Stratified microbial communities are highly sensitive towards multiple combined global change factors, revealing antagonistic and synergistic effects

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Abstract

Microbial communities in many ecosystems are facing a broad range of global change scenarios, resulting in microbial changes and possibly regime shifts with unknown ecological consequences. While the influence of single stressors is already described in numerous studies, the effects of multiple stressors working simultaneously are still poorly understood. In this study, we used 240 highly replicable oxic/anoxic aquatic lab micro-ecosystems to understand the influence of four stressors (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 stressors on different levels of organization, ranging from species abundance to community and ecosystem parameters. Our data reveal that (i) combination of specific stressors can change the biological consequence and direction compared to single stressors in all levels of organisation (ii), effects of stressor combinations are modified by temperature, and (iii) that the number of stressors applied also lead to significant changes. In sum, our study confirmed the need of investigating multiple stressors working simultaneously across different ecological levels of organisation.

Introduction

Microbial communities, as drivers of many ecosystems, must face increasing amounts and strengths of anthropogenic global change (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 an ongoing temperature increase (Wu et al. 2011) force populations and whole ecosystems to develop in different ways compared to less or unaffected ones. For decades, many studies analysed and proved 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 biogeochemically cycles and ecological function of the affected habitat (Gruber 2011; Suleiman et al. 2021b).

While most studies so far focused on one global change stressor, recent studies (Christensen et al. 2006; Rillig et al. 2019; Suleiman et al. 2021a) indicated that multiple stressors applied simultaneously do not always show additive effects, which would be the sum of the individual stressors. Rather, they demonstrate that interaction effects can occur. These can be synergistic (combined effect greater than the sum of the effect of individual stressors) or antagonistic (combined effect less than the sum of the effect of individual stressors). Furthermore, it was shown, that not just the character of stressor, but also the number of factors combined plays a crucial role (Rillig et al. 2019). Nevertheless, the amount of studies testing three or more stressors for an experimental system in the lab remains very low (Rillig et al. 2019), which highlights the need of investigating combined effects on experimental ecosystems, and is a critical aspect of the research field of climate change microbiology (Hutchins et al. 2019).

The limited number of studies with three or more stressors may be in part due to the logistical demands of such experiments and the complexity of interpreting their results. For example, to assess all interactions, one needs a fully-factorial experimental design, and so experiments can very quickly become large. Regarding interpretation, it can be difficult to understand the meaning of interactions among more than three stressors, 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 stressors, 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 stressors and combinations of stressors act across levels of ecological organization, from individuals to ecosystem (Galic et al. 2018). For example, Galic et al (2018) in a case study of a model of amphipod feeding behavior, showed that responses to multiple stressors 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 unobserved) 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, is the relationship between a biological variable and the number of drivers dependent on the level of ecological organization of the biological variable. And do the strength and nature of the interaction effects vary with level of ecological organization.

Microbial networks of aquatic ecosystems are complex (Christensen et al. 2006; DAVIS et al. 2010; Faust and Raes 2012), consisting of feedback reactions of numerous abiotic and biotic interactions (Singh et al. 2009), with and within specific functional microbial groups (Bush et al. 2017; Richardson et al. 2018). Recently, previous work indicated that these networks are sensitive to environmental change (Christensen et al. 2006; Shade et al. 2011, 2012; Suleiman et al. 2021b, a), identifying these habitats as appropriate systems for study and understanding influences of global changes.

In this work, we applied four different global change stressors (fertilizer, glyphosate, metal pollution, antibiotics, and temperature) in all combinations possible with increasing temperature (20 °C, 24 °C, and 28 °C), on a recently developed and highly replicable stratified aquatic microbial lab system, resulting in a total number of 240 analysed experimental micro-ecosystems. 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). We hypothesize that (i) depending on the combinations of stressors the ecosystems will be driven in different directions and (ii) increasing temperatures can change the influence of combined stressors (iii) increasing number of stressors goes along with significant effects.

Material and methods

Experimental set up

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, the 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 3.5 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. Incubation took place for 23 days (about three and a half weeks) at 20 °C, 24 °C and 28 °C, respectively.

Multiple stressor treatments

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

127 [“Control","F","G","M","A","FG","FM","FA","GM","GA","MA","FGM","FGA","GMA","FMA",

128 "FGMA"] * 5 replicates * 3 Temperatures

129 = 240 micro-ecosystems

130 For the stressor treatments, the following chemical compounds were added to the micro-

131 ecosystems 2 hours after the assembly of the microecosystems:

132 - $NH_4H_2PO_4$ in final concentration of 0.01 % as fertilizer (F), like used in a previous

133 study (Suleiman et al., 2021)

134 - Glyphosate in final concentration of 0.0001 % (G) like used in a recent study of (da

135 Costa et al. 2021)

136 - Trace element solution SL-10 final concentration 10x higher than recommended for

137 metal pollution (M)

138 - Mixture of penicillin and ampicillin (0,05 mg/100 mL each) in final concentration of

139 0.000005 % for antibiotic treatment (A), since such amounts were detected in

140 natural aquatic habitats (Xu et al. 2018b)

141 Stressors were applied to the micro-ecosystems only on day 0; there were no further press

142 or pulse perturbations.

143 ## Sampling and full length 16S rRNA sequencing

144

145 After incubation for 23 days, the whole micro-ecosystem was centrifuged for 5 min at 10.000

146 rpm (sediment and water column together). DNA was extracted using ZymoBIOMICS DNA

147 Miniprep Kit (ZymoResearch), following the manufacturer’s instructions. Full-length 16S rRNA

148 gene amplification was performed using the primer pair 27F forward primer (5’-

149 AGRGTTYGATYMTGGCTCAG-3’) and 1592R reverse primer (5’-RGYTACCTTGTTACGACTT-3’),

150 like reported previously (Suleiman et al., 2021).

