Contrasting resistance and resilience to light variation of the coupled oxic and anoxic components of an experimental microbial ecosystem

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Competing interests

The authors declare no competing financial interests.

Abstract

Understanding how microbial communities of aquatic ecosystems respond to environmental change remains a critical challenge in microbial ecology. In this study, we used phototrophic oxic-anoxic micro-ecosystems to understand how the functioning and diversity of aerobic and anaerobic lake analog communities is affected by light deprivation. Continuous measurements were performed to describe oxygen dynamics (mean/min/max/amplitude) and time-series of full-length 16S rRNA sequencing were used to quantify changes in alpha- and beta-diversity. In the top oxic layer, oxygen concentration decreased significantly under light deprivation, but showed resilience in mean, minimum and maximum after light conditions were restored. Only the amplitude of diurnal fluctuations in oxygen concentrations did not recover fully, and instead tended to remain lower in treated ecosystems. Alpha-diversity of the top oxic layer communities showed a delayed increase after light conditions were restored, and was not resilient. In contrast, alpha-diversity of the anoxic bottom layer communities increased due to the stressor, but was resilient. Community composition changed significantly during light deprivation, showed resilience in the oxic layer and lack of resilience in the anoxic layer. Alpha-diversity and the amplitude of oxygen within and among treatments were strongly correlated, suggesting that higher diversity could lead to less variable oxygen concentrations, or vice versa. Our experiment showed that light deprivation induces multifaceted responses of

26 community function and structure, hence focusing on a single stability component could
27 potentially be misleading.
28

29 **Introduction**

30 Microbial communities are critical components of ecosystems, driving their development and
31 functioning since billions of years. Over these extreme time-scales, microbes have tackled
32 major transitions, such as the increasing oxygenation of Earth's atmosphere. The success of
33 microbial life is to a large degree explained by being highly adaptable to a variety of
34 environmental conditions [1, 2]. Even slight changes in conditions can lead to taxonomical and
35 functional community shifts, in turn being important indicators of microbial community
36 stability, or lack thereof [3]. Communities can react towards disturbances by showing
37 resistance, resilience, functional redundancy or alternate stable states [4]. Observing responses
38 on the functional and compositional level and how they are related can provide much needed
39 insights about the mechanisms of microbial community stability [5]. How functioning and
40 stability are mediated by the diversity of an ecosystem has been addressed with controlled
41 experiments [6–8], however, these assume random species loss, while disturbances often affect
42 biodiversity non-randomly [9].

43 Lake ecosystems are facing a broad range of stressors [10], including temperature-
44 increase, fertilizer-intake [11] chemical pollutants [12], or increasing microplastic pollution
45 [13]. One factor that is still not investigated well is light deprivation of lake ecosystems, which
46 can occur indirectly due to biomass formation on aquatic surfaces, including blooms of algae
47 [14] and biofilms[15], as well as plant biomass growth. A broad range of functional aerobic
48 and anerobic microbial groups are highly dependent on light, including cyanobacteria in the
49 upper water column and phototrophic sulfur bacteria in the lower water column. Hence light
50 deprivation could have a large effect on microbial community composition and ecosystem

51 properties such as the oxygen concentration in lake ecosystems [16] due to tight coupling of
52 light, microbial respiration and cyanobacterial O_2 production.
53 Here we studied the response of mixed aerobic-anaerobic communities to light
54 deprivation within a single ecosystem with a recently developed dynamic phototrophic oxic-
55 anoxic micro-ecosystem [17]. These micro-ecosystems are analogs of freshwater ecosystems,
56 like lakes and ponds, which harbor highly diverse functional groups of microorganisms, tightly
57 connected through the oxygen state of the aquatic environment, which are dependent on light
58 for oxygenic and anoxygenic photosynthesis.
59
60 **Material and methods**
61 Sediment and water samples were taken from a pond (Zurich, Switzerland, 47°23'51.2"N
62 8°32'33.3"E) and eight micro-ecosystems were set-up as reported previously [17]. In short, 1.5
63 cm sediment (with 0.5 % crystalline cellulose, 0.5 % methyl-cellulose, 1 % $CaSO_4$, 0.2 %
64 $CaCO_3$, 0.01 % $NH_4H_2PO_4$) were covered with 16 mL pond water and incubated for 35 days
65 at 24 °C under a light-dark-cycle of 16:8 h (gradient of light). Continuous non-invasive oxygen
66 measurements (PreSens Precision Sensing GmbH, Germany) were performed every 5 min at
67 the bottom liquid part (1.5 cm above sediment) and top liquid part (2 cm below surface) of each
68 column (Supplementary Fig. S1a). After incubating the eight columns for eight days, four
69 columns were covered tightly with aluminum foil and incubated in darkness for seven days
70 (stressor-treatment). After the treatment, incubation continued with the standard light
71 conditions, like the control group, for another 20 days. 500 µL liquid sample were taken on
72 day 8 (prior-to-stressor), day 15 (stressor-sample), day 19 (short-term recovery) and day 35
73 (long-term recovery), respectively, at the height of the top and bottom oxygen sensor. DNA
74 extraction and 16S rRNA full-length sequencing (PacBio) were performed as reported
75 previously [17]. Raw sequencing data were transcribed to Amplicon Sequence Variants (ASV)

with Dada2 [18], and analysis of alpha- and beta-diversity were performed with the R packages phyloseq [19] and vegan [20].

