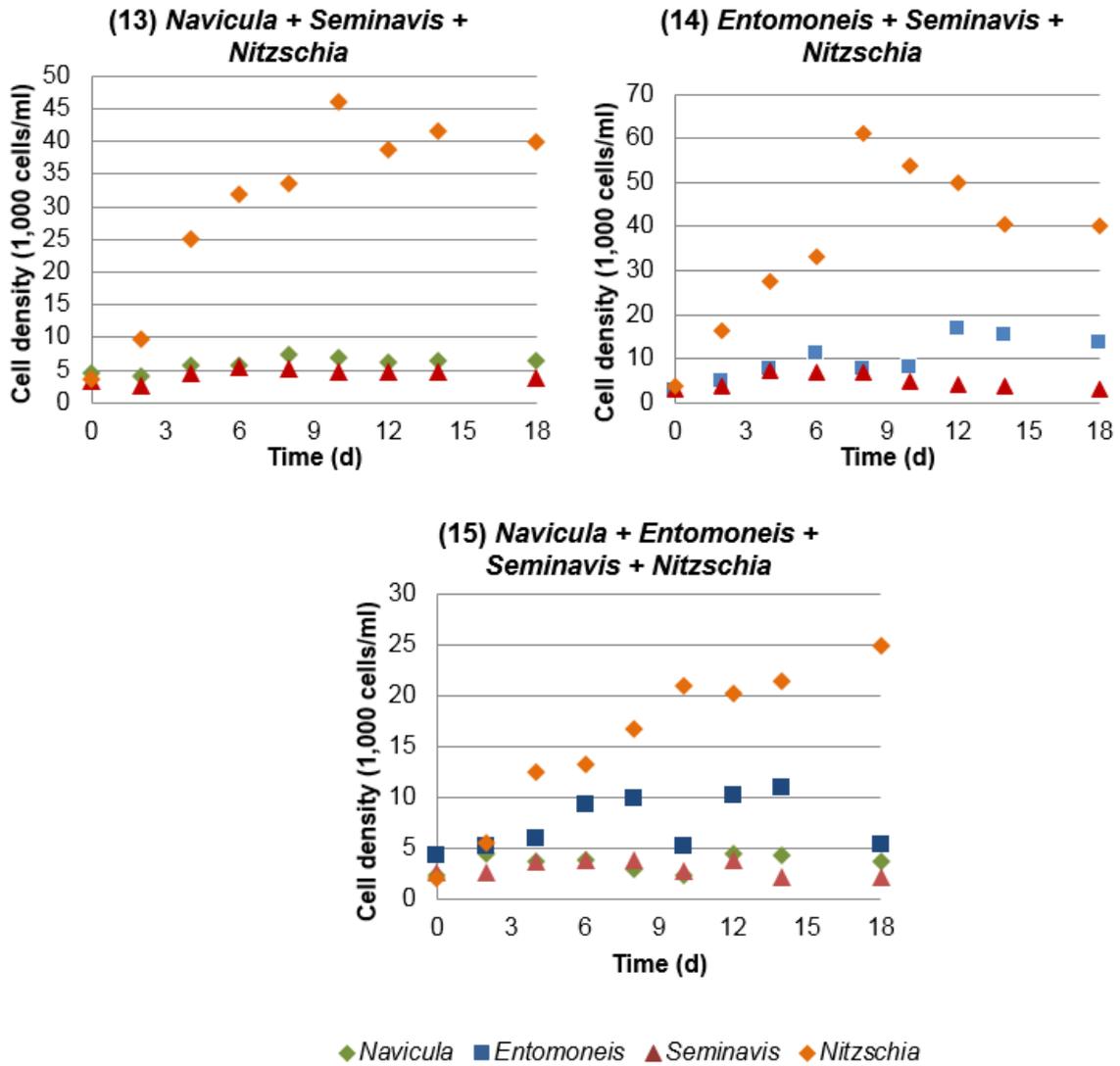


(6) *Navicula + Seminavis*



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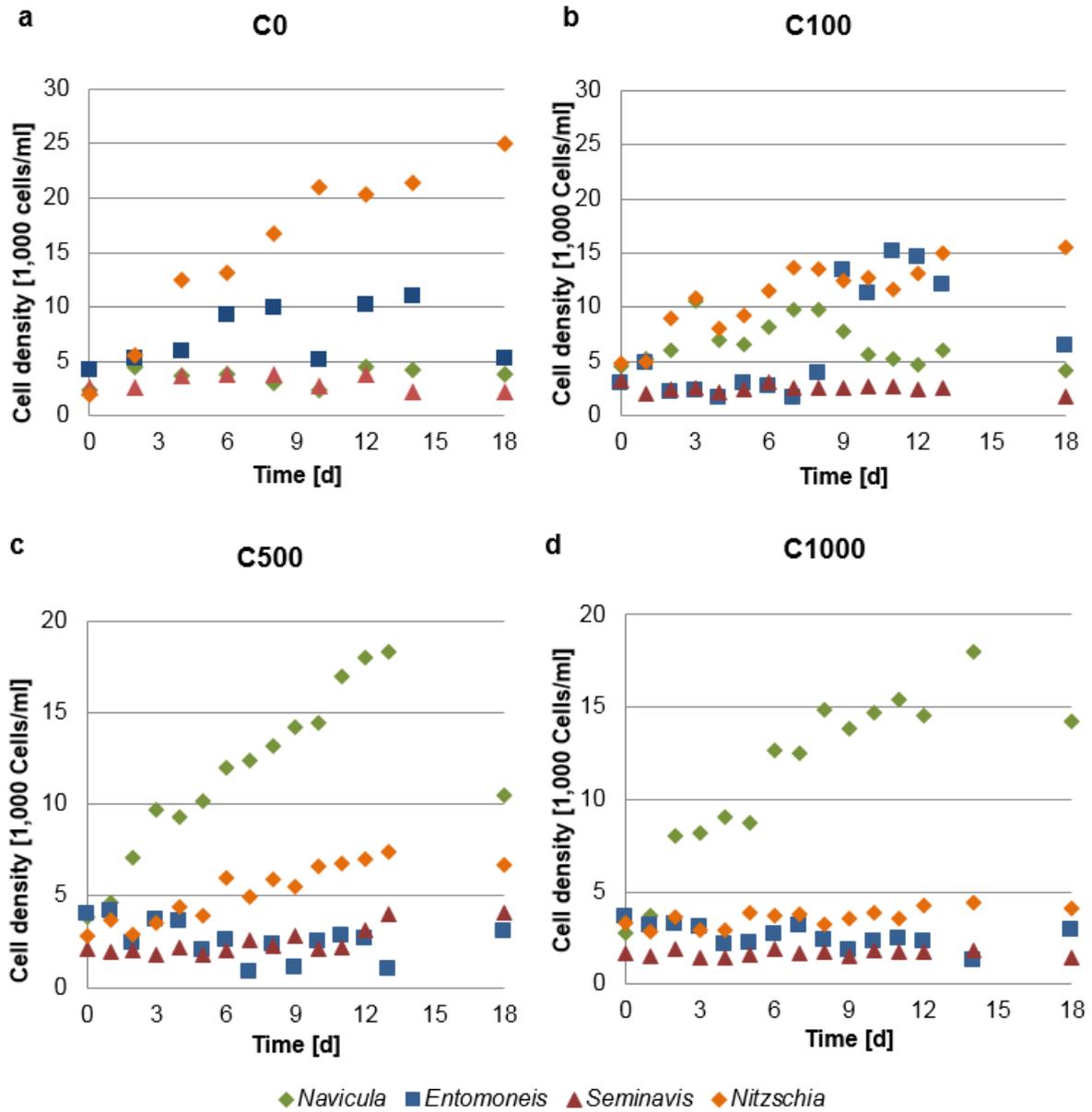




3439

3440 **Fig. S2:** Cell density per species in the random assembly experiment. Treatments 1-15 (n=12) represent all
 3441 possible random species combinations. Colour codes refer to the species *Navicula arenaria* (green),
 3442 *Entomoneis paludosa* (blue), *Seminavis robusta* (red) and *Nitzschia sp.* (orange).

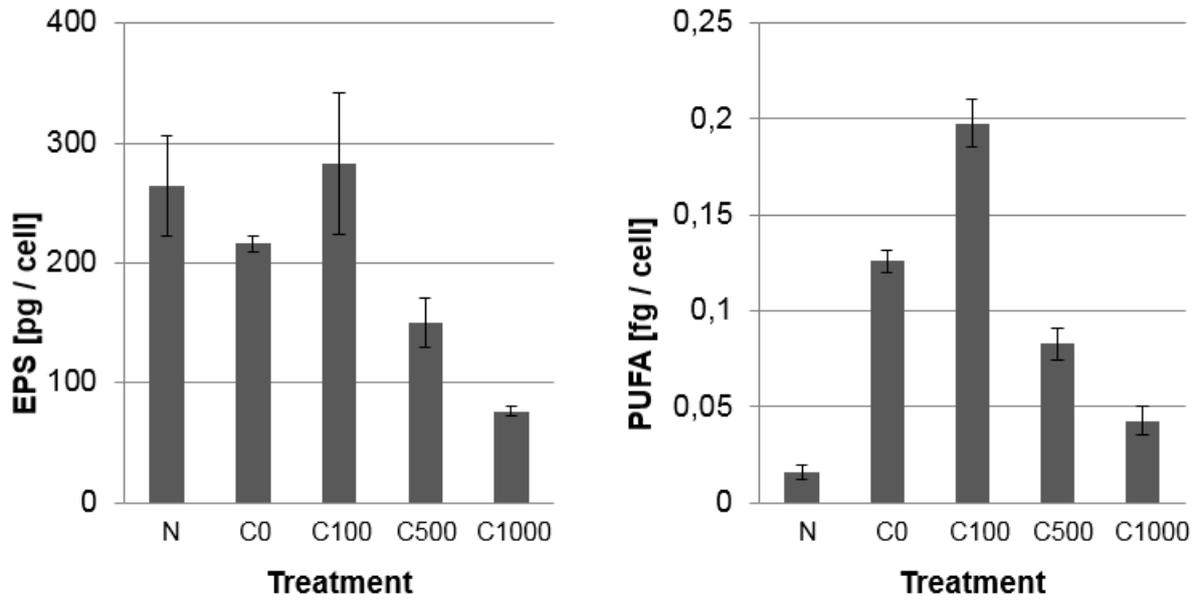
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3445 **Fig. S3:** Cell density per diatom species in the atrazine experiment. Treatments C0, C100, C500 and C1000
 3446 (0, 100, 500 and 1000 µg/l atrazine, n=18) are indicated in panels a, b, c and d respectively. Colour codes
 3447 refer to the species *Navicula arenaria* (green), *Entomoneis paludosa* (blue), *Seminavis robusta* (brown) and
 3448 *Nitzschia sp.* (orange).

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3451 **Fig. S4:** EPS (a) and PUFA (b) concentrations normalized to cell density in monocultures of *Navicula*
 3452 *arenaria* and atrazine-exposed communities. Treatments: N = *Navicula arenaria* monoculture, C0, C100,
 3453 C500 and C1000 = full species community exposed to 0, 100, 500 and 1000 $\mu\text{g/l}$ atrazine. Treatments C500
 3454 and C1000 were dominated by *Navicula arenaria* (s. Fig. S3). EPS: n=3, PUFA: n=2

3455 **Addendum II – Supporting Material Chapter 3**

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Species	Acc. Nr.	K [10 ³ cells/ml]	Biovolume [µm ³]	S:V	Length [µm]	Chl. a [F0]	TN [%]	TOC [%]	Mix 0.1	Mix 0,6	PE [Fv/Fm]	EPS total [mg/ml]	EPS / cell [ng/cell]	FA total [µg/ml]	FA / cell [pg/cell]
<i>Cylindrotheca fusiformis</i>	DCG 0423	997 (+- 32)	67 (+- 7)	1.57 (+- 0.08)	18.25 (+- 1.0)	0.34 (+- 0.05)	6.02 (+- 1.2)	41.67 (+- 0.3)	0.91 (+- 0.1)	1.00 (+- 0.1)	0.27 (+- 0.02)	1.32 (+- 0.01)	3.16 (+- 0.07)	931.10 (+- 310)	294.78 (+- 103)
<i>Nitzschia sp. 1</i>	DCG 0483	170 (+- 26)	380 (+- 48)	0.61 (+- 0.04)	26.15 (+- 2.1)	0.27 (+- 0.09)	NA	17.71	0.89 (+- 0.2)	1.13 (+- 0.4)	0.46 (+- 0.02)	1.28 (+- 0.07)	16.56 (+- 2.86)	3.67 (+- 2.34)	58.84 (+- 23)
<i>Nitzschia acicularis</i>	DCG 0459	412 (+- 46)	517 (+- 68)	0.54 (+- 0.03)	33.01 (+- 2.5)	0.42 (+- 0.07)	7.26 (+- 0.6)	33.20 (+- 2.5)	1.22 (+- 0.1)	1.10 (+- 0.1)	0.56 (+- 0.02)	1.96 (+- 0.13)	9.99 (+- 0.32)	7.00 (+- 1.63)	39.24 (+- 8)
<i>Amphora lineolata</i>	DCG 0478	121 (+- 25)	778 (+- 123)	0.93 (+- 0.05)	26.39 (+- 1.5)	0.30 (+- 0.09)	9.03	43.84	0.18 (+- 0.1)	0.81 (+- 0.3)	0.64 (+- 0.04)	3.61 (+- 0.31)	89.09 (+- 0.72)	29.99 (+- 5.22)	767.20 (+- 228)
<i>Cylindrotheca closterium</i>		141 (+- 20)	345 (+- 60)	0.86 (+- 0.06)	25.46 (+- 1.0)	0.43 (+- 0.03)	5.28 (+- 1.7)	14.74 (+- 16)	2.36 (+- 0.3)	3.82 (+- 1.1)	0.53 (+- 0.04)	3.39 (+- 0.34)	67.02 (+- 4.48)	19.02 (+- 2.31)	313.14 (+- 35)
<i>Gyrosigma sp. 2</i>	DCG 0491	42 (+- 6)	2081 (+- 241)	0.34 (+- 0.02)	44.58 (+- 2.2)	1.45 (+- 0.24)	7.41 (+- 2.8)	17.59 (+- 3.4)	1.87 (+- 0.5)	1.85 (+- 0.4)	0.64 (+- 0.01)	1.89 (+- 0.21)	157.19 (+- 25.46)	10.99 (+- 3.62)	820.91 (+- 207)
<i>Navicula arenaria str. A2</i>	DCG 0487	58 (+- 11)	4466 (+- 388)	0.39 (+- 0.02)	39.49 (+- 2.8)	1.72 (+- 0.35)	2.86	22.19	0.16 (+- 0.1)	0.61 (+- 0.3)	0.52 (+- 0.04)	3.35 (+- 0.39)	279.30 (+- 15.80)	10.90 (+- 4.91)	782.41 (+- 155)
<i>Astartiella bahusiensis</i>	DCG 0469	86 (+- 28)	1552 (+- 239)	0.59 (+- 0.05)	33.19 (+- 0.3)	2.71 (+- 1.22)	7.49 (+- 2.9)	31.90 (+- 8.9)	0.89 (+- 0.2)	0.92 (+- 0.2)	0.63 (+- 0.02)	2.88 (+- 0.51)	84.10 (+- 9.75)	21.34 (+- 2.59)	687.92 (+- 41)
<i>Nitzschia sp. 2</i>	DCG 0421	234 (+- 22)	650 (+- 46)	0.40 (+- 0.02)	23.69 (+- 1.4)	1.46 (+- 0.20)	6.51 (+- 0.2)	36.66 (+- 2.9)	0.98 (+- 0.3)	1.08 (+- 0.3)	0.65 (+- 0.01)	3.12 (+- 0.69)	76.48 (+- 15.01)	49.51 (+- 5.13)	895.53 (+- 182)
<i>Gyrosigma sp. 1</i>	DCG 0468	120 (+- 12)	2147 (+- 428)	0.35 (+- 0.04)	56.08 (+- 3.4)	1.14 (+- 0.19)	6.13 (+- 1.2)	32.29 (+- 1.4)	0.41 (+- 0.1)	0.90 (+- 0.3)	0.51 (+- 0.03)	0.57 (+- 0.24)	11.74 (+- 5.38)	12.22 (+- 4.79)	247.84 (+- 85)
<i>Navicula arenaria str. A7</i>	DCG 0489	67 (+- 10)	3120 (+- 519)	0.46 (+- 0.04)	39.80 (+- 0.4)	1.62 (+- 0.37)	8.69 (+- 1.2)	25.19 (+- 6.4)	0.78 (+- 0.4)	0.38 (+- 0.2)	0.45 (+- 0.04)	2.39 (+- 0.13)	80.98 (+- 6.31)	8.95 (+- 1.41)	282.64 (+- 24)
<i>Navicula digitoradiata</i>	DCG 0490	38 (+- 6)	5738 (+- 640)	0.34 (+- 0.02)	35.50 (+- 1.0)	2.39 (+- 0.72)	13.90	17.71	1.40 (+- 0.5)	1.05 (+- 0.3)	0.49 (+- 0.01)	3.91 (+- 0.79)	254.63 (+- 51.01)	7.93 (+- 1.60)	508.04 (+- 46)

Navicula sp. 2	DCG 0671	258 (+ 34)	587 (+ 113)	0.79 (+ 0.05)	21.46 (+ 1.6)	0.65 (+ 0.12)	8.10 (+ 0.1)	31.14 (+ 1.4)	0.79 (+ 0.3)	0.79 (+ 0.2)	0.37 (+ 0.03)	2.16 (+ 0.46)	17.31 (+ 3.41)	14.89 (+ 9.04)	144.67 (+ 87)
Amphora sp. 1	DCG 0481	69 (+ 16)	533 (+ 87)	1.01 (+ 0.06)	24.21 (+ 1.3)	1.58 (+ 0.38)	11.45 (+ 2.9)	21.25 (+ 15)	0.75 (+ 0.2)	0.42 (+ 0.2)	0.58 (+ 0.05)	3.56 (+ 0.27)	158.77 (+ 29.13)	5.52 (+ 0.31)	215.01 (+ 58)
Biremis ambigua	DCG 0480	15 (+ 3)	4108 (+ 593)	0.37 (+ 0.02)	29.39 (+ 1.5)	2.99 (+ 0.66)	14.21	28.18	1.25 (+ 0.4)	1.50 (+ 0.7)	0.43 (+ 0.05)	1.51 (+ 0.12)	203.54 (+ 29.55)	2.24 (+ 0.41)	377.72 (+ 57)
Entomoneis sp.	DCG 0466	45 (+ 12)	10932 (+ 2767)	0.28 (+ 0.03)	41.53 (+ 3.3)	2.97 (+ 0.71)	17.52 (+ 10)	22.79 (+ 9.9)	1.01 (+ 0.3)	0.96 (+ 0.2)	0.60 (+ 0.02)	0.59 (+ 0.03)	39.20 (+ 8.05)	20.78 (+ 3.27)	1292.26 (+ 489)
Navicula sp. 1		302 (+ 46)	303 (+ 80)	1.02 (+ 0.12)	19.06 (+ 0.5)	1.11 (+ 0.24)	7.37 (+ 1.5)	26.21 (+ 1.9)	1.40 (+ 1.0)	1.54 (+ 0.6)	0.51 (+ 0.02)	2.19 (+ 0.49)	16.29 (+ 4.47)	8.86 (+ 7.91)	67.91 (+ 60)
Amphora sp. 2	DCG 0672	132 (+ 7)	1052 (+ 183)	0.88 (+ 0.10)	24.57 (+ 1.2)	2.22 (+ 0.12)	7.97 (+ 0.5)	28.24 (+ 11)	1.09 (+ 0.2)	0.78 (+ 0.2)	0.56 (+ 0.03)	0.67 (+ 0.2)	11.03 (+ 3.35)	5.69 (+ 0.42)	78.96 (+ 7)

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Table S1: Listing of the 18 diatom strains used in the experiments. From left to right are indicated: species name, strain accession number (Acc. Nr.) in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (<http://bccm.belspo.be>), carrying capacity K (n=6), traits and functional contribution as determined in the control treatments of the atrazine experiment: biovolume (n=12), surface to volume ratio (S:V, n=12), length (n=12), chlorophyll content (Chl. a, n=6; minimum chlorophyll fluorescence signal F_0 per 10^6 cells), % total nitrogen (TN, n=2 or 1), % total organic carbon (TOC, n=2 or 1), mixotrophy (Mix0.1 and Mix0.6, n=6, change of carrying capacity in the presence of 0.1 mM and 0.6 mM Glucose), photosynthetic efficiency (PE, n=6, ratio of variable to maximum fluorescence), EPS (n=3) per ml culture and per cell, polyunsaturated fatty acids (FA, n=2) per ml culture and per cell.

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Substance	Theoretical concentration [$\mu\text{g/l}$]	Measured concentration [$\mu\text{g/l}$]
Control	0	<1
Atrazine	50	48
Atrazine	100	91
Atrazine	200	190
Atrazine	500	542
Atrazine	1000	1080
Copper	30	29
Copper	90	94
Copper	270	271
Copper	810	826

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3469 **Table S2:** Experimental atrazine and copper treatments with indication of theoretical and verified atrazine
3470 and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of
3471 quantification : < 1,0 $\mu\text{g/L}$ atrazine and 2.6 $\mu\text{g/L}$ copper for 10 ml samples).

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3473

Response	Data	Mod	Predictors	Slope	s.e.	T	P	AIC	Log lik.	Validity
Numerical: Carrying capacity	Cu	A	Biovolume	0.03	0.003	8.82	<0.0001	1275	-634	Yes
			Mix	-31.6	23.4	-1.35	0.20			
	Atr	A	Mix	144.0	30.0	4.76	0.0003	190	-91.9	Yes
Functional: Photosynthetic efficiency	Cu	A	Biovolume	0.12	0.01	6.73	<0.0001	1582	-787	Yes
			Mix	-110.9	115.8	-0.96	0.35			
	Atr	A	Biovolume	0.18	0.01	13.87	<0.0001	1480	-736	No
			Mix	188.7	102.2	1.85	0.08			
		B	<i>Biovolume</i>	0.11	0.02	5.76	<0.0001	1399	-695	Yes
			Mix	187.7	51.8	3.62	<i>0.0028</i>			
Functional: EPS	Cu	A	Biovolume	0.056	0.007	6.75	<0.0001	607	-300	Yes
			Mix	-57.2	42.1	-1.36	0.20			
	Atr	A	Mix	-46.7	31.9	-1.47	0.16	192	-92.8	Yes
Functional: Fatty acids	Cu	A	Biovolume	0.043	0.006	6.62	<0.0001	352	-171	No
			Mix	-11.1	19.8	-0.56	0.58			
		B	<i>Biovolume</i>	0.051	0.011	4.47	0.0001	344	-167	Yes
			Mix	-17.4	17.0	-1.02	0.33			
	Atr	A	Biovolume	0.028	0.008	3.52	<i>0.0014</i>	418	-205	Yes
			Mix	-26.1	34.6	-0.76	0.46			

3475

3476 **Table S3:** Results of generalized least squares models predicting diatom numerical and functional response
3477 to copper and atrazine from traits; 'Response' denotes the response variable: numerical or functional
3478 response in terms of photosynthetic efficiency, EPS or fatty acid production; 'Data' denotes the data on
3479 which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); 'Mod' indicates
3480 if the model was fitted without (Model A) or with (Model B) variance structure; Predictors are biovolume
3481 and mixotrophic response to 0.6 mM Glucose concentrations (Mix); 'Slope' indicates the relation between
3482 predictors and numerical and functional response to copper and atrazine; 's.e.' is the standard error on the
3483 estimated slopes; 'T' and 'P' denote the t- and p-values, bold values are statistically significant, italic values
3484 are statistically significant over the whole dataset but not if outlier species are omitted (model fits without
3485 outlier species are then indicated in Table S4); 'AIC' is the Akaike information criterion, 'Log lik' the log-
3486 likelihood ratio. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not
3487 ('no'). If 'no', models were refitted ('Model B') with a variance structure allowing the residuals to change
3488 with the predictor (the predictor along which residuals were allowed to vary is marked in italic). Only best-
3489 fitting models with uncorrelated predictors are shown (p<0.05, correlation factor < 0.5, sequential
3490 removing of predictors, evaluation of model fit by AIC and log likelihood); Additional model fits with traits
3491 that also predicted numerical and functional response to copper and atrazine, but were correlated to
3492 biovolume and mixotrophy (correlation factor > 0.5), are indicated in Table S4.

3493

3494

Response	Data	Mod	Predictors	Slope	s.e.	T	P	AIC	Log lik.	Validity
Numerical: Carrying capacity	Cu'	B	Biovolume	0.04	0.006	7.08	<0.0001	1199	-595	Yes
			Mix	-28.0	26.8	-1.04	0.31			
	Cu	B	SV	-206	37	-5.53	<0.0001	1272	-630	Yes
			Chl. a	5.44	11.01	0.49	0.62			
			Mix	-40.6	30.2	-1.34	0.20			
		B	Length	6.80	0.67	10.21	<0.0001	1236	-612	Yes
			Chl. a	21.1	7.95	2.65	0.0093			
			Mix	-28.3	9.80	-2.89	0.0119			
	Atr'	A	Mix	161.7	64.1	2.52	0.0244	177	-85.6	No
		B	Mix	281.9	47.4	4.89	0.0002	178	-85.2	Yes
Atr''	A	Biovolume	0.005	0.009	0.54	0.59	1260	-627	Yes	
Functional: Photosynthetic efficiency	Cu	B	SV	-668	145.1	-4.61	<0.0001	1589	-788	Yes
			Chl. a	-89.0	44.7	-1.99	0.049			
			Mix	-139	117.3	-1.19	0.25			
		B	Length	21.2	3.71	5.71	<0.0001	1587	-787	Yes
			Chl. a	-49.5	41.1	-1.20	0.23			
			Mix	-131	111.3	-1.18	0.26			
	Cu'	B	Biovolume	0.11	0.02	5.26	<0.0001	1486	-738	Yes
			Mix	-212	116.9	-1.81	0.09			
	Atr''	B	Biovolume	0.09	0.02	6.37	<0.0001	1287	-639	Yes
			Mix	185.7	51.9	3.58	<i>0.0034</i>			
	Atr	B	SV	-336	42.7	7.99	<0.0001	1465	-727	Yes
			Mix	129.4	59.7	2.17	<i>0.0478</i>			
		B	Length	14.7	4.19	3.51	0.0007	1433	-710	Yes
Chl. a			81.2	25.1	3.24	0.0017				
Mix			180.7	61.7	2.93	<i>0.0017</i>				
Functional: EPS	Cu	A	SV	-390	97.1	4.02	0.0002	607	-300	Yes
			Chl. a	-14.5	32.6	-0.44	0.66			
			Mix	-71.4	60.8	-1.17	0.26			
	A	Length	8.13	2.14	3.50	0.0011	608	-299	Yes	
		Chl. a	25.4	31.2	0.81	0.42				
		Mix	-54.3	59.5	-0.91	0.388				
Cu'	A	Biovolume	0.058	0.013	4.50	0.0001	580	-287	Yes	
		Mix	-56.6	45.2	-1.25	0.23				
Functional: Fatty acids	Cu	A	SV	-336	93.5	-3.60	0.0013	336	-163	Yes
			Chl. a	50.0	19.9	2.51	0.0185			
			Mix	-2.42	32.3	0.07	0.94			
		B	Length	4.02	1.41	2.85	0.0083	336	-162	Yes
			Chl. a	28.0	22.0	1.27	0.22			
			Mix	-27.5	21.1	-1.30	0.22			

Cu'	B	<i>Biovolume</i>	0.046	0.011	4.17	0.0013	316	-153	Yes
		Mix	-20.9	14.7	-1.43	0.18			
Atr''	B	Biovolume	0.005	0.011	0.46	0.65	385	-188	Yes
		Mix0.6	-33.5	31.6	-1.06	0.31			

3496

3497 **Table S4:** Results of generalized least squares models predicting numerical and functional response to
3498 copper and atrazine from traits; 'Response' denotes the response variable: numerical and functional
3499 response in terms of photosynthetic efficiency, EPS or fatty acid production; 'Data' denotes the data on
3500 which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); the copper
3501 experiment without the largest species (*Entomoneis* sp., (Cu')), the atrazine experiment without the most
3502 mixotrophic species (*Cylindrotheca closterium*, (Atr')), and the atrazine experiment without the largest
3503 species (*Entomoneis* sp., (Atr'')); 'Mod' indicates if the model was fitted without (Model A) or with (Model
3504 B) variance structure; Predictors are biovolume, surface to volume ratio (SV), chlorophyll a content (Chl. a),
3505 Length and mixotrophic response to 0.6 mM Glucose concentrations (Mix); 'Slope' indicates the relation
3506 between predictors and numerical and functional response to copper and atrazine; 's.e.' is the standard
3507 error on the estimated slopes; Only models with uncorrelated predictors that predicted numerical and
3508 functional response are shown ($p < 0.05$, correlation factor < 0.5 , sequential adding and removing of
3509 predictors, evaluation of model fit by AIC and log likelihood ratio); 'T' and 'P' denote the t- and p-values,
3510 bold values are statistically significant, italic values are statistically significant over the whole dataset but
3511 not if outlier species are omitted; 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood.
3512 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no',
3513 models were refitted ('Model B') with a variance structure allowing the residuals to change with the
3514 predictor (the predictor along which residuals were allowed to vary is marked in italic).

