Dissecting Impacts of Host-microbiome Interactions on Phytoplankton Microbiome Assembly

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in the University of Michigan
2024

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Dedication

To my wife, Esther, who gave me love and strength to explore my life.

Acknowledgements

This accomplishment would not have been possible without the continuous encouragement and support from many people.

Thanks to my committee members, Vincent Denef, Annette Ostling, Donald Zack, and Luis Zaman, for their insightful feedback and encouragement. I am deeply grateful to my advisor, Vincent, for demonstrating patience, flexibility, and understanding. He helped me develop my abilities as a scientist, provided ample space to explore, always responded promptly whenever I needed help, and reminded me of the passion for science. I am grateful to Annette for her kindness, support, understanding, inspiring my interest in population and community ecology, and assisting with many fellowship applications. I am thankful to Don for helping me think precisely and critically. His compliments and support were invaluable to me. I am appreciative of Luis for his creativity and kindness, which has inspired me to think outside the box in fascinating ways. Engaging in research discussions with him has been both enjoyable and enriching. I also want to express my gratitude to my previous committee member, Fernanda Valdovinos, for her invaluable assistance in developing my research.

Special thanks to current and former members of the Denef and Duhaime labs: Dylan Baker, Eric Bastien, Rachel Cable, Nikesh Dahal, Melissa Duhaime, Jacob Evans, Madelyn Foulkes, Skylar Har, Sara Jackrel, Marvin Yu-Chen Lin, Morgan Lindback, Lizy Michaelson, Kathryn Schmidt, and AJ Wing, for bringing me joy, wisdom, and care over these six years. I am

also grateful to the Klausmeier-Litchman lab for opening their lab meetings to me, allowing me to learn and be inspired.

Deepest thanks to my parents, sister, and friends in the USA and Taiwan for all the joy and happiness they have brought into my life. I am also grateful for the research grants from the University of Michigan Department of Ecology & Evolutionary Biology, Rackham Graduate School, Integrated Training in Microbial Systems program, and Ministry of Education Taiwan that made this work possible.

All the support I received empowered me, ensuring my well-being, and allowing me to achieve this milestone.

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Abstract

Almost all eukaryotic organisms, from microscopic algae to humans, are host to closely associated communities of microorganisms, called microbiomes, that are important for host health. Host-microbiome interactions constitute a critical ecological process that impacts the functions of both the host and the microbial community, yet much remains to be learned about the nature, mechanisms, and impacts of these interactions. This dissertation aims to extend the current understanding of how host-microbiome interactions influence microbiome assembly by using phytoplankton as a model host system. It does so by dissecting the effects of innate host selection and host-microbiome feedback on phytoplankton microbiome assembly. Innate host selection implies that the microbiome is formed solely based on the phytoplankton-produced dissolved organic matter (DOM) composition as innately produced by the host in function of host species or physiological state. Conversely, host-microbiome feedback refers to how the microbiome's presence modifies host DOM production, thereby influencing ecological selection applied by the host on the microbiome. Throughout this dissertation, I utilized the freshwater microalga *Chlorella sorokiniana* and its associated microbiome as a model system. In Chapter 2, focusing on the overall microbiome impacts, I found that innate host selection and host-microbiome feedback led to divergent microbiome compositions while collectively maintaining microbiome richness. However, the host-microbiome feedback reduced microbiome evenness. These findings inspired the hypothesis that the two effects selected for different microbes: innate host selection favored passive symbionts thriving on host-derived DOM, whereas the host-

microbiome feedback favored active symbionts capable of triggering direct host-microbe interactions to their advantage. To test this hypothesis, Chapter 3 identified two bacterial species from *C. sorokiniana*'s microbiome that thrived better under different treatments: (i) solely innate host selection, representing passive symbionts, and (ii) in the presence of host-microbiome feedback, representing active symbionts. Further analysis of metabolic dynamics revealed that the active symbiont adjusted its metabolic status significantly between treatments, potentially synthesizing chemical signals to facilitate direct interactions with the host. Additionally, the host's gene expression profile indicated a higher expression of metabolic functions related to the biosynthesis of plant-microbe mutualistic interaction compounds (e.g., flavonoids) only when co-cultured with the active symbiont. In Chapter 4, expanding into environmental factors, I investigated how nutrient supply affects the impacts of innate host selection and host-microbiome feedback on microbiome assembly using a microbiome of seven phytoplankton-associated bacteria. The results showed divergent microbiome compositions between the two effects and among different nitrogen supplies. Particularly, the presence of host-microbiome feedback revealed a specific bacterium hindering host growth, resulting in only two bacterial species establishing the microbiome at the lowest nitrogen level. However, this antagonistic bacterium was suppressed by the host at the highest nitrogen level, allowing for a more diverse microbiome (six species). Overall, this dissertation demonstrated that microbiome assembly is shaped by both host selection and host-microbiome feedback, with the two effects selecting for different microbiomes that can be influenced by the external nitrogen supply levels.

Chapter 1 : Introduction

Most eukaryotic organisms harbor a specific collection of microbes that is distinct from the microbial communities in their surrounding environment. Emerging studies have shown a strong association between microbiome composition and host fitness in response to changing environments and competition, underscoring how the shaping of the microbiome would influence the health and survival of the host (Bjorbækmo et al. 2020; Apprill 2017; Hou et al. 2022; Voolstra et al. 2024; Cirri and Pohnert 2019). Host-microbiome interaction contribute to the biodiversity in natural ecosystems by creating unique ecological niches that sustain microbes with diverse symbiotic strategies and functions (Seabloom et al. 2023; Weiland-Bräuer 2021). This helps address classic ecological questions such as "How do ecosystems harbor diverse species?" and "How do species coexist?" (Sutherland et al. 2013; Kremer and Klausmeier 2013). From an evolutionary perspective, the co-evolution between hosts and specific microbes results in the species-specific association that determines host selection on its microbiome (Koskella and Bergelson 2020; O'Brien et al. 2019). Therefore, understanding how host-microbiome interactions shape the microbiome contributes to ecological and evolutionary studies, as well as applications for promoting and maintaining the health of most eukaryotic organisms. However, our understanding of host-microbiome interactions in microbiome assembly remains limited, especially for host organisms beyond humans and animals.

The important role of phytoplankton microbiome

My dissertation focuses on the host-microbiome system of phytoplankton. Phytoplankton are the foundation of aquatic ecosystems by being the principal primary producers (Field et al. 1998). Phytoplankton house their microbiomes in an area surrounding their cell(s), either attached or not, and this habitat is called the phycosphere. The dissolved organic matter (DOM) phytoplankton are known to release and serves as a rich nutrient and energy source for these microbes (Dow 2021; Seymour et al. 2017). In addition to its implicit importance, the phytoplankton-microbiome system can also serve as a model to understand host-microbiome systems in which the microbiome is shaped through host secretions, and where the microbiome is exposed to an open environment. Examples are plant microbiomes, skin microbiomes, and coral microbiomes (Voolstra et al. 2024; Byrd, Belkaid, and Segre 2018; Müller et al. 2016).

Contrasting bacteria-free phytoplankton to the same phytoplankton with a microbiome recruited from natural bacterial communities has shown that the microbiome affects phytoplankton competition fitness (Jackrel et al. 2020), growth rate and carrying capacity (Lian, Baker et al. 2022), adaptation to the changing environment (Ahern et al. 2021; Costas-Selas et al. 2024; Astafyeva et al. 2022) , and the chemical compounds of phytoplankton cells (Fuentes et al. 2016; Krohn et al. 2022) exemplifying the significant influence of microbiomes on their phytoplankton hosts. Given their important role in natural ecosystems and ease of use in laboratory and field experiments, phytoplankton has a long history as a model system in ecology, including for understanding how the microbiome affects host physiology (Olofsson et al. 2022; Costas-Selas et al. 2024) and interactions between hosts (Jackrel et al. 2020). Considering the important role of phytoplankton in food web and nutrient cycle functioning (Field et al. 1998; Litchman, Edwards, and Klausmeier 2015), and the impact of the microbiome on phytoplankton functioning, a better understanding of what shapes phytoplankton microbiome composition is

important. Such insights may also have practical implications as phytoplankton are used as a feed source, biofuel precursor, potential medicine source, and agent in wastewater treatment. Indeed, a variety of studies have pointed out the impact of the microbiome on outcomes in industrial applications (Lian et al. 2018; Krohn et al. 2022; Morris et al. 2022).

Phytoplankton-bacteria interactions and its impact on phytoplankton microbiome assembly

The center of phytoplankton-bacteria interaction is the exchange of metabolites. Phytoplankton fix carbon dioxide from the atmosphere and release part of the biomolecules they synthesize in the form of DOM to the environment. There it serves as a critical carbon and energy resource for microbes (Seymour et al. 2017). In return, bacteria inhabiting the phycosphere and using DOM, can re-mineralize phytoplankton waste products, regenerating CO_2 and dissolved nutrients in a form available for phytoplankton uptake. In addition, bacteria can provide a range of growth factors such as vitamins and growth hormones, antibiotics to suppress other bacteria, as well as factors that negatively impact the host (Cirri and Pohnert 2019; Ramanan et al. 2016). The composition of the DOM determines which microbial groups can be established in the phytoplankton microbiome based on bacterial ability to import and metabolize the DOM. Phytoplankton DOM composition varies among species and genotypes (Ahern et al. 2021; Cordone et al. 2022; Jackrel et al. 2021a; Becker et al. 2014) and can change during different growth phases and environmental stressors (Thornton 2014; McNabney et al. 2023; Aigner et al. 2022). Emphasizing the importance of DOM composition for microbiome assembly, varying microbiome composition was also found to be associated with these phytoplankton biological properties (Sison-Mangus et al. 2016; Costas-Selas et al. 2024). These

studies suggest that intrinsic host factors dominate the process of microbiome assembly to support the host's needs.

However, the microbiome itself can impact microbiome composition as well. Specifically, the presence of some specific microbes often plays a disproportionate impact on the microbiome composition. The most explicit evidence of microbiome-directed microbiome assembly came from examples in which the presence of a microbiome triggered the phytoplankton host to alter its physiology and select beneficial bacteria over other microbial members (Shibl et al. 2020; Blifernez-Klassen et al. 2021). This suggests that some microbes can alter host-mediated selection of the microbiome. In the phytoplankton-bacteria system, these specific microbes may include members with the ability to synthesize plant growth hormones, cofactors (e.g., siderophores and vitamins), or other chemical signaling molecules (Dow 2021; Droop 2007; Seymour et al. 2017; Zhu et al. 2022; Cirri and Pohnert 2019). These microbes do not rely on phytoplankton DOM that is innately produced by the host in the absence of bacteria, the composition of which depends solely on phytoplankton biological properties. Instead, these groups of microbes exhibit a direct interaction with the phytoplankton with signaling molecules, triggering the emergence of host-microbiome feedback, which leads to a modified composition of the microbiome to their benefit (Shibl et al. 2020; Olofsson et al. 2022).

While studies focused on both innate host control and to a lesser extent host-microbiome feedback have been carried out, we lack studies dissecting their impact on microbiome assembly simultaneously. It is important to understand the interplay between these two effects, because they appear to select for different functional groups of microbes that can lead to different microbiome compositions and functions. Moreover, each effect may respond differently to a changing environment and thus a better understanding of the various forces shaping microbiome

assembly is important to predict the implications of a changing environment. By contrasting these two forces in microbiome assembly, we can start to understand the mechanisms that underpin host-microbiome interactions, which opens the door to better ways to manage microbiomes and their impacts on their hosts. We presume that innate host selection would favor bacteria able to consume innately produced host DOM while the host-microbiome feedback effect would select for those microbes with the ability to synthesize secondary metabolites (e.g., hormone and vitamins) and other signaling chemicals to interact with the phytoplankton host. Taking advantage of the planktonic feature of phytoplankton, innate host selection can be separated from host-microbiome feedback by separating bacteria-free phytoplankton cells from their spent medium that is rich in the phytoplankton-produced DOM. This can be challenging in the other systems for which our system serves as a model.

Finally, most host-microbiome interaction studies focus either on specific pairwise host-microbe interactions with detailed physiological and metabolic understanding, or on the broader impact of various factors on the whole microbiome taxonomic composition but often without delving into the underlying mechanisms (Mars Brisbin et al. 2022; Droop 2007; Dow 2021). Integrated studies that bridge the linkage between populations and complex communities, as well as between selection on microbiome taxonomy and functions are needed.

The conditional host-microbe interactions

Determining how host and microbe interact is one of the keys to understanding its impact on microbiome formation; however, host-microbe interactions can be context-dependent (Gould et al. 2018; Sharp and Foster 2022). That is, the kind of host-microbe interactions (e.g., mutualistic, agnostic, or commensalism) and the strength and tightness of the interactions can be

altered by other factors, such as the physiological state of the partners (Wang et al. 2015), and the change in environmental conditions (Cheng, Zhang, and He 2019; Ahn and Hayes 2021). This increases the challenge of predicting the microbiome composition, especially in dynamic natural systems. Studies on the impact of environmental change on host-microbiome interactions and microbiome formation are merging, particularly in the context of human-caused global change. In the phytoplankton microbiome system, some research suggests that elevated temperatures enhance the mutualistic relationship between phytoplankton and bacteria (Arandia-Gorostidi et al. 2022). Conversely, other studies indicate that warming disrupts these mutualistic indications due to nutrient limitations caused by increased algal bloom formation under warmer conditions (Cordone et al. 2022). Nutritional status also plays a critical role in shaping phytoplankton-bacteria interactions. Mutualistic phytoplankton-bacteria relationships are ubiquitous in oligotrophic environments, where such interactions confer higher fitness for survival (Hernandez et al. 2009; Gonzalez and Bashan 2000). Conversely, limited nutrition can lead to competition between phytoplankton and bacteria (Cao et al. 2020; Bertrand et al. 2015), while strong mutualistic interactions are observed under high-nutrient conditions (Danger et al. 2007; Ramanan et al. 2016). The environmentally triggered shift in phytoplankton-bacteria interaction could therefore have different impacts on innate host selection and host-microbiome feedback, and how each of them shapes the microbiome composition under changing environmental conditions. Namely, innate host selection would be more affected by the direct environmental effect on the host and its produced DOM composition, while the host-microbiome feedback involves shifts in the resulting intrinsic host selection, shifts in the composition of the environmental microbiome, and shifts in the biotic interactions in function of changing environmental conditions.

Overview of dissertation

My dissertation explored the impact of two host-microbiome interaction effects on both whole microbiome assembly and selection on specific functional groups of bacteria, as well as considering their impact in the context of a variable environment.

In **Chapter II**, I investigated whether innate host selection and host-microbiome feedback effects exhibit the same or different impact on microbiome assembly. Specifically, I exposed the phytoplankton microbiome to varying degrees of the relative impact of these two effects and examined the consequences of microbiome taxonomic composition and diversity. In **Chapter III**, I focuses on the interactions of phytoplankton hosts and two bacterial symbionts with distinct growth patterns under innate host selection and host-microbiome feedback effect. I examined both phytoplankton and bacteria physiological (growth) and metabolic status (gene expression) to explore evidence for the underlying mechanism of each effect selected for the different functional groups of microbes. Finally, in **Chapter IV**, I investigated the impact of the interplay between nutrient supply and two host-microbiome interaction effects on shaping the composition of a synthetic microbiome, which was composed of seven phytoplankton-associated bacteria, as well as how community-level dynamics could be predicted by either the fitness of the individual bacteria or their individual fitness effect on the host.

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Chapter 2 : Dissecting Host-microbiome Interaction Effects on Phytoplankton Microbiome Composition and Diversity[1]

Abstract

Phytoplankton and their associated microbiomes of heterotrophic bacteria are foundational to primary production, energy transfer, and biogeochemical cycling in aquatic systems. While it is known that these microbiomes are shaped by host-released dissolved organic matter (DOM), the extent to which dynamic phytoplankton-bacteria interactions shape bacterial community assembly remains to be examined. Here, we investigated the effects of two mechanisms in host-microbiome interactions on phytoplankton bacterial microbiome formation: (i) innate host selection and (ii) host-microbiome feedback. For the former, phytoplankton-produced DOM composition is based solely on the host's properties (species or physiological state); for the latter, the presence of the microbiome modifies host DOM production. The microbiome of *Chlorella sorokiniana* was extracted and exposed to six ratios of the two effects. We found that microbiome composition changed along with the six host-microbiome feedback versus innate host selection ratios, with the highest compositional distance between communities under the strongest and the weakest ratio of the two effects. This indicates that each mechanism selects for different bacterial species. In addition, our findings showed that when both selective

[1]Published as: Yang JW, Denef VJ. Dissecting host-microbiome interaction effects on phytoplankton microbiome composition and diversity. *Environ Microbiol* 2024; 26(2):e16585.

forces were applied, it led to a higher community richness, while host-microbiome feedback alone reduces community evenness due to its strong species-specific selection.

Introduction

Host-microbiome interactions are ubiquitous and critical to both host health and the functioning of the associated bacterial microbiome. In terrestrial systems, evidence is mounting that bacterial microbiomes can fundamentally change plant or animal host fitness (Lau and Lennon 2012; Rosshart et al. 2017), physiology and behavior (Coleman-Derr and Tringe 2014; Sampson and Mazmanian 2015), and even competitive and trophic interactions (Oliver, Smith, and Russell 2014; Siefert et al. 2018). In the aquatic systems we focus on, bacterially dominated microbiomes of phytoplankton are a key system of interest as they modify phytoplankton productivity, competitive interactions, and survival (Cirri and Pohnert 2019; Jackrel et al. 2021; Schmidt et al. 2020; Seymour et al. 2017). It is important to consider the impact of interactions between phytoplankton and their microbiomes on plankton community assembly as (1) the role of phytoplankton as primary producers means effects of host-microbiome interactions on host community dynamics can cascade through the ecosystem, and (2) bacteria are the main driver of decomposition, nutrient dynamics, and energy flow (Azam et al. 1983), and as such any effects from phytoplankton-bacteria interactions on bacterial community assembly could lead to ecosystem-level impacts.

Across all systems, while bacterial community assembly has been extensively focused on (Nemergut et al. 2013), our understanding of the impact of host-microbiome interactions on microbiome assembly remains limited, which we aim to address using phytoplankton-bacteria symbioses as our study system. At the center of the interactions between phytoplankton species

and their bacterial microbiomes is the exchange of metabolites. Phytoplankton produce and release dissolved organic matter (DOM) that sustains its microbiome as well as parts of the bacterioplankton community. Bacterial community composition shifts associated with changes in phytoplankton community composition, species, genotypes, and bloom phase (Tada et al. 2017; Zhou et al. 2018; Pinhassi et al. 2004; Kimbrel et al. 2019) have been suggested to relate to differences in phytoplankton-produced DOM availability. These field observations have been supported by laboratory experiments that showed a bacterial community composition shift upon the introduction of different phytoplankton species or different phytoplankton-produced compounds (Tada et al. 2017; Fu et al. 2020; Kieft et al. 2021; Kimbrel et al. 2019).

While host control is critical in shaping the bacterial community, it is also well-known that the bacterial microbiome can influence the production and composition of phytoplankton-produced DOM. As indirect evidence, phytoplankton symbiotic bacteria were found to promote phytoplankton growth through synthesizing growth-promoting factors (e.g., siderophores, vitamins, and hormones) and increase phytoplankton nutrient availability via nutrient remineralization (Ramanan et al. 2016; Mühlenbruch et al. 2018). More direct evidence for this idea is that the presence of phytoplankton-associated bacterial isolates or a natural bacterial community can trigger a change in phytoplankton metabolism and the composition of DOM that they produce (Bruckner et al. 2008; Seyedsayamdost et al. 2011; Sison-Mangus et al. 2016; Uchimiya et al. 2022). Such changes in phytoplankton DOM composition also led to the selection of beneficial bacteria (Shibl et al. 2020). In this case, a feedback loop between the host and the microbiome changes what the innately produced DOM's effect on microbiome community assembly would otherwise have been. These results suggest that microbiomes may alter the ecological selection of their own community by interacting with the phytoplankton host.

Although both host selection that depends on the biological properties of the host (e.g. host species, genotypes, or growth phase) and the host-microbiome feedback (resulting from host-bacteria interactions) are often mentioned as important mechanisms in phytoplankton bacterial microbiome formation, we lack explicit tests examining the impact from each mechanism simultaneously. As a first step to address this question, we exposed a phytoplankton-associated bacterial community, after dissociation from its host, to culture conditions at six degrees of initial host-microbiomes feedback effect relative to innate host selection (hereafter feedback-to-innate index) and investigated the consequences on the composition and diversity of the phytoplankton microbiome community. We used *Chlorella sorokiniana* and its microbiome as a model study system due to *C. sorokiniana* rapid growth, ease of cultivation, and extensive use for ecological and industrial research (Steichen et al. 2020; Ziganshina et al. 2022). We hypothesized that host-microbiome feedback and host-innate selection select different bacterial species, resulting in the phytoplankton microbiome having a different community composition in function of the feedback-to-innate index applied. This is because host-microbiome feedback would select for bacterial taxa that influence the host-produced DOM composition through direct host-microbe interactions, creating a feedback loop that alters bacterial composition, while host-innate selection would favor strong competitors on the innately produced phytoplankton DOM. Furthermore, we aimed to identify the key phytoplankton-associated taxonomic groups that were more or less favored by the two ecological selection mechanisms. Finally, we predicted a higher alpha-diversity (richness, Shannon diversity, and evenness) under intermediate feedback-to-innate indices as a result of a higher possibility of coexistence and more even composition for different bacterial functional groups (host-DOM influencer versus strong competitors on innate host-DOM).

Materials and Methods

Feedback-to-innate manipulation experiment

The axenic and xenic *Chlorella sorokiniana* culture (UTEX 2805) were rendered from 2017 (Schmidt et al. 2020) and in 2018 (Jackrel et al. 2021), respectively. The first step was to extract the microbiome from the xenic *C. sorokiniana* culture we generated above (Step 1 in **Figure 2.1**). The starting xenic *C. sorokiniana* culture was subsampled and collected for 16S rRNA gene sequencing as the original phytoplankton microbiome (two replicates). Then, the extracted microbiome was inoculated to culture with six feedback-to-innate indices. To create six feedback-to-innate indices (**Figure 2.1** Step 2), an axenic *C. sorokiniana* culture was separated into the axenic host (resuspended in fresh culture medium) and host-produced DOM (without phytoplankton cells). We then created six feedback-to-innate indices by mixing 0-ml and 50-ml, 2.5-ml and 47.5-ml, 12.5-ml and 37.5-ml, 25-ml and 25-ml, 37.5-ml and 12.5-ml, and 50-ml and 0-ml of the host cells only and the host DOM only medium, respectively; which represented the 0%, 5%, 25%, 50%, 75%, and 100% feedback-to-innate index, respectively. The 100 % indicates the ratio relative to the strongest host-microbiome feedback (the highest initial host density) that we can create in this experiment. This is with an assumption that, with a given microbiome density, a higher axenic host density would lead to a stronger host-microbiome feedback effect due to a higher host-bacterial encounter rate (a higher host-bacteria density ratio). All treatments were conducted in five replicates. After microbiomes (extracted in Step 1) were inoculated into six feedback-to-innate indices (which were created in Step 2), we prevented the effects from accumulated metabolites, nutrient depletion, and ensured phytoplankton remained in the exponential growth phase during incubation, by transferring 5% of 2-day-old cultures to fresh medium with the same feedback-to-innate indices every other day until day 12 (**Figure 2.1** Step

3). Fresh axenic *C. sorokiniana* cultures were prepared for creating fresh media at each of the six feedback-to-innate indices for each transfer to avoid any effects of DOM or cell storage. We note that in an effort to maintain the intended ratio of selective effects we reset media conditions every two days and set the initial phytoplankton density low enough so that phytoplankton remained in the exponential growth phase and did not reach steady state during the two-day period between condition resets (**Figure SI 2.1**). However, we acknowledge that consumption of innately produced DOM and phytoplankton growth likely increased host-microbiome feedback effects in the two days between condition resets. Similarly, some accumulation of DOM in the 5% of the old culture that is used as the inoculum for the transfer, means we cannot achieve a full 100% initial host-microbiome effect after the start of the experiment. Nonetheless, a gradient of effect ratios is maintained through the experiment. A total of 187 microbiome samples were collected, including the original xenic *C. sorokiniana* culture (before dissociation of the microbiome), a microbiome sample from each of the six feedback-to-innate indices at day 0, and 180 samples from day 2 to day 12 (six feedback-to-innate indices with five replicates and 6 collections). The detailed protocol of this experiment is provided in Appendix A: Supplementary information for Chapter 2.

Sequencing and bioinformatics

Each pellet was resuspended in 90-µl 1x PBS (0.2-µm filtered and autoclaved). We added 5-µl lysozyme solution (50 mg/ml) and 100-ul Qiagen ATL buffer and incubated at 37 °C for 30 min, followed by the protocol of the Qiagen DNeasy Blood & Tissue Kit Qiagen, Hilden, Germany). The extracted DNA samples were sent to the University of Michigan Center for Microbial Systems to carry out 16S rRNA gene library preparation and sequencing. The V4

region of the 16S rRNA gene was amplified with 515F/806R primers (Walters et al. 2016) and sequenced on a 2 x 250 Illumina MiSeq v2 run. RTA V1.17.28 and MCS V2.2.0 software were used to generate data. Raw fastq files were uploaded on NCBI sequence read archive with BioProject number PRJNA941033.

Raw sequences were processed with DADA2 (Callahan et al. 2016) (with q2-dada2 v1.20.0) by using the Quantitative Insights Into Microbial Ecology 2 (QIIME 2 v2021.11) pipeline (Bolyen et al. 2019). The primers were removed (the forward and reverse primers were trimmed at position 19 and 20, respectively), forward and reverse reads were trimmed at positions 220 and 160, respectively, where the mean quality score start to drop below 30, followed by errors correction, merging read pairs (at least 12 bases overlapped), removing possible PCR chimeras (consensus method), and generating the ribosomal amplicon sequence variants (ASVs) representative sequences and tables. We assigned taxonomy with a pre-trained Naive Bayes classifier that was trained on Silva 138 99% OTUs from 515/F806R region of sequences (Quast et al. 2013; Bokulich et al. 2018; Robeson et al. 2020). ASVs classified as "Bacteria_unclassified", chloroplast, and mitochondrion were removed. The filtered representative sequences were then blasted against the NCBI database (blast+ v2.12.0; (Camacho et al. 2009)) and removed ASVs containing the keywords chlorella, mitochondrion, and chloroplast in the NCBI descriptions. Finally, we removed microbiomes with fewer than 2,000 bacterial reads to ensure a robust analysis of community composition (for the majority of samples, the number of ASVs levels off at the 2,000-read cut-off in the rarefaction curve; **Figure SI 2.2**). This left 178 communities to be used in the subsequent analyses.

Statistical analysis

The principal coordinate analysis based on Bray-Curtis distance across all microbiome communities was used to reveal microbiome composition change along the feedback-to-innate indices and times. The ASV table was subsampled 100 times at 2,000 reads to calculate the mean Bray-Curtis distances between each pair of communities. The first two axes of the Principal Coordinates Analysis (PCoA) ordination based on the Bray-Curtis distance were calculated, where the microbiomes of each sampling day were plotted in separate panels (**Figure 2.2**). The analyses above were calculated by using the "vegan" version 2.6-1 R package (Oksanen et al. 2022). This allowed us to evaluate how microbiome compositional distance among different feedback-to-innate indices changed with time. To identify potential key bacterial species in the host-microbiome interactions, the Pearson's correlation coefficient with Bonferroni correction on p-values between each ASV's relative abundance versus increasing feedback-to-innate indexes was conducted. In the main results, we only included ASVs that were found in the original microbiome, with a significant response (Pearson's correlation coefficient, $p < 0.05$) and more than 1% of total reads across 30 microbiomes collected on Day 12 after rarefaction at 2,000 reads (equivalent to > 600 reads). Finally, for estimating community alpha diversity, the ASV table was rarefied at 2,000 reads to measure observed richness, evenness, and Shannon diversity indices. Richness was calculated by summing the number of ASVs for each sample. Evenness was calculated by dividing the inverse Simpson index by richness. The first and second polynomial regressions were conducted to evaluate the relationships of each diversity index versus the feedback-to-innate index. The first and second polynomial regression, Pearson correlation coefficient analysis were conducted by using the "stats" version 4.2.2 R package (R

Core Team, 2022). R scripts and data are provided online (https://github.com/jinnyyang/Feedback-Innate-exp).

Additional experiment assessing the assumption made in the feedback-to-innate manipulation experiment

An additional experiment was conducted due to two concerns from the feedback-to-innate manipulation experiment. Firstly, increasing the feedback-to-innate index, which was manipulated by increasing the relative volume of washed phytoplankton host resuspended in the fresh medium to innate host DOM, not only increased the host-microbiome feedback effect but also the dilution of innate host DOM by fresh medium. Thus, any changes in microbiome composition may not be driven by the relative impact of the two tested mechanisms (host-microbiome feedback versus innate host selection) but simply due to the dilution of innate host DOM. Therefore, we tested if microbiome assembly showed a divergent response to the dilution on innate host DOM with fresh medium versus increasing feedback-to-innate index. The microbiome was extracted from a xenic *C. sorokiniana* culture, inoculated into the following treatments, and followed by semi-continuous incubation (collected and transferred every two days): (i) 100% innate host DOM, (ii) two degrees of dilution on innate host DOM with fresh medium (50%-to-50% and 95%-to-5% of fresh medium-to-innate host DOM), and (iii) two feedback-to-innate indices (50%-to-50% and 95%-to-5% of washed phytoplankton-to-innate host DOM).

