Predicting the effects of multiple global change drivers on microbial communities remains challenging

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Abstract

Microbial communities in many ecosystems are facing a broad range of global change drivers, such as nutrient enrichment, chemical pollution, and temperature change. These drivers can cause changes in the abundance of taxa, the composition of communities, and the properties of ecosystems. While the influence of single drivers is already described in numerous studies, the effect and predictability of multiple drivers changing simultaneously is still poorly understood. In this study, we used 240 highly replicable oxic/anoxic aquatic lab micro-ecosystems and four drivers (fertilizer, glyphosate, metal pollution, antibiotics) in all possible combinations at three different temperatures (20 °C, 24 °C, and 28 °C) to shed light into consequences of multiple drivers on different levels of organization, ranging from species abundance to community and ecosystem parameters. We found (i) that at all levels of ecological organisation, combinations of drivers can change the biological consequence and direction of effect compared to single drivers (ii), that effects of combinations are further modified by temperature, (iii) that a larger number of drivers occurring simultaneously is often quite closely related to their effect size, and (iv) that there is little evidence that any of these effects are associated with the level of ecological organisation of the state variable. These findings suggest that, at least in this experimental ecosystem approximating a stratified aquatic ecosystem, there may be relatively little scope for predicting the effects of

combinations of drivers from the effects of individual drivers, or by accounting for the level of ecological organisation in question, though there may be some scope for prediction based on the number of drivers that are occurring simultaneous. A priority, though also a considerable challenge, is to extend such research to consider continuous variation in the magnitude of multiple drivers acting together.

Introduction

Microbial communities are key components of many ecosystems, and yet must face many anthropogenic global change drivers (Christensen et al. 2006; Jackson et al. 2016). Confrontation with fertilizer (Suleiman et al. 2017) and metal pollution (Xu et al. 2018a), pesticides like glyphosate(Solomon and Thompson 2003; Relyea 2009), antibiotics (Xu et al. 2018b) and temperature increase (Wu et al. 2011) force populations and whole ecosystems to develop in different ways compared to less or unaffected ones (Tylianakis et al. 2008). Studies show that even slight changes in environmental conditions can lead to large taxonomic and functional microbial change, and even to regime shifts, with potential long-term consequences for biogeochemical cycles and ecological function of the affected habitat (Gruber 2011; Suleiman et al. 2021b).

While many studies have focused on one global change driver, recent studies (Christensen et al. 2006; Rillig et al. 2019; Suleiman et al. 2021a) indicated that the combined effect of multiple drivers applied simultaneously is often not equal to the sum of the effects of each individual driver. Rather, they demonstrate that interactive effects can occur. These can be synergistic (combined effect greater than the sum of the effect of individual drivers) or antagonistic (combined effect less than the sum of the effect of individual drivers).

Furthermore, not just the type of driver, but also the number of factors combined plays a crucial role (Rillig et al. 2019). Nevertheless, the number of studies testing three or more drivers remains very low (Rillig et al. 2019), which highlights the need for further investigations of combined effects. Combining this with the importance of understanding drivers of change in microbial communities is a critical aspect of the emerging research field of climate/global change microbiology (Hutchins et al. 2019).

The limited number of studies with three or more drivers may be in part due to the logistical demands of the required experiments and the complexity of interpreting their results. For example, to assess all interactions a fully-factorial experimental design is needed which quickly leads to large experiments. Regarding interpretation, it can be difficult to understand the meaning of interactions among more than three drivers, such that even if one would conduct a fully factorial experiment, traditional methods for interpreting interactions (such as interaction plots) may be insufficient. Some studies have instead focused on the effect of variation in the number of drivers, and have applied only a subsample of all possible combinations (e.g. Brennan and Collins 2015; Rillig et al. 2019).

Another gap in understanding is how drivers and combinations of drivers act across levels of ecological organization from individuals to ecosystems (Galic et al. 2018). For example, in a case study of a model of amphipod feeding behavior Galic et al (2018) showed that responses to multiple drivers at the individual level were not consistent with those at higher levels of organization. In their case-study, the nature of this inconsistency would lead to underestimation of effects at population and ecosystem levels if effects at the individual level were assumed to hold across levels of organisation.

These gaps in knowledge are problematic. One reason is that interactions can be source of ecological surprises, i.e., if we assume additivity we can be surprised if there are

interactions (Christensen et al 2006). Also, unless there are some generalities about interactions among drivers, we will never be able to predict the effect of a new (previously unstudied) combination of drivers, so interactions will also then be a surprise (Christensen et al 2006). Hence, we and others (e.g., Simmons et al 2021, Rillig et al 2018) are interested in discovering if there are any general patterns that will allow us to predict the effects of combinations of environmental changes. This report is about our search for signals of general patterns in the effects of combinations of environmental drivers. For example, whether the relationship between a biological variable and the number of drivers is dependent on the level of ecological organization of the biological variable and whether the strength and nature of the interaction effects varies with level of ecological organization.

Microbial communities in aquatic ecosystems are complex (Chistensen et al. 2006; Davis et al. 2010; Faust and Raes 2012), consisting of feedback among numerous abiotic and biotic interactions (Singh et al. 2009), among and within functional microbial groups (Bush et al. 2017; Richardson et al. 2018). Recent work indicates that these ecosystems are sensitive to environmental change (Christensen et al. 2006; Shade et al. 2011, 2012; Suleiman et al. 2021b, a), identifying them as appropriate systems for the study of the influences of global changes.

In this work, we applied four different global change drivers (fertilizer, glyphosate, metal pollution, and antibiotics) together with increasing temperatures (20 °C, 24 °C, and 28 °C) in all combinations possible, on a recently developed and highly replicable stratified aquatic microbial lab system. Our experiment includes the analysis of several abiotic (oxygen, total nitrogen, total organic carbon, pH) and biotic variables (diversity richness, microbial community composition, genera abundances), since recent studies have shown that various levels of ecosystems can be affected (Weithoff et al. 2000; Shade et al. 2011, 2012; Suleiman

et al. 2021b) and in order to examine if effects show predictable variation across levels of organisation. We hypothesize that (i) combinations of drivers will have non-additive effects on system properties, (ii) the non-additive effects will be different depending on the temperature, and (iii) increasing the number of drivers applied will cause a systematic change in system properties.

Material and methods

Experimental ecosystems

Incubation of stratified microbial communities was performed in micro-ecosystems consisting of standard glass test tubes (4 mL volume). These test tubes were closed with plastic lids, with a small whole (0.5 mm) that allowed gas exchange between the headspace and the atmosphere. Each micro-ecosystem consisted of sediment and water samples taken in May from a small pond in Zurich, Switzerland (47°23'51.2"N 8°32'33.3"E, temperature of 19 °C, pH of 7) at a depth of 25 cm. Sediment was homogenized (30 min mixing) and subsequently supplemented with sterile 0.25 % crystalline cellulose, 0.25 % methyl-cellulose, 0.5 % $CaSO_4$, 0.1% $CaCO_3$, and 0.005 % $NH_4H_2PO_4$. Glass tubes were filled with a 3 mm layer of supplemented sediment and covered with 3.4 mL pond water (with 0.005 % $NH_4H_2PO_4$), resulting in 500 µL headspace. Microecosystems were incubated at room temperature for 2 hours to let sediment settle, before the different treatments were applied. The experiment involved incubation for 23 days at either 20 °C, 24 °C or 28 °C.

Experimental design

The four drivers, namely $NH_4H_2PO_4$-fertilizer (F), Glyphosate (G), metal pollution (M) and antibiotics (A), each had two levels. Furthermore, control micro-ecosystems (C) were used without application of any drivers and the experiment was carried out at 20 °C, 24 °C and 28 °C under a dark-light cycle of 8:16 h. The temperature treatment and four driver treatments were applied in a fully factorial design. Five replicates (Burgess et al. 2022) per treatment combination resulted in 240 micro-ecosystems, according to the following design:

[“Control","F","G","M","A","FG","FM","FA","GM","GA","MA","FGM","FGA","GMA","FMA", "FGMA"] * 5 replicates * 3 Temperatures

= 240 micro-ecosystems.