151 PCR products were visualized on an 1 % (w/v) agarose gel and were pooled for
152 sequencing in equal concentrations. PCR product pools were purified using AMPure®PB beads
153 (PacBio). Sequencing was performed at the Functional Genomic Center Zürich, Switzerland,
154 and performed using SMRT Technology (PacBio) as reported previously (on three SMRT
155 chips). Sequencing data quality were checked using the PacBio SMRT Link software.
156
157 ## Bioinformatics
158
159 Sequencing data were filtered based on primer sequences, length (1300-1600 bp), quality,
160 error rates and chimeras usind *dada2* (Callahan et al. 2016). The final sequence table was
161 aligned using SILVA ribosomal RNA database (Quast et al. 2012), using version 138 (non-
162 redundant dataset 99). A phyloseq object was created using the *phyloseq* package (McMurdie
163 and Holmes 2013), consisting of amplicon sequence variant (ASV) table, taxonomy table and
164 sample data. For further analysis, the R packages *phyloseq* (McMurdie and Holmes 2013) and
165 *vegan* (Oksanen et al. 2019) were used.
166 In order to compare the effects of the multiple stressors, we analysed various
167 response variables, namely community composition based on NMDS1, NMDS2, Shannon
168 richness, oxygen, TN, TOC, and abundances of specific genera and functional groups.
169 Microbial community composition was quantified using NMDS (non-metric
170 multidimensional scaling) based on Bray-Curtis scores with the *metaMDS* function of the
171 vegan R package (Oksanen et al. 2019), with three dimensions used (k=3, try=100). Shannon
172 richness was calculated using *phyloseq* package (McMurdie and Holmes 2013). Functional
173 groups were constructed by filtering for cyanobacterial and sulfur-related species, which
174 resulted in a reduced dataset consisting of members of the order of *Cyanobacteriales*,
175 *Chromatiales*, *Synechococcales*, *Chlorobiales*, *Leptolyngbyales*, *Desulfobulbales*,

176 *Desulfovibrionales*, *Limnotrichales* and *Campylobacterales*. Detailed scripts and a phyloseq
177 with all sequences are available on github (https://github.com/Marcel2907/multiple-
178 stressor).

179 ### Measurement of abiotic factors
180
181 Oxygen concentration was measured 1.5 cm below the water/air interphase using an
182 oxygen dipping probe (PreSens, Germany) at the last day of incubation. pH was measured in
183 the supernatant of the centrifuged micro-ecosystem, as well as total nitrogen (TN) and total
184 organic carbon using a TOC analyser.

185 ## Results
186
187 We first results about the microbial community and genera abundances of the 240 micro-
188 ecosystems (Fig. 1), then, the impact of stressor combination as well as number of stressors
189 were calculated for diversity richness (Shannon), community structure (NMDS1), community
190 structure of a subpopulation, as well oxygen and pH (Fig. 2). Additional abiotic and biotic
191 variables were investigated (NMDS2, total nitrogen, total carbon) and can be find in detail in
192 the Supplementary Fig. 3. The summary results of all variables (species, community,
193 ecosystem) analysed based on the impact of stressor combination can be found in Fig. 3, and
194 the influence of number of stressors in Fig. 4.

195 In total, 20'319 unique ASVs were identified after running the dada2 pipeline. After
196 filtering to retain ASVs that appear at least in a rel. abundance of 0.1 % in at least one sample,
197 the total number of reads decreased to 5830 taxa. The control micro-ecosystems consisted
198 of mainly cyanobacteria (e.g. *Phormidaceae*) and phototrophic sulfur bacteria (specially
199 *Chromatiaceae*) (Fig. 1 a + b, Supplementary Fig. 1). Depending on the stressor combination
200 and temperature, the microbial communities and genera abundances showed strong

differences compared to the controls. Micro-ecosystems facing stressors of glyphosate and metal pollution, as a single stressor or in combination, were still dominated by members of *Phormidiaceae* and *Chromatiaceae* like the controls, but in all remaining treatments the abundance of members of these families was strongly decreased (Supplementary Fig. 1).

Also on genus level, we observed shifts in microbial composition depending on stressor combination and temperature (Fig. 1 b). The cyanobacterial genus *Tychonema* is highly abundant in metal pollution treatment, and with increasing temperature also in glyphosate + metal pollution treatments (20 % at 28 °C, 7 % at 20 °C). *Chromatium*, in contrast, appears in several treatments and reached highest rel. abundance at 20 °C when confronted with G:M (20 %), but vanished with increasing temperature in all treatments (< 5%). Statistical analysis of the relative abundance dependent on stressor combinations and temperature were performed on genus level for *Tychonema*, *Sulfuricurvum* and *Chromatium*, as representative species for Cyanobacteria and sulfur-depending bacteria (Fig. 3). Statistical analysis confirmed significant changes of relative species abundance depending on stressors, their combination and the temperature, but also depended on number of stressors applied (Fig. 4).