Secondly, we used the original microbiome associated with our cultures of *C. sorokiniana* as a proxy of the microbiome when under natural host-microbiome interaction conditions. This was based on the assumption that the original microbiome, even after being

dissociated from its host and growing under semi-continuous incubation, can return to its original assembly of the microbiome in the presence of an originally axenic *C. sorokiniana* culture. To test this assumption, we inoculated the microbiome extracted from the xenic *C. sorokiniana* culture into axenic *C. sorokiniana* and maintained it using the same semi-continuous incubation procedure as the main experiment to mimic regular host-microbiome interaction conditions. This means that every two days, 5 % of the culture was transferred to axenic *C. sorokiniana* growing in COMBO medium, but without the steps separating the phytoplankton from the innately produced DOM. An illustration of this additional experiment is shown in **Figure SI 2.8**.

Results

Phytoplankton microbiome compositional distance under six feedback-to-innate indices

In **Figure 2.1**, the compositional distance between different feedback-to-innate indices increased with time and stabilized at Day 6, where the community composition showed a continuous change in the function of increasing feedback-to-innate indices, with a largest compositional distance between communities subjected to 100% and 0% feedback-to-innate indices. This pattern remained consistent at the end of the experimental period on Day 12. When comparing the microbiome compositional differences among feedback-to-innate indices on Day 12, pairwise PERMANOVA showed no significant difference between microbiomes under 0% and 5% as well as 50% and 75% feedback-to-innate indices (pairwise PERMANOVA p-values = 0.27 and 0.12, respectively; **Table SI 2.1**). In addition, we found significant differences between microbiomes between 25% and 50%, as well as between 25% and 75% feedback-to-innate indices (pairwise PERMANOVA p-value = 0.007 and 0.009, respectively; **Table SI 2.1**). When taking the original microbiome into account, we found that it was significantly different from

0%, 5%, and 100% feedback-to-innate indices (pairwise PERMANOVA p-value = 0.046, 0.045, and 0.042, respectively; **Table SI 2.1**) but not significant difference from microbiomes under 25%, 50% and 75% feedback-to-innate indices (pairwise PERMANOVA p-value = 0.059, 0.053, and 0.059, respectively; **Table SI 2.1**). We acknowledge the potential effect of other photoautotrophs (e.g., cyanobacteria) on our findings. However, we only found a very small portion of reads from Cyanobacteria (only 5 reads across all samples after 2,000-read rarefaction). Hence, we assumed little impact from these photoautotrophs in our study.

Identifying key taxa of the phytoplankton microbiome that were favored by host-microbiome feedback versus innate host selection

Firstly, we analyzed the original xenic *C. sorokiniana* microbiome composition on the D5 level classification based on the SILVA database (genus level). The top 10 taxa (summing across multiple ASVs with the same taxonomic classification) in the original host-associated microbiome after rarefaction at 2,000 reads were *Ottowia* (occupied 26.9% of reads), *Sphingobacteriales* NS11-12 marine group (13.9%), *Novosphingobium* (12.4%), *Chitinophagaceae* (8.2%), *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (hereafter *A-N-P-R; 7.4%)*, *Bacillales* (7.1%), *Methylophilus* (4.4%), *Cyclobacteriacea* (3.7%), *Neoasaia* (2.6%), and *Alphaproteobacteria* (2.4%; **Figure 2.3**).

Secondly, we analyzed relative abundance changes in function of the feedback-to-innate index at Day 12 for each ASV; we focused on ASVs that were in the original microbiome and with a significant response to feedback-to-innate index, which occupied an average of 37% reads in the original *C. sorokiniana* microbiome. ASVs of which the relative abundance showed a significant positive correlation (Pearson's correlation coefficient adjusted p-value < 0.05) with

higher feedback-to-innate indices and with > 1% of total reads across 30 microbiomes belonged to the genus *Inquilinus, Methylophilus,* family *Chitinophagaceae, and class Bacteroidia* (ranked by Pearson's correlation coefficient = 0.51, 0.48, 0.87, and 0.56 respectively; **Figure 2.4** and **Table S2.1**). ASVs that had a relative abundance that showed a significant negative correlation with higher feedback-to-innate indices and with > 1% of total reads across 30 microbiomes belonged to the *Chryseobacterium and Ottowia* (ranked by Pearson's correlation coefficient = -0.85 and -0.55, respectively; **Figure 2.4** and **Table S2.1**). Finally, *A-N-P-R and Novosphingobium* included ASVs that either significantly increased or decreased with higher feedback-to-innate indices (**Figure 2.4** and **Table S2.1**).

Phytoplankton microbiome alpha-diversity under six feedback-to-innate indices

We focused on the diversity of the phytoplankton microbiome on Day 12, which represented quasi-equilibrium conditions existing from day 6 to 12 and, being the final day of the experiment, represented the longest selection by the various host-microbiome feedback vs innate host selection effects. The relationship between each diversity index (richness, evenness, and Shannon diversity) in the function of the feedback-to-innate index was evaluated by the first and second-order polynomial regressions (**Figure 2.5**). We found that different diversity properties showed different relationships with the feedback-to-innate index. Richness showed no significant linear relationship (**Figure 2.5a**; with p-value > 0.1), but a significant hump-shape relationship with the feedback-to-innate index (with p-value <0.001). Secondly, evenness linearly decreased with a higher host-microbiome feedback effect (**Figure 2.5b**; with p-value < 0.001). The assumptions of this linear regression were satisfied (**Figure SI 2.9**). Finally, Shannon diversity decreased with the initial host-microbiome feedback effect (**Figure 2.5c**; with linear regression p

< 0.001). In addition, their relationship also fit the second-order polynomial regression which indicates the intermediate feedback-to-innate index showed a higher Shannon diversity (with p-value <0.001). Correlations on other days were provided in **Figure SI 2.10**, in which we found consistent correlations on Day 10 but less pronounced correlations on other days which may be due to the microbiome being in a transitional state.

Results from the additional experiment validating our assumptions

In the additional experiment, a large compositional distance was found between 95%-to-5% Feedback-to-DOM versus 95%-to-5% medium-to-DOM treatments based on the first axis of PCoA starting on Day 4 (**Figure IS 2.11**). This indicated that our experiment, rather than just diluting the innate host DOM, did manipulate the host-microbiome feedback effect on microbiome composition. In addition, the microbiome that grew with the *C. sorokiniana* culture under semi-continuous incubation became more similar to the original phytoplankton microbiome with time and overlapped on Day 10 (**Figure IS 2.11**). This indicated that the original microbiome can be returned to its original assembly under regular host-microbiome interaction conditions after being disassociated from the host and grown under semi-continuous incubation.

Discussion

Interactions between hosts and bacteria are important for the community assembly of the microbiome, but questions remain about the underlying mechanisms (Coyte, Schluter, and Foster 2015; Zimmermann-Kogadeeva 2021). We focused on the phytoplankton microbiome system as it is a system of high relevance considering the foundational role of phytoplankton in aquatic

food webs (Field et al. 1998; Smriga et al. 2016) and as it has been used extensively as a model system in ecology (Litchman et al. 2007; Cardinale et al. 2009; Behrenfeld et al. 2021). Central to our work was addressing the extent to which host factor-based selection is strictly innate, i.e. based on intrinsic host traits, or dynamically altered by interactions with the microbiome, meaning the microbiome would be involved in its own community assembly.

Host-microbiome feedback and innate host selection exert significant but different impacts on phytoplankton microbiome formation

By manipulating the feedback-to-innate index and investigating its impact on the phytoplankton microbiome, we found diverging selection when only host DOM produced under axenic conditions was provided (innate host selection) compared to when the live host was present (and host-microbiome feedback effects could occur). This indicates that both host-microbiome feedback and innate host selection are adequate to affect microbiome assembly, and each mechanism is enriching for different taxa. Since close feedback-to-innate indices sometimes result in similar microbiome composition (e.g., 0% versus 5% and 50% versus 75%), there are thresholds of phytoplankton host density to exert enough strength of host-microbiome feedback effect to be reflected in the microbiome composition. As we found that the original phytoplankton microbiome composition (16S rRNA gene sequencing of the xenic phytoplankton culture) was closer to the intermediate, instead of 100% feedback-to-innate index, where accumulating DOM is removed every two days, the phytoplankton microbiome appears to be shaped by a mixture of these two mechanisms.

Our findings, which highlight the differential impact on microbiome composition and diversity resulting from innate host selection and host-microbiome feedback effects, are

consistent with previous studies suggesting host control on microbiome assembly and that such control can be modified by host-microbiome interactions (Mühlenbruch et al. 2018; Shibl et al. 2020). When considering host control, which we tested by manipulating innate host selection microbiome bacterial community composition and diversity are known to change in function of the phytoplankton species, genotypes, bloom phase, and phytoplankton community composition indicates a potential role for phytoplankton host selection on their bacterial microbiome in natural systems (Zhou et al. 2018; Sison-Mangus et al. 2016; Krohn-Molt et al. 2017; Cordone et al. 2022). Further, phytoplankton metabolites composition varies between growth stages, where the exponential growth phase is marked by the presence of free amino acids while during the early stationary phase sugar alcohols, mono- and disaccharides are more abundant (Kuhlisch et al. 2020). These changes in phytoplankton-derived metabolites can potentially affect its bacterial microbiome formation as well, as direct experimental evidence has shown that bacterial community composition can be predicted by the mixture of known phytoplankton exometabolites (Fu et al. 2020). When considering what is known about how host-microbiome interactions can modify bacterial community assembly, which tested by manipulating the host-microbiome feedback effect in the current study, shifts in phytoplankton physiology, metabolism, and thus exudate composition is often found after introducing the bacterial community to an axenic host (Bruckner et al. 2008; Seymour et al. 2017; Sison-Mangus et al. 2016; Uchimiya et al. 2022). The most direct support for this idea was found in the observation of a transcriptional and metabolic shift in a diatom after introducing the natural bacterial community. This triggered the diatom to secrete unique secondary metabolites that favored beneficial bacteria and hindered the growth of opportunistic bacteria in the microbiome (Shibl et al. 2020). Our study built on this previous work by providing another explicit test of the idea that

both innate host control and host-microbiome feedback simultaneously shape microbiome assembly.

Identifying key bacterial taxa in host-microbiome interactions

We found 10 ASVs belonging to 8 different genera within the original *C. sorokiniana* microbiome were favored by host-microbiome feedback or innate host selection (**Figure 2.3** and **2.4**). Among them, we found that *Inquilinus, Methylophilus,* and *Chitinophagaceae* included bacterial taxa that were enriched by host-microbiome feedback (**Figure 2.4**), suggesting that these bacteria are likely the main driver of or somehow benefited from host-microbiome feedback. Although the mechanisms that allowed these taxa to be favored by host-microbiome feedback remain to be resolved, *Azospirallales* (*Inquilinus*), and *Methylobacteriaceae* (*Methylophilus*) are potential nitrogen fixers that could drive the positive feedback loop by directly providing nutrients to the host (Chen et al. 2019; Miyamoto, Kawahara, and Minamisawa 2004; de Lajudie et al. 2019). Furthermore, Chitinophagaceae are known for their ability to degrade the complex organic matter of roots and plant exudates (Hester et al. 2018; Rosenberg 2014). By contrast, *Chryseobacterium* and *Ottowia* were enriched with higher innate-host selection (**Figure 2.4**). This would indicate these bacteria were favored by the innate phytoplankton exudates, possibly due to their ability to use a variety of DOM. Finally, we found that *Novosphingobium* and A-N-P-R contained ASVs with not only significant but divergent responses to increasing the feedback-to-innate index (**Figure 2.4**). Specifically, members of A-N-P-R are well-known drivers in plant-soil feedback that can provide nutrients and hormones that are essential for plant growth, and some have been shown to defend their plant host against

pathogens (Angel et al. 2016; Hester et al. 2018; Rosenberg 2014; Cernava et al. 2017; Upadhyay et al. 2022; Ochieno et al. 2021).

The effect of the interplay between host-microbiome feedback and innate host selection on phytoplankton bacterial microbiome diversity

Considering the fact that innate host selection can be modified by host-microbiome feedbacks and that each mechanism exerts divergent selection on bacterial taxa, we argue that the relative strength of each mechanism can result in different phytoplankton microbiome diversity. We expected higher diversity when both mechanisms were applied assuming that bacterial species favored by each mechanism would be more likely to coexist. Indeed, we found a higher richness under intermediate feedback-to-innate indices; yet, the pattern was weak (**Figure 2.5a**). In addition, an unexpected decrease of evenness with an increasing feedback-to-innate index was perhaps caused by a strong species-specific selection from the host-microbiome feedback effect alone (**Figure 2.5b**).

To our knowledge, it remains unclear how stronger host-microbiome interactions, which may relate to higher host density, affect bacterial community diversity as conflicting results have been found. In field observations, bacterial richness showed no significance (Berry et al. 2017) or a positive relationship with phytoplankton density (Zhou et al. 2018; Woodhouse et al. 2016). Simpson diversity was found to decrease during a phytoplankton bloom (Zhou et al. 2018). Moreover, divergent responses in diversity to increasing phytoplankton host density among bacterial taxa have been reported. For example, the Simpson diversity of Alphaproteobacteria displays a hump-shaped relationship while Betaproteobacteria and Bacteroidetes showed a positive relationship with increasing phytoplankton density (Berry et al. 2017). In our case, we

found that Alphaproteobacteria richness showed a consistent hump-shaped relationship while Gammaproteobacteria richness showed a negative relationship with increasing feedback-to-innate index. As for evenness, only Bacteroide evenness consistently decreased with the feedback-to-innate index (**Figures SI 2.12 and 2.13**).

Beyond the phytoplankton-microbiome system

Using a phytoplankton-microbiome system, our study showed how different mechanisms in host-microbiome interactions shape bacterial community assembly differently, and how host-microbiome feedback can modify innate host control to affect bacterial community composition and diversity. As both the composition and diversity of the microbiome affect bacterial microbiome functions (Jasinska et al. 2020; Hooper et al. 2005), this may in turn affect how the microbiome impacts its host. Our findings suggest that the interplay between innate host control and host-microbiome feedback should be considered to understand microbiome assembly. We posit that this is true regardless of the host-microbiome system of interest. For skin microbiomes, coral microbiomes, and the plant rhizosphere, host secretions are the main carbon and energy source, and potential host-microbiome feedback effects on host secretion composition have been observed (Boxberger et al. 2021; Oppen and Blackall 2019; Chen, Fischbach, and Belkaid 2018). Hence, there likely are strong analogies to the host-microbiome interactions we observed in the phytoplankton-microbiome system. For example, skin microbiome composition is highly associated with the gender, age, and area of the host, indicating the impact of innate host control on microbiome composition; on the other hand, microbiomes are important to host skin homeostasis, immune system maintenance, and wound repair, which may indicate host-microbiome feedbacks (Boxberger et al. 2021). In contrast, our findings likely do not translate

well to digestive tract microbiomes, where diet is the dominating factor shaping microbiome assembly (Valdes et al. 2018). Despite this limitation, the planktonic nature of phytoplankton and bacteria and their short generation times make them an ideal system for experimental validation of ecological and evolutionary theories relevant to complex host-microbiome systems (Litchman et al. 2007; Cardinale et al. 2009).

Caveats and limitations

Here, we acknowledge some limitations of our study. First, we are aware that by their nature as unicellular organisms, phytoplankton, unless they grow in a colonial form, lack the extensive physical structure to harbor bacteria in a similar way as animal or plant phyllosphere or rhizosphere microbiomes do. In addition, phytoplankton hosts likely respond to bacterial microbiomes faster than multicellular organisms do due to their shorter generation time and a higher host-to-bacteria abundance ratio. Potential weaker host control and more active and frequent host-microbiome interactions could occur in the phytoplankton-bacteria symbiosis than in animal- or/and plant-microbiome systems.

Second, symbiont phenotypes may vary under different environmental conditions and with different phytoplankton strains/species. For example, obligate mutualistic interaction can become weaker outside of the adapted temperature range (Zhang et al. 2019; Kishimoto et al. 2020). In addition, a nutrient-limited condition would foster different mutualistic interactions in comparison to the nutrient-rich conditions we used; for example, it has been reported that phytoplankton abandons its nitrogen-fixing partner when environmental nitrogen is abundant (Hay et al. 2004). Furthermore, the composition of the phytoplankton microbiome was observed to be host-specific, even when selected from the same natural bacterial community source

(Jackrel et al., 2021). Moreover, different phytoplankton species can vary in their metabolic capacities and phenotype (e.g., mixotrophic versus autotrophic growth; Piasecka et al. 2020). Together, these facts suggest that host-microbe interactions and their phenotypes may vary among different phytoplankton species. This in turn may influence the selection of bacterial taxa and the relative importance of host selection versus host-microbiome feedback effects on microbiome assembly. In our study, experiments were conducted at one temperature in a nutrient-rich medium with a specific phytoplankton species. Hence, results may diverge under nutrient-limited conditions, different temperatures and diverse phytoplankton species/strains.

Third, we did not distinguish the response of the particle-associated and free-living bacterial communities, which have been found to differ in the assemblages in nature (Allgaier and Grossart 2006). Instead, we looked at the community as a whole. A focus on particle-associated bacteria may be warranted as they play an important role in the remineralization of organic matter, which is one of the potential forces to drive the bacteria-phytoplankton mutualistic interaction (Parveen et al. 2013). Particle-associated bacteria also exert a more direct interaction with their host due to their physical attachment to the phytoplankton surface, as has been suggested before (Schweitzer-Natan et al. 2019; Ahern et al. 2021; Arandia-Gorostidi et al. 2022). Consequently, particle-associated bacteria may have a stronger association with host-microbiome feedback effects compared to free-living bacterial communities. However, it is worth noting that free-living and particle-associated communities (based on sequential filtration on 3 and 0.22-micron filters) in laboratory cultures showed similar responses and significant overlap in the microbiome in composition in the microbiome recruitment study that generated the microbiomes that we used here (Jackrel et al. 2021).

Finally, there is a possibility that the observed host-microbiome feedback effects resulted from phytoplankton-microbiome competition for nutrients. However, such competitive interactions are typically triggered by limited nutrient conditions (Ratnarajah et al. 2020), whereas we refreshed our cultures with nutrient-rich COMBO media every two days. Nevertheless, further examination of nutrient levels during culture incubations would be required to confirm our assumption.

Conclusion and outlook

We showed how the interplay between host-microbiome feedback and innate host selection influences phytoplankton microbiome formation. Each mechanism showed different impacts on microbiome community composition by favoring different species. As for the impact on microbiome community diversity, bacterial richness was higher with the presence of both mechanisms while evenness decreased with increasing host-microbiome effect. In addition, the original phytoplankton microbiome was most similar to the microbiome when both mechanisms were applied, indicating that the natural phytoplankton microbiome is shaped by both forces rather than host-microbiome feedback or innate host control alone. Together, our results suggest that comprehending the factors and mechanisms involved in phytoplankton microbiome formation requires awareness of the distinct impacts arising from innate host control and feedback loops driven by the microbiome, given that each has significant but distinct impacts on microbiome composition and diversity. Knowing that these two effects are at play allows us to evaluate whether they respond similarly or differently to extrinsic driving factors, for example a changing temperature. We already know microbiome composition affects phytoplankton host fitness, and our work generates a practical and theoretical framework to understand and predict

phytoplankton responses to a changing world. In addition, our findings provide valuable insights extending beyond the phytoplankton microbiomes, as host-microbiome interactions are likely to shape microbiome community assembly across any system where host secretions play an important role in microbiome assembly.

Acknowledgments

We thank Madelyn Foulkes for her assistance to prepare the experiment. The high-throughput sequencing was performed by The University of Michigan Microbiome Core. This work was funded by the University of Michigan, including funds from the Rackham Graduate School Graduate Student Research Grant, the Department of Ecology and Evolutionary Biology Research Award Program, and the LSA Associate Professor Research Fund.

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Figure 2.1 Illustration of feedback-to-innate manipulation experiments.

Step 1: Phytoplankton microbiome communities were detached from a xenic *C. sorokiniana* culture and inoculated into media with six indices of initial host-microbiome feedback vs. innate host selection effects. Step 2: to create six feedback-to-innate indices, axenic phytoplankton hosts and host-produced DOM were separated by centrifuging the axenic *C. sorokiniana* culture. The supernatant that contains host-produced DOM (with 1.2-μm filtration) is for exerting the host-innate selection. The pellets that contain axenic *C. sorokiniana* were washed and resuspended in fresh medium and used for exerting the host-microbiome feedback. Washed axenic hosts and host-produced DOM were mixed with different ratios to create six degrees of feedback-to-innate indices with a total volume of 50 ml for each in five replicates. Step 3: Two days after *C. sorokiniana* microbiome inoculation, 5% of each culture was transferred to a fresh medium with the same feedback-to-innate effect. In addition, each microbiome community was collected from the remaining 95% culture by centrifugation into a pellet (supernatant removed) for later DNA extraction and 16S rRNA gene sequencing. A total of five transfers were conducted in a 12-day experimental period.

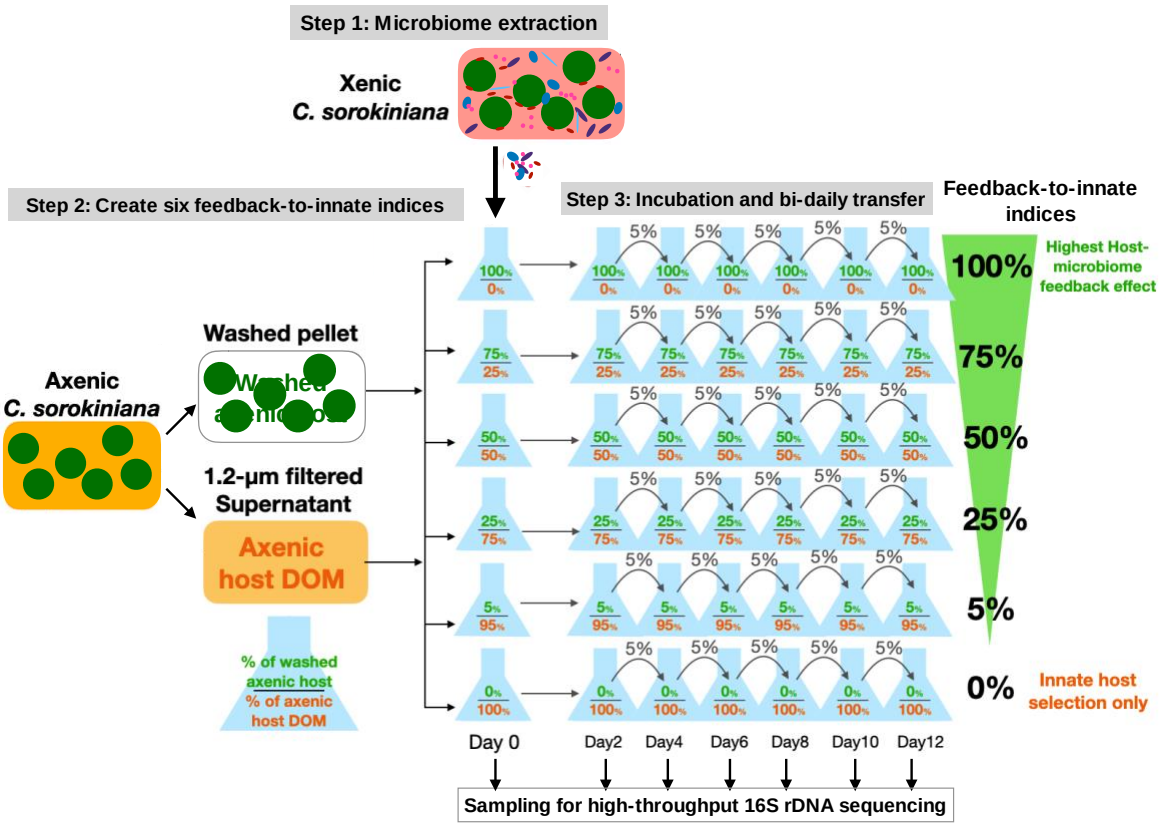


Figure 2.2 Principal Coordinates Analysis (PCoA) ordination based on Bray-Curtis dissimilarities between phytoplankton microbiome communities in the function of feedback-to-innate index and time.

Symbols represent the microbiome communities under 0% (yellow open circles), 5% (orange closed triangles), 25% (red open triangles), 50% (brown closed triangles), 75% (blue open squares), and 100% feedback-to-innate index (close green square), respectively. Star symbols represent the original bacterial microbiome communities at Day 0 (microbiome prior to detachment procedure from *C. sorokiniana*). Variance explained was 21.9% and 14.1% for the first two PCoA axes.

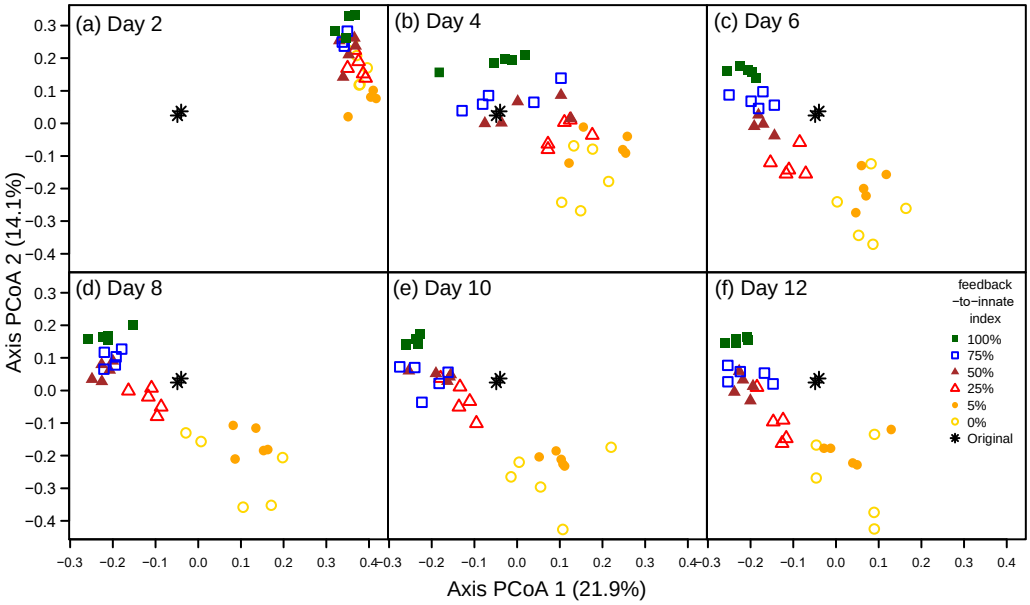


Figure 2.3 The mean of original xenic *C. sorokiniana* and Day 12 microbiome compositions. Each block represents an ASV.

Blocks enclosed by solid lines represent ASVs show a significant (positive or negative) response in relative abundance to an increasing feedback-to-innate gradient (as shown in Figure 4) and with > 1% total number of reads across all 30 2,000-read rarefied microbiome samples (60,000 of total reads). Blocks enclosed by dotted represent no response in relative abundance to an increasing feedback-to-innate gradient. ASVs with the same color were the same Genus, which in the legend were annotated with Genus/Family/Class levels. The communities were rarefied at 2,000 reads. A-N-P-R refers to the genus Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium. UA refers to the genus uncultured anaerobic ammonium-oxidizing bacterium.

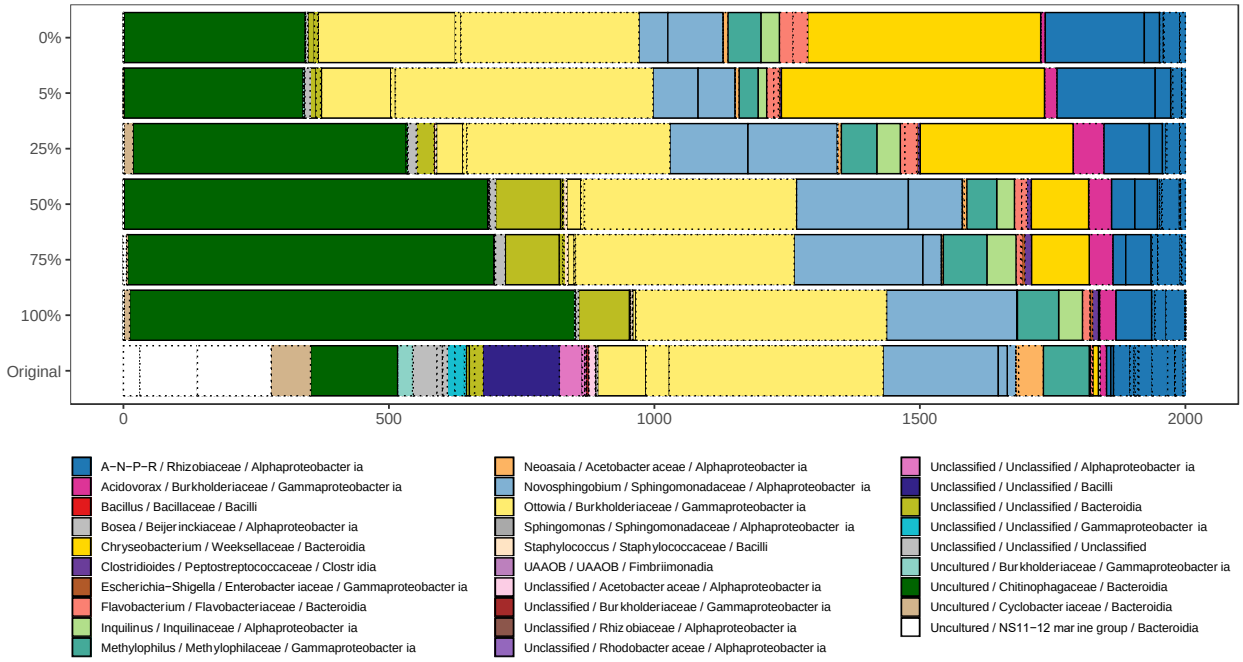


Figure 2.4 Correlation coefficient relationship between each ASV's relative abundance with initial host-microbiome feedback effect on day 12, grouped based on their genus-level classification (with Family and Class levels in parentheses).