For the driver treatments, the following chemical compounds were added to the micro-ecosystems two hours after the assembly of the micro-ecosystems:

- $NH_4H_2PO_4$ in final concentration of 0.01 % as fertilizer (F), as used in a previous study (Suleiman et al., 2021)
- Glyphosate in final concentration of 0.0001 % (G) as used in a recent study (da Costa et al. 2021)
- For metal pollution: DSMZ Trace element solution SL-10 final concentration 10x higher than recommended (find medium composition here https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium320.pdf) (M)
- Mixture of penicillin and ampicillin (0,05 mg/100 mL each) in final concentration of 0.000005 % for antibiotic treatment (A), since such amounts were detected in natural aquatic habitats (Xu et al. 2018b)

151 Drivers were applied to the micro-ecosystems only on day 0; there were no further press or
152 pulse perturbations.

153 ## Microbial community composition

154

155 After incubation for 23 days, the whole micro-ecosystem was centrifuged for 5 min at 10'000
156 rpm (sediment and water column together). DNA was extracted using ZymoBIOMICS DNA
157 Miniprep Kit (ZymoResearch), following the manufacturer's instructions. Full-length 16S rRNA
158 gene amplification was performed using the primer pair 27F forward primer (5'-
159 AGRGTTYGATYMTGGCTCAG-3') and 1592R reverse primer (5'-RGYTACCTTGTTACGACTT-3'),
160 as reported previously (Suleiman et al., 2021).

161 PCR products were visualized on an 1 % (w/v) agarose gel and were pooled for
162 sequencing in equal concentrations. PCR product pools were purified using AMPure®PB beads
163 (PacBio). Sequencing was performed at the Functional Genomic Center Zürich, Switzerland,
164 and performed using SMRT Technology (PacBio) as reported previously (Suleiman et al.
165 2021a) (on three SMRT chips). Sequencing data quality were checked using the PacBio SMRT
166 Link software.

167

168 Sequencing data were filtered based on primer sequences, length (1300-1600 bp),
169 quality, error rates and chimeras using the R-package *dada2* (Callahan et al. 2016). The final
170 sequence table was aligned using SILVA ribosomal RNA database (Quast et al. 2012), using
171 version 138 (non-redundant dataset 99). A phyloseq object was created using the *phyloseq* R-
172 package (McMurdie and Holmes 2013), consisting of amplicon sequence variant (ASV) table,
173 taxonomy table and sample data. For further analysis, the R-packages *phyloseq* (McMurdie
174 and Holmes 2013) and *vegan* (Oksanen et al. 2019) were used.

To compare the effects of the multiple drivers, we analysed various response variables, namely community composition based on non-metric multidimensional scaling NMDS, Shannon richness, oxygen, total nitrogen TN, total organic carbon TOC, and abundances of specific genera and functional groups.

Microbial community composition was quantified using NMDS based on Bray-Curtis scores with the *metaMDS* function of the vegan R-package (Oksanen et al. 2019), with three dimensions used (k=3, try=100). Shannon richness was calculated using the *phyloseq* package (McMurdie and Holmes 2013). Functional groups were constructed by filtering for cyanobacterial and sulfur-related species, which resulted in a reduced dataset consisting of members of the orders *Cyanobacteriales*, *Chromatiales*, *Synechococcales*, *Chlorobiales*, *Leptolyngbyales*, *Desulfobulbales*, *Desulfovibrionales*, *Limnotrichales* and *Campylobacterales*. The community of these members were used as a subset for NMDS analysis in this study. Detailed scripts and a phyloseq with all sequences are available in the github repository https://github.com/Marcel2907/multiple-stressor and on zenodo (DOI 10.5281/zenodo.5948600).

Measurement of abiotic factors

Oxygen concentration was measured 1.5 cm below the water/air interphase using an oxygen dipping probe (PreSens, Germany) at the last day of incubation. pH was measured in the supernatant of the centrifuged micro-ecosystemTotal nitrogen (TN) and total organic carbon (TOC) were measured in the supernatant using a TOC analyser.

Data analysis

For every response variable (see above) we carried out two linear models, one to investigate the relation between the response variable and the various drivers and their combinations and one to investigate how the response depended on the number of drivers applied. To ensure that modelling assumptions were met, we carried out the following response variable transformations, which are listed in detail in the deposited R script. For total nitrogen we took the square root, for total carbon we took the squared inverse, for *Tychonema* we used a logit transformation and for *Sulfuricurvum* we took the cubic root. For the variables not listed here no data transformations were necessary. Further, for total nitrogen concentration we excluded 13 data points due to measurement problems. For the analyses of the effect of the number of applied drivers, all response variables were further standardized to render them more comparable. Moreover, in the cases of pH and total nitrogen we included the variable fertilizer as a covariate, as it strongly influenced these response variables. In all models we included all possible covariate interactions.

Results

We first show the results regarding the microbial community and genera abundances of the 240 micro-ecosystems (Fig. 1). We then show the impact of driver combination and of number of applied drivers on the response variables microbial richness (Shannon), community structure (NMDS1), community structure of a subset of the taxa, oxygen and pH (Fig. 2). Additional abiotic and biotic variables were investigated (NMDS2, total nitrogen, total carbon) and can be found in detail in Supplementary Fig. 3. The summary results of all variables (species, community, ecosystem) analysed based on the impact of driver combination can be

223 found in Fig. 3, and the influence of number of drivers in Fig. 4. Finally, Fig. 5 illustrates one
224 of the observed three-way interactions among drivers.

225 In total, 20’319 unique ASVs were identified after running the dada2 pipeline. After
226 filtering to retain only ASVs with relative abundance of at least 0.1 % in at least one sample,
227 the total number of ASVs decreased to 5’830 taxa. The control micro-ecosystems consisted
228 of mainly cyanobacteria (e.g., *Phormidaceae*) and phototrophic sulfur bacteria (specially
229 *Chromatiaceae*) (Fig. 1 a&b, Supplementary Fig. 1). Depending on the driver combination and
230 temperature, the microbial communities and genera abundances showed strong differences
231 compared to the controls. Micro-ecosystems with glyphosate and metal pollution, each as a
232 single driver or in combination, were still dominated by members of *Phormidiaceae* and
233 *Chromatiaceae*, but in all remaining treatments the abundance of members of these families
234 was lower (Supplementary Fig. 1).

235

236 Also on genus level, we observed shifts in microbial composition due to driver
237 combination and temperature (Fig. 1 b). The cyanobacterial genus *Tychonema* was highly
238 abundant in the metal pollution only treatment, and with increasing temperature also in
239 glyphosate+metal pollution treatments (20 % at 28 °C, 7 % at 20 °C). *Chromatium*, in contrast,
240 appeared in several treatments and reached highest relative abundance at 20 °C when
241 confronted with G:M (20 %), but vanished with increasing temperature in all treatments (<
242 5%). Statistical analysis of the dependence of relative abundance on driver combinations and
243 temperature were performed on genus level for *Tychonema* and *Sulfuricurvum*, as
244 representative species for high-abundance and low-abundance taxa (Fig. 3). Statistical
245 analysis confirmed significant changes of relative species abundance caused by individual
246 drivers, due to combination of drivers, and due to temperature.

