Biodiversity and ecosystem functioning decoupled: invariant ecosystem functioning despite non-random reductions in consumer diversity

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Most research that demonstrates enhancement and stabilization of ecosystem functioning due to biodiversity is based on biodiversity manipulations within one trophic level and measuring changes in ecosystem functions provided by that same trophic level. However, it is less understood whether and how modifications of biodiversity at one trophic level propagate vertically to affect those functions supplied by connected trophic levels or by the whole ecosystem. Moreover, most experimental designs in biodiversity–ecosystem functioning research assume random species loss, which may be of little relevance to non-randomly assembled communities. Here, we used data from a published ecotoxicological experiment in which an insecticide gradient was applied as an environmental filter to shape consumer biodiversity. We tested how non-random consumer diversity loss affected gross primary production (an ecosystem function provided by producers) and respiration (an ecosystem function provided by the ecosystem as whole) in species-rich multitrophic freshwater communities (total of 128 macroinvertebrate and 59 zooplankton species across treatments). The insecticide decreased and destabilized macroinvertebrate and, to a lesser extent, zooplankton diversity. However, these effects on biodiversity neither affected nor destabilized any of the two studied ecosystem functions. The main reason for this result was that species susceptible to environmental filtering were different from those most strongly contributing to ecosystem functioning. The insecticide negatively affected the most abundant species, whereas much less abundant species had the strongest effects on ecosystem functioning. The latter finding may be explained by differences in body size and feeding guild membership. Our results indicate that biodiversity modifications within one trophic level induced by non-random species loss do not necessarily translate into changes in ecosystem functioning supported by other trophic levels or by the whole community in the case of limited overlap between sensitivity and functionality.

Research on the effects of biodiversity on ecosystem functioning is inspired, in part, by the observed global decline of biodiversity. The majority of experimental studies suggest that biodiversity (B) both enhances and stabilizes ecosystem functioning (EF; Jiang and Pu 2009, Cardinale et al. 2013, Gross et al. 2014; but see Petchey et al. 2002, Polley et al. 2007). However, most of these studies create a biodiversity gradient via random assembly of species into communities (Loreau and Hector 2001, Wilsey and Polley 2004, Reich et al. 2012, de Mazancourt et al. 2013). Yet, understanding

the functional effects of realistic non-random species loss is more relevant to conservation science (Solan et al. 2004, Srivastava and Vellend 2005, Polley et al. 2007, Bracken and Low 2012, Mensens et al. 2015).

Another characteristic of the experiments used in biodiversity–ecosystem functioning (BEF) research to date is their focus on a single trophic level, mostly terrestrial primary producers in grasslands (Loreau and Hector 2001, Wilsey and Polley 2004, Reich et al. 2012, Cardinale et al. 2013). Only a few studies considered the possible implications of biodiversity modifications at one trophic level for the ecosystem functions provided by other levels by experimentally manipulating the biodiversity of the targeted trophic level (Srivastava and Vellend 2005, Haddad et al. 2011, Bracken and Low 2012). To understand the implications of the biodiversity changes in nature, one needs to test whether non-random

species loss within one trophic level impairs functionality and diversity in vertically connected communities.

In nature, realistic species loss is driven by anthropogenic stressors, such as habitat fragmentation, pollution, and species invasion (Lawler et al. 2006). Chemical pollution represents one of the most understudied stressors in biological conservation (Lawler et al. 2006) despite demonstrated negative effects on biodiversity and ecosystem functioning at local and regional levels (Schaefer et al. 2007, Beketov et al. 2013, Viaene et al. 2013). We argue that community-level experiments in ecotoxicology offer a unique opportunity to study BEF questions in more realistic settings for two reasons. First, pesticides are designed to act as specific stressors, targeting certain species or taxa within communities, which leads to non-random modifications of the biodiversity of those communities (McMahon et al. 2012, Halstead et al. 2014). Thus, the toxicant application in these experiments creates a biodiversity gradient via non-random species loss (Viaene et al. 2013, De Laender et al. 2014). Second, as these experiments are designed to assess the risk for entire aquatic communities, species abundances are monitored across trophic levels, allowing us to test for vertical propagation of biodiversity changes.

In this study we ask whether insecticide-induced reductions of consumer diversity in experimental freshwater ditches affect gross primary production (GPP) and ecosystem respiration. GPP is an ecosystem function carried out by primary producers, i.e. by a vertically connected trophic level, whereas respiration is a function provided by the whole community – primary producers as well as consumer trophic levels. To this end, we re-analyzed data from a previously conducted ecotoxicological experiment (Van den Brink et al. 1996, Van Wijngaarden et al. 1996, Kersting and Van den Brink 1997) with application of the insecticide chlorpyrifos in species-rich multitrophic freshwater communities (a total of 128 macroinvertebrate and 59 zooplankton species across the treatments). Chlorpyrifos is an insecticide inhibiting the activity of the enzyme acetylcholinesterase, which leads to the over-stimulation of nerve impulses (Brock et al. 2000). Consequently, chlorpyrifos is detrimental to macroinvertebrates and, to a lesser extent, to zooplankton, but does not directly affect primary producers. In fact, chlorpyrifos application is known to cause algal blooms following the release of producers from consumer grazing pressure (Hurlbert 1975, Butcher et al. 1977, Van den Brink et al. 2002, Fleeger et al. 2003). Such an algal bloom was also observed in the ecotoxicological experiment from which we used the data in this study, confirming the presence of strong vertical interactions (Kersting and Van den Brink 1997). Therefore, we expected indirect effects of chlorpyrifos on GPP and respiration. We completed our analyses by proposing a mechanism connecting the insecticide-induced biodiversity gradient to these two ecosystem functions considered.