All 30 microbiome samples were rarefied at 2,000 reads before the analysis. ASVs with significant positive correlations with the feedback-to-innate gradient would suggest an association with host-microbiome feedback processes; conversely, ASVs with significant negative correlations would indicate their role as a strong competitor on innate phytoplankton DOM. The green and orange points represent ASVs that increased and decreased with increasing feedback-to-innate indices, respectively. Only ASVs with significant responses (with Pearson's correlation coefficient, adjusted-p value < 0.05; Table S2), with > 1% total number of reads across all 30 2,000-read rarefied microbiome samples (60,000 of total reads). *A-N-P-R* refers to the genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium.*

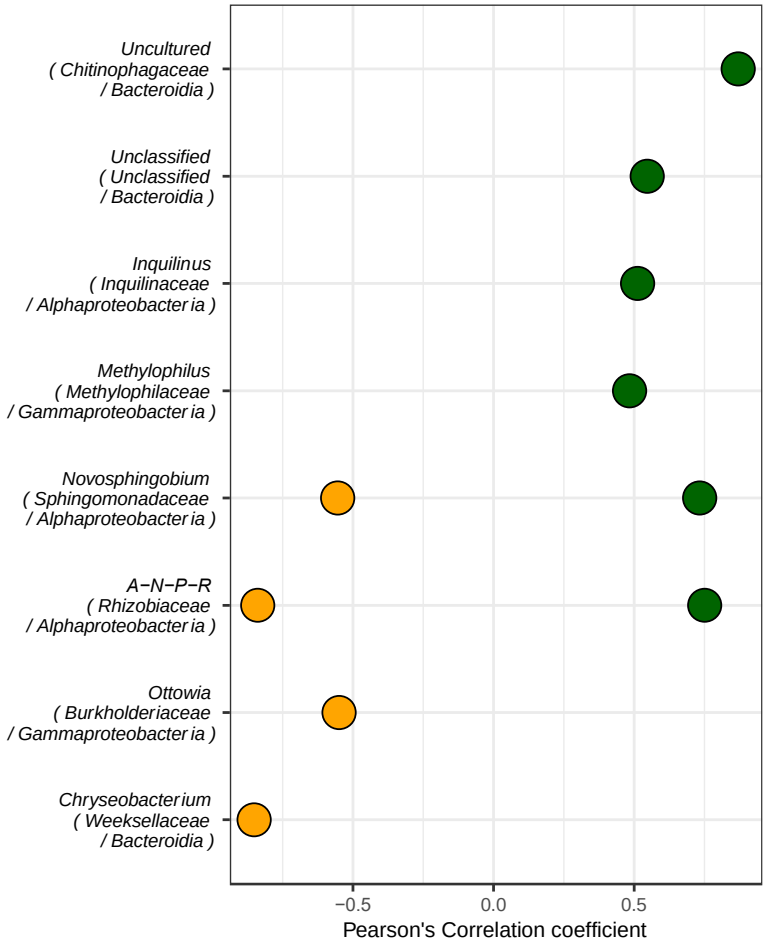
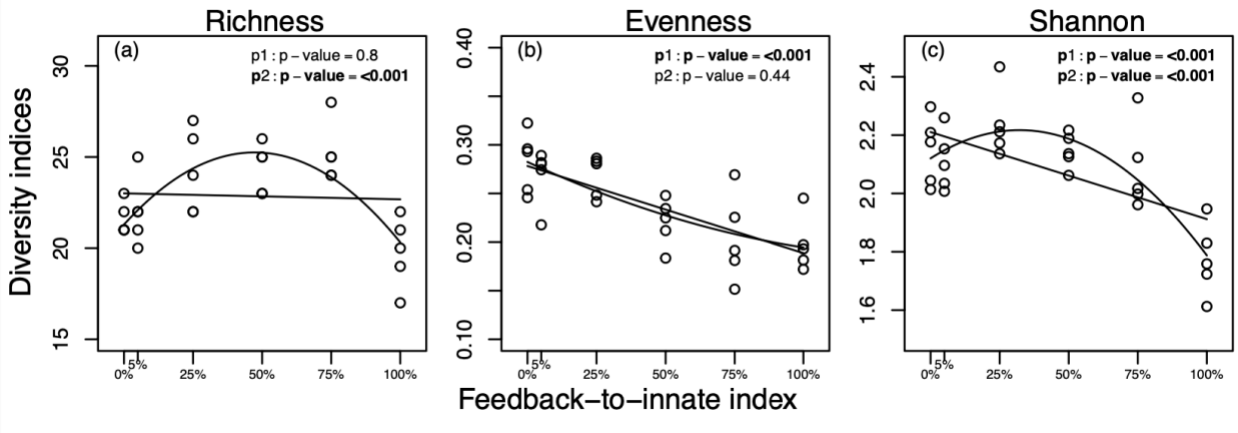


Figure 2.5 The relationship between phytoplankton bacterial microbiome diversity versus feedback-to-innate index at Day 12.

Day 12 was included as it was the final day of the experiment, representing the longest selection by the various feedback-to-innate indices. The linear and non-linear lines are the first (p1) and the second (p2) degree polynomial regression, respectively. Significant p-values ($p < 0.05$) were shown in bold.



Supplementary Information for Chapter 2

Materials and Methods details

Study model species and sources

A unialgal *Chlorella sorokiniana* culture (UTEX 2805) was obtained from the University of Texas Culture Collection of Algae in 2011 and maintained on agar slants at 30 μmol · m^{-2} · s^{-1} at 15°C on COMBO medium (Jackrel et al. 2021; Kilham et al. 1998). The culture was rendered axenic in 2017 (Schmidt et al. 2020). In 2018, we generated xenic *C. sorokiniana* cultures through a recolonization experiment that allowed complex microbial communities from pond water from the University of Michigan's E. S. George Reserve (ESGR; Pinckney, MI, USA) to colonize the axenic *C. sorokiniana* culture (Jackrel et al. 2021). In this previous study, we used three different source communities (triplicates for each source) to obtain three xenic cultures shaped by the *C. sorokiniana* host and to lesser extent the source community. Both axenic and xenic *C. sorokiniana* cultures were maintained in laboratory slant cultures under a light intensity no higher than 30 μmol · m^{-2} · s^{-1} at 15°C on COMBO agar medium and be reinoculated to fresh medium every four to six months. Our experiments were carried out between July 10 and August 6, 2021.

Extracting microbiomes and preparing the initial xenic phytoplankton culture

Axenic and the three xenic *C. sorokiniana* cultures from different source bacterial communities (triplicates from the same community source were combined into a single community) were inoculated into 100-ml liquid COMBO medium from agar slant cultures and incubated on a table shaker set to a continuous 80 RPM with 16:8 light/dark cycle with a light intensity of 80 μmol · m^{-2} · s^{-1} at 20 °C. After 10 days, we extracted the microbiomes from the three xenic *C. sorokiniana* cultures, combined and reintroduced them into a 100-mL fresh axenic *C. sorokiniana* culture (as the original phytoplankton culture), and allowed reassociation and growth for 10 days as the starting xenic *C. sorokiniana* culture. The microbiomes were extracted by sonicating 35-ml of each xenic *C. sorokiniana* culture in a water bath twice at 120 W for 30 seconds, with a 60 second interval. Next, we filtered the culture through a 1.2-μm pore-size filter to remove the phytoplankton hosts. The filtrate (containing the microbiome) was then centrifuged at 2,500 x g for 10 min, the supernatant was mostly removed with the remaining ~1-ml liquid containing the concentrated microbiome. Three ~1-ml concentrated microbiomes (from three different source bacterial communities) were then combined and inoculated in a 100-ml fresh axenic *C. sorokiniana* culture (with ~ 5 x 10^4 cells/mL at the exponential growth phase). The sonication conditions were selected based on a preliminary test using a range of conditions as it resulted in the highest levels of cell detachment from the phytoplankton host while minimizing lethal effects on bacteria by screening for the highest number of colonies that can grow on the R2A (Teknova, USA) agar plates after extraction (**Figure SI 2.14).**

Extracting the microbiome for the main experiment

The first step of the main experiment was to extract the microbiome from the xenic culture we generated above (Step 1 in **Figure 2.1**). The 100-mL starting xenic *C. sorokiniana* culture was inoculated to 1,800-mL of fresh COMBO medium and grown to a density of ~2 x 10^5 cells/mL. Two ~50 mL of the culture were collected for 16S rRNA gene sequencing as the original phytoplankton microbiome (two replicates) by centrifuging at 2,500 x g for 15 min into a pellet, removing the supernatant, and storing the pellets at -20°C for later DNA extraction.

Then, the microbiome was extracted and collected from the remaining (~1,700 mL) culture following the microbiome extraction protocol described in the previous section. The extracted microbiome was washed with fresh COMBO medium to remove remaining metabolites by centrifuging at 2,500 x g for 10 min, removing the supernatant, and resuspending the pellet in 1-mL sterile COMBO medium. This washing process was conducted twice. Overall, the whole microbiome extraction and washing processes were repeated six times, processing ~280-mL starting xenic culture per time, to create six washed microbiome pellets to set up the main experiment. The remaining culture was streaked onto two agar slant tubes (made with COMBO medium) as storage and grew under no higher than 30 μmol · m^{-2} · s^{-1} at 15°C, which were later used for the additional experiment described below.

Manipulation of host-microbiome feedback versus host-innate selection effect (feedback-to-innate manipulation experiment)

The second step was to create six feedback-to-innate indices (**Figure 2.1** Step 2). An axenic *C. sorokiniana* culture was centrifuged at 900 x g for 5 min to separate the axenic host and host-produced DOM. The supernatant was 1.2-μm filtered to obtain algal DOM secretions produced in the absence of a microbiome, to be used as the material for innate host selection. For manipulating the host-microbiome feedback effect, the axenic algal pellets were washed twice by resuspending in fresh COMBO medium, centrifuging and removing the supernatant. The washed pellet was then resuspended with a total of ~1,300-ml fresh COMBO medium to obtain axenic host cells without algal-secreted DOM, with density ~2 x 10^5 cells/mL. We created six feedback-to-innate indices by mixing 0-ml and 50-ml, 2.5-ml and 47.5-ml, 12.5-ml and 37.5-ml, 25-ml and 25-ml, 37.5-ml and 12.5-ml and 50-ml and 0-ml of the host cells only and the host DOM only medium, respectively; which represented the 0%, 5%, 25%, 50%, 75%, and 100% feedback-to-innate index, respectively. The % indicates the ratio relative to the strongest host-microbiome feedback (the highest host density) that we can create in this experiment. This is with an assumption that, with a given microbiome density, a higher axenic host density would lead to a stronger host-microbiome feedback effect due to a higher host-bacterial encounter rate (a higher host-bacteria density ratio). All treatments were conducted in five replicates, incubated on a table shaker set to a continuous 80 RPM with 16:8 light/dark cycle under a light intensity of ~ 80 μmol · m^{-2} · s^{-1} at 20 °C.

After microbiomes (extracted in Step 1) were inoculated into six feedback-to-innate indices (which were created in Step 2), we prevented the effects from accumulated metabolites, nutrient depletion, and ensured phytoplankton remained in the exponential growth phase during incubation, by transferring 5% (2.5 ml) of 2-day-old cultures to fresh medium with the same feedback-to-innate indices every other day until day 12 (**Figure 2.1** Step 3). Fresh axenic *C. sorokiniana* cultures were prepared for creating fresh media at each of the six feedback-to-innate indices for each transfer to avoid any effects of DOM or cell storage. To maintain the fresh axenic *C. sorokiniana* culture at exponential growth phase (a density of ~ 2 x 10^5 cells/mL; **Figure S2.6),** it was diluted 5 % by adding fresh COMBO medium every two days. In addition, the axenic *C. sorokiniana* culture was observed by epifluorescence microscopy to check for contamination and health conditions before being used. A total of 187 microbiome samples were collected, including the original xenic *C. sorokiniana* culture (before dissociation of the microbiome), a microbiome sample from each of the six feedback-to-innate indices at day 0, and 180 samples from day 2 to day 12 (six feedback-to-innate indices with five replicates and 6 collections). Microbiome samples were collected by centrifuging the remainder (~ 47.5 mL) of

each culture at 2,500 x g for 10 min into a pellet, with the supernatant removed and the pellets stored at -20 °C until DNA extraction.

Table SI 2.1 Statistical results of pairwise permutational multivariate analysis of variance (PERMANOVA).

p-values shown in boldface indicate the two comparing microbiomes were significantly compositional different. “Original” indicates the original xenic *C. sorokiniana* microbiome. 999 times permutation was conducted.

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Pairwise comparison between feedback-to-innate indices	Sum Of Squares	R2	p-value
0%-5%	0.04	0.14	0.269
0%-25%	0.20	0.44	**0.011**
0%-50%	0.42	0.66	**0.008**
0%-75%	0.47	0.65	**0.012**
0%-100%	0.72	0.78	**0.007**
5%-25%	0.14	0.50	**0.006**
5%-50%	0.31	0.75	**0.009**
5%-75%	0.34	0.71	**0.008**
5%-100%	0.53	0.85	**0.009**
25%-50%	0.09	0.43	**0.007**
25%-75%	0.13	0.43	**0.009**
25%-100%	0.28	0.70	**0.007**
50%-75%	0.03	0.18	0.123
50%-100%	0.08	0.51	**0.012**
75%-100%	0.06	0.34	**0.014**
Original-0%	0.52	0.75	**0.046**
Original-5%	0.43	0.87	**0.045**
Original-25%	0.37	0.81	0.059
Original-50%	0.38	0.89	0.053
Original-75%	0.36	0.81	0.059
Original-100%	0.39	0.91	**0.042**

Table SI 2.2 Pearson's correlation coefficient between ASV's relative abundance and increasing feedback-to-innate indexes based on microbiome collected on Day 12.

A total of 30 microbiomes and rarefied at 2,000 reads (6 feedback-to-innate treatments with 5 replicates and a total number of 60,000 reads) were collected on Day 12. Only ASVs with significant correlation are shown. A-N-P-R indicates Genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. P-values were adjusted by Bonferroni correction (Adjusted p-value). *We excluded ASVs in the main result (Figure 4) that were not found in the original microbiome likely cross-contamination at the microbiome core (ASV5, ASV12, ASV13, and ASV14), and ASVs with <600 read counts (1% total number of reads) to reduce overinterpreting patterns that may have been due to stochastic effects (ASV4, ASV5, ASV8, ASV9, ASV11, ASV12, AS13, ASV14, ASV15, ASV16, ASV19 and ASV20). **ASV number of reads across 30 samples collected on Day 12 after rarefaction at 2,000 reads.

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	Genus	Family	Order	Class	Correlation coefficient	Adjusted p-value	**Number of reads
ASV1	uncultured	*Chitinophagaceae*	*Chitinophagales*	*Bacteroidia*	0.87	<0.001	17189
ASV2	*A-N-P-R*	*Rhizobiaceae*	*Rhizobiales*	*Alphaproteobacteria*	0.751	<0.001	1152
ASV3	*Novosphingobium*	*Sphingomonadaceae*	*Sphingomonadales*	*Alphaproteobacteria*	0.733	<0.001	5009
*ASV4	*A-N-P-R*	*Rhizobiaceae*	*Rhizobiales*	*Alphaproteobacteria*	0.717	<0.001	207
*ASV5	*Clostridioides*	*Peptostreptococcaceae*	*Clostridiales*	*Clostridia*	0.589	0.001	177
ASV6	Unclassified	Unclassified	Unclassified	*Bacteroidia*	0.547	0.002	1662
ASV7	*Inquilinus*	*Inquilinaceae*	*Azospirillales*	*Alphaproteobacteria*	0.512	0.004	1193
*ASV8	Unclassified	*NS11-12 marine group*	*Sphingobacteriales*	*Bacteroidia*	0.51	0.005	39
*ASV9	*A-N-P-R*	*Rhizobiaceae*	*Rhizobiales*	*Alphaproteobacteria*	0.504	0.005	124
ASV10	*Methylophilus*	*Methylophilaceae*	*Betaproteobacteriales*	*Gammaproteobacteria*	0.484	0.007	1981
*ASV11	*Sphingomonas*	*Sphingomonadaceae*	*Sphingomonadales*	*Alphaproteobacteria*	0.481	0.007	18
*ASV12	*Escherichia-Shigella*	*Enterobacteriaceae*	*Enterobacteriales*	*Gammaproteobacteria*	0.479	0.007	84
*ASV13	*Staphylococcus*	*Staphylococcaceae*	*Bacillales*	*Bacilli*	0.436	0.016	99
*ASV14	*Flavobacterium*	*Flavobacteriaceae*	*Flavobacteriales*	*Bacteroidia*	-0.365	0.047	321
*ASV15	uncultured	*Burkholderiaceae*	*Betaproteobacteriales*	*Gammaproteobacteria*	-0.405	0.027	27
*ASV16	*A-N-P-R*	*Rhizobiaceae*	*Rhizobiales*	*Alphaproteobacteria*	-0.473	0.008	195
ASV17	*Ottowia*	*Burkholderiaceae*	*Betaproteobacteriales*	*Gammaproteobacteria*	-0.549	0.002	2407
ASV18	*Novosphingobium*	*Sphingomonadaceae*	*Sphingomonadales*	*Alphaproteobacteria*	-0.554	0.002	2379
*ASV19	*Ottowia*	*Burkholderiaceae*	*Betaproteobacteriales*	*Gammaproteobacteria*	-0.695	<0.001	166
*ASV20	*Neoasaia*	*Acetobacteraceae*	*Acetobacterales*	*Alphaproteobacteria*	-0.762	<0.001	162
ASV21	*A-N-P-R*	*Rhizobiaceae*	*Rhizobiales*	*Alphaproteobacteria*	-0.839	<0.001	2575
ASV22	*Chryseobacterium*	*Weeksellaceae*	*Flavobacteriales*	*Bacteroidia*	-0.852	<0.001	7150

Figure SI 2.6 Xenic phytoplankton growth. Three 50-ml Xenic *Chlorella sorokiniana* cultures were established.

Two 10-μL aliquots were subsampled every two days from each 50-ml *C. sorokiniana* culture and algal cells were counted using a hemocytometer (average of duplicate count for each sample). The results demonstrated that the phytoplankton was in the exponential growth phase within the four-day period spanning from Day 2 to Day 6, reaching a density ranging from ~ 2 to 8 x 10^5 phytoplankton cells/mL. Therefore, to maintain the phytoplankton at an exponential growth phase throughout the two-day incubation period in the feedback-to-innate manipulation experiment, we began our cultures with the highest phytoplankton density (100% feedback-to-innate index) with an initial concentration of ~ 2 x 10^5 phytoplankton cells/mL on Day 0 and at every transfer that occurred at two-day intervals between Day 2 and 12. The boxes represented the Interquartile range between the 1st and 3rd quartiles. The horizontal lines in the boxes represent the median. The dotted lines out of the boxes represent the range of estimates between the minimum (bottom) and maximum (top) values, excluding the outlier.

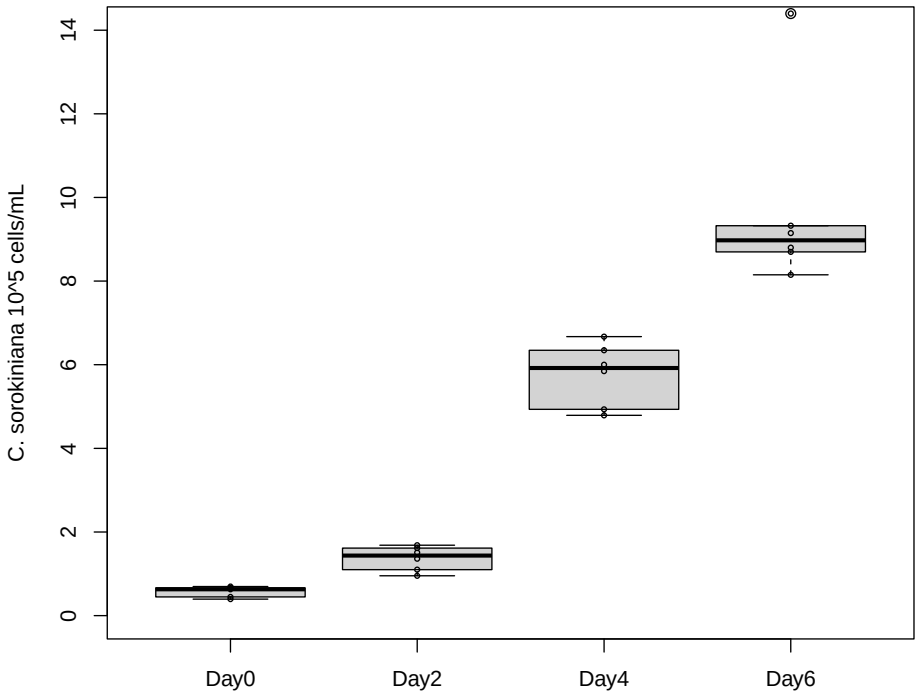


Figure SI 2.7 Rarefaction plot before (a) and after (b) 2,000 reads rarefaction.

The plots encompass data from 187 microbiome communities used in the current study.

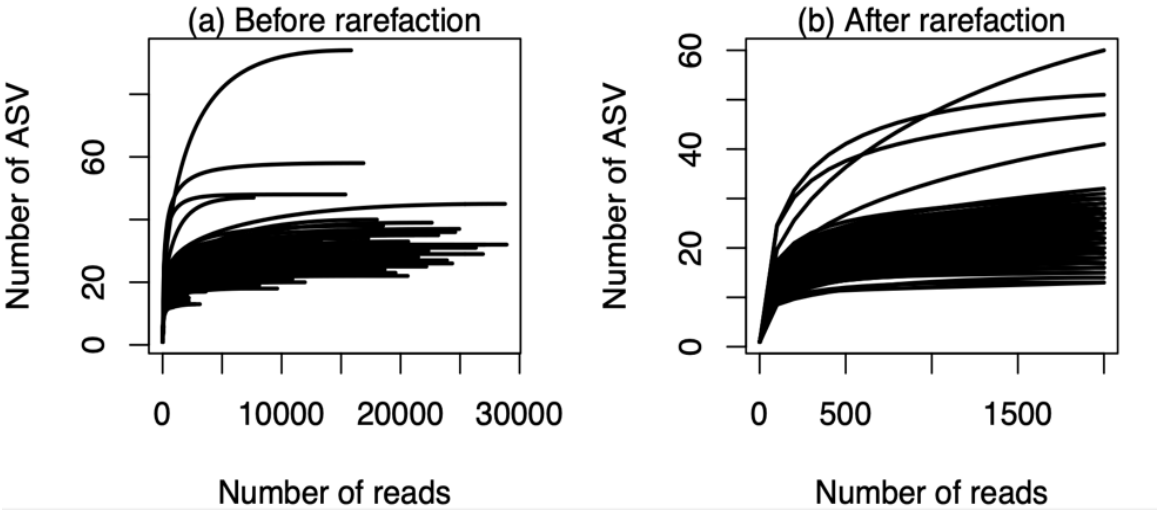


Figure SI 2.8 Illustration of the additional experiment.

The phytoplankton microbiome was detached from a xenic *C. sorokiniana* culture and inoculated into media with 100 % innate phytoplankton DOM, two mixtures of innate phytoplankton DOM with the washed host (~2 x 10^5 cells/mL) or with fresh medium, or an axenic phytoplankton culture. 50 ml volume cultures with three replicates were conducted for each treatment. Two days after *C. sorokiniana* microbiome inoculation, 5% of each culture was transferred to a fresh medium with the same DOM-to-host, DOM-to-medium medium, or axenic culture. In addition, each microbiome community was collected from the remaining culture by centrifugation into a pellet (supernatant removed) for later DNA extraction and 16S rRNA gene sequencing. A total of four transfers were conducted in a 10-day experimental period.

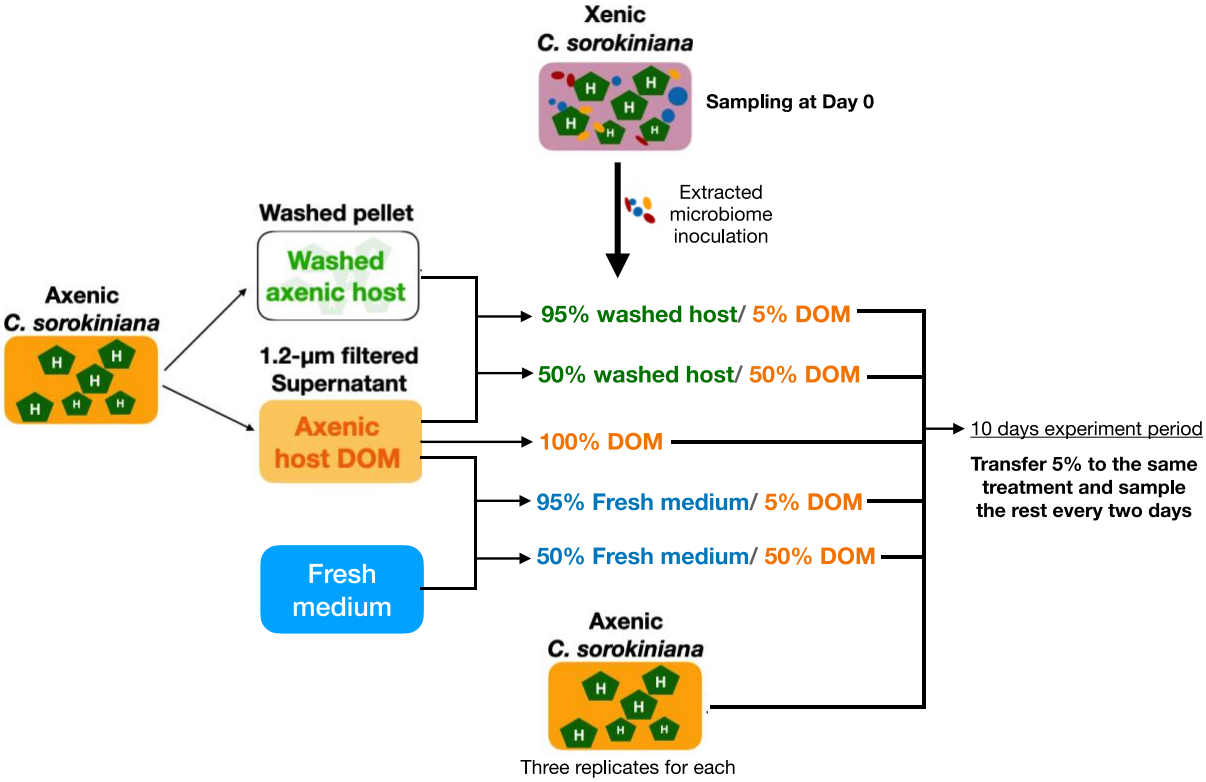


Figure SI 2.9 Linear regression assumptions test.

We tested if the relationship between microbiome evenness and feedback-to-innate indices on Day 12 satisfied the linear regression assumptions. Three assumptions were tested: (i) No relationship between residuals-fitted values, (ii) the mean of residuals is zero and (iii) residuals are independent from each other (not autocorrelated). Durbin Watson test (D-W) was conducted to test the third assumption by using the R package "car" (Reference: Fox, J. (2016) Applied Regression Analysis and Generalized Linear Models, Third Edition. Sage.). Our results satisfied all three assumptions: (i) no relationship between residuals and fitted values, (ii) the mean of residuals is ~ 0, and (iii) no significant autocorrelation was detected (D-W test p-value > 0.05).