Species	EC50 [$\mu\text{g/l}$]			
	Carrying capacity	Photosynthetic efficiency	EPS / cell	Fatty acids / cell
<i>Cylindrotheca fusiformis</i>	217.19 (3.15) <i>54.94 (10.01)</i>	221.29 (13.46) <i>75.77 (27.98)</i>	220.05 (101.35) <i>40.91 (8.03)</i>	23.62 (21.22)
<i>Nitzschia</i> sp. 1	137.09 (40.09) <i>96.46 (22.94)</i>	231.41 (42.18) <i>712.15 (65.91)</i>	478.08 (229.89) <i>157.14 (56.86)</i>	95.71 (3.90) <i>84.03 (7.80)</i>
<i>Nitzschia acicularis</i>	213.31 (33.58) <i>281.12 (44.41)</i>	292.72 (23.14) <i>1511.18 (299.76)</i>	112.91 (10.87) <i>531.48 (172.67)</i>	236.54 (11.01) <i>117.07 (79.21)</i>
<i>Amphora lineolata</i>	175.08 (18.32) <i>287.76 (131.17)</i>	391.50 (44.71) <i>703.83 (98.68)</i>	192.31 (157.25) <i>178.38 (47.59)</i>	66.54 (2.04) <i>107.58 (18.31)</i>
<i>Cylindrotheca closterium</i>	652.28 (171.90) <i>39.14 (10.50)</i>	987.78 (65.51) <i>256.40 (54.26)</i>	59.59 (7.64) <i>31.19 (0.94)</i>	4.78 (3.33) <i>13.71 (7.75)</i>
<i>Gyrosigma</i> sp. 2	453.41 (159.01) <i>271.42 (98.76)</i>	499.60 (32.72) <i>730.73 (15.75)</i>	216.74 (88.37)	85.50 (79.62) <i>325.09 (115.22)</i>
<i>Navicula arenaria</i> str. A2	349.01 (128.63) <i>219.22 (50.81)</i>	1144.84 (257.17) <i>1005.53 (325.52)</i>	176.89 (16.70) <i>581.77 (41.40)</i>	209.07 (57.34) <i>179.27 (1.95)</i>
<i>Astartiella bahusiensis</i>	328.79 (84.83) <i>243.28 (54.76)</i>	296.67 (26.56) <i>479.63 (118.04)</i>	172.73 (27.45) <i>158.30 (35.39)</i>	325.68 (18.47) <i>163.35 (137.67)</i>
<i>Nitzschia</i> sp. 2	161.77 (49.61) <i>64.78 (17.83)</i>	292.78 (34.05) <i>434.83 (78.93)</i>	76.94 (57.10) <i>237.49 (54.50)</i>	47.63 (29.93) <i>89.58 (32.25)</i>
<i>Gyrosigma</i> sp. 1	337.01 (72.29) <i>180.63 (58.23)</i>	759.70 (41.81) <i>403.43 (22.28)</i>	253.62 (154.76) <i>175.52 (42.58)</i>	134.51 (26.60) <i>95.28 (3.14)</i>
<i>Navicula arenaria</i> str. A7	171.36 (32.67) <i>348.17 (152.21)</i>	275.68 (42.65) <i>1267.94 (370.70)</i>	379.90 (54.36) <i>515.57 (197.42)</i>	68.40 (5.79) <i>288.86 (4.92)</i>
<i>Navicula digitoradiata</i>	217.51 (120.77) <i>258.05 (87.62)</i>	616.91 (77.63) <i>955.84 (102.73)</i>	184.35 (64.45) <i>380.21 (230.53)</i>	138.98 (57.13)
<i>Navicula</i> sp. 2	57.61 (16.99) <i>70.01 (26.09)</i>	446.60 (89.33) <i>157.66 (18.38)</i>	189.20 (12.23) <i>258.57 (104.69)</i>	52.52 (66.30) <i>189.24 (1.36)</i>
<i>Amphora</i> sp. 1	230.13 (124.85) <i>132.97 (46.56)</i>	551.16 (182.05) <i>698.06 (29.55)</i>	113.50 (42.56) <i>190.75 (85.02)</i>	205.04 (0.12) <i>80.79 (91.09)</i>
<i>Biremis ambigua</i>	415.03 (76.71) <i>134.41 (32.92)</i>	581.31 (96.54) <i>144.12 (27.81)</i>	178.98 (85.62) <i>205.49 (85.05)</i>	119.23 (22.55)
<i>Entomoneis</i> sp.	329.94 (230.12) <i>396.63 (61.51)</i>	2676.35 (746.98) <i>1934.08 (488.57)</i>	251.21 (98.69) <i>792.79 (75.41)</i>	507.03 (185.09) <i>502.39 (243.05)</i>
<i>Navicula</i> sp. 1	400.87 (101.04) <i>35.12 (12.05)</i>	404.32 (48.76) <i>175.19 (13.18)</i>	155.33 (28.56) <i>140.05 (33.51)</i>	262.52 (231.74) <i>3.36 (0.75)</i>
<i>Amphora</i> sp. 2	<i>148.73 (15.07)</i>	<i>626.61 (33.71)</i>		<i>100.27 (9.66)</i>
Average	288.32 (143.79) <i>181.27 (111.43)</i>	627.70 (589.20) <i>631.83 (504.96)</i>	200.71 (103.06) <i>299.87 (212.88)</i>	150.72 (129.87) <i>141.89 (124.90)</i>

3516

3517 **Table S5:** Atrazine and copper EC50s obtained for carrying capacity (n=6), photosynthetic efficiency (n=6),
3518 fatty acid (n=2) and EPS production (n=3) of 18 benthic diatom strains. The first line refers to the results
3519 with atrazine, the second with copper (italics). Standard deviations are indicated in brackets.

3520

3521

Function	Data	Mod	Predictors	Slope	s.e.	T	P	AIC	Log lik.	Validity
Photo-synthetic efficiency	Cu	A	EC50 D	2.7e-4	7.8e-5	3.44	0.0008	-166	86	Yes
		A	EC50 PE	6.7e-5	1.8e-5	3.69	0.0004	-165	86	No
		B	EC50 PE	4.0e-5	1.1e-5	3.52	0.0006	-178	93	Yes
	Atr	A	EC50D	4.8e-5	6.3e-5	-0.76	0.45	-123	65	Yes
			EC50 PE	3.3e-5	1.8e-5	1.84	0.07			
EPS	Cu	A	EC50 D	0.091	0.026	3.48	0.0010	489	-241	Yes
		A	EC50 EPS	0.039	0.014	2.71	0.0093	439	-217	Yes
	Atr	A	EC50 D	0.011	0.078	0.14	0.89	606	-299	Yes
			EC50 EPS	-0.121	0.106	-1.14	0.26			
Fatty acids	Cu	A	EC50 D	-0.043	0.220	-0.19	0.84	392	-193	Yes
		A	EC50 FA	-0.011	0.187	-0.06	0.95	380	-187	Yes
	Atr	A	EC50 D	0.515	0.383	1.34	0.19	492	-242	Yes
			EC50 FA	0.764	0.457	1.67	0.11			

3522

3523 **Table S6:** Results of generalized least squared models relating functional contribution to numerical and
3524 functional response to copper and atrazine. 'Function' denotes the response variable: photosynthetic
3525 efficiency and fatty acid and EPS production per cell in unstressed conditions; 'Data' denotes the data on
3526 which the models are based: the copper experiment (Cu) or the atrazine experiment (Atr); 'Mod' indicates
3527 if the model was fitted without (Model A) or with (Model B) variance structure; Predictors are numerical
3528 response (EC50 D) and functional response to copper and atrazine (EC50 PE for photosynthetic efficiency,
3529 EC50 EPS for EPS, EC50 FA for fatty acids); if uncorrelated, numerical and functional response were
3530 included as predictors in a multiple regression, if numerical and functional response were correlated,
3531 separate regressions were fitted; 'Slope' indicates the relation between numerical and functional response
3532 to copper and atrazine and functioning under control conditions; 's.e.' is the standard error on the estimated
3533 slopes; 'T' and 'P' denote the t- and p-values, bold values are statistically significant; 'AIC' is the Akaike
3534 information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if residuals were homogeneous and
3535 normally distributed ('yes') or not ('no'). If 'no', models were refitted ('Model B') with a variance structure
3536 allowing the residuals to change with the predictor (the predictor along which residuals were allowed to
3537 vary is marked in italic).

3538

3539

3540

FR	Data	Mod	Predictor	Slope	s.e.	T	P	AIC	Log lik.	Validity
Primary Production: Photosynth. efficiency	Cu	A	EC50 K	3.11	0.29	10.75	<0.0001	1575	-784	Yes
		B	EC50 K	3.20	0.30	10.51	<0.0001	1535	-763	Yes
	Atr	A	EC50 K	1.03	0.34	3.05	0.0029	1582	-788	Yes
Sediment stabilization: EPS	Cu	A	EC50 K	1.26	0.23	5.50	<0.0001	630	-312	Yes
	Atr	A	EC50 K	-0.27	0.10	-2.74	0.0085	632	-313	Yes
		B	EC50 K	-0.21	0.04	-5.44	<0.0001	620	-306	Yes
Energy content: Fatty acids	Cu	A	EC50 K	0.78	0.18	4.40	0.0001	365	-180	No
		B	EC50 K	0.67	0.19	3.52	0.0015	360	-176	Yes
	Atr	A	EC50 K	-0.11	0.15	-0.78	0.44	431	-212	No
		B	EC50 K	-0.26	0.11	-2.32	0.0266	429	-211	Yes

3541

3542 **Table S7:** Results of generalized least squared models relating numerical and functional response. 'FR'
 3543 denotes the response variable: functional response to copper or atrazine in terms of photosynthetic
 3544 efficiency, fatty acid or EPS production; 'Data' denotes the data on which the models are based: the copper
 3545 experiment (Cu) or the atrazine experiment (Atr); 'Mod' indicates if the model was fitted without (Model A)
 3546 or with (Model B) variance structure; Predictors are numerical response (the EC50 of carrying capacity K)
 3547 to copper and atrazine respectively; 'Slope' indicates the relation between numerical and functional
 3548 response; 's.e.' is the standard error on the estimated slopes; 'T' and 'P' denote the t- and p-values, bold
 3549 values are statistically significant; 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood.
 3550 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no',
 3551 models were refitted ('Model B') with a variance structure allowing the residuals to change with the
 3552 predictor (the predictor along which residuals were allowed to vary is marked in italic).

Species	Biovolume Control		Biovolume Atrazine		T	P
<i>Cylindrotheca fusiformis</i>	68	(+ 20)	84	(+ 23)	-1.87	0.08
<i>Nitzschia</i> sp. 1	380	(+ 81)	406	(+ 102)	-0.14	0.88
<i>Nitzschia acicularis</i>	514	(+ 80)	508	(+ 127)	0.13	0.90
<i>Amphora lineolata</i>	527	(+ 91)	553	(+ 113)	-0.64	0.53
<i>Cylindrotheca</i> sp. 2	340	(+ 94)	281	(+ 64)	1.78	0.09
<i>Gyrosigma</i> sp. 2	2078	(+ 252)	2013	(+ 179)	0.73	0.47
<i>Navicula arenaria</i> A2	4447	(+ 670)	4510	(+ 687)	-0.23	0.82
<i>Astartiella bahusiensis</i>	1540	(+ 303)	1794	(+ 832)	-0.99	0.34
<i>Nitzschia</i> sp. 2	643	(+ 99)	593	(+ 171)	0.88	0.39
<i>Gyrosigma</i> sp. 1	2134	(+ 458)	2134	(+ 411)	<0.01	0.99
<i>Navicula arenaria</i> A7	3131	(+ 717)	3074	(+ 427)	0.23	0.82
<i>Navicula digitoradiata</i>	5753	(+ 694)	5316	(+ 762)	1.47	0.16
<i>Navicula</i> sp. 2	568	(+ 102)	585	(+ 145)	-0.33	0.75
<i>Amphora</i> sp. 1	527	(+ 91)	553	(+ 113)	-0.64	0.53
<i>Biremis ambigua</i>	4082	(+ 575)	3872	(+ 773)	0.76	0.46
<i>Entomoneis</i> sp.	11124	(+ 2654)	11976	(+ 2108)	-0.87	0.39
<i>Navicula</i> sp. 1	304	(+ 80)	342	(+ 50)	-1.41	0.18

3553

3554 **Table S8 a:** Biovolume per species at the end of the experiment, in control and atrazine (500 µg/L)
3555 treatments. T and P denote the t- and p-values from a two-sided t-test testing differences in biovolume in
3556 control vs. atrazine treatments, bold values are statistically significant (p<0.05).
3557

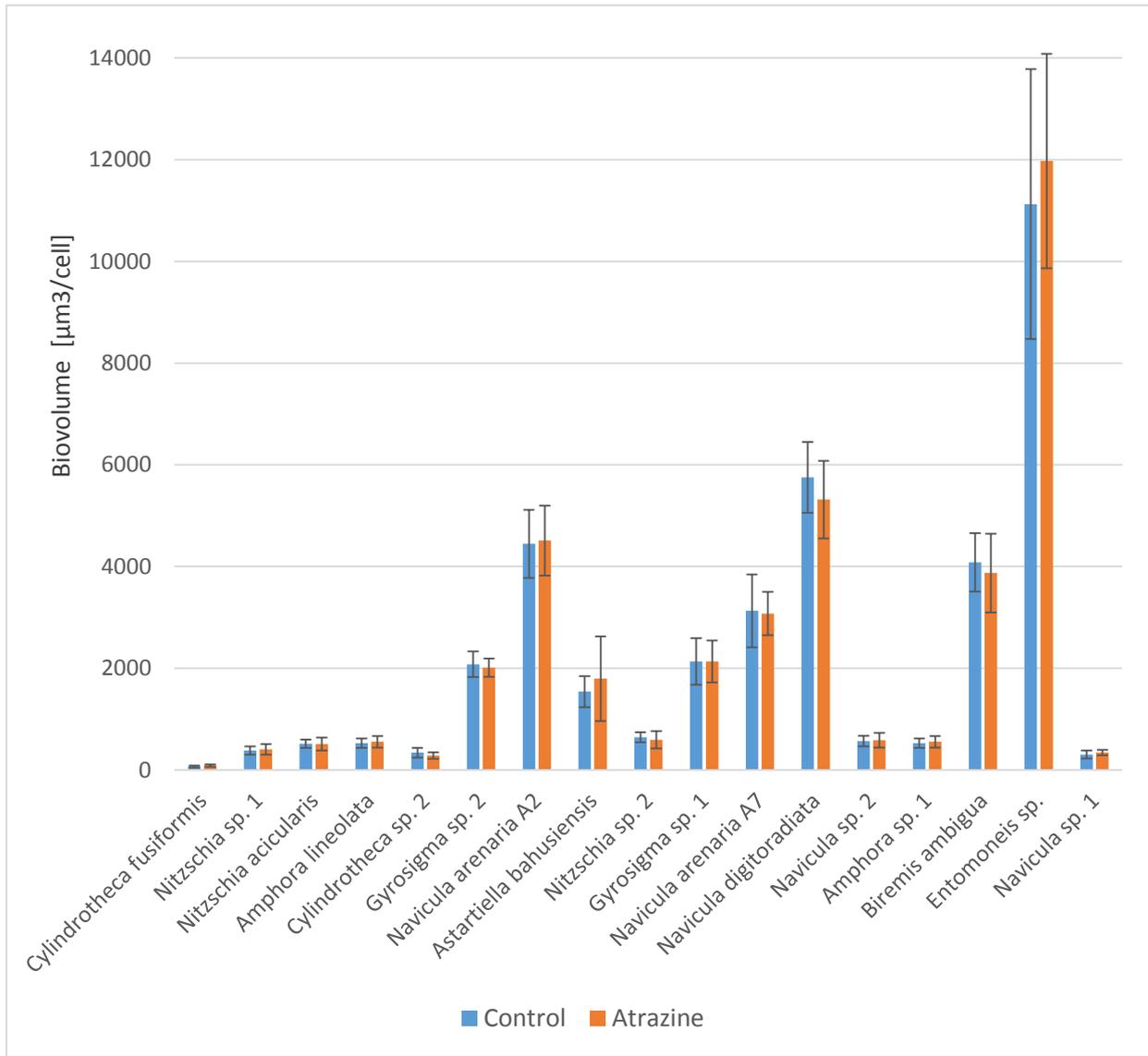
Species	Biovolume Control		Biovolume Cu		T	P
<i>Cylindrotheca fusiformis</i>	149	(+ 57)	122	(+ 44)	-1.30	0.21
<i>Nitzschia</i> sp. 1	366	(+ 95)	438	(+ 88)	-1.79	0.09
<i>Nitzschia acicularis</i>	622	(+ 144)	712	(+ 189)	-1.31	0.20
<i>Amphora lineolata</i>	967	(+ 247)	1080	(+ 213)	-1.20	0.25
<i>Cylindrotheca</i> sp. 2	334	(+ 64)	342	(+ 127)	-0.20	0.84
<i>Gyrosigma</i> sp. 2	2873	(+ 767)	2522	(+ 613)	1.24	0.23
<i>Navicula arenaria</i> A2	4214	(+ 723)	4139	(+ 672)	0.26	0.79
<i>Astartiella bahusiensis</i>	1418	(+ 242)	1779	(+ 140)	-4.48	0.0003
<i>Nitzschia</i> sp. 2	612	(+ 141)	702	(+ 160)	-1.45	0.16
<i>Gyrosigma</i> sp. 1	1722	(+ 509)	1782	(+ 454)	-0.30	0.76
<i>Navicula arenaria</i> A7	5077	(+ 1204)	4146	(+ 828)	1.45	0.17
<i>Navicula digitoradiata</i>	5698	(+ 1155)	5359	(+ 1015)	0.76	0.18
<i>Navicula</i> sp. 2	884	(+ 96)	780	(+ 154)	1.98	0.06
<i>Amphora</i> sp. 1	739	(+ 147)	740	(+ 114)	-0.02	0.98
<i>Biremis ambigua</i>	2650	(+ 482)	2843	(+ 707)	-0.78	0.45
<i>Entomoneis</i> sp.	10621	(+ 2743)	11607	(+ 1786)	-1.04	0.31
<i>Navicula</i> sp. 1	270	(+ 43)	313	(+ 93)	-1.45	0.17
<i>Amphora</i> sp. 2	739	(+ 147)	740	(+ 114)	-0.02	0.98

3558

3559 **Table S8 b:** Biovolume per species at the end of the experiment, in control and copper (270 µg/L)
 3560 treatments. T and P denote the t- and p-values from a two-sided t-test testing differences in biovolume in
 3561 control vs. atrazine treatments, bold values are statistically significant (p<0.05).

3562

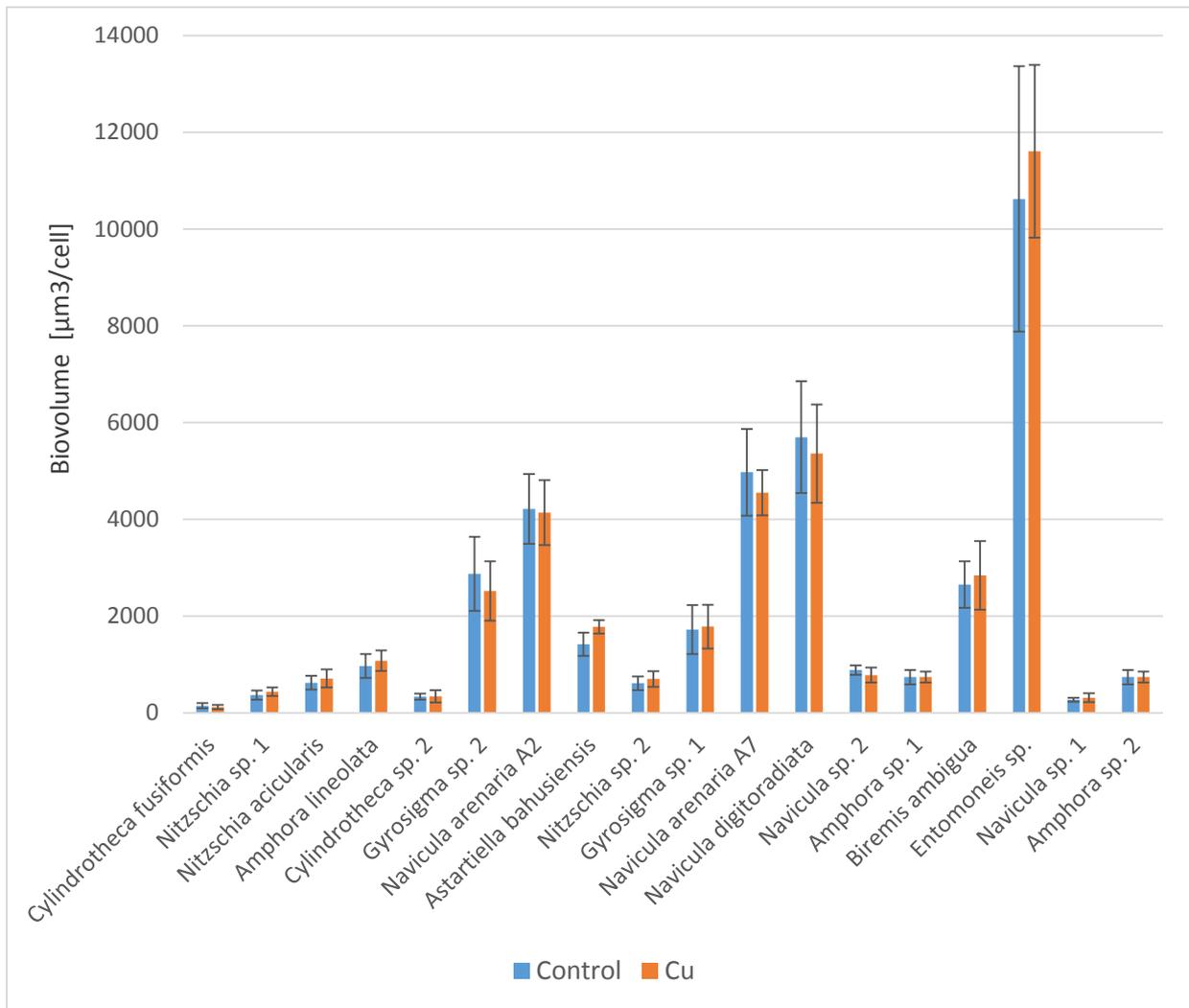
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3565 **Fig. S1 a:** Average biovolume per species in control (blue bars) and atrazine (500 µg/L, red bars) treatments