In order to compare the effects of the light treatment we analysed seven response variables (daily mean, maximum, minimum, and amplitude of oxygen concentration, alpha diversity (Shannon index), and two components of microbial community composition). Microbial community composition was quantified using NMDS (non-metric multidimensional scaling) based on Bray-Curtis distances with the *metaMDS* function of the vegan R package [20], with two dimensions used (giving the previously mentioned two components of microbial community composition).

Each of the seven response variables was analysed separately at each time point it was measured at. At each time point, we calculated the mean of each treatment group (control group and light deprivation group), the difference between these means, and the 95% confidence interval of this difference (assuming normally distributed errors). We then visually inspected if the confidence interval included zero, and if so judged there to be no difference between the treatment and control group, and if so that there was a difference. We say that a response variable was resistant to the treatment if the 95% confidence interval of the difference between control and treatment included zero at the end of the treatment (day 15 and 19) (or not resistant if the 95% CI did not include zero). We say that a response variable was resilient to the treatment if the 95% confidence interval of the difference between control and treatment included zero at the end of the experiment (day 35) (or not resilient if the 95% CI did not include zero). Although the oxygen measurements are plotted and analysed for each day, we only based assessments of resistance and resilience on the same days as for community composition (days 15, 19, 35). This reduces the number of comparison, which reduces any issues associated with multiple testing, and also reduces any issues associated with temporal

100 autocorellation / repeated measured. Detailed scripts are available on zenodo
101 (10.5281/zenodo.5195092) and sequencing raw reads are available on NCBI (PRJNA731625).
102

103 **Results and discussion**

104 Each of the eight micro-ecosystems showed oxygen-based stratification (Fig. 1a) and microbial
105 community compositions characteristic for layered lake ecosystems, with aerobic communities
106 in the oxic top liquid part (diverse members of *Gammaproteobacteria*, *Bacteroidia*,
107 *Cyanobacteriia*) and (phototrophic) sulfur bacteria in the anoxic bottom layer (*Chlorobium*,
108 *Chlorobaculum, Magnetospirillum, Sulfuricurvum*) (Supplementary Fig. S1+2).

109 During the first week, the oxygen concentration of the top layer of the eight micro-
110 ecosystems increased during the light- and decreased during dark-phase, resulting in
111 comparable behavior of the mean-, max- and min-oxygen concentration, as well as the
112 amplitude (Fig. 1, day 0-8). During light deprivation (day 8-15) the disturbed columns showed
113 a significant decrease in total oxygen concentration to microaerophilic conditions (Fig. 1b, day
114 8-15). After normal light conditions were restored, oxygen concentration increased, showing
115 resilience until the end of the experiment for mean, minimum and maximum, but not for the
116 amplitude, which was higher in the controls (Fig. 1c). Therefore, light deprivation had a lasting
117 impact on the oxygen amplitude, which stayed within a narrower range of values for
118 communities that experienced the stressor, while untreated ones were more variable. A single
119 replicate showed a higher oxygen concentration at the end of the experiment. Exclusion of that
120 microcosm affected the estimated differences in oxygen concentration (i.e., resistance and
121 resilience), but did not qualitatively change the results. Exclusion also had no effect on the
122 conclusions of the analyses of microbial community composition. Since the bottom layer
123 turned completely anoxic within 2 days, we did not detect any effect of light deprivation on the
124 oxygen pattern there.