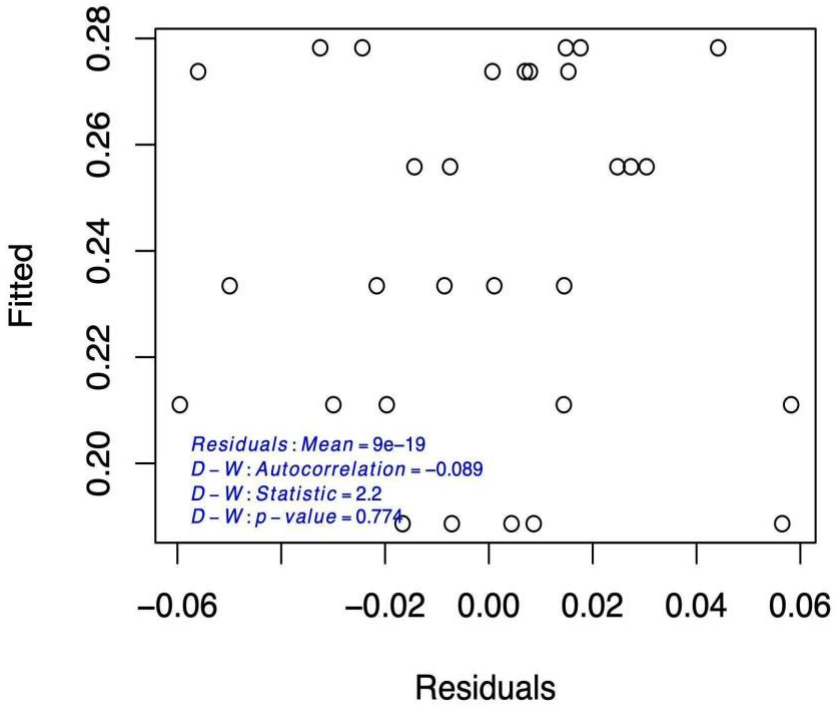


Figure SI 2.10 The relationship between phytoplankton bacterial microbiome diversity versus initial host-microbiome feedback effect.

The black and blue lines are the first (p1) and the second (p2) degree polynomial regression, respectively. The solid and the dash lines indicate the significant ($p < 0.05$) and non-significant ($p > 0.05$) regression,

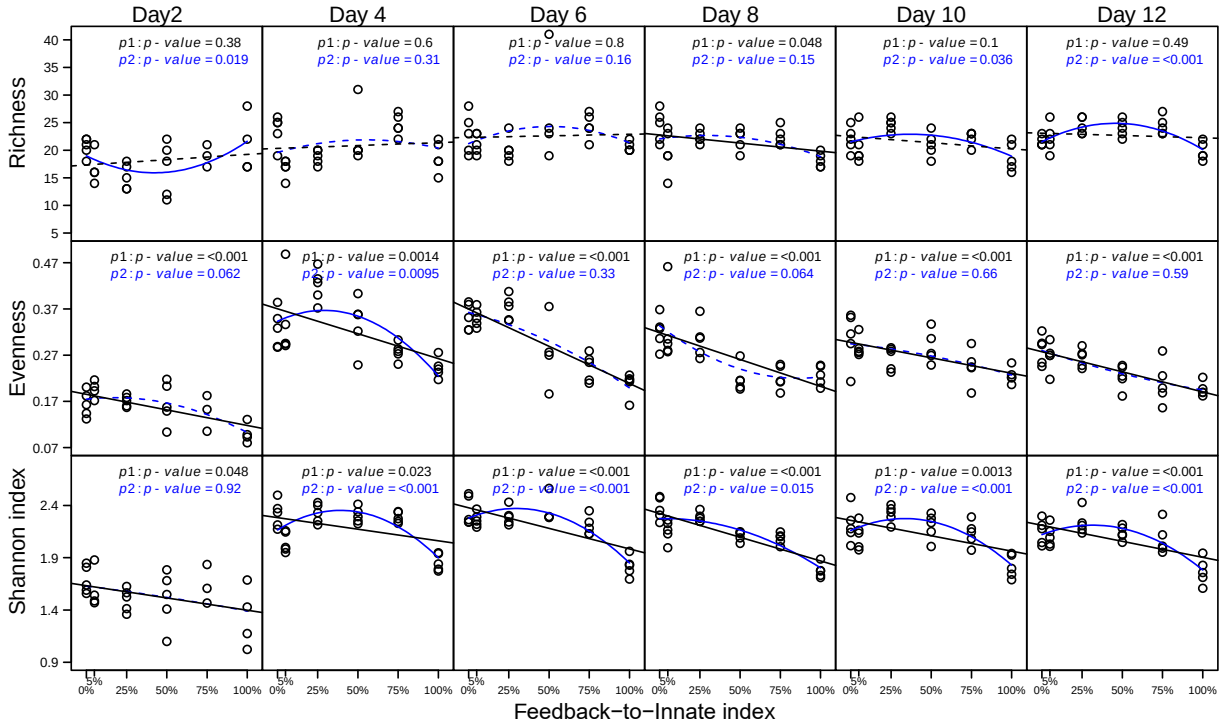


Figure SI 2.11 Principal Coordinates Analysis (PCoA) ordination based on Bray-Curtis dissimilarities among microbiome communities in the additional experiment.

Feedback-to-DOM indicates the volume ratio of washed *C. sorokiniana* versus innate host DOM (which is the same as feedback-to-innate index). Medium-to-DOM indicates the ratio of the volume of fresh COMBO medium versus innate host DOM. Symbols represent the microbiome communities under 95%-to-5% Feedback-to-DOM (black squares), 50%-to-50% Feedback-to-DOM (grey squares), 95%-to-5% Medium-to-DOM (black triangles), 50%-to-50% Medium-to-DOM (grey triangles), and 100% DOM (open triangles). Star symbols represent the original microbiome communities at Day 0 (microbiome prior to detachment from *C. sorokiniana*) and the open circles represent the microbiome that grew under regular host-microbiome interaction conditions (inoculated in axenic *C. sorokiniana* culture). Except for the original microbiome, all treatments were grown under semi-continuous incubation (process of collecting and transferring cultures every two days). Variance explained was 38.4% and 13.8% for the first two PCoA axes.

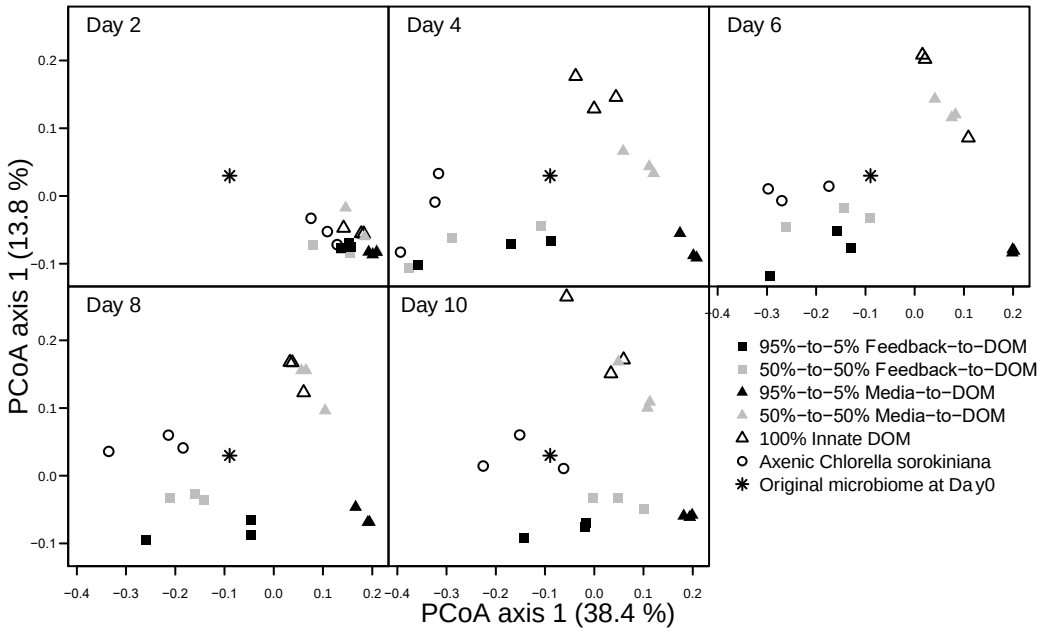


Figure SI 2.12 The relationship between phytoplankton bacterial microbiome richness versus feedback-to-innate index in function of select phyla on day 10 and 12.

The black and blue lines are the first (p1) and the second (p2) degree polynomial regression, respectively. The solid and the dash lines indicate the significant ($p < 0.05$) and non-significant ($p > 0.05$) regression.

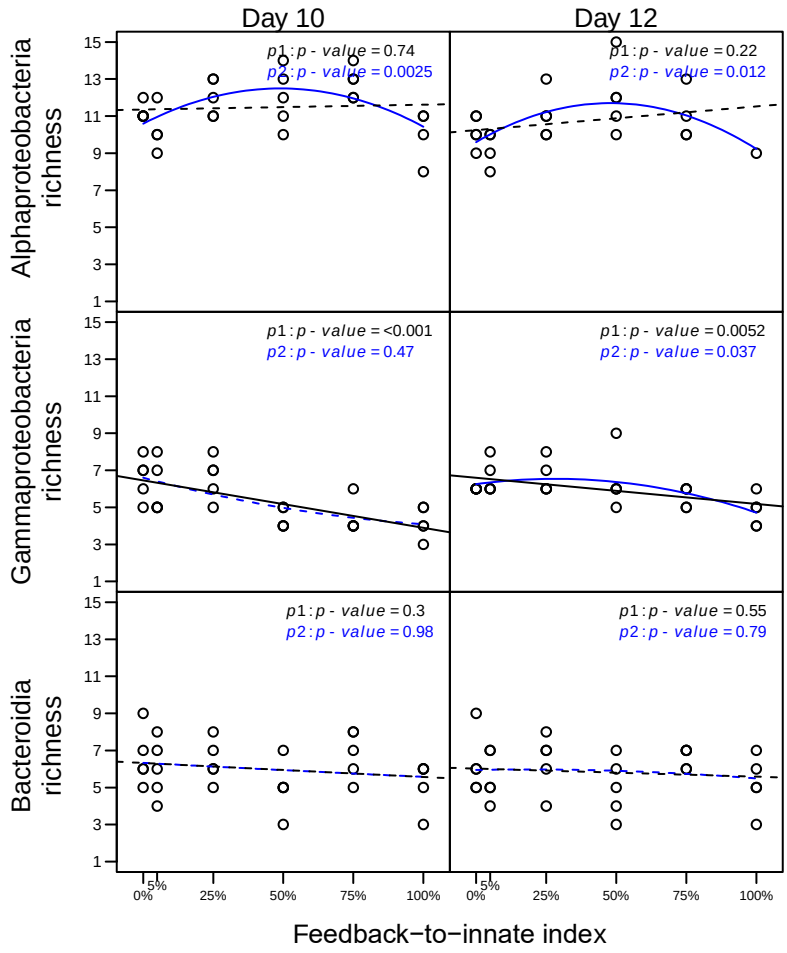


Figure SI 2.13 The relationship between phytoplankton bacterial microbiome evenness versus feedback-to-innate index in function of select phyla on day 10 and 12.

The black and blue lines are the first (p1) and the second (p2) degree polynomial regression, respectively. The solid and the dash lines indicate the significant ($p < 0.05$) and non-significant ($p > 0.05$) regression.

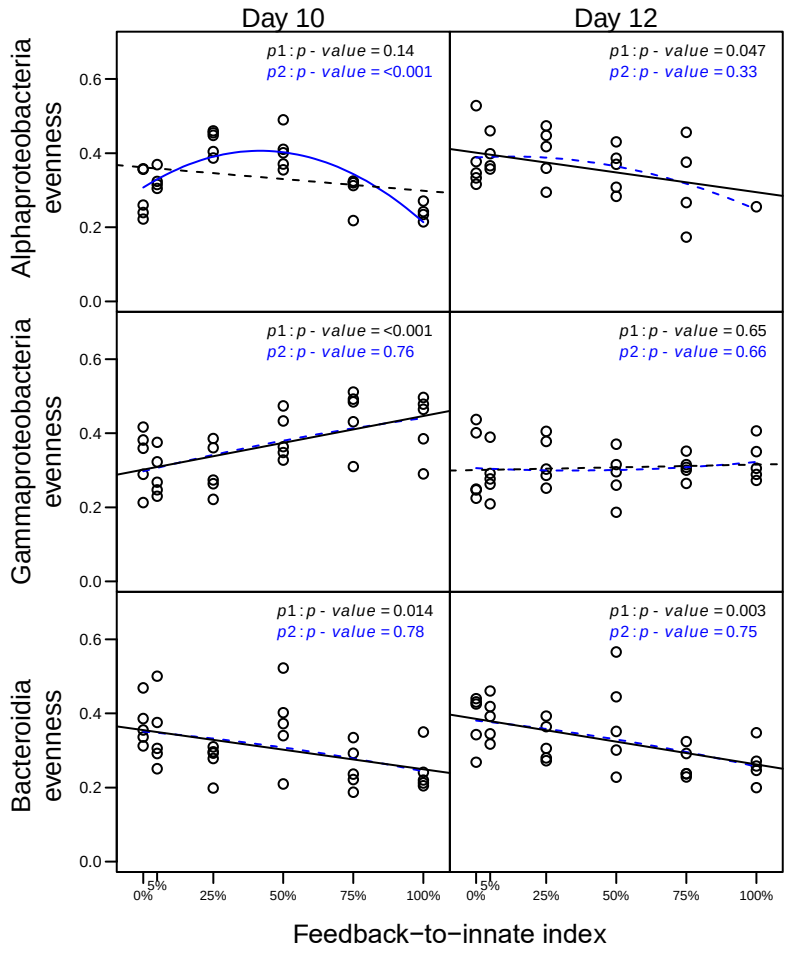
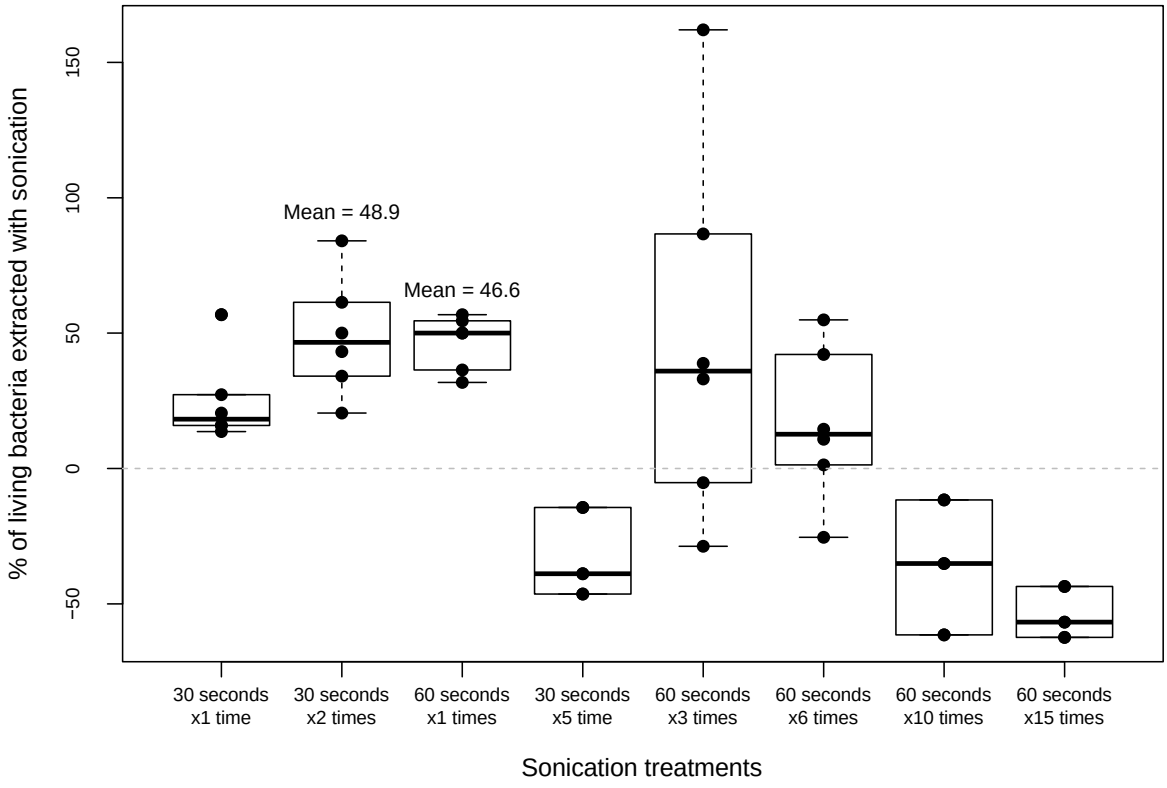


Figure SI 2.14 Percentage of living bacteria detached from C. sonokiniana under different sonication conditions.

Xenic C. sorokiniana cultures were treated under eight different sonication conditions and the extracted bacteria were collected by 1.2-µm filtration to remove phytoplankton host cells. The number of extracted and living bacteria was quantified using spread plates with R2A agar (Teknova, USA) and counting the number of colony-forming units (CFU). The percentage of living bacteria detached under each sonication treatment was calculated by: ((Number of CFU with sonication - Number of CFU without sonication) / Number of CFU without sonication) x 100. Eight sonication treatments were tested: 30 seconds for 1-, 2-, or 5-times sonication, and 60 seconds for 1-, 3-, 6-, 10- or 15-times sonication. All with a 60-second rest interval between each sonication. The boxes represented the Interquartile range between the 1st and 3rd quartiles. The horizontal lines in the boxes represent the median. The dotted lines out of the boxes represent the range of estimates between the minimum (bottom) and maximum (top) values, excluding the outlier (e.g., one estimate from treatment 30 seconds x1 time).



Chapter 3 : Dissecting the Population Dynamics and Gene Expression Patterns of Two Contrasting Modes of Host-microbe Interaction

Abstract

Most eukaryotic species host a microbiome that comprises a diversity of microbial symbionts marked by varying modes of interaction with the host. While some microbial symbionts are selected based on one-way, passive interactions in which bacteria obtain resources from the host or its diet (innate host selection), others establish two-way or active interactions that alter host physiology (host-microbe feedback). Here, we aim to obtain a detailed understanding of these two interaction modes from both a physiological (growth patterns) and metabolic (gene expression and functions) perspective. The green microalga host, *Chlorella sorokiniana*, and two associated bacteria were used as the study system. A *Curvibacter* sp. grew best when provided with the spent medium of axenic *C. sorokiniana (innate host selection)*; by contrast, a *Roseomonas* sp. grew best in the presence of live cells of *C. sorokiniana (host-microbe feedback condition)*. Hence, *Curvibacter* sp. was a passive symbiont, as its growth depends on exudates the host released in absence of bacteria, and *Roseomonas* sp. was an active player due to its ability to enhance its fitness through interacting with the host. These observations aligned with much larger shifts in gene expression between innate vs. host-microbe feedback conditions for *Roseomonas* sp. compared to *Curvibacter* sp. Furthermore, we found that function of the biosynthesis and metabolism of secondary metabolites, that potentially inferring the bi-directional phytoplankton-bacteria interactions, were relatively more expressed

in *Roseomonas* sp. and in *C. sorokiniana* when they were co-cultured, contrasting with co-culturing with *Curvibacter* sp.

Introduction

Most eukaryotic species host a microbiome that is essential to the survival and health of the host organism (Bjørrisen 1988; Apprill 2017; Kinross, Darzi, and Nicholson 2011; Hou et al. 2022; Voolstra et al. 2024). Research has been revealing intrinsic host-microbiome interactions and a strong correlation between microbiome taxonomic composition and the biology of the host (Costas-Selas et al. 2024; Kembel and Mueller 2014; Gould et al. 2018; Ahern et al. 2021; Gupta and Nair 2020; Arandia-Gorostidi et al. 2022). This highlights the important role of host-microbiome interactions in determining which microbial taxa can persist within the microbiome. However, it remains a subject of debate whether the taxonomic composition of the microbiome directly impacts the functions it provides to the host (Robinson, Bohannan, and Young 2010; Neu, Allen, and Roy 2021). Here, we focus on the selection of microbiome members based on their interaction modes during host-microbiome interactions. The host itself often serves as the dominant driving force of microbiome formation (Ferrer-González et al. 2021; Barak-Gavish et al. 2023). Additionally, beyond the unidirectional selection from the host on the microbiome, several studies have observed dynamic feedback interactions between the host and its microbiome, leading to the microbiome itself impacting how the host shapes microbiome composition (Yang and Denef 2024; Shibl et al. 2020; Olofsson et al. 2022). The different selection impacts from these uni- and bi-directional host-microbiome interactions (host control versus feedback driven by specific microbes) act together to maintain microbiome composition and diversity (Yang and Denef 2024). This diversity, in turn, ensures the stability and resilience

of microbiome functional contributions to the host (Lozupone et al. 2012). Yet, our understanding of how uni- and bi-direction host-microbiome interactions differ from each other remains limited.

In this study, we used a phytoplankton species and two members of its microbiome as a model system to contrast uni- and bi-directional host microbiome interactions. Phytoplankton house their microbes within the outer layer of cells, enveloped by a concentrated zone of phytoplankton-produced dissolved organic matter (DOM) an area known as the phycosphere (Kilham et al. 1998; Cirri and Pohnert 2019; Seymour et al. 2017) . Phytoplankton-microbiome systems therefore can serve as a model to understand host-microbiome systems that shape microbiomes through host secretion, such as plant microbiomes, skin microbiomes and coral microbiomes (Müller et al. 2016; Voolstra et al. 2024; Byrd, Belkaid, and Segre 2018). Phytoplankton have a long history as a model system in ecology, including for understanding how the microbiome affects host physiology (Olofsson et al. 2022; Costas-Selas et al. 2024) interactions between hosts (Jackrel et al. 2020). Considering the important role of phytoplankton for food web and nutrient cycle functioning (Field et al. 1998; Litchman et al. 2007), and the impact of the microbiome on phytoplankton functioning, a better understanding of what shapes phytoplankton microbiome composition is important. Moreover, DOM produced by phytoplankton is key in shaping the environmental microbial community not directly associated with phytoplankton hosts, and any host-microbiome interactions that affect the production of DOM will affect the entire microbial community and the biogeochemical cycles they drive (Bertrand et al. 2015). Finally, for industrial applications, where phytoplankton is used as feed source, biofuel precursor, and potential medicine source, a variety of studies have pointed out the impact of the microbiome on outcomes (Lian et al. 2018; Morris et al. 2022).

Recent studies have highlighted the two contrasting modes of interaction between a phytoplankton host and bacterial species in their microbiome. On the one hand, microbiomes can be passively selected by the DOM composition that is determined by the host's innate genetic make-up and growth state. For example, in field observations, the composition of microbiomes is often found correlated to phytoplankton species/genotype and physiological status (e.g., growth phase, under a changing environment, under stress and competition fitness (Steinrücken et al. 2023; Baker et al. 2022; Ahern et al. 2021). The central role of DOM in shaping phytoplankton microbiome composition revealed how composition can be altered and even predicted based on added phytoplankton metabolites (Tada et al. 2017; Kieft et al. 2021; Fu et al. 2020; Kimbrel et al. 2019). On the other hand, while phytoplankton innately produce a mixture of DOM molecules in absence of a microbiome, some microbes in the phycosphere are able to actively interact with the host and modify host physiological status and the metabolites released into the phycosphere. For instance, exposing axenic phytoplankton to natural bacterial communities or certain microbial species were found to change the phytoplankton gene expression and DOM composition (KleinJan et al. 2023; Frischkorn et al. 2017). This leads to a feedback effect that selects for a microbiome distinct from what host originally selected. The most direct evidence supporting this idea was a study that showed how microbiome-triggered metabolites produced by a diatom host favored mutualistic over algicidal bacteria in the microbiome (Shibl et al. 2020). In summary, how host-microbiome interactions shape the phytoplankton microbiome involves the interplay between two interaction modes, (i) a unidirectional interaction mode, where the microbiome is shaped passively by innately produced host DOM, and (ii) a bidirectional interaction mode where some microbiome members can actively interact with the host, altering host physiology and the produced DOM composition to their benefit. However, there are only a

limited number of studies that have characterized these two interaction modes. In addition, most relevant studies characterize the two selection modes at a community scale, with limited insight into the different modes of interactions underlying the community-level effects.

In a previous study, we showed how "innate host selection" (microbiome selected by spent medium from an axenic algal culture) and "host-microbiome feedback effects" (presence of live algal cells) led to different microbiome compositions (Yang and Denef 2024), inspiring a hypothesis of the existence of trade-offs between microbes being favored by either effect. Building upon this study, we designed the current study to contrast, at a population dynamic and gene expression level, the selection process by uni- and bi-directional interaction modes, using two bacterial populations isolated from the host with distinct interaction modes with the host. We hypothesized that, (i) the symbiont with the uni-directional interaction mode would be favored by innate host selection and have limited impacts on host gene expression as it prefers to use DOM that the host innately produced and is unable to modify host produced DOM through direct host-microbe interactions. On the contrary, (ii) the symbiont with the bidirectional interaction mode would be favored by host-microbiome feedback conditions and would alter host gene expression more. We also hypothesized that different sets of gene, which referring potential different function be exhibited, will be expressed in presence of host cells vs. DOM only for the symbiont with the two-way interaction mode. The rationale is that the active symbiont possesses the ability to directly interact with the host; for example, by synthesizing chemical signals, to trigger the host to synthesize and release different DOM that promote its growth or hinder the growth of its competitors.

To test our hypothesis, we used the *Chlorella sorokiniana* as the host model system (Bashan et al. 2016). The bacteria were isolated from a *C. sorokiniana* microbiome that was

recruited from natural pond bacterial communities. From a subset of isolates tested, we selected the two bacterial isolates with the most distinct growth patterns under innate host selection versus host-microbiome feedback effects, to have one representative of each symbiont type (passive and active, respectively). Host and bacterial growth responses were examined in presence of axenically produced DOM (bacteria only) and live host cells, and gene expression profiles were compared to identify the extent of gene expression reprogramming and the prioritized metabolic pathways in host and bacterial symbionts.

Methods

Bacterial and microalgal growth dynamics experiments

C. sorokiniana (UTEX 2805) was rendered axenic in 2011 (Jackrel et al. 2020) and maintained on COMBO media slants (Kilham et al. 1998) under a light intensity no higher than 30 μmol · m^{-2}· s^{-1} at 15°C and be reinoculated to fresh medium every four to six months since then. Microbiomes were recruited to this axenic culture from natural ponds in 2018 (Jackrel et al. 2021b). The xenic culture we obtained isolates from in this study was generated in 2021 from those initial microbiomes and was also used in our previous experiments to identify the innate and feedback selective effects (Yang and Denef 2024). In search for bacteria representative of passive and active symbiosis, we selected six bacteria isolated from xenic *C. sorokiniana* on R2A solid media (Teknova, Hollister, CA, USA) based on their distinctive colony morphology (to enable easy tracking of growth of each culture when in co-culture) and tested their growth response under innate host selection (*Treatment Innate*) or host-microbiome feedback (*Treatment Feedback*). To create two treatments, axenic *C. sorokiniana* was harvested during exponential growth phase (~2 x 10^5 cells/mL) in COMBO medium, and centrifuged at 900 x g for 5 min. The

supernatant was collected and filtered through 1.2-um pore-size filters to remove any remaining cells. This filtered spent medium was used for *Treatment Innate*, which contains only dissolved organic matter (DOM) innately produced by the host without any direct microbe-host interactions. For creating *Treatment Feedback*, the pelleted *C. sorokiniana* cells were washed in fresh COMBO medium to remove remaining spent medium and resuspended in fresh COMBO medium at ~2 x 10^5 cells/mL. This medium contains only axenic host cells without any initial DOM. To minimize the impact of remnants of the liquid R2A medium, which the bacterial isolates were grown in, on the microalgal host, each 1 mL bacterial R2A culture was washed twice by centrifuged at 2,500 x g for 10 min, spent medium removed, and resuspended in 1 mL fresh COMBO medium. The final resuspended bacterial isolates were inoculated into each treatment after 1,000 times dilution into five 1-ml replicate cultures using 48-well plates (Fisher Scientific, Hampton, NH, USA). The plates were cultured under a continuous 80 RPM rotation with a 16:8 light/dark cycle under a light intensity of ~ 80 μmol · m^{-2}· s^{-1} at 20 °C in a Minitron incubator (Infors HT, Bottmingen, Switzerland). To track bacterial growth, 10-μl of each replicate was sampled at Day 0, 2, 4, 6, 8, 10, and 12. Colony forming units (CFU) were counted using drop plate technique (Herigstad, Hamilton, and Heersink 2001), where each 10-μl subsample was 10^{-2}, 10^{-3} and, 10^{-4} diluted using R2A liquid medium, and three 10-μl replicate drops of each dilution were dropped on R2A agar plates. The plates were incubated at room temperature in the dark.

After two representative bacterial symbionts were identified, we repeated the experiment with a larger culture volume that would be needed to obtain sufficient RNA for transcriptomic analysis. We grew the *Curvibacter* sp. and *Roseomonas* sp. in *Treatment Innate* and *Treatment Feedback* following the aforementioned procedure in 100-mL volume in 125-mL glass

erlenmeyer flasks in triplicate. In addition to mono-bacterial cultures, we also grew mixed-bacteria cultures in each treatment condition to investigate potential effects on bacterial growth patterns from microbe-microbe interactions. In the larger volume experiments 10-ul of each culture were collected on day 0, 5, 8, 12, and 19 after inoculation to track bacterial growth. In addition, to examine host response to either bacterial symbiont, triplicate controls of the axenic microalga without initial DOM were grown in fresh COMBO medium. To track *C. sorokiniana population dynamics*, 1-mL of each culture from *Treatment Feedback* and the control treatment were collected and the relative fluorescence intensity unit (RFU) of Chlorophyll-a (with the excitation and emission wavelengths of 465 and 680 nanometers) were measured as a proxy of microalgal density using a Synergy H1 microplate reader (Bio Tek, Winooski, VT, USA).