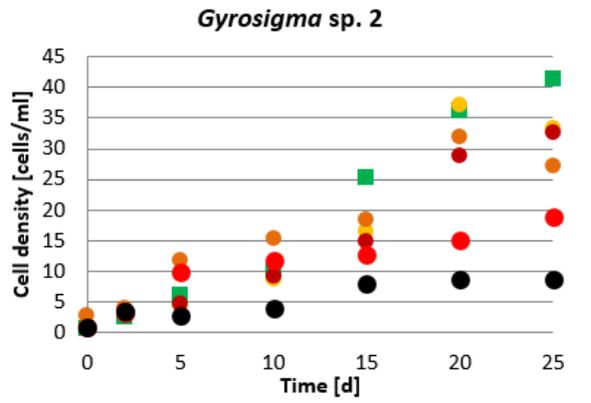
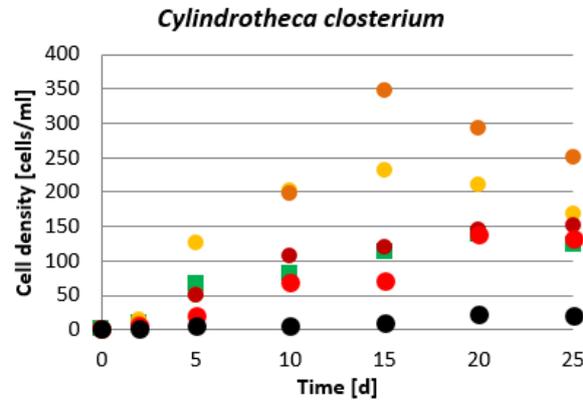
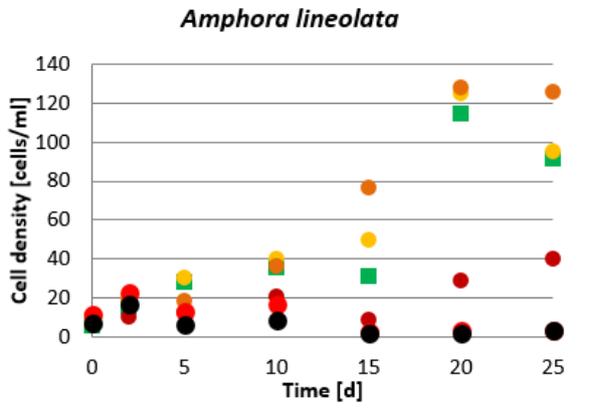
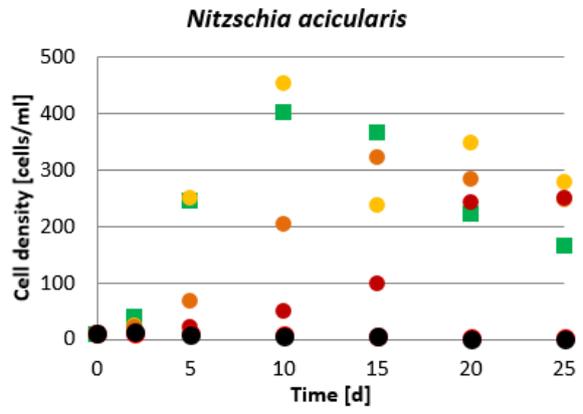
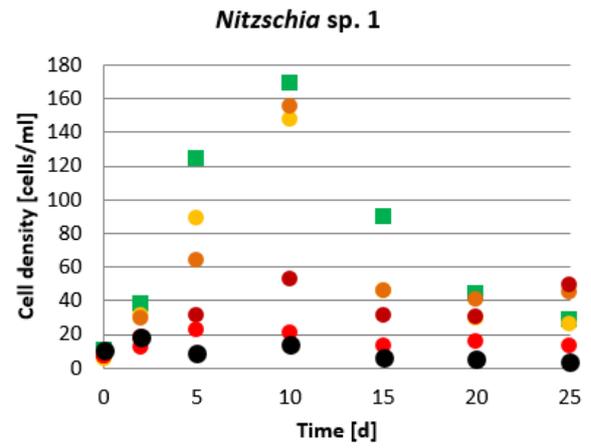
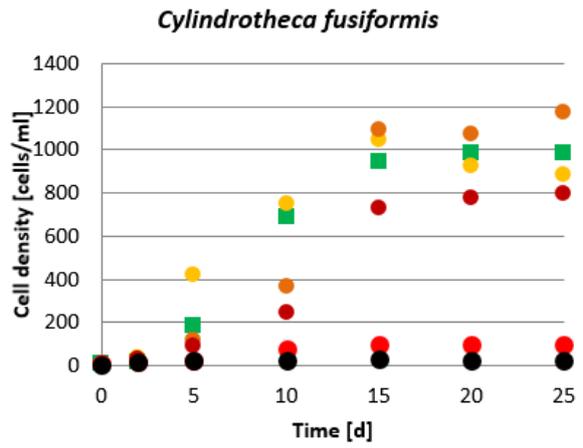
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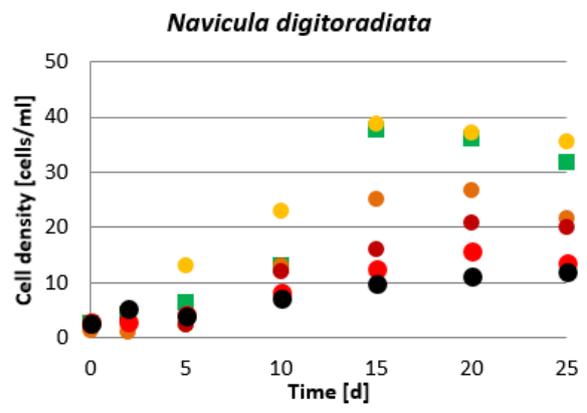
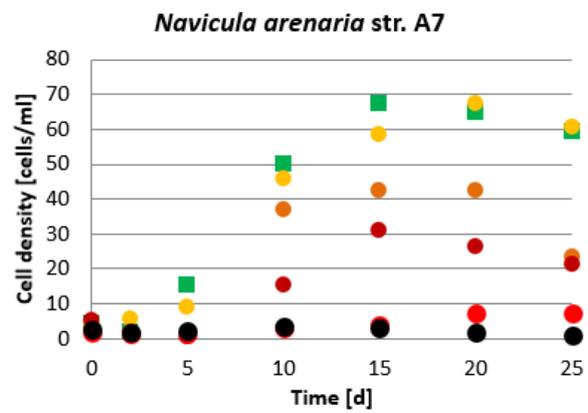
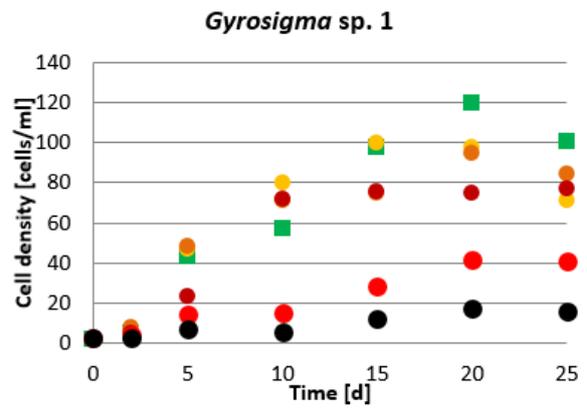
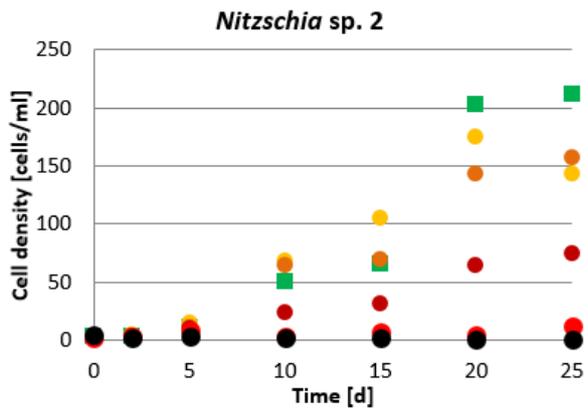
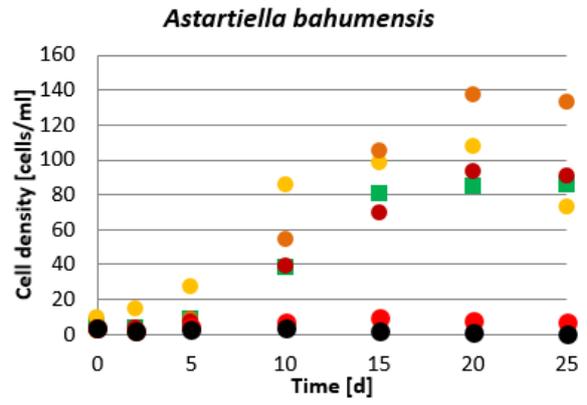
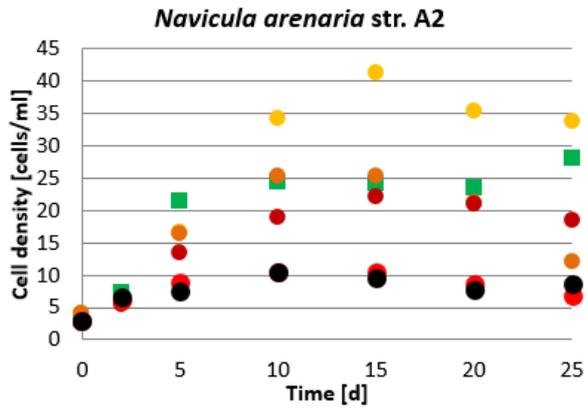
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3568 **Fig. S1 b.** Average biovolume per species in control (blue bars) and copper (270 µg/L, red bars) treatments

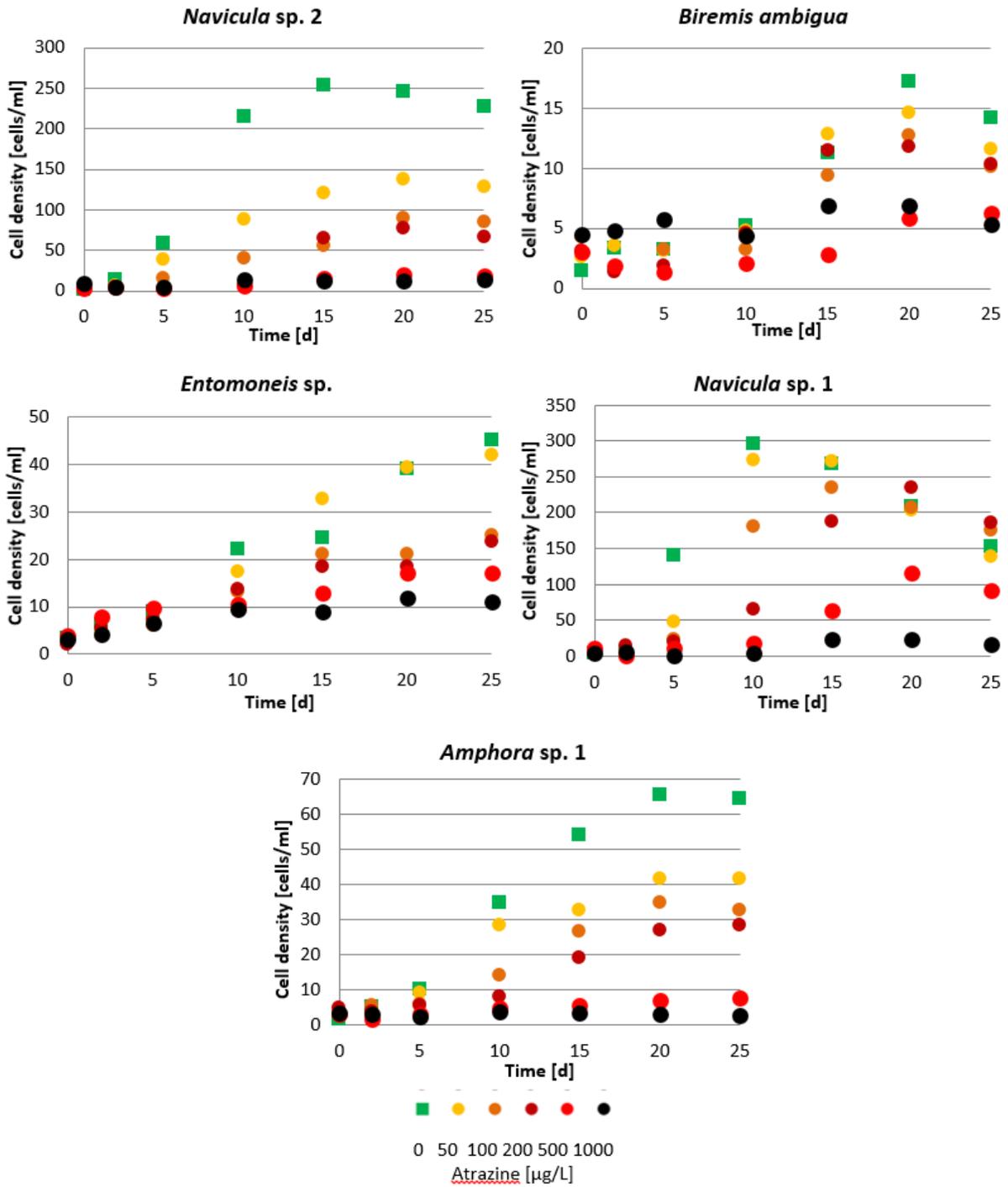
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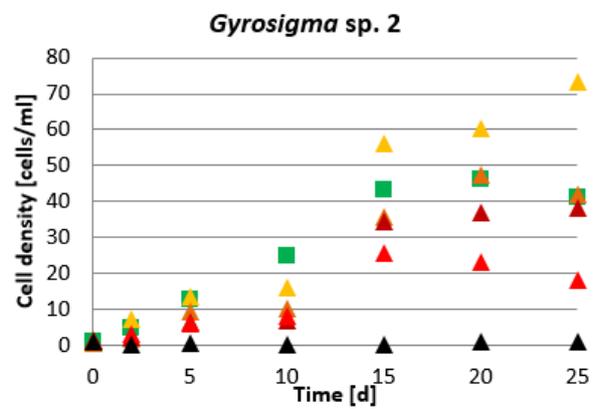
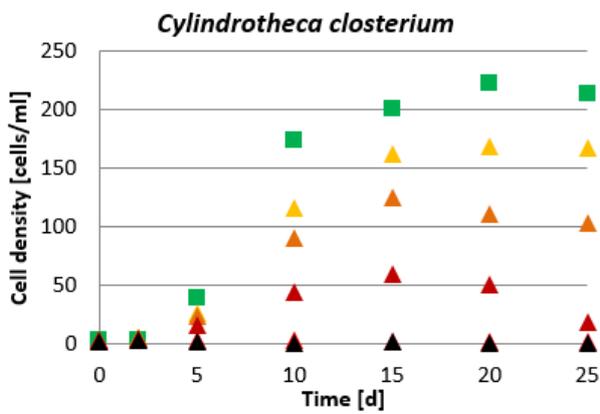
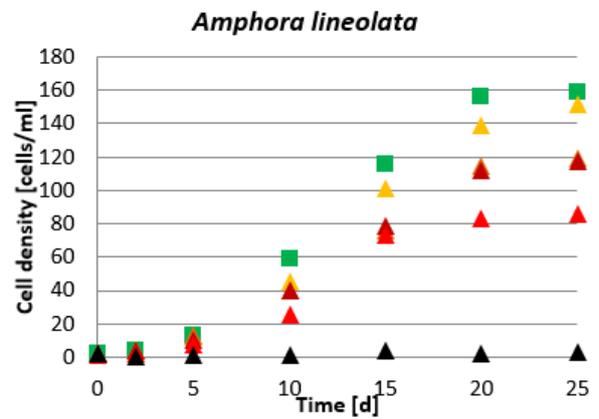
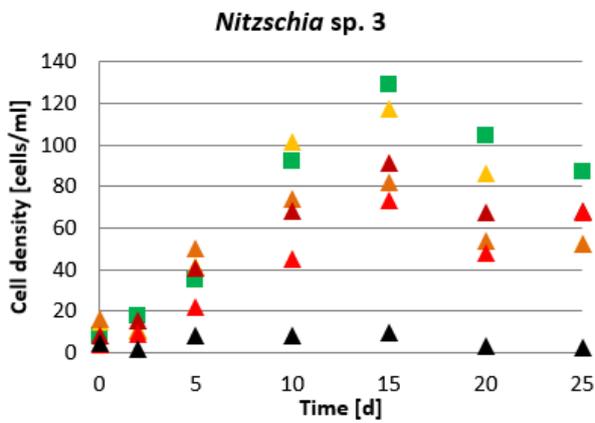
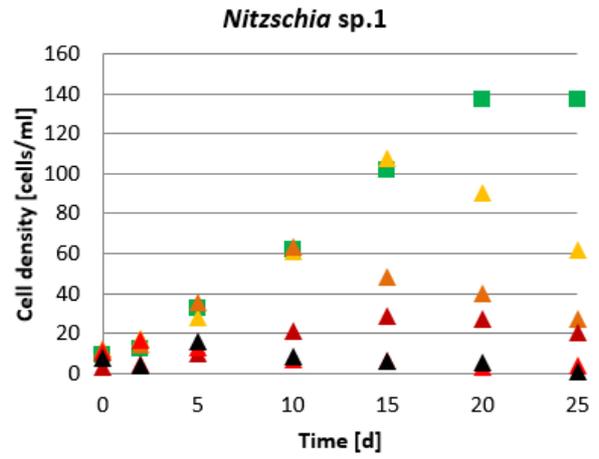
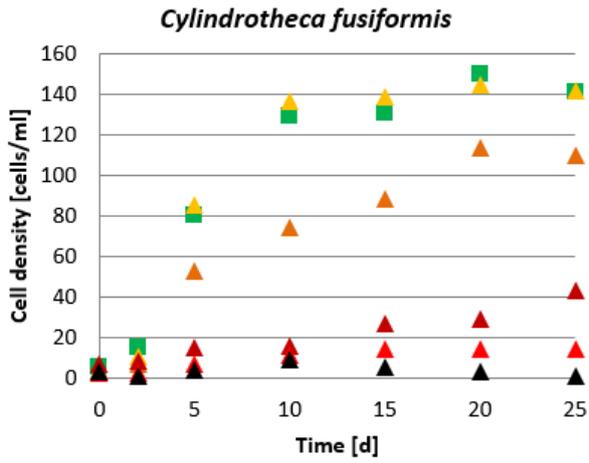


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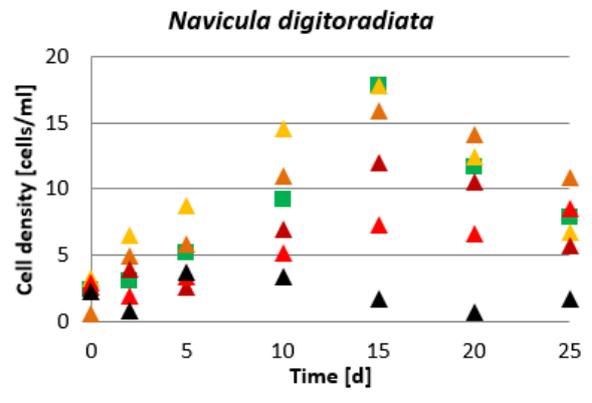
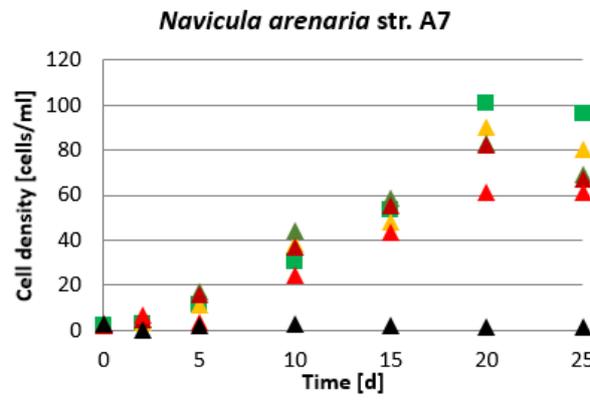
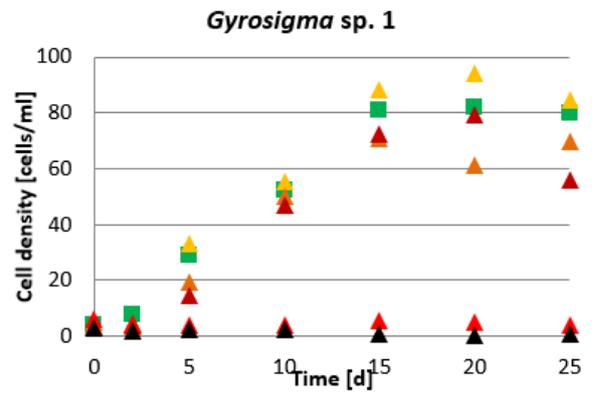
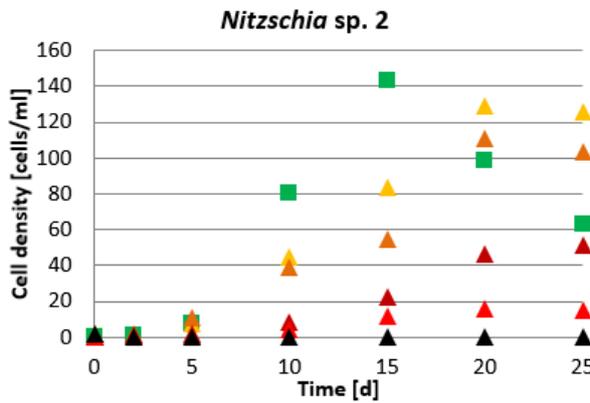
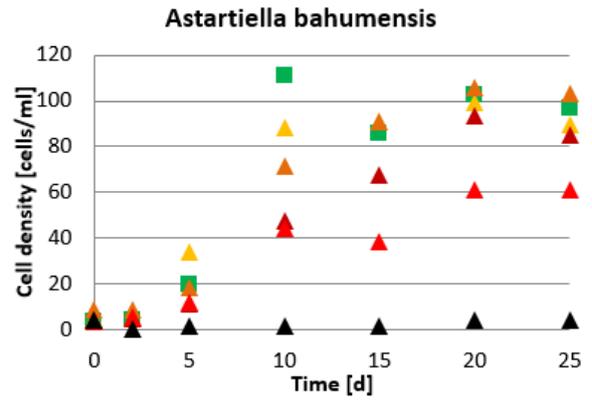
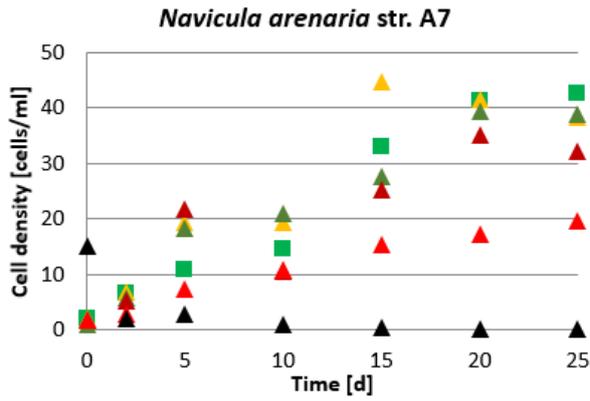


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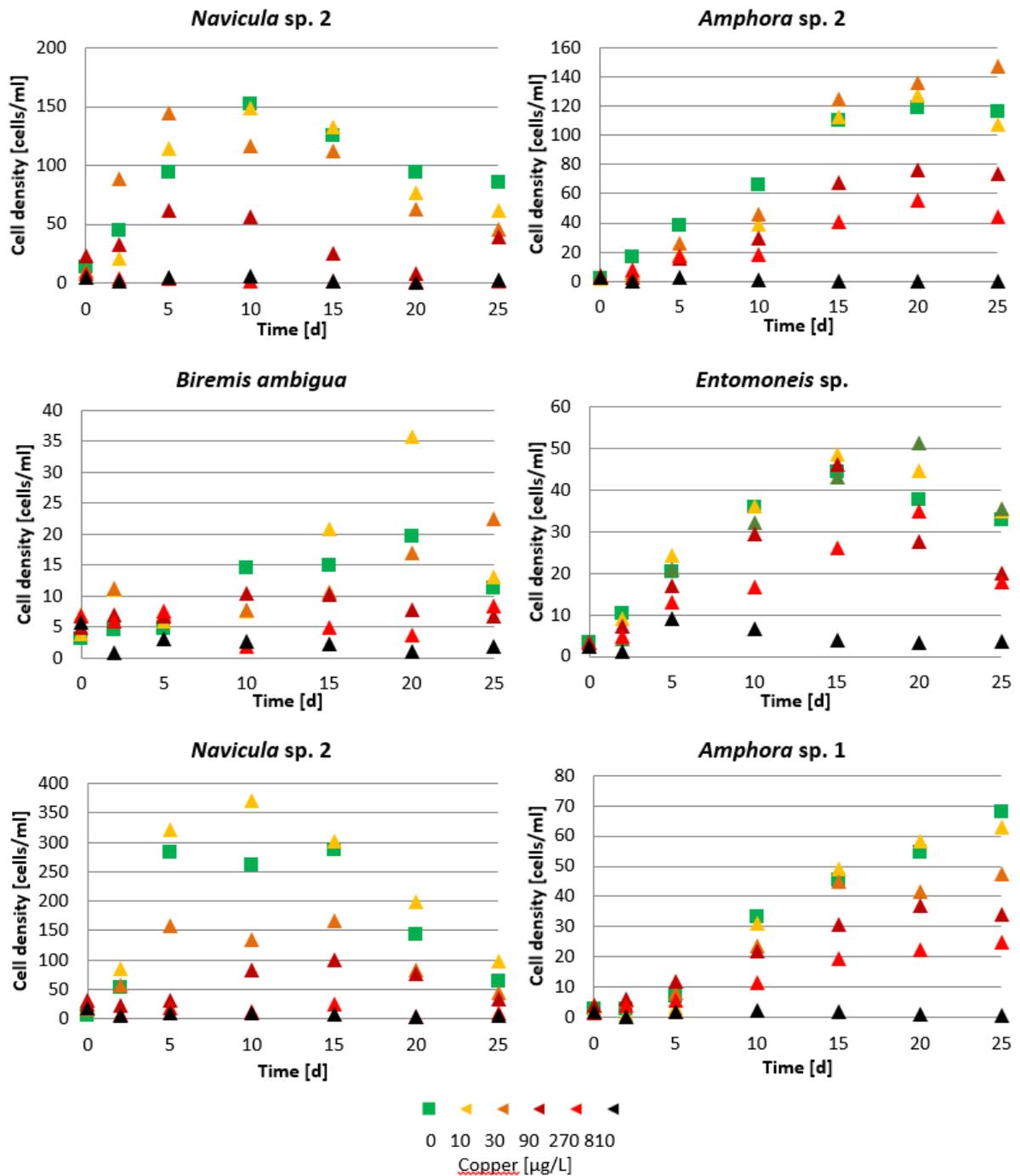
3573 **Fig. S2 a:** Cell densities per species in the atrazine experiment. Colour codes refer the atrazine
 3574 concentrations (green = control, yellow = 50 µg/L, orange = 100 µg/L, dark red = 200 µg/L, red = 500 µg/L,
 3575 black = 1000 µg/L).
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Fig. S2 b: Cell densities per species in the copper experiment. Colour codes refer the copper concentrations (green = control, yellow = 10 µg/L, orange = 30 µg/L, dark red = 90 µg/L, red = 270 µg/L, black = 810 µg/L).

3584 **Addendum III – Supporting Material Chapter 4**

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Community	Species	Acc. Nr.	Biovolume total [10 ³ mm ³]	Biovolume / cell [µm ³]	EPS total [mg/ml]	EPS / biomass [mg/mm ³]
A	<i>Nitzschia</i> sp. 1	DCG 0483	31.46 (+- 3.09)	380 (+- 48)	0.25 (+- 0.01)	7.90 (+- 0.49)
	<i>Nitzschia acicularis</i>	DCG 0459	25.39 (+- 3.08)	517 (+- 68)	0.31 (+- 0.01)	12.43 (+- 1.76)
	<i>Amphora lineolata</i>	DCG 0478	18.47 (+- 2.82)	867 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)
	<i>Cylindrotheca closterium</i>		35.68 (+- 2.81)	345 (+- 60)	0.32 (+- 0.03)	9.16 (+- 1.68)
	<i>Gyrosigma</i> sp. 2	DCG 0491	43.52 (13.67)	2081 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)
	<i>Navicula arenaria</i>	DCG 0487	78.88 (18.50)	5644 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)
B	<i>Gyrosigma</i> sp. 1	DCG 0468	20.62 (+- 5.49)	2147 (+- 509)	1.56 (+- 0.42)	75.88 (+- 5.15)
	<i>Entomoneis</i> sp.	DCG 0466	87.72 (+- 9.33)	10932 (+- 2743)	0.16 (+- 0.02)	1.93 (+- 0.30)
	<i>Navicula arenaria</i>	DCG 0487	78.88 (18.50)	5644 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)
	<i>Amphora lineolata</i>	DCG 0478	18.47 (+- 2.82)	867 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)
	<i>Gyrosigma</i> sp. 2	DCG 0491	43.52 (13.67)	2081 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)
	<i>Navicula digitoradiata</i>	DCG 0490	36.32 (+- 6.86)	5738 (+- 528)	1.36 (+- 0.31)	39.19 (+- 14.76)

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Table S1: Listing of the two diatom communities used in the experiments. From left to right are indicated: species name, strain accession number ('Acc. Nr. ') in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (<http://bccm.belspo.be>), biovolume (total and per cell) and EPS production (total and per biomass) in monoculture (all n=3).

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Substance	Theoretical concentration [µg/l]	Measured Concentration [µg/l]
Control	<1	<2.6
Atrazine	200	160
Atrazine	500	542
Copper	200	271
Copper	500	508

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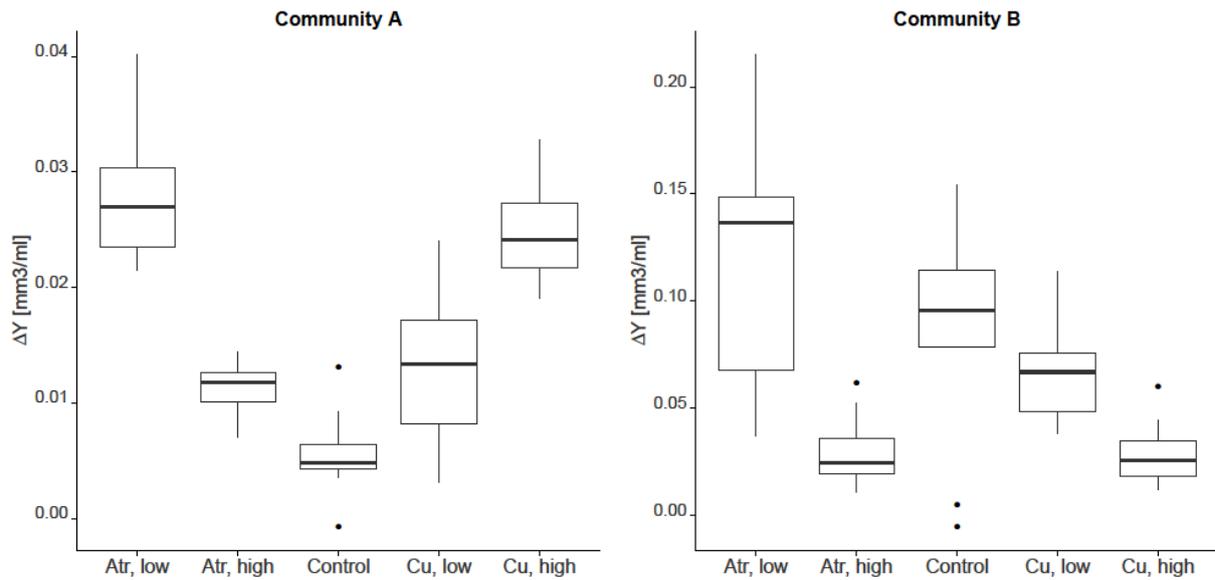
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Table S2: Experimental atrazine and copper treatments with indication of theoretical and verified atrazine and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of quantification : < 1,0 µg/L atrazine and 2.6 µg/L copper for 10 ml samples).