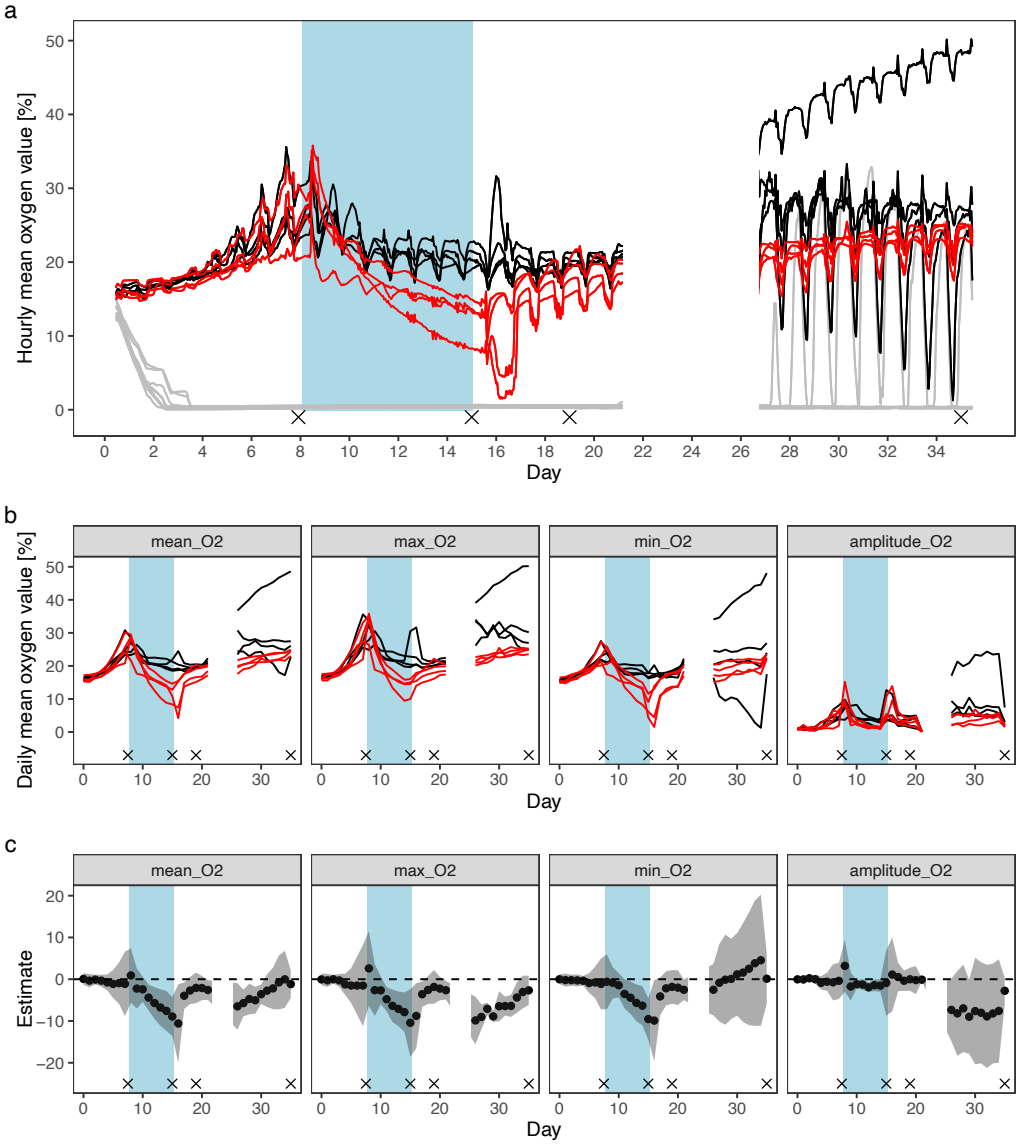

Fig. 1 Dynamics and functional response of oxygen in the micro-ecosystems. Oxygen concentration was recorded every 5 minutes. **(a)** Hourly mean of the oxygen concentration of the top sensors (line) and bottom sensors (grey) of the eight micro-ecosystems. **(b)** Daily-mean oxygen concentration (illustrated as mean, maximum, minimum and amplitudes) of the top sensors of the eight micro-ecosystems. **(c)** Estimates of difference in the mean and 95% CI of the estimate of the oxygen concentration treatment vs. controls. Black lines represent controls, red lines represent columns incubated in darkness from day 8-15 (blue area). Gray ribbons show the 95% confidence intervals. Crosses show the sampling days for analysing microbial communities of the top and bottom part. A recording error caused the missing oxygen data from day 23-27.

Alpha diversity was differentially affected by light deprivation in the top and bottom level (Fig. 2): The richness of the anaerobic bottom communities was immediately increased by light deprivation (Day 15, Fig. 2a+b) and stayed significantly different after normal light conditions were restored in the short-term recovery sample, but showed resilience (i.e. return to control values) in the long-term. In contrast, the alpha-diversity of the aerobic top layer communities was not immediately affected by the stressor, showed marginally significant differences in the short-term recovery samples but stayed significantly changed in the long-term (Fig. 2c+d).

Non-metric multidimensional scaling revealed significantly different composition of top and bottom layer communities within a single micro-ecosystem, but the top and bottom composition converged during light deprivation (Supplementary Fig. S3). This convergence is most likely due to the loss of the light- and oxygen-gradient during the disturbance, indicating how stressors can directly influence microbial communities by affecting stratification processes in an ecosystem.

To understand the dynamic changes of community composition, we analysed time series of NMDS1 and NMDS2 (Fig. 2b). For the bottom community, the NMDS1 score was immediately affected by light deprivation but showed resilience, while NMDS2 was affected in delayed fashion and remained significantly changed (Fig. 2e-h). The top layer communities were affected immediately based on NMDS1 and NMDS2, but both showed resilience at the last sampling (Fig. 2i-l).