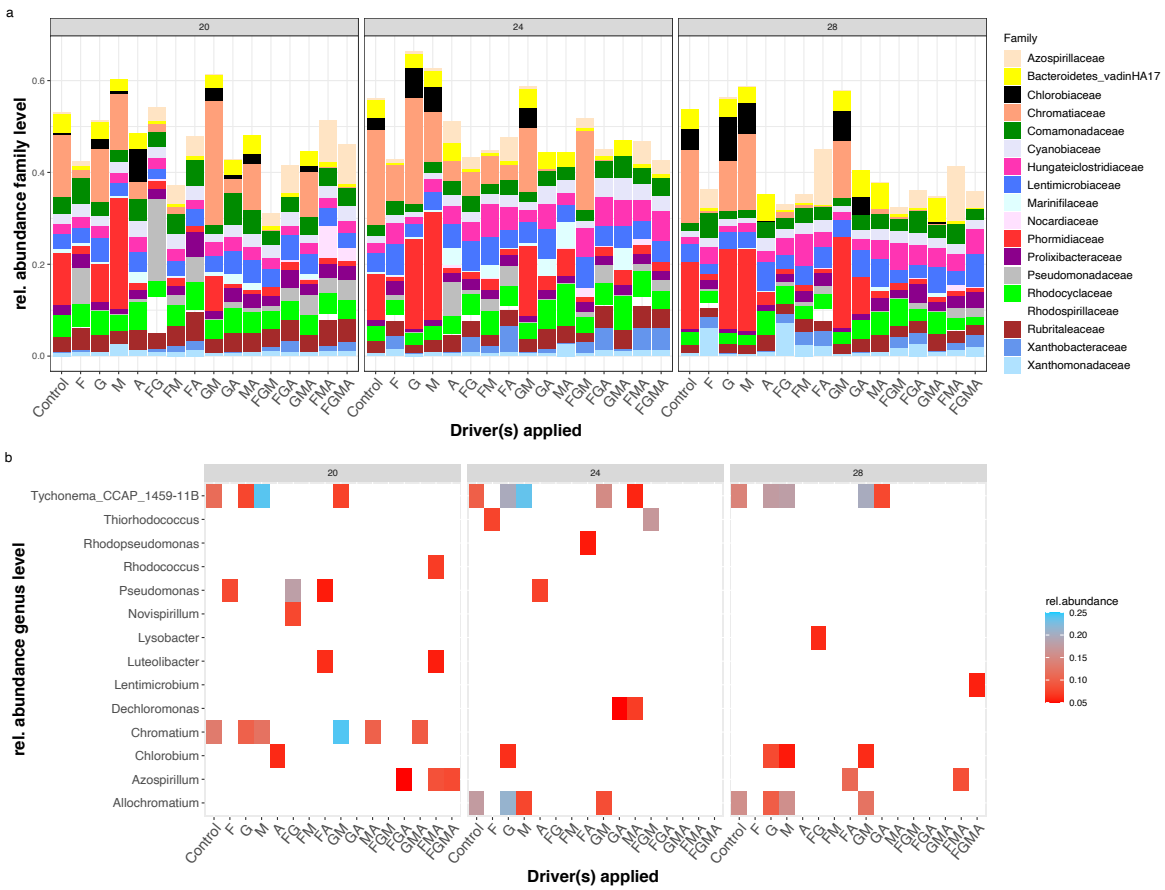

Fig. 1 Effects of drivers and driver combinations on microbial community composition and genera abundance. (a) Microbial community composition (mean per 5 replicates) at family level. Taxa with rel. abundance > 5 % shown. **(b)** Relative abundance of specific genera with rel. abundance > 5 % for each driver combination at each of the three temperatures. White color represents a relative abundance < 5 %.

ASV diversity of the micro-ecosystems changed depending on the combination of drivers (Fig. 2a+b), resulting in Shannon index values between 3 and 7, with the majority of samples between 5 and 6, indicating a high diversity in most of the micro-ecosystems. Several significant driver treatments and combinations effects occurred, namely temperature increase (F-statistic = 6.8, p-value = 0.001), fertilizer (F-statistic = 16.0, p-value < 0.001), antibiotics (F-statistic = 26.3, p-value < 0.001), as well as combination of fertilizer+antibiotics (F-statistic = 17.9, p-value < 0.001) and fertilizer+antibiotics+glyphosate (F-statistic = 4.8, p-value = 0.030) (Fig. 2a). All significant driver combinations had a positive effect on diversity, except the temperature increase to 28 °C and the fertilizer+antibiotic combination treatment (Fig. 3). Interestingly, the negative effect of fertilizer+antibiotic was reversed to a positive

264 effect again by adding glyphosate, while the treatment of glyphosate alone had no impact
265 (Fig. 2a, Fig. 3). When analysing the effect of number of drivers and temperature increase on
266 diversity, just temperature was identified to have a significant effect, but the number of
267 drivers had no impact (Fig. 2b, Fig. 4).

268 Fertilizer addition was a key driver of microbial community composition (quantified
269 via NMDS). (Fig. 2b+c, Supplementary Figure 2). Numerous driver combinations showed
270 significant effects on NMDS score 1 and NMDS score 2 (Fig. 2c for NMDS1, Supplementary Fig.
271 3 for NMDS2, Fig. 3 for both), ranging from single to four-way effects. Different impact
272 directions (positive or negative) can be identified depending on the driver combinations (Fig.
273 2c, Fig. 3). The three-way interaction of fertilizer+antibiotics+temperature on NMDS1 score is
274 a good example how combination of drivers can change the impact direction (Fig. 5): When
275 no fertilizer was present, the NMDS1 score was negative, while in presence of fertilizer, the
276 NMDS1 score was positive. Additionally the combination of antibiotics and temperature
277 changed the magnitude of the NMDS1 score. NMDS1 score was affected by number of drivers
278 (F-statistic = 82.8, p-value < 0.001) (Fig. 2d, Fig. 4), and NMDS2 score was affected by both
279 number of drivers (F-statistic = 12.7, p-value < 0.001) and temperature (F-statistic = 27.8, p-
280 value < 0.001, Supplementary Figure 2b, Fig. 4). For both NMDS scores there was no
281 interaction between number of drivers and temperature.

282 Analysis of the community of the subset of cyanobacteria, phototrophic sulfur bacteria
283 and sulfate-reducing bacteria confirmed the trends observed for NMDS of all ASVs (Fig. 2e for
284 NMDS1, Supplementary Fig. 3 for NMDS2, Fig. 3 for both), but showed slight differences on
285 three-factorial and four-factorial driver combinations.

286 Oxygen concentration was highly dependent on the driver combinations used, and
287 various strong effects were identified (Fig. 2g), both positive and negative in direction. All

288 drivers combined had a negative effect on oxygen concentration (F-statistic = 4.3, p-value =
289 0.040, non-standardised estimate -6.1 %). The number of drivers applied had a strong effect
290 on the oxygen concentration (F-statistic = 18.8, p-value < 0.001), as well as temperature (F-
291 statistic = 17.9, p-value < 0.001), and the interaction between temperature and number of
292 drivers (F-statistic = 3.3, p-value = 0.040, Fig. 2h, Fig. 4).

293 The pH of the micro-ecosystems was influenced by numerous treatment
294 combinations, but the strongest influences was identified as fertilizer addition, which
295 decreased the pH from 8.5 to 6.5 (F-statistic = 3.2×10^3, p-value < 0.001, Fig. 2i). Driver
296 combinations that include fertilizer addition had lower magnitude though were of the same
297 sign (Fig. 3). Number of drivers had a negative effect on pH (F-statistic = 4.4, p-value = 0.037),
298 with the clearest effect at 20 °C and no fertilizer used, in which case pH decreased the more
299 drivers were applied (Fig. 2j, Fig. 4).

300 Effects on total nitrogen were driven by addition of fertilizer (F-statistic = 7.4×10^4, p-
301 value < 0.001), but also numerous other combinations had a significant effect (Fig. 3,
302 Supplementary Fig. 3). Furthermore, number of drivers strongly affected the total nitrogen
303 concentration (F-statistic = 67.8, p-value < 0.001). Total carbon concentration was
304 significantly affected by fertilizer (F-statistic = 31.9, p-value = 0.005), metal pollution (F-
305 statistic = 9.9, p-value = 0.002) and temperature (F-statistic = 23.4, p-value < 0.001), as well
306 as by antibiotics (F-statistic = 9.2, p-value = 0.003) and numerous combinations (Fig. 3,
307 Supplementary Fig. 3). Furthermore, for the concentration of total carbon there was a
308 significant interaction between number of drivers and temperature (F-statistic = 3.5, p-value
309 = 0.032), with TC increasing with number of applied drivers at 28°C (Fig. 4, note that the
310 response variable is the square inverse of total carbon).