3600 **Fig. S1:** The net biodiversity effect in two benthic diatom communities (A and B) exposed to low and high
 3601 (200 and 500 $\mu\text{g/L}$) concentrations of copper and atrazine. ΔY is the difference between the biomass yield
 3602 observed in diatom communities and the yield expected from the individual species' monocultures.
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Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	PLR
A	Atr, low	0.0038	0.0005	6.96	<0.0001						
	Atr, high	0.0016	0.0005	3.06	0.0040	1	-405	208	Yes		
	Cu, low	0.0012	0.0005	2.15	0.0373						
	Cu, high	0.0043	0.0005	7.98	<0.0001					7.23	0.1244
	Atr, low	0.0038	0.0005	8.04	<0.0001						
	Atr, high	0.0017	0.0055	3.42	0.0014	2	-403	212	Yes		
	Cu, low	0.0012	0.0006	1.88	0.0667						
	Cu, high	0.0043	0.0003	12.00	<0.0001						
B	Atr, low	-0.0072	0.0016	-4.55	<0.0001						
	Atr, high	-0.0125	0.0016	-7.88	<0.0001	1	-319	165	Yes		
	Cu, low	-0.0215	0.0016	-13.50	<0.0001						
	Cu, high	-0.0134	0.0016	-8.41	<0.0001					15.28	0.0042
	Atr, low	-0.0072	0.0219	-3.31	0.0019						
	Atr, high	-0.0125	0.0147	-8.55	<0.0001	2	-326	173	Yes		
	Cu, low	-0.0215	0.0167	-12.84	<0.0001						
	Cu, high	-0.0134	0.0148	-9.01	<0.0001						

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3607 **Table S3:** Results of generalized least squared models predicting changes in the dominance effect under
 3608 copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom community (A
 3609 or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to: high or low
 3610 (200 or 500 $\mu\text{g/L}$) copper and atrazine. 'Slope' indicates the relation between the dominance effect and

3611 treatment type, i.e. the difference of the dominance effect (in mm³/ml) at each stress level compared to the
 3612 control. 's.e.' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and p-values, bold values
 3613 are statistically significant. 'Mod' indicates if the model was fitted without (Model 1) or with (Model 2) a
 3614 variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood. 'Validity' denotes if
 3615 residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no', models were refitted
 3616 ('Model 2') with a variance structure allowing the residuals to change according to treatment type. 'LR' is
 3617 the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

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Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	PLR
A	Atr, low	0.0191	0.0022	8.67	<0.0001						
	Atr, high	0.0040	0.0022	1.80	0.0799	1	-293	152	Yes		
	Cu, low	0.0060	0.0022	2.73	0.0094						
	Cu, high	0.0148	0.0022	6.73	<0.0001					9.31	0.0537
	Atr, low	0.0191	0.0024	7.83	<0.0001						
	Atr, high	0.0040	0.0014	2.92	0.0057	2	-294	157	Yes		
	Cu, low	0.0060	0.0021	2.88	0.0064						
	Cu, high	0.0148	0.0019	7.64	<0.0001						
B	Atr, low	0.0390	0.0178	2.19	0.0300						
	Atr, high	-0.0417	0.0178	-2.34	0.0242	1	-125	69	No		
	Cu, low	0.0029	0.0178	0.16	0.8707						
	Cu, high	-0.0421	0.0178	-2.37	0.0229					21.91	0.0002
	Atr, low	0.0390	0.0258	1.51	0.1392						
	Atr, high	-0.0417	0.0177	-2.36	0.0234	2	-139	79	Yes		
	Cu, low	0.0029	0.0184	0.16	0.8749						
	Cu, high	-0.0421	0.0175	-2.40	0.0210						

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3620 **Table S4:** Results of generalized least squared models predicting changes in the complementarity effect
 3621 under copper and atrazine stress compared to control conditions. 'Com' denotes for which diatom
 3622 community (A or B) the models were fitted. 'Stress' denotes which type of stress diatoms were exposed to:
 3623 high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the
 3624 complementarity effect and treatment type, i.e. the difference of the complementarity effect (in mm³/ml) at
 3625 each stress level compared to the control. 's.e.' is the standard error on the estimated slopes. 'T' and 'P'
 3626 denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted
 3627 without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik'
 3628 the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not
 3629 ('no'). If 'no', models were refitted ('Model 2') with a variance structure allowing the residuals to change
 3630 according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-
 3631 value.

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Com	Effect	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	TIC	Atr, low	0.0189	0.0026	7.08	<0.0001	1	-278	145	Yes	3.99	0.4076
		Atr, high	0.0090	0.0026	3.27	0.0022						
		Cu, low	0.0072	0.0026	2.72	0.0096						
		Cu, high	0.0141	0.0026	5.32	<0.0001						
		Atr, low	0.0189	0.0027	6.99	<0.0001	2	-274	147	Yes		
		Atr, high	0.0090	0.0021	4.18	0.0002						
		Cu, low	0.0072	0.0021	3.44	0.0014						
		Cu, high	0.0141	0.0025	5.68	<0.0001						
	TDC	Atr, low	0.0004	0.0011	0.34	0.7318	1	-349	180	No	32.73	<0.0001
		Atr, high	-0.0047	0.0011	-4.21	0.0001						
		Cu, low	-0.0012	0.0011	-1.06	0.2964						
		Cu, high	0.0008	0.0011	0.68	0.5012						
		Atr, low	0.0004	0.0006	0.69	0.4907	2	-372	196	Yes		
		Atr, high	-0.0047	0.0013	-3.55	0.0010						
		Cu, low	-0.0012	0.0004	-2.78	0.0082						
		Cu, high	0.0008	0.0010	0.78	0.4402						
B	TIC	Atr, low	0.0391	0.0292	1.34	0.1885	1	-86	49	Yes	19.16	0.0007
		Atr, high	-0.018	0.0292	-0.60	0.5529						
		Cu, low	0.1567	0.0292	5.36	<0.0001						
		Cu, high	0.0311	0.0292	1.06	0.2938						
		Atr, low	0.0391	0.0184	2.12	0.0400	2	-97	59	Yes		
		Atr, high	-0.018	0.0151	-1.16	0.2539						
		Cu, low	0.1567	0.0337	4.64	<0.0001						
		Cu, high	0.0311	0.0306	1.02	0.3150						
	TDC	Atr, low	-0.0002	0.0233	-0.01	0.9940	1	-104	58	No	51.18	<0.0001
		Atr, high	-0.0242	0.0233	-1.04	0.3063						
		Cu, low	-0.1538	0.0233	-6.60	<0.0001						
		Cu, high	-0.0732	0.0233	-3.14	0.0032						
		Atr, low	-0.0002	0.0087	-0.02	0.9837	2	-147	84	Yes		
		Atr, high	-0.0242	0.0047	-5.18	<0.0001						
		Cu, low	-0.1538	0.0279	-5.52	<0.0001						
		Cu, high	-0.0732	0.0230	-3.18	0.0028						

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3636 **Table S5:** Results of generalized least squared models predicting changes in two-way complementarity
3637 (TIC) and one-way complementarity (TDC) under copper and atrazine stress compared to control
3638 conditions. 'Com' denotes for which diatom community (A or B) the models were fitted. 'Effect' indicates
3639 the complementarity effect (TIC or TDC). 'Stress' denotes which type of stress diatoms were exposed to:
3640 high or low (200 or 500 µg/L) copper and atrazine. 'Slope' indicates the relation between the respective
3641 complementarity effect and treatment type, i.e. the difference of the complementarity effect (in mm³/ml) at

3642 each stress level compared to the control. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P'
 3643 denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted
 3644 without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik'
 3645 the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not
 3646 ('no'). If 'no', models were refitted (('Model 2') with a variance structure allowing the residuals to change
 3647 according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-
 3648 value.

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Com	Stress	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr	1.2e-3	3.7e-4	3.16	0.0158	1	-44	25	Yes	0.43	0.81
		9.1e-4	3.3e-4	2.76	0.0282	2	-40	25	Yes		
	Cu	-2.6e-4	4.2e-4	-0.64	0.5452	1	-36	21	Yes	3.18	0.20
		1.1e-4	1.9e-4	0.55	0.5964	2	-37	23	Yes		
B	Atr	1.4e-3	5.1e-3	0.28	0.7884	1	-14	10	Yes	5.04	0.08
		-6.5e-3	2.2e-3	3.03	0.0192	2	-15	12	Yes		
	Cu	1.2e-2	3.6e-3	3.96	0.0115	1	-5.0	5.5	Yes	3.66	0.16
		2.1e-2	2.7e-3	7.59	0.0001	2	-6.0	8.0	Yes		

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3653 **Table S6:** Results of generalized least squared models predicting changes in two-way complementarity
 3654 (TIC) from diatom EPS production. 'Com' denotes for which diatom community (A or B) the models were
 3655 fitted. 'Stress' denotes which type of stress diatoms were exposed to: copper (Cu) and atrazine (Atr). Models
 3656 were fitted over the three concentrations of each stressor (control, 200 and 500 µg/L). 'Slope' indicates the
 3657 relation between EPS production and TIC. 's.e. ' is the standard error on the estimated slopes. 'T' and 'P'
 3658 denote the t- and p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted
 3659 without (Model 1) or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik'
 3660 the log-likelihood. 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not
 3661 ('no'). If 'no', models were refitted (('Model 2') with a variance structure allowing the residuals to change
 3662 according to treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-
 3663 value.

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Com	Stress	Pred	Slope	s.e.	T	P	Mod	AIC	Log lik.	Validity	LR	P LR
A	Atr	Tol	-0.27	0.21	-1.30	0.2240	1	20.0	-7.0	Yes	<0.01	0.95
			-0.27	0.21	-1.30	0.2213	2	22.0	-7.0	Yes		
	Yield	-0.23	0.16	-1.38	0.1983	1	20.3	-7.2	No	0.01	0.92	
		-0.23	0.16	-1.39	0.1937	2	-22.3	-7.1	No			
	Cu	Tol	-0.19	0.25	-0.78	0.4557	1	29.2	-11.6	Yes	0.40	0.53
			-0.21	0.26	-0.79	0.4501	2	30.9	-11.4	Yes		
		Yield	-0.25	0.23	-1.10	0.2982	1	28.8	-11.4	Yes	0.44	0.51
			-0.27	0.24	-1.13	0.2841	2	30.4	-11.2	Yes		
B	Atr	Tol	-0.41	0.31	-1.33	0.2107	1	21.2	-7.6	Yes	0.19	0.66
			-0.44	0.30	-1.47	0.1735	2	23.0	-7.5	Yes		
	Yield	-0.21	0.23	-0.89	0.3960	1	22.8	-8.4	Yes	0.11	0.75	
		-0.22	0.23	-0.97	0.3540	2	24.7	-8.3	Yes			
	Cu	Tol	-0.78	0.21	-3.80	0.0035	1	19.3	-6.9	Yes	0.21	0.65
			-0.80	0.20	-3.99	0.0026	2	21.2	-6.6	Yes		
		Yield	-0.54	0.18	-2.99	0.0136	1	22.4	-8.2	Yes	0.08	0.77
			-0.55	0.18	-3.07	0.0118	2	24.3	-8.2	Yes		

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3667 **Table S7:** Results of generalized least squared models predicting the deviation of observed relative yield
3668 from observed frequency of diatoms grown in atrazine- and copper-exposed communities from the species'
3669 monoculture yields and stress tolerance. 'Com' denotes for which diatom community (A or B) the models
3670 were fitted. 'Stress' denotes which type of stress diatoms were exposed to: copper (Cu) and atrazine (Atr).
3671 Models were fitted over the two concentrations of each stressor (200 and 500 µg/L). 'Pred' denotes the
3672 predictor: species monoculture yield ('Yield') or stress tolerance ('Tol'). 'Slope' indicates the relation
3673 between the deviation of the species' observed yield from their observed frequency and their monoculture
3674 yield and stress tolerance. 's.e.' is the standard error on the estimated slopes. 'T' and 'P' denote the t- and
3675 p-values, bold values are statistically significant. 'Mod' indicates if the model was fitted without (Model 1)
3676 or with (Model 2) a variance structure. 'AIC' is the Akaike information criterion, 'Log lik' the log-likelihood.
3677 'Validity' denotes if residuals were homogeneous and normally distributed ('yes') or not ('no'). If 'no',
3678 models were refitted ('Model 2') with a variance structure allowing the residuals to change according to
3679 treatment type. 'LR' is the likelihood ratio of model 1 vs. model 2, P LR the corresponding p-value.

3680

3681 **Addendum IV – Supporting Material Chapter 5**

3682

Species	Acc. Nr.	Biovolume total [10 ³ mm ³]	Biovolume / cell [µm ³]	EPS total [mg/ml]	EPS / biomass [mg/mm ³]	EFA total [µg/ml]	EFA / biomass [µg / mm ³]
<i>Nitzschia</i> sp.	DCG 0483	31.46 (+- 3.09)	380.34 (+- 48)	0.25 (+- 0.01)	7.90 (+- 0.49)	5.80 (+- 0.58)	184.47 (+- 4.31)
<i>Nitzschia acicularis</i>	DCG 0459	25.39 (+- 3.08)	517.58 (+- 68)	0.31 (+- 0.01)	12.43 (+- 1.76)	2.53 (+- 0.63)	98.67 (+- 13.62)
<i>Amphora lineolata</i>	DCG 0478	18.47 (+- 2.82)	867.75 (+- 247)	0.30 (+- 0.01)	16.65 (+- 3.02)	0.33 (+- 0.08)	18.34 (+- 7.59)
<i>Cylindrotheca closterium</i>		35.68 (+- 2.81)	345.20 (+- 60)	0.32 (+- 0.03)	9.16 (+- 1.68)	0.46 (+- 0.17)	13.17 (+- 5.24)
<i>Gyrosigma</i> sp.	DCG 0491	43.52 (+-13.67)	2081.56 (+- 241)	0.27 (+- 0.11)	6.47 (+- 2.92)	0.70 (+- 0.21)	16.23 (+- 3.44)
<i>Navicula arenaria</i>	DCG 0487	78.88 (+-18.50)	5644.09 (+- 723)	0.18 (+- 0.01)	2.37 (+- 0.49)	3.29 (+- 0.81)	42.13 (+- 8.27)

3683 **Table S1:** Listing of the six diatom strains used in the experiments. From left to right are indicated: species name, strain accession number ('Acc. Nr. ') in the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (<http://bccm.belspo.be>), biovolume (total and per cell), EPS and EFA production (total and per biovolume) in monoculture (all n=3).

3688

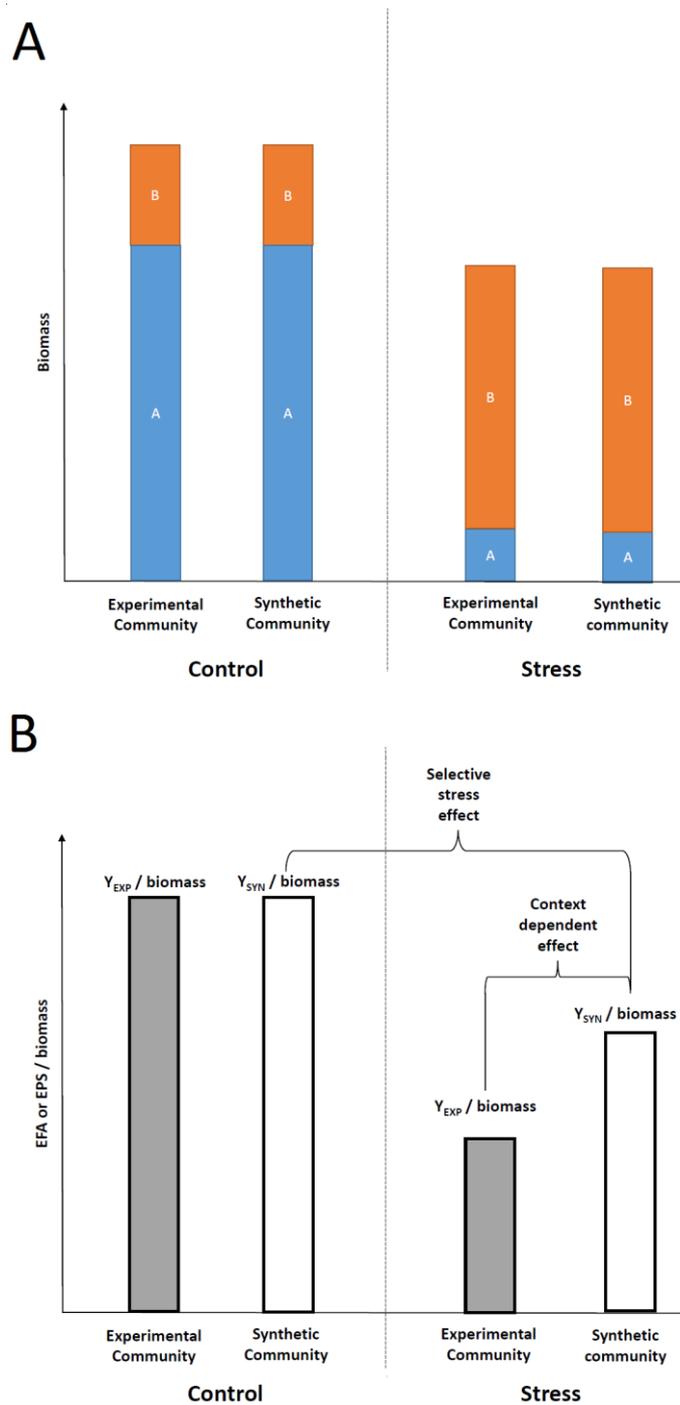
3689

Substance	Theoretical concentration [µg/l]	Measured Concentration [µg/l]
Control	<1	<2.6
Atrazine	200	160
Atrazine	500	542
Copper	200	271
Copper	500	508

3690 **Table S2:** Experimental atrazine and copper treatments with indication of theoretical and verified atrazine and copper concentrations as determined by GC-MS and graphite furnace AAS respectively (Limits of quantification : < 1,0 µg/L atrazine and 2.6 µg/L copper for 10 ml samples).

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3695



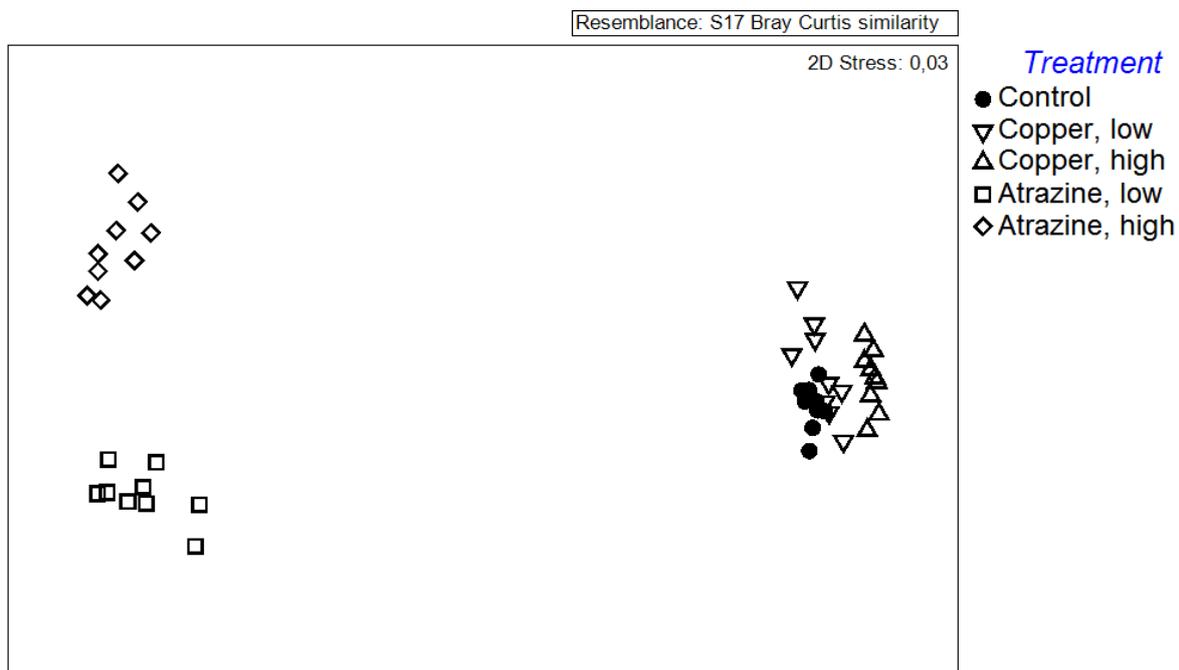
3696

3697 **Fig. S1:** Quantification of selective and context-dependent stress effects by comparison of the EFA and EPS
 3698 production in experimental and synthetic communities. For the sake of clarity, this example uses a
 3699 community with only two species (A and B) and only one stress level. Panel A shows the community
 3700 structure of experimental and synthetic communities. In the experimental community, stress induces a
 3701 change in biomass and community structure (dominance of species B under stress). The synthetic
 3702 community reflects the biomass and community structure observed in the corresponding experimental
 3703 community. Panel B shows the EFA or EPS production per unit biomass in the experimental community
 3704 (Y_{EXP}) and in the synthetic community (Y_{SYN}). Y_{SYN} is calculated by multiplying each species' EFA or EPS
 3705 production in unstressed monocultures with its biomass share in the corresponding experimental
 3706 community. Y_{SYN} thus reflects the EFA or EPS production expected in a community without stress exposure
 3707 and species interactions, but which has the same biomass and community structure as induced by stress.
 3708 Selective stress effects are quantified by comparing synthetic communities reflecting the community

3709 structure under stress and under control conditions. Confounding effects of direct stress or changes in
 3710 species interactions are eliminated, since Y_{SYN} of both communities is calculated from unstressed
 3711 monocultures. Context-dependent effects are quantified by comparing experimental and synthetic
 3712 communities of the same community structure. Confounding effects of community structure are eliminated,
 3713 and differences in Y_{EXP} and Y_{SYN} are attributed to species interactions or direct stress effects on Y_{EXP} . In this
 3714 example, Y_{EXP} is lower under stress than in the control. This is due to both context-dependent stress effects
 3715 ($Y_{EXP} \text{ stress} < Y_{SYN} \text{ stress}$) and selective stress effects ($Y_{SYN} \text{ stress} < Y_{SYN} \text{ control}$). Here, stress would thus
 3716 reduce diatom EFA or EPS production by reducing the species' EFA or EPS output, as well as by causing
 3717 dominance by an unproductive species.

3718

3719



3720
 3721 **Fig. S2:** Non-metric multidimensional scaling (Bray-Curtis similarity) of untransformed relative (%)
 3722 biomass data of communities from unstressed conditions and exposed to low (200 µg/L) and high (500
 3723 µg/L) atrazine and copper concentrations.
 3724

Treatments	Dissimilarity	Species	Contribution
Copper, low	14.49	<i>Navicula arenaria</i>	33.25
		<i>Nitzschia acicularis</i>	33.00
		<i>Gyrosigma</i> sp.	10.92
Copper, high	18.30	<i>Navicula arenaria</i>	28.56
		<i>Nitzschia</i> sp.	19.60
		<i>Nitzschia acicularis</i>	16.51
Atrazine, low	69.79	<i>Cylindrotheca closterium</i>	49.63
		<i>Nitzschia acicularis</i>	28.45
		<i>Navicula arenaria</i>	12.71
Atrazine, high	75.97	<i>Nitzschia acicularis</i>	39.20
		<i>Cylindrotheca closterium</i>	25.23
		<i>Navicula arenaria</i>	24.79

3725

3726 **Table S3.** Results of dissimilarity percentage (SIMPER) analysis of biomass composition of diatom
 3727 communities grown exposed to copper and atrazine compared to control communities. Analysis was run on
 3728 the untransformed biomass data. 'Treatments' indicates the treatment whose biomass composition is
 3729 compared to the control: low (200 µg/L) and high (500 µg/L) atrazine and copper. 'Dissimilarity' indicates
 3730 the average dissimilarity (in %) of biomass composition of the respective treatment and the control.
 3731 'Species' indicates the three species contributing most to dissimilarities with the control biomass
 3732 composition. 'Contribution' indicates the average contribution (in %) of each species to dissimilarity from
 3733 the control biomass composition.
 3734

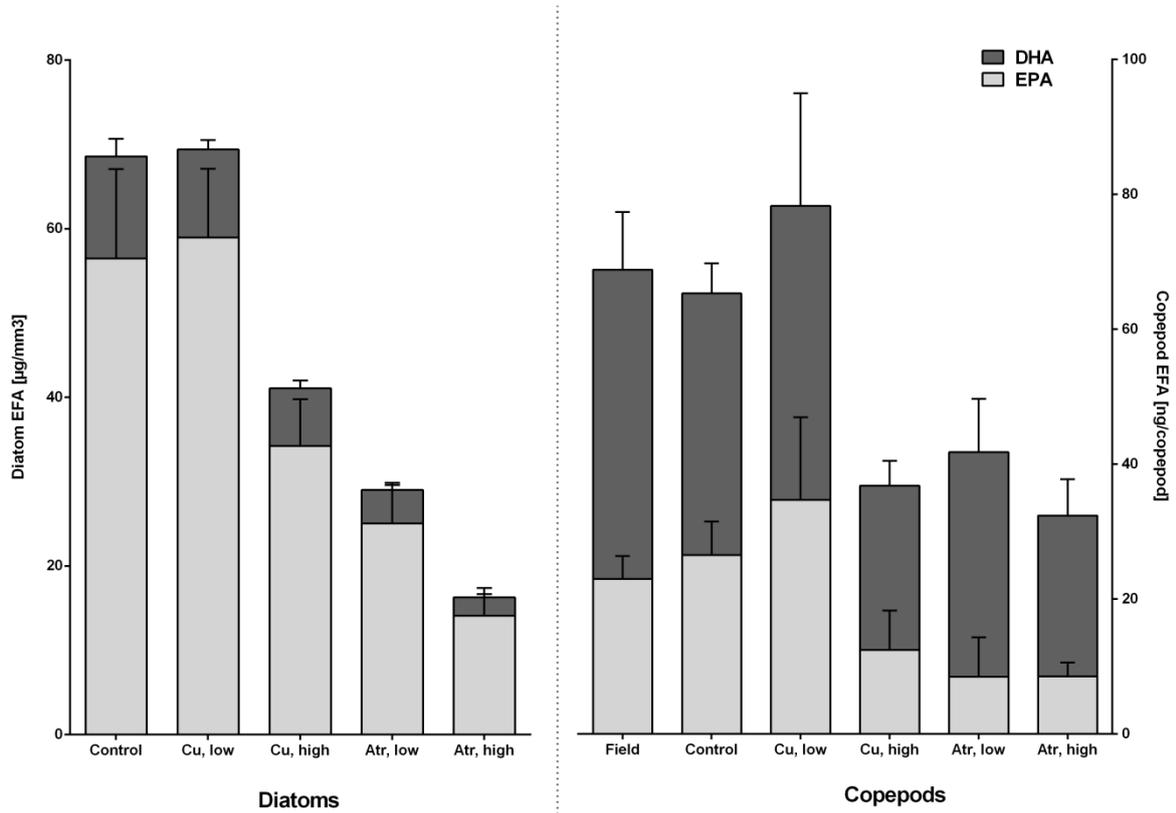
3735

Treatment	Similarity	Species	Mean	Contribution
Control	91.52	<i>Nitzschia acicularis</i>	20.01	48.02
		<i>Navicula arenaria</i>	15.17	32.56
		<i>Nitzschia sp.</i>	2.78	5.93
Copper, low	84.07	<i>Nitzschia acicularis</i>	18.30	49.25
		<i>Navicula arenaria</i>	12.69	33.26
		<i>Nitzschia sp.</i>	2.70	7.47
Copper, high	90.06	<i>Nitzschia acicularis</i>	20.96	62.52
		<i>Navicula arenaria</i>	11.62	33.10
Atrazine, low	88.32	<i>Cylindrotheca closterium</i>	36.15	76.27
		<i>Navicula arenaria</i>	7.44	9.85
		<i>Gyrosigma sp.</i>	4.78	3.78
Atrazine, high	86.27	<i>Cylindrotheca closterium</i>	15.68	74.22
		<i>Gyrosigma sp.</i>	2.76	11.90
		<i>Navicula arenaria</i>	2.46	9.06

3736 **Table S4:** Results of similarity percentage (SIMPER) analysis of biomass composition of diatom
 3737 communities grown under unstressed conditions as well as copper and atrazine exposure. Analysis was run
 3738 on the untransformed biomass data. 'Treatment' indicates the growth conditions: control, low (200 µg/L)
 3739 and high (500 µg/L) atrazine and copper. 'Similarity' indicates the average similarity (in %) of biomass
 3740 composition within the nine replicates of each treatment. 'Species' indicates the species contributing most
 3741 to similarities within treatments (only species with a mean biomass > 2 x 10³ µm³ are reported). 'Mean'
 3742 indicates the mean biomass (in 10³ µm³) of species within treatments. 'Contribution' indicates the
 3743 contribution (in %) of each species to similarities within treatments.
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Fig. S3: EPA and DHA content of diatom communities exposed to low (200 µg/L) and high (500 µg/L) atrazine ('Atr') and copper ('Cu') concentrations, and EPA and DHA content of *Microarthridion littorale* collected in the field and after 10d of feeding on the diatom diet that was subject to the stressors (see left part of the figure for corresponding abbreviations of the treatments).