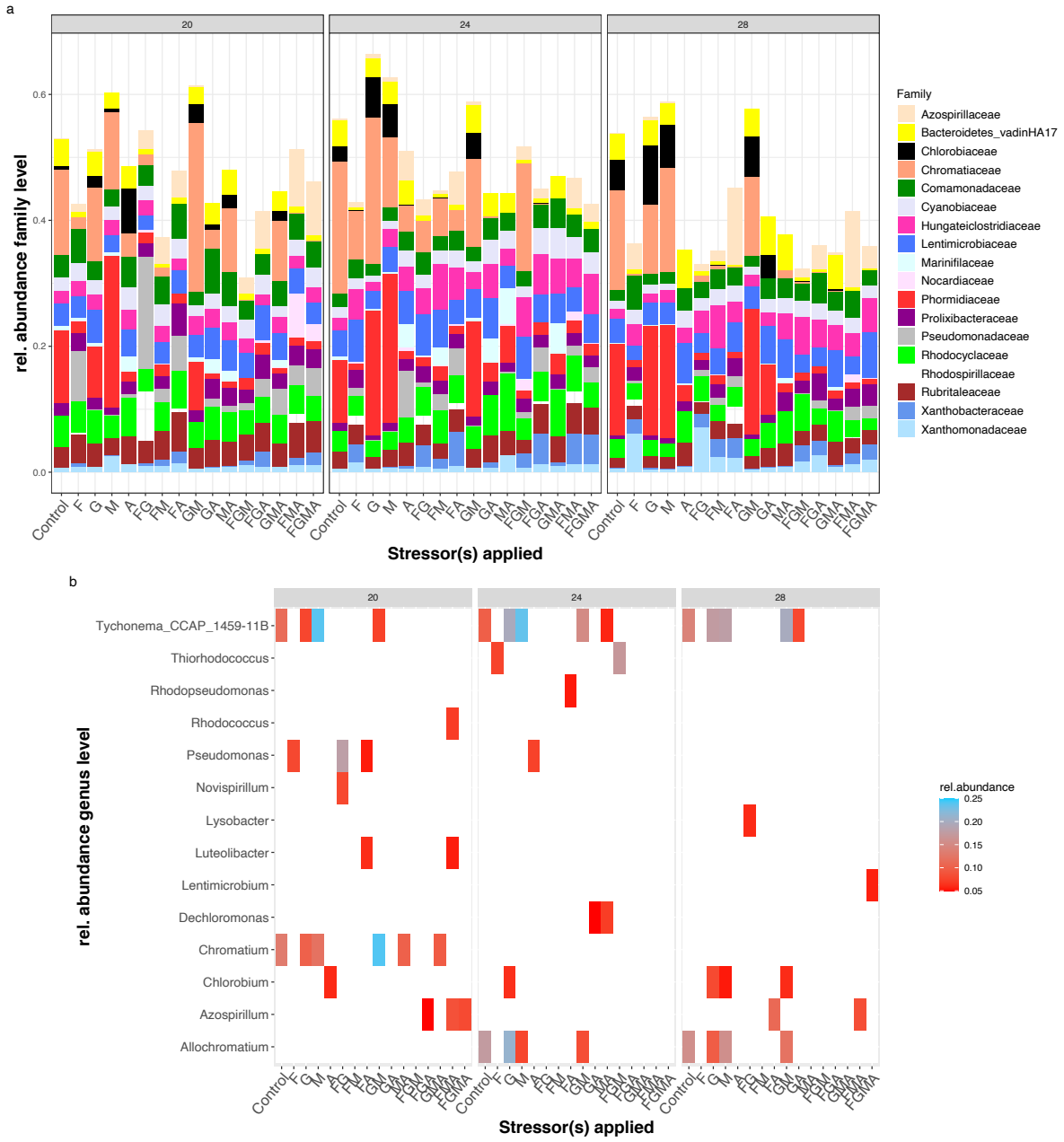


Fig. 1 Overview of microbial communities and genera abundance depending on temperature and stressors combined. (a) Microbial community composition (mean per 5 replicates) on family level. Taxa with rel. abundance > 5 % shown. **(b)** Relative abundance of specific genera with rel. abundance > 5 % for each stressor 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 stressors (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 stressor treatments and combinatios were identified, namely temperature

230 increase (p 1.41e-03), fertilizer (p 9.32e-05), antibiotics (p 7.85e-07), as well as combination
231 of fertilizer+antibiotics (p 3.82e-05) and fertilizer+antibiotics+glyphosate (p 3.02e-02) (Fig.
232 2a). All listed significant stressor combinations had a positive effect on diversity, except of
233 temperature increase to 28 °C and fertilizer:antibiotic treatment (Fig. 3). Interestingly, the
234 negative effect of fertilizer:antibiotic was reversed to a positive effect again by adding
235 glyphosate, while the treatment of glyphosate alone had no impact (Fig. 2a, Fig. 3). When
236 analysing the effect of number of stressors and temperature increase on diversity, just
237 temperature was identified to have a significant effect, but the number of stressors had no
238 impact (Fig. 2b, Fig. 4).

239 The influence of multiple stressors on microbial community composition was analysed
240 using NMDS, and fertilizer was identified as a key stressor for shifting microbial communities
241 (NMDS1 p 5.11e-77) (Fig. 2b+c, Supplementary Figure 2). Numerous stressor combinations
242 showed significant differences for NMDS score 1 and NMDS score 2 compared to the controls
243 (Fig. 2c for NMDS1, Supplementary Fig. 3 for NMDS2, Fig. 3 for both), ranging from single to
244 four-factorial combined stressors. Different impact directions (positive or negative score
245 change) of the estimates can be identified depending on the stressor combinations (Fig. 2c,
246 Fig. 3). The three-way interaction of fertilizer+antibiotics+temperature on NMDS1 score is a
247 good example how combination of stressors can change the impact direction (Fig. 5): When
248 no fertilizer was present, the NMDS1 score was negative, while in presence of fertilizer, the
249 NMDS1 score was positive. The combination of antibiotics and temperature changed
250 additionally the magnitude of the NMDS1 score. NMDS1 and NMDS2 scores were affected by
251 number of stressors (p 5.07e-17 and p 3.96e-04, respectivley), but not by total number of
252 stressors interacting with temperature (Fig. 2d, Fig. 4). The number of stressors did also affect
253 NMDS1, but not NMDS2 scores (Fig. 2f, Fig. 4).

254 Analysis of the subset of cyanobacteria, phototrophic sulfur bacteria and sulfate-
255 reducing bacteria confirmed the trends observed for NMDS of the whole data set (Fig. 2e for
256 NMDS1, Supplementary Fig. 3 for NMDS2, Fig. 3 for both), but showed slight differences on
257 three-factorial and four-factorial stressor combinations.

258 Oxygen concentration was highly dependent on the stressor combinations used, and
259 various strong effects were identified (Fig. 2g), both positive and negative in direction. All
260 stressors combined had a negative effect on oxygen concentration (p 4.04e-02, non-
261 standardised estimate - 6.1). The number of stressors applied had a strong effect on the
262 oxygen concentration (p 3.02e-05), as well as temperature (p 4.7e-08) and the interaction of
263 temperature + number of factors (p 0.04) (Fig. 2h, Fig. 4).