Material and methods

Experimental data

A gradient of chlorpyrifos concentrations (0.1, 0.9, 6, 44 µg l^{-1}) was applied to outdoor freshwater ditches (length = 40 m, width = 3.4 m, water column depth = 0.5 m), with two replicates per concentration level. Four ditches were not manipulated and represent the control replicates. All ditches had been populated with macrophytes more than two years prior to the experiment to let the floral and faunal communities typical of drainage ditches in the Netherlands develop (Van Wijngaarden et al. 1996). The ditches contained a typical invertebrate community for oligotrophic drainage ditches. The zooplankton community was dominated by ciliates, rotifers, cyclopods, ostracods and the cladocerans *Daphnia galeata* and *Simocephalus vetulus*. The invertebrate community was dominated by ephemeroptera (*Caenis horaria* and *Cloeon dipterum*), the phantom midge *Chaoborus obscuripes*, oligochaetes, the snails *Armiger crista* and *Potamopyrgus antipodarum*, the trichopteran *Mystacides longicornis/nigra*, Coenagrionidae and the macrocrustaceans *Gammarus pulex* and *Asellus aquaticus*. Zooplankton was sampled weekly from the beginning of the experiment (application of chlorpyrifos, week 0) to week three, and from then on sampling continued on a bi-weekly basis until week 24, when the sampling was terminated due to the onset of winter. Macroinvertebrates were sampled by means of artificial substrate in weeks 0, 1, 2, 4 and then on a monthly basis until week 24. Individuals in zooplankton and macroinvertebrate samples were identified to the species level whenever possible and counted. Chlorpyrifos was sprayed over as a commercially-used Dursban 4E, in a pulse treatment. Dissipation of 50% of the insecticide was observed on the first day, and 97% dissipation was observed after 28 days for all treatments (Van Wijngaarden et al. 1996). The details on the experiment and community data collection can be found in Van Wijngaarden et al. (1996). Dissolved oxygen was measured daily and ecosystem respiration and gross primary production (GPP) were estimated daily from the oxygen mass balance equation as described in Kersting and Van den Brink (1997). For our analyses, we took weekly averages of the two ecosystem functions.

Statistical analyses

Effect of treatment on the mean and variance of biodiversity and ecosystem functioning

At each available time point we calculated 13 biodiversity indices per community (zooplankton and macroinvertebrates), including indices describing biodiversity per se and indices focusing on evenness (Supplementary material Appendix 1 Table A1). Because many biodiversity indices are strongly correlated with each other (Supplementary material Appendix 1 Fig. A1–A2), we chose to present only a subset of indices in the main text and to present the results on the other eight indices in the Supplementary material Appendix 1 Fig. A3). More precisely, in the main text, we focus on five biodiversity indices: three represent biodiversity per se (Shannon–Wiener index, Simpson index and species richness), and two represent evenness (Pielou and McIntosh evenness). Species richness gives most weight to rare species, Simpson index gives most weight to the abundant species, and Shannon–Wiener index has an intermediate position (Hill 1973, Jost 2006). The two indices based on evenness are the most commonly used in biodiversity studies.

Previous analyses (Van den Brink and Ter Braak 1999) have demonstrated the decline of arthropod abundances in the overall community during the first three weeks following the chlorpyrifos application (the strength of the effect on the community increased along the insecticide concentration gradient), and its subsequent recovery (see Fig. 3 in Van den Brink and Ter Braak 1999 for the principal response curves). Therefore, we split the total duration of the experiment into two periods: the exposure period (when most population abundances declined according to Van den Brink and Ter Braak 1999; week 0–3 inclusive) and the post exposure period (when the populations started recovering; week 4–24). We then tested whether the temporal mean, temporal standard deviation and temporal coefficient of variation of the five biodiversity indices and two ecosystem functions differed between both periods. To test how robust our results were to the choice of the week at which to split the experiment duration into two periods, we conducted a sensitivity analysis by using a different time point to split the experiment: with the first period lasting 0–5 weeks.

To assess the effect of chlorpyrifos on the mean, standard deviation (SD) and coefficient of variation (CV) of the biodiversity indices, we calculated the temporal mean, temporal SD and temporal CV of each biodiversity index per period (exposure and post exposure), per community (zooplankton and macroinvertebrates) and per ditch. Because the number of data points available differed between the exposure and post exposure periods, we randomly selected three and five data points in the post exposure period for macroinvertebrates and zooplankton communities, respectively. None of the temporal means, SDs and CVs was equal to 0, so that we did not have to add a certain threshold or process the values in any other way. Next, following Gross et al. (2014), we regressed the log-transformed mean, SD and CV of the biodiversity indices on the insecticide concentration:

$$Ln\,(Mean\,BD) \sim \beta_{\mu BD} \times Ins\,Concentr$$
$$Ln\,(SD\,BD) \sim \beta_{\sigma BD} \times Ins\,Concentr$$
$$Ln\,(CV\,BD) \sim \beta_{CVBD} \times Ins\,Concentr$$

