

## Preview

# pH and buffering capacity: Fundamental yet underappreciated drivers of algal-bacterial interactions

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Understanding microbial interactions in native habitats has been difficult given the complexity of such environments. Using state-of-the-art microfluidics to examine >100,000 cultures of algae and bacteria across hundreds of media conditions, a study published in this issue of *Cell Systems*<sup>1</sup> found that environmental pH and buffering capacity are critical modulators of phototroph-heterotroph interactions.

Microbes are fundamental to life on Earth. Their influence spans from the global scale, where they drive essential biogeochemical cycles,<sup>2</sup> to the individual scale, where they play vital roles in plant, animal, and human health.<sup>3</sup> Although the importance of microbes has long been recognized, we are only beginning to more definitively understand how microbial interactions can be impacted by a shared environment.

Many microbial interactions are based on nutrient cross-feeding. For example, microalgae that are foundational to marine food webs engage in complex metabolic interactions with heterotrophic bacteria: algae often provide photosynthetic products to bacteria, which in turn provide iron, CO<sub>2</sub>, vitamins, and nitrogenous compounds to algae.<sup>4</sup> Together, algae and bacteria directly influence nutrient cycling and higher-level ecosystem functions, and elucidating how environmental conditions affect the interactions of these taxa is, ultimately, essential for understanding their impact on global primary production and ecosystem stability.

Environmental factors can have a strong influence on microbial interactions.<sup>5</sup> However, the dynamic and complex nature of microbial habitats poses significant challenges in studying their effects. Whereas top-down approaches to interrogating microbial systems face the challenge of understanding emergent complexity and integrating lower-level

system variables across higher scales, bottom-up approaches that focus on carefully examining a specific subset of interactions at a time are inherently too time-consuming for exhaustive characterization.<sup>6</sup> The vast number of environmental variables and conditions that characterize microbial habitats make it impractical to explore all possible combinations in great detail. Moreover, the need for sufficient replication to achieve statistically meaningful results adds another layer of experimental challenge.

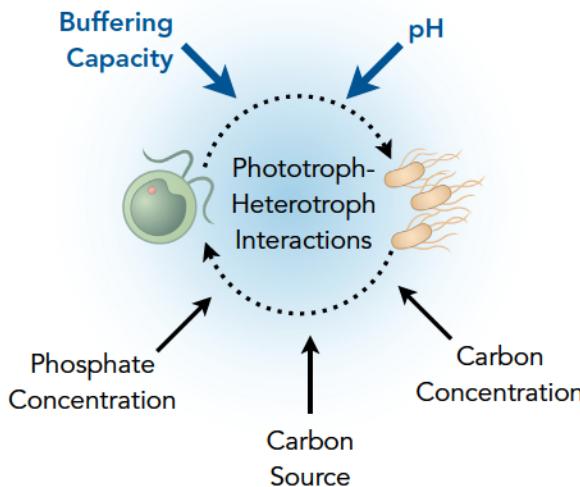
In this issue of *Cell Systems*, Gopalakrishnappa et al.<sup>1</sup> tackled these challenges by leveraging a high-throughput microfluidics platform and a simple regression model to investigate algal-bacterial interactions at an unprecedented scale, examining over 100,000 cultures across more than 500 environmental conditions. This approach enabled an extraordinary scope of analysis at a sufficiently granular level to deduce how specific environmental variables affect microbial dynamics. Their work reveals how key abiotic factors, particularly pH and buffering capacity (i.e., the ability of the environment to withstand changing pH), can shape the availability of nutrients central to cross-feeding interactions.

Using two well-studied microbial representatives of phototrophic and heterotrophic metabolic strategies, the unicellular alga *Chlamydomonas reinhardtii* and the bacterium *Escherichia coli*, the authors

explored how a range of pH values (6.1–7.5), buffering capacities (0–0.35 mM), and other environmental variables influence their interactions (Figure 1). pH is a primary environmental driver of bacterial community composition and is hypothesized to regulate nutrient availability in bacteria and modulate their abundance.<sup>7</sup> Microbes such as bacteria can “niche construct” and change the pH of their environment, which in turn can alter the growth of interacting partners.<sup>8</sup>

The authors found a general pattern showing that pH and buffering capacity strongly shape algal-bacterial interactions. For instance, at higher concentrations of glycerol, the bacteria grew well in conditions of lower pH and higher buffering capacity, which the authors refer to as a “permissive” environment. By contrast, bacterial yields were poor at higher pH and lower buffering capacity, a “stressful” environment. When co-cultured with algae, bacteria had growth yields that were greater under the stressful condition than under the permissive one. As pointed out by the authors, these results are consistent with the stress-gradient hypothesis, which posits that interactions tend to be beneficial in stressful conditions but competitive in nonstressful environments.<sup>5</sup> Higher buffering capacity could help to prevent pH fluctuations that derive from (1) bacterial carbon metabolism that may lead to acidification of the medium; (2) algal assimilation of ammonium, which also decreases pH; or





**Figure 1.** Graphic summary of the impact of environmental factors on the interaction between algae (*C. reinhardtii*) and bacteria (*E. coli*) studied by Gopalakrishnappa et al.<sup>1</sup> Among the variables studied (including carbon identity and concentration and phosphate concentration), pH and buffering capacity were found to critically influence algal-bacterial interactions. Additional environmental factors may further modulate these interactions.

(3) photosynthesis, which increases pH due to the assimilation of dissolved CO<sub>2</sub> in the medium. Thus, buffering capacity may be a fundamental environmental variable that shapes microbial communities. This idea remains largely unexplored but merits consideration in efforts to understand the structure and function of natural microbiomes, such as those associated with plant roots or the human gut.

