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

Marcel Suleiman, Frank Pennekamp, Yves Choffat and Owen Petchey

Department of Evolutionary Biology and Environmental Studies, University of Zurich

Corresponding author: Marcel Suleiman, marcel.suleiman@uzh.ch, Tel: +41 44 635 4773

Competing interests

The authors declare no competing financial interests.

Abstract

Understanding how microbial communities as key drivers of global biogeochemical cycles respond to environmental change remains a critical challenge in microbial ecology. In this study, we used phototrophic oxic-anoxic micro-ecosystems to understand how aerobic and anaerobic lake analog communities responded towards stressful light removal. Continuous oxygen measurements and four snapshots of full-length 16S rRNA sequencing were performed to detect responses of oxygen concentration, and of alpha and beta diversity. In the top layer, oxygen concentration decreased significantly under light limitation, but showed almost complete resilience after normal light conditions were restored, while the bottom layer remained anoxic throughout the experiment. Microbial communities, however, differed in their response behavior: alpha-diversity of the aerobic communities showed a delayed response after light conditions were restored, and their composition was not resilient during the duration of the experiment. In contrast, alpha-diversity of the anaerobic bottom water communities increased due to the stressor and was resilient. Beta-diversity changed significantly during light removal, showed resilience for the aerobic communities, but stayed significantly affected for the anaerobic communities. We conclude that whole-ecosystem responses and several time-points are needed to fully understand the impact of stressors on microbial ecosystems, since resistance/resilience can differ among and within abiotic and biotic ecosystem components.

26 **Main text**

27 Microbial communities are critical components of any ecosystem, driving their development
28 and functioning since billions of years. Over these extreme time-scales, microbes have tackled
29 major transitions, such as the increasing oxygenation of Earth's atmosphere. The success of
30 microbial life is to a large degree explained by being highly adaptable to a variety of
31 environmental conditions [1, 2]. Even slight changes in conditions can lead to taxonomical and
32 functional community shifts, in turn being important indicators of microbial community
33 stability, or lack thereof [3]. Communities can react towards disturbances by showing
34 resistance, resilience, functional redundancy or alternate stable states [4]. Importantly,
35 observing these responses on the functional and compositional level can provide insights about
36 the potential mechanisms of microbial community stability [5].

37 To understand microbial response behavior of aerobic and anaerobic communities
38 within one ecosystem to a major disturbance, we study functional and compositional responses
39 to light removal in recently developed dynamic (phototrophic) oxic-anoxic micro-ecosystems
40 [6]. These micro-ecosystems are analogs of aquatic inland ecosystems, like lakes and ponds,
41 which harbor highly diverse functional groups of microorganisms that are dependent on light
42 for oxygenic and anoxygenic photosynthesis. These aerobic and anaerobic functional groups
43 are tightly connected through the oxygen state of the aquatic environment [7]. We analysed the
44 responses of aerobic and anaerobic aquatic communities (4 snapshots of 16S rRNA full-length
45 sequencing) in combination with high frequency and resolution measurements of the oxygen
46 state of the system.

47 Sediment and water samples were taken from a pond (Zurich, Switzerland, 47°23'51.2"N
48 8°32'33.3"E) and eight micro-ecosystems were set-up as reported previously [6]. In short, 1.5
49 cm sediment (with 0.5 % crystalline cellulose, 0.5 % methyl-cellulose, 1 % $CaSO_4$, 0.2 %
50 $CaCO_3$, 0.01 % $NH_4H_2PO_4$) were covered with 16 mL pond water and incubated for 35 days

at 24 °C under a light-dark-cycle of 16:8 h (gradient of light). Continuous non-invasive oxygen measurements (PreSens Precision Sensing GmbH, Germany) were performed every 5 min at the bottom liquid part (1.5 cm above sediment) and top liquid part (2 cm below surface) of each column (Supplementary Fig. S1a). After incubating the eight columns for eight days, four columns were covered tightly with aluminum foil and incubated in darkness for seven days (stressor-treatment). After the treatment, incubation continued with the standard light conditions (like control group) for another 20 days. 500 µL liquid sample were taken on day 8 (prior-to-stressor), day 15 (stressor-sample), day 19 (short-term recovery) and day 35 (long-term recovery), respectively, at the height of the top and bottom oxygen sensor. DNA extraction and 16S rRNA full-length sequencing (PacBio) were performed as reported previously [6]. Raw sequencing data were transcribed to Amplicon Sequence Variants (ASV) with Dada2 [8], and analysis of alpha- and beta-diversity were performed with the R packages phyloseq [9] and vegan [10]. Estimates of oxygen and microbial data were calculated by applying t-tests for each time/sample point. Scripts are available on zenodo (10.5281/zenodo.5195092) and raw reads are available on NCBI (PRJNA731625).