264 The pH of the micro-ecosystems was influenced by numerous treatment
265 combinations, but the strongest influences was identified as fertilizer addition, which
266 decreased the pH from 8.5 to 6.5 (p 4.76e-118). Stressor combinations that include fertilizer
267 addition had lower magnitude though were of the same sign (Fig. 3).Number of stressors had
268 a negative effect on pH, though this was mostly due to the strong influence of fertilizer and
269 its greater probability of occurrence in treatment combinations with more stressors applied
270 (Fig. 4).

271 Effects on total nitrogen were driven by addition of fertilizer (p 1.58e-65), and this was
272 not changed by addition of other stressors (Fig. 3, Supplementary Fig. 3). Furthermore,
273 number of stressors was highly affecting the total nitrogen concentration (p 4.09e-12), which
274 again can be explained by fertilizer addition alone. Total carbon concentration was
275 significantly negative affected by fertilizer (p 0.006), metal pollution (p 0.025) and
276 temperature (p 0.029) (Fig. 3, Supplementary Fig. 3).

277

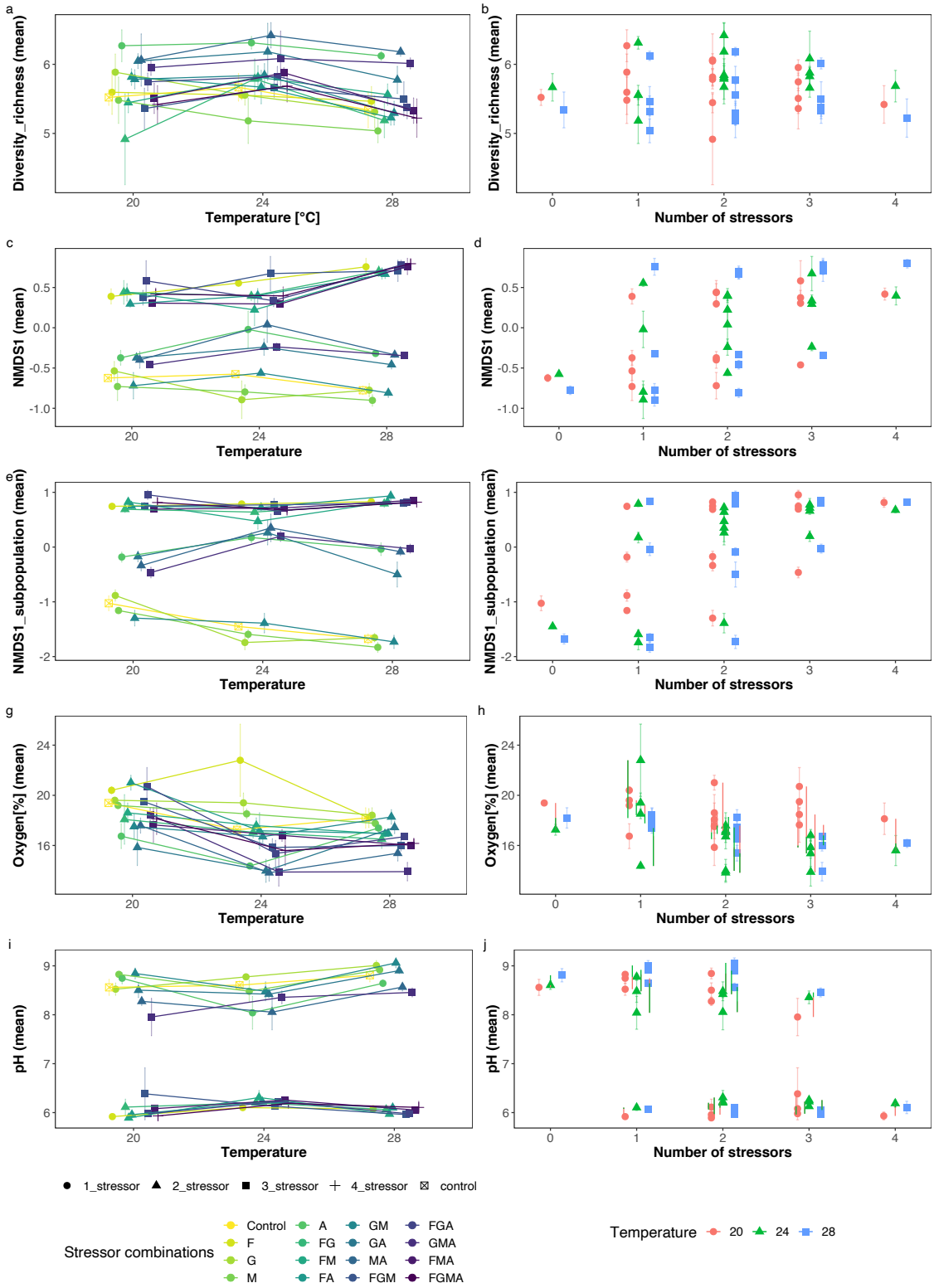


Fig. 2 Effects of stressor combination and number of stressors on temperature applied for microbial community and ecosystem variables. Shannon diversity index in dependence on (a) temperature and stressor combination and (b) temperature and number of stressors. NMDS1 scores in dependence on (c) temperature and stressor combination and (d) temperature and number of stressors. NMDS1_subpopulation scores dependence on (e) temperature and stressor combination and (f) temperature and number of stressors. Oxygen concentration in dependence on (g) temperature and stressor combination and (h) temperature and number of stressors. pH scores in dependence on (i) temperature and stressor combination and (j) temperature and number of stressors. Standard errors are shown (n = 5 replicates).