3752

3753 **Addendum V – Acute toxicity of copper to harpacticoid copepods**

3754

3755 Addendum V reports the results of single-species toxicity tests, which measured the tolerance of
3756 five harpacticoid copepod species to direct copper exposure.

3757 Harpacticoid copepods were collected from sediments at the Paulina intertidal flat (SW
3758 Netherlands, 51° 21'N, 3°43'E). Five harpacticoid copepod species (*Microarthridion littorale*,
3759 *Stenhelia* sp., *Platychelipus littoralis*, *Nannopus palustris* and one species of the family
3760 Ectinosomatidae) were extracted alive from the sediment using a mixed technique of sediment
3761 decantation and extraction via white light attraction. Adult specimens were randomly collected
3762 with a glass pasteur pipette using a Wild M5 binocular. Copepods were washed 3 times over a 38
3763 µm sieve and placed in glass jars with filtered and autoclaved natural seawater overnight in order
3764 to empty their intestines prior to the start of the experiment.

3765 Copepod sensitivity to direct copper exposure was quantified by exposing the five harpacticoid
3766 species collected at the sampling site for 48h to a concentration gradient of 0 to 2430 µg/L copper
3767 (Table S1). The experiment was conducted in glass jars containing 100 ml of filtered and
3768 autoclaved seawater in a climate room at 15±1 °C with a 12:12 h light:dark cycle and 40 to 50
3769 µmol photons m⁻² s⁻¹. During the experiment, harpacticoids were offered 0.5 mm³ of an equal mix
3770 of the six diatom species used as food source in Chapter 5 (*Nitzschia* sp., *N. acicularis*, *C. closterium*,
3771 *A. lineolata*, *Gyrosigma* sp., *N. arenaria*).

Cu [µg/l]	Replicates (copepod individuals per replicate)				
	<i>Microarthridion littorale</i>	<i>Stenhelia sp.</i>	<i>Nannopus palustris</i>	Ectinosomatidae sp.	<i>Platychelipus littoralis</i>
0	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
10	5 (15)				5 (30)
30	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
90	5 (15)				5 (30)
270	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)
810	5 (15)	5 (15)	5 (15)		5 (30)
2430	5 (15)	5 (15)	5 (15)	5 (15)	5 (30)

3772
3773 **Table S1:** Experimental copper treatments with indication of the number of replicates per treatment and
3774 the number of individuals per replicate (in brackets) for each copepod species (*Microarthridion littorale*,
3775 *Stenhelia* sp., *Nannopus palustris*, Ectinosomatidae sp., *Platychelipus littoralis*)

3776

3777 Copepod sensitivity to direct copper exposure was quantified as the 48-h median lethal
3778 concentration (LC50), i.e. the effective copper concentration inducing a 50% mortality of each

3779 harpacticoid species. LC50 values were calculated by means of a three-parameter log-logistic
 3780 model (Equation 1) and a three-parameter Weibull model in two different parameterisations
 3781 (Equations 2 and 3) as described in Ritz & Streibig (2005).

3782 $Y = d / (1 + \exp\{b[\log x - \log e]\})$ [1]

3783 $Y = d(1 - \exp\{-\exp[b(\log x - \log e)]\})$ [2]

3784 $Y = d(\exp\{-\exp[b(\log x - e)]\})$ [3]

3785 *Y* represents the response variable (surviving harpacticoid individuals), *b* the relative slope of the
 3786 curve, *d* the upper limit, *e* the inflection point, and *x* the copper concentration. Dose-response
 3787 models and LC50 values were computed in R 3.0.1. using RStudio (R Development Core Team
 3788 2016) and the package drc (Ritz and Streibig 2005).

3789
 3790 The five harpacticoid copepod species differed in their sensitivity to direct copper exposure, with
 3791 LC50 values ranging from 387 µg/L (*N. palustris*) to 1475 µg/L (*Stenhelia* sp.). *M. littorale*, being
 3792 the most abundant copepod species at the sampling site representing ~ 90% of all collected
 3793 individuals, was the second most copper-sensitive species with an LC50 of 546 µg/L (Table S2).

Species	LC50	Surviving individuals per treatment (µg/L Cu)						
		Control	10	30	90	270	810	2430
<i>Microarthridion littorale</i>	546.43 (+78.38)	65	67	68	66	48	18	14
<i>Stenhelia</i> sp..	1474.60 (+235.58)	71		70		57	55	14
<i>Nannopus palustris</i>	386.83 (+160.89)	67		67		40	23	0
Ectinosomatidae sp.	696.88 (+188.42)	66		50		48	33	2
<i>Platychelipus littoralis</i>	879.28 (+260.09)	134	133	107	116	115	67	12

3794
 3795 **Table S2:** Copper 48h-LC50 values obtained for five harpacticoid copepod species (standard deviations are
 3796 indicated in brackets). Each treatment had five replicates, with 15 individuals (30 for *Platychelipus littoralis*)
 3797 harpacticoid individuals per replicate. Surviving individuals are indicated as sum of the five replicates.
 3798

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3800 **References**

- 3801 Abdulkadir, S., and M. Tsuchiya. 2008. One-step method for quantitative and qualitative analysis
3802 of fatty acids in marine animal samples. *Journal of Experimental Marine Biology and Ecology*
3803 354:1–8.
- 3804 Albert, C. H., W. Thuiller, N. G. Yoccoz, A. Soudant, F. Boucher, P. Saccone, and S. Lavorel. 2010.
3805 Intraspecific functional variability: Extent, structure and sources of variation. *Journal of*
3806 *Ecology* 98:604–613.
- 3807 Alheit, J., and W. Scheibel. 1982. Benthic harpacticoids as a food source for fish. *Marine Biology*
3808 70:141–147.
- 3809 Andersen, B. S., J. D. Carl, P. Grønkjær, and J. G. Støttrup. 2005. Feeding ecology and growth of age
3810 0 year *Platichthys flesus* (L.) in a vegetated and a bare sand habitat in a nutrient rich fjord.
3811 *Journal of Fish Biology* 66:531–552.
- 3812 Anderson, P. J., and J. F. Piatt. 1999. Community reorganisation in the Gulf of Alaska following
3813 ocean climate regime shift. *Marine Ecology Progress Series* 189:117–123.
- 3814 Arendt, K. E., S. H. Jónasdóttir, P. J. Hansen, and S. Gärtner. 2005. Effects of dietary fatty acids on
3815 the reproductive success of the calanoid copepod *Temora longicornis*. *Marine Biology*
3816 146:513–530.
- 3817 Arndt, C., and U. Sommer. 2014. Effect of algal species and concentration on development and fatty
3818 acid composition of two harpacticoid copepods, *Tisbe* sp. and *Tachidius discipes*, and a
3819 discussion about their suitability for marine fish larvae. *Aquaculture Nutrition* 20:44–59.
- 3820 Arts, M. T., and B. C. Wainmann. 1999. *Lipids in Freshwater Ecosystems*. Springer, New York.
- 3821 Backhaus, T., J. Snape, and J. Lazorchak. 2012. The impact of chemical pollution on biodiversity
3822 and ecosystem services: The need for an improved understanding. *Integrated Environmental*
3823 *Assessment and Management* 8:575–576.
- 3824 Baert, J. M., C. R. Janssen, K. Sabbe, and F. De Laender. 2016. Per capita interactions and stress
3825 tolerance drive stress-induced changes in biodiversity effects on ecosystem functions.
3826 *Nature Communications* 7.
- 3827 Baird, D. J., and P. J. Van den Brink. 2007. Using biological traits to predict species sensitivity to
3828 toxic substances. *Ecotoxicology and Environmental Safety* 67:296–301.

- 3829 Ball, B. A., M. D. Hunter, J. S. Kominoski, C. M. Swan, and M. A. Bradford. 2008. Consequences of
3830 non-random species loss for decomposition dynamics: Experimental evidence for additive
3831 and non-additive effects. *Journal of Ecology* 96:303–313.
- 3832 Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid.
3833 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and
3834 services. *Ecology Letters* 9:1146–56.
- 3835 Barnett, A., V. Méléder, L. Blommaert, B. Lepetit, P. Gaudin, W. Vyverman, K. Sabbe, C. Dupuy, and
3836 J. Lavaud. 2015. Growth form defines physiological photoprotective capacity in intertidal
3837 benthic diatoms. *International Society for Microbial Ecology* 9:1–14.
- 3838 Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L.
3839 McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth
3840 mass extinction already arrived? *Nature* 471:51–57.
- 3841 Bejarano, A. C., and G. T. Chandler. 2003. Reproductive and developmental effects of atrazine on
3842 the estuarine meiobenthic copepod *Amphiascus tenuiremis*. *Environmental Toxicology and*
3843 *Chemistry / SETAC* 22:3009–16.
- 3844 Bejarano, A. C., P. L. Pennington, M. E. Delorenzo, and G. T. Chandler. 2005. Atrazine effects on
3845 meiobenthic assemblages of a modular estuarine mesocosm. *Marine Pollution Bulletin*
3846 50:1398–1404.
- 3847 Beketov, M. A., K. Foit, R. B. Schäfer, C. A. Schriever, A. Sacchi, E. Capri, J. Biggs, C. Wells, and M.
3848 Liess. 2009. SPEAR indicates pesticide effects in streams - Comparative use of species- and
3849 family-level biomonitoring data. *Environmental Pollution* 157:1841–1848.
- 3850 Bell, M., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems:
3851 general pathways and new directions. Page 211–236 in M. Arts, M. Brett, and M. Kainz,
3852 editors. *Lipids in aquatic ecosystems*. Springer, Dordrecht.
- 3853 Bell, M. V., J. R. Dick, T. R. Anderson, and D. W. Pond. 2007. Application of liposome and stable
3854 isotope tracer techniques to study polyunsaturated fatty acid biosynthesis in marine
3855 zooplankton. *Journal of Plankton Research* 29:417–422.
- 3856 Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate
3857 change on the future of biodiversity. *Ecology Letters* 15:365–377.
- 3858 Benbrook, C. M. 2016. Trends in glyphosate herbicide use in the United States and globally.
3859 *Environmental Sciences Europe* 28:3.

- 3860 Bérard, A., and C. Benninghoff. 2001. Pollution-induced community tolerance (PICT) and seasonal
3861 variations in the sensitivity of phytoplankton to atrazine in nanocosms. *Chemosphere*
3862 45:427–437.
- 3863 Bertness, M. D., and R. M. Callaway. 1994. Positive interactions in communities. *Trends in Ecology*
3864 & *Evolution* 9:191–193.
- 3865 Bester, K., H. Hühnerfuss, U. Brockmann, and H. J. Rick. 1995. Biological effects of triazine herbicide
3866 contamination on marine phytoplankton. *Archives of Environmental Contamination and*
3867 *Toxicology* 29:277–283.
- 3868 Bianchini, A., S. E. G. Martins, and I. F. Barcarolli. 2004. Mechanism of acute copper toxicity in
3869 euryhaline crustaceans: implications for the Biotic Ligand Model. *International Congress*
3870 *Series* 1275:189–194.
- 3871 Böger, P., B. Matthes, and J. Schmalfuß. 2000. Towards the primary target of chloroacetamides –
3872 new findings pave the way. *Pest Management Science* 56:497–508.
- 3873 Bolam, S. G., T. F. Fernandes, and M. Huxham. 2002. Diversity, biomass, and ecosystem processes
3874 in the marine benthos. *Ecological Monographs* 72:599–615.
- 3875 Bopp, L., O. Aumont, P. Cadule, S. Alvain, and M. Gehlen. 2005. Response of diatoms distribution to
3876 global warming and potential implications: A global model study. *Geophysical Research*
3877 *Letters* 32:L19606.
- 3878 Borer, A. E. T., E. W. Seabloom, J. B. Shurin, K. E. Anderson, C. A. Blanchette, S. D. Cooper, and B. S.
3879 Halpern. 2005. What determines the strength of a trophic cascade? *Ecology* 86:528–537.
- 3880 Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations (with discussion). *Journal of the*
3881 *Royal Statistical Society B* 26:211–252.
- 3882 Bracken, M. E. S., S. E. Friberg, C. a Gonzalez-Dorantes, and S. L. Williams. 2008. Functional
3883 consequences of realistic biodiversity changes in a marine ecosystem. *Proceedings of the*
3884 *National Academy of Sciences of the United States of America* 105:924–928.
- 3885 Brain, P., and R. Cousens. 1989. An equation to describe dose response where there is stimulation
3886 of growth at low doses. *Weed Research* 29:93–96.
- 3887 Brett, M. T., C. Mu, A. P. Ballantyne, J. L. Ravet, C. R. Goldman, and D. C. Müller-Navarra. 2006.
3888 *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography*.
3889 51:2428–2437.

- 3890 Brett, M. T., and D. C. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic
3891 foodweb processes. *Freshwater Biology* 38:483–499.
- 3892 Bunker, D. E., F. De Clerck, J. C. Bradford, R. K. Colwell, I. Perfecto, O. L. Phillips, M. Sankaran, and
3893 S. Naeem. 2005. Species loss and aboveground carbon storage in a tropical forest. *Science*
3894 310:1029–1031.
- 3895 Van den Brink, P. J., A. C. Alexander, M. Desrosiers, W. Goedkoop, P. L. M. Goethals, M. Liess, and S.
3896 D. Dyer. 2011. Traits-based approaches in bioassessment and ecological risk assessment:
3897 Strengths, weaknesses, opportunities and threats. *Integrated Environmental Assessment*
3898 and Management 7:198–208.
- 3899 De Brouwer, J. F. C., S. Bjelic, E. M. G. T. De Deckere, and L. J. Stal. 2000. Interplay between biology
3900 and sedimentology in a mudflat (Biezelingse Ham, Westerschelde, The Netherlands).
3901 *Continental Shelf Research* 20:1159–1177.
- 3902 Bruno, J. F., K. E. Boyer, J. E. Duffy, and S. C. Lee. 2008. Relative And Interactive Effects Of Plant And
3903 Grazer Richness In A Benthic Marine Community. *Ecology* 89:2518–2528.
- 3904 Bruno, J. F., S. C. Lee, J. S. Kertesz, R. C. Carpenter, Z. T. Long, and J. Emmett Duffy. 2006. Partitioning
3905 the effects of algal species identity and richness on benthic marine primary production. *Oikos*
3906 115:170–178.
- 3907 Buchwalter, D. B., D. J. Cain, C. A. Martin, L. Xie, S. N. Luoma, and T. Garland. 2008. Aquatic insect
3908 ecophysiological traits reveal phylogenetically based differences in dissolved cadmium
3909 susceptibility. *Proceedings of the National Academy of Sciences of the United States of*
3910 *America* 105:8321–6.
- 3911 Buffan-Dubau, E., and K. R. Carman. 2000. Diel feeding behavior of meiofauna and their
3912 relationships with microalgal resources. *Limnology and Oceanography* 45:381–395.
- 3913 Burns, C. W., M. T. Brett, and M. Schallenberg. 2011. A comparison of the trophic transfer of fatty
3914 acids in freshwater plankton by cladocerans and calanoid copepods. *Freshwater Biology*
3915 56:889–903.
- 3916 Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: Functional diversity and
3917 the maintenance of ecological processes and services. *Journal of Applied Ecology* 48:1079–
3918 1087.
- 3919 Cagliari, A., R. Margis, F. dos S. Maraschin, A. C. Turchetto-Zolet, G. Loss, and M. Margis-Pinheiro.
3920 2011. Biosynthesis of Triacylglycerols (TAGs) in plants and algae. *International Journal of*

- 3921 Plant Biology 2:e10.
- 3922 Cannon, W. B. 1926. Physiological regulation of normal states: some tentative postulates
3923 concerning biological homeostatics. Page 91 in A. Petit, editor. A. Charles Richet: ses amis, ses
3924 collègues, ses élèves. Éditions Médicales.
- 3925 Carafa, R., J. Wollgast, E. Canuti, J. Ligthart, S. Dueri, G. Hanke, S. J. Eisenreich, P. Viaroli, and J. M.
3926 Zaldívar. 2007. Seasonal variations of selected herbicides and related metabolites in water,
3927 sediment, seaweed and clams in the Sacca di Goro coastal lagoon (Northern Adriatic).
3928 Chemosphere 69:1625–1637.
- 3929 Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. Nature
3930 472:86–89.
- 3931 Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace,
3932 D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S.
3933 Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–
3934 67.
- 3935 Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I.
3936 O'Connor, and A. Gonzalez. 2011. The functional role of producer diversity in ecosystems.
3937 American Journal of Botany 98:572–92.
- 3938 Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem
3939 functioning through interspecific facilitation. Nature 415:426–9.
- 3940 Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau.
3941 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature
3942 443:989–92.
- 3943 Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J.
3944 J. Weis. 2007. Impacts of plant diversity on biomass production increase through time
3945 because of species complementarity. Proceedings of the National Academy of Sciences of the
3946 United States of America 104:18123–8.
- 3947 Carlisle, D. M., and W. H. Clements. 2005. Leaf litter breakdown, microbial respiration and
3948 shredder production in metal-polluted streams. Freshwater Biology 50:380–390.
- 3949 Carlton, A. N. C. and J. T. 1998. Accelerating invasion Rate in a Highly Invaded Estuary. Science
3950 279:3.

- 3951 Carroll, I. T., B. J. Cardinale, and R. M. Nisbet. 2011. Niche and fitness differences relate the
3952 maintenance of diversity to ecosystem function. *Ecology* 92:1157–1165.
- 3953 Casula, P., A. Wilby, and M. B. Thomas. 2006. Understanding biodiversity effects on prey in multi-
3954 enemy systems. *Ecology Letters* 9:995–1004.
- 3955 CBD. 2010. Strategic Plan for Biodiversity 2011-2020, including Aichi Biodiversity Targets.
3956 Convention on Biological Diversity, Montreal, QC.
- 3957 Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015.
3958 Accelerated modern human – induced species losses: entering the sixth mass extinction.
3959 *Sciences Advances* 1:1–5.
- 3960 Cedergreen, N., C. Ritz, and J. C. Streibig. 2005. Improved empirical models describing hormesis.
3961 *Environmental Toxicology and Chemistry* 24:3166–72.
- 3962 Chapin, F. S. I., E. Schulze, and H. Mooney. 1992. Biodiversity and ecosystem processes. *Trends in*
3963 *Ecology and Evolution* 7:677–680.
- 3964 Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S.
3965 Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing
3966 biodiversity. *Nature* 405:234–42.
- 3967 Chapin, F. S. I., P. . Matson, and H. A. Mooney. 2002. Principles of terrestrial ecosystem ecology.
3968 Springer, New York.
- 3969 Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nisbet,
3970 and T. J. Case. 2002. The interaction between predation and competition. *Ecology Letters*
3971 5:302–315.
- 3972 Chepurnov, V. A., D. G. Mann, P. Von Dassow, P. Vanormelingen, J. Gillard, D. Inzé, K. Sabbe, and W.
3973 Vyverman. 2008. In search of new tractable diatoms for experimental biology. *BioEssays*
3974 30:692–702.
- 3975 Chia, M. A., A. T. Lombardi, M. D. G. G. Melão, and C. C. Parrish. 2013a. Effects of cadmium and
3976 nitrogen on lipid composition of *Chlorella vulgaris* (Trebouxiophyceae, Chlorophyta).
3977 *European Journal of Phycology* 48:1–11.
- 3978 Chia, M. A., A. T. Lombardi, M. da G. G. Melao, and C. C. Parrish. 2013b. Lipid composition of
3979 *Chlorella vulgaris* (Trebouxiophyceae) as a function of different cadmium and phosphate
3980 concentrations. *Aquatic Toxicology* 128–129:171–182.

- 3981 Chirnside, A. E. M., W. F. Ritter, and M. Radosevich. 2007. Isolation of a selected microbial
3982 consortium from a pesticide-contaminated mix-load site soil capable of degrading the
3983 herbicides atrazine and alachlor. *Soil Biology and Biochemistry* 39:3056–3065.
- 3984 Cid, A., C. Herrero, E. Torres, and J. Abalde. 1995. Copper toxicity on the marine microalga
3985 *Phaeodactylum tricornutum*: effects on photosynthesis and related parameters. *Aquatic*
3986 *Toxicology* 1.
- 3987 Clarke, K., and R. Gorley. 2006. PRIMER v6: user manual/ tutorial. PRIMER-E, Plymouth.
- 3988 Clements, W. H., and J. R. Rohr. 2009. Community responses to contaminants: using basic
3989 ecological principles to predict ecotoxicological effects. *Environmental Toxicology and*
3990 *Chemistry* 28:1789.
- 3991 Cleveland, J. S., M. J. Perry, D. A. Kiefer, and M. C. Talbot. 1989. Maximal quantum yield of
3992 photosynthesis in the northwest Sargasso Sea. *Journal of Marine Research* 47:869–886.
- 3993 Cnudde, C., T. Moens, E. Werbrouck, G. Lepoint, D. Van Gansbeke, and M. De Troch. 2015.
3994 Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope
3995 composition and fatty acid profiles. *Marine Ecology Progress Series* 524:225–239.
- 3996 Cnudde, C., A. Willems, K. Van Hoorde, W. Vyverman, T. Moens, and M. De Troch. 2011. Effect of
3997 food preservation on the grazing behavior and on the gut flora of the harpacticoid copepod
3998 *Paramphiascella fulvofasciata*. *Journal of Experimental Marine Biology and Ecology* 407:63–
3999 69.
- 4000 Cobbett, C., and P. Goldsbrough. 2002. Phytochelatins and metallothioneins: roles in heavy metal
4001 detoxification and homeostasis. *Annual Reviews in Plant Biology* 53:159–182.
- 4002 Consalvey, M., R. G. Perkins, D. M. Paterson, and G. J. C. Underwood. 2005. PAM Fluorescence: a
4003 beginners guide for benthic diatomists. *Diatom Research* 20:1–22.
- 4004 Covich, A. P., M. C. Austen, F. Bärlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, O.
4005 Dangles, M. Solan, M. O. Gessner, B. Statzner, and B. Moss. 2004. The role of biodiversity in
4006 the functioning of freshwater and marine benthic ecosystems. *BioScience* 54:767.
- 4007 Davies, T. W., S. R. Jenkins, R. Kingham, J. Kenworthy, S. J. Hawkins, and J. G. Hiddink. 2011.
4008 Dominance, biomass and extinction resistance determine the consequences of biodiversity
4009 loss for multiple coastal ecosystem processes. *PloS ONE* 6:e28362.
- 4010 Debenest, T., E. Pinelli, M. Coste, J. Silvestre, N. Mazzella, C. Madigou, and F. Delmas. 2009.

- 4011 Sensitivity of freshwater periphytic diatoms to agricultural herbicides. *Aquatic Toxicology*
4012 93:11–17.
- 4013 Debenest, T., J. Silvestre, M. Coste, and E. Pinelli. 2010. Effects of pesticides on freshwater diatoms.
4014 *Reviews of Environmental Contamination and Toxicology* 203:87–103.
- 4015 Decho, A. W. 1990. Microbial exopolymer secretions in ocean environments - their role(s) in food
4016 webs and marine processes. *Oceanography and Marine Biology* 78:73–153.
- 4017 Decho, A. W. 2000. Microbial biofilms in intertidal systems: an overview. *Continental Shelf*
4018 *Research* 20:1257–1273.
- 4019 Decho, A. W., and J. W. Fleeger. 1988. Ontogenetic feeding shifts in the meiobenthic harpacticoid
4020 copepod *Nitocra lacustris*. *Marine Biology* 97:191–197.
- 4021 DeLorenzo, M. E., G. I. Scott, and P. E. Ross. 1999. Effects of the agricultural pesticides atrazine,
4022 deethylatrazine, endosulfan, and chlorpyrifos on an estuarine microbial food web.
4023 *Environmental Toxicology and Chemistry* 18:2824–2835.
- 4024 DeLorenzo, M. E., G. I. Scott, and P. E. Ross. 2001. Toxicity of pesticides to aquatic microorganisms:
4025 a review. *Environmental Toxicology and Chemistry* 20:84–98.
- 4026 Deutschman, D. H. 2001. Design and analysis of biodiversity field experiments. *Ecological*
4027 *Research* 16:833–843.
- 4028 Diaz, S., and M. Cabido. 2001. Vive la difference: plant functional diversity matters to ecosystem
4029 processes: plant functional diversity matters to ecosystem processes. *Trends in Ecology and*
4030 *Evolution* 16:646–655.
- 4031 Díaz, S., A. J. Symstad, F. Stuart Chapin, D. a. Wardle, and L. F. Huenneke. 2003. Functional diversity
4032 revealed by removal experiments. *Trends in Ecology & Evolution* 18:140–146.
- 4033 Dorigo, U., and C. Lebouranger. 2001. A pulse-amplitude modulated fluorescence-based method
4034 for assessing the effects of photosystem II herbicides on freshwater periphyton. *Journal of*
4035 *Applied Phycology* 13:509–515.
- 4036 Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014.
4037 Assemblage time series reveal biodiversity change but not systematic loss. *Science* 344:296–
4038 9.
- 4039 Downing, A. L., and M. a. Leibold. 2002. Ecosystem consequences of species richness and
4040 composition in pond food webs. *Nature* 416:837–841.