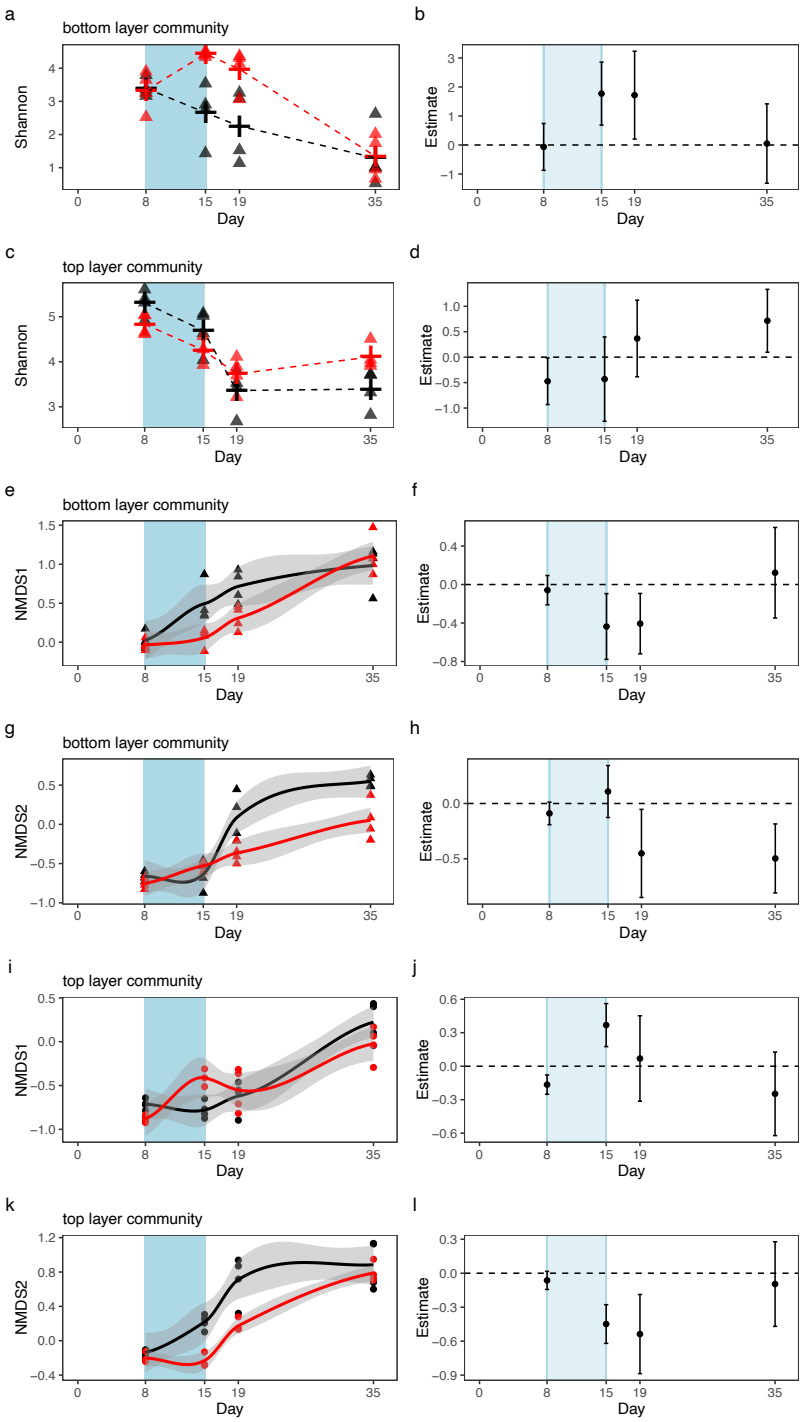

Fig. 2 Resistance and resilience of the microbial communities based on full-length 16S RNA sequencing. **(a)** Alpha-diversity based on Shannon index of the bottom water communities of the eight micro-ecosystems. Cross: mean. **(b)** Estimated difference between treatments and 95% confidence interval for the alpha diversity analysis for the bottom water communities. **(c)** Alpha-diversity based on Shannon index of the top water communities of the eight micro-ecosystems. Cross: mean. **(d)** Estimated difference between treatments and 95% confidence interval for the alpha diversity analysis for the top water communities. **(e,g,i,k)** Beta-diversity analysis based on NMDS1 and NMDS2 score for the bottom and top water communities, respectively. **(f,h,j,l)** Estimated difference between treatments and 95% confidence interval for the beta diversity (based on NMDS1 and NMDS2 scores) as response parameters based on NMDS1 score for the bottom and top water communities. Black lines represent controls, red lines represent columns incubated in darkness from day 8-15 (blue area). Gray ribbons show the 95% confidence intervals.

171 In order to understand possible relationships between diversity and stability, we examined the
172 correlation of alpha diversity and the oxygen amplitude of the last incubation day 35 (Fig. 3).
173 We found a strong negative correlation between alpha-diversity and amplitude of diurnal
174 oxygen concentration ($n=8$, $t= -5.1134$, $p = 0.002$). This correlation holds when excluding the
175 replicate with high mean oxygen concentration, very high amplitude of oxygen variation (~
176 7.4), and low diversity (SI 2.8) ($n=7$, $t= -2.6$, $p = 0.04$) (Supplementary Figure S4). This finding
177 is in line with the insurance hypothesis of biodiversity, that higher biodiversity will cause lower
178 temporal variation in aggregate properties of communities and of ecosystem states, such as
179 oxygen concentration [6]. However, in our experiment diversity was not manipulated, but was
180 rather an outcome of the factors such as environmental conditions (including the light
181 treatment) and interspecific interactions. Accordingly, we cannot be sure if the higher diversity
182 observed in the light deprivation treatment was the cause of the greater stability (lower
183 amplitude) of the oxygen concentration in these communities. Also, since, we did not
184 manipulate the amplitude of the oxygen fluctuations, we cannot say if this was responsible for
185 the observed differences in alpha diversity. Further studies manipulating the diversity of
186 organisms in the ecosystem and separately the magnitude of fluctuations in environmental
187 conditions such as oxygen concentration would be needed to assign causation and understand
188 the likely feedback between organismal diversity and environmental fluctuations.