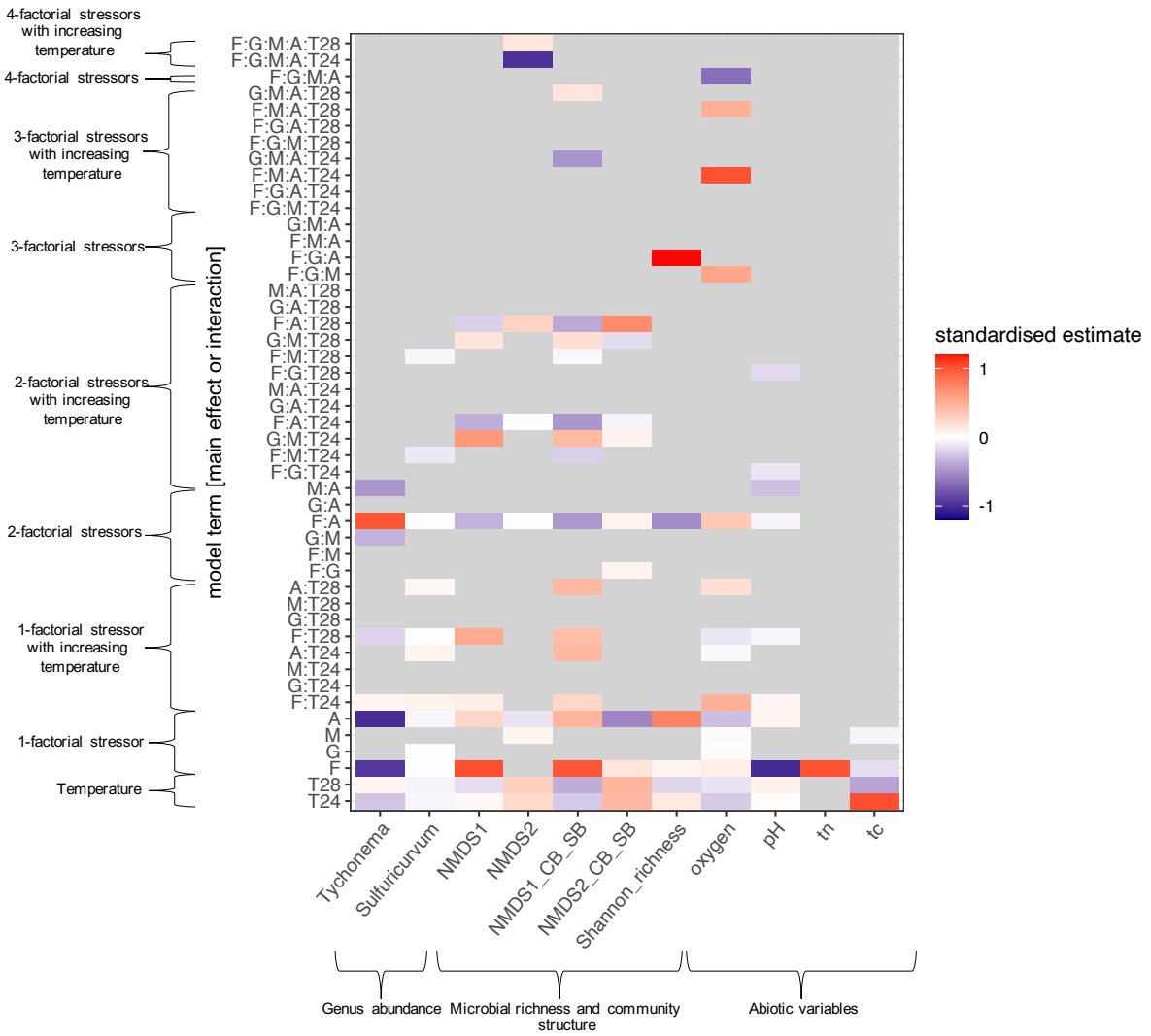


Fig. 3 Summary of statistical analyses of all variables analysed.
We analysed how 12 variables (grouped by genus appearance, microbial richness and community structure and abiotic factors) are affected by four factors applied in all combinations possible and additionally at three different temperatures. Coefficient intervals (t-test) are shown for significant affected stressor combination (F-test p-value < 0.05). Color scheme illustrates the direction of the influence by the stressor (based on estimate positive or negative, estimates of each variable were standardized by dividing by highest estimate value. Grey: not significant based on f-test (p value > 0.05). tn= total nitrogen, tc= total carbon.

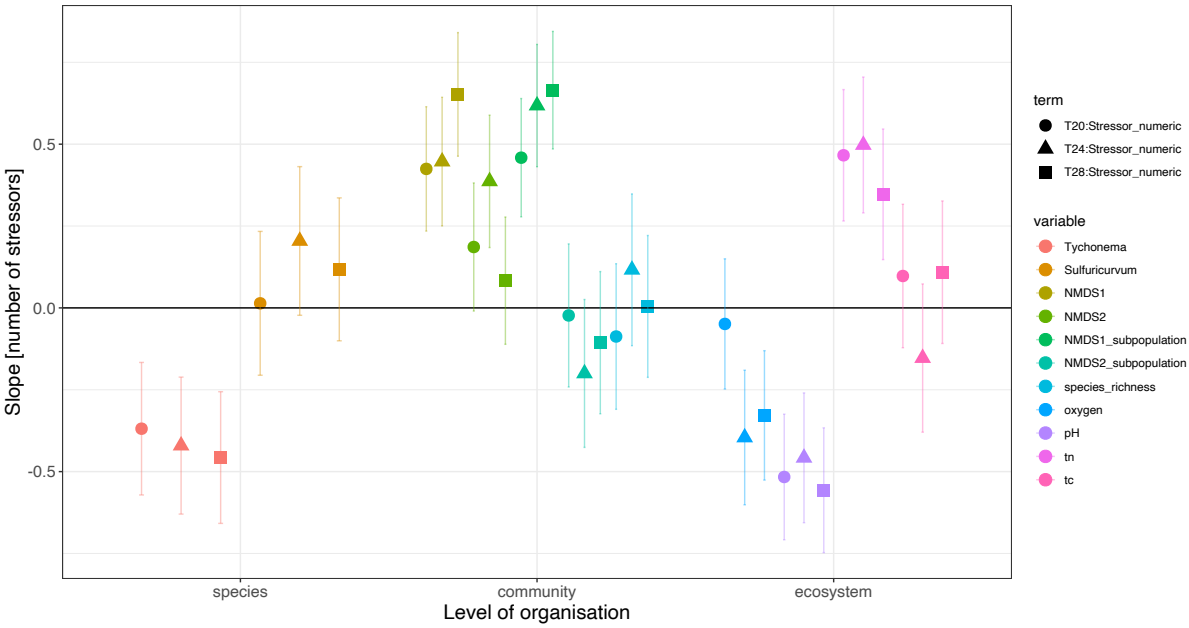


Fig. 4 Slope of effect of number of stressors over temperature for all level or organisation (species, community, ecosystem). 95 % confidence intervals are shown. tn=total nitrogen, tc=total carbon.