Sample collection, RNA and DNA extraction, library preparation, and sequencing

The taxa of the six tested bacterial isolates were identified using Sanger sequencing of the 16S rRNA gene. For DNA extraction, each isolated colony was resuspended in 90-µL 1× PBS (0.2-µm filtered and autoclaved) and lysed by adding 5-µL lysozyme solution (50 mg/mL) and 100-µL Qiagen ATL buffer and incubated at 37°C for 30 min, followed by the protocol of the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The 16S rRNA gene was amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg et al. 1991a). 1-ul of template DNA was mixed with 10-ul of NEBNEXT 2X Master Mix (New England Biolabs, Ipswich, MA, USA) and 9 ul of Nuclease Free water. The PCR cycling condition consisted of an initial denaturation at 95°C for 5 minutes, 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, and a final extension step at 72°C for 5 minutes.

The PCR products are stained with GelRed GelRed (Biotium, Fremont, CA, USA) and examined with gel electrophoresis using a 1% agarose gel. The qualified PCR products were sent out for PCR purification, quantification, and Sanger sequencing (Eurofins, Louisville, KY, USA). The low-quality base-pair was trimmed and taxonomic classification was obtained using blastn (Altschul et al., 1990) against the nucleotide collection (nr/nt) database available from National Center for Biotechnology Information (NCBI), with the best hit taxon used.

After ensuring the two representative symbionts exhibited consistent growth patterns in large volume compared to those identified in the initial small volume experiments, the large volume experiment was repeated for transcriptomic analysis. This time, without subsampling at different times to track cell growth, the whole each 100-ml culture was collected after 5 days of incubation in *Treatment Innate* and 12 days of incubation in *Treatment Feedback* (Figure 3.1). The incubation time was selected to capture optimal gene expression that can reflect the distinctive growth pattern of different interaction modes while also maintaining a sufficiently high cell density to yield enough RNA samples for sequencing. The collected cultures were filtered through 0.2 *u*m pore-size PES filters (Sigma Millipore, Burlington, MA, USA). For RNA extraction, in brief, cells were washed down from the filter and homogenized using QiaShredder Kits (Qiagen, Hilden, Germany). RNAs were extracted and purified using the RNeasy Micro Kit (Qiagen, Hilden, Germany) following the standard kit protocol. We quantified RNA using the Qubit RNA Broad Range Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The pure RNA samples were sent to the University of Michigan Center for Advanced Genomic core to carry out library preparation, including rRNA depletion using QIAseq FastSelect 5S/16S/23S Kit and rRNA Plant Kits (Qiagen, Hilden, Germany) for samples containing bacteria and microalgae, respectively. Samples were sequenced using the NovaSeq S4 x 300 cycle platform

(Illumina, San Diego, CA, USA) with 12.5% of the flow cell used, evenly distributed across all 15 samples.

To obtain a bacterial genomic reference for identifying genes and mapping RNA transcripts to, whole genome sequencing was conducted on the *Curvibacter sp.* and *Roseomonas sp.* isolates. Each isolated colony was resuspended in 90-µL 1× PBS (0.2-µm filtered and autoclaved) and lysed by adding 5-µL lysozyme solution (50 mg/mL) and 100-µL Qiagen ATL buffer and incubated at 37°C for 30 min, followed by the protocol of the Qiagen DNeasy Blood & Tissue Kit Qiagen, Hilden, Germany). The extracted DNA was sent to the University of Michigan Center for Microbial Systems to carry out library preparation using plexWell Plus 24 Library Preparation Kit (seqWell, USA) and sequenced using an Illumina MiSeq 2x150 V2 flow cell with 30X coverage yield. Raw fastq files of RNA and whole genome sequencing were uploaded on NCBI sequence read archive with BioProject number XXXX.

Bioinformatics

For bacteria, the whole genome sequences of *Curvibacter sp.* and *Roseomonas sp.* were used as genome reference for RNA-sequences mapping. To prepare the genome reference, the reads were examined with fastqQC v0.12.1 (Andrews, 2010) and the adapters and low-quality base-pair (<20Q) were trimmed using Trimmomatics v0.39 (Bolger, Lohse, and Usadel 2014) with parameter "LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:36". The trimmed whole genome sequences were *de novo* assembled using SPAdes v3.13.5 (Bankevich et al. 2012) followed by gene prediction using Prodigal (Hyatt et al. 2010). Functional annotation was assigned using Hidden Markov Models (HMMs)-based searches against Pfam (Finn et al. 2016) and using Diamond (Buchfink, Xie, and Huson 2015) to search against Clusters of

Orthologous Groups (COG; Tatusov et al. 2003) databases using the Anvi'o pipeline (v8, Eren et al. 2013). The predicted gene calls were used for RNA mapping. The raw RNA seq were trimmed with aforementioned criteria and mapped using STAR (Dobin et al. 2013), followed by creating and visualizing gene count tables using SAMtools (Danecek et al. 2021) and BEDtools (Quinlan and Hall 2010).

For *C. sorokinana*, RNA reads from all treatments and replicates were *de novo* assembled and used as the transcript reference for RNA mapping. The raw RNA-sequences were trimmed with aforementioned criteria, *de novo* assembled using Trinity (Grabherr et al. 2011)and genes predicted using TransDecoder (Haas et al. 2013). The potential contaminants and non-coding regions were eliminated, where we only kept transcripts with translated amino acid sequences that hit to the "Green algae" (NCBI:txid3041) in non-redundant protein sequences database available on NCBI using blastp (Altschul et al., 1990). These transcripts were used for transcript reference for mapping trimmed RNA reads using Bowtie2 (Langmead and Salzberg 2012) with default parameters. For functional annotation, KOfamScan (Aramaki et al. 2020) were used to search against the KEGG database (Kanehisa et al. 2016), respectively.

In Yang and Denef 2024, the whole microbiome was exposed to 6 degrees of Feedback-to-Innate effect, where the microbiome composition was revealed by the high-throughput sequencing on 16S rRNA gene V4 region. To identify representative ASVs to the *Curvibacter* sp and *Roseomonas* sp tested in the current study, we used blastn to align the ASV sequences to the V1-V9 regions of 16S rRNA gene sequences obtained from Sanger sequencing of the two isolates.

Data analysis

To examine the compositional distance of the gene expression profiles between treatments, a principal component (PC) analyses using variance stabilizing transformed transcript count tables were conducted separately for each species. The differentially expressed genes were also identified using DEseq2 (Love, Huber, and Anders 2014) by comparing gene expression between *Treatment Feedback* relative to *Treatment Innate* for bacteria. In this case, the log2 Fold Change (LFC) of each gene represents their relative expression level between treatments, where positive values indicate genes that were relatively more expressed under *Treatment Feedback*, and negative value refers to genes that were relatively more expressed under *Treatment Innate*. The differentially expressed genes (DE genes) were genes with LFC > 1 or < -1 and adjusted p-value <=0.05. For microalgae, the LFC values were calculated to compare the gene expression profiles of algae co-cultured with either bacterium relative to the axenic microalgal condition. Positive LFC values indicate that gene expression was higher when co-cultured with the bacteria, and negative LFC values indicate that gene expression was higher in the axenic state. The DE transcripts were transcripts with > 1 or < -1 LFC and adjusted p-value <=0.05.

For functional analysis, for bacteria, in addition to the general COG categories that including annotations in the COG database and the Pfam Gene Ontology (GO) category, we also investigated the function of DOM-related transporter using a curated list of DOM transporters based on COG annotations (Poretsky et al., 2010). The Pfam annotation was mapped to GO terms using the R package "ragp" (Dragićević et al. 2020) and manually assigned to COG categories. For microalgae, we focus on annotation results from the KEGG database and the KO accession numbers were assigned to subcategories using the R package "clusterProfiler" (Wu et al. 2021). Fisher's exact test (with <= 10 gene annotations) and Chi-square test (with > 10 gene annotations) were conducted to identify over- or under-expressed functions using the R package

"state". All analyses were carried out using the R program (version 4.3.2, R Core Team, 2022). The R scripts, original data and all annotation results are provided on Github: XXXX.

Results

Bacterial and microalgal population dynamics in batch culture

We selected six bacteria isolated from *C. sorokiniana* based on their distinctive colony morphology to enable easy tracking of growth of each culture when in co-culture and tested their growth response under Treatment Innate (with host innate DOM but no host cells) and Treatment Feedback (with host cells after removal of previously produced host innate DOM). Among them, we found that a *Curvibacter* sp. and *Roseomonas* sp. showed the most distinct growth patterns under these two treatments (**Figure SI 3.1**). The *Curvibacter* sp. grew relatively fast under Treatment Innate but with a longer lag time to achieve the same density under Treatment Feedback. On the contrary, a *Roseomonas* sp. did not grow much under Treatment Innate but grew much faster and achieved higher density under Treatment Feedback. We therefore took *Curvibacter sp.* to represent a passive symbiont with one-way interaction mode and *Roseomonas sp.* to represent an active symbiont with two-way interaction mode. We grew *Curvibacter* sp. and *Roseomonas* sp. under two treatments again, but this time with both mono-culture and mixed culture conditions in a larger volume (100-mL). We again found that the two symbionts showed distinct growth patterns between two treatments, where *Curvibacter* sp. dominated under Treatment Innate and *Roseomonas* sp. dominated the Treatment feedback, regardless of the mono- or mixed-culture condition (**Figure 3.1**). For *C. sorokiniana*, there was no difference between axenic status and a co-culture with *Curvibacter* On the other hand, when co-cultured with *Roseomonas* sp. *or the mix of both bacterial species, C. sorokiniana* leveled off at lower density compared to the axenic status.

The relative abundance of two tested bacteria under degree of Feedback-to-Innate effect in Yang and Denef 2024

We also examined the dynamic of *Curvibacter* sp. and *Roseomonas* sp. relative abundance at the whole microbiome level using results from our previous study. We found three and two ASVs that represent the *Curvibacter* sp. and *Roseomonas* sp., with > 97% identity. We found that the *Curvibacter* sp. showed a hump-shape, while *Roseomonas* sp. decreased with the increasing Feedback-to-Innate effect (**Figure SI 3.6**).

Changes *in bacterial and microalgal host gene expression profiles*

Firstly, we explored the variability in gene expression profiles of both bacterial symbionts and microalgal hosts across treatments by conducting a Principal Component Analysis on the variance-stabilized transformed transcript count table. We found a clear clustering based on *Treatment Innate* and *Feedback* for both bacteria, with 89% and 98% variance explained by the first PC axis for *Curvibacter sp.* and *Roseomonase sp*, respectively (**Figures 3.2a and 3.2b**). In addition, we found that the gene compositional distance between two clusters was significantly higher in *Roseomonase sp.* than in *Curvibacter sp.* (**Figure SI 3.6**). For *C. sorokiniana*, the gene expression profiles of the axenic condition were more similar when co-cultured with *Curvibacter sp.* than when co-cultured with *Roseomonas sp.*, although the distance difference was small (**Figure 3.2c and Figure SI 3.6**). In addition, we found that when co-cultured with *Curvibacter* sp. *C. sorokiniana* showed a higher number of transcripts that were also detected in axenic condition (1694 transcripts) compared to when co-cultured with *Roseomonas* sp. (800 transcripts; **Figure 3.2d**). There was also a higher number of unique

transcripts detected when co-cultured with *Curvibacter* sp. (2314 transcripts) than *Roseomonas* sp. (2031 transcripts).

Secondly, for bacteria, we compared the number of differentially expressed (DE) genes between Treatment Innate and Treatment Feedback. For *Curvibacter sp.*, 7,870 genes were identified in the genome and 334 DE genes were more highly expressed under Treatment Innate while only 13 DE genes were more highly expressed under *Treatment Feedbac*k (**Table 3.1**). For *Roseomonas sp*., among a total of 6,345 genes identified in the genome, 1,572 and 1,402 of DE genes were more highly expressed under Treatment Innate and Treatment Feedback, respectively (**Figure 3.3b**). For *C. sorokiniana*, a total of 23,510 transcripts remained after filtering the *de novo* assembly. When comparing its relative transcript levels when co-cultured with either bacteria to its axenic state, we found 3,024 and 2,390 DE transcripts were more highly expressed when co-cultured with *Curvibacter sp.* and *Roseomonas sp.*, respectively. Transcripts only detected in one treatment made up the largest proportion of these DE transcripts. When comparing the relative transcripts levels for C. sorokiniana when co-cultured with *Curvibacter sp.* vs. with *Roseomonas sp.*, we observed 3,171 and 2,447 more highly expressed DE transcripts, respectively, again including uniquely detected transcripts in each condition.

Functional annotation of DE genes

For functional analysis, we focus on selected DE genes (DE genes with PC loading > 0.01 or <-0.01). For *the 347 DE Curvibacter* sp. genes 146 (42.1 %) were annotated by either Pfam (77) and/or COG (111). For *Roseomonas* sp, among 2,974 DE genes, 1,517 (51.0 %) were annotated by either Pfam (763) and/or COG databases (1,040). For *C. sorokiniana*, where the KEGG database had the highest annotation rate, out of a total of 2,685 selected DE genes, 2,247

were annotated when co-cultured with *Curvibacter* sp. (83.7%); and out of a total of 2,394 selected DE genes, 2,037 were annotated when co-cultured with *Roseomonas* sp. (85%).

Functional expression of bacteria and microalgal host

Here, we focus on functions with fraction of DE genes that were significantly higher under each treatment in *Roseomonas* sp relative to their proportion in the reference genome (**Figure 3.3**). Functions with significantly higher gene expression levels were: "Transporter" (3.27 % and 4.18% for Treatment Feedback and Innate), "Translation, ribosomal structure and biogenesis" (9.19% and 8.86% for Treatment Feedback and Innate), and "Energy production and conversion" (11.71 % and 12.35 % for Treatment Feedback and Innate). Functions that were only more expressed under Treatment Feedback were "Transcription" (3.27 %) and "Secondary metabolites biosynthesis, transport and catabolism" (3.27 %). Functions that were only more expressed under Treatment Innate were "Nucleotide transport and metabolism" (3.29%) ," General function prediction only" (8.17%), and "Cell wall/membrane/envelope biogenesis" (9.26%).

For *C. sorokiniana*, we found 8 functions were significantly more and less expressed under both treatments (**Figure 3.4**), including "Xenobiotic biodegradation and metabolism", "Nucleotide metabolism", "Metabolism of terpenoids and polyketides", "Metabolism of amino acids", "Metabolism of cofactors and vitamins", "Lipid metabolism", "Glycan biosynthesis and metabolism", and "Biosynthesis of other secondary metabolites". While functions "Nucleotide metabolism" (2.1 %) were only more expressed when co-cultured with *Rosoemonas sp*. Functions "Membrane transport" (0.3%) were only more expressed when co-cultured with *Curvibacter* sp.

Discussion

Host-microbiome interactions play a critical role in microbiome community assembly, determining which functional groups of microbiota can establish within the microbiome as well as the functions that the microbiome provides to the host organism (Mohamed et al. 2023; Song et al. 2020; Gupta and Nair 2020). Our study aimed to explore in detail two contrasting modes of interaction occurring between individual microbes and their host: (i) a uni-directional interaction mode in which the symbiont is passively selected by the host, and (ii) a bi-directional interaction mode in which the symbiont actively interacts with the host, which in turn affects the selective forces by which the host shapes its microbiome. We previously showed how these two interaction modes lead to different microbiome compositions at the community level and act simultaneously in sustaining microbiome diversity (Yang and Denef 2024). However, the underlying mechanisms remain unclear, which led us to seek insights from single microbial symbiont-host interactions to characterize these two interaction modes between host and microbe populations in more detail.

From a collection of isolates, we selected those with the most contrasting modes of interaction: where a *Curvibacter* sp. outcompeted the *Roseomonas* sp. in Treatment Innate and the *Roseomonas* sp. outcompeting the *Curvibacter* sp. in Treatment Feedback. Despite of the fact that one is outcompeting the other under either effects, they were co-occurred in the microbiome of C. *sorokiniana* that they were isolated from. While this would appear to be contradictory data, we showed in our previous study that both innate and feedback selection are acting simultaneously to explain coverall community composition (Yang and Denef, 2024), and together lead to more species being able to coexist than when one selective force is acting alone. Hence, their co-occurrence in the microbiome is not that surprising. In addition, in a more

complex community context, indirect effects can affect coexistence as well (Daskin and Alford 2012; Davitt, Chen, and Rudgers 2011). The distinctive growth patterns between these two bacteria are also consistent with our hypothesis stemming from this previous study that a microbiome is formed by some bacteria that are passively selected by uni-directional interaction by the host (the passive symbiont, *Curvibacter* sp.), whereas some bacteria drive bi-directional interaction modes that "engineer" host physiology and thus the produced DOM composition (the active symbiont, *Roseomonas* sp.).

To understand what underpins these different interaction modes, we applied transcriptomic analyses to the host and its symbionts. Based on the observed population dynamics of each bacterium in each treatment, we found a significant different gene expression profile for both bacterium between treatment. Specifically, the gene expression profile difference for *Roseomonas* sp. is larger than for *Curvibacter* sp. (**Figure 3.2** and **SI 3.6**). These findings were in line with the idea of two different modes of bacterial-host interactions, with *Roseomonas* sp. experienced a more significant change in gene expression than *Curvibacter* sp. What was less expected is that we only found a slightly larger distance between axenic host gene expression and when co-cultured with *Roseomonas* sp. than with *Curvibacter* sp (**Figure SI 3.6**). While this finding is consistent with our expectation that *Roseomonas* sp. as an active player would trigger a larger difference on the host, but the effect was weak.

Even more surprising was that we found a lower number of DE transcripts relative to the axenic condition when co-cultured with *Roseomonas* sp. (2,390 DE genes) than *Curivabcter* sp. (3,024 DE genes). To seek a possible reason for the unexpected findings, we examined the number of genes overlapped among different co-cultured conditions in *C. sorokiniana* (**Figure 3.2d**). While there were a higher number of genes overlapped between axenic conditions and co-

cultured with *Curivabcter* sp. compared to *Roseomonas* sp., which contribute to a smaller gene expressed profile distance between them. This was driven primarily by the higher number of transcripts that were only detected (and hence labeled as DE transcripts) when co-cultured with *Curvibacter* sp. (2,314 genes) compared to *Roseomonas* sp. (2,031 genes). Potentially, this may indicate that the host tries to exert an antagonistic effect on *Curvibacter* sp, which suppresses *Curvibacter* sp. growth (**Figure 3.1**), as the *Curvibacter* sp. did not grow as well as on axenically produced DOM even though there should have been a steady supply of host DOM released into the media. The underlying mechanism remains unclear.

To investigate the potential metabolites exchange between active symbiont and host that drive the bi-dirctional interaction, we investigated which functions of DE genes were more prioritized in *Roseomonas* sp. when under uni- than bi-directional host-microbiome interactions, as well as in *C. sorokiniana* when co-cultured with *Roseomonas* sp than *Curvibacter* sp. We found that functions related to secondary biosynthesis metabolites were significantly more expressed in *Roseomonas* sp. under Treatment Feedback than Treatment Innate, as well as in *C. sorokiniana* when co-cultured with *Roseomona* sp. relative to the axenic condition (**Figures 3.1** and **3.4**). Consistent with our expectation that, during the bi-directional interactions, bacteria would produce signal chemicals (Dow 2021; Amin et al 2015) and host growth hormones (Cirri and Pohnert 2019) that trigger a change in host physiological and metabolic status. For example, we found that "Flavonoid biosynthesis" and "Isoflavones biosynthesis" were only significantly more expressed when co-cultured with *Roseomonas* sp. (**Figure S3.8**), where the flavonoid is one of the most studied metabolites class that stimulates plant growth promoting rhizobacteria (Ghitti et al. 2024). Furthermore, we found that functions that were more expressed when co-cultured with *Curivabcter* sp. were all related to antibiotic and toxin biosynthesis (Figure S4).

This was in line with the aforementioned finding of host agnostic effect on the growth of *Curvibacter* sp. However, we noticed many of these functions were bacterial-specific functions (e.g., the biosynthesis of staurosporine, novobiocin, monobactam, and aflatoxin), which a follow-up check on the filtration step on green algae transcripts and KEGG database will be conducted.

Our study used controlled experiments to demonstrate the physiological and transcriptomic dynamic in microalgae and bacterial symbionts under uni- and bi-directional host-microbiome interactions. However, we notice some caveats regarding our findings. Firstly, the growth phase affects gene expression status (Veselovsky et al. 2022; Rolfe et al. 2012). To capture a time point during growth where we observed clear effects of the bacterial symbionts on host growth meant that we collected the *Curvibacter sp*. cells during different growth phases between treatments, where Treatment Innate was collected at the exponential to stationary phase, while Treatment Feedback was collected at stationary to death state (**Figure 3.1**). We, therefore, acknowledge a divergence in gene expression profile between treatments can potentially be influenced by the growth phase difference. But we also notice that we found relatively few DE genes in *Curvibacter* sp. when contrasting the two treatments, which indicated that the impact of growth phase on gene expression was small. Secondly, the host-microbiome interactions can vary under different environments (Chen et al. 2019; Ochieno et al. 2021). However, our experiments were only conducted under a constant environmental condition with a high nutrient supply. We acknowledge potential distinct results when the environment changes.

Finally, we observed discrepancies between results obtained from mono-culture experiments and those at the microbiome level. For example, our monoculture of *Roseomonas* sp. exhibited enhanced growth due to a host-microbiome feedback effect. However, in the microbiome, we noted a decrease in the relative abundance of Roseomonas sp. with increasing

Feedback-to-Innate index (Yang and Denef 2024). Furthermore, we observed partially consistent findings with *Curvibacter* sp. In the microbiome, its relative abundance decreased from moderate to high Feedback-to-Innate indices, aligning with our observation in mono-culture experiments; however, its relative abundance decreased when at the highest innate host effect, contrasting to our mono-culture result that it is favored by host innate DOM. This contrasting outcome underscores the complexity of microbial interactions within the microbiome, where the findings from mono-culture settings are not representative enough to predict outcomes in complex community contexts. Nevertheless, we also found consistent patterns between the mono-culture and complex microbiome levels. Specifically, *Curvibacter* sp. exhibited rapid growth and higher abundance, whereas *Roseomonas* sp. was characterized as a slow grower and relatively rare within the microbiome. Despite these caveats, our study does contribute to gaining a deeper understanding of what underpins contrasting modes of host-microbe interactions.

Our study demonstrates how two types of effects—innate host selection and host-microbiome feedback—favor bacteria with different symbiotic functions (passive and active symbionts). This provides an example of how host-microbiome interactions shape and maintain microbiome composition and diversity. While not representative of all host-microbiome systems, the phytoplankton microbiome is a particularly good model for microbiomes that are shaped by host secretions, such as in plant, skin, and coral microbiomes (Boxberger et al. 2021; Voolstra et al. 2024; Müller et al. 2016). The planktonic feature and interaction via metabolic exchanges allowed us to manipulate and examine the separate effects of two interaction modes on the microbiome, which is challenging to conduct in other systems. Our observation brought up the idea of the trade-off between functional groups of bacteria being selected by each host-microbiome interaction mode. For example, while *Curvibacter* sp. was favored by DOM that the

host innately produced, it comes with a lack of ability to drive bi-directional host-microbiome interaction to increase its fitness. On the contrary, albeit *Roseomonas* sp. was a weak symbiont under uni-directional interactions, it was able to shift the competition outcome with *Curvibacter* sp. under the bi-directional interaction modes. We observe a different strategy allocation between *Curvibacter* sp. with fast-growing features and genes set central nutrition utility versus *Roseomonas* sp. being slow-growing but with the ability to express functionally diverse genes that involve both utilizing host DOM and synthesizing chemical signals to interact with hosts. This negative relationship between growth ability and gene expression diversity aligned with the trade-offs between growth and bet-hedging suggested by previous studies (Kim et al., 2020; Groot et al. 2023).

Acknowledgments

The high-throughput sequencing was performed by The University of Michigan Microbiome Core and Advanced Genomic Core. This work was funded by the University of Michigan, including funds from the Rackham Graduate School Graduate Student Research Grant, the Department of Ecology and Evolutionary Biology Research Award Program, The Integrated Training in Microbial Systems program, and the LSA Associate Professor Research Fund.

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Table 3.1 Number of total genes and DE genes in bacteria and microalgae under different treatments.

Number of total genes and DE genes (with adjusted p-value <0.05 and abs(log2FoldChange) >= 1 for bacteria and >= 5 for *C. sorokiniana*) that are relatively upregulated under different treatments.

Organisms	# of total genes/transcripts	Comparison	Treatments	# of DE genes/transcripts that were more highly expressed
Curvibacter sp.	7870	Treatment Innate versus Treatment Feedback	Innate	334
			Feedback	13
Roseomonas sp.	6345		Innate	1572
			Feedback	1402
C. sorokiniana	23510	*+Curvibacter sp.* versus Axenic control	*+Curvibacter sp.*	3024
			Axenic	15
		+Roseomonas sp. versus Axenic control	*+Roseomonas sp.*	2390
			Axenic	45
		+Curvibacter sp. versus +Roseomonas sp.	*+Curvibacter sp.*	3171
			+Roseomonas sp.	2447

Figure 3.1 Bacterial and microalgal host growth dynamics.

Bacterial growth in Treatment Feedback in (a) monoculture and (b) mixed culture and in Treatment Innate in (c) monoculture and (d) mixed culture, and (e) microalgal host growth when co-cultured with either bacterium, both bacteria, and without bacteria (axenic state). The vertical blue dashed lines indicate the sampling time for RNA sequencing. The bacteria were counted by plate counts (CFU) and microalgal growth was measured based on Chlorophyll-a fluorescence (RFU). In the bacterial growth curves, the black and pink points and lines present the growth of *Curvibacter sp.* and *Roseomonas sp.* In the microalgal host growth curve, the black, pink, brown, and green points and lines indicate the growth of microalga co-cultured with, *Curvibacter sp.*, *Roseomonas sp.*, mixed of two bacterium and axenic control.

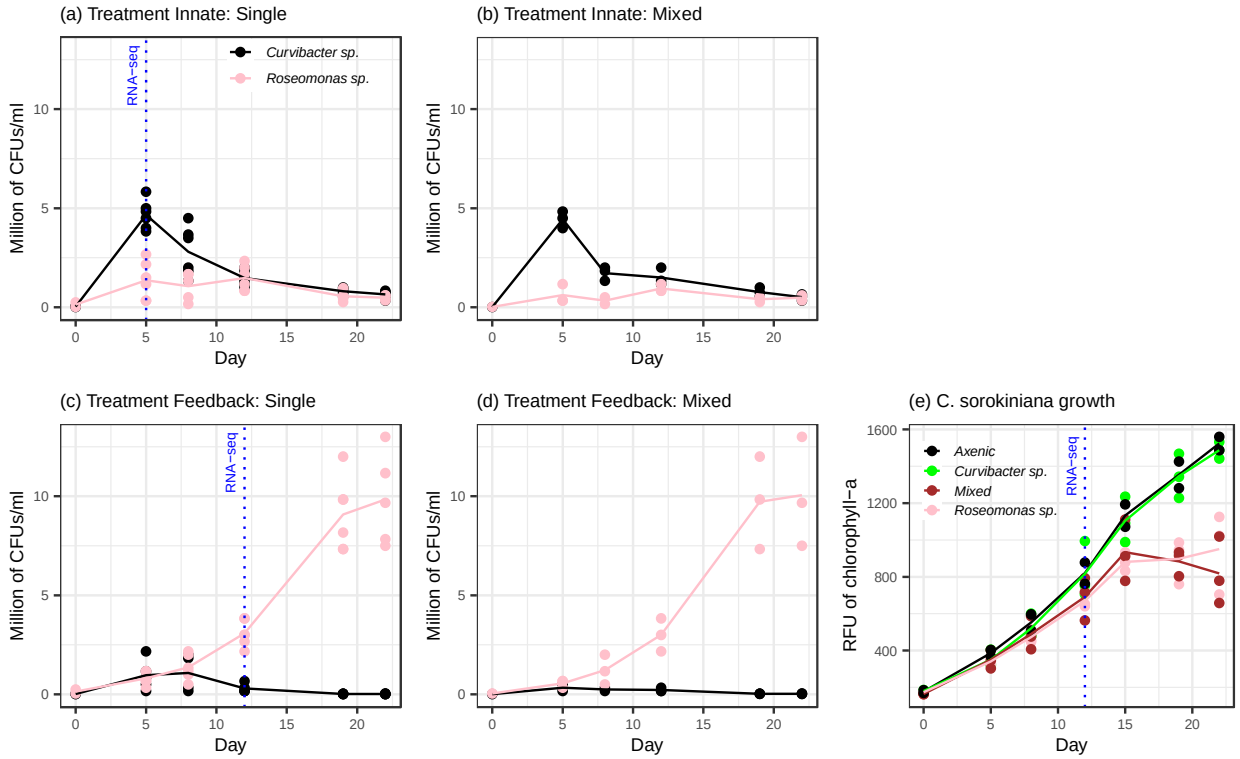


Figure 3.2 Principal component (PC) analysis revealing the gene expression profile differences between treatments for (a) *Curvibacter sp.*, (b) *Roseomonas sp.* and (c) *C. sorokiniana*, and (d) transcript overlap analysis of C. *sorokiniana*.