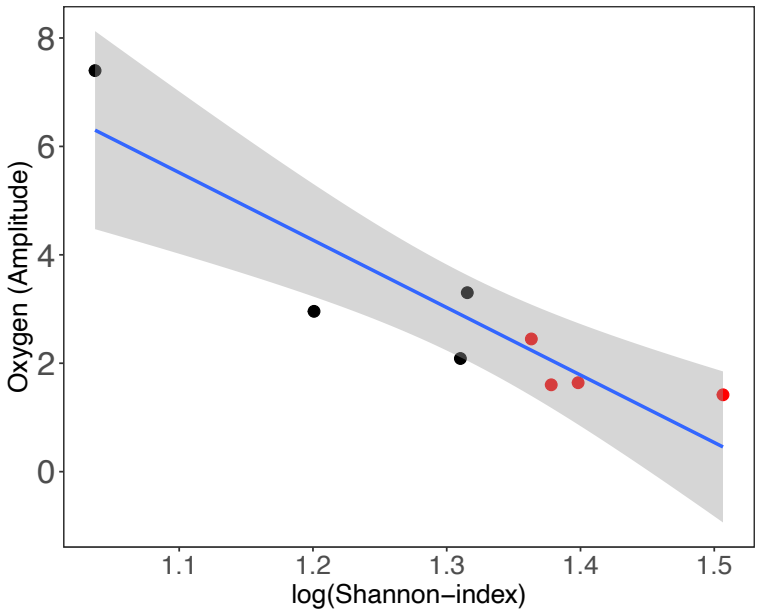

Fig. 3 Relationship between the amplitude of daily fluctuations in oxygen concentration and Shannon diversity of top layer communities the long-term recovery sample (day 35). Black symbols represent controls, red symbols represent treated columns. Gray ribbons show the 95% confidence intervals.: Pearson's product-moment correlation: p-value=0.002, correlation coefficients= -0.90.

Our experiment showed that light deprivation induces multifaceted responses of community function and structure at multiple temporal scales. We found that light deprivation has differential effects on the coupled communities in ecosystems: (i) light deprivation had a strong effect on microbial function, richness and composition, but most responses were resilient in the long run (ii); For non-resilient aspects, we found that the richness and the amplitude of oxygen concentration were related. We suggest that non-random effects of light deprivation leads to a change in richness, with subsequently affects the oxygen amplitude, but this proposed causal relationship needs experimental testing (iii); recovery and resilience operate at different time scales and hence several time points of analysis are needed for making robust conclusions about microbial resistance and resilience [21]. Focusing on just a single aspect of community stability may hence be misleading, since a stressor can affect multiple stability components differently [22, 23].

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Competing interests

The authors declare no competing financial interests.

Author contributions

OLP and MS planned the experimental set-up. MS performed all experiments in the lab. MS performed the up- and downstream bioinformatics. FP supported the data analysis. YC provided technical support. OLP, MS and FP drafted the manuscript. All authors confirmed the final version of the manuscript.

References

1. Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, et al. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 2013; **94**: 714–725.
2. Evans SE, Wallenstein MD. Climate change alters ecological strategies of soil bacteria. *Ecol Lett* 2014; **17**: 155–164.
3. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of microbial community resistance and resilience. *Front Microbiol* 2012; **3**: 1–19.
4. Allison SD, Martiny JBH. Resistance, resilience, and redundancy in microbial communities. *Light Evol* 2009; **2**: 149–166.
5. Philippot L, Griffiths BS, Langenheder S. Microbial Community Resilience across Ecosystems and Multiple Disturbances. *Microbiol Mol Biol Rev* 2021; **85**: e00026-20.
6. Yachi S, Loreau M. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proc Natl Acad Sci* 1999; **96**: 1463–1468.
7. Fox JW. The intermediate disturbance hypothesis should be abandoned. *Trends Ecol Evol* 2013; **28**: 86–92.
8. Huston MA. Disturbance, productivity, and species diversity: empiricism vs. logic in ecological theory. *Ecology* 2014; **95**: 2382–2396.
9. De Laender F, Rohr JR, Ashauer R, Baird DJ, Berger U, Eisenhauer N, et al. Reintroducing Environmental Change Drivers in Biodiversity–Ecosystem Functioning Research. *Trends Ecol Evol* 2016; **31**: 905–915.
10. Chaudhari S, Felfelani F, Shin S, Pokhrel Y. Climate and anthropogenic contributions to the desiccation of the second largest saline lake in the twentieth century. *J Hydrol* 2018; **560**: 342–353.
11. Gao W, Swaney DP, Hong B, Howarth RW, Liu Y, Guo H. Evaluating anthropogenic