311

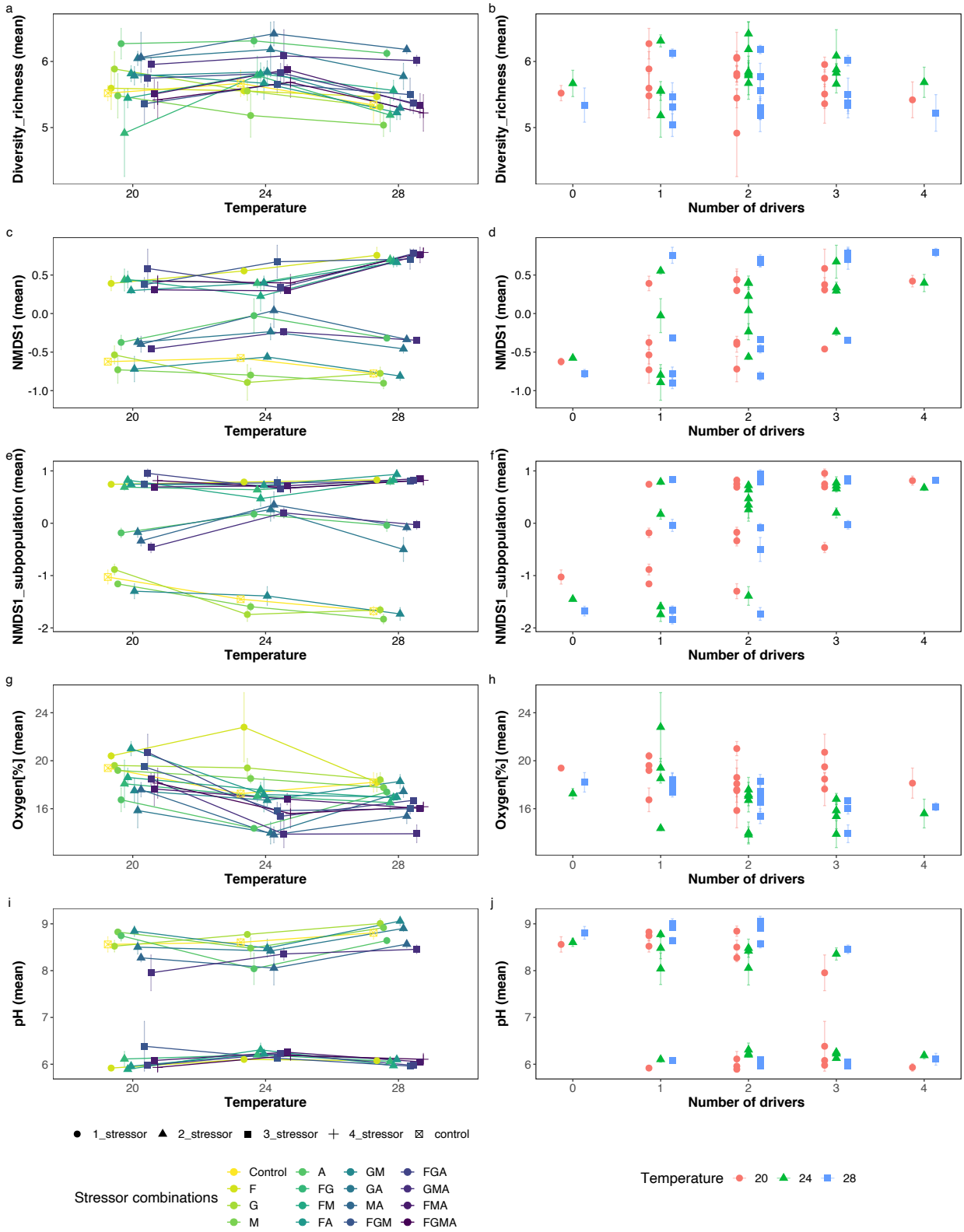

Fig. 2 Effects of driver combination, number of drivers, and temperature on microbial community and ecosystem variables. Each row of the figure contains two graphs, both of which concern the same response variable, e.g., first row (a) and (b) concern Shannon diversity index. The left column of figures shows the effects of temperature and driver combination. The right column shows the effects of temperature and number of drivers. Symbols are the mean of the five replicates, and errors bars show the standard error of the mean.

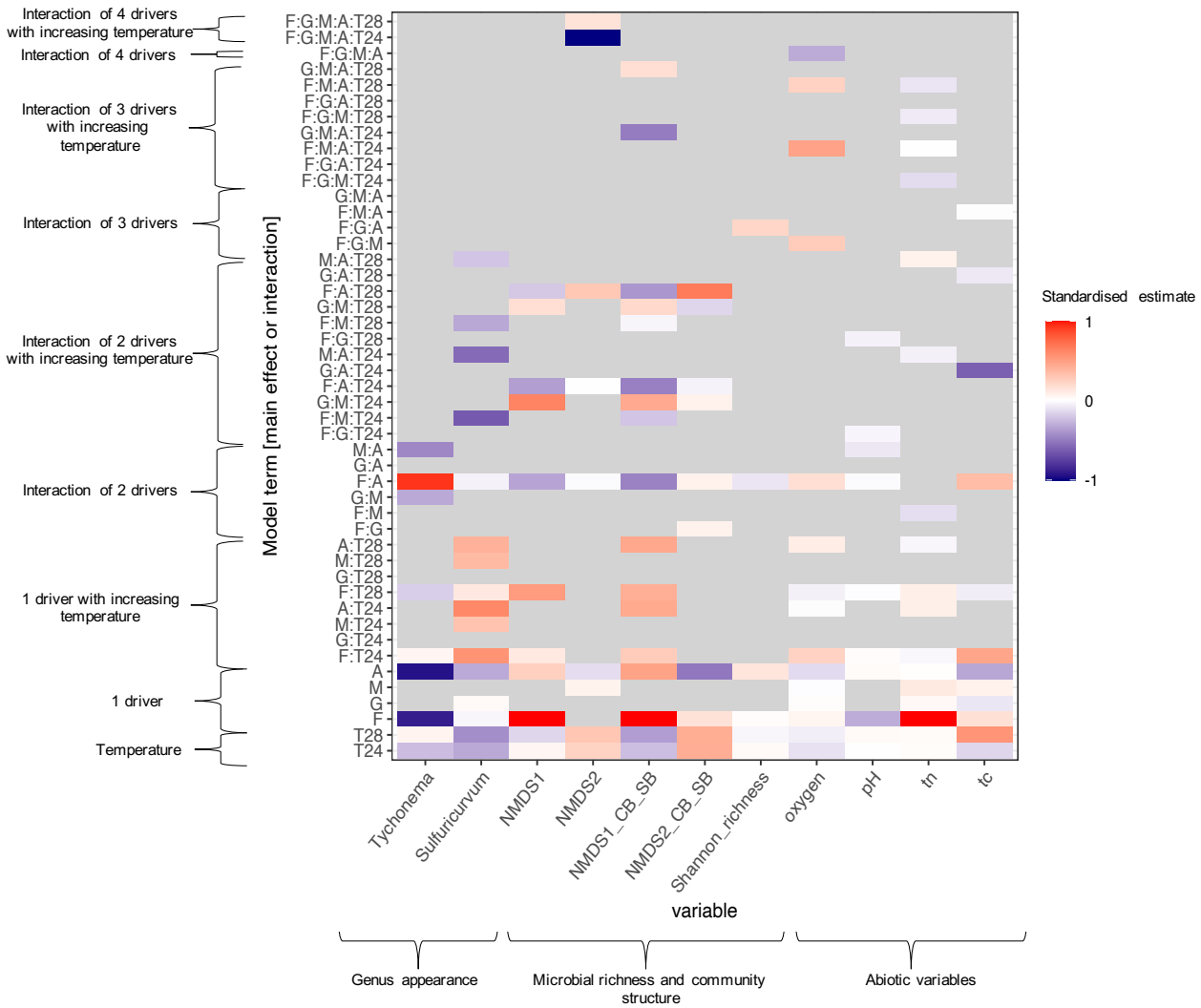

Fig. 3 Summary of statistical analyses of all variables analysed. Eleven variables are shown in the columns of the heat map, (grouped by genus appearance, microbial richness and community structure and abiotic factors). Rows show the estimated coefficients of the single, one-way, two-way, three-way, and four-way interaction terms. Grey cells indicate a response variable and coefficient pairs for which the coefficients were not significantly different from zero (t-test p-value > 0.05), otherwise the diverging colour palette illustrates the direction of the influence by the driver or interaction of drivers (estimates of each variable were standardized by dividing by largest absolute value of the estimates in each variable). See the main text for the applied response variable transformations. tn = total nitrogen, tc= total carbon.