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

During the first week, the oxygen concentrations of the top layer of the eight micro-ecosystems increased during the light- and decreased during dark-phases, resulting in comparable behavior of the mean-, max- and min-oxygen concentration, as well as the amplitude (Fig. 1, day 0-8). During light removal, the disturbed columns showed a significant decrease in total oxygen concentration to microaerophilic conditions (Fig. 1b, day 8-15). After

normal light conditions were restored, oxygen concentration increased, showing resilience until the end of the experiment (Fig. 1c). Since the bottom layer turned completely anoxic within 2 days, no effect of light removal could be detected. A single replicate showed a higher oxygen concentration at the end of the experiment. Exclusion of that microcosm affected the estimated differences in oxygen concentration (i.e., resistance and resilience), but did not qualitatively change the results. Exclusion had no effect on the conclusions of the microbial dynamics.

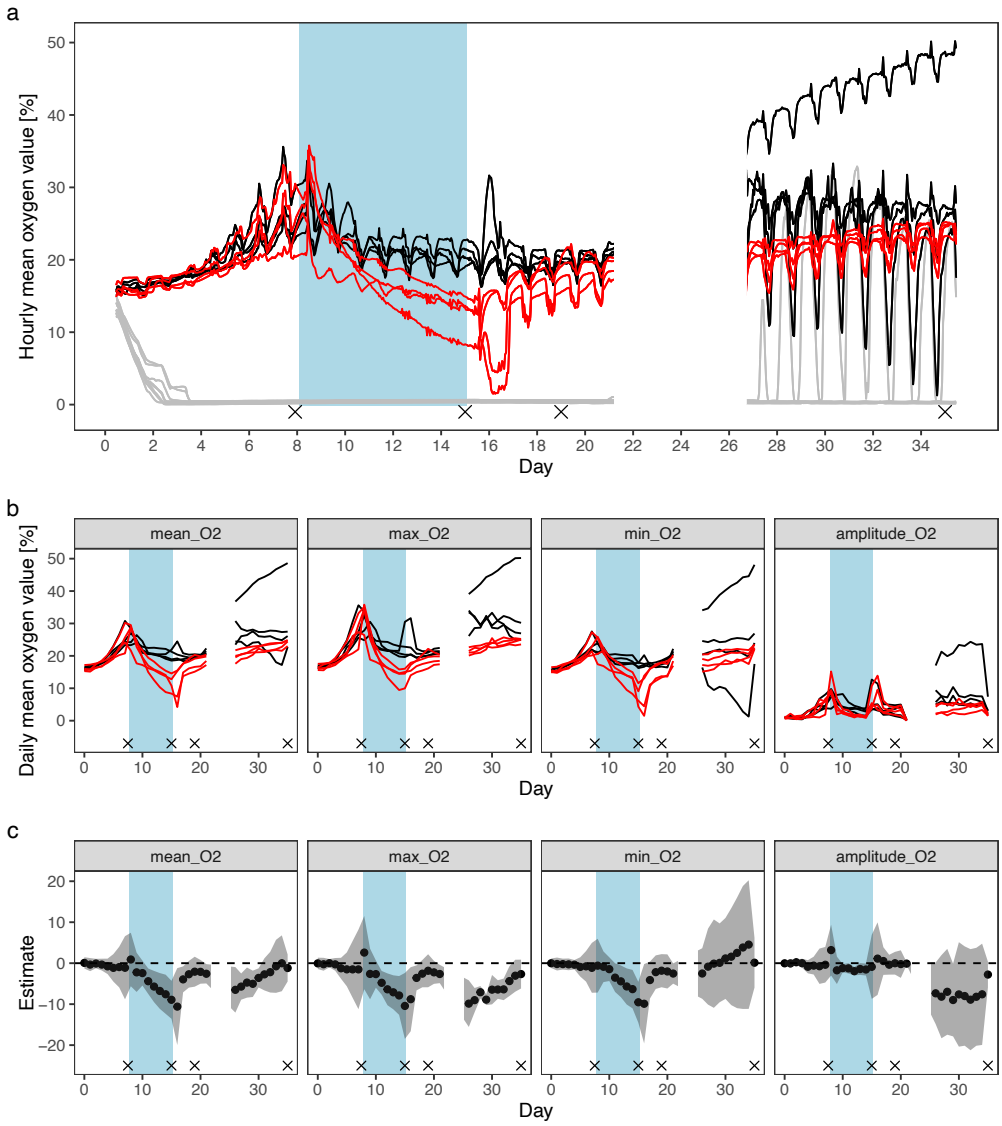

Fig. 1 Dynamics and functional response of oxygen in the micro-ecosystems. Oxygen concentration was recorded in 5 min intervals. **(a)** Hourly mean of the oxygen concentration of the top sensors (line) and bottom sensors (grey) of the eight micro-ecosystems. **(b)** Daily-mean oxygen concentration (illustrated as mean, max, min and amplitudes) of the top sensors of the eight micro-ecosystems. **(c)** Estimates (t-test per time-point) 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. Recording error caused lack of oxygen data from day 23-27.

Microbial alpha diversity was differentially affected by light removal in the top and bottom level (Fig. 2): The richness of anaerobic bottom communities was immediately increased by the stressor (Day 15, Fig. 2a+b), stayed significantly different after stressor-removal in the short-term recovery sample, but showed resilience in the long-term recovery sample. 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 recovery sample (Fig. 2c+d). While light removal caused a loss of biodiversity in the top water communities, it increased the biodiversity of the anaerobic bottom communities.

Non-metric multidimensional scaling revealed significantly different composition of top and bottom layer communities within a single micro-ecosystem, which, during light removal, converged in their respective columns (Supplementary Fig. S3). This convergence is most likely due to the loss of the light- and oxygen-gradient during the disturbance. This finding indicates how stressors can influence microbial communities directly 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 but showed resilience, while NMDS2 was affected later 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).