259 N inputs to diverse lake basins: A case study of three Chinese lakes. *Ambio* 2015; **44**:
260 635–646.
261 12. Li C, Yang L, Shi M, Liu G. Persistent organic pollutants in typical lake ecosystems.
262 *Ecotoxicol Environ Saf* 2019; **180**: 668–678.
263 13. Andersson B, Anderson JM. Lateral heterogeneity in the distribution of chlorophyll-
264 protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim*
265 *Biophys Acta* 1980; **593**: 427–440.
266 14. Sun S, Wang F, Li C, Qin S, Zhou M, Ding L, et al. Emerging challenges: Massive
267 green algae blooms in the Yellow Sea. *Nat Preced* 2008.
268 15. Jones JI, Young JO, Eaton JW, Moss B. The influence of nutrient loading, dissolved
269 inorganic carbon and higher trophic levels on the interaction between submerged
270 plants and periphyton. *J Ecol* 2002; **90**: 12–24.
271 16. Bush T, Diao M, Allen RJ, Sinnige R, Muyzer G, Huisman J. Oxic-Anoxic regime
272 shifts mediated by feedbacks between biogeochemical processes and microbial
273 community dynamics. *Nat Commun* 2017; **8**.
274 17. Suleiman M, Choffat Y, Daugaard U, Petchey OL. Large and interacting effects of
275 temperature and nutrient addition on stratified microbial ecosystems in a small,
276 replicated, and liquid-dominated Winogradsky column approach. *Microbiologyopen*
277 2021; **10**: e1189.
278 18. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:
279 High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**:
280 581–583.
281 19. McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive
282 Analysis and Graphics of Microbiome Census Data. *PLoS One* 2013; **8**.
283 20. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, et al. Package

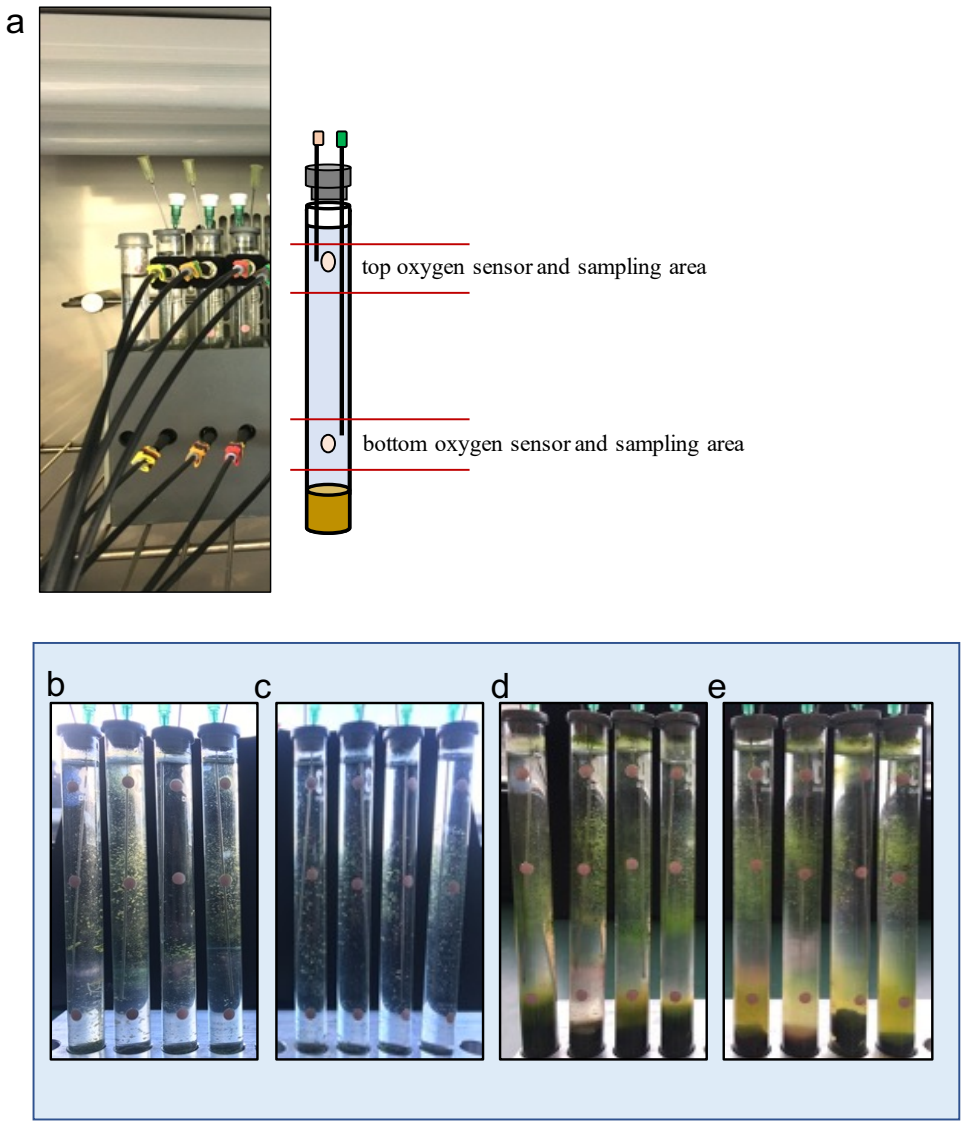
'vegan' Title Community Ecology Package. *Community Ecol Packag* 2019; **2**: 1–297.

21. Garnier A, Pennekamp F, Lemoine M, Petchey OL. Temporal scale dependent interactions between multiple environmental disturbances in microcosm ecosystems. *Glob Chang Biol* 2017; **23**: 5237–5248.