where *Mean BD*, *SD BD* and *CV BD* are the mean, standard deviation and coefficient of variation of each biodiversity index, respectively. *InsConcentr* is the chlorpyrifos concentration and the beta coefficients $\beta_{\mu BD}$, $\beta_{\sigma BD}$ and $\beta_{CV\,BD}$ are the slopes measuring the effects of insecticide concentration on the mean, SD and CV of each biodiversity index, respectively. Model diagnostics (normality of the residuals, absence of the patterns in residuals when plotted against the predictor) showed satisfying results for such models (Supplementary material Appendix 1 Fig. A4). We then plotted slopes measuring the effects of the insecticide concentration on the mean of biodiversity indices, i.e. the $\beta_{\mu BD}$'s, on the x-axis and the corresponding slopes measuring the effects of the insecticide concentration on the SD, i.e. the $\beta_{\sigma BD}$'s, on the y-axis (schematized in Fig. 1). Based on the previously reported negative effect of insecticide on biodiversity in the studied system (Van den Brink et al. 1996), we expected that an increase in insecticide concentration would decrease the mean of biodiversity and increase its SD. This would lead to the decline and destabilization of biodiversity, corresponding
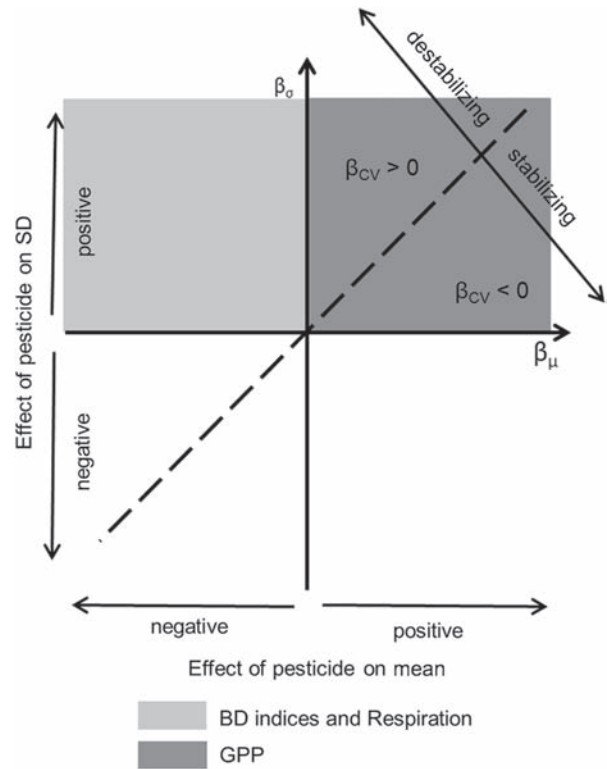

Figure 1. Schematic representation of the method suggested by Gross et al. (2014) for partitioning the insecticide effect on stability into the effects on the mean (x-axis) and SD (y-axis) of the metric of interest. The 1:1 line corresponds to stability maintenance; beta coefficients above and below this line represent insecticide-induced destabilization and stabilization of the metric of interest, respectively. We hypothesize that the insecticide concentration gradient will result in lower mean and higher SD of biodiversity indices (for more details see Methods), corresponding to beta coefficients falling into the 2nd quadrant (light grey). Similarly, an increase of insecticide concentration is hypothesized to decrease the mean and increase the SD of respiration, resulting in beta coefficients falling in the 2nd quadrant (light grey). In case of GPP, we hypothesize its mean and SD to increase with an increase in the insecticide concentration (for more details see Methods), resulting in beta coefficients located in the 1st quadrant (dark grey).

to points falling in the 2nd quadrant in Fig. 1. We assessed the statistical significance of insecticide impact on mean, SD and CV of each biodiversity index per community and per period by bootstrap sampling, in which $\beta_{\mu BD}$, $\beta_{\sigma BD}$ and $\beta_{CV\,BD}$ obtained from regressions were compared with their estimated sampling distributions obtained from 10 000 bootstrap samples.

To assess the effect of chlorpyrifos on the mean, SD and CV of the two ecosystem functions, we calculated the temporal mean, temporal SD and temporal CV of both ecosystem functions per period (exposure and post exposure) and ditch. Analogously to biodiversity, we regressed the log-transformed mean, SD and CV of the ecosystem functions on the insecticide concentration:

$$Ln\,(Mean\,EF) \sim \beta_{\mu EF} \times Ins\,Concentr$$
$$Ln\,(SD\,EF) \sim \beta_{\sigma EF} \times Ins\,Concentr$$
$$Ln\,(CV\,EF) \sim \beta_{CV\,EF} \times Ins\,Concentr$$

where *Mean EF*, *SD EF* and *CV EF* are the mean, standard deviation and coefficient of variation of each ecosystem function, respectively. The beta coefficients $\beta_{\mu EF}$, $\beta_{\sigma EF}$ and $\beta_{CV\ EF}$ are the slopes measuring the effects of insecticide concentration on the mean, SD and CV of each ecosystem function, respectively. The slopes were plotted exactly as for the biodiversity analysis. Because a negative effect of insecticide application on ecosystem respiration was previously reported (Kersting and Van den Brink 1997), we hypothesized that an increase in the chlorpyrifos concentration would lead to a decrease in the mean of respiration accompanied by an increase in its SD, which would result in slopes located in the 2nd quadrant of the plot (Fig. 1). Contrary to the effect on respiration, the effect on GPP is expected to be mediated by the indirect effects on producers, resulting in an increase of GPP along the insecticide concentration gradient. Indeed, in the highest chlorpyrifos treatments, the GPP was previously reported to increase (Kersting and Van den Brink 1997). This, associated with the hypothesized increase of the SD of this ecosystem function, would result in slopes located in the 1st quadrant of the plot (Fig. 1). The statistical significance of insecticide effects on mean, SD and CV of each ecosystem function per period was estimated as for the biodiversity indices, i.e. with bootstrap sampling.

Relationships between rarity, functionality and sensitivity

To understand the connection between insecticide effects on consumer diversity (and its stability) and producer/ecosystem functioning, we conducted two types of analyses on a per species basis using presence–absence data for each consumer species. This allowed us to assess the sensitivity and functionality of rare and common species. First, we used a generalized linear model with binomial error distribution and logit link (logistic regression) to assess which species were significantly affected by insecticide application:

$$Prob\ Pres \sim \frac{1}{1+e^{-(\beta_{PA}\times Ins\ Consentr)}}$$

where *ProbPres* is the probability of a species being present, the beta coefficient β_{PA} is the slope measuring the effect of insecticide concentration on the presence of each species. Second, we used ANOVA tests per species to assess how the presence/absence of the species affected the two ecosystem functions. The values of both ecosystem functions were standardized prior to the analyses (as [EF – Mean EF]/SD EF per ditch). Both types of tests were conducted separately for the exposure and the post exposure period. To test for correlations between the effects of insecticide application on species presence and the effects of species presence on ecosystem functioning, we used Spearman correlation on the corresponding slopes. Next, for each species, we retained the slopes that were significant in at least one of the model types to infer which species were most affected by the treatment and which were affecting GPP (and respiration) the most. We then plotted these slopes for each species next to its relative abundance (calculated as the proportional abundance across time in control ditches). Such a plot demonstrates how the rare and common species are, on the one hand, affected by insecticide and, on the other hand, affect ecosystem functioning.

