

Nectar microbes: the hidden partners linking plants and animals
“Microbes have remained for long as silent third partners in plant-animal interactions.”



Sergio Quevedo-Caraballo¹, Clara de Vega², Bart Lievens³, Sergio Álvarez-Pérez⁴

¹ Centro de Astrobiología (CAB, INTA-CSIC), 28850 Torrejón de Ardoz, Spain.

² Departamento de Biología Vegetal y Ecología, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain.

³ Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems, KU Leuven, B-3001 Leuven, Belgium.

⁴ Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, 28933 Móstoles, Spain.

The Floral Nectar Microbiome

Storyline

Flowers are more than just pretty! They are like thrilling secret worlds. Inside them, lots of tiny living creatures, called microbes, can live. One of the most exciting places is the nectar, the sweet liquid secreted by the floral nectaries, which flowers produce to attract pollinators, such as bees, butterflies, hummingbirds, bats, and even lizards! But nectar isn't an easy place to live for microbes. It's full of sugar (which makes life hard because it pulls water away), it has very few nutrients like amino acids, and sometimes the plant adds toxic chemicals to keep intruders away. Still, some yeasts and bacteria are clever enough to survive there.

Nectar microbes are found on every continent, from the flowers in your garden to tropical forests, from high mountains to coastal areas. Microbial presence in floral parts other than nectar (e.g., petals, sepals, stamens, stigmas, styles, ovaries, and pollen; *Figure 1*) can also occur, but this has been studied in much less detail and will not be covered in this chapter.

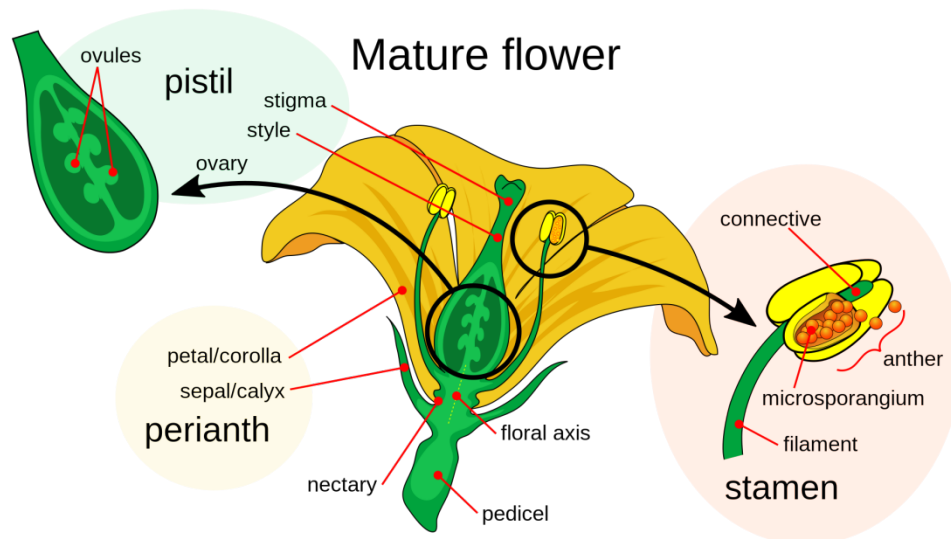


Figure 1: Typical parts of a flower (source: <https://en.wikipedia.org/wiki/Flower>).