22. Pennekamp F, Pontarp M, Tabi A, Altermatt F, Alther R, Choffat Y, et al. Biodiversity increases and decreases ecosystem stability. *Nature* 2018; **563**: 109–112.

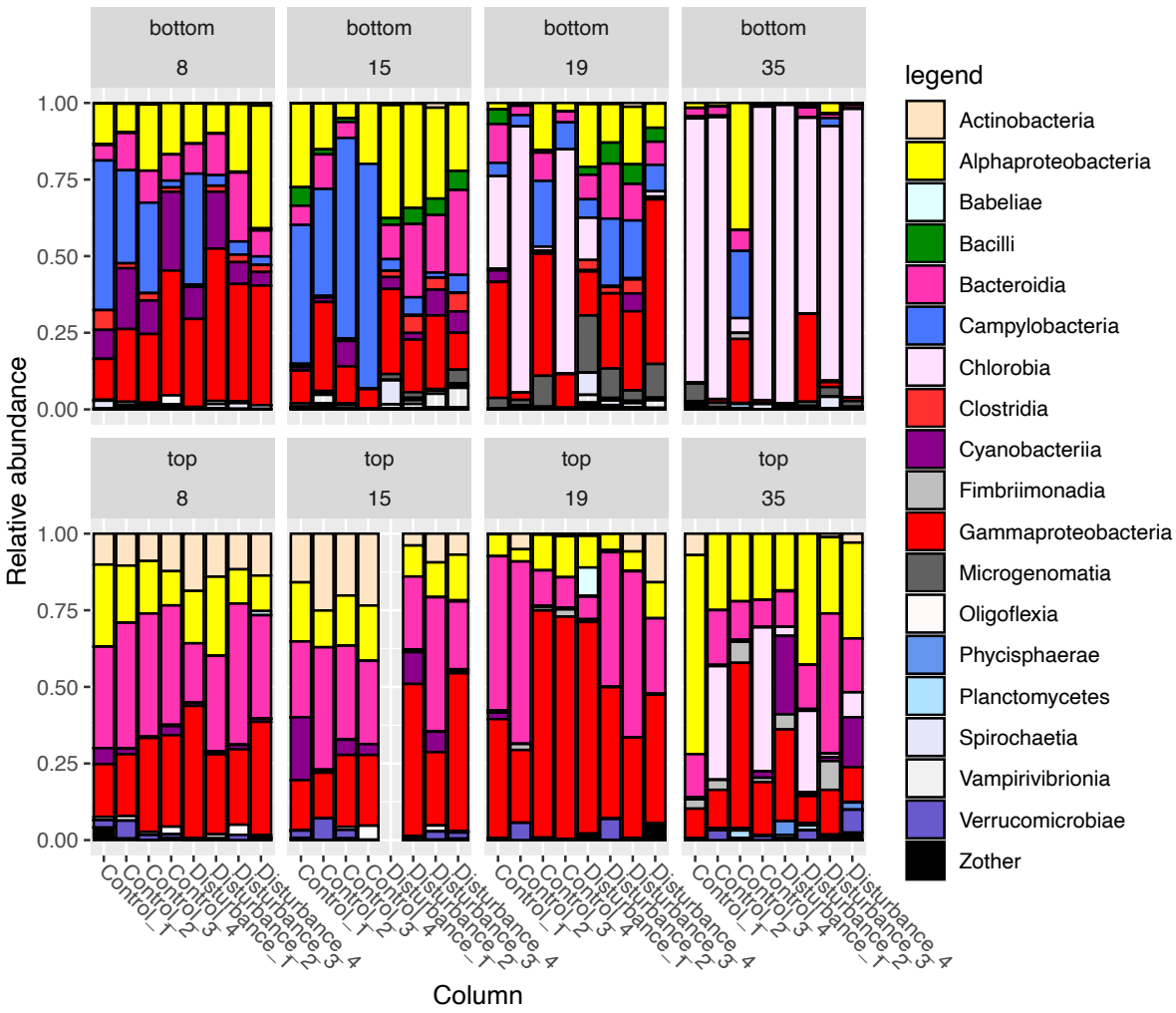
23. Baert JM, De Laender F, Sabbe K, Janssen CR. Biodiversity increases functional and compositional resistance, but decreases resilience in phytoplankton communities. *Ecology* 2016; **97**: 3433–3440.