For both bacteria, green and yellow points indicate gene expression profiles under Treatment Feedback and Treatment Feedback, respectively. For microalgae, the green, pink, and black points represent the axenic state, the co-culture with *Roseomonas* sp., and the co-culture with *Curvibacter sp*. The percentages in the X and Y axis labels show the proportion of variation explained by the first and second PC. The number in the gene overlap analysis indicates the number of gene that were presence each category, with a total number of 23,510 genes.

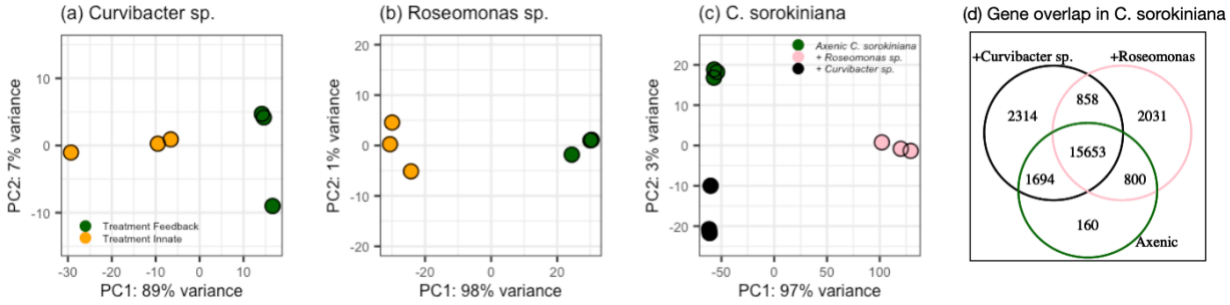


Figure 3.3 *Roseomonas* sp. gene expression of function related to host-microbiome interaction and DOM-related transporter of reference database and DE genes that are more expressed under Treatment Feedback and Treatment Innate.

The % showed the fraction of genes hit to each category, with the exact number of counts in the brackets. The asterisk indicates significant over-expression functions ($p < 0.05$) based on Fisher's extract test or Chi-square test.

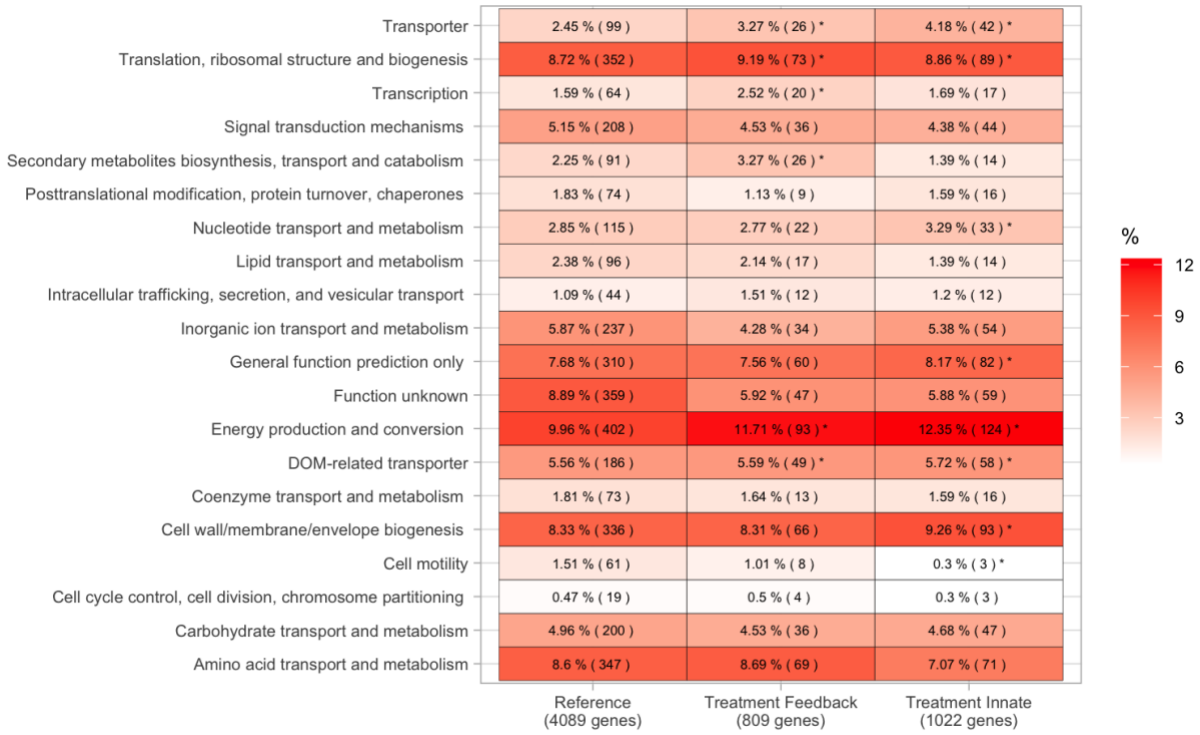
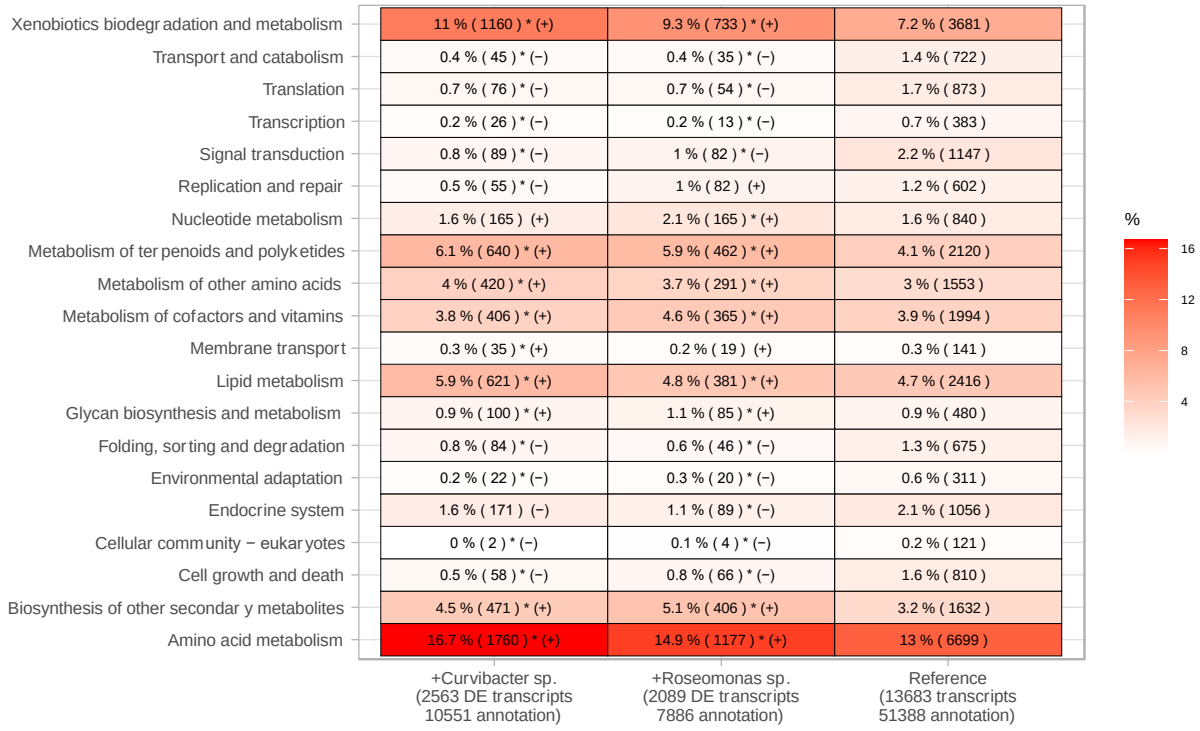


Figure 3.4 The fraction of DE transcripts annotation to function in *C. sorokiniana* co-cultured with *Roseomonas* sp. or *Curvibacter* sp. relative to the axenic condition.

The (+) and (-) symbols indicate over- or under-expressed functions and with an asterisk indicates the expression was significant ($p < 0.05$) based on Fisher's extract test or Chi-square test. Only pathways with significant over- or under-expression that were more expressed when co-cultured with Curvibacter sp. or Roseomonas sp. relative to axenic conditions were shown.



Supplementary information for Chapter 3

Figure SI 3.5 Growth dynamic of six selected bacteria isolated from *C. sorokiniana* under Treatment Innate and Treatment Feedback.

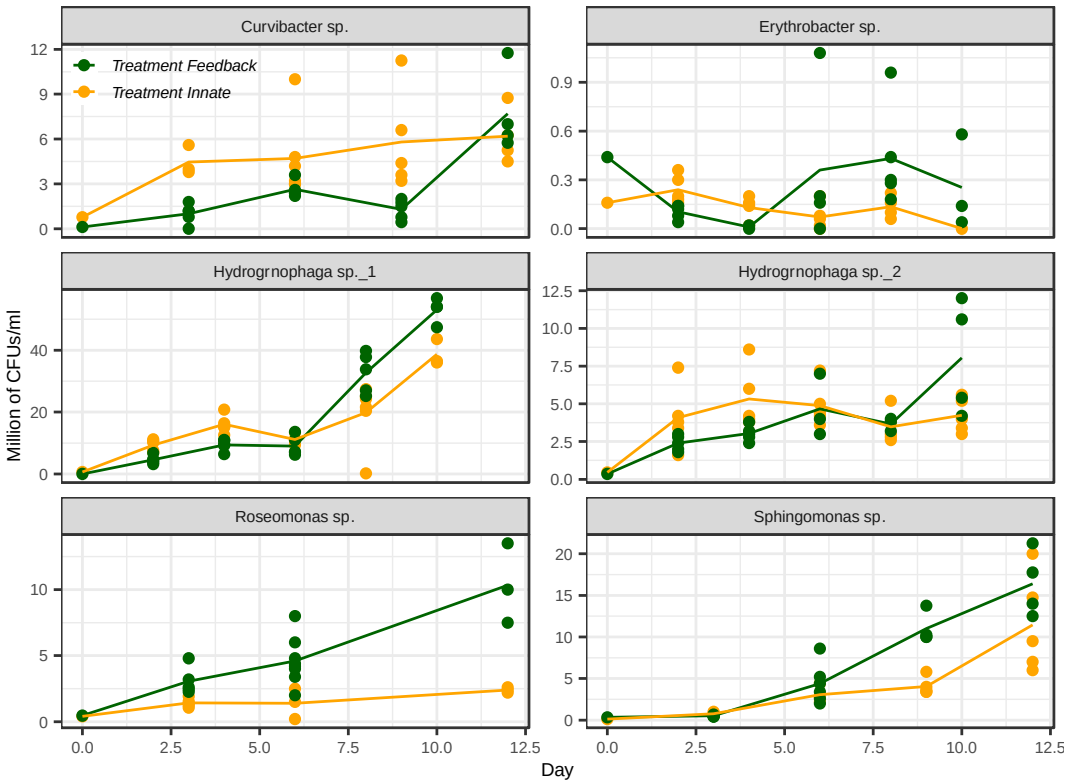


Figure SI 3.6 The dynamic of *Roseomonas* sp. and *Curvibacter* sp. relative abundance in the microbiome when incubated under degree of Feedback-to-innate index from Yang and Denef, 2024.

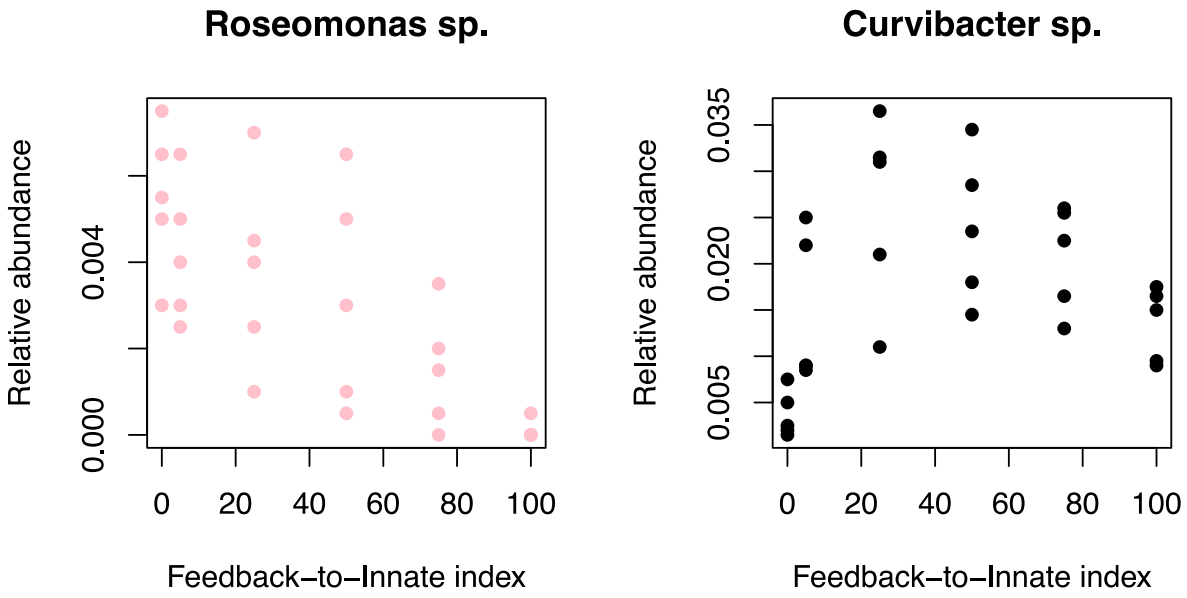


Figure SI 3.7. The Euclidean distance on variance stabilizing transformed gene/transcript count tables of (a) Bacteria between Treatment Innate and Feedback and (b) C. sorokiniana when co-culture with Curvibacter sp. (CC), Roseomonas sp. (CR) and axenic condition (C).

Each treatment with three replicates. The red letters above boxplot are groups with significantly different Euclidean distance between comparisons. It was determined by Kruskal-Wallis test followed by the pairwise T test. The Bonferroni correction method was used for adjusting P-values. The height of the box represents the middle 50% of the data, the whiskers extend to 1.5 times the interquartile range from the first or third quartile, the middle line in each box is the medium.

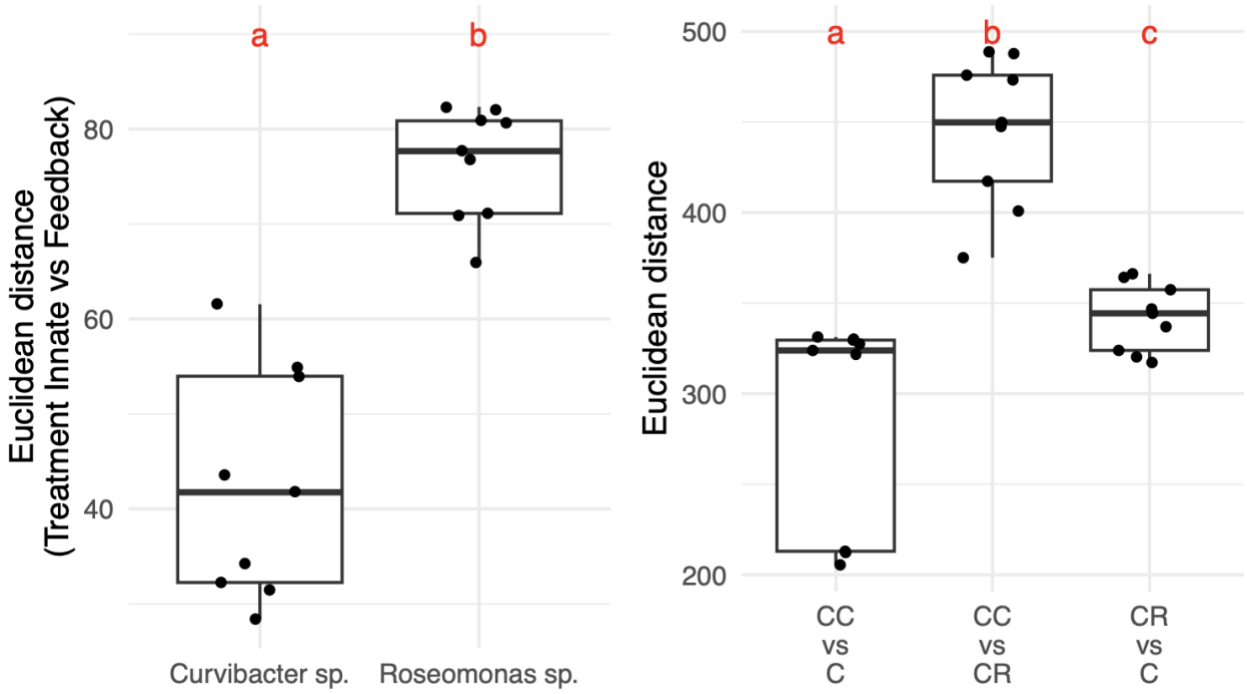
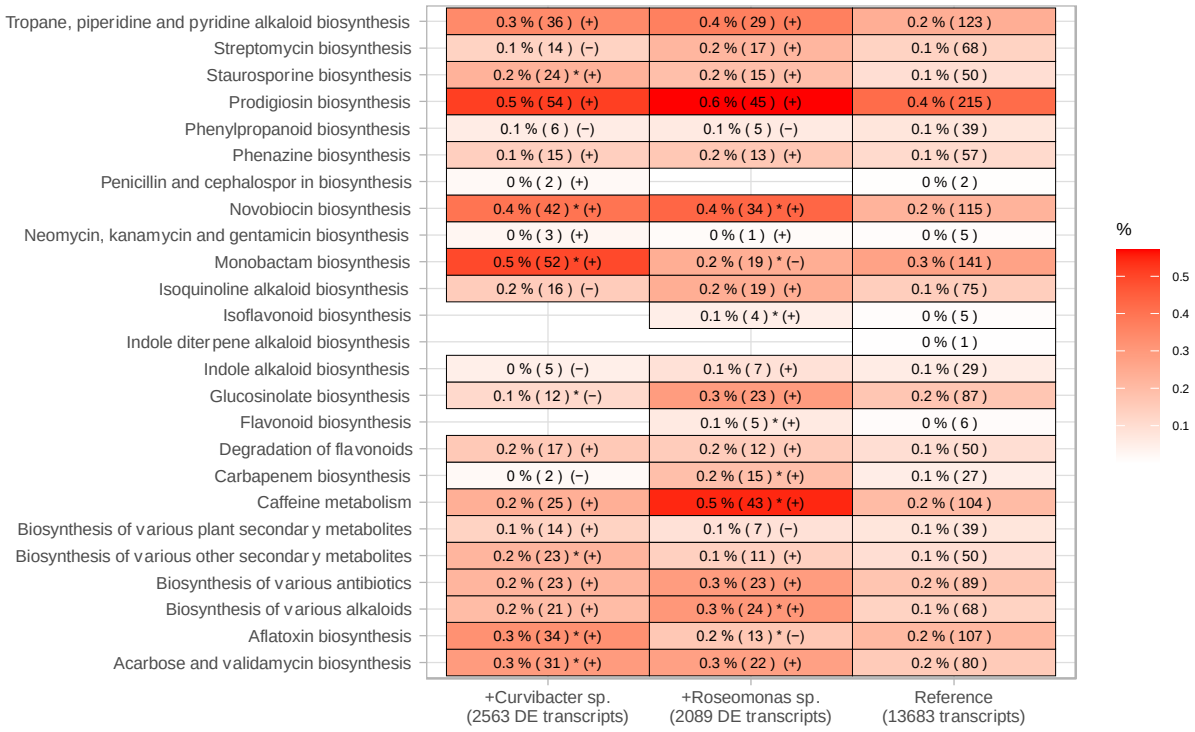


Figure SI 3.8 The fraction of DE transcripts annotation to function category "metabolism of secondary metabolites" in *C. sorokiniana* co-cultured with *Roseomonas* sp. or *Curvibacter* sp. relative to the axenic condition.

The (+) and (-) symbols indicate over- or under-expressed functions and asterisks indicates the expression was significant ($p < 0.05$) based on Fisher's extract test.



Chapter 4 : Dissecting Interplays of Host-Microbiome Interactions and Nitrogen Supply on Phytoplankton Microbiome Composition

Abstract

Microbiomes are shaped by innate host properties, host-microbiome feedback, and environmental conditions, influencing their composition and function provided to the host. Previous studies identified two forms of host selection effects on algal microbiomes that lead to distinct microbiome composition: innate host selection via host-produced dissolved organic matter (DOM) and host-microbiome feedback where microbes affect how the host shapes the microbiome. It is also known that individual host-microbe interactions can shift with environmental conditions. However, it remains unclear how these individual changes in interactions affect overall microbiome composition and impacts on host fitness. We used the green alga *C. sorokiniana* and a synthetic microbiome of seven associated bacteria as a host-microbiome model system. We examined microbiome composition, and how it relates to individual bacterial growth rates and host fitness effects under two host-microbiome interaction modes across three nitrogen supply levels. Our findings show distinct microbiome composition under innate host selection versus host-microbiome feedback, and in function of nutrient supply. These shifts were more pronounced when host-microbiome feedback was present. At lower nitrogen levels, we observed strong competition between phytoplankton and bacteria, whereas at high nitrogen levels, these antagonistic bacteria were suppressed, allowing for more bacteria to establish in the microbiome. We observed a positive relationship between monoculture growth rates and bacterial density in the microbiome only when under high nutrients. Similarly, the

relationship between bacterial impact on host fitness and microbiome density was negative under low nutrient conditions but weakened or disappeared under higher nutrient supplies. Notably, these shifts in relationships were more pronounced under host-microbiome feedback compared to innate host selection. These findings highlight how host-microbiome feedback can amplify competitive effects under lower nutrient conditions and alleviate the competition effect under higher nutrient levels that allow more bacteria to coexist in the microbiome.

Introduction

Microbes that are harbored by eukaryotic organisms are collectively called microbiomes. A variety of studies have indicated a strong association between having specific microbes in the microbiome and its effect on host growth and competitive fitness, highlighting the potential impact of microbiome composition on host health and survival (Lau and Lennon 2012; Rosshart et al. 2017; Coleman-Derr and Tringe 2014; Sampson and Mazmanian 2015; Oliver, Smith, and Russell 2014; Siefert et al. 2018). The composition of the microbiome, along with the functions it potentially provides to the host, is dynamically shaped by host selection (Xiong et al. 2021; Davitt, Chen, and Rudgers 2011; Arandia-Gorostidi et al. 2022), host-microbe interactions (Cirri and Pohnert 2019; Seymour et al. 2017), and the external environment (Ahn and Hayes 2021; Chong-Neto, D'amato, and Rosário Filho 2022). In the context of human-caused global change, interest has been growing in how aquatic nutrient concentration, temperature, and CO_2 levels influence microbiome assembly. In addition, emerging studies have revealed shifts in host selection on microbiome (Trivedi et al. 2022), and changing environment can alter the host-microbe interaction types (e.g., shift to antagonistic or mutualistic interactions) (Rogalski et al., 2021; Paull, LaFonte, and Johnson 2012) and strength (e.g., stronger associated or disrupted)

(Ramanan et al. 2016; Courboulès et al. 2022). These would indicate that besides direct environmental effects on the microbiome (e.g., changing microbiome growth rate and strength of competition), the shift in host-microbe interactions can shift the microbiome composition and functioning.

For the phytoplankton system we focus on, released dissolved organic matter (DOM) serves as a nutrient and energy source for microbes. Consequently, phytoplankton attract and harbor a microbiome attached to and in the boundary layer surrounding phytoplankton cells (Fu et al. 2020; Cirri and Pohnert 2019). As these microbiomes are exposed to the open environment environmental factors may influence the interaction between phytoplankton and bacteria, albeit with varying outcomes. For instance, some research suggests that elevated temperatures enhance the mutualistic relationship between phytoplankton and bacteria by increasing metabolites exchange between them (Arandia-Gorostidi et al. 2022). Conversely, other studies indicate that warming disrupts these mutualistic indications due to nutrient limitations caused by increased algal bloom formation under warmer conditions (Courboulès et al. 2022). Nutritional status also plays a critical role in shaping phytoplankton-bacteria interactions. Mutualistic phytoplankton-bacteria relationships are ubiquitous in oligotrophic environments, in which such interactions confer higher fitness for survival (Hernandez et al. 2009; Gonzalez and Bashan 2000). Conversely, limited nutrition can lead to competition between phytoplankton and bacteria (Cao et al. 2020; Ratnarajah et al. 2021), whereas strong mutualistic interactions are observed under high-nutrient conditions (Danger et al. 2007; Ramanan et al. 2016). These inconsistent findings can be attributed to the net cost and benefit for the host gain from these interactions, which depends on the tightness of species association (e.g., obligate or facultative mutualistic interactions; Hale and Valdovinos 2021; Leftwich, Edgington, and Chapman, 2020) and the

presence of other biological interactions (e.g., pathogens; (Cao et al. 2020; Ratnarajah et al. 2021)

In previous studies, we demonstrated that innate host selection (host control on the microbiome with innately produced DOM) and host-microbiome feedback effect (host-microbiome feedback altering the DOM composition that the host produces) during host-microbiome interactions selected for distinct groups of bacteria, favoring those either influenced by innate host-derived DOM or by direct interaction with the host (Yang and Denef, *in preparation*). These interaction modes collectively contribute to maintaining overall microbiome diversity (Yang and Denef 2024). Counterintuitively, we observed that certain bacteria that had negative fitness effects on the host were favored under the host-microbiome feedback effect (Yang and Denef, in preparation). As these experiments were conducted under conditions of high-nutrient medium and stable incubation temperatures, reducing nutrient supply to levels closer to those found in natural environments may yield different results, either the host selecting for beneficial bacteria, or fitness effects on the host of certain bacterium shifting in function of nutrient supply. Alternatively, bacteria favored by the host-microbiome feedback effect may be parasitic, driving the host to select for them despite these negative fitness effects.

Under innate host selection, the influence of nutrient supply is primarily determined by its effect on the composition and amount of phytoplankton-derived DOM. Nutrient limitation may result in lower production of DOM by the host with lower nutritional value and diversity (Thornton 2014; Livanou et al. 2017), such as a reduction in the release of nitrogen-rich compounds. Thus, we hypothesize that favored bacteria under each nutrient can be determined by their growth rate (which is shaped by nutrient acquisition capacities) in monoculture on the same DOM, in part based on previous studies that showed that bacterial community composition

was associated with the composition of host metabolites (Ferrer-González et al. 2021; Fu et al. 2020). In contrast, the host-microbiome feedback effect with bi-directional host-microbe interaction relies more on the tightness of species associations and the net cost and benefit gained from the host. Limited nutrient supply triggers competition between the host and microbiome. As nutrient supply increases mutualistic interactions can be promoted, up to a point where surplus nutrients may lower the host benefits gained from the mutualistic interaction. We, therefore, expect that nutrient levels will lead to a shift in microbiome composition, one that is larger when the host is present than when the microbiome is merely supplied with DOM produced by the axenic host under different nutrient supplies. We also expected the nature of the interactions to change in function of nutrient supplies, with the most positive impacts on the host at intermediary nutrient concentrations.

To test these hypotheses, we used *Chlorella sorokiniana* as a host model system. Each bacterium was isolated from a *C. sorokiniana* culture with a microbiome that was originally recruited from a natural pond. While phosphorus is more often limiting in freshwater systems (Correll 1999), we focused on nitrogen (N) as the limiting nutrient in this study as we have previously shown N resource limitation to be more affected by the presence of a microbiome (Baker et al. 2022), and N being central to many secondary metabolites involved in interspecies interactions (Singh and Singh 2018). For Treatment Innate, the synthetic microbiome was inoculated into spent medium of *C. sorokiniana* that contains the rich DOM it produced innately, and after all host cells were removed so as to establish only unidirectional host-microbiome interactions (microbiome consuming host DOM). For Treatment Feedback, the microbiome was inoculated into fresh medium with washed *C. sorokiniana* cells without initial DOM to set up allow for bi-directional host-microbiome interactions to shape microbiome selection. In addition,

the growth rate of each bacterium in monoculture was determined under Treatment Innate and Feedback, as well as the impact of each bacterium on *C. sorokiniana* growth rate and carrying capacity. We evaluated how microbiome composition and the extent to which monoculture growth dynamics and impacts on host growth could predict microbiome composition under each treatment in function of nitrogen supply.

Methods

Microalgae and bacterial source, bacterial isolation, and species identification

C. sorokiniana (UTEX 2805) was rendered axenic in 2011 (Jackrel et al. 2020) and maintained on COMBO media slants (Kilham et al. 1998) under a light intensity no higher than 30 μmol · m^{-1} · s^{-1} at 15°C and reinoculated to fresh medium every four to six months since then. Microbiomes were recruited to this axenic culture from natural ponds in 2018 (Jackrel et al. 2021b). The xenic culture we obtained isolates from in this study was generated in 2021 from those initial microbiomes and was also used in our previous experiments identifying the innate and feedback selective effects (Yang and Denef, 2024).