Beyond this general pattern, the study revealed that the dependence of algal-bacterial interactions on pH and buffering capacity can be further modulated by the specific carbon source available. For instance, bacterial yields in co-cultures were higher with galactose than glucose as carbon concentrations and buffering capacity were increased. A hierarchical clustering of the carbon sources by their impact on co-culture growth revealed that glycerol is most similar to glucose and galactose is most similar to pyruvate; acetate (the only carbon source that can be used by the alga) showed no strong correlation with any other carbon source. While bacterial growth on glucose and glycerol significantly lowered pH, growth on galactose, pyruvate, and acetate did not impact pH. Thus, the authors concluded that bacterial carbon metabolism drives pH changes in co-culture, which could explain the critical influence of buffering capacity in the interaction.

All the aforementioned insights were based on a surprisingly simple statistical framework and linear regression analyses of the experimental data. It would be interesting to see how effective this straightforward approach might be in predicting the impact of environmental changes not easily accounted for in consumer-resource models of interspecies interactions in more complex communities and ecosystems.

Interestingly, the authors also found that co-culturing with algae consistently inhibited bacterial growth and resulted in a dispersal of bacterial aggregates. This phenomenon was previously reported,<sup>9</sup> yet the underlying mechanism remains unclear, but it may stem from the release of specific signaling molecules. An emerging view is that algae and bacteria produce interkingdom signaling molecules to modulate their interactions; these include acyl-homoserine-lactone-related bacterial quorum-sensing compounds, auxins, and extracellular polymeric substances.<sup>10</sup> Future studies focusing on the signaling pathways between algae and bacteria, particularly those that engage in interactions in the wild, may elucidate the underlying cross-talk mechanisms responsible for these general patterns of bacterial growth inhibition and aggregate dispersal by algae.

The use of droplet microfluidics allows for mass screening analysis. As

the authors rightly point out, it is possible that confinement within droplets may not accurately reflect the dynamics of interactions in open or well-mixed environments. Moreover, the permeabilities of such droplets to nutrients and metabolic products would be important experimental variables to characterize for new microbial systems to be studied using this technology. Future studies to benchmark results from microfluidics-based experiments against those from more traditional batch culture experiments will be important, although the former would be useful for screening purposes regardless. In addition to studying synthetic communities in a massively parallel fashion, it will be exciting to see to what degree Gopalakrishnappa et al.'s approach could be applied to reveal novel microbial interactions by reducing or subsetting complex natural microbiomes.

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#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### REFERENCES

1. Gopalakrishnappa, C., Li, Z., and Kuehn, S. (2024). Environmental modulators of alga-bacteria interactions at scale. *Cell Syst* 15, 838–853.e13. <https://doi.org/10.1016/j.cels.2024.08.002>.
2. Bertrand, J.-C., Bonin, P., Caumette, P., Gattuso, J.-P., Grégori, G., Guyoneaud, R., Le Roux, X., Matheron, R., and Poly, F. (2015). Biogeochemical Cycles. In *Environmental Microbiology: Fundamentals and Applications: Microbial Ecology*, J.-C. Bertrand, P. Caumette, P. Lebaron, R. Matheron, P. Normand, and T. Sime-Ngando, eds. (Springer Netherlands), pp. 511–617. [https://doi.org/10.1007/978-94-017-9118-2\\_14](https://doi.org/10.1007/978-94-017-9118-2_14).
3. Ma, L.-C., Zhao, H.-Q., Wu, L.B., Cheng, Z.-L., and Liu, C. (2023). Impact of the microbiome on human, animal, and environmental health from a One Health perspective. *Sci. One Health* 2, 100037. <https://doi.org/10.1016/j.soh.2023.100037>.
4. Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., and Kim, H.-S. (2016). Algae-bacteria interactions: Evolution, ecology and emerging applications. *Biotechnol. Adv.* 34, 14–29. <https://doi.org/10.1016/j.biotechadv.2015.12.003>.
5. Piccardi, P., Vessman, B., and Mitri, S. (2019). Toxicity drives facilitation between 4 bacterial species. *Proc. Natl. Acad. Sci. USA* 116, 15979–15984. <https://doi.org/10.1073/pnas.1906172116>.

6. Gralka, M. (2023). Searching for principles of microbial ecology across levels of biological organization. *Integr. Comp. Biol.* 63, 1520–1531. <https://doi.org/10.1093/icb/icad060>.
7. Zhalnina, K., Dias, R., de Quadros, P.D., Davis-Richardson, A., Camargo, F.A.O., Clark, I.M., McGrath, S.P., Hirsch, P.R., and Triplett, E.W. (2015). Soil pH determines microbial diversity and composition in the park grass experiment. *Microb. Ecol.* 69, 395–406. <https://doi.org/10.1007/s00248-014-0530-2>.
8. Ratzke, C., and Gore, J. (2018). Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS Biol.* 16, e2004248. <https://doi.org/10.1371/journal.pbio.2004248>.
9. Mickalide, H., and Kuehn, S. (2019). Higher-order interaction between species inhibits bacterial invasion of a phototroph-predator microbial community. *Cell Syst.* 9, 521–533.e10. <https://doi.org/10.1016/j.cels.2019.11.004>.
10. Burgunder-Delamare, B., Shetty, P., Vuong, T., and Mittag, M. (2024). Exchange or Eliminate: The Secrets of Algal-Bacterial Relationships. *Plants* 13, 829. <https://doi.org/10.3390/plants13060829>.