OIST E&E PREreview Journal Club, "Frequency of disturbance alters diversity, function, and underlying assembly mechanisms of complex bacterial communities"

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Frequency of disturbance alters diversity, function, and underlying assembly mechanisms of complex bacterial communities

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Understanding the effects of disturbance on ecosystem function and diversity has many potential applications in microbial ecology and human disease biology. In this paper, the authors tackled the long-standing question of how different disturbance frequencies affect bacterial community diversity and function. To do so, activated-sludge communities within laboratory-scale microcosms were exposed to toxic 3-chloroaniline (3-CA) at varying frequencies. Ecosystem function and community diversity were measured weekly by measuring biomass and organic carbon, ammonia, and toxin removal as proxies for ecosystem function and T-RFLP 16S rRNA gene fingerprinting and shotgun metagenomics were performed to examine variation in bacterial diversity and community composition. This work is an excellent example of integrating genomic and functional analysis, thereby providing a more thorough understanding of the effects of disturbance frequency on microbial community diversity and function. Interestingly, both genetic methods yielded similar results, suggesting that the less expensive gene fingerprinting method could be sufficient when sequencing resources are limited. We particularly commend the use of multiple alpha-diversity measurements and the inclusion of abundance-related indices, which are less method dependent and allow results to be compared between studies. Ultimately, the authors propose the "Intermediate Stochasticity Hypothesis," which suggests that stochastic processes produce higher diversity assemblages at intermediate disturbance frequencies while deterministic processes produce lower diversity assemblages at low and high disturbance frequencies. Overall, this paper is a fascinating and substantial contribution to microbial ecology. There are, however, a few issues that we feel could be improved in future versions of the manuscript.

Major concerns:

This comment is unique to the preprint. The manuscript references multiple figures available in the sup-

plementary materials, but these materials were not made available as part of the preprint. This hindered our ability to understand the fine points of the experiments. We encourage the authors to upload the supplementary materials to bioRxiv.

1. Figure 2 is an integral figure to the manuscript because it showcases the effects of 3-CA disturbance frequencies on community performance, namely organic carbon and toxin removal (plots A, C) and nitrification products (plots B, D). In the Materials and Methods section (lines 353-356), the authors state that these parameters were measure weekly, which leads to the assumption that data is available for days 7, 15, 21, and 35, even though only the data from days 7 and 35 are included in the figure (is there T0 data?). The results section refers to supplementary figures S2 and S3 in addition to Figure 2, so these supplementals may portray the data of interest. However, since these data are so important to the overall conclusions, we believe it should be available in the main text. One way to accomplish this could be to have one plot per variable with time on the x-axis and different colors for each disturbance frequency. The number of plots could be reduced by not including Volatile Suspended Solids (VSS) results in the main text.

In Figure 2A, the COD removal and 3-CA removal is not monotonously decreasing relative to the disturbance frequency (specifically, level 2 and 4). We figured that this was due to the number of days since the disturbance being different for each disturbance frequency at measurement time on day 7. We encourage the authors to mention and explain this in the text, as this was a puzzling feature of the results for us for some time. It also calls into question the appropriateness of the weekly measurements, especially given that some disturbance level will be highly correlated to this rhythm of measurement (level 1 disturbance will always happen on the same day of the measurements, while level 2 and others will drift).

2. Along with disturbance frequency, varied intensity and duration of disturbance and differing sampling frequencies (e.g- data collection every two days or bi-weekly, larger spread of intermediate disturbance levels) might produce a different pattern of microbial community diversity and function. Questions we can ask are: would the system reach the observed IDH pattern at an early stage? Would the intermediate levels still follow the IDH model? We would be very interested in the authors opinions on these topics, perhaps in the discussion section.

Minor concerns:

1. When discussing disturbance frequencies and 'levels' throughout the manuscript, consistency of language is key. These different treatment 'levels' are misleading if described as disturbance levels since this description can be interpreted as disturbance intensity if not read carefully. Clarity of language surrounding disturbance manipulation is really important for specific understanding and placing the study in the wider context of studies of disturbance. We suggest changing 'levels' to frequency/ies' throughout.

2. We suggest including T0 data in the NMDS plot in Figure 1B. However, we were not able to understand why two different ordination methods were used in Figure 1 and suggest using only one method (NMDS or PCoA). The plot could be combined into one, if color represents disturbance frequency and shape represents time.

3. The frequency of measurements implies sampling with replacement (but this was not mentioned in the methods section), we would like to see a description of how replacement was achieved and discussion of what the impacts of replacement may have been. We are also interested in the implications of scaling up the microcosm size and varying initial conditions to reproduce and expand the experimental design for further work testing the new model.

4. Since the Results section appears before the Materials and Methods section in this manuscript, we suggest writing out the full names of abbreviated terms in the Results section so that readers can read sections in the order they appear and know what abbreviations represent.

5. The symbols and colors chosen for the figures made it difficult to interpret the figures in many cases. For example, in figure 3, L4 and L6 are both represented by light grey squares that are very difficult to discern in the legend and are not visible in the plot (the plot may have been changed without updating the legend?). To make it easier to interpret figures, we suggest choosing one color scheme for all figures and keeping the colors for each disturbance level consistent throughout all figures. Additionally, if points are overlapping, we suggest increasing the alpha (transparency) of the points. Finally, it would be significantly easier and quicker to interpret the figures if legends were incorporated into the figures.

6. Although, at several points in the paper, the authors reference the softwares used, and sometimes, the corresponding parameters, we would encourage the authors to share both the raw data (the performance indicators in addition to the raw sequences, which are available on NCBI) and the accompanying code used to analyze it (github or similar site). In some cases the citations are missing for the relevant packages or software. Sharing the code would shed light on the details of some procedures that are not made explicit in the manuscript, and increase the reproducibility of the experiment.

This comment is unique to the preprint. The layout of the preprint, which we understand is likely the result of the requirements of a submission format, makes understanding the figures rather challenging. For future preprint submissions, we encourage the authors to consider associating the figures with their titles and captions, and to put the figures inline, close to the relevant parts of the text.

Overall, it was a great pleasure reading this interesting and exciting work and we are extremely grateful that the authors posted it as a preprint on bioRxiv. We sincerely hope that our comments are useful to the authors and we look forward to reading the final version when it is published.

Very best wishes,

The OIST Ecology and Evolution Preprint Journal Club