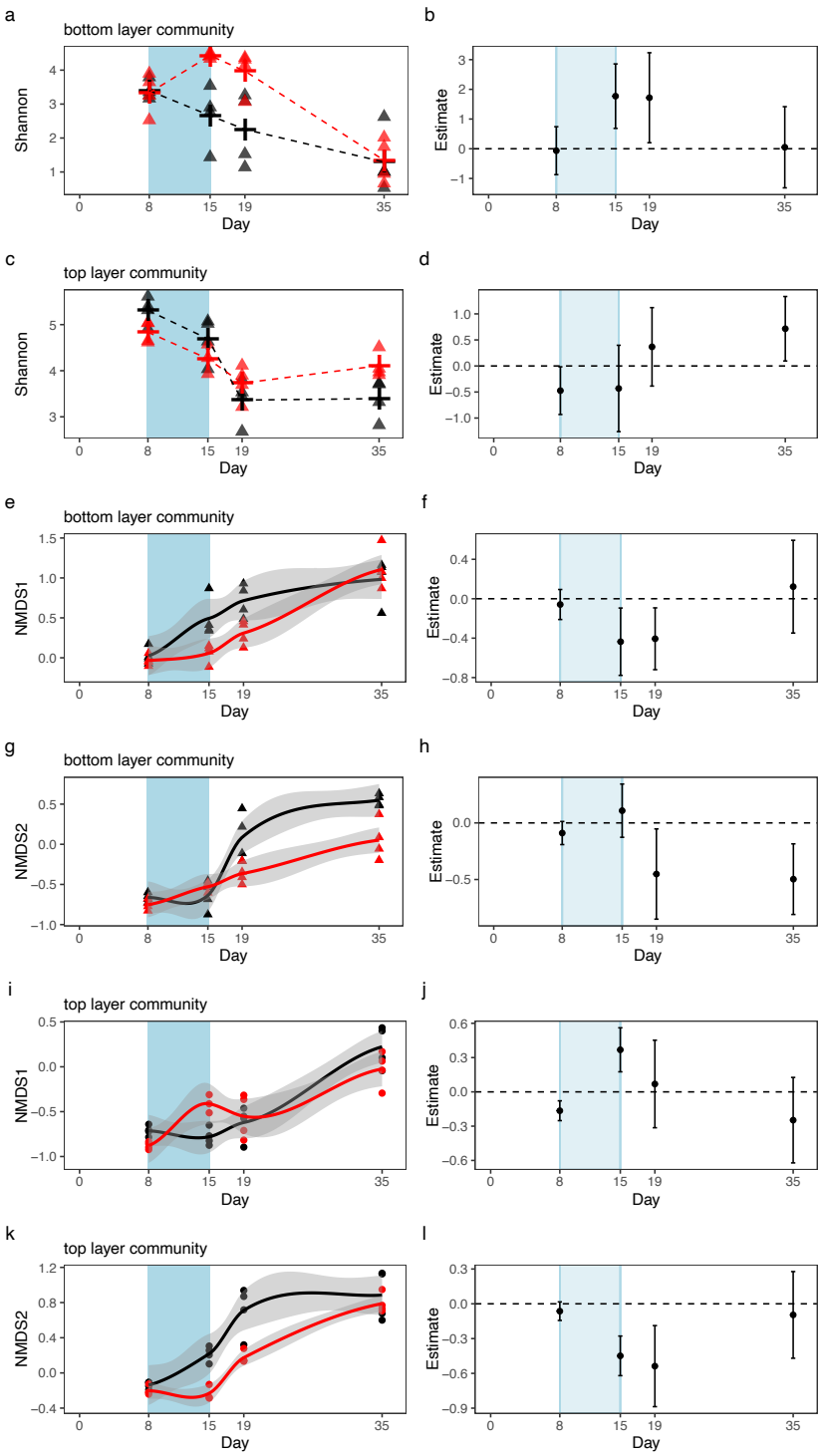

Fig. 2 Resistance and resilience of the microbial communities based on full-length 16S RNA sequencing. **(a)** Alpha-diversity based on Shannon index of the bottom water communities of the eight micro-ecosystems. Cross: mean. **(b)** Calculated estimates (t-test per time-point) as response parameters of 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)** Calculated estimates (t-test per time-point) as response parameters of 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)** Calculated estimates (t-test per time-point) of 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.

Our experiment showed that microbial community responses are multifaceted and a focus on just one aspect of community stability may be misleading, since a stressor can affect different types of stability [11, 12]. In addition, we revealed important aspects about the responses of microbial communities to light removal: (i) resistance and resilience of aerobic and anaerobic communities within one ecosystem can differ strongly (ii); differential effects may unfold through time and hence several time points of analysis are needed for making robust conclusions about microbial resistance and resilience (iii); direct stressors like light removal can trigger indirect stressor (decreased oxygen) whose effects could be more important than the direct stressor. In conclusion, fully grasping the multifaceted responses of diverse and spatially heterogeneous communities is needed to foresee the impact of stressors on microbial ecosystems.

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Competing interests

The authors declare no competing financial interests.

Author contributions

OP 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. OP, MS and FP d