- 4041 Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for
4042 determination of sugars and related substances. *Analytical Chemistry* 28:350–356.
- 4043 Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. *Ecology Letters*
4044 6:680–687.
- 4045 Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault, and M. Loreau. 2007. The
4046 functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology*
4047 *Letters* 10:522–38.
- 4048 Dunstan, G. A., J. K. Volkman, S. M. Barrett, J. M. Leroi, and S. W. Jeffrey. 1993. Essential
4049 polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochemistry*
4050 35:155–161.
- 4051 Eder, K. 1995. Gas chromatographic analysis of fatty acid methyl esters. *Journal of*
4052 *Chromatography B: Biomedical Sciences and Applications* 671:113–131.
- 4053 Eisenhauer, N., A. D. Barnes, S. Cesarz, D. Craven, O. Ferlian, F. Gottschall, J. Hines, A. Sendek, J.
4054 Siebert, M. P. Thakur, and M. Türke. 2016. Biodiversity-ecosystem function experiments
4055 reveal the mechanisms underlying the consequences of biodiversity change in real world
4056 ecosystems. *Journal of Vegetation Science*:1–10.
- 4057 von Elert, E. 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using
4058 a new method to enrich food algae with single fatty acids. *Limnology and Oceanography*
4059 47:1764–1773.
- 4060 El-Sheekh, M. M., H. M. Kotkat, and O. H. E. Hammouda. 1994. Effect of atrazine herbicide on
4061 growth, photosynthesis, protein synthesis, and fatty acid composition in the unicellular
4062 green alga *Chlorella kessleri*. *Ecotoxicology and Environmental Safety* 29:349–358.
- 4063 Emmerson, M. C., M. Solan, C. Emes, D. M. Paterson, and D. Raffaelli. 2001. Consistent patterns and
4064 the idiosyncratic effects of biodiversity in marine ecosystems. *Nature* 411:73–7.
- 4065 EU. 2001. Strategy for a future chemicals policy. Brussels.
- 4066 European Marine Board. 2013. Linking oceans and human health: a strategic research priority for
4067 Europe. Ostend, Belgium.
- 4068 Fargione, J., D. Tilman, R. Dybzinski, J. H. R. Lambers, C. Clark, W. S. Harpole, J. M. H. Knops, P. B.
4069 Reich, and M. Loreau. 2007. From selection to complementarity: shifts in the causes of
4070 biodiversity-productivity relationships in a long-term biodiversity experiment. *Proceedings*

- 4071 of the Royal Society B 274:871–6.
- 4072 Fernandes, I., C. Pascoal, and F. Cássio. 2011. Intraspecific traits change biodiversity effects on
4073 ecosystem functioning under metal stress. *Oecologia* 166:1019–1028.
- 4074 Fernandes, J. C., and F. S. Henriques. 1991. Biochemical, physiological, and structural effects of
4075 excess copper in plants. *The Botanical Review* 57:246–273.
- 4076 Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. A. V Rees, and J. A. Raven. 2010. Phytoplankton in a
4077 changing world: cell size and elemental stoichiometry. *Journal of Plankton Research* 32.
- 4078 Fischer, B. B., F. Pomati, and R. I. L. Eggen. 2013. The toxicity of chemical pollutants in dynamic
4079 natural systems: The challenge of integrating environmental factors and biological
4080 complexity. *Science of the Total Environment* 449:253–259.
- 4081 Fisher, N. S., and D. Frood. 1980. Heavy metals and marine diatoms: Influence of dissolved organic
4082 compounds on toxicity and selection for metal tolerance among four species. *Marine Biology*
4083 59:85–93.
- 4084 Fleeger, J. W., K. R. Carman, and R. M. Nisbet. 2003. Indirect effects of contaminants in aquatic
4085 ecosystems. *Science of the Total Environment* 317:207–233.
- 4086 Fontana, S., J. Jokela, and F. Pomati. 2014. Opportunities and challenges in deriving phytoplankton
4087 diversity measures from individual trait-based data obtained by scanning flow-cytometry.
4088 *Frontiers in Microbiology* 5:1–12.
- 4089 Forget-Leray, J., I. Landriau, C. Minier, and F. Leboulenger. 2005. Impact of endocrine toxicants on
4090 survival, development, and reproduction of the estuarine copepod *Eurytemora affinis*
4091 (Poppe). *Ecotoxicology and Environmental Safety* 60:288–294.
- 4092 Forster, R. M., V. Créach, K. Sabbe, W. Vyverman, and L. J. Stal. 2006. Biodiversity-ecosystem
4093 function relationship in microphytobenthic diatoms of the Westerschelde estuary. *Marine*
4094 *Ecology Progress Series* 311:191–201.
- 4095 Fox, J. W. 2004. Effects of algal and herbivore diversity on the partitioning of biomass within and
4096 among trophic levels. *Ecology* 85:549–559.
- 4097 Fox, J. W. 2005. Interpreting the ‘selection effect’ of biodiversity on ecosystem function. *Ecology*
4098 *Letters* 8:846–856.
- 4099 Fox, J. W. 2006. Using the price equation to partition the effects of biodiversity loss on ecosystem
4100 function. *Ecology* 87:2687–2696.

- 4101 Fox, J. W., and W. S. Harpole. 2008. Revealing how species loss affects ecosystem function : the
4102 trait-based price equation partition. *Ecology* 89:269–279.
- 4103 Fugère, V., P. Andino, R. Espinosa, F. Anthelme, D. Jacobsen, and O. Dangles. 2012. Testing the
4104 stress-gradient hypothesis with aquatic detritivorous invertebrates: insights for
4105 biodiversity-ecosystem functioning research. *The Journal of Animal Ecology* 81:1259–67.
- 4106 Galgani, F., N. Piel, and F. Vincent. 1999. A simple procedure for polymerase chain reaction of the
4107 PSBA gene in algae: Application to the screening of mutations conferring atrazine resistance
4108 and discrimination of natural populations of *Porphyra linearis*. *Comparative Biochemistry
4109 and Physiology – Part B: Biochemistry and Molecular Biology* 124:363–369.
- 4110 Galloway, R. E., and L. J. Mets. 1984. Atrazine, bromacil, and diuron resistance in *Chlamydomonas*.
4111 A single non-Mendelian genetic locus controls the structure of the thylakoid binding site.
4112 *Plant Physiology* 74:469–474.
- 4113 Gamfeldt, L., H. Hillebrand, and P. R. Jonsson. 2005. Species richness changes across two trophic
4114 levels simultaneously affect prey and consumer biomass. *Ecology Letters* 8:696–703.
- 4115 Gamfeldt, L., H. Hillebrand, and P. R. Jonsson. 2008. Multiple functions increase the importance of
4116 biodiversity for overall ecosystem functioning. *Ecology* 89:1223–1231.
- 4117 Gamfeldt, L., J. S. Lefcheck, J. E. K. Byrnes, B. J. Cardinale, J. E. Duffy, and J. N. Griffin. 2015. Marine
4118 biodiversity and ecosystem functioning: what’s known and what’s next? *Oikos* 124:252–265.
- 4119 Gamfeldt, L., T. Snäll, R. Bagchi, M. Jonsson, L. Gustafsson, P. Kjellander, M. C. Ruiz-Jaen, M. Fröberg,
4120 J. Stendahl, C. D. Philipson, G. Mikusiński, E. Andersson, B. Westerlund, H. Andrén, F. Moberg,
4121 J. Moen, and J. Bengtsson. 2013. Higher levels of multiple ecosystem services are found in
4122 forests with more tree species. *Nature Communications* 4:1340.
- 4123 Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, P. Ceryngier, J.
4124 Liira, T. Tscharntke, C. Winqvist, S. Eggers, R. Bommarco, T. Pärt, V. Bretagnolle, M.
4125 Plantegenest, L. W. Clement, C. Dennis, C. Palmer, J. J. Oñate, I. Guerrero, V. Hawro, T. Aavik,
4126 C. Thies, A. Flohre, S. Hänke, C. Fischer, P. W. Goedhart, and P. Inchausti. 2010. Persistent
4127 negative effects of pesticides on biodiversity and biological control potential on European
4128 farmland. *Basic and Applied Ecology* 11:97–105.
- 4129 Gekeler, W., E. Grill, E. L. Winnacker, and M. H. Zenk. 1988. Algae sequester heavy metals via
4130 synthesis of phytochelatin complexes. *Archives of Microbiology* 150:197–202.
- 4131 Gerbersdorf, S. U., R. Bittner, H. Lubarsky, W. Manz, and D. M. Paterson. 2009a. Microbial

- 4132 assemblages as ecosystem engineers of sediment stability. *Journal of Soils and Sediments*
4133 9:640–652.
- 4134 Gerbersdorf, S. U., B. Westrich, and D. M. Paterson. 2009b. Microbial extracellular polymeric
4135 substances (EPS) in fresh water sediments. *Microbial Ecology* 58:334–349.
- 4136 Gessner, M. O., and J. Hines. 2012. Stress as a modifier of biodiversity effects on ecosystem
4137 processes? *Journal of Animal Ecology* 81:1143–1145.
- 4138 Giles, K. L., R. D. Madden, R. Stockland, M. E. Payton, and J. W. Dillwith. 2002. Host plants affect
4139 predator fitness via the nutritional value of herbivore prey: Investigation of a plant-aphid-
4140 ladybeetle system. *BioControl* 47:1–21.
- 4141 Giller, P. S., H. Hillebrand, U.-G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie,
4142 B. Malmqvist, M. T. Monaghan, P. J. Morin, and G. O'Mullan. 2004. Biodiversity effects on
4143 ecosystem functioning: emerging issues and their experimental test in aquatic environments.
4144 *Oikos* 104:423–436.
- 4145 Gladyshev, M. I., N. N. Sushchik, O. V. Anishchenko, O. N. Makhutova, V. I. Kolmakov, G. S. Kalachova,
4146 A. A. Kolmakova, and O. P. Dubovskaya. 2011. Efficiency of transfer of essential
4147 polyunsaturated fatty acids versus organic carbon from producers to consumers in a
4148 eutrophic reservoir. *Oecologia* 165:521–531.
- 4149 Godbold, J. A., M. Solan, and K. Killham. 2009. Consumer and resource diversity effects on marine
4150 macroalgal decomposition. *Oikos* 118:77–86.
- 4151 Goldstein, D. S., and I. J. Kopin. 2007. Evolution of concepts of stress. *Stress* 10:109–20.
- 4152 Gonçalves, A. L., A. V. Lirio, J. Pratas, and C. Canhoto. 2011. Uranium contaminated water does not
4153 affect microbial activity but decreases feeding by the shredder *Sericostoma vittatum*.
4154 *Fundamental and Applied Limnology / Archiv für Hydrobiologie* 179:17–25.
- 4155 Gonzalez, A., B. J. Cardinale, G. R. H. Allington, J. Byrnes, K. A. Endsley, D. G. Brown, D. U. Hooper, F.
4156 Isbell, M. I. O'Connor, and M. Loreau. 2016. Estimating local biodiversity change: A critique
4157 of papers claiming no net loss of local diversity. *Ecology* 97:1949–1960.
- 4158 Graymore, M., F. Stagnitti, and G. Allinson. 2001. Impacts of atrazine in aquatic ecosystems.
4159 *Environment International* 26:483–95.
- 4160 Griffin, J. N., J. E. K. Byrnes, and B. J. Cardinale. 2013. Effects of predator richness on prey
4161 suppression: a meta-analysis. *Ecology* 94:2180–2187.

- 4162 Griffin, J. N., V. Méndez, A. F. Johnson, S. R. Jenkins, and A. Foggo. 2009. Functional diversity
4163 predicts overyielding effect of species combination on primary productivity. *Oikos* 118:37–
4164 44.
- 4165 Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance
4166 to ecological and evolutionary. *The American Naturalist* 111:1169–1194.
- 4167 Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects.
4168 *Journal of Ecology* 86:902/910.
- 4169 De Groot, R. S., M. A. Wilson, and R. M. J. Boumans. 2002. A typology for the classification,
4170 description and valuation of ecosystem functions, goods and services. *Ecological Economics*
4171 41:1–20.
- 4172 Gross, K., and B. J. Cardinale. 2005. The functional consequences of random vs. ordered species
4173 extinctions. *Ecology Letters* 8:409–418.
- 4174 Guénard, G., P. C. Von Der Ohe, D. De Zwart, P. Legendre, and S. Lek. 2011. Using phylogenetic
4175 information to predict species tolerances to toxic chemicals. *Ecological Applications*
4176 21:3178–3190.
- 4177 Guillard, R. L. 1975. Culture of Phytoplankton for Feeding Marine Invertebrates. Pages 29–60 *in*
4178 W. Smith and M. Chanley, editors. *Culture of Marine Invertebrate Animals SE - 3*. CHAP,
4179 Springer. New York.
- 4180 Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2016. The importance of high-quality algal food
4181 sources in stream food webs - current status and future perspectives. *Freshwater Biology*
4182 61:815–831.
- 4183 Guschina, I. A., and J. L. Harwood. 2006. Lipids and lipid metabolism in eukaryotic algae. *Progress*
4184 *in Lipid Research* 45:160–186.
- 4185 Guschina, I. A., and J. L. Harwood. 2009. Algal lipids and effect of the environment on their
4186 biochemistry. Pages 1–24 *in* M. T. Arts, M. T. Brett, and M. J. Kainz, editors. *Lipids in aquatic*
4187 *ecosystems*. Springer, New York.
- 4188 Gustafsson, C., and C. Boström. 2011. Biodiversity influences ecosystem functioning in aquatic
4189 angiosperm communities. *Oikos* 120:1037–1046.
- 4190 Haddad, N. M., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant
4191 species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters*

- 4192 12:1029–1039.
- 4193 Hall, A., A. H. Fielding, and M. Butler. 1979. Mechanisms of copper tolerance in the marine fouling
4194 alga *Ectocarpus siliculosus* - Evidence for an exclusion mechanism. *Marine Biology* 54:195–
4195 199.
- 4196 Hall, L. W., M. C. Ziegenfuss, R. D. Anderson, and D. P. Tierney. 1995. The influence of salinity on
4197 the chronic toxicity of atrazine to an estuarine copepod: Implications for development of an
4198 estuarine chronic criterion. *Archives of Environmental Contamination and Toxicology*
4199 28:344–348.
- 4200 Halpern, B. S., K. A. Selkoe, F. Micheli, and C. V. Kappel. 2007. Evaluating and ranking the
4201 vulnerability of global marine ecosystems to anthropogenic threats. *Conservation Biology*
4202 21:1301–1315.
- 4203 Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey,
4204 C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig,
4205 M. Spalding, R. Steneck, and R. Watson. 2008. A global map of human impact on marine
4206 ecosystems. *Science* 319:948–953.
- 4207 Halstead, N. T., T. A. McMahon, S. A. Johnson, T. R. Raffel, J. M. Romansic, P. W. Crumrine, and J. R.
4208 Rohr. 2014. Community ecology theory predicts the effects of agrochemical mixtures on
4209 aquatic biodiversity and ecosystem properties. *Ecology Letters* 17:932–941.
- 4210 Hänsch, R., and R. R. Mendel. 2009. Physiological functions of mineral micronutrients (Cu, Zn, Mn,
4211 Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology* 12:259–266.
- 4212 Hartig, P., K. Wolfstein, S. Lippemeier, and F. Colijn. 1998. Photosynthetic activity of natural
4213 microphytobenthos populations measured by fluorescence. *Marine Ecology Progress Series*
4214 166:53–62.
- 4215 Harwood, J. L., and I. A. Guschina. 2009. The versatility of algae and their lipid metabolism.
4216 *Biochimie* 91:679–684.
- 4217 Hautier, Y., D. Tilman, F. Isbell, E. W. Seabloom, E. T. Borer, and P. B. Reich. 2015. Anthropogenic
4218 environmental changes affect ecosystem stability via biodiversity. *Science* 348:336–340.
- 4219 Hawlena, D., and O. J. Schmitz. 2010. Physiological stress as a fundamental mechanism linking
4220 predation to ecosystem functioning. *The American Naturalist* 176:537–556.
- 4221 Hayes, T. B., L. L. Anderson, V. R. Beasley, S. R. De Solla, T. Iguchi, H. Ingraham, P. Kestemont, J.

- 4222 Kniewald, Z. Kniewald, V. S. Langlois, E. H. Luque, K. A. McCoy, M. Muñoz-De-Toro, T. Oka, C.
4223 A. Oliveira, F. Orton, S. Ruby, M. Suzawa, L. E. Tavera-Mendoza, V. L. Trudeau, A. B. Victor-
4224 Costa, and E. Willingham. 2011. Demasculinization and feminization of male gonads by
4225 atrazine: Consistent effects across vertebrate classes. *Journal of Steroid Biochemistry and*
4226 *Molecular Biology* 127:64–73.
- 4227 He, Q., M. D. Bertness, and A. H. Altieri. 2013. Global shifts towards positive species interactions
4228 with increasing environmental stress. *Ecology Letters* 16:695–706.
- 4229 Hector, A., and R. Bagchi. 2007. Biodiversity and ecosystem multifunctionality. *Nature* 448:188–
4230 90.
- 4231 Hector, A., T. Bell, J. Connolly, J. Finn, J. W. Fox, L. Kirwan, M. Loreau, J. McLaren, B. Schmid, and A.
4232 Weigelt. 2009. The analysis of biodiversity experiments: from pattern towards mechanism.
4233 Pages 94–104 in S. Naeem, editor. *Biodiversity, ecosystem functioning, and human*
4234 *wellbeing: an ecological and economic perspective*. Oxford University Press, Oxford.
- 4235 Hector, A., J. Joshi, S. Lawler, E. Spehn, and A. Wilby. 2001. Conservation implications of the link
4236 between biodiversity and ecosystem functioning. *Oecologia* 129:624–628.
- 4237 Hector, A., B. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. Finn, H.
4238 Freitas, P. Giller, J. Good, and R. Harris. 2000. No consistent effect of plant diversity on
4239 productivity: response. *Science* 289:1255a.
- 4240 Hector, A., C. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. a Finn,
4241 H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, J. Joshi, A. Jumpponen, C.
4242 Körner, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, J. S.
4243 Pereira, A. Prinz, D. J. Read, M. Scherer-lorenzen, E. D. Schulze, A.-. S. D. Siamantziouras, E. M.
4244 Spehn, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 1999. Plant
4245 diversity and productivity experiments in European grasslands. *Science* 286:1123–1127.
- 4246 van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A.
4247 Wiemken, and I. R. Sanders. 1999. 'Sampling effect', a problem in biodiversity manipulation?
4248 A reply to David A. Wardle. *Oikos* 87:408–410.
- 4249 Hellebust, J. A., and J. Lewin. 1977. Heterotrophy. Pages 169–197 in D. Werner, editor. *The biology*
4250 *of diatoms*. Botanical Monographs. University of California Press, Berkeley.
- 4251 Heuner, M., A. Silinski, J. Schoelynck, T. J. Bouma, S. Puijalon, P. Troch, E. Fuchs, B. Schröder, U.
4252 Schröder, P. Meire, and S. Temmerman. 2015. Ecosystem engineering by plants on wave-

- 4253 exposed intertidal flats is governed by relationships between effect and response traits. *PLoS*
4254 *ONE* 10:1–18.
- 4255 Hicks, N., M. T. Bulling, M. Solan, D. Raffaelli, P. C. L. White, and D. M. Paterson. 2011. Impact of
4256 biodiversity-climate futures on primary production and metabolism in a model benthic
4257 estuarine system. *BMC Ecology* 11:7.
- 4258 Hiddink, J. G., T. Wynter Davies, M. Perkins, M. Machairopoulou, and S. P. Neill. 2009. Context
4259 dependency of relationships between biodiversity and ecosystem functioning is different for
4260 multiple ecosystem functions. *Oikos* 118:1892–1900.
- 4261 Hillebrand, H., D. M. Bennett, and M. W. Cadotte. 2008. Consequences of dominance: a review of
4262 evenness effects on local and regional ecosystem processes. *Ecology* 89:1510–1520.
- 4263 Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollinger, and T. Zohary. 1999. Biovolume
4264 calculation for pelagic and benthic microalgae. *Journal of Phycology* 424:403–424.
- 4265 Hillebrand, H., and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and
4266 progress in functional biodiversity research. *Ecology Letters* 12:1405–19.
- 4267 Hillebrand, H., B. Worm, and H. K. Lotze. 2000. Marine microbenthic community structure related
4268 by nitrogen loading and grazing pressure. *Marine Ecology Progress Series* 204:27–38.
- 4269 Hobbs, R. J. 1992. Is biodiversity important for ecosystem functioning - implications for research
4270 and management. Page 211- Biodiversity of mediterranean ecosystems in Australia. Perth,
4271 Australia.
- 4272 Hodapp, D., H. Hillebrand, B. Blasius, and A. B. Ryabov. 2016. Environmental and trait variability
4273 constrain community structure and the biodiversity-productivity relationship. *Ecology*
4274 97:1463–1474.
- 4275 Hooper, D., F. I. Chapin, J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. Lawton, D. Lodge, M. Loreau, S.
4276 Naeem, B. Schmid, H. Setälä, A. Symstad, J. Vandermeer, and D. Wardle. 2005. Effects of
4277 biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological*
4278 *Monographs* 75:3–35.
- 4279 Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. a Hungate, K. L. Matulich, A. Gonzalez, J.
4280 E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as
4281 a major driver of ecosystem change. *Nature* 486:105–8.
- 4282 Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models.

- 4283 Biometrical Journal 50:346--363.
- 4284 Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological
4285 consequences of genetic diversity. *Ecology Letters* 11:609–623.
- 4286 Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem
4287 function of biodiversity. *Oecologia* 110:449–460.
- 4288 Huston, M. A., L. W. Aarssen, M. P. Austin, B. S. Cade, J. D. Fridley, E. Garnier, J. P. Grime, J. Hodgson,
4289 W. K. Lauenroth, K. Thompson, J. H. Vandermeer, and D. A. Wardle. 2000. No consistent effect
4290 of plant diversity on productivity. *Science* 289:1255a–1255.
- 4291 Imhoff, M., L. Bounoua, T. Ricketts, C. Loucks, R. Harriss, and W. T. Lawrence. 2004. Global patterns
4292 in human consumption of net primary production. *Nature* 429:870–873.
- 4293 InVEST. 2016. InVEST - Integrated Valuation of Ecosystem Services and Tradeoffs:
4294 <http://www.naturalcapitalproject.org/invest/>. Natural Capital Project.
- 4295 IPBES. 2016. Scenarios and models of biodiversity and ecosystem services - summary for
4296 policymakers. Bonn, Germany.
- 4297 Isbell, F. I., H. W. Polley, and B. J. Wilsey. 2009. Biodiversity, productivity and the temporal stability
4298 of productivity: Patterns and processes. *Ecology Letters* 12:443–451.
- 4299 Isbell, F., V. Calcagno, A. Hector, J. Connolly, W. S. Harpole, P. B. Reich, M. Scherer-Lorenzen, B.
4300 Schmid, D. Tilman, J. van Ruijven, A. Weigelt, B. J. Wilsey, E. S. Zavaleta, and M. Loreau. 2011.
4301 High plant diversity is needed to maintain ecosystem services. *Nature* 477:199–202.
- 4302 Islam, M. S., and M. Tanaka. 2004. Impacts of pollution on coastal and marine ecosystems including
4303 coastal and marine fisheries and approach for management: A review and synthesis. *Marine*
4304 *Pollution Bulletin* 48:624–649.
- 4305 Ives, A. R., and B. J. Cardinale. 2004. Food-web interactions govern the resistance of communities
4306 after non-random extinctions. *Nature* 429:174–177.
- 4307 Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. *Science* 58:58–63.
- 4308 Jiang, L. 2007. Negative selection effects suppress relationships between bacterial diversity and
4309 ecosystem functioning. *Ecology* 88:1075–1085.
- 4310 Jiang, L., Z. Pu, and D. R. Nemergut. 2008. On the importance of the negative selection effect for the
4311 relationship between biodiversity and ecosystem functioning. *Oikos* 117:488–493.

- 4312 Jodice, P. G. R., D. D. Roby, K. R. Turco, R. M. Suryan, D. B. Irons, J. F. Piatt, M. T. Shultz, D. G.
4313 Roseneau, A. B. Kettle, and J. A. Anthony. 2006. Assessing the nutritional stress hypothesis:
4314 Relative influence of diet quantity and quality on seabird productivity. *Marine Ecology*
4315 *Progress Series* 325:267–279.
- 4316 Johnston, E. L., and D. A. Roberts. 2009. Contaminants reduce the richness and evenness of marine
4317 communities: a review and meta-analysis. *Environmental Pollution* 157:1745–52.
- 4318 Jonsson, M., O. Dangles, B. Malmqvist, and F. Guérol. 2002. Simulating species loss following
4319 perturbation: assessing the effects on process rates. *Proceedings of the Royal Society B*
4320 269:1047–52.
- 4321 Jüttner, I., A. Peither, J. P. Lay, A. Kettrup, and S. J. Ormerod. 1995. An outdoor mesocosm study to
4322 assess ecotoxicological effects of atrazine on a natural plankton community. *Archives of*
4323 *Environmental Contamination and Toxicology* 29:435–441.
- 4324 Kainz, M. J., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web
4325 and their ecological role for higher trophic levels. *Limnology and Oceanography* 49:1784–
4326 1793.
- 4327 Kareiva, P., H. Tallis, T. H. Ricketts, G. C. Daily, and S. Polasky. 2011. *Natural capital: theory and*
4328 *practice of mapping ecosystem services*. Oxford University Press, Oxford.
- 4329 Karlson, A. M. L., F. J. A. Nascimento, J. Näslund, and R. Elmgren. 2010. Higher diversity of deposit-
4330 feeding macrofauna enhances phytodetritus processing. *Ecology* 91:1414–1423.
- 4331 Kasai, F., and T. Hanazato. 1995. Effects of the triazine herbicide, simetryn, on freshwater plankton
4332 communities in experimental ponds. *Environmental Pollution* 89:197–202.
- 4333 Kennish, M. J. 1996. *Practical handbook of estuarine and marine pollution*. CRC Press, Boca Raton,
4334 Florida, USA.
- 4335 Kirwan, L., A. Lüscher, M. T. Sebastià, J. a. Finn, R. P. Collins, C. Porqueddu, A. Helgadottir, O. H.
4336 Baadshaug, C. Brophy, C. Coran, S. Dalmannsdóttir, I. Delgado, A. Elgersma, M. Fothergill, B.
4337 E. Frankow-Lindberg, P. Golinski, P. Grieu, a. M. Gustavsson, M. Höglind, O. Huguenin-Elie, C.
4338 Iliadis, M. Jørgensen, Z. Kadziulienė, T. Karyotis, T. Lunnan, M. Malengier, S. Maltoni, V. Meyer,
4339 D. Nyfeler, P. Nykanen-Kurki, J. Parente, H. J. Smit, U. Thumm, and J. Connolly. 2007. Evenness
4340 drives consistent diversity effects in intensive grassland systems across 28 European sites.
4341 *Journal of Ecology* 95:530–539.
- 4342 Kitaysky, A. S., E. V. Kitaiskaia, J. F. Piatt, and J. C. Wingfield. 2006. A mechanistic link between chick

- 4343 diet and decline in seabirds? Proceedings. Biological sciences / The Royal Society 273:445–
4344 450.
- 4345 Knauert, S. 2008. Toxicity of pesticides and their mixture to primary producers.
- 4346 Knauert, S., and K. Knauer. 2008. The role of reactive oxygen species in copper toxicity to two
4347 freshwater green algae. Journal of Phycology 44:311–319.
- 4348 Koelmans, A. A., A. Van der Heijde, L. M. Knijgg, and R. H. Aalderink. 2001. Integrated modelling of
4349 eutrophication and organic contaminant fate & effects in aquatic ecosystems. A review.
4350 Water Research 35:3517–3536.
- 4351 Koide, R. T., C. Fernandez, and G. Malcolm. 2014. Determining place and process: functional traits
4352 of ectomycorrhizal fungi that affect both community structure and ecosystem function. New
4353 Phytologist 201:433–439.
- 4354 Koricheva, J., C. P. H. Mulder, B. Schmid, J. Joshi, and K. Huss-Danell. 2000. Numerical responses of
4355 different trophic groups of invertebrates to manipulations of plant diversity in grasslands.
4356 Oecologia 125:271–282.
- 4357 Körner, C. 2003. Limitation and stress - always or never? Journal of Vegetation Science 14:141–
4358 143.
- 4359 Koski, M., W. K. Breteler, and N. Schogt. 1998. Effect of food quality on rate of growth and
4360 development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Marine
4361 Ecology Progress Series 170:169–187.
- 4362 Kromkamp, J., C. Barranguet, and J. Peene. 1998. Determination of microphytobenthos PSII
4363 quantum efficiency and photosynthetic activity by means of variable chlorophyll
4364 fluorescence. Marine Ecology Progress Series 162:45–55.
- 4365 Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lürling, C. S. Reynolds, and M.
4366 Scheffer. 2010. A morphological classification capturing functional variation in
4367 phytoplankton. Freshwater Biology 55:614–627.
- 4368 De Laender, F., C. J. Melian, R. Bindler, P. J. Van den Brink, M. Daam, H. Roussel, J. Juselius, D.
4369 Verschuren, and C. R. Janssen. 2014. The contribution of intra- and interspecific tolerance
4370 variability to biodiversity changes along toxicity gradients. Ecology Letters 17:72–81.
- 4371 De Laender, F., J. R. Rohr, R. Ashauer, D. J. Baird, U. Berger, N. Eisenhauer, V. Grimm, U. Hommen,
4372 L. Maltby, C. J. Meliàn, F. Pomati, I. Roessink, V. Radchuk, and P. J. van den Brink. 2016. Re-

- 4373 introducing environmental change drivers in biodiversity-ecosystem functioning research.
4374 Trends in Ecology and Evolution Evolution accepted.
- 4375 De Laender, F., K. Soetaert, and J. J. Middelburg. 2010. Inferring chemical effects on carbon flows
4376 in aquatic food webs: methodology and case study. *Environmental Pollution* 158:1775–82.
- 4377 Lage, O. M., A. M. Parente, H. M. V. M. Soares, M. T. S. D. Vasconcelos, and R. Salema. 1994. Some
4378 effects of copper on the dinoflagellates *Amphidinium carterae* and *Prorocentrum micans* in
4379 batch culture. *European Journal of Phycology* 29:253–260.
- 4380 Lahive, E., J. O' Halloran, and M. A. K. Jansen. 2011. Differential sensitivity of four Lemnaceae
4381 species to zinc sulphate. *Environmental and Experimental Botany* 71:25–33.
- 4382 Larigauderie, A., and H. A. Mooney. 2010. The Intergovernmental science-policy Platform on
4383 Biodiversity and Ecosystem Services: moving a step closer to an IPCC-like mechanism for
4384 biodiversity. *Current Opinion in Environmental Sustainability* 2:9–14.
- 4385 Larras, F., A. Bouchez, F. Rimet, and B. Montuelle. 2012. Using bioassays and species sensitivity
4386 distributions to assess herbicide toxicity towards benthic diatoms. *PLoS ONE* 7:e44458.
- 4387 Larras, F., V. Gregorio, A. Bouchez, B. Montuelle, and N. Chèvre. 2016. Comparison of specific
4388 versus literature species sensitivity distributions for herbicides risk assessment.
4389 *Environmental Science and Pollution Research* 23:3042–3052.
- 4390 Larras, F., F. Keck, B. Montuelle, F. Rimet, and A. Bouchez. 2014. Linking diatom sensitivity to
4391 herbicides to phylogeny: A step forward for biomonitoring? *Environmental Science &*
4392 *Technology* 48:1921–1930.
- 4393 Larsen, T. H., N. M. Williams, and C. Kremen. 2005. Extinction order and altered community
4394 structure rapidly disrupt ecosystem functioning. *Ecology Letters* 8:538–47.
- 4395 Lau, D. C. P., K. M. Y. Leung, and D. Dudgeon. 2009. Evidence of rapid shifts in the trophic base of
4396 lotic predators using experimental dietary manipulations and assimilation-based analyses.
4397 *Oecologia* 159:767–776.
- 4398 Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem
4399 functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* 16:545–556.
- 4400 Lawler, J. J., J. E. Aukema, J. B. Grant, B. S. Halpern, P. Kareiva, C. R. Nelson, K. Ohleth, J. D. Olden, M.
4401 A. Schlaepfer, B. R. Silliman, and P. Zaradic. 2006. Conservation Science: a 20-year report
4402 card. *Frontiers in Ecology and the Environment* 4:473–480.