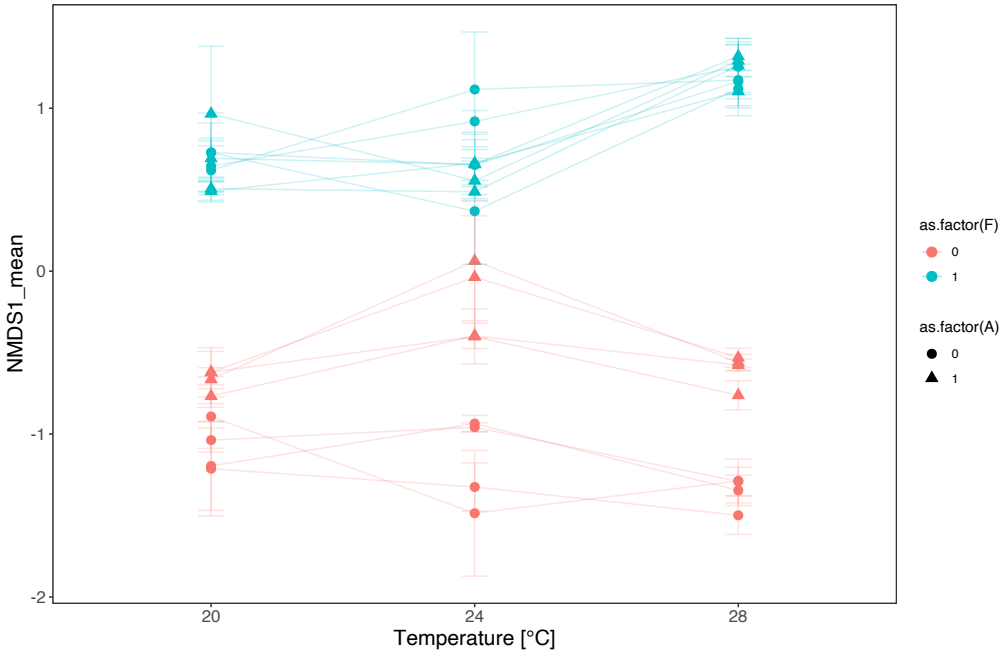


Fig. 5 Visualization of the significant stressor (interactions) of temperature, fertilizer and antibiotics on NMDS1. NMDS1 is affected by the presence of fertilizer, the presence of antibiotics and temperature, but also by the interactions of fertilizer-antibiotics and Fertilizer-antibiotics-temperature.