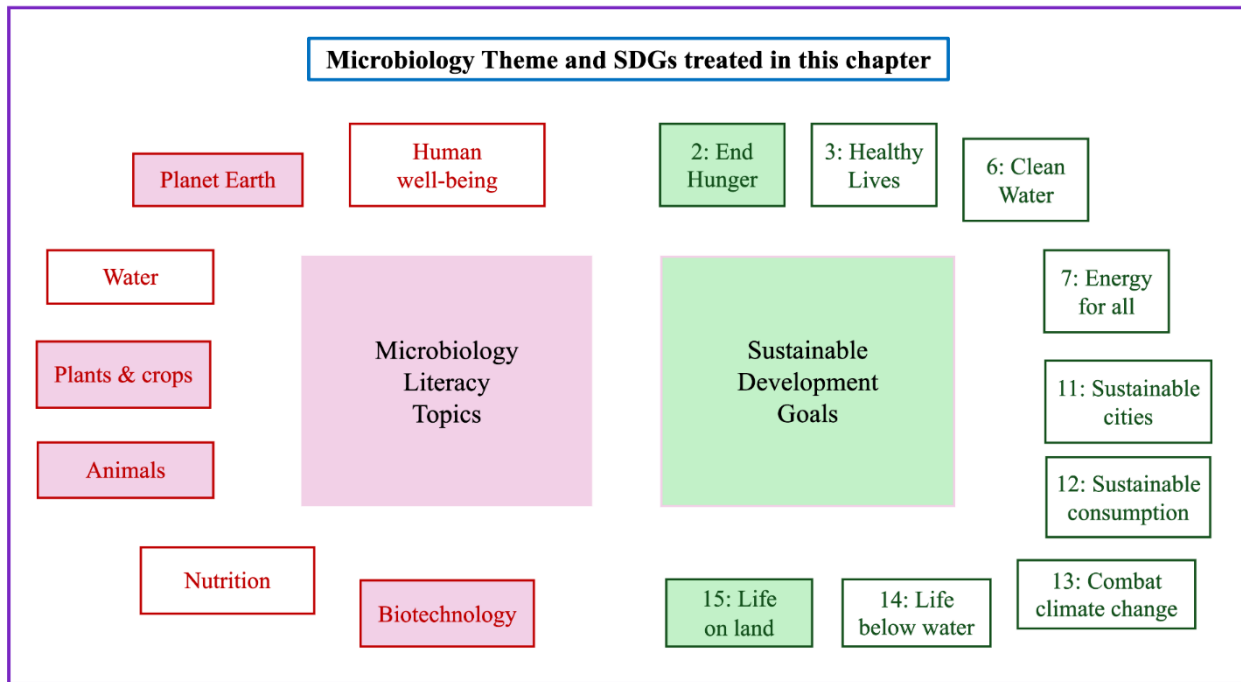
While nectar is assumed to be clean (sterile) when the flower first opens, this sweet secretion often becomes rapidly colonized by microbes, sometimes reaching extremely high cell densities (up to one million cells per cubic millimetre (cells/mm^3) for yeasts and ten million cells/mm^3 for bacteria!). As a result, floral nectar often turns into a thick, sugary soup of microorganisms that plants offer to pollinating insects and other floral visitors.

Recent evidence suggests that this tripartite system (plant-microbe-animal) may be of great ecological importance, as it could help pollination success for a wide variety of plants and contribute to the plants' and pollinators' **fitness**. Additionally, there is an increasing interest in

A learner-centric microbiology education framework

using nectar microbes in various sustainable agricultural applications, including biological pest control and improved pollination, as well as in ecological research.

The Microbiology and Societal Context



Microbiology: plant- and animal-associated microbes; microbial ecology. *Context*: flower pollination; plant-animal interactions. *Sustainability issues*: use of nectar microbes in biological pest control and improved pollination; reduction in the use of toxic agrochemicals; biotechnological potential.

The Plant-Nectar Microbe-Animal system

1. How do microbes arrive to floral nectar?

Nectar-inhabiting microbes originate from various sources, including:

- ✓ environment, such as the surrounding air and raindrops;
- ✓ plant surfaces, especially the surface of leaves and other floral parts;
- ✓ but MAINLY the body (generally mouthparts) of pollinators and other flower-visiting animals (e.g., **nectar robbers**, who feed on nectar through holes bitten in flowers, rather than by entering through the flowers' natural openings. In this way, they get the sugary nectar without pollinating the flower).

Depending on their origin, the microbes arriving into floral nectar are often categorized into two distinct groups:

A learner-centric microbiology education framework

- i. **habitat generalists**: microbes that originate from different environmental sources, show no specific adaptations to nectar conditions, and appear at low densities;
- ii. **nectar specialists**: those microbial species that are mainly dispersed from flower to flower by animal visitors (mostly insects but also humming birds and other animals) and typically show much higher levels of specialization to live and survive in flower nectar. They commonly exert a strong influence on nectar properties.

2. What do microbes do in floral nectar?

Microbes can alter the floral nectar's chemistry in multiple ways. For example, they consume the sugars and amino acids in nectar, thus altering nectar composition, potentially affecting pollinator foraging behaviour. Additionally, nectar microbes can lower the pH of nectar and convert it into an acidic solution, altering its taste and inhibiting the growth of other microorganisms. The activity of nectar microbes can also cause flowers to increase their internal temperature. Finally, nectar microorganisms can also alter the floral scent by releasing a wide variety of **volatile organic compounds (VOCs)** that insects and other animals can sense.