Potential mechanisms underlying rarity–functionality relationship

We suggested two tentative and non-mutually exclusive mechanisms responsible for the higher functionality of the rare species: 1) the individual body mass of the rare species is higher than that of the abundant species, so that they have higher per capita effect on the ecosystem functioning; and 2) the rare and abundant species represent different feeding guilds, so that the rare species belong to the feeding guild that is more functionally important. To test the mechanism regarding the individual body mass, we used a general linear model on the log-transformed body mass (to achieve normality) of the species that belong to both communities (zooplankton and macroinvertebrates). A categorical variable (*Rare*) distinguishing between rare and abundant species was used as an explanatory variable:

$$Ln\ (Body\ Mass) \sim \beta_R \times Rare$$

where *BodyMass* is a species body mass, and the beta coefficient β_R is the slope measuring the effect of the species being rare or abundant on the species body mass. The species were classified into either rare or abundant based on their proportional abundances, calculated across time for control ditches only. We used 10^{-4} as a threshold proportional abundance value for zooplankton and 10^{-2} for macroinvertebrates. This threshold divides species into rare (proportional abundance lower than the threshold) and abundant (proportional abundance higher than the threshold). The individual dry body masses (g) were extracted from the literature (Supplementary material Appendix 1 Table A2). For zooplankton body mass estimates were available for all species, whereas for macroinvertebrates body mass estimates were available for four out of ten abundant species and eleven out of 21 rare species. We therefore limited the above-described analyses only to the species for which body mass estimates were available from the literature data. To test the mechanism implying the differential impact of the feeding guild on the functionality of the species, we used χ^2-test to assess whether there is a significant difference in the feeding guilds of the rare versus abundant species separately for each community.

All analyses were conducted with R 3.0.2 software (<www.r-project.org>).

Results

Effects of insecticide on biodiversity and its stability

In the exposure period, the insecticide significantly decreased the mean zooplankton richness ($\beta_\mu = -0.009$, bootstrap SE (BSE) = 3×10^{-5}, two-tailed bootstrap p = 0.008, Fig. 2A) and increased its CV ($\beta_{CV} = 0.017$, BSE = 6.8×10^{-5}, p = 0.003). Most other indices were not affected (Supplementary material Appendix 1 Fig. A3a–b, Table A3). In the post exposure period the insecticide significantly decreased the mean of zooplankton species richness ($\beta_\mu = -0.008$, BSE = 3.5×10^{-5}, p = 0.014), however the mean of most other indices was not significantly affected (Supplementary material Appendix 1 Fig. A3a–b, Table A3). Although the insecticide did not significantly affect SD of
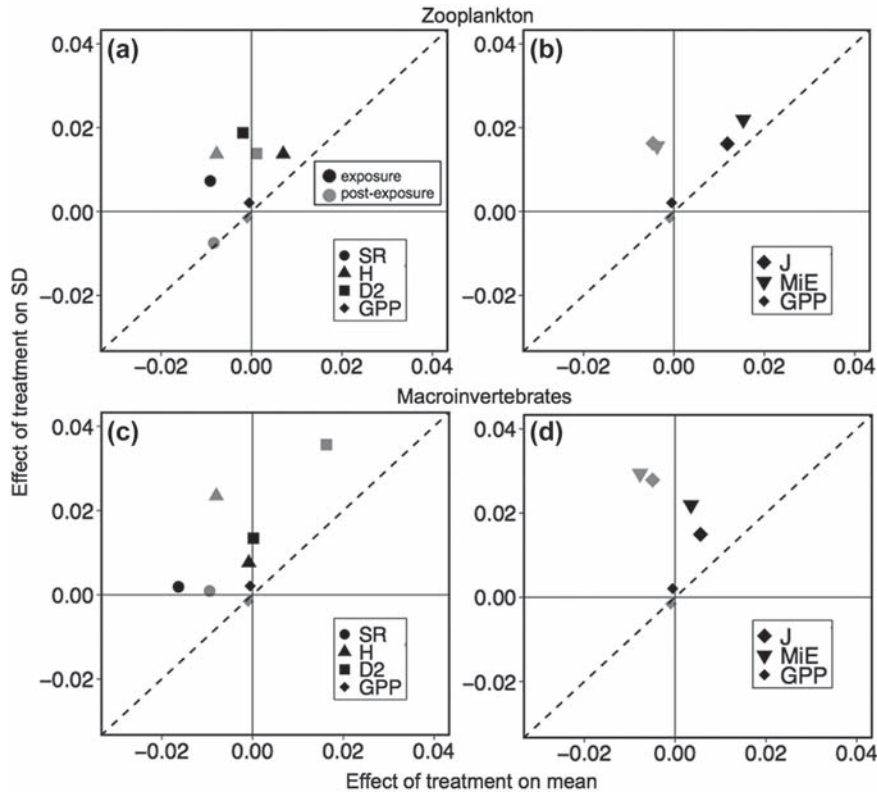
Figure 2. Effect of chlorpyrifos on the mean, SD and coefficient of variation of zooplankton (a, b); macroinvertebrate community (c, d); and gross primary production (a–d) for exposure and post exposure phases. The effect on biodiversity is measured in terms of biodiversity indices per se (a, c) and evenness indices (b, d). The dashed 1:1 line represents the stability threshold, with the points above the line representing destabilization of the metrics, and below the line –stabilization of the metrics, as exemplified on Fig. 1. Abbreviations are as follows: SR - species richness, H – Shannon–Wiener index, D2 - Simpson index, J - Pielou evenness, MiE - McIntosh evenness, GPP - gross primary production.

any of biodiversity indices, several of them were destabilized (Fig. 2a–b): Shannon index ($\beta_{CV} = 0.02$, BSE = 1×10^{-4}, p = 0.03), Pielou ($\beta_{CV} = 0.02$, BSE = 9.7×10^{-5}, p = 0.02) and McIntosh evenness ($\beta_{CV} = 0.02$, BSE = 9.7×10^{-5}, p = 0.03; for others see Supplementary material Appendix 1 Table A3).