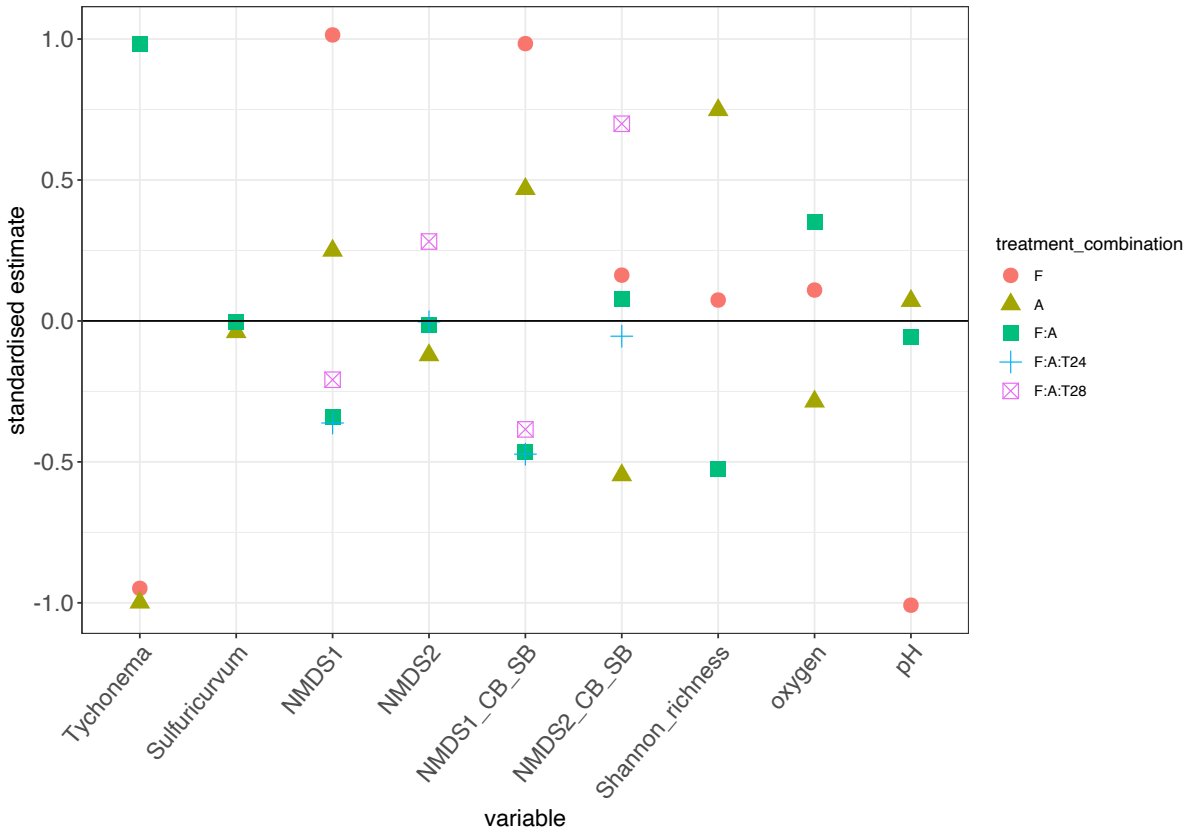
Discussion

Our study revealed strong evidence of effects of the combination and the total number of stressor on taxa abundances, community composition, and ecosystem properties, and therefore confirmed recent findings that multiple stressors are highly important to study (Rillig et al. 2019). The addition of a stressor, and/or temperature increase, could change the biological consequence of already applied stressors, even when the added stressor applied alone had no effect (Fig. 3). We observed three trends here: The addition of a further stressor can increase (e.g. F:A:T28), decrease (e.g. F:T28) or turn the whole direction of stressors which were already applied (e.g. F:A on *Tychonema* abundance, Fig. 6). This demonstrates clearly the need of analysing multiple stressors in all possible combinations. Furthermore, our study also confirmed that the number of stressors applied alone can have an effect on microbial communities and thereby provides some hope that number of stressors can be used as a predictor, though it is clear that individual stressor effects can greatly contributed to the effect of number of stressors (Rillig et al. 2019). The effect of number of stressors can be negatively (e.g. *Tychonema* abundance) or positively (e.g. total nitrogen), which highlights again the need of analysing various ecological response variables of species, microbial communities and the whole ecosystem.

While fertilizer, metal pollution and antibiotics had numerous effects when applied as a single stressor, glyphosate did not show this trend. This finding is probably concentration dependent and should be regarded with caution. Nevertheless, it is even more interesting that when adding glyphosate to more stressors it can have an effect (see e.g. diversity richness). Strong interactions were detected for antibiotics and fertilizer, and interestingly, these stressors revealed in combination different biological consequences compared to

334 applied as a single stressor, and temperature could also change the effects of the fertilizer

335 antibiotic stressor duo again (Fig. 6).



336

337 **Fig. 6 Effects of fertilizer, antibiotics and their combination at 20 °C and elevated temperature on species, microbial**
338 **community and ecosystem variables.** Just significant treatments are shown (F-test p value < 0.05). Total nitrogen and total
339 carbon are not listed in this figure because they were not significantly affected by F, A, and F:A.

340

341 The effect of temperature is in our view highly important to notice, since it is

342 predicting that observed biological consequences caused by global change can change with

343 on-going global warming. An increase of temperature can lead to new interactions among

344 stressor combinations (F:A with increasing temperature led again to significant affected

345 variables) or can lead to significant effects that were not observed at 20 °C (G:M, G:M:24 and

346 G:M:28 for NMDS1).

347 Our study also contributes to close the gap of understanding how stressors and their

348 combination act across various levels of organisation, since our results revealed that

individuals can be affected differently compared to abiotic factors of the ecosystem or the microbial community composition. Therefore, our study is in line with the findings of Galic et al. (2018) who reported already differences of the influence of multiple stressors across levels of organisation. Interestingly, in our study, some treatment combinations, like fertilizer and antibiotics working simultaneously, showed significant influence across most of the different levels of organisation (except total nitrogen and total carbon).

Stressors and how they are affecting ecosystems and communities are concentration-dependent. In this study, we did used only two levels of stressor concentration and added the stressor in a single pulse treatment. More information about specific concentration thresholds and press disturbances are important to study and may clarify some observed trends, as well as the timing of the stressor applied. In addition, responses of functional aspects of the aquatic microbial communities should be investigated in future studies, which could shed light into activated and deactivated pathways and enzymes used in microbial communities to react to changing environmental conditions.

In sum, our study confirmed the need of analysing the effects of multiple stressors on microbial communities, and indicate further that a broad range of levels of organisation (species, microbial community, ecosystem) should be analysed due to its variation of significant effects. Furthermore, our study showed that combination of specific stressors can change the biological consequence and direction compared to single stressors in all levels of organisation and that the effects of stressor combinations can be modified by temperature.

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489
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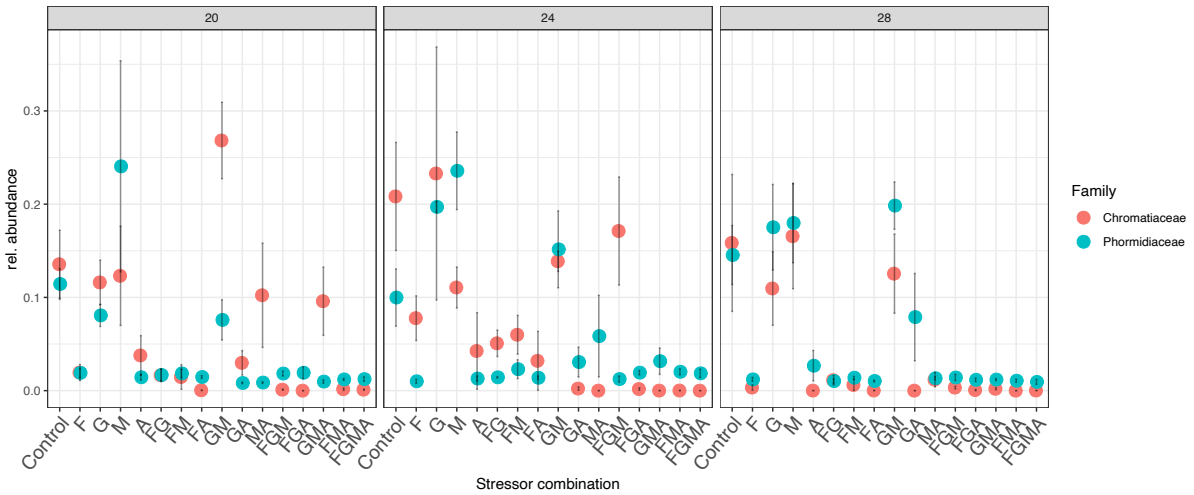
496
497
498 **Author contribution**
499
500 OLP and MS planned the experimental set-up. MS performed all experiments in the lab. MS
501 performed the up- and downstream bioinformatics. MS and OLP performed the data analysis.
502 YC provided technical support and performed the TOC analysis. XZ performed the DNA
503 extractions and provided technical support. OLP and MS drafted the manuscript. All authors
504 confirmed the final version of the manuscript.