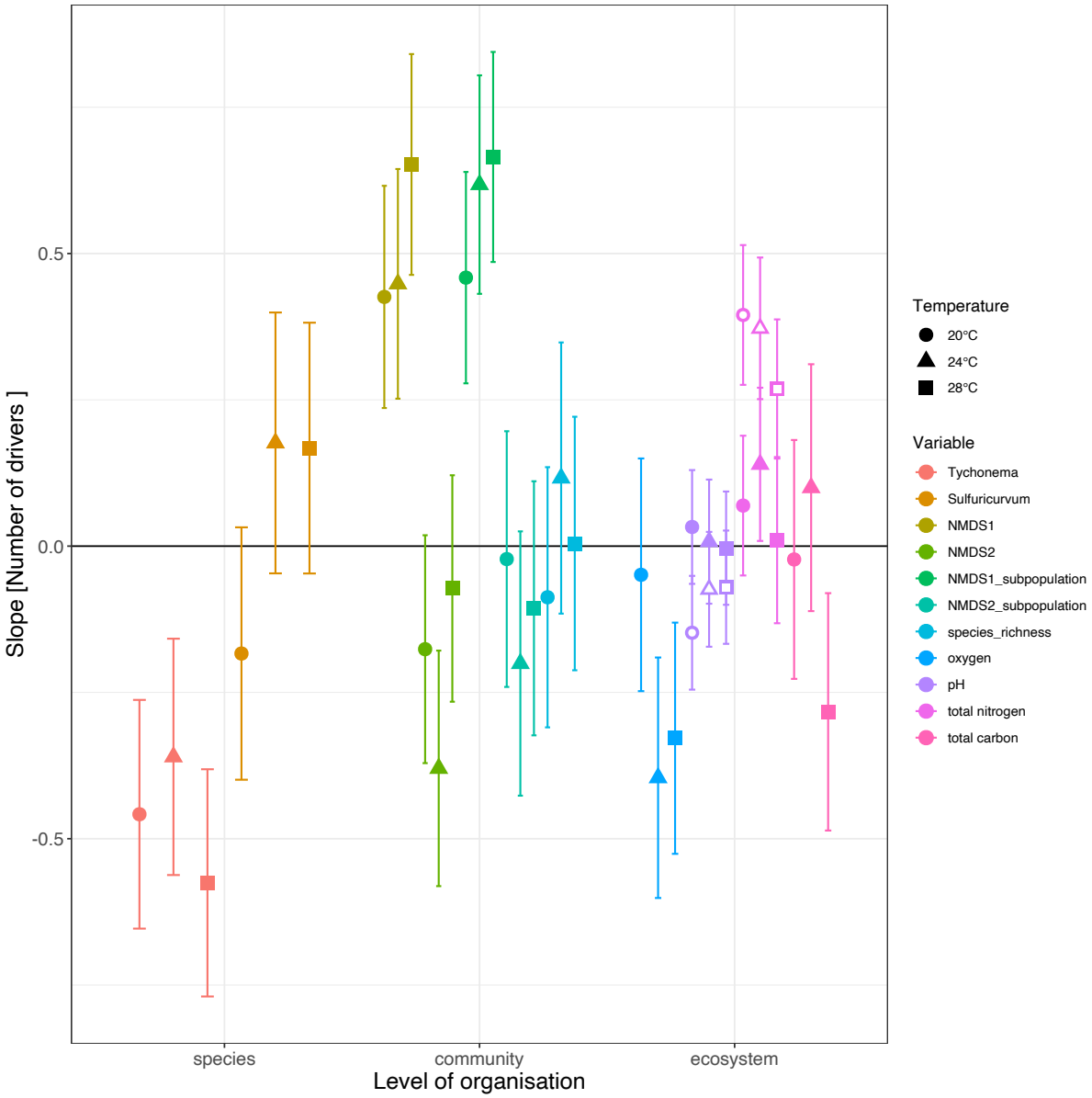

Fig. 4 Effect size of number of drivers for each response variable at each temperature. Means and 95 % confidence intervals are shown. For the variables of pH and total nitrogen, fertilizer was used as a covariate due to its strong influence. Filled total nitrogen and pH labels: fertilizer was used as a driver. Empty total nitrogen and pH labels: fertilizer was excluded as driver. See the main text for the applied response variable transformations.

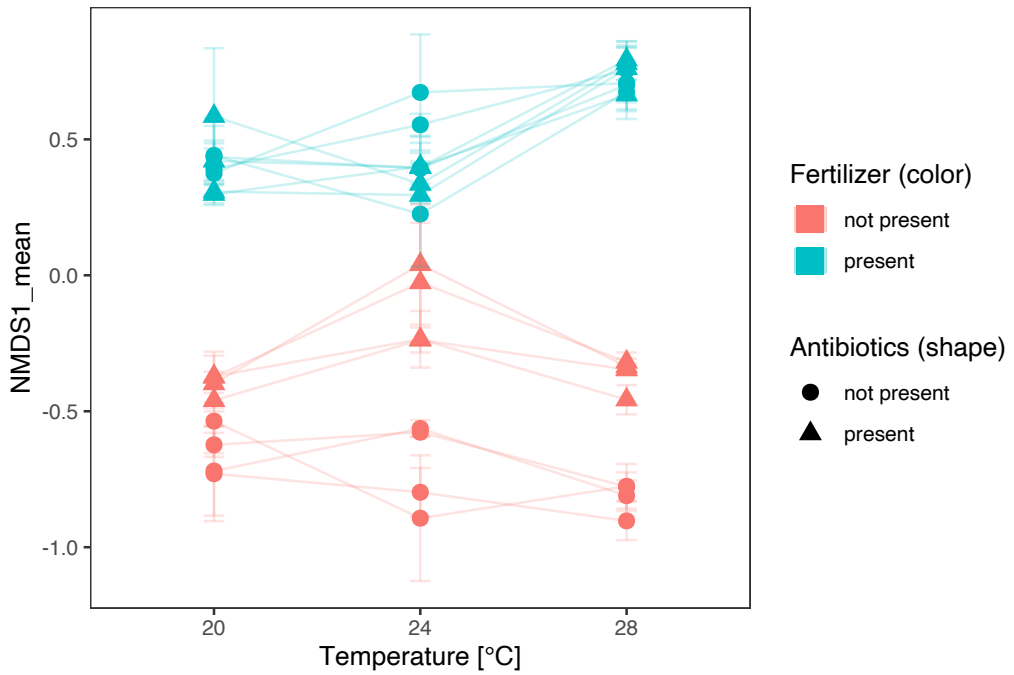

Fig. 5 Visualization of the significant driver interactions of temperature, fertilizer and antibiotics on NMDS1. NMDS1 is affected by the presence of fertilizer, the presence of antibiotics and temperature, but also by the interactions of fertilizer+antibiotics and fertilizer+antibiotics+temperature. Means (n=5) are shown. Error bars represent standard error.