- 4403 Lefcheck, J. S., J. E. K. Byrnes, F. Isbell, L. Gamfeldt, J. N. Griffin, N. Eisenhauer, M. J. S. Hensel, A.
4404 Hector, B. J. Cardinale, and J. E. Duffy. 2015. Biodiversity enhances ecosystem
4405 multifunctionality across trophic levels and habitats. *Nature Communications* 6:6936.
- 4406 Legrand, H., O. Herlory, J. M. Guarini, G. F. Blanchard, and P. Richard. 2006. Inhibition of
4407 microphytobenthic photosynthesis by the herbicides atrazine and diuron. *Cahiers de*
4408 *Biologie Marine* 47:39–45.
- 4409 Lennon, J. T., and B. K. Lehmkuhl. 2016. A trait-based approach to bacterial biofilms in soil.
4410 *Environmental Microbiology* 18:2732–2742.
- 4411 Lepš, J. 2004. What do the biodiversity experiments tell us about consequences of plant species
4412 loss in the real world? *Basic and Applied Ecology* 5:529–534.
- 4413 Levy, J. L., J. L. Stauber, and D. F. Jolley. 2007. Sensitivity of marine microalgae to copper: The effect
4414 of biotic factors on copper adsorption and toxicity. *Science of the Total Environment*
4415 387:141–154.
- 4416 Lewin, J., and J. A. Hellebust. 1975. Heterotrophic nutrition of the marine pennate diatom *Nitzschia*
4417 *angularis* var. *affinis*. *Canadian Journal of Microbiology* 21:1335–1342.
- 4418 Lewis, O. T. 2009. Biodiversity change and ecosystem function in tropical forests. *Basic and*
4419 *Applied Ecology* 10:97–102.
- 4420 Li, M., C. Hu, Q. Zhu, L. Chen, Z. Kong, and Z. Liu. 2006. Copper and zinc induction of lipid
4421 peroxidation and effects on antioxidant enzyme activities in the microalga *Pavlova viridis*
4422 (Prymnesiophyceae). *Chemosphere* 62:565–572.
- 4423 Liebig, M., G. Schmidt, D. Bontje, B. W. Kooi, G. Streck, W. Traunspurger, and T. Knacker. 2008.
4424 Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor
4425 microcosm. *Aquatic Toxicology* 88:102–110.
- 4426 Liess, M., and P. C. Von Der Ohe. 2005. Analyzing effects of pesticides on invertebrate communities
4427 in streams. *Environmental Toxicology and Chemistry* 24:954–965.
- 4428 Litchman, E., and C. A. Klausmeier. 2008. Trait-based community ecology of phytoplankton.
4429 *Annual Review of Ecology, Evolution, and Systematics* 39:615–639.
- 4430 Litchman, E., C. A. Klausmeier, O. M. Schofield, and P. G. Falkowski. 2007. The role of functional
4431 traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to
4432 ecosystem level. *Ecology Letters* 10:1170–1181.

- 4433 Litchman, E., P. de Tezanos Pinto, K. F. Edwards, C. A. Klausmeier, C. T. Kremer, and M. K. Thomas.
4434 2015. Global biogeochemical impacts of phytoplankton: A trait-based perspective. *Journal of*
4435 *Ecology* 103:1384–1396.
- 4436 Litzow, M. a, K. M. Bailey, F. G. Prahl, and R. Heintz. 2006. Climate regime shifts and reorganization
4437 of fish communities: the essential fatty acid limitation hypothesis. *Marine Ecology Progress*
4438 *Series* 315:1–11.
- 4439 Liu, H., and E. J. Buskey. 2000. Hypersalinity enhances the production of Extracellular Polymeric
4440 Substance (EPS) in the Texas brown tide alga, *Aureoumbra lagunsensis* (Pelagophyceae).
4441 *Journal of Phycology* 36:71–77.
- 4442 Lockert, C. K., K. D. Hoagland, and B. D. Siegfried. 2006. Comparative sensitivity of freshwater algae
4443 to atrazine. *Bulletin of Environmental Contamination and Toxicology* 76:73–79.
- 4444 Long, Z. T., S. R. Fegley, and C. H. Peterson. 2013. Fertilization and plant diversity accelerate
4445 primary succession and restoration of dune communities. *Plant Ecology* 214:1419–1429.
- 4446 Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos*
4447 91:3–17.
- 4448 Loreau, M. 2010. Linking biodiversity and ecosystems: towards a unifying ecological theory.
4449 *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*
4450 365:49–60.
- 4451 Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity
4452 experiments. *Nature* 412:72–6.
- 4453 Loreau, M., S. Naeem, and P. Inchausti. 2001a. Biodiversity and ecosystem functioning: the
4454 emergence of a synthetic ecological framework. Pages 3–11 *in* Biodiversity and ecosystem
4455 functioning: synthesis and perspectives. Oxford University Press, Oxford.
- 4456 Loreau, M., S. Naeem, and P. Inchausti. 2002. Biodiversity and ecosystem functioning. Synthesis
4457 and perspectives. Oxford University Press, Oxford.
- 4458 Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D.
4459 Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001b. Biodiversity and ecosystem
4460 functioning : current knowledge and future challenges. *Science* 294:804–808.
- 4461 Lotze, H. K., H. S. Lenihan, B. J. Bourque, R. H. Bradbury, R. G. Cooke, M. C. Kay, S. M. Kidwell, M. X.
4462 Kirby, C. H. Peterson, J. B. C. Jackson, and M. Bay. 2006. Depletion, degradation, and recovery

- 4463 potential of estuaries and coastal seas. *Science* 312:1806–1809.
- 4464 Lubchenco, J., A. M. Olson, L. B. Brubaker, S. R. Carpenter, M. M. Holland, S. P. Hubbell, S. A. Levin,
4465 J. A. Macmahon, A. Pamela, J. M. Melillo, H. A. Mooney, C. H. Peterson, H. R. Pulliam, A. Leslie,
4466 P. J. Regal, and P. G. Risser. 1991. The Sustainable biosphere initiative: an ecological research
4467 agenda. *Ecology* 72:371–412.
- 4468 Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient
4469 hypothesis for competition and facilitation in plant communities. *Journal of Ecology* 97:199–
4470 205.
- 4471 Maestre, F. T., M. a Bowker, C. Escolar, M. D. Puche, S. Soliveres, S. Maltez-Mouro, P. García-Palacios,
4472 A. P. Castillo-Monroy, I. Martínez, and A. Escudero. 2010. Do biotic interactions modulate
4473 ecosystem functioning along stress gradients? Insights from semi-arid plant and biological
4474 soil crust communities. *Philosophical Transactions of the Royal Society of London. Series B,*
4475 *Biological sciences* 365:2057–2070.
- 4476 Maestre, F. T., J. L. Quero, N. J. Gotelli, A. Escudero, V. Ochoa, M. Delgado-Baquerizo, M. García-
4477 Gómez, M. a Bowker, S. Soliveres, C. Escolar, P. García-Palacios, M. Berdugo, E. Valencia, B.
4478 Gozalo, A. Gallardo, L. Aguilera, T. Arredondo, J. Blones, B. Boeken, D. Bran, A. a Conceição, O.
4479 Cabrera, M. Chaieb, M. Derak, D. J. Eldridge, C. I. Espinosa, A. Florentino, J. Gaitán, M. G. Gatica,
4480 W. Ghiloufi, S. Gómez-González, J. R. Gutiérrez, R. M. Hernández, X. Huang, E. Huber-
4481 Sannwald, M. Jankju, M. Miriti, J. Monerris, R. L. Mau, E. Morici, K. Naseri, A. Ospina, V. Polo,
4482 A. Prina, E. Pucheta, D. a Ramírez-Collantes, R. Romão, M. Tighe, C. Torres-Díaz, J. Val, J. P.
4483 Veiga, D. Wang, and E. Zaady. 2012. Plant species richness and ecosystem multifunctionality
4484 in global drylands. *Science* 335:214–8.
- 4485 Magnusson, M., K. Heimann, and A. P. Negri. 2008. Comparative effects of herbicides on
4486 photosynthesis and growth of tropical estuarine microalgae. *Marine Pollution Bulletin*
4487 56:1545–1552.
- 4488 Malaj, E., P. C. von der Ohe, M. Grote, R. Kühne, C. P. Mondy, P. Usseglio-Polatera, W. Brack, and R.
4489 B. Schäfer. 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the
4490 continental scale. *Proceedings of the National Academy of Sciences of the United States of*
4491 *America* 111:9549–54.
- 4492 Malmqvist, B., and S. Rundle. 2002. Threats to the running water ecosystems of the world.
4493 *Environmental Conservation* 29:134–153.
- 4494 Manimaran, K., P. Karthikeyan, S. Ashokkumar, V. A. Prabu, and P. Sampathkumar. 2012. Effect of

- 4495 copper on growth and enzyme activities of marine diatom, *Odontella mobiliensis*. Bulletin of
4496 Environmental Contamination and Toxicology 88:30–37.
- 4497 Manzoni, S., R. B. Jackson, J. a Trofymow, and A. Porporato. 2008. The global stoichiometry of litter
4498 nitrogen mineralization. Science 321:684–686.
- 4499 Martin-Jézéquel, V., M. Hildebrand, and M. A. Brzezinski. 2000. Silicon metabolism in diatoms:
4500 implications for growth. Journal of Phycology 36:821–840.
- 4501 Martín-López, B., C. Montes, and J. Benayas. 2007. The non-economic motives behind the
4502 willingness to pay for biodiversity conservation. Biological Conservation 139:67–82.
- 4503 Masmoudi, S., N. Nguyen-Deroche, A. Caruso, H. Ayadi, A. Morant-Manceau, G. Tremblin, M.
4504 Bertrand, and B. Schoefs. 2013. Cadmium, copper, sodium and zinc effects on diatoms: from
4505 heaven to hell — a Review. Cryptogamie, Algologie 34:185–225.
- 4506 McGill, B. J., M. Dornelas, N. J. Gotelli, and A. E. Magurran. 2015. Fifteen forms of biodiversity trend
4507 in the anthropocene. Trends in Ecology and Evolution 30:104.
- 4508 McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem
4509 predictability. Nature 390:162–165.
- 4510 McIntyre, P. B., L. E. Jones, A. S. Flecker, and M. J. Vanni. 2007. Fish extinctions alter nutrient
4511 recycling in tropical freshwaters. Proceedings of the National Academy of Sciences
4512 104:4461–4466.
- 4513 McKinney, M. L. 1997. Extinction Vulnerability and Selectivity: Combining ecological and
4514 paleontological views. Annual Review of Ecology and Systematics 28:495–516.
- 4515 McLachlan, D. H., C. Brownlee, A. R. Taylor, R. J. Geider, and G. J. C. Underwood. 2009. Light-induced
4516 motile responses of the estuarine benthic diatoms *Navicula perminuta* and *Cylindrotheca*
4517 *closterium* (bacillariophyceae). Journal of Phycology 45:592–599.
- 4518 McMahan, T. A., N. T. Halstead, S. Johnson, T. R. Raffel, J. M. Romansic, P. W. Crumrine, and J. R.
4519 Rohr. 2012. Fungicide-induced declines of freshwater biodiversity modify ecosystem
4520 functions and services. Ecology Letters 15:714–722.
- 4521 McQuoid, M. R., and L. A. Hobson. 1996. Diatom resting stages. Journal of Phycology 32:889–902.
- 4522 McWilliam, R. A., and D. J. Baird. 2002. Postexposure feeding depression: a new toxicity endpoint
4523 for use in laboratory studies with *Daphnia magna*. Environmental Toxicology and Chemistry
4524 21:1198–1205.

- 4525 Mensens, C., F. De Laender, C. R. Janssen, K. Sabbe, and M. De Troch. 2015. Stressor-induced
4526 biodiversity gradients: revisiting biodiversity-ecosystem functioning relationships. *Oikos*
4527 124:677–684.
- 4528 Miao, A.-J., W.-X. Wang, and P. Juneau. 2005. Comparison of Cd, Cu, and Zn toxic effects on four
4529 marine phytoplankton by pulse-amplitude-modulated fluorometry. *Environmental*
4530 *Toxicology and Chemistry* 24:2603.
- 4531 Millennium Ecosystem Assessment. 2005. *Ecosystems and human well-being: biodiversity*
4532 *synthesis*. Washington DC.
- 4533 Millward, R. N., and A. Grant. 2000. Pollution-induced tolerance to copper of nematode
4534 communities in the severely contaminated restronguet creek and adjacent estuaries,
4535 Cornwall, United Kingdom. *Environmental Toxicology and Chemistry* 19:454–461.
- 4536 Moens, T., C. Luyten, J. J. Middelburg, P. M. J. Herman, and M. Vincx. 2002. Tracing organic matter
4537 sources of estuarine tidal flat nematodes with stable carbon isotopes. *Marine Ecology*
4538 *Progress Series* 234:127–137.
- 4539 Moens, T., A. M. Vafeiadou, E. De Geyter, P. Vanormelingen, K. Sabbe, and M. De Troch. 2014.
4540 Diatom feeding across trophic guilds in tidal flat nematodes, and the importance of diatom
4541 cell size. *Journal of Sea Research* 92:125–133.
- 4542 Moens, T., and M. Vincx. 1997. Observations on the feeding ecology of estuarine nematodes.
4543 *Journal of the Marine Biological Association of the UK* 77:211–227.
- 4544 Molnar, J. L., R. L. Gamboa, C. Revenga, and M. D. Spalding. 2008. Assessing the global threat of
4545 invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* 6:485–
4546 492.
- 4547 Montagna, P. A., G. F. Blanchard, and A. Dinet. 1995. Effect of production and biomass of intertidal
4548 microphytobenthos on meiofaunal grazing rates. *Journal of Experimental Marine Biology*
4549 *and Ecology* 185:149–165.
- 4550 Mooney, H. A. 2002. The debate on the role of biodiversity in ecosystem functioning. Pages 12–17
4551 *in* M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning*.
4552 Oxford University Press, Oxford.
- 4553 Moran, X. A. G., A. Lopez-Urrutia, A. Calvo-Diaz, and W. K. W. Li. 2010. Increasing importance of
4554 small phytoplankton in a warmer ocean. *Global Change Biology* 16:1137–1144.

- 4555 Morelli, E., and G. Scarano. 2004. Copper-induced changes of non-protein thiols and antioxidant
4556 enzymes in the marine microalga *Phaeodactylum tricornutum*. *Plant Science* 167:289–296.
- 4557 Morgan-Kiss, R. M., J. C. Prisco, T. Pocock, L. Gudynaite-Savitch, and N. P. A. Huner. 2006.
4558 Adaptation and acclimation of photosynthetic microorganisms to permanently cold
4559 environments. *Microbiology and Molecular Biology Reviews* 70:222–252.
- 4560 Mulder, C. P., D. D. Uliassi, and D. F. Doak. 2001. Physical stress and diversity-productivity
4561 relationships: the role of positive interactions. *Proceedings of the National Academy of
4562 Sciences of the United States of America* 98:6704–6708.
- 4563 Mullan Crain, C., and M. D. Bertness. 2006. Ecosystem engineering across environmental
4564 gradients: Implications for conservation and management. *Bioscience* 56:211–218.
- 4565 Müller-Navarra, D. C. 1995. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in
4566 nature. *Archiv für Hydrobiologie* 3:297–307.
- 4567 Müller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty
4568 acid predicts carbon transfer between primary producers and consumers. *Nature* 403:74–
4569 77.
- 4570 Murray-Gulde, C. L., J. E. Heatley, A. L. Schwartzman, and J. H. Rodgers. 2002. Algicidal effectiveness
4571 of Clearigate, Cutrine-Plus, and copper sulfate and margins of safety associated with their
4572 use. *Archives of Environmental Contamination and Toxicology* 43:19–27.
- 4573 Naeem, S. 1999. Ecology - power behind diversity's throne. *Nature* 401:653–654.
- 4574 Naeem, S. 2008. Advancing realism in biodiversity research. *Trends in Ecology and Evolution*
4575 23:414–6.
- 4576 Naeem, S., D. E. Bunker, A. Hector, M. Loreau, and C. Perrings. 2009. Biodiversity, ecosystem
4577 functioning, and human wellbeing: an ecological and economic perspective. Oxford
4578 University Press, Oxford.
- 4579 Naeem, S., J. E. Duffy, and E. Zavaleta. 2012. The functions of biological diversity in an age of
4580 extinction. *Science* 336:1401–1406.
- 4581 Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining
4582 biodiversity can alter the performance of ecosystems. *Nature* 368:734–736.
- 4583 Naeem, S., and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning:
4584 deriving solutions to a seemingly insormountable problem. *Ecology Letters* 6:567–579.

- 4585 Naidoo, R., and T. H. Ricketts. 2006. Mapping the economic costs and benefits of conservation.
4586 PLoS Biology 4:2153–2164.
- 4587 Nanton, D. A., and J. D. Castell. 1998. The effects of dietary fatty acids on the fatty acid composition
4588 of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae.
4589 Aquaculture 163:251–261.
- 4590 Neumann-Lee, L. A. 2016. Anthropogenic and natural stressors and their effect on immunity,
4591 reproduction and the stress response. Utah State University.
- 4592 Niquil, N., G. Kerleguer, D. Leguerrier, P. Richard, H. Legrand, C. Dupuy, P. Y. Pascal, and C. Bacher.
4593 2006. How would the loss of production due to an herbicide have repercussions in the food
4594 web of an intertidal mudflat? Sensitivity analysis of an inverse model for Brouage mudflat,
4595 Marennes-Oléron Bay, France. Cahiers de Biologie Marine 47:63–71.
- 4596 Noppe, H., A. Ghekiere, T. Verslycke, E. De Wulf, K. Verheyden, E. Monteyne, K. Polfliet, P. van
4597 Caeter, C. R. Janssen, and H. F. De Brabander. 2007. Distribution and ecotoxicity of
4598 chlorotriazines in the Scheldt Estuary (B-NI). Environmental Pollution (Barking, Essex :
4599 1987) 147:668–76.
- 4600 Norberg, J. 2004. Biodiversity and ecosystem functioning: a complex adaptive systems approach.
4601 Limnology and Oceanography 49:1269–1277.
- 4602 O'Connor, N. E., M. E. S. Bracken, T. P. Crowe, and I. Donohue. 2015. Nutrient enrichment alters the
4603 consequences of species loss. Journal of Ecology 103:862–870.
- 4604 O'Connor, N. E., and T. P. Crowe. 2005. Biodiversity loss and ecosystem functioning: distinguishing
4605 between number and identity of species. Ecology 86:1783–1796.
- 4606 Österblom, H., O. Olsson, T. Blenckner, and R. W. Furness. 2008. Junk-food in marine ecosystems.
4607 Oikos 117:967–977.
- 4608 Pacala, S., and A. P. Kinzig. 2002. Introduction to theory and the common ecosystem model. Pages
4609 169–174 in A. P. Kinzig, S. Pacala, and D. Tilman, editors. The functional consequences of
4610 biodiversity: empirical progress and theoretical extensions. Princeton University Press,
4611 Princeton, New Jersey.
- 4612 Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse
4613 ecosystems. Trends in Ecology and Evolution 14:483–488.
- 4614 Pakeman, R. J. 2011. Multivariate identification of plant functional response and effect traits in an

- 4615 agricultural landscape. *Ecology* 92:1353–1365.
- 4616 Parrish, C. C. 2009. Essential fatty acids in aquatic food webs. Pages 309–326 in M. T. Arts, M. T.
4617 Brett, and M. Kainz, editors. *Lipids in aquatic ecosystems*. Springer, New York.
- 4618 Pasari, J. R., T. Levi, E. S. Zavaleta, N. K. Tonthat, S. L. Milam, N. Chinnam, W. Margolin, M. a
4619 Schumacher, S. Sitch, M. A. White, and H. Hashimoto. 2013. Several scales of biodiversity
4620 affect ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*
4621 110:15163–15163.
- 4622 Payne, M. F., R. J. Rippingale, and R. B. Longmore. 1998. Growth and survival of juvenile pipefish
4623 (*Stigmatopora argus*) fed live copepods with high and low HUFA content. *Aquaculture*
4624 167:237–245.
- 4625 Pennington, P., J. Daugomah, A. Colbert, M. Fulton, P. Key, B. Thompson, E. Strozier, and G. Scott.
4626 2001. Analysis of pesticide runoff from mid-Texas estuaries and risk assessment
4627 implications for marine phytoplankton. *Journal of Environmental Science and Health, Part B*
4628 36:1–14.
- 4629 Pereira, H. M., P. W. Leadley, V. Proença, R. Alkemade, J. P. W. Scharlemann, J. F. Fernandez-
4630 Manjarrés, M. B. Araújo, P. Balvanera, R. Biggs, W. W. L. Cheung, L. Chini, H. D. Cooper, E. L.
4631 Gilman, S. Guénette, G. C. Hurtt, H. P. Huntington, G. M. Mace, T. Oberdorff, C. Revenga, P.
4632 Rodrigues, R. J. Scholes, U. R. Sumaila, and M. Walpole. 2010. Scenarios for global biodiversity
4633 in the 21st century. *Science* 330:1496–501.
- 4634 Petchey, O. L., T. Casey, L. Jiang, P. T. McPhearson, and J. Price. 2002. Species richness,
4635 environmental fluctuations, and temporal change in total community biomass. *Oikos*
4636 99:231–240.
- 4637 Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: Back to basics and looking forward.
4638 *Ecology Letters* 9:741–758.
- 4639 Petchey, O. L., K. J. Gaston, A. L. Downing, G. G. Mittelbach, L. Persson, C. F. Steiner, P. H. Warren,
4640 and G. Woodward. 2004. Species loss and the structure and functioning of multitrophic
4641 aquatic systems. *Oikos* 104:467–478.
- 4642 Peterson, H. G., C. Boutin, P. A. Martin, K. E. Freemark, N. J. Ruecker, and M. J. Moody. 1994. Aquatic
4643 phyto-toxicity of 23 pesticides applied at expected environmental concentrations. *Aquatic*
4644 *Toxicology* 28:275–292.
- 4645 Piepho, M., M. T. Arts, and A. Wacker. 2012. Species-specific variation in fatty acid concentrations

- 4646 of four phytoplankton species: Does phosphorus supply influence the effect of light intensity
4647 or temperature? *Journal of Phycology* 48:64–73.
- 4648 Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. *Science*
4649 269:347–350.
- 4650 Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2015. nlme: linear and nonlinear
4651 mixed effects models.
- 4652 Pinho, G. L. L., M. S. Pedroso, S. C. Rodrigues, S. S. de Souza, and A. Bianchini. 2007. Physiological
4653 effects of copper in the euryhaline copepod *Acartia tonsa*: waterborne versus waterborne
4654 plus dietborne exposure. *Aquatic Toxicology* 84:62–70.
- 4655 Pistocchi, R., F. Guerrini, V. Balboni, and L. Boni. 1997. Copper toxicity and carbohydrate
4656 production in the microalgae *Cylindrotheca fusiformis* and *Gymnodinium* sp.. *European*
4657 *Journal of Phycology* 32:125–132.
- 4658 Pomati, F., N. J. B. Kraft, T. Posch, B. Eugster, J. Jokela, and B. W. Ibelings. 2013. Individual cell based
4659 traits obtained by scanning flow-cytometry show selection by biotic and abiotic
4660 environmental factors during a phytoplankton spring bloom. *PLoS ONE* 8:e71677.
- 4661 Pomati, F., B. Matthews, J. Jokela, A. Schildknecht, and B. W. Ibelings. 2012. Effects of re-
4662 oligotrophication and climate warming on plankton richness and community stability in a
4663 deep mesotrophic lake. *Oikos* 121:1317–1327.
- 4664 Pomati, F., and L. Nizzetto. 2013. Assessing triclosan-induced ecological and trans-generational
4665 effects in natural phytoplankton communities: a trait-based field method. *Ecotoxicology*
4666 22:779–794.
- 4667 Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, S. Mills, G. Daily, J. C. Castilla, J.
4668 Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. *Bioscience* 46:609–
4669 620.
- 4670 Purvis, A., and A. Hector. 2000. Getting the measure of well-being. *Nature* 405:212–219.
- 4671 Quigg, A., J. R. Reinfelder, and N. S. Fisher. 2006. Copper uptake kinetics in diverse marine
4672 phytoplankton. *Limnology and Oceanography* 51:893–899.
- 4673 Quijas, S., B. Schmid, and P. Balvanera. 2010. Plant diversity enhances provision of ecosystem
4674 services: A new synthesis. *Basic and Applied Ecology* 11:582–593.
- 4675 Rabaut, M., K. Guilini, G. Van Hoey, M. Vincx, and S. Degraer. 2007. A bio-engineered soft-bottom

- 4676 environment: The impact of *Lanice conchilega* on the benthic species-specific densities and
4677 community structure. *Estuarine, Coastal and Shelf Science* 75:525–536.
- 4678 Radchuk, V., F. De Laender, P. J. Van den Brink, and V. Grimm. 2016. Biodiversity and ecosystem
4679 functioning decoupled: invariant ecosystem functioning despite non-random reductions in
4680 consumer diversity. *Oikos* 125:424–433.
- 4681 Raffaelli, D. 2004. How extinction patterns affect ecosystems. *Ecology* 306:5–6.
- 4682 Raffaelli, D. 2006. Biodiversity and ecosystem functioning: issues of scale and trophic complexity.
4683 *Marine Ecology Progress Series* 311:285–294.
- 4684 Raffaelli, D., M. van der Heijden, W. van der Putten, E. Kennedy, J. Koricheva, G. Lacroix, J. Mikola,
4685 L. Persson, O. Petchey, and D. A. Wardle. 2002. Multi-trophic processes and ecosystem
4686 function. Pages 147–154 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and*
4687 *ecosystem functioning*. Oxford University Press, Oxford.
- 4688 Real, M., I. Munoz, H. Guasch, E. Navarro, and S. Sabater. 2003. The effect of copper exposure on a
4689 simple aquatic food chain. *Aquatic Toxicology* 63:283–291.
- 4690 Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn, and N. Eisenhauer. 2012.
4691 Impacts of biodiversity loss escalate through time as redundancy fades. *Science* 336:589–
4692 592.
- 4693 Reiss, J., J. R. Bridle, J. M. Montoya, and G. Woodward. 2009. Emerging horizons in biodiversity and
4694 ecosystem functioning research. *Trends in Ecology and Evolution* 24:505–514.
- 4695 Relyea, R., and J. Hoverman. 2006. Assessing the ecology in ecotoxicology: a review and synthesis
4696 in freshwater systems. *Ecology Letters* 9:1157–1171.
- 4697 Rengel, Z. 1999. Heavy metals as essential nutrients. Pages 231–251 in M. N. V. Prasad and J.
4698 Hagemayer, editors. *Heavy metal stress in plants*. Springer, Berlin, Heidelberg.
- 4699 Reusch, T. B. H., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after climatic
4700 extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences*
4701 102:2826–2831.
- 4702 Rhee, J. S., I. T. Yu, B. M. Kim, C. B. Jeong, K. W. Lee, M. J. Kim, S. J. Lee, G. S. Park, and J. S. Lee. 2013.
4703 Copper induces apoptotic cell death through reactive oxygen species-triggered oxidative
4704 stress in the intertidal copepod *Tigriopus japonicus*. *Aquatic Toxicology* 132–133:182–189.
- 4705 Rijstenbil, J. W., J. W. M. Derksen, L. J. A. Gerringa, T. C. W. Poortvliet, A. Sandee, M. Vandenberg, J.