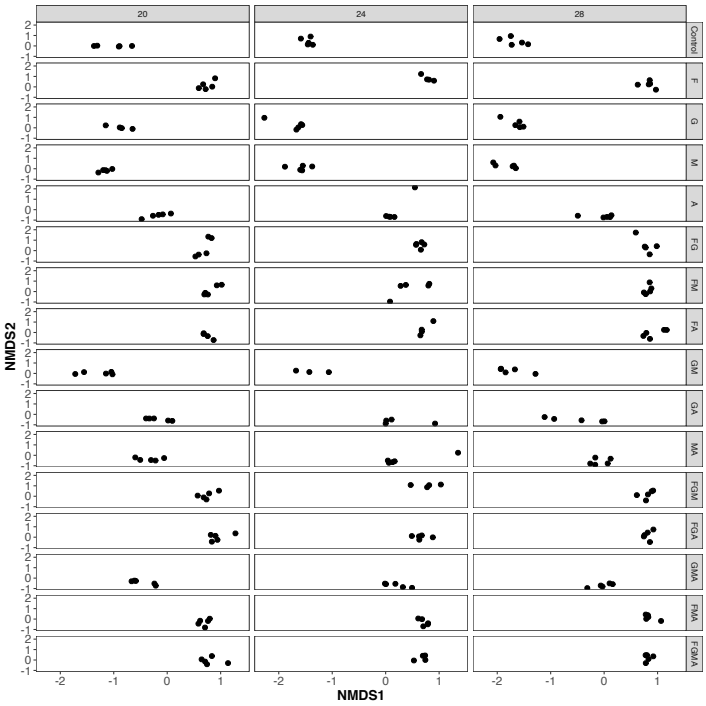
Competing interests

The authors declare no competing financial interests.

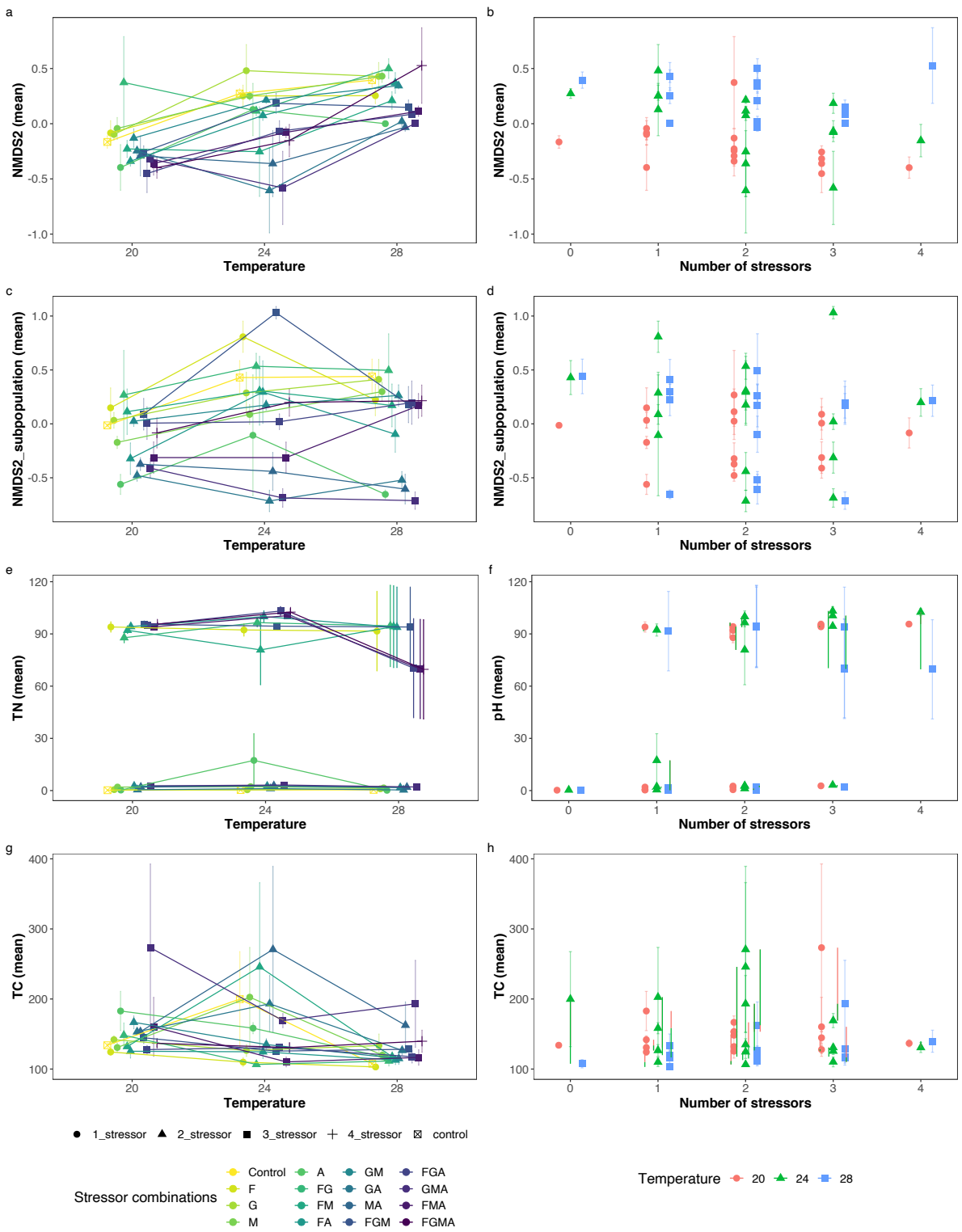
Supplementary Information



Supplementary Fig. 1 Relative abundance of Chromatiaceae and Phormidiaceae in the micro-ecosystem depending on stressor 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 stressor combinations. Stress is 0.16



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