Discussion

Our study revealed compelling evidence of effects of the combination and the total number of drivers on taxa abundances, community composition, and ecosystem properties, and such that multiple drivers acting in combination can have important consequences across levels of ecological organisation (Rillig et al. 2019). Specifically, the addition of a driver, and/or temperature increase, could change the biological consequence of already applied drivers, even when the added driver applied alone had no effect (Fig. 3). We observed three trends here: The addition of a further driver can increase (e.g. F:A:T28), decrease (e.g. F:T28) or reverse the effect of the drivers which were already applied (e.g. F:A on *Tychonema* abundance, Fig. 6). This demonstrates clearly the need of analysing multiple drivers in all combinations. Furthermore, our study also confirmed that the number of drivers occurring together can influence microbial communities and thereby provide some hope that number of drivers can be used as a useful predictor, though individual driver effects can greatly

353 contribute to the effect of number of drivers (Rillig et al. 2019). Furthermore, the effect of the
354 number of drivers can be negative (e.g., *Tychonema* abundance) or positive (e.g. total
355 nitrogen) and of different magnitudes, which highlights again the need of analysing various
356 ecological response variables of species, microbial communities, and the whole ecosystem.
357 While there may be some patterns across levels of organisation, e.g., the largest effects of
358 number of drivers were observed at species and community levels of organisation (greater
359 than 0.5 or less than -0.5 standardised effect size), the general absence of such patterns limits
360 the scope for explanation and prediction of effect of multiple drivers across levels of
361 organisation.

362 While fertilizer, metal pollution and antibiotics had detectable effects when applied
363 as single drivers, glyphosate did not. This finding is likely quite specific to the concentration
364 of treatments used and should be regarded with caution. Nevertheless, it is even more
365 interesting that when adding glyphosate to other drivers it did have an effect (see e.g.,
366 diversity richness, Fig. 3). Strong interactions were detected for antibiotics and fertilizer, and
367 interestingly, these drivers revealed in combination different biological consequences
368 compared to applied as a single driver, and temperature could also change the effects of the
369 fertilizer-antibiotic driver duo again (Fig. 6).

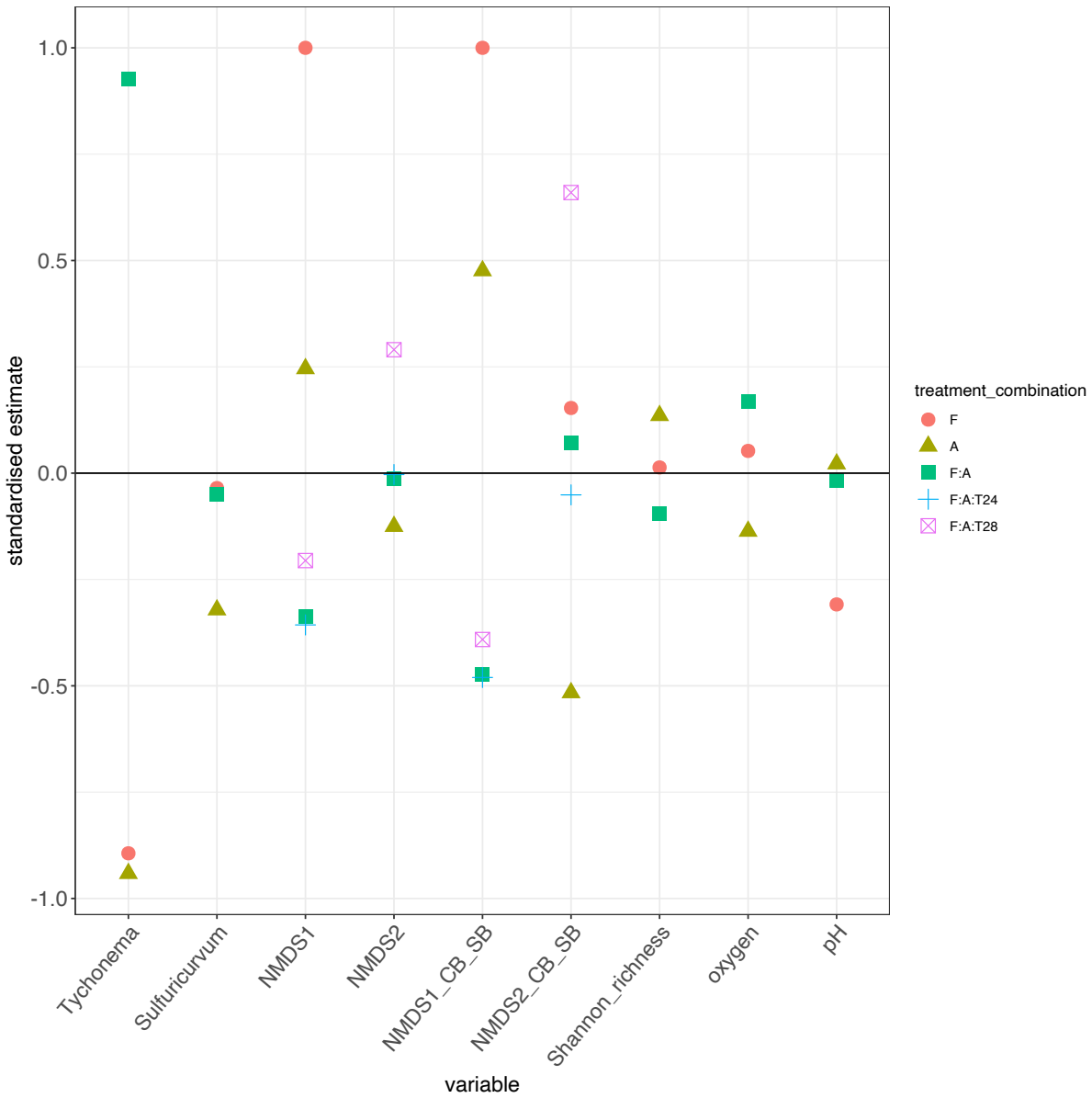

Fig. 6 Effects of fertilizer, antibiotics and their combination at 20, 24, and 28 °C ASV relative abundance, microbial composition, and ecosystem variables. Only significant treatments / treatment combinations are shown (F-test p value < 0.05). Total nitrogen and total carbon are not listed in this figure because they were not significantly affected by F, A, and F:A.

The effect of temperature is in our view important to note, since it predicts that observed biological consequences caused by global change can change with on-going global warming. An increase of temperature can lead to new interactions among driver combinations (F:A with increasing temperature led again to significant affected variables) or

380 can lead to significant effects that were not observed at 20 °C (G:M, G:M:24 and G:M:28 for
381 NMDS1).

382 Our study also contributes to narrowing the gap of understanding of how drivers and
383 their combination act across various levels of organisation. Our results revealed that
384 individual taxa can be affected differently compared to abiotic factors of the ecosystem or
385 the microbial community composition. Therefore, our study is in line with the findings of Galic
386 et al. (2018) who reported differences of the influence of multiple drivers across levels of
387 organisation. Interestingly, in our study, some treatment combinations, like fertilizer and
388 antibiotics, showed significant influence across most variables and the three levels of
389 organisation (except total nitrogen and total carbon), though even then the sign of the
390 interaction term was sometimes negative and sometimes positive.

391

392 Effects of drivers, such as those we manipulated, are of course concentration-
393 dependent. In this study, we used only two levels of driver concentration and added the driver
394 in a single pulse treatment. More information about specific concentration thresholds and
395 press disturbances are important to study and may clarify some observed trends, as well as
396 the timing of the driver applied. In addition, responses of functional (metabolic) aspects of
397 the aquatic microbial communities should be investigated in future studies, which could shed
398 light into activated and deactivated pathways and enzymes used in microbial communities to
399 react to changing environmental conditions.