In the exposure period, the insecticide decreased the mean of macroinvertebrate species richness ($\beta_{\mu} = -0.016$, BSE = 5.5×10^{-5}, p = 0) and increased the mean of Pielou evenness ($\beta_{\mu} = 0.006$, BSE = 2.7×10^{-5}, p = 0.017; Fig. 2c–d). Mean of several other indices was also affected, however the insecticide did not significantly affect either SD or CV of most biodiversity indices (Fig. 2c–d, Supplementary material Appendix 1 Table A3). Contrary, in the post exposure period, the mean of most biodiversity indices decreased due to the insecticide application (Fig. 2c–d, Supplementary material Appendix 1 Fig. A3, Table A3). This, together with an increase in SD of several indices (Simpson index: $\beta_{\sigma} = 0.04$, BSE = 1.8×10^{-4}, p = 0.02; McIntosh evenness: $\beta_{\sigma} = 0.03$, BSE = 1.5×10^{-4}, p = 0.05) resulted in destabilization of most of them (Fig. 2c–d, Supplementary material Appendix 1 Fig. A3, Table A3).

These results were qualitatively unaffected by the point at which the duration of the complete experiment was split into two periods (Supplementary material Appendix 1 Fig. A5, Table A4).

Consequences of insecticide-induced biodiversity shifts for ecosystem functioning

Contrary to our expectations, the mean (exposure: $\beta_{\mu} = -0.005$, BSE = 1.8×10^{-5}, p = 0.74; post exposure: $\beta_{\mu} = -0.001$, BSE = 1.5×10^{-5}, p = 0.59), SD (exposure: $\beta_{\sigma} = 0.002$, BSE = 5×10^{-5}, p = 0.71; post exposure: $\beta_{\sigma} = -0.002$, BSE = 3.4×10^{-5}, p = 0.69) and CV of GPP (exposure: $\beta_{CV} = 0.0026$, BSE = 4.9×10^{-5}, p = 0.61; post exposure: $\beta_{CV} = -0.0006$, BSE = 2.7×10^{-5}, p = 0.83) were not significantly affected by the insecticide (Fig. 2, Supplementary material Appendix 1 Fig. A6). Similarly, the insecticide did not significantly affect the mean, SD and CV of respiration in any of the periods (Supplementary material Appendix 1 Table A5). Obtained results were not affected by the point at which the duration of the experiment was split into two periods (Supplementary material Appendix 1 Fig. A7, Table A5). Consequently, the increasing insecticide concentration did not destabilize any of the two ecosystem functions inspected.

Species rarity, functionality and sensitivity

The insecticide application had a negative impact on the species presence in most of the cases where the effect of the insecticide was significant (Fig. 3, 4). Most of the species
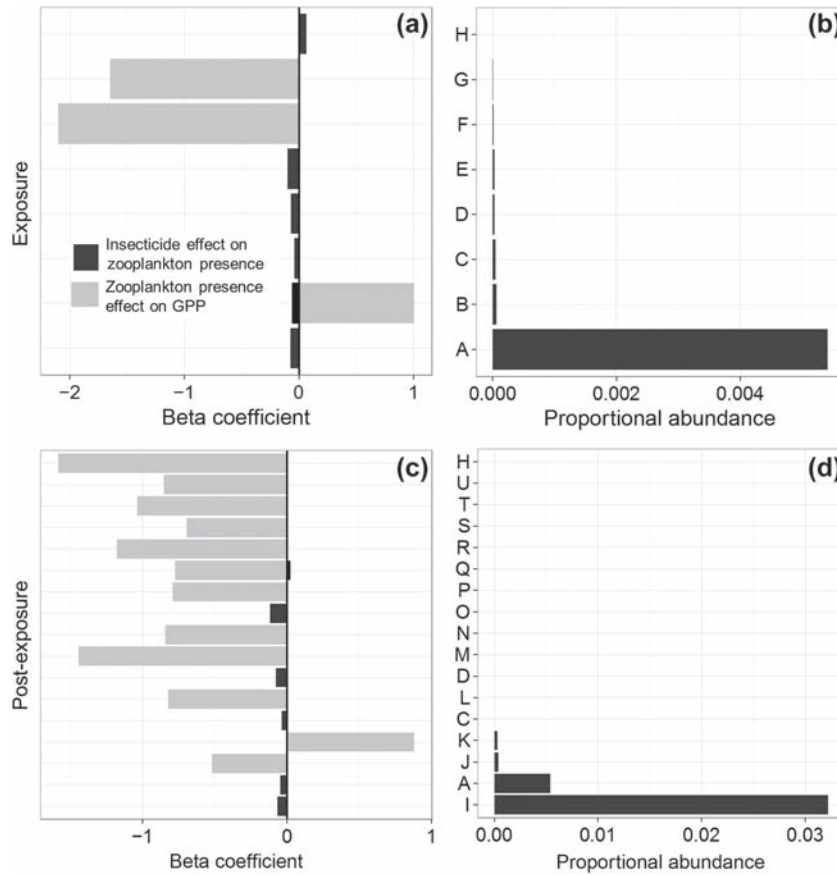
Figure 3. Slopes measuring the effect of chlorpyrifos application on the presence of zooplankton species (black) and of zooplankton species presence on GPP (grey) in exposure (a) and post exposure (c) period. Only slopes that were significant (p-value < 0.05) are shown. Relative abundance of each species (calculated using control ditches across time) is shown in (b) and (d). Species abbreviations are as follows: A - *Nauplius* larvae, B - *Ostracoda* sp., C - *Daphnia longispina*, D - *Simocephalus vetulus*, E - *Copepoda* spp., F - *Trichocerca porcellus*, G - *Mytilina ventralis*, H - *Squatinella muticum*, I - *Strombidium viride*, J - *Ascomorpha* sp., K - *Lecane quadridentata*, L - *Lecane* sp., M - *Chydorus sphaericus*, N - *Alona affinis*, O - *Ceriodaphnia pulchella*, P - *Alona guttata*, Q - *Stylaria lacustris*, R - *Alona rectangula*, S - *Alonella exigua*, T - *Acroperus harpae*, U - *Pleuroxus aduncus*.