Seven bacterial isolates with distinctive colony morphology were chosen to enable easy tracking of growth of each culture when in a synthetic microbiome. To identify bacterial species by sequencing, for DNA extraction, each isolated colony was resuspended in 90-μL 1× PBS (0.2-μm filtered and autoclaved) and lysed by adding 5-μL lysozyme solution (50 mg/mL) and 100-μL Qiagen ATL buffer and incubated at 37°C for 30 min, followed by the protocol of the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The 16S rRNA gene was amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg et al. 1991b). 1-ul of template DNA was

mixed with 10-ul of NEBNEXT 2X Master Mix (New England Biolabs, Ipswich, MA, USA) and 9 ul of Nuclease Free water. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, and a final extension step at 72°C for 5 minutes. The PCR products were stained with GelRed (Biotium, Fremont, CA, USA) and examined with gel electrophoresis using a 1% agarose gel. The qualified PCR products were sent out for PCR purification, quantification, and Sanger sequencing (Eurofins, Louisville, KY, USA). Low quality base-pairs were trimmed and taxonomic classification was obtained using blastn (Altschul et al., 1990) against the nucleotide collection (nr/nt) database available from National Center for Biotechnology Information (NCBI), with the best hit taxon used.

Semi-continuous experiment with synthetic microbiome and each bacterium

Seven bacterial isolates were mixed and exposed to Treatment Innate (with host innate DOM but no host cells) and Treatment Feedback (with host cells after removal of previously produced host innate DOM) in function of three nitrate supplies, followed by a semi-continuous incubation. Axenic C. sorokiniana was first grown in full nitrate supplied (12.6 N mg/L) COMBO medium until exponential growth phase, then harvested at exponential growth phase (~2 x 105 cells/mL) and 1-ml of culture was transferred to 100-ml of COMBO medium with 0.14, 0.98 and 4.2 N mg/L of nitrate supply, which represent the oligotrophic, eutrophic and polluted environment (Quirós 2003). To deplete the extra nitrogen from the original full nitrate supply COMBO medium in the medium and stored in C. sorokiniana cells, 30% of these cultures was transferred to fresh medium at matching nitrate supply levels after two days of incubation. This process was conducted three times.

To create two host selection treatments, each axenic microalga culture was harvested and centrifuged at 900 x g for 5 min. The supernatant was collected and filtered through 1.2-um pore-size filters to remove any remaining cells. This filtered spent medium was used for *Treatment Innate,* which contained only dissolved organic matter (DOM) innately produced by the host without any direct microbe-host interactions. For creating *Treatment Feedback*, the pelleted *C. sorokiniana* cells were washed in fresh COMBO medium to remove remaining spent medium and resuspended in fresh COMBO medium at ~2 x 10^5 cells/mL. This medium contained only axenic host cells without any initial DOM.

For preparing the bacterial isolates culture, each bacterium was incubated in liquid R2A medium to exponential growth. To minimize the impact of remnants of the liquid R2A medium on the microalgal host, each 1 mL bacterial R2A culture was washed twice by centrifuging at 2,500 x g for 10 min, removing the spent medium, and resuspension in 1 mL fresh COMBO medium (at each nitrogen supply level). The final resuspended bacterial isolates were mixed and inoculated into each treatment after 1,000 times dilution into six 1-ml replicate cultures using 48-well plates (Fisher Scientific, Hampton, NH, USA). The plates were cultured under a continuous 80 RPM rotation with a 16:8 light/dark cycle under a light intensity of ~ 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 20 °C in a Minitron incubator (Infors HT, Bottmingen, Switzerland). After two days of initial incubation, we conducted six daily 30 % (300-µl) transfer of old cultured into 700-µl of fresh medium for Treatment Feedback at each nitrogen level or to 700-µl of fresh filtered *C. sorokiniana* spent medium from the axenic *C. sorokiniana* culture at each nitrogen level.

To track growth of *C. sorokiniana* in Treatment Feedback, relative fluorescence intensity units (RFU) of Chlorophyll-a (with the excitation and emission wavelengths of 465 and 680 nm) were measured as a proxy of microalgal density using a Synergy H1 microplate reader (Bio Tek,

Winooski, VT, USA). To reveal the microbiome composition, three 10-µl replicates of each culture were sampled at Day 6 (with three replicates; **Figure SI 4.5**) and 9 (final day, with all six replicates; **Figure 4.1**). Colony forming units (CFU) were counted using drop plate technique (Herigstad, Hamilton, and Heersink 2001), where each 10-µl subsample was 10^{-2}, $10^{-3}$and, 10^{-4}diluted using R2A liquid medium, and three 10-µl replicate drops of each dilution were dropped on R2A agar plates. The plates were incubated at room temperature in the dark.

Bacterial direct effect on algal fitness

To examine how each bacterium affects microalgal growth rate during the semi-continuous experiment, the aforementioned semi-continuous experiment was repeated but with each isolate inoculated separately, only Chlorophyll-a were measured, and with five replicates. To quantify bacterial effect on microalgal carrying capacity, axenic microalgae across three nitrogen supplies were exposed to each bacterium, after washing and diluting the culture 1,000 times, followed by eight days of incubation during which growth was tracked using Chlorophyll-a measurements. Five replicates were conducted. All cultures were incubated under the same conditions as the experiment with the synthetic community.

Bacterial growth rate in monoculture

The preparation of bacterial isolates, Treatment Innate and Feedback across three nitrogen supplies used the same protocols as the other experiments. To provide time for microalgae-bacteria reassociation, bacterial inoculation was followed by allowing the cultures to grow for 48 hours, followed by two 30% daily transfers (to fresh algal DOM for Treatment Innate and to fresh COMBO medium for Treatment Feedback). After incubation for 24 hours

after the second transfer, 10 % (100-uL) of the cultures were transferred to fresh host DOM (Treatment Innate) or COMBO medium (Treatment innate) for the actual experiment. Bacterial density was measured at the start and after 48-hours, using the aforementioned drop plate technique.

Statistical analysis

The microbiome composition was differentiated with ANOSIM and PERMANOVA, and variation across samples was visualized with PCoA plots based on Bray-Curtis dissimilarity. For bacterial effect on host fitness, we picked the microalgal growth at the final transfer in the semi-continuous experiment with each bacterium inoculated separately. The host growth rate was calculated with an equation based on the cultures being in the exponential growth phase: ln(24 hours growth/initial density)/24 hours (Fernández-Martínez, Javelle, and Hoskisson 2024). The carrying capacity was estimated by fitting a logistic model to microalgal growth in a 8-day batch incubation experiment using R package "growthcurve" package (Petzoldt et al. 2022). To test if bacteria have significant impact on microalgal host fitness, the growth rate and carrying capacity of the host when co-cultured with each bacterium were compared with an axis condition with ANOVA and the Tukey test for paired comparisons. The correlation between bacterial density in microbiome versus bacterial growth rate in monocultures or bacterial direct effect on host fitness were analyzed with a linear-mixed model (LMM), with the former as the dependent variable, the latter as independent variables and bacterial species as random effect. To investigate the correlation based on the relative response or impact among seven bacterial isolates, the values were scaled across different bacteria before the LMM analysis. When investigating the above two relationships within one nutrient supply level, the Pearson's correlation coefficient and its p-

value were calculated. All above statistical analyses were conducted using R package “vegan” (Oksanen et al. 2022) and ”stats” (R Core Team 2023).

Results

Microbiome composition in Treatment Innate and Treatment Feedback in function of three different nitrogen supply levels

We found clear clusters of microbiome composition under Treatment Innate and Treatment Feedback and across three different nitrogen supply levels at Day 6 (after 3 daily transfer) and final day (**Figure 4.1** and **SI 4.5**), with both PERMONOVA p-value <0.05 for all pairwise comparison (**Table SI 4.1**). In Treatment Feedback at the two lower nutrient levels, we observed that the microalgae exhibited a reduction in growth across transfers (**Figure SI 4.6**). In order to identify which bacteria drove this, we repeated the semi-continuous incubation experiment but with each isolate inoculated separately, and we found one bacteria, *Curivibacter* sp., showed a negative effect on host growth under 0.14 and 0.98 N mg/L nitrogen supplies (**Figure S4.7**). It was one of the two bacteria that were observed in the microbiome at the lowest nitrogen supply level (**Figure 4.1**). As for the impact on microbiome diversity, we found that the number of bacteria establishing the microbiome from low to high nutrient supply (0.14, 0.98 and 4.2 N mg/L) was two, four and six bacterial species in Treatment Feedback, and five, six, and five bacterial species in Treatment Innate (**Figure 4.1**).

The relationship between bacterial relative growth rate in monoculture and relative density in microbiome

Base on LMM analysis, setting bacteria as random effect, we found a significant positive relationship between bacterial relative growth rate in monoculture and relative density in a microbiome when under both Treatment Feedback and Innate (Correlation coefficient of fixed effect: 0.27 and 0.15, respectively and both p-value <0.001). However, when we investigated the relationship within the same nitrogen supply, this significant positive relationship was absent when with 0.14 and 0.98 N mg/L supplies under Treatment Feedback, and 0.14 N and 4.2 N mg/L under Treatment Innate (with Pearson's correlation coefficient all p-value > 0.08, **Figure S4.7**).

Bacterial impact on algal growth and its relationship with relative density in microbiome

Among seven tested bacteria, we only found *Curvibcater* sp. significantly reduced microalgal growth rate under 0.14 N and 0.98 N mg/L nutrient supply and carrying capacity under all nutrient supply (**Figure 4.2**), compared to microalgal axenic conditions. Microalgal host inoculated with *Emiticicia* sp. *Erythrobacter* sp. *Hydrigebiphaga* sp. *Parahrinhrimera* sp., *Roseomonas* sp., and *Sphingomonas* sp. showed no significant difference in carrying capacity and growth rate from the axenic condition, regardless of nitrogen level. Nevertheless, I noticed that *Erythrobacter* sp. and *Sphingomonas* sp. showed relatively more positive effects on microalgal host carrying capacity, especially under a higher nitrogen supply.

We found that bacterial effect on microalgal growth rate was negatively correlated to bacterial relative density in the microbiome under both Treatment Feedback and Treatment Innate (Correlation coefficient of fixed effect: -0.38 and -0.28, respectively; both p-value < 0.001; **Figure 4.4**). However, this relationship was absent when under the highest nitrogen

supplies, 4.2 N mg/L, under both Treatment Feedback and Innate (Peason's correlation coefficient (r) = 0.11 and 0.12, respectively, with both p-value > 0.05; **Figure SI 4.8**).

In addition, we also found that bacterial effect on microalgal carrying capacity was negative correlated to bacterial relative density in the microbiome under both Treatment Feedback and Treatment Innate (Correlation coefficient of fixed effect: -0.60 and -0.55, respectively; both p-value < 0.001; **Figure 4.4**). This time, the negative relationship remains but becomes weaker with increasing nitrogen supplies. The correlation coefficient was shifted from -0.76, to -0.55, to -0.34 from low to high nitrogen supply under Treatment Feedback, and from -0.62, to -0.42, to -0.39 from low to high nitrogen supply under Treatment Innate (with p-value all <0.001; **Figure SI 4.9**).

Discussion

Microbiome assembly is shaped by innate host control, host-microbe feedback effects, and abiotic environmental conditions, including temperature and nutrient levels (Davitt, Chen, and Rudgers 2011; Arandia-Gorostidi et al. 2022; Cirri and Pohnert 2019; Seymour et al. 2017; Ahn and Hayes 2021; Chong-Neto, D'amato, and Rosário Filho 2022). While each of these three factors is important in shaping microbiome composition, it remains unclear what the impacts of their interplay are. In addition, most studies focus on either the whole microbiome or specific host-bacteria pairs (Yang and Denef 2024; Fu et al. 2020; Shibl et al. 2020), and studies at intermediate levels of complexity using synthetic consortia are lacking, despite their utility to gain insights into how pairwise interactions translate to higher levels of complexity (Chang and Baji 2023). Here, we investigated the compositional shift of a synthetic microbiome of 7 bacterial members of the *C. sorokiniana* microbiome when exposed to Treatment Innate (innate

host control) and Feedback (presence of host-microbiome feedback effects) in function of three different nitrogen supply levels. Furthermore, to investigate the link between the population and community levels, we estimated bacterial growth in monoculture conditions and tested if it related to the composition of the microbiome. Finally, we examined the impact of each bacterium on the algal host, and examined if it is related to the microbiome composition, to establish the extent to which host impacts drive how the host selects its microbiome under different nitrogen supply levels.

We found that host control by innately produced DOM and host-microbiome feedback effects led to distinct microbiome (**Figure 4.1**) and that composition changed in function of nitrogen supply levels, a shift that was more pronounced under host-microbiome feedback effect conditions. When nitrogen supply was low, competition for resources strongly dominated microbiome assembly, as there were only two fast-growing bacterial species, *Curvibacter* sp. and *Hydrogenophaga* sp., establishing the microbiome. The role of competition, primarily by *Curvibacter sp.*, was further demonstrated by the microalgal host growth being so negatively affected that the growth rate was lower than the semi-continuous dilution rate when under the two lower nutrition supplies (**Figure SI 4.6** and **SI 4.7**). This indicated a strong microbe-microbe and host-microbe competition resulting in strong bacterial competitors dominating the community. On the other hand, when the nitrogen supply was high, there was a higher number (6) of bacteria establishing the microbiome and a relatively consistent microalgal growth rate was observed during the semi-continuous experiment, regardless of mixed or mono-culture inoculation (**Figure SI 4.6** and **SI 4.7**). This indicated that sufficient nutrition can lower the competition stress allowing for a higher diversity of species to coexist in the microbiome.

While the ability to sustain more diversity with increased nitrogen supply may simply be due to relieving ecological selection, host-microbiome feedback may play a role as well in the higher levels of diversity at higher nitrogen levels. This was based on our findings when comparing the microbiome composition and diversity between Treatment Feedback and Innate. The abundance of *Curvibacter* sp. increased with nutrient supply in the Innate Treatment, whereas its density remained constant in the Feedback Treatment, while other bacteria increased in abundance, suggesting an antagonistic effect on *Curvibacter* sp. growth when the presence of the host. In this context, it is also interesting to note that the microalgal growth-promoting effect by beneficial bacteria increased with increasing nutrition supply. This was particularly true for *Sphingomonas sp*., for which we observed the highest positive effect on host fitness, and which only established in the microbiome when in the presence of the host under the highest two nutrition supply levels (Figure 1 and Figure 3). In contrast, at low nutrient supply levels, we observed fewer bacterial species coexisting under the Feedback Treatment compared to the Innate Treatment. This again indicates that the host plays a role in excluding other bacteria, including beneficial bacteria, from the microbiome, as competition for limited resources (nitrogen in this case) dominated community interactions at low nitrogen supply levels. Our findings are in line with studies that observed that lower nutrient levels trigger host-microbe competition (Cao et al., 2020; Liu et al., 2012) while higher nutrient levels can sustain more solid mutualistic interaction (Danger et al., 2007; Ramanan et al., 2016). Our study extended the results from these previous studies by showing that this phenomenon is primarily driven by shifts in host-microbiome feedback effects in the function of nutrient supply levels, rather than solely by the host's response to environmental changes without effects from the microbiome itself, seen

that higher levels of diversity could be maintained at low nutrient levels when the grown on axenic host-produced DOM in absence of the host.

We found that the predictive power of bacterial growth rate in mono-culture on the final microbiome composition varied under two host-microbiome interaction effects and at different nitrogen supply levels. Although we found a positive relationship when considering all data together (**Figure 4.2**) when considering each nutrient level separately we found no relationship under the lowest and intermediate nitrogen supply levels in the host-microbiome feedback effect treatment, and the lowest and highest nitrogen supply in the innate host selection treatment (**Figure SI 4.7**). When nutrients are not limiting, it makes sense that the fastest-growing species is more dominant. When nutrients are limited, ecological theory predicts the species with the lowest R^*, the concentration at which the net growth rate reaches zero, to prevail (Tilman 1981). Hence, the absence of a relationship between monoculture growth rate and relative abundance in the mixed community at the lower nutrient levels is logical, again pointing to the important role of competitive interactions in shaping community composition. Again, the contrast between treatment feedback and innate indicates that when the host is present, competitive interactions are more pronounced at low nutrient concentrations, but less so when nutrients are bountiful, potentially due to host-microbiome feedback effects lessening competition.

We found that bacterial effects on host fitness also correlated with the microbiome composition (**Figure 4.4**). However, this relationship was opposite to what we may have expected when assuming that the host can select for species it favors, as bacteria with more negative effect on host fitness resulted in a higher density in the microbiome. Again, this supports the idea that competition for resources is central to microbiome community assembly, at least under the conditions and with the set of species that we included in this study. However, we

also found that this negative relationship was absent (host growth rate as fitness) or weaker (host carrying capacity as fitness) with an increasing nitrogen supply (**Figure SI 4.9** and **SI 4.10**). Notably, the degree of shift in this relationship across different nitrogen supplies was larger under host-microbiome feedback effect than innate host selection, again pointing at an ability for host-microbiome feedback effects to add to the factors that shape microbiome assembly. This aligns with the aforementioned findings that host-microbiome feedback intensifies competition exclusion under limited nutrients but mitigates competition effects when nutrient supply is sufficient.

Caveats

Culturing and laboratory experiments allow us to test and isolate the specific impacts of each host presence/absence and nutrient supply; however, it was limited in its ability to represent reality and the complexity of the natural environment. Firstly, most of the microbes in the microbiome are challenging to culture. Although the microbiome we used was originally from the natural pond water, we did not necessarily work with bacterial species with dominant roles in the host-microbiome interactions. Secondly, our experiments did not capture many factors in the complex nature, such as dynamic abiotic factors (e.g., changing nutrient, temperature and light intensity), a massive microbial diversity pool from which species can continuously be recruited to the microbiome, and the presence of other interactions such as competition, predation, and viral infection on both phytoplankton and microbes, all of which can potentially affect the host-microbiome interactions. For example, the more stress and dynamic environment with the combination of reducing competition effect from strong competitor microbe on the phytoplankton (e.g., due to massive microbial pool and predation selection on fast-growing

microbial species), the relative benefit from mutualistic interaction for the phytoplankton would become greater and a more solid mutualistic interaction would be established. Future work on incorporating higher-order levels of complexity is needed. This can be achieved by diversifying the range of microbial isolates used and exploring a broader spectrum of environmental factors. Additionally, conducting experiments in natural settings with phytoplankton and bacterial communities under *in situ* incubation setups will provide a more realistic understanding of how the two host-microbiome interaction effects shape the microbiome and their response under changing environments.

Conclusion

Our study showed that different host-microbiome interactions displayed different responses to a changing nutrient supply on microbiome composition and diversity. Specifically, we found that the host-microbiome feedback effect amplified the impact of competitive exclusion when nutrients were limited, while it helped alleviate competition effects when nutrient supply was high, presumably by suppressing the dominant bacteria and sustaining a higher diversity of bacteria to establish the microbiome. Under conditions where bacterial growth rate as monocultures (under the same conditions) was predictive, the relationship was positive, while the impact on host fitness was negatively correlated, together indicating that competitive interactions dominated the factors that shaped community assembly. These findings provide insights into how microbiomes are shaped by host-microbiome interactions under varying environmental conditions and are the foundation for more extensive work that determines the influence of human activity-induced global change within ecosystems. The understanding of the ecological interactions that shape microbiome community assembly is also

of value to understanding general principles of what shapes microbiome community assembly across systems, particularly for systems where host secretions are key and in environments where microbiomes are exposed to external environmental factors (Boxberger et al. 2021; Mohamed et al. 2023; Davitt, Chen, and Rudgers 2011).

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Table 4.1 Microbiome composition under Treatment Feedback and Innate across three nitrogen levels.

(A) Bar chart displaying the natural log-transformed density of each bacterial species within the microbiome under Treatment Feedback and Innate across three nitrogen levels. Each bar represents the mean density, with error bars indicating the standard error of the mean. (B) Principal Coordinates Analysis (PCoA) plot illustrating the microbiome composition based on Bray-Curtis distance under Treatment Feedback and Innate across three nitrogen levels.

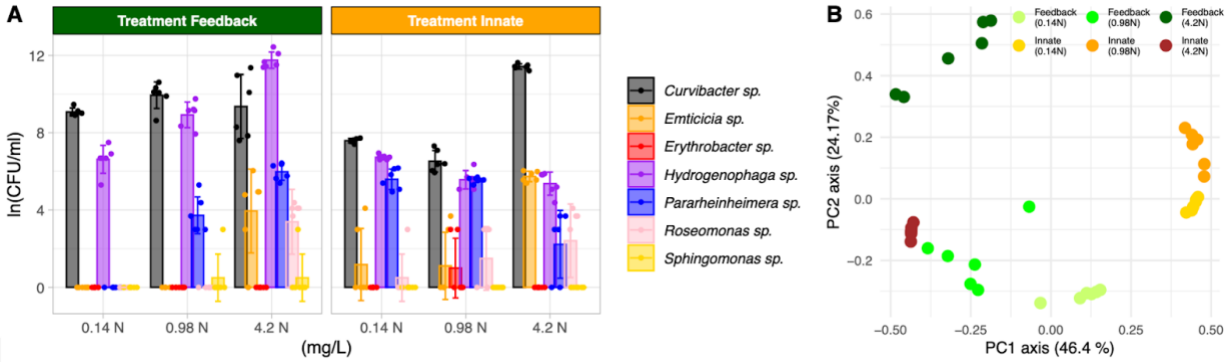


Figure 4.1 Relationship between bacterial relative growth rate in monoculture and bacterial relative final density in microbiome across two treatments and three nitrogen supplies.

The relative growth rate is the growth rate scaled across bacterial species under the same treatment and nitrogen supply, and the relative density in microbiome is the bacterial density in microbiome scaled across seven bacteria under the same treatment and nitrogen supply. Each point on the scatter plot represents the mean of bacterial growth rate and final density, with error bars indicating standard deviation. Linear Mixed Effects Model (LMM) analysis was performed with bacterial species specified as a random effect.

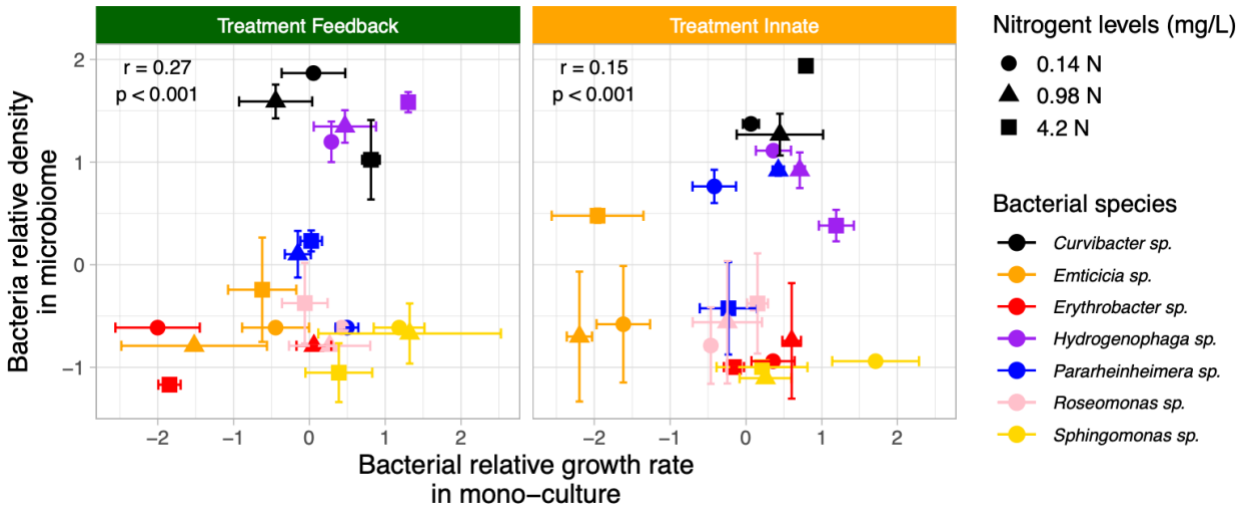


Figure 4.2 Host relative growth rate and carrying capacity with different inoculation under different nitrogen supplies.

The growth rate and carrying capacity of microalgae were scaled across eight different inoculations (axenic and inoculated with each bacteria) as relative growth rate and carrying capacity. The box spans from the first to third quartile, representing the middle 50% of the data. The whiskers extend from the edges of the box to the minimum and maximum values. The asterisk indicates a significant different from axenic control based on Tukey's test.

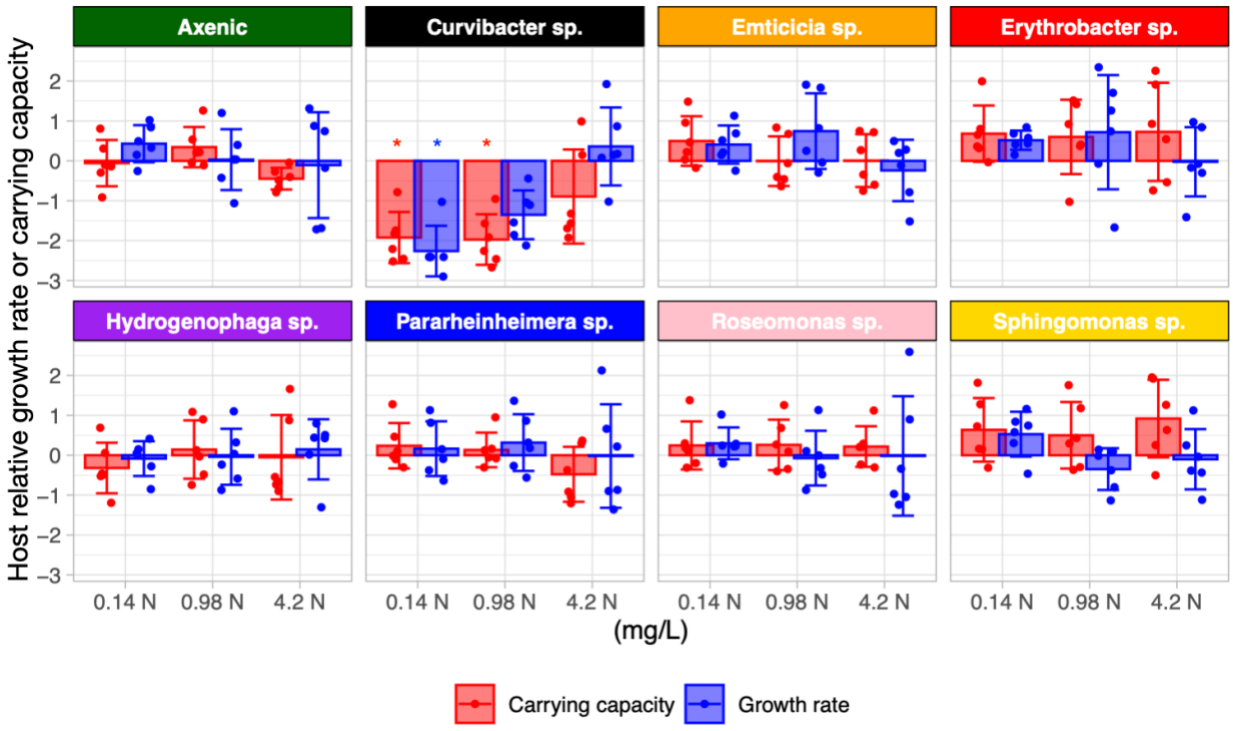
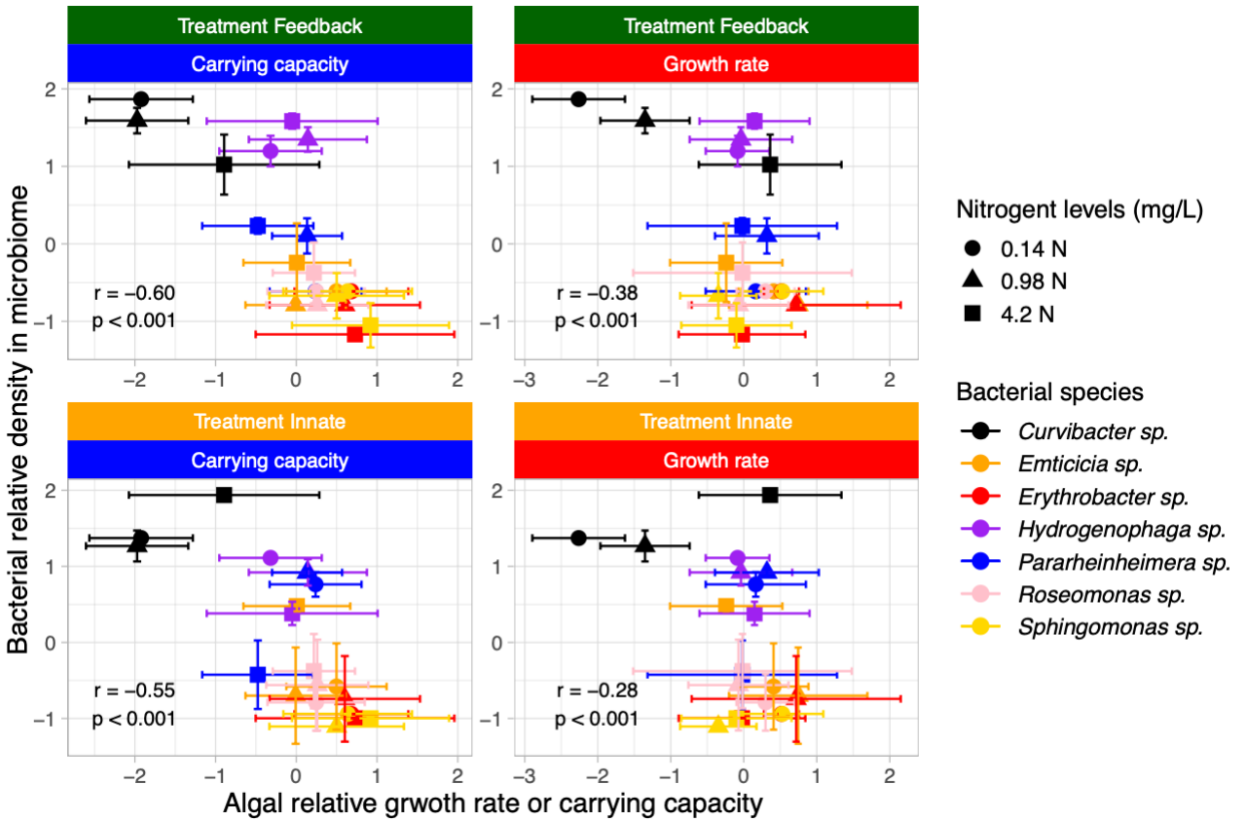


Figure 4.3 The relationship between the effect of bacterial species on algal host growth and their relative density within the microbiome.