400 In sum, our study confirmed the need to research the effects of multiple drivers on
401 microbial communities and indicate further that a broad range of levels of organisation
402 (species, community, ecosystem) should be analysed due unique sets of effect and
403 interactions across these and the different variables within them. Furthermore, our study

showed that combination of specific drivers can change the biological consequence and direction compared to single drivers at all levels of organisation and that the effects of driver combinations can be modified by temperature. Overall, the lack of patterns in effect sizes across levels of organisation, and with respect to the number of drivers, represents lack of evidence for these to be useful in the prediction of the effects of combinations of global change drivers on ecological communities.

References

Brennan G, Collins S (2015) Growth responses of a green alga to multiple environmental drivers. Nat Clim Chang 5:892–897. https://doi.org/10.1038/nclimate2682

Bush T, Diao M, Allen RJ, et al (2017) Oxic-Anoxic regime shifts mediated by feedbacks between biogeochemical processes and microbial community dynamics. Nat Commun 8:. https://doi.org/10.1038/s41467-017-00912-x

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Christensen MR, Graham MD, Vinebrooke RD, et al (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob Chang Biol 12:2316–2322. https://doi.org/https://doi.org/10.1111/j.1365-2486.2006.01257.x

da Costa N, Fugère V, Hébert M-P, et al (2021) Resistance, resilience, and functional redundancy of freshwater bacterioplankton communities facing a gradient of agricultural stressors in a mesocosm experiment. Mol Ecol 30:4771–4788. https://doi.org/https://doi.org/10.1111/mec.16100

Davis J, Sim L, Chambers J (2010) Multiple stressors and regime shifts in shallow aquatic ecosystems in antipodean landscapes. Freshw Biol 55:5–18. https://doi.org/https://doi.org/10.1111/j.1365-2427.2009.02376.x

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550. https://doi.org/10.1038/nrmicro2832

Galic N, Sullivan LL, Grimm V, Forbes VE (2018) When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. Ecol Lett 21:568–577. https://doi.org/https://doi.org/10.1111/ele.12923

Gruber N (2011) Warming up, turning sour, losing breath: ocean biogeochemistry under global change. Philos Trans R Soc A Math Phys Eng Sci 369:1980–1996. https://doi.org/10.1098/rsta.2011.0003

Hutchins DA, Jansson JK, Remais J V, et al (2019) Climate change microbiology — problems and perspectives. Nat Rev Microbiol 17:391–396. https://doi.org/10.1038/s41579-019-0178-5

Jackson MC, Loewen CJG, Vinebrooke RD, Chimimba CT (2016) Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob Chang Biol 22:180–189. https://doi.org/https://doi.org/10.1111/gcb.13028

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8:. https://doi.org/10.1371/journal.pone.0061217

Oksanen J, Blanchet FG, Friendly M, et al (2019) Package "vegan" Title Community Ecology Package. Community Ecol Packag 2:1–297

Quast C, Pruesse E, Yilmaz P, et al (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219

Relyea RA (2009) A cocktail of contaminants: How mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159:363–376. https://doi.org/10.1007/s00442-008-1213-9

Richardson J, Miller C, Maberly SC, et al (2018) Effects of multiple stressors on cyanobacteria abundance vary with lake type. Glob Chang Biol 24:5044–5055. https://doi.org/https://doi.org/10.1111/gcb.14396

Rillig MC, Ryo M, Lehmann A, et al (2019) The role of multiple global change factors in driving soil functions and microbial biodiversity. Science (80-) 366:886–890. https://doi.org/10.1126/science.aay2832

Shade A, Read JS, Welkie DG, et al (2011) Resistance, resilience and recovery: Aquatic bacterial dynamics after water column disturbance. Environ Microbiol 13:2752–2767. https://doi.org/10.1111/j.1462-2920.2011.02546.x

Shade A, Read JS, Youngblut ND, et al (2012) Lake microbial communities are resilient after a whole-ecosystem disturbancefile:///Users/marcel/Downloads/10.1007_PL00008896-

citation.ris. ISME J 6:2153–2167. https://doi.org/10.1038/ismej.2012.56

Singh BK, Dawson LA, Macdonald CA, Buckland SM (2009) Impact of biotic and abiotic interaction on soil microbial communities and functions: A field study. Appl Soil Ecol 41:239–248. https://doi.org/https://doi.org/10.1016/j.apsoil.2008.10.003

Solomon KR, Thompson DG (2003) Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J Toxicol Environ Heal - Part B Crit Rev 6:289–324. https://doi.org/10.1080/10937400306468

Suleiman M, Brandt FB, Brenzinger K, et al (2017) Potential N2O Emissions from the Tanks of Bromeliads Suggest an Additional Source of N2O in the Neotropics. Microb Ecol 73:751–754. https://doi.org/10.1007/s00248-016-0903-9

Suleiman M, Choffat Y, Daugaard U, Petchey OL (2021a) Large and interacting effects of temperature and nutrient addition on stratified microbial ecosystems in a small, replicated, and liquid-dominated Winogradsky column approach. Microbiologyopen 10:e1189. https://doi.org/https://doi.org/10.1002/mbo3.1189

Suleiman M, Pennekamp F, Choffat Y, Petchey OL (2021b) Contrasting resistance and resilience to light variation of the coupled oxic and anoxic components of an experimental microbial ecosystem. bioRxiv. https://doi.org/10.1101/2021.08.13.456201

Weithoff G, Lorke A, Walz N (2000) Effects of water-column mixing on bacteria, phytoplankton, and rotifers under different levels of herbivory in a shallow eutrophic lake. Oecologia 125:91–100. https://doi.org/10.1007/PL00008896

Wu Z, Dijkstra P, Koch GW, et al (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Glob Chang Biol 17:927–942. https://doi.org/https://doi.org/10.1111/j.1365-2486.2010.02302.x

Xu J, Chen Y, Zheng L, et al (2018a) Assessment of heavy metal pollution in the sediment of the main tributaries of Dongting Lake, China. Water (Switzerland) 10:1–16. https://doi.org/10.3390/w10081060

Xu Z, Li T, Bi J, Wang C (2018b) Spatiotemporal heterogeneity of antibiotic pollution and ecological risk assessment in Taihu Lake Basin, China. Sci Total Environ 643:12–20. https://doi.org/10.1016/j.scitotenv.2018.06.175

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536
537 **Author contribution**
538
539 OLP and MS planned the experimental set-up. MS performed all experiments in the lab. MS
540 performed the up- and downstream bioinformatics. MS, OLP and UD performed the data
541 analysis. UD supported the data transformation and statistical models. YC provided technical
542 support and performed the TOC analysis. XZ performed the DNA extractions and provided
543 technical support. OLP, MS and UD drafted the manuscript. All authors confirmed the final
544 version of the manuscript.