3. How do nectar microbes interact with flower-visiting animals?

Animals can detect in some way (e.g., through VOCs, nectar's taste or even temperature) microbial presence in flowers and adapt their foraging behaviour accordingly. Thus, the microbial-mediated changes in nectar's chemistry might increase or decrease, depending on the case, floral attractiveness to pollinators and other animal visitors of flowers.

Nectar microbes are carried from flower to flower by pollinators and other floral visitors, and some insects may even serve as the overwintering site of nectar specialists between consecutive flowering seasons. Some of these microbes live symbiotically within the insect and shelter in its gut. In return, insect-associated microbes seem to play an important role in insect host physiology and nutrition, in different ways, for example:

- a) by providing digestive enzymes and essential nutrients that insects cannot produce by themselves;
- b) by participating in the detoxification of toxic plant metabolites that are present in the insect's diet;
- c) by producing antibiotics or changing nectar physicochemical properties, hindering the growth of other microbes that act as insect pathogens.

4. How do nectar microbes interact with plants?

The way in which nectar microbes affect plants is still not completely clear. Different studies indicate that it depends both on the plant species and on the type of microbe and the associated pollinator. While some plants have been shown to produce more fruits and seeds when their nectar is colonised by yeasts or bacteria, other plants do not seem to be affected, and there are even plant species for which the presence of nectar microbes can be detrimental. It also remains unclear how plants distinguish between benign or nectar microbes, which cause no

A learner-centric microbiology education framework

harm, and pathogenic microorganisms that exploit nectaries as a gateway to invade other plant tissues. The study of nectar microbes and their role in plant life is still relatively new, and there is much left to uncover about this fascinating hidden world.

5. *How do nectar microbes interact with each other?*

Different nectar microbial species can interact with each other in multiple ways, including:

- a) competition for nectar nutrients;
- b) positive nutritional interactions, such as the exchange of metabolites between different microbial species (**cross-feeding**);
- c) habitat modification, due to the microbe-mediated changes in nectar chemistry, which, depending on the case, might prevent or facilitate the growth of other species;
- d) **antibiosis**, a special type of habitat modification in which some microbes release toxic metabolites or cell components that inhibit or prevent the growth of other microbes.

Additionally, it is essential to consider that the arrival order and initial abundance of each microbial species in floral nectar can affect, either positively or negatively, the growth of other species arriving afterwards. There is compelling evidence demonstrating that so-called “**priority effects**”, in which the order and timing of microbial arrival strongly influence who can establish later, are a major force shaping nectar microbial communities.

Nectar Microbes and their use in Sustainable Agriculture and Biotechnology

1. *How can nectar microbes be used in sustainable agriculture?*

Nectar microbes can be used in sustainable agriculture in at least two ways:

- To develop non-toxic alternatives to traditional chemical pest control.
Microorganisms can alter the scent profile of floral nectar, which, in turn, may prevent the arrival of pest insects to plants and/or attract their natural enemies. Besides, it has also been proposed to use nectar microbes in the biological control of other microorganisms causing disease to plants (i.e., pathogenic bacteria and fungi and other phytopathogens).
- To improve the pollination of major crop plants.
There are studies indicating that the presence of certain microbial species in the floral nectar of specific crop plants, such as pear trees and almond trees, may allow a higher rate of pollinator visits, which in turn translates into greater fruit and seed production. Accordingly, there is a growing interest in using nectar microbes to improve pollination in major crops.

2. *Do nectar microbes have any biotechnological interest?*

Yes, nectar microbes have an as yet unexplored biotechnological potential. For example, the natural sugars in floral nectar are broken down by nectar microbes, producing ethanol and

other alcohols that could be used as **biofuels** if the process were carried out in **bioreactors**. Moreover, some nectar yeasts might be used to obtain specific flavours in beer and other fermented foods and beverages, and the wide variety of VOCs produced by nectar microbes could be exploited in diverse industries.

3. Which factors hinder the use of nectar microbes in sustainable agriculture and biotechnology?

The successful use of nectar microbes in agriculture and biotechnology depends on multiple factors that determine the optimal growth and performance of these microorganisms in the field and under industrial conditions. After all, this is a very recent field of research and there are still many experiments to be carried out before these microbes can be useful for human purposes. Besides, the release of nectar microbes to the field (e.g., for pest control and improved pollination) raises several ecological and ethical issues, which include the possible side effects on non-target insects (i.e., those that are not considered to be pests), such as the modification of their normal microbiota and/or their foraging preferences.