All variables were scaled across seven bacterial species under the same treatments and nitrogen supplies. Each point on the scatter plot represents the mean of bacterial growth rate and final density, with error bars indicating standard deviation. Linear Mixed Effects Model (LMM) analysis was performed with bacterial species specified as a random effect.



Supplementary information for Chapter 4

Table SI 4.2 Statistical results of pairwise permutational multivariate analysis of variance (PERMANOVA) on microbiome under Treatment Feedback and Innate across three nutrient supplies.

999 times permutation was conducted. “F” and “I” refers to microbiome under Treatment Feedback and Innate, respectively. The number indicates different nitrogen supplies, where 1: 0.14, 2: 0.98, and 3: 4.2 N mg/L. For example, F1 refers to microbiome under Treatment Feedback with 0.14 N mg/L supplies.

Comparison between treatments		Sum Of Squares	R^2	F	p-value
F1	F2	0.77	0.66	19.5	0.005
F1	F3	2.36	0.87	64.591	0.003
F1	I1	0.97	0.92	113.21	0.003
F1	I2	1.83	0.86	63.536	0.002
F1	I3	1.98	0.96	238.13	0.003
F2	F3	1.53	0.7	23.831	0.002
F2	I1	1.89	0.48	52.209	0.002
F2	I2	2.32	0.8	40.934	0.003
F2	I3	1.03	0.74	28.58	0.002
F3	I1	2.6	0.87	78.318	0.005
F3	I2	2.63	0.83	49.126	0.001
F3	I3	1.81	0.84	54.946	0.006
I1	I2	0.5	0.66	19.625	0.003
I1	I3	2.69	0.98	541.22	0.006
I2	I3	2.74	0.92	108.45	0.005

Table SI 4.3 Results of linear-mixed model (LMM) analysis with bacteria specified as a random effect.

GR refers to bacteria or microalgal host growth rate and K refers to microalgal host carrying capacity.

Independent variables	Dependent variables: relative density in microbiome	Estimate	Std. Error	Degree of freedom	t value	P-value	AIC	BIC
Bacterial GR in Treatment Innate	in Treatment Innate	0.15	0.05	376	3.03	<0.001	1061.47	1077.21
Bacterial GR in Treatment Feedback	in Treatment Feedback	0.27	0.05	376	5.41	<0.001	1042.33	1058.07
Host K	in Treatment Feedback	-0.60	0.03	752	-19.17	<0.001	1919.81	1938.32
Host GR	in Treatment Feedback	-0.38	0.03	754	-10.98	<0.001	2057.07	2075.58
Host K	In Treatment Innate	-0.55	0.03	752	-16.70	<0.001	1981.96	2000.47
Host GR	In Treatment Innate	-0.28	0.04	754	-8.01	<0.001	2107.40	2125.91

Figure SI 4.4 The composition of synthetic microbiome at initial (Day 0) and after three 30% daily transfers (Day 6) in a semi-continuous experiment across different nitrogen supplies.

Bar chart displaying the natural log-transformed density of each bacterial species within the microbiome under Treatment Feedback and Innate across nitrogen levels. Each bar represents the mean density, with error bars indicating the standard error of the mean.

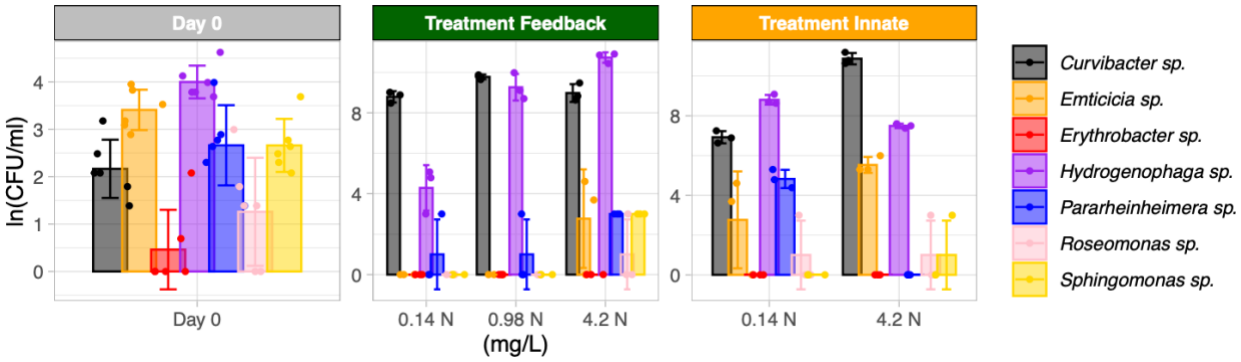


Figure SI 4.5 Growth of *C. sorokiniana* during semi-continue experiment inoculated with synthetic microbiome over seven 30% daily transfers (T1-T7) across three nitrogen supplies.

The relative fluorescence intensity units (RFU) of Chlorophyll-a was measured as a proxy of microalgal density. Lines are the linear regression lines.

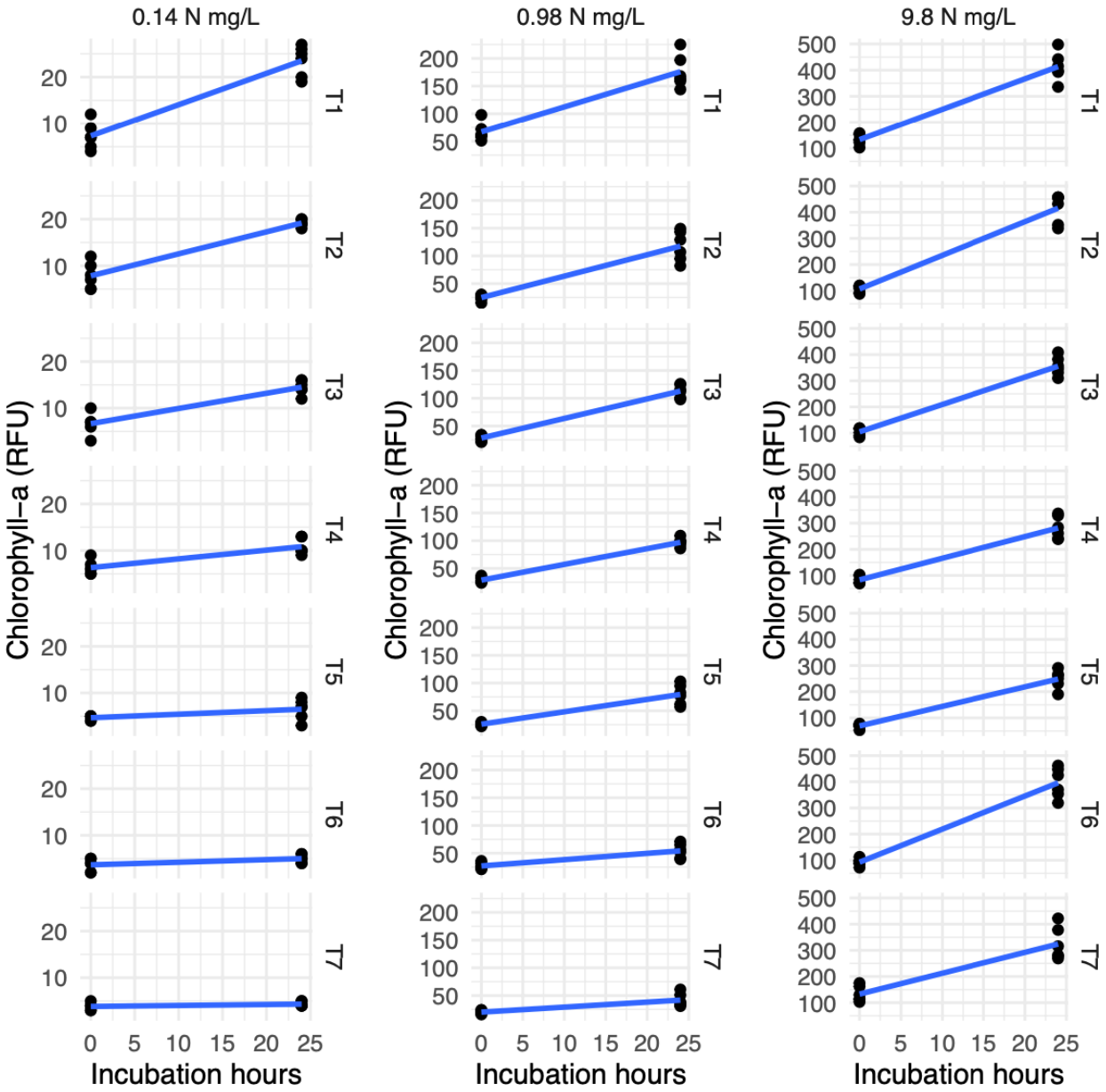


Figure SI 4.6 Growth of *C. sorokiniana* during semi-continue experiment inoculated with each bacterial isolates over seven 30% daily transfers (T1-T7) across three nitrogen supplies.

The relative fluorescence intensity units (RFU) of Chlorophyll-a was measured as a proxy of microalgal density. Different colors of points and lines indicate different bacterial inoculated and regression lines. There was accidently over-diluted on the second transfer (T2), therefore, we grew 48 hours after the 3rd transfer. Nevertheless, it remained enough to show each bacterial effect on microalgal growth.

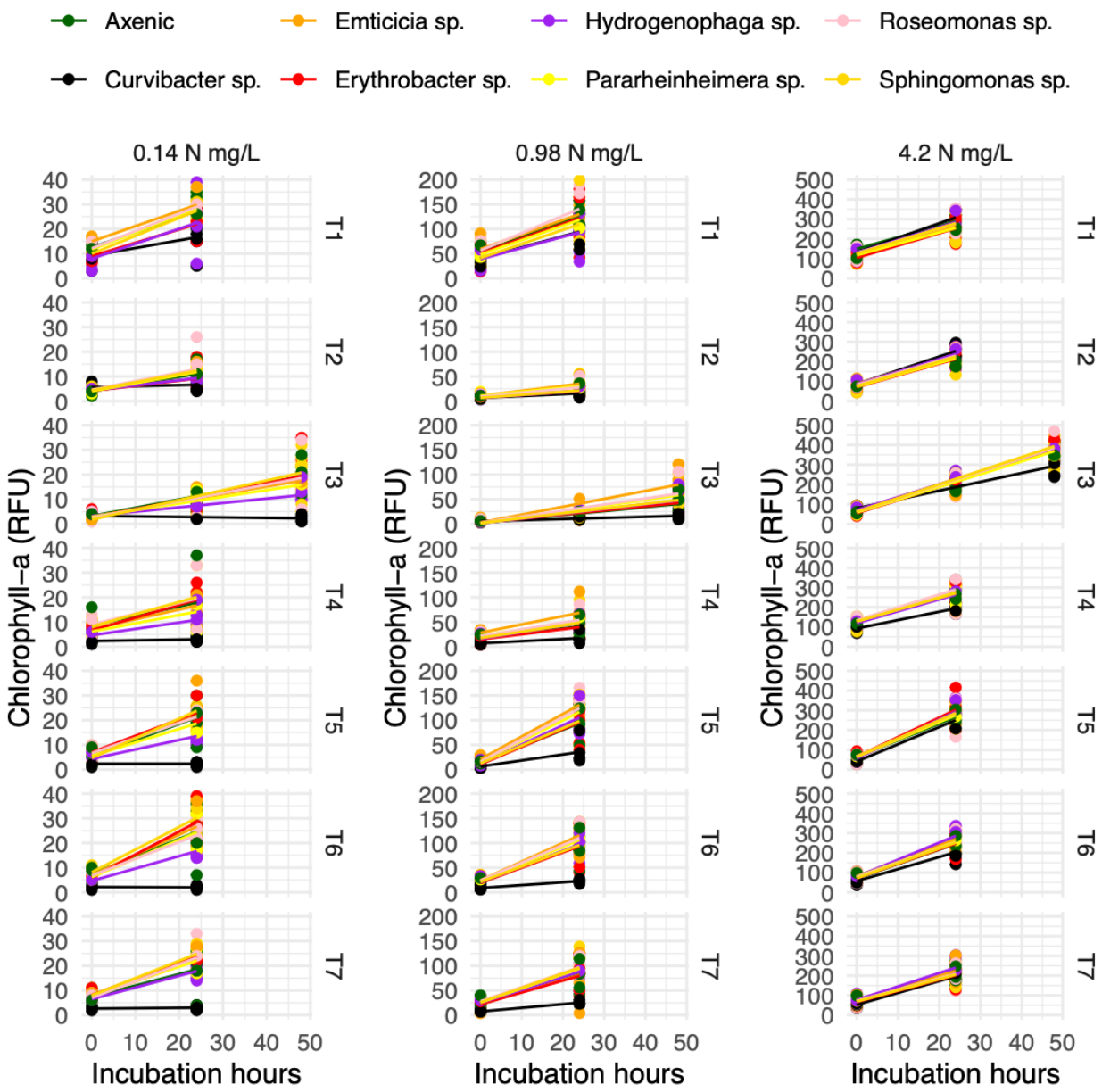


Figure SI 4.7 Relationship between bacterial growth rate in monoculture and bacterial density in microbiome under each nitrogen level.

Both variables were scaled across seven bacterial species under the same treatments and nitrogen supplies. The values are Pearson's correlation coefficient (r) and p-value (p). Each point on the scatter plot represents the mean of bacterial growth rate and final density, with error bars indicating standard deviation.

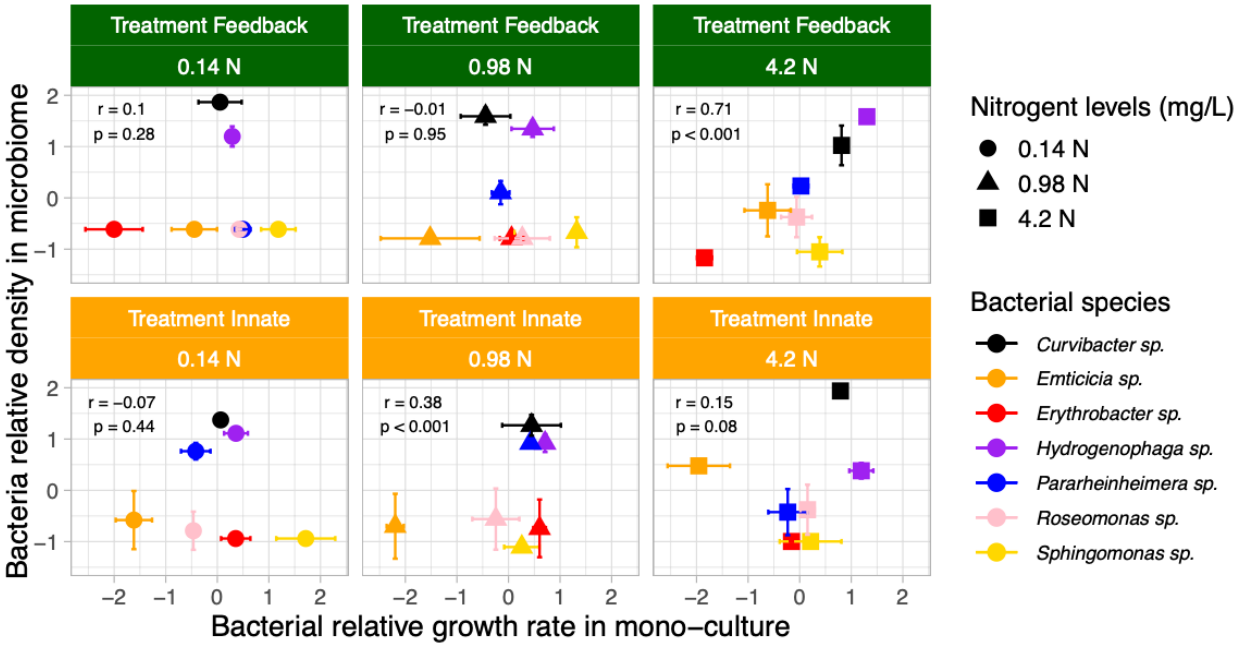


Figure SI 4.8 Relationship between bacterial effect on host growth rate and bacterial density in microbiome under each nitrogen level.

Both variables were scaled across seven bacterial species under the same treatments and nitrogen supplies. The values are Pearson's correlation coefficient (r) and p-value (p). Each point on the scatter plot represents the mean of bacterial growth rate and final density, with error bars indicating standard deviation.

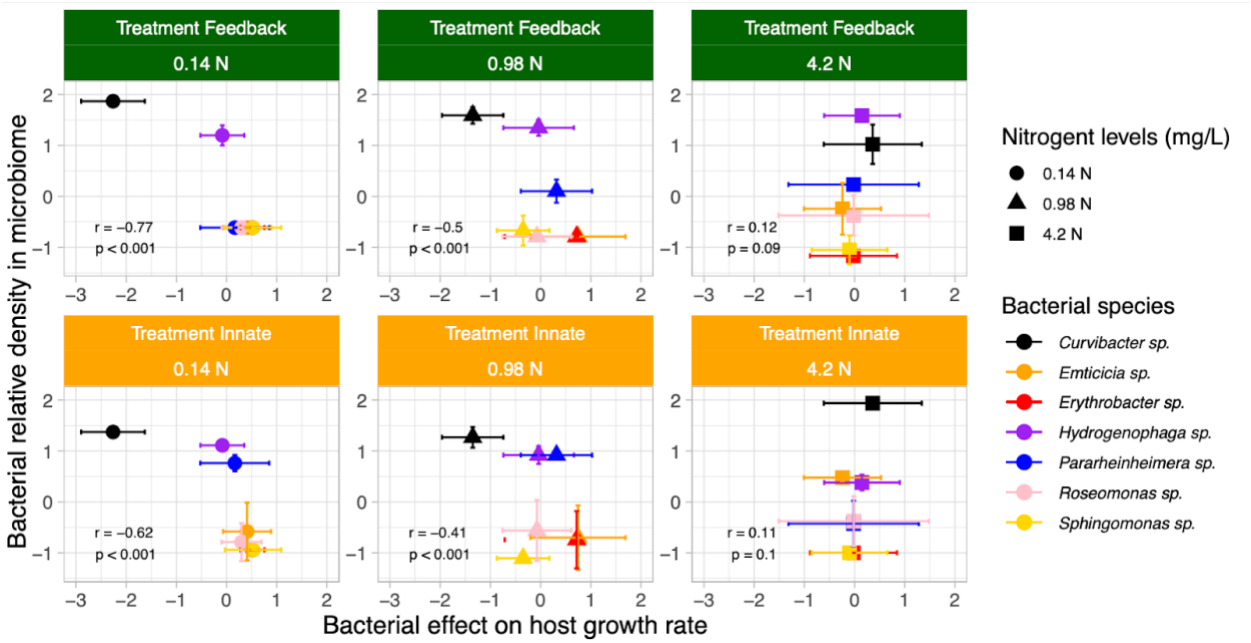
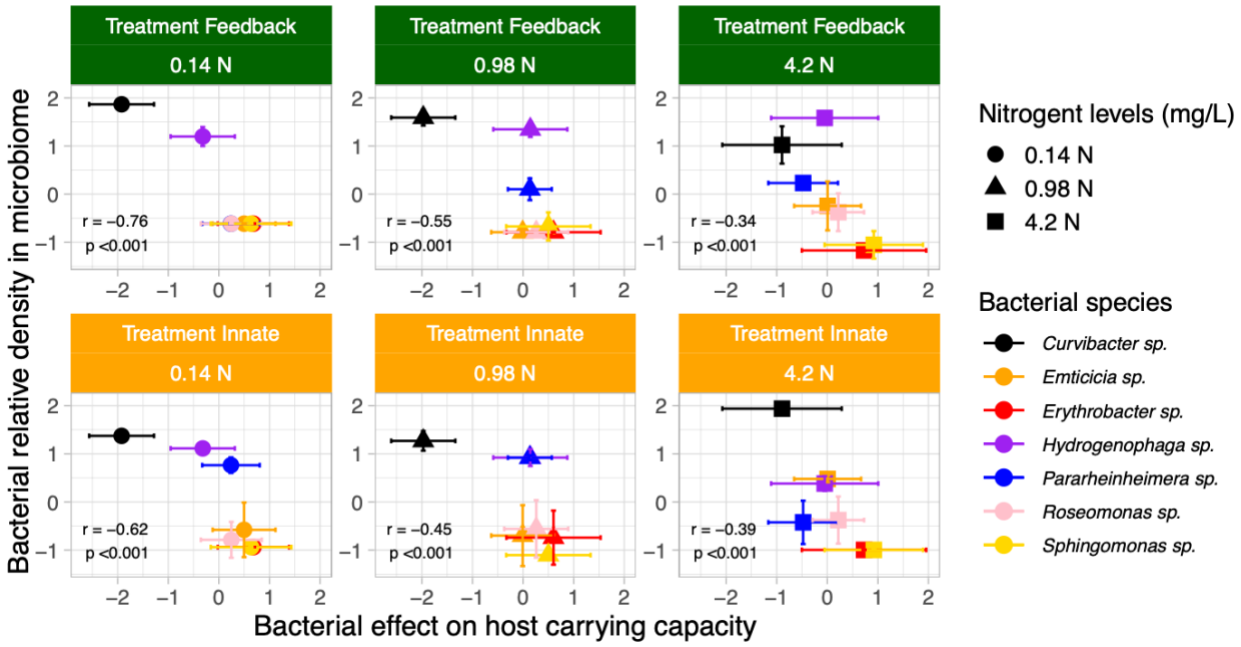


Figure SI 4.9 Relationship between bacterial effect on host carrying capacity and bacterial density in microbiome under each nitrogen level.

Both variables were scaled across seven bacterial species under the same treatments and nitrogen supplies. The values are Pearson's correlation coefficient (r) and p-value (p). Each point on the scatter plot represents the mean of bacterial growth rate and final density, with error bars indicating standard deviation.



Chapter 5 : Conclusion

Reflection

In my dissertation, I used laboratory approaches to manipulate the impact of innate host selection and host-microbiome feedback on the microbiome assembly of *C. sorokiniana*. I investigated the consequences on microbiome composition and diversity, the selection of different functional groups of bacterial populations, and how nutrient supply alters their selection on the microbiome. The goal of my dissertation was to resolve how the complex host-microbiome interactions shape the microbiome and to bridge (a) the linkage between population and community levels and (b) the stable laboratory environment and dynamic environment conditions in natural systems.

In **Chapter II**, I exposed the microalgal microbiome to degrees of the relative effect of innate host selection and host-microbiome feedback effect and examined the dynamic of microbiome community composition and diversity. I found that the two effects result in different microbiome composition and diversity, and together maintain the coexistence of diverse species in the microbiome. The findings suggested that the two effects select for different functional groups of bacteria, where I hypothesized innate host selection would favor passive symbionts that targeted phytoplankton innate DOM but did not directly interact with the host, and the host-microbiome feedback effect favored active symbionts that have the ability to trigger the host to modify its produced DOM to its the symbiont's benefit.

In **Chapter III**, I tested the hypothesis inspired by Chapter II. I focused on a system of the host and two bacterial populations representing the two symbiont types and investigated their dynamic in physiological (growth pattern) and metabolic status (gene expression) between innate host selection and host-microbiome feedback effect. Comparing growth and gene expression between the two effects, I found a passive symbiont grew better under innate host selection and showed a smaller shift in gene expression shift than the active symbiont. The active symbiont grew better under the host-microbiome feedback effect condition and displayed a larger shift in gene expression profile, along with a significantly higher functional expression of biosynthesis and metabolism of secondary metabolites under the host-microbiome feedback effect. The phytoplankton host also showed a larger gene expression profile shift when co-cultured with an active player than a passive player relative to the axenic condition. Although both symbionts induced significant expression of secondary metabolites biosynthesis in the phytoplankton, co-cultured with the passive symbiont only led to antibiotic biosynthesis, while co-cultured with the active symbiont resulted in the biosynthesis of compounds (flavonoid and isoflavones) that could be involved in promoting associated microbes. The shift in phytoplankton host gene expression profile and metabolic functions that associated with the co-cultured bacteria's ability on triggering the bi-directional host-microbiome feedback effect matches to how host-microbiome feedback affect result in different selection on microbiome from the innate host selection. This strong species-specific mode of host-microbe interaction supports the idea of intricate levels of coevolution between the host and specific microbiome members.

Finally in **Chapter IV**, I further considered the impact of nutrient supply on how the two host-microbiome interactions shape the microbiome composition. This time, I used a synthetic microbiome of seven phytoplankton-associated bacteria and measured the composition of the

microbiome and the growth of the individual bacteria under innate host selection and host-microbiome feedback effect across three different nitrogen supply levels. The impact on host fitness by each bacteria was also examined. This allowed us to test to what extent microbiome composition was more driven by host selection in function of the benefit each bacterium confers or the ability of the individual bacteria to acquire and compete for resources. In line with **Chapter II,** I found that the two host-microbiome interaction effects led to a distinct microbiome composition. This composition was strongly affected by nitrogen supply levels, and the impact of nutrient supply levels was larger when host-microbiome feedback effects shaped the microbiome than when microbes competed for the innately produced DOM in absence of host cells. At lower nitrogen levels, we observed strong competition between phytoplankton and bacteria, while at high nitrogen levels, these antagonistic bacteria were suppressed, allowing a more diverse microbiome to be established. Monoculture growth rates correlated best with bacteria relative density in the microbiome under innate host selection conditions. These findings support the hypothesis that host-microbiome interactions shift with changing nutrient supplies, influencing microbiome composition differently from when solely shaped by DOM innately produced by the host.

Some unknowns and potential limitations

My studies were confined to the laboratory and culturing experiments. This allowed a relatively easy manipulation approach and quantification on host and microbiome response at both community and population levels. However, I acknowledge two main limitations in this experimental setting. Firstly, most of the microbes are not readily culturable. Although the microbiome I used was originally from the natural pond water, I did not necessarily work with

bacterial species with dominant roles in the host-microbiome interactions. Secondly, while experimental control allowed me to test and isolate the specific impacts of the two host selection modes under varying nitrogen supply levels, it was still limited in its ability to represent the complexity of the natural environment. Factors I was not able to include were dynamic abiotic factors (e.g., changing nutrient, temperature and light intensity), a massive microbial diversity pool from which species can continuously be recruited to the microbiome, and the presence of other interactions such as competition, predation, and viral infection on both phytoplankton and microbes, all of which can potentially affect the host-microbiome interactions.

Hence, future work should focus on incorporating higher-order levels of complexity. This can be achieved by diversifying the range of microbial isolates used and exploring a broader spectrum of environmental factors. Additionally, conducting experiments in natural settings with phytoplankton and bacterial communities under *in situ* incubation setups will provide a more realistic understanding of how the two host-microbiome interaction effects shape the microbiome and their response under changing environments.

Synthesis

Many microbiome studies have focused on understanding how environment vs. host effects shape microbiome composition. While my studies add to this understanding, my dissertation went beyond what typically has been done by dissecting host selection into innate host selection and host-microbiome feedback effects. These two effects turned out to enact contrasting selective forces on the microbiome, favoring distinct species. Moreover, by comparing with the community assembled after long-term ecological selection without the experimental manipulations I carried out, the community composition indicated that both effects

act simultaneously and together maintain a higher diversity of species in the microbiome relative to each force independently. This provides an example of the mechanisms by which a large diversity of microbes can coexist in a microbiome, adding a response to the classic ecological question of "Why can some many species coexist in a community?". My work provides a new line of evidence of the presence of bacterial species-specific host-microbe co-evolutionary dynamics that can influence overall microbiome assembly. Specifically, I showed how host-microbiome feedback effects can exert a strong species-specific selection and how specific bacteria have the ability to trigger these feedback effects through a bi-directional host-microbe interaction versus other bacteria that merely consume what the host innately provides. Finally, the shift of host-microbe interactions across different nutrient supply levels indicated the importance of considering conditions more in line with natural environmental conditions to more fully understand which forces shape microbiome assembly. While competitive interactions were predominating community assembly at low nutrient levels more in line with gradients found in nature, the degree of community shift in function of nutrient levels was larger under host-microbiome feedback effect than innate host selection. This indicated that the microbiome's response to a changing environment is driven not only by direct impacts of the environment on DOM produced by the host and competitive interactions between microbiome species, but also by shifts in microbe-microbe interactions mediated by the host. My findings provide valuable insights for future studies that want to assess and model the effects of environmental change on host-microbiome interactions and their impact on microbiome assembly and potentially the functions microbiomes provide to their hosts and the ecosystem.