- 4706 Vandrie, and J. A. Wijnholds. 1994. Oxidative stress induced by copper: defense and damage
4707 in the marine planktonic diatom *Ditylum brightwellii*, grown in continuous cultures with high
4708 and low zinc levels. *Marine Biology* 119:583–590.
- 4709 Ritz, C., and J. C. Streibig. 2005. Bioassay analysis using R. *Journal of Statistical Software* 12:1–22.
- 4710 Robinson, N. J. 1989. Algal metallothioneins: secondary metabolites and proteins. *Journal of*
4711 *Applied Phycology* 1:5–18.
- 4712 Roger, F., A. Godhe, and L. Gamfeldt. 2012. Genetic diversity and ecosystem functioning in the face
4713 of multiple stressors. *PLoS ONE* 7.
- 4714 Rohr, J. R., and P. W. Crumrine. 2005. Effects of an herbicide and an insecticide on pond community
4715 structure and processes. *Ecological Applications* 15:1135–1147.
- 4716 Rohr, J. R., J. L. Kerby, and A. Sih. 2006. Community ecology as a framework for predicting
4717 contaminant effects. *Trends in Ecology and Evolution* 21:606–613.
- 4718 Rosen, D. A. S., and A. W. Trites. 2005. Examining the potential for nutritional stress in young
4719 Steller sea lions: physiological effects of prey composition. *Journal of Comparative*
4720 *Physiology B: Biochemical, Systemic, and Environmental Physiology* 175:265–273.
- 4721 Rubach, M. N., D. J. Baird, M. C. Boerwinkel, S. J. Maund, I. Roessink, and P. J. Van Den Brink. 2012.
4722 Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide
4723 chlorpyrifos. *Ecotoxicology* 21:2088–2101.
- 4724 Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F.
4725 Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N.
4726 L. Poff, M. T. Skykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Global biodiversity
4727 scenarios for the Year 2100. *Science* 287:1770–1774.
- 4728 Sanpera-Calbet, I., I. Ylla, A. M. Romani, S. Sabater, and I. Muñoz. 2016. Biochemical quality of basal
4729 resources in a forested stream: effects of nutrient enrichment. *Aquatic Sciences*:1–14.
- 4730 Sargent, J. R., J. G. Bell, M. V. Bell, R. J. Henderson, and D. R. Tocher. 1995. Requirement criteria for
4731 essential fatty acids. *Journal of Applied Ichthyology* 11:183–198.
- 4732 Sax, D. F., and S. D. Gaines. 2003. Species diversity: from global decreases to local increases. *Trends*
4733 *in Ecology and Evolution* 18:561–566.
- 4734 Schabhüttl, S., P. Hingsamer, G. Weigelhofer, T. Hein, A. Weigert, and M. Striebel. 2013.
4735 Temperature and species richness effects in phytoplankton communities. *Oecologia*

- 4736 171:527–536.
- 4737 Scherber, C., N. Eisenhauer, W. W. Weisser, B. Schmid, W. Voigt, M. Fischer, E.-D. Schulze, C.
4738 Roscher, A. Weigelt, E. Allan, H. Beßler, M. Bonkowski, N. Buchmann, F. Buscot, L. W. Clement,
4739 A. Ebeling, C. Engels, S. Halle, I. Kertscher, A.-M. Klein, R. Koller, S. König, E. Kowalski, V.
4740 Kummer, A. Kuu, M. Lange, D. Lauterbach, C. Middelhoff, V. D. Migunova, A. Milcu, R. Müller,
4741 S. Partsch, J. S. Petermann, C. Renker, T. Rottstock, A. Sabais, S. Scheu, J. Schumacher, V. M.
4742 Temperton, and T. Tschardtke. 2010. Bottom-up effects of plant diversity on multitrophic
4743 interactions in a biodiversity experiment. *Nature* 468:553–556.
- 4744 Schimel, J., T. C. Balsler, and M. Wallenstein. 2007. Microbial stress-response physiology and its
4745 implications for ecosystem function. *Ecology* 88:1386–1394.
- 4746 Schläpfer, F., A. B. Pfisterer, and B. Schmid. 2005. Non-random species extinction and plant
4747 production: implications for ecosystem functioning. *Journal of Applied Ecology* 42:13–24.
- 4748 Schläpfer, F., and B. Schmid. 1999. Ecosystem effects of biodiversity: a classification of hypotheses
4749 and exploration of empirical results. *Ecological Applications* 9:893–912.
- 4750 Schmidtke, A., U. Gaedke, and G. Weithof. 2010. A mechanistic basis for underyielding in
4751 phytoplankton communities. *Ecology* 91:212–221.
- 4752 Schneider, C. a, W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image
4753 analysis. *Nature Methods* 9:671–675.
- 4754 Schwarzman, M., and M. Wilson. 2009. New science for chemicals policy. *Science* 326:1065–1066.
- 4755 Siebenkäs, A., J. Schumacher, and C. Roscher. 2016. Effects in experimental grass-forb mixtures.
4756 *PLoS ONE* 11:1–21.
- 4757 Smith, D. J., and G. J. C. Underwood. 2000. The production of extracellular carbohydrates by
4758 estuarine benthic diatoms: the effects of growth phase and light and dark treatment. *Journal*
4759 *of Phycology* 36:321–333.
- 4760 Smith, M. D., and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non-
4761 random species loss. *Ecology Letters* 6:509–517.
- 4762 Snelgrove, P. V. R. 1999. Getting to the bottom of marine biodiversity: Sedimentary habitats: Ocean
4763 bottoms are the most widespread habitat on Earth and support high biodiversity and key
4764 ecosystem services. *BioScience* 49:129–138.
- 4765 Solan, M., B. J. Cardinale, A. L. Downing, K. a M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004.

- 4766 Extinction and ecosystem function in the marine benthos. *Science* 306:1177–80.
- 4767 Solan, M., J. A. Godbold, A. Symstad, D. F. Flynn, and D. E. Bunker. 2009. Biodiversity-ecosystem
4768 function research and biodiversity futures: early bird catches the worm or a day late and a
4769 dollar short. Pages 30–45 in S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings,
4770 editors. *Biodiversity, ecosystem functioning, and human wellbeing: an ecological and
4771 economic perspective*. Oxford University Press, Oxford.
- 4772 Soliveres, S., F. van der Plas, P. Manning, D. Prati, M. M. Gossner, S. C. Renner, F. Alt, H. Arndt, V.
4773 Baumgartner, J. Binkenstein, K. Birkhofer, S. Blaser, N. Blüthgen, S. Boch, S. Böhm, C. Börschig,
4774 F. Buscot, T. Diekötter, J. Heinze, N. Hölzel, K. Jung, V. H. Klaus, T. Kleinebecker, S. Klemmer,
4775 J. Krauss, M. Lange, E. K. Morris, J. Müller, Y. Oelmann, J. Overmann, E. Pašalić, M. C. Rillig, H.
4776 M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, J. Sikorski, S. A. Socher, E. F.
4777 Solly, I. Sonnemann, E. Sorkau, J. Steckel, I. Steffan-Dewenter, B. Stempfhuber, M. Tschapka,
4778 M. Türke, P. C. Venter, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, V.
4779 Wolters, T. Wubet, S. Wurst, M. Fischer, and E. Allan. 2016. Biodiversity at multiple trophic
4780 levels is needed for ecosystem multifunctionality. *Nature* 536:456–459.
- 4781 Solomon, K. R., J. a Carr, L. H. Du Preez, J. P. Giesy, R. J. Kendall, E. E. Smith, and G. J. Van Der Kraak.
4782 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. *Critical
4783 Reviews in Toxicology* 38:721–72.
- 4784 Souffreau, C., P. Vanormelingen, E. Verleyen, K. Sabbe, and W. Vyverman. 2010. Tolerance of
4785 benthic diatoms from temperate aquatic and terrestrial habitats to experimental desiccation
4786 and temperature stress. *Phycologia* 49:309–324.
- 4787 Špaèková, I., and J. Lepš. 2001. Procedure for separating the selection effect from other effects in
4788 diversity-productivity relationship. *Ecology Letters* 4:585–594.
- 4789 Speybroeck, J., D. Bonte, W. Courtens, T. Gheskiere, P. Grootaert, J.-P. Maelfait, S. Provoost, K.
4790 Sabbe, E. W. M. Stienen, V. Van Lancker, W. Van Landuyt, M. Vincx, and S. Degraer. 2008. The
4791 Belgian sandy beach ecosystem: a review. *Marine Ecology* 29:171–185.
- 4792 Spijkerman, E., D. Barua, A. Gerloff-Elias, J. Kern, U. Gaedke, and S. A. Heckathorn. 2007. Stress
4793 responses and metal tolerance of *Chlamydomonas acidophila* in metal-enriched lake water
4794 and artificial medium. *Extremophiles* 11:551–562.
- 4795 Srivastava, D. S. 2002. The role of conservation in expanding biodiversity research. *Oikos* 98:351–
4796 360.

- 4797 Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P. Munguia, T.
4798 Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms useful model
4799 systems for ecology? *Trends in Ecology and Evolution* 19:379–384.
- 4800 Srivastava, D. S., and M. Vellend. 2005. Biodiversity - ecosystem functioning research: is it relevant
4801 to conservation? *Annual Review of Ecology, Evolution, and Systematics* 36:267–294.
- 4802 Staats, N., L. J. Stal, and L. R. Mur. 2000a. Exopolysaccharide production by the epipellic diatom
4803 *Cylindrotheca closterium*: effects of nutrient conditions. *Journal of Experimental Marine*
4804 *Biology and Ecology* 249:13–27.
- 4805 Staats, N., L. J. Stal, B. de Winder, and L. R. Mur. 2000b. Oxygenic photosynthesis as driving process
4806 in exopolysaccharide production of benthic diatoms. *Marine Ecology Progress Series*
4807 193:261–269.
- 4808 Stachova, T., P. Fibich, and J. Leps. 2013. Plant density affects measures of biodiversity effects.
4809 *Journal of Plant Ecology* 6:1–11.
- 4810 Stachowicz, J., J. Bruno, and J. Duffy. 2007. Understanding the effects of marine biodiversity on
4811 communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics*
4812 38:739–766.
- 4813 Stachowicz, J. J., R. J. Best, M. E. S. Bracken, and M. H. Graham. 2008a. Complementarity in marine
4814 biodiversity manipulations: reconciling divergent evidence from field and mesocosm
4815 experiments. *Proceedings of the National Academy of Sciences of the United States of*
4816 *America* 105:18842–7.
- 4817 Stachowicz, J. J., M. Graham, M. E. S. Bracken, and A. I. Szoboszlai. 2008b. Diversity enhances cover
4818 and stability of seaweed assemblages: the role of heterogeneity and time. *Ecology* 89:3008–
4819 3019.
- 4820 Stauber, J. L., and C. M. Davies. 2000. Use and limitations of microbial bioassays for assessing
4821 copper bioavailability in the aquatic environment. *Environmental Reviews* 8:255–301.
- 4822 Stauber, J. L., and T. M. Florence. 1989. The effect of culture medium on metal toxicity to the marine
4823 diatom *Nitzschia closterium* and the freshwater green alga *Chlorella pyrenoidosa*. *Water*
4824 *Research* 23:907–911.
- 4825 Steudel, B., A. Hector, T. Friedl, C. Löffke, M. Lorenz, M. Wesche, and M. Kessler. 2012. Biodiversity
4826 effects on ecosystem functioning change along environmental stress gradients. *Ecology*
4827 *Letters* 15:1397–1405.

- 4828 Stockenreiter, M., A. K. Graber, F. Haupt, and H. Stibor. 2012. The effect of species diversity on lipid
4829 production by micro-algal communities. *Journal of Applied Phycology* 24:45–54.
- 4830 Suding, K. N., S. Lavorel, F. S. Chapin, J. H. C. Cornelissen, S. Díaz, E. Garnier, D. Goldberg, D. U.
4831 Hooper, S. T. Jackson, and M. L. Navas. 2008. Scaling environmental change through the
4832 community-level: a trait-based response-and-effect framework for plants. *Global Change*
4833 *Biology* 14:1125–1140.
- 4834 Symstad, A. J., and D. Tilman. 2001. Diversity loss, recruitment limitation, and ecosystem
4835 functioning: lessons learned from a removal experiment. *Oikos* 92:424–435.
- 4836 Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala, and M. T. Brett. 2013. Fatty acid
4837 composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in
4838 22 genera and in seven classes. *Aquatic Microbial Ecology* 71:165–178.
- 4839 Tang, M., and E. Kristensen. 2007. Impact of microphytobenthos and macroinfauna on temporal
4840 variation of benthic metabolism in shallow coastal sediments. *Journal of Experimental*
4841 *Marine Biology and Ecology* 349:99–112.
- 4842 Temperton, V. M., P. N. Mwangi, M. Scherer-Lorenzen, B. Schmid, and N. Buchmann. 2007. Positive
4843 interactions between nitrogen-fixing legumes and four different neighbouring species in a
4844 biodiversity experiment. *Oecologia* 151:190–205.
- 4845 Teoh, M. L., S. M. Phang, and W. L. Chu. 2013. Response of Antarctic, temperate, and tropical
4846 microalgae to temperature stress. *Journal of Applied Phycology* 25:285–297.
- 4847 Thebaut, E., and M. Loreau. 2005. Trophic interactions and the relationship between species. *The*
4848 *American Naturalist* 166:E95–E114.
- 4849 Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. *Nature* 367:363–365.
- 4850 Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and ecosystem functioning. *Annual*
4851 *Review of Ecology, Evolution, and Systematics* 45:471–493.
- 4852 Tilman, D., C. L. Lehman, and K. T. Thomson. 1997. Plant diversity and ecosystem productivity:
4853 Theoretical considerations. *Proceedings of the National Academy of Sciences* 94:1857–1861.
- 4854 Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and
4855 productivity in a long-term grassland experiment. *Science* 294:843–845.
- 4856 Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity
4857 in grassland ecosystems. *Nature* 379:718–720.

- 4858 Tolhurst, T. J., B. Jesus, V. Brotas, and D. M. Paterson. 2003. Diatom migration and sediment
4859 armouring — an example from the Tagus Estuary, Portugal. Pages 183–193 in M. B. Jones, A.
4860 Ingólfsson, E. Ólafsson, G. V Helgason, K. Gunnarsson, and J. Svavarsson, editors. Migrations
4861 and Dispersal of Marine Organisms SE - 20. CHAP, Springer Netherlands.
- 4862 Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2007. Trophic relations in a stream food web:
4863 importance of fatty acids for macroinvertebrate consumers. *Journal of the North American*
4864 *Benthological Society* 26:509–522.
- 4865 Trites, A. W., A. J. Miller, H. D. G. Maschner, M. A. Alexander, S. J. Bograd, J. A. Calder, A. Capotondi,
4866 K. O. Coyle, E. Di Lorenzo, B. P. Finney, E. J. Gregr, C. E. Grosch, S. R. Hare, G. L. Hunt, J. Jahncke,
4867 N. B. Kachel, H. J. Kim, C. Ladd, N. J. Mantua, C. Marzban, W. Maslowski, R. Mendelssohn, D. J.
4868 Neilson, S. R. Okkonen, J. E. Overland, K. L. Reedy-Maschner, T. C. Royer, F. B. Schwing, J. X. L.
4869 Wang, and A. J. Winship. 2007. Bottom-up forcing and the decline of Steller sea lions
4870 (*Eumetopias jubatus*) in Alaska: assessing the ocean climate hypothesis. *Fisheries*
4871 *Oceanography* 16:46–67.
- 4872 De Troch, M., P. Boeckx, C. Cnudde, D. Van Gansbeke, A. Vanreusel, M. Vincx, and M. Caramujo.
4873 2012a. Bioconversion of fatty acids at the basis of marine food webs: insights from a
4874 compound-specific stable isotope analysis. *Marine Ecology Progress Series* 465:53–67.
- 4875 De Troch, M., M. Grego, V. A. Chepurnov, and M. Vincx. 2007. Food patch size, food concentration
4876 and grazing efficiency of the harpacticoid *Paramphiascella fulvofasciata* (Crustacea,
4877 Copepoda). *Journal of Experimental Marine Biology and Ecology* 343:210–216.
- 4878 De Troch, M., J. Mees, and E. Wakwabi. 1998. Diets of abundant fishes from beach seine catches in
4879 seagrass beds of a tropical bay (Gazi Bay, Kenya). *Belgian Journal of Zoology* 128:135–154.
- 4880 De Troch, M., M. B. Steinarsdóttir, V. Chepurnov, and E. Ólafsson. 2005. Grazing on diatoms by
4881 harpacticoid copepods: species-specific density-dependent uptake and microbial gardening.
4882 *Aquatic Microbial Ecology* 39:135–144.
- 4883 De Troch, M., I. Vergaerde, C. Cnudde, P. Vanormelingen, W. Vyverman, and M. Vincx. 2012b. The
4884 taste of diatoms: the role of diatom growth phase characteristics and associated bacteria for
4885 benthic copepod grazing. *Aquatic Microbial Ecology* 67:47–58.
- 4886 Tuchman, N. C., M. a. Schollett, S. T. Rier, and P. Geddes. 2006. Differential heterotrophic utilization
4887 of organic compounds by diatoms and bacteria under light and dark conditions.
4888 *Hydrobiologia* 561:167–177.

- 4889 Turner, R. K., and M. Schaafsma. 2015. Coastal zones ecosystem services: from science to values
4890 and decision making. R. K. Turner and M. Schaafsma, editors. Springer.
- 4891 Tylianakis, J. M., T. A. Rand, A. Kahmen, A. M. Klein, N. Buchmann, J. Perner, and T. Tschardt.
4892 2008. Resource heterogeneity moderates the biodiversity-function relationship in real world
4893 ecosystems. *PLoS Biology* 6:0947–0956.
- 4894 Underwood, G. J. C., and J. Kromkamp. 1999. Primary production by phytoplankton and
4895 microphytobenthos in estuaries. Pages 93–153 in D. Nedwell and D. Raffaelli, editors.
4896 *Advances in ecological research*. Elsevier.
- 4897 Underwood, G. J. C., and D. M. Paterson. 2003. The importance of extracellular carbohydrate
4898 production by marine epipelagic diatoms. *Advances in Botanical Research* 40:183–240.
- 4899 Underwood, G., J. Phillips, and K. Saunders. 1998. Distribution of estuarine benthic diatom species
4900 along salinity and nutrient gradients. *European Journal of Phycology* 33:173–183.
- 4901 Vanellander, B., A. De Wever, N. Van Oostende, P. Kaewnuratchadasorn, P. Vanormelingen, F.
4902 Hendrickx, K. Sabbe, and W. Vyverman. 2009. Complementarity effects drive positive
4903 diversity effects on biomass production in experimental benthic diatom biofilms. *Journal of*
4904 *Ecology* 97:1075–1082.
- 4905 Vansteenbrugge, L., K. Hostens, B. Vanhove, A. De Backer, L. De Clippele, and M. De Troch. 2016.
4906 Trophic ecology of *Mnemiopsis leidyi* in the southern North Sea: a biomarker approach.
4907 *Marine Biology* 163:1–17.
- 4908 Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Springer, Berlin.
- 4909 Venail, P. A., R. C. Maclean, C. N. Meynard, and N. Mouquet. 2010. Dispersal scales up the
4910 biodiversity-productivity relationship in an experimental source-sink metacommunity.
4911 *Proceedings of the Royal Society B: Biological Sciences* 277:2339–2345.
- 4912 Vinebrooke, R. D., K. L. Cottingham, J. Norberg, M. Scheffer, S. I. Dodson, S. C. Maberly, and U.
4913 Sommer. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the
4914 role of species co-tolerance. Pages 451–457 *Oikos*.
- 4915 Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the
4916 concept of trait be functional! *Oikos* 116:882–892.
- 4917 Vitousek, P., and D. Hooper. 1993. Biodiversity and ecosystem function. Pages 3–14 in E. D. Schulze
4918 and H. Mooney, editors. Springer, Berlin.

- 4919 Vitousek, P. M., H. a Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth' s
4920 ecosystems. *Science* 277:494–499.
- 4921 Wagg, C., S. F. Bender, F. Widmer, and M. G. A. van der Heijden. 2014. Soil biodiversity and soil
4922 community composition determine ecosystem multifunctionality. *Proceedings of the*
4923 *National Academy of Sciences of the United States of America* 111:5266–70.
- 4924 Wanless, S., M. P. Harris, P. Redman, and J. R. Speakman. 2005. Low energy values of fish as a
4925 probable cause of a major seabird breeding failure in the North Sea. *Marine Ecology Progress*
4926 *Series* 294:1–8.
- 4927 Wainman, B. C., R. E. Smith, H. Rai, and J. A. Furgal. 1999. Irradiance and lipid production in natural
4928 algal populations. Pages 45–70 *in* M. T. Arts and B. C. Wainmann, editors. *Lipids in freshwater*
4929 *ecosystems*. Springer, New York.
- 4930 Waldbusser, G. G., R. L. Marinelli, R. B. Whitlatch, and P. T. Visscher. 2004. The effects of infaunal
4931 biodiversity on biogeochemistry of coastal marine sediments. *Limnology and Oceanography*
4932 49:1482–1492.
- 4933 Wang, J., C. B. Zhang, T. Chen, and W. H. Li. 2013. From selection to complementarity: the shift
4934 along the abiotic stress gradient in a controlled biodiversity experiment. *Oecologia* 171:227–
4935 235.
- 4936 Wang, Y., and M. Frei. 2011. Stressed food - The impact of abiotic environmental stresses on crop
4937 quality. *Agriculture, Ecosystems and Environment* 141:271–286.
- 4938 Wardle, D. A. 1999. Is 'sampling effect' a problem for experiments investigating biodiversity-
4939 ecosystem function relationships? *Oikos* 87:403–407.
- 4940 Wardle, D. A. 2016. Do experiments exploring plant diversity-ecosystem functioning relationships
4941 inform how biodiversity loss impacts natural ecosystems? *Journal of Vegetation Science*
4942 27:646–653.
- 4943 Weisshaar, H., G. Retzlaff, and P. B. Boger. 1988. Chloroacetamide inhibition of fatty acid synthesis.
4944 *Pesticide Biochemistry and Physiology* 32:212–216.
- 4945 Welsh, P. G., J. L. Parrott, D. G. Dixon, P. V. Hodson, D. J. Spry, and G. Mierle. 1996. Estimating acute
4946 copper toxicity to larval fathead minnow (*Pimphales promelas*) in soft water from
4947 measurements of dissolved organic carbon, calcium, and pH. *Canadian Journal of Fisheries*
4948 *and Aquatic Sciences* 53:1263–1271.

- 4949 Wendt-Rasch, L., P. J. Van Den Brink, S. J. H. Crum, and P. Woin. 2004. The effects of a pesticide
4950 mixture on aquatic ecosystems differing in trophic status: responses of the macrophyte
4951 *Myriophyllum spicatum* and the periphytic algal community. *Ecotoxicology and*
4952 *Environmental Safety* 57:383–398.
- 4953 Werbrouck, E., D. Van Gansbeke, A. Vanreusel, C. Mensens, and M. De Troch. 2016. Temperature-
4954 induced changes in fatty acid dynamics of the intertidal grazer *Platychelipus littoralis*
4955 (Crustacea, Copepoda, Harpacticoida): Insights from a short-term feeding experiment.
4956 *Journal of Thermal Biology* 57:44–53.
- 4957 Wilcove, D. S., and L. L. Master. 2005. How many endangered species are there in the United States?
4958 *Frontiers in Ecology and the Environment* 3:414.
- 4959 Wilsey, B. J., and H. W. Polley. 2004. Realistically low species evenness does not alter grassland
4960 species-richness-productivity relationships. *Ecology* 85:2693–2700.
- 4961 Wittebolle, L., M. Marzorati, L. Clement, A. Balloi, D. Daffonchio, P. De Vos, K. Heylen, W. Verstraete,
4962 and N. Boon. 2009. Initial community evenness favours functionality under selective stress.
4963 *Nature* 458:623–6.
- 4964 Wolfstein, K., and L. J. Stal. 2002. Production of extracellular polymeric substances (EPS) by
4965 benthic diatoms: Effect of irradiance and temperature. *Marine Ecology Progress Series*
4966 236:13–22.
- 4967 Wood, R. J., S. M. Mitrovic, and B. J. Kefford. 2014. Determining the relative sensitivity of benthic
4968 diatoms to atrazine using rapid toxicity testing: A novel method. *The Science of the Total*
4969 *Environment* 485–486C:421–427.
- 4970 Worm et al., B. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314:787–
4971 790.
- 4972 Wu, J., S. Chang, and K. Chen. 1995. Enhancement of intracellular proline level in cells of *Anacystis*
4973 *nidulans* (cyanobacteria) exposed to deleterious concentrations of copper. *Journal of*
4974 *Phycology* 379:376–379.
- 4975 Wyckmans, M., V. a. Chepurnov, A. Vanreusel, and M. De Troch. 2007. Effects of food diversity on
4976 diatom selection by harpacticoid copepods. *Journal of Experimental Marine Biology and*
4977 *Ecology* 345:119–128.
- 4978 Yarmin, F. 1995. Biodiversity, ethics and international law. *International Affairs* 721:529–546.

- 4979 Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland
4980 resistance to biological invaders. *Science* 306:1175–7.
- 4981 Zhang, Y., H. Y. H. Chen, and P. B. Reich. 2012. Forest productivity increases with evenness, species
4982 richness and trait variation: a global meta-analysis. *Journal of Ecology* 100:742–749.
- 4983 Zwart, J. A., C. T. Solomon, and S. E. Jones. 2015. Phytoplankton traits predict ecosystem function
4984 in a global set of lakes. *Ecology* 96:2257–2264.