rafted the manuscript. All authors confirmed the final version of the manuscript.

172 **References**

173 1. Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK, et al. Microbial
174 abundance and composition influence litter decomposition response to environmental
175 change. *Ecology* 2013; **94**: 714–725.
176 2. Evans SE, Wallenstein MD. Climate change alters ecological strategies of soil
177 bacteria. *Ecol Lett* 2014; **17**: 155–164.
178 3. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals
179 of microbial community resistance and resilience. *Front Microbiol* 2012; **3**: 1–19.
180 4. Allison SD, Martiny JBH. Resistance, resilience, and redundancy in microbial
181 communities. *Light Evol* 2009; **2**: 149–166.
182 5. Philippot L, Griffiths BS, Langenheder S. Microbial Community Resilience across
183 Ecosystems and Multiple Disturbances. *Microbiol Mol Biol Rev* 2021; **85**: e00026-20.
184 6. Suleiman M, Choffat Y, Daugaard U, Petchey OL. Large and interacting effects of
185 temperature and nutrient addition on stratified microbial ecosystems in a small,
186 replicated, and liquid-dominated Winogradsky column approach. *Microbiologyopen*
187 2021; **10**: e1189.
188 7. Bush T, Diao M, Allen RJ, Sinnige R, Muyzer G, Huisman J. Oxic-Anoxic regime
189 shifts mediated by feedbacks between biogeochemical processes and microbial
190 community dynamics. *Nat Commun* 2017; **8**.
191 8. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:
192 High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; **13**:
193 581–583.
194 9. McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive
195 Analysis and Graphics of Microbiome Census Data. *PLoS One* 2013; **8**.
196 10. 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.

11. 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.

12. 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.