545 **Competing interests**

546 The authors declare no competing financial interests.

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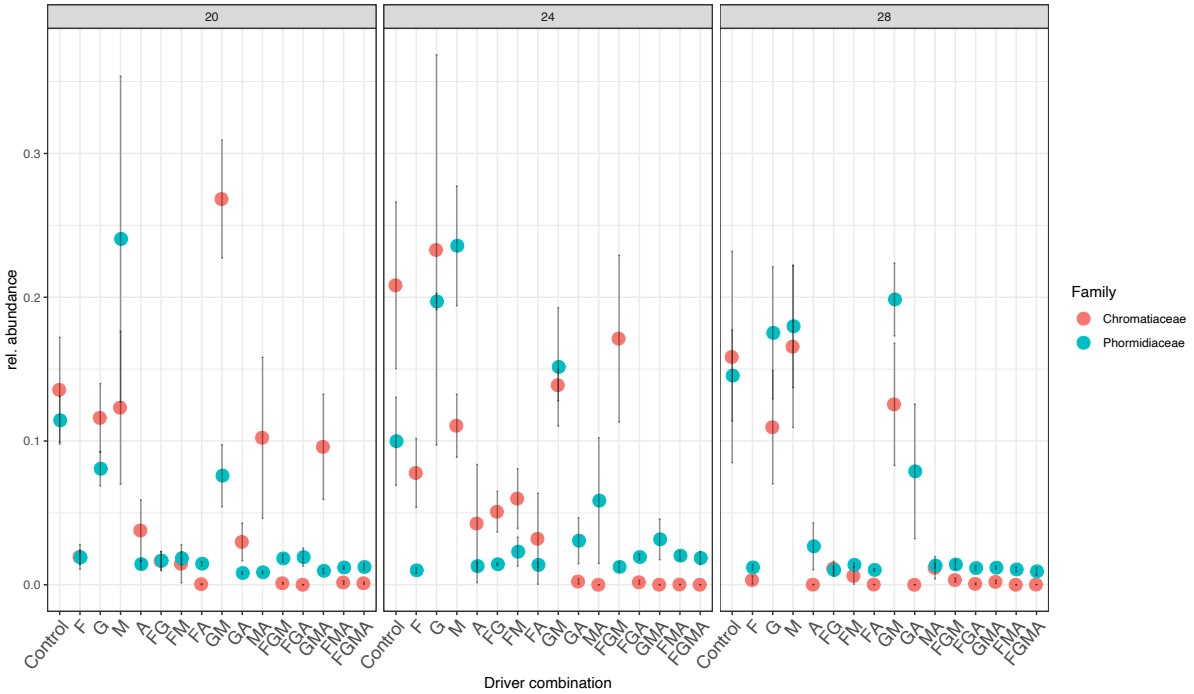
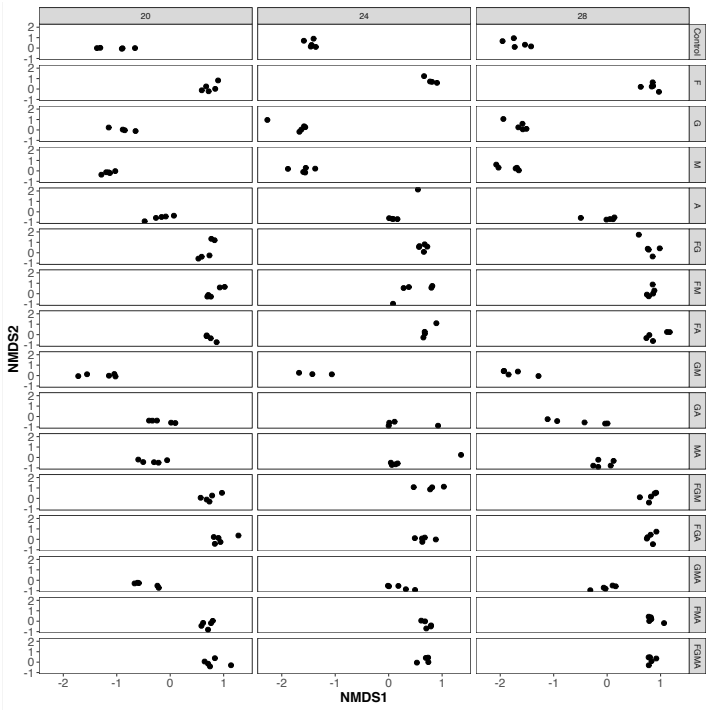
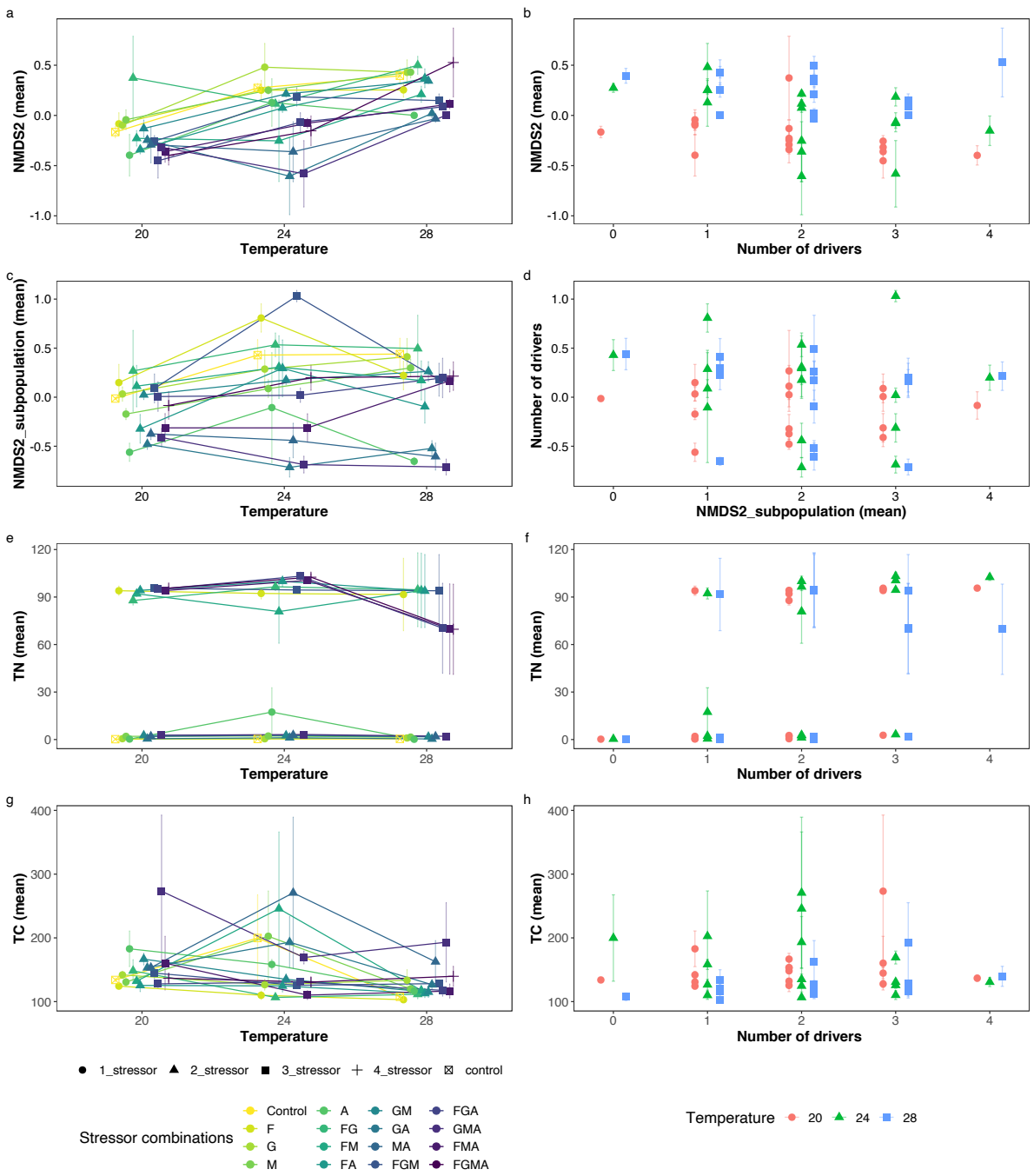
Supplementary Information



Supplementary Fig. 1 Relative abundance of *Chromatiaceae* and *Phormidiaceae* in the micro-ecosystem depending on driver combination and temperature. Mean is shown by the circle, standard errors are shown, n=5)



Supplementary Fig. 2 Beta-diversity based on NMDS analysis (Bray-Curtis) in relation to temperature and driver combinations. Stress is 0.16



Supplementary Fig. 3 Effects of driver combination and number of drivers on temperature applied for microbial community and ecosystem variables. NMDS2 scores varying with (a) temperature and driver combination and (b) temperature and number of drivers. NMDS2_subpopulation scores varying with (c) temperature and driver combination and (d) temperature and number of drivers. Total nitrogen concentration varying with (e) temperature and driver combination and (f) temperature and number of drivers. Total carbon concentration varying with (g) temperature and driver combination and (h) temperature and number of drivers. Standard errors are shown (n = 5 replicates).