Nectar Microbes in Ecological Research

1. Can microbes be used in ecological research?

Yes, of course they can! Although much of the current knowledge about community assembly and other ecological topics has been acquired through the study of various “macroorganisms”, primarily animals and plants, in recent years it has been highlighted the great potential of microorganisms as model systems for teaching and research in ecology.

2. What characteristics of nectar microorganisms make them suitable for ecological research?

When flowers open, the nectar initially sterile gets rapidly colonized by diverse species of yeasts and bacteria. Most of these microorganisms are part of the natural microbiota of insects and other floral visitors, which are responsible for dispersing nectar microbial communities. Besides, flowers only last a short time (from just a few hours to a few days), their nectar can vary a lot in its chemistry, and plants are often scattered irregularly across natural habitats. All of this makes flower–nectar systems highly dynamic and constantly changing.

Nectar is a hotspot of interactions and an underexplored habitat where dozens of new microbial species are being discovered. The study of these microbial communities allows for the analysis of nectar as an **environmental filter** and provides relevant information on how competition and **synergy** determine the spatiotemporal distribution of microorganisms. Floral nectar microbial communities present qualities very suitable for ecological research due to the short **generation times** of most of their members, their relative simplicity compared to other natural microbiomes (e.g., **rhizosphere**) and their organization in a well-defined **hierarchical structure** of increasing complexity (nectaries within flowers, flowers within individual plants, plants within populations, etc.), thus allowing **multi-scale** approaches.

3. Which types of ecological questions can be addressed using nectar microbes?

All these characteristics have sparked interest in using floral nectar microbiota as a model system for the study and teaching of various ecological topics, such as pollination ecology, inter-species interactions, community ecology, ecosystem functioning, **dispersal**, **competitive exclusion**, **historical contingency**, and **metacommunity dynamics** (island ecology) (Figure 2).

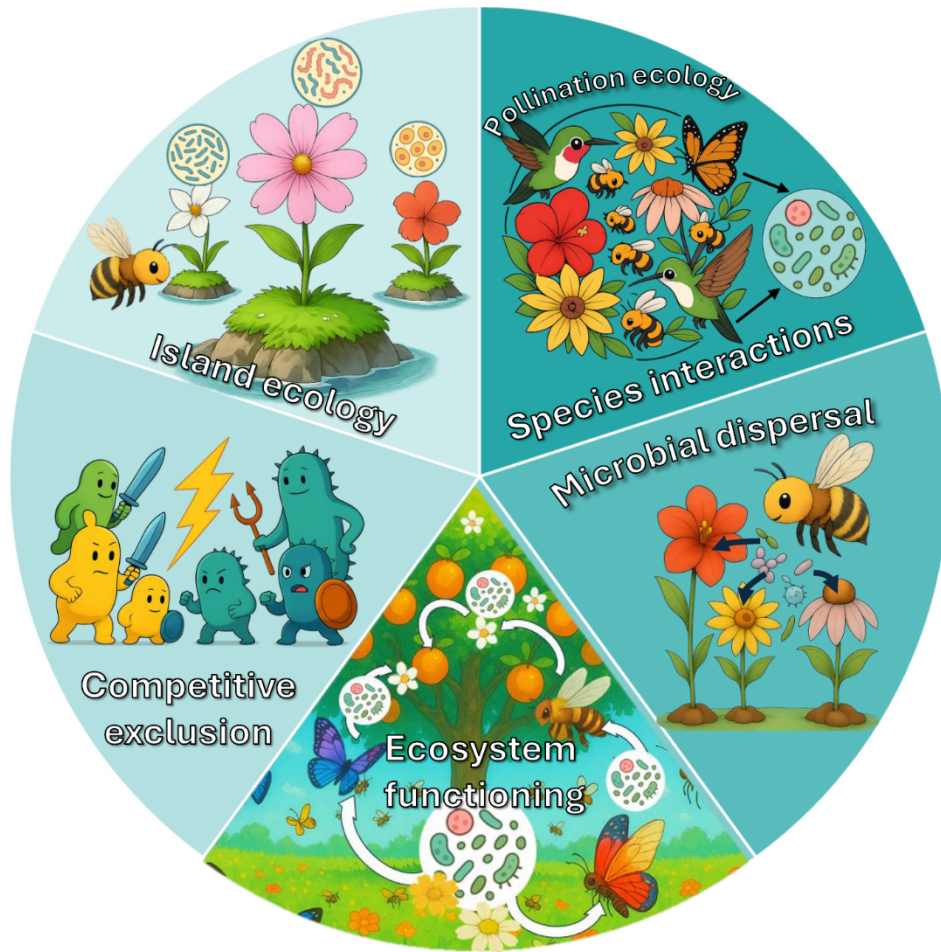


Figure 2: Some of the main ecological issues can be addressed using nectar microbes (source: own production, using artificial intelligence tools with Microsoft Copilot).