whose presence was affected by insecticide application were among the most abundant and they had little overlap with the species whose presence significantly affected GPP (Fig. 3, 4; and respiration: Supplementary material Appendix 1 Fig. A8–A9). The species whose presence significantly affected GPP (and respiration, Supplementary material Appendix 1 Fig. A8–A9) were much less abundant than the species susceptible to insecticide application. In the zooplankton community, 6 out of 59 species (10.2%) were significantly affected by the insecticide in both exposure and post exposure period. In the macroinvertebrate community, 9 out of 128 species (7%) were significantly affected by the insecticide in the exposure and 16 species (12.5%) were significantly affected in the post exposure period. Regarding the number of species that had a significant effect on the ecosystem functions, 3 (5.1%) and 12 (20.3%) zooplankton species had a significant impact on GPP in exposure and post exposure period respectively. Similarly, 3 (5.1%) and 10 (17%) zooplankton species had a significant impact on respiration in exposure and post exposure period respectively. For macroinvertebrates, 3 (2.3%) and 19 (14.8%) species had a significant effect on GPP in exposure and post exposure periods respectively; and 7 (5.5 %) and 16 (12.5 %) species had a significant

impact on respiration in exposure and post exposure period, respectively. Spearman correlation between the slopes measuring the effect of insecticide application on species presence and slopes measuring the effect of species presence on ecosystem functions (using betas from all models, irrespective of whether they were significant or not) was significantly negative in most cases for macroinvertebrates (exposure period: with respiration, $\rho(N = 52) = -0.40$, $p = 0.003$; with GPP, $\rho(N = 52) = -0.13$, $p = 0.35$; post exposure period: with respiration, $\rho(N = 79) = -0.36$, $p = 0.001$; with GPP, $\rho(N = 79) = -0.24$, $p = 0.031$). For the zooplankton community, the only significant correlation was between slopes measuring the effect of insecticide application on species presence and slopes measuring the effect of species presence on respiration in the exposure period ($\rho(N = 36) = -0.36$, $p = 0.031$).