Supplementary Information

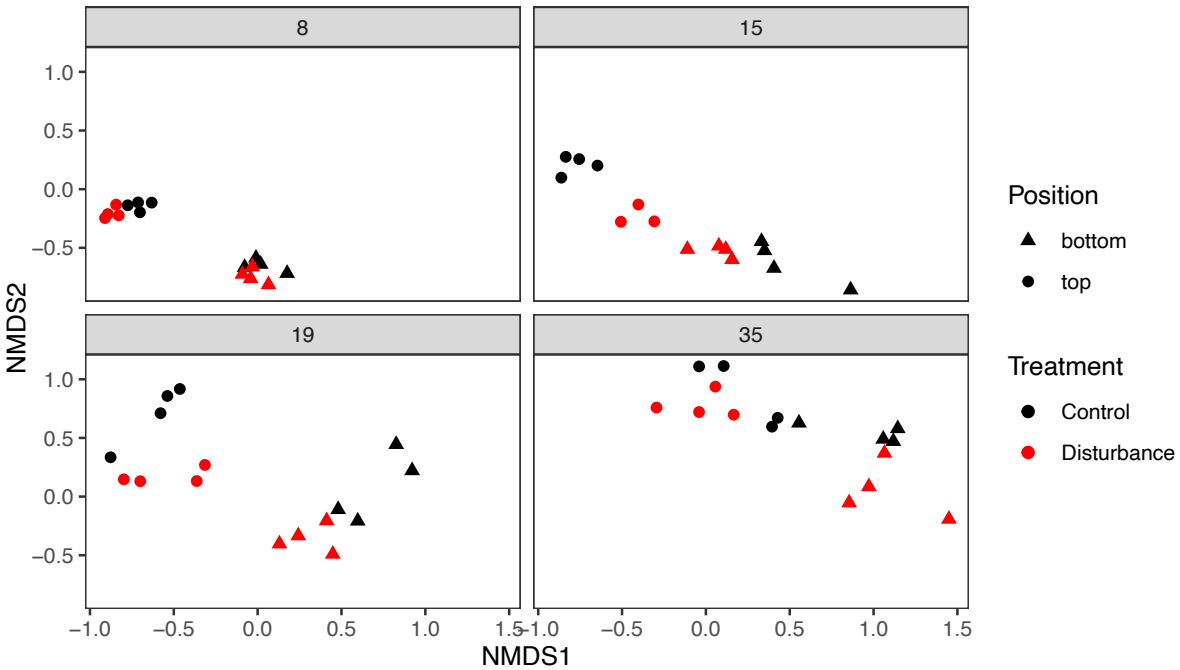


Supplementary Fig. S1 Setup and macroscopic appearance of the micro-ecosystems.

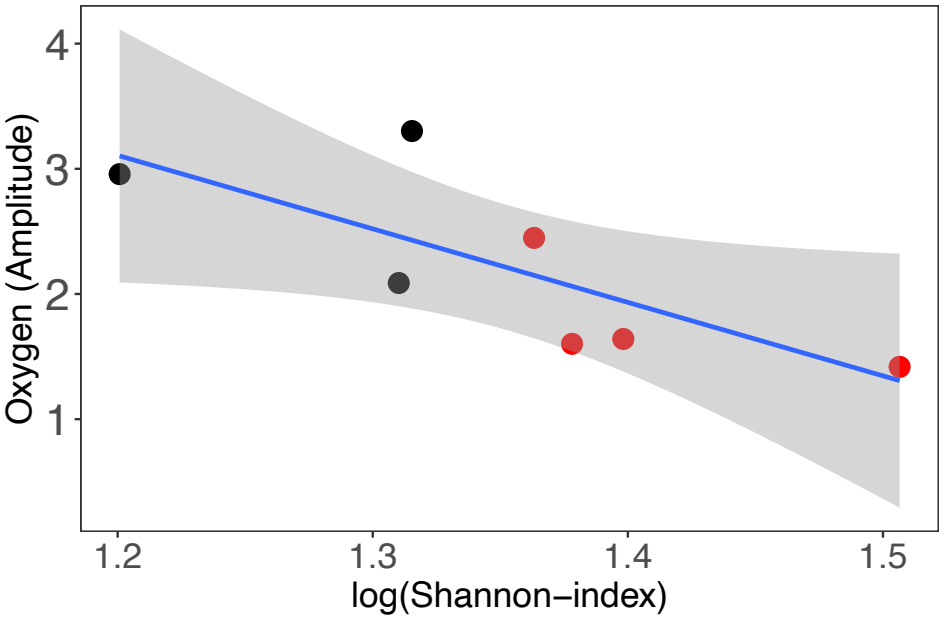
(a) Technical setup (exemplary cutout) of the micro-ecosystems and the automatic oxygen-measurements at the top and bottom sensors. Oxygen was measured in 5 min intervals. Samples were taken at day 8 (prior stressor sample), day 15 (stressor sample), day 19 (short-term recovery sample) and day 33 (long-term recovery sample) at height of the top and bottom sensor, respectively. (b) Control micro-ecosystems on day 15. (c) Stressor-applied micro-ecosystems on day 15. (d) Control micro-ecosystems on day 35. (e) Stressor-applied micro-ecosystems on day 35.



Supplementary Fig. S2 Microbial community composition of the micro-ecosystems. Relative abundance of microbial community composition (rel. abundance > 0.05 %) on class level for the top and bottom community of the prior-stressor sample (day 8), stressor-sample (day 15), short-term recovery sample (day 19) and long-term recovery sample (day 35).



Supplementary Fig. S3 NMDS analysis of the top and bottom communities of the prior-stressor sample (day 8), stressor-sample (day 15), short-term recovery sample (day 19) and long-term recovery sample (day 35). Stress is 0.16.



Supplementary Fig. S4 Relationship between the amplitude of daily fluctuations in oxygen concentration and Shannon diversity of top layer communities without the high amplitude-low diversity replicate in the long-term recovery sample (day 35). Black symbols represent controls, red symbols represent treated columns. Gray ribbons show the 95% confidence intervals. Pearson's product-moment correlation: p-value=0.047, correlation coefficients = -0.76.