Potential mechanisms linking high functionality to rarity

The body mass of the rare species was not significantly higher than that of the more abundant species either for zooplankton ($p = 0.642$, Fig. 5a) or for macroinvertebrates ($p = 0.0505$, Fig. 5b).
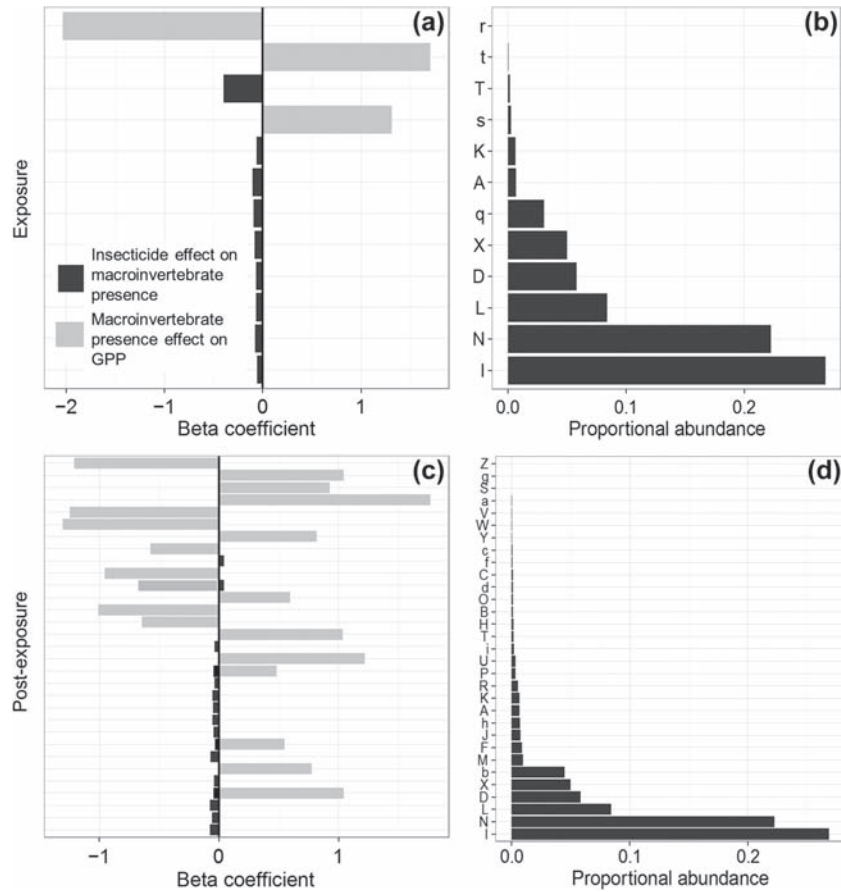
Figure 4. Slopes measuring the effect of chlorpyrifos application on the presence of macroinvertebrate species (black) and of macroinvertebrate species presence on GPP (grey) in exposure (a) and post exposure (c) period. Only slopes that were significant (p-value < 0.05) are shown. Relative abundance of each species (calculated using control ditches across time) is shown in (b) and (d). Species abbreviations are as follows: A - *Ablabesmyia phatta/monilis*, B - Aeschnidae, C - *Agrypnia/Dasystegia/Phryganea* complex, D - *Armiger crista*, F - *Asellus aquaticus*, H - *Bothromesostoma* sp., I - *Caenis horaria*, J - *Caenis luctuosa*, K - Ceratopogonidae, L - *Chaoborus obscuripes*, M - *Chironomus* sp., N - *Cloeon dipterum*, O - *Glossiphonia complanata*, P - *Helobdella stagnalis*, R - Hydracarina, S - *Hyphydrus ovatus*, T - *Hygrotus versicolor*, U - *Laccophilus minutus*, V - *Lymnaea stagnalis*, W - *Microtendipes chloris*, X - *Mystacides longicornis/nigra*, Y - *Notonecta glauca*, Z - *Notonecta obliqua*, a - *Oecetes lacustris*, b - *Potamopyrgus antipodarum*, c - *Procladius* sp., d - *Psectrocladius obvius*, f - *Radix peregra*, g - *Sigara striata*, h - Sphaeriidae, i - *Tanytarsus* sp., q - Coenagrionidae, r - *Corixa panzeri*, s - *Gammarus pulex*, t - *Haliplus confinis*.
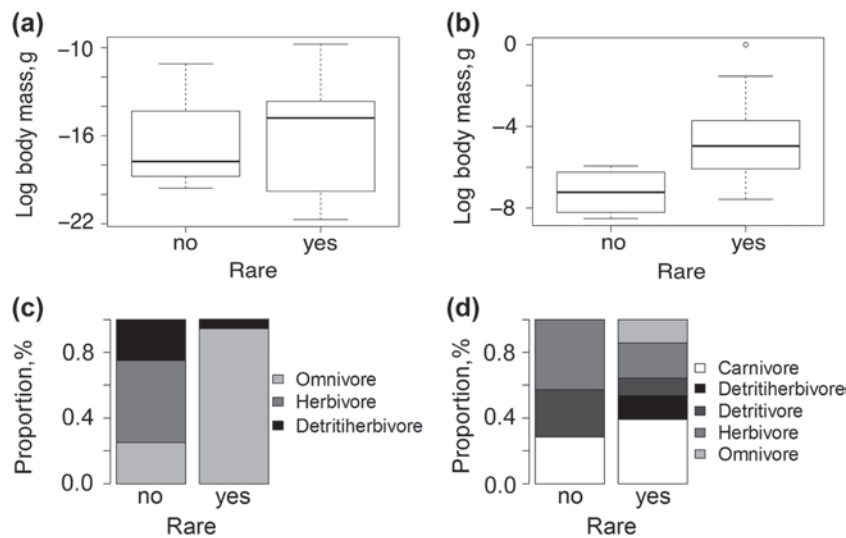

Figure 5. Logarithm-transformed body masses of rare versus abundant species in (a) zooplankton (18 rare and 4 abundant species) and (b) macroinvertebrate (11 rare and 4 abundant species) communities. Feeding guilds of rare and abundant species belonging to (c) zooplankton and (d) macroinvertebrates.

For zooplankton species, the feeding guilds differed significantly between the rare and abundant species (χ^2 (2, N = 22) = 12.29, p = 0.0021). The majority of the rare species were omnivores, whereas most of abundant species were represented by herbivores and detritiherbivores (Fig. 5c). For macroinvertebrates, no significant patterns were found (χ^2 (4, N = 35) = 4.42, p = 0.3958).

Discussion

Here, we benefited from an ecotoxicological experiment conducted previously whereby the application of an environmental filter (insecticide) resulted in non-random biodiversity loss. This enabled us to study whether a consumer diversity gradient in a multitrophic system changed the ecosystem functioning provided by either a connected trophic level or the ecosystem as a whole. We looked not only at the mean of ecosystem functioning, but were primarily interested in its stability, quantified here as variability (coefficient of variation). We applied a recently developed method (Gross et al. 2014) to disentangle the effects of insecticide treatment on the mean and standard deviation of both metrics of interest to us: consumer diversity and ecosystem functioning provided by either producers solely or by the overall community. As expected, insecticide application decreased and destabilized consumer diversity with more pronounced effects on macroinvertebrates than on zooplankton. However, such a gradient of consumer diversity did not affect either the mean or the stability of ecosystem functions (GPP and respiration). We demonstrated that the decoupling of consumer diversity and ecosystem functioning provided by connected trophic levels is likely due to the correlation between species sensitivity to the insecticide and species impact on ecosystem functioning being negative or absent.

Insecticide application negatively affected both zooplankton and macroinvertebrate communities, however its effect on macroinvertebrates was more pronounced, in agreement with its mode of action. As expected, chlorpyrifos negatively affected the mean and stability of biodiversity indices. We found a stronger destabilization of macroinvertebrate diversity in the post exposure compared to the exposure period. Such a delayed response was not found for zooplankton, which may possibly be due to their shorter life span than that of macroinvertebrates. The observed stronger insecticide effect on post exposure period was more pronounced for macroinvertebrate evenness, indicating that the abundances of species were changing repeatedly and to a great extent in the post exposure period.

To understand all these different effects, a mechanistic framework is needed. Such a framework based on functional traits was suggested in order to understand the processes behind the human-induced biodiversity modifications and the resulting changes in the temporal mean and stability of ecosystem functioning (Hooper et al. 2002, Díaz et al. 2013). To ensure the continuity of ecosystem provisioning under the constant pressure of multiple anthropogenic stressors, functional traits were suggested to be differentiated into response traits that measure species sensitivity to a certain environmental stressor, and effect traits that reflect

a contribution of the species to a certain ecosystem function (Violle et al. 2007, Suding et al. 2008, Díaz et al. 2013).

A correlation between the functional response traits, i.e. traits related to extinction risk on the one hand, and the traits affecting ecosystem functioning on the other hand, was suggested to be key to understanding the mechanisms underlying BEF relationships (Srivastava and Vellend 2005, Suding et al. 2008, Díaz et al. 2013). For example, Larsen et al. (2005) demonstrated that the body size of bees was positively correlated with extinction risk caused by agricultural intensification on the one hand, and with the pollination efficiency on the other hand. Therefore, such a correlation between response and effect traits makes pollination a fragile ecosystem function, prone to decline via negative effects of agriculture on bees. Similarly, Solan et al. (2004) developed a set of models parameterized with the data on marine benthos and demonstrated how an effect trait (sediment bioturbation) may decline in different ways depending on the alternative species extinction scenarios. Mensens et al. (2015) recently found sensitivity for a herbicide to correlate positively with functionality in mudflat diatom assemblages, resulting in steeper BEF slopes than expected from random community assembly BEF experiments.

Here, by using a freshwater system, we further support the importance of measuring covariation in species response traits (i.e. species extinction risk) with species effect traits. In our case, species sensitivity to insecticide correlated negatively (or not correlated in the case of zooplankton) with the effects these species had on ecosystem functioning. Although rare species are on average more prone to go extinct due to their higher susceptibility to environmental and demographic stochasticity (Srivastava and Vellend 2005), in this particular case more abundant species appeared to be more sensitive to the effect of a specific stressor used here (chlorpyrifos). We therefore demonstrated that the common assumption of rarity being a proxy to species' vulnerability may not hold when rare species are less sensitive.

Our attempt to test two mechanisms that are tentatively responsible for the higher functionality of the rare species indicated that the mechanisms behind this pattern most likely differ for the two communities. For zooplankton, the differential functionality between rare and common species seems to be (at least partly) due to the difference in the feeding guilds to which those species belong. The rare species are predominantly represented by omnivores, a feeding guild that seems to be more influential to the measured ecosystem functions than the other feeding guilds. An effect of individual body masses on per capita functioning was not supported by our data, despite recent demonstrations in other systems (Ruesink and Srivastava 2001, Larsen et al. 2005, 2008, Reiss et al. 2011, Norkko et al. 2013, Séguin et al. 2014). A larger sample size would have allowed more thoroughly testing for such an effect, which was at least suggested for macroinvertebrates.

Our study sheds the light on the BEF relationship in communities shaped by non-random species loss. However, we cannot pinpoint the mechanistic relationship between consumer diversity and the ecosystem functions we considered. The plausible connection between consumer diversity and ecosystem functions considered could include one or both of the following mechanisms. First, macroinvertebrates

may affect oxygen balance via litter breakdown by detritivores. Detritivore abundances plotted across time provide support for this mechanism: we observed abrupt declines of detritivore abundances from week 1 on in treatment ditches, followed by very slow recoveries (Supplementary material Appendix 1 Fig. A10). Second, reduced zooplankton grazing pressure on algae may potentially lead to algal blooms, which in turn will also affect oxygen balance. In support of this mechanism, the herbivore abundances were largely suppressed in the first 10 weeks after insecticide application in the high concentration treatments (6 and 44 µg of chlorpyrifos, Supplementary material Appendix 1 Fig. A10). Moreover, an original study of this experiment reported an increase in epiphyton algae in the high concentration treatments, leading to algal blooms (Kersting and Van den Brink 1997).

The data we used was not produced by a BEF design, which poses certain constraints on how the results can be interpreted. On the one hand, the experimental design considered here cannot separate the direct effects of insecticide addition on ecosystem functioning, e.g. via effects on organismal physiology, and the indirect effects that are mediated by biodiversity (Relyea and Hoverman 2006). On the other hand, the open-air ditches containing complex food-webs with multiple trophic levels allowed us to study how vertical interactions and biodiversity changes combine in shaping EF against a backdrop of seasonal changes (Kersting and Van den Brink 1997). Kersting and Van den Brink (1997) showed that the respiration values were lower between May and August in the ditches with high insecticide concentration, indicating interacting effects of seasonality and insecticide on this ecosystem function that deserve closer inspection. This demonstrates that re-analyses of previously collected data, as was performed here, can be used to formulate hypotheses that can further be tested with purposefully designed and carefully planned experiments.

This ecotoxicological experiment gave us an excellent opportunity to study BEF relationships in more environmentally realistic settings, whereby non-random species loss occurs in a multitrophic species-rich system. However, such proximity to natural conditions comes at a cost of increased complexity and an increased number of links between species not only within the same trophic level but also with the species at other trophic levels. Presumably, the rich food web structure complicated our understanding of the mechanisms involved in the effects of biodiversity changes at one trophic level on ecosystem functions provided by other trophic level(s) (GPP and respiration). Nevertheless, our study underlines the need to consider not only classical measures of biodiversity (such as species richness, Pielou evenness and Shannon index) but also use functional-based measures of biodiversity, as this grants more mechanistic understanding of the processes governing BEF relationships. Such a combined use of different biodiversity measures facilitates understanding of the implications of on-going anthropogenically driven biodiversity loss to the ecosystem functioning. We encourage conducting similar analyses, by either re-using already existing data or carefully designing and conducting planned experiments, in other model systems to facilitate an enhanced understanding of BEF relationships across the trophic levels in environmentally realistic settings.

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Supplementary material (available online as Appendix oik.02220 at < www.oikosjournal.org/readers/appendix >). Appendix 1.