Pupil participation activities:

1. Creating a field of artificial flowers

- Activity: Have students go to the field and collect nectar (e.g., with a glass capillary or pipette tip) from flowers of different plant species. Then, the students should examine the nectar drops under the microscope at a magnification of $\times 400$ or more,

A learner-centric microbiology education framework

preferably after staining the samples with methylene blue, 30% lactophenol cotton blue or another dye. Finally, the students will draw the different types of microbes they observe (the shapes are very diverse, spherical, forming chains, or “airplanes”).

- Other possible tasks:
 - The students might compare the microbial diversity they observe in nectar samples from freshly opened flowers vs. older flowers.
 - Some nectar samples might be placed in sugar water (e.g., 1.5 g of table sugar (sucrose) in 10 millilitres of water). After two or more days, the students should observe how the samples smell of fermentation in comparison with a control group of uninoculated samples (i.e., without any nectar microbe) that should not smell of anything.
- Goal: This activity reinforces the concepts of microbial colonization of floral nectar, microbial diversity and VOC production.

2. Research project on nectar microbes

- Activity: Assign students a research project on one of the following topics:
 - interspecies competition
 - **mutualism**
 - colonization of **island-like habitats**
 - impact of fungicide use on the elimination of nectar microbes (loss of biodiversity)
 - potential interactions between nectar yeasts and bacteria

They should propose a theory related to the proposed topic and design an experiment to test it using nectar microbes.

- Goal: This activity enhances students’ research and writing skills while broadening their understanding of diverse ecological processes.

3. Case study analysis

- Activity: Divide students into groups and assign each group a case study about: i) a farm that uses nectar microbes to prevent crop pests; or ii) a farm using chemical-based approaches for pest control.
- Task: Each group should identify the challenges the farm faced, the techniques they used to overcome them and the potential outcomes (benefits, environmental impacts, etc.). Then, the students will present their findings to the class.
- Goal: This activity encourages critical thinking about the real-world application of nectar microbes and how they can contribute to environmental protection and food

security.

The evidence base, further reading and teaching aids

Materials for further reading

1. Plant-nectar microbe-animal system

- Aleklett, K., Hart, M., Shade, A. (2014). “The microbial ecology of flowers: an emerging frontier in phyllosphere research.” *Botany*, 92, 253-266.
- Álvarez-Pérez, S., Lievens, B., Fukami, T. (2019). “Yeast-bacterium interactions: the next frontier in nectar research.” *Trends in Plant Science*, 24(5), 393-401.
- Barberis, M., Nepi, M., & Galloni, M. (2024). “Floral nectar: fifty years of new ecological perspectives beyond pollinator reward.” *Perspectives in Plant Ecology, Evolution and Systematics*, 62, 125764.
- Chappell, C. R., & Fukami, T. (2018). “Nectar yeasts: a natural microcosm for ecology.” *Yeast*, 35, 417-423.
- Jacquemyn, H., Pozo, M. I., Álvarez-Pérez, S., Lievens, B., & Fukami, T. (2021). “Yeast-nectar interactions: metacommunities and effects on pollinators.” *Current Opinion in Insect Science*, 44, 35-40.
- Klaps, J., Lievens, B., & Álvarez-Pérez, S. (2020). “Towards a better understanding of the role of nectar-inhabiting yeasts in plant-animal interactions.” *Fungal Biology and Biotechnology*, 7, 1.
- Lignon, V. A., Mas, F., Jones, E. E., Kaiser, C., & Dhimi, M. K. (2025). “The floral interface: a playground for interactions between insect pollinators, microbes, and plants.” *New Zealand Journal of Zoology*, 52, 218-237.
- Martin, V. N., Schaeffer, R. N., & Fukami, T. (2022). Potential effects of nectar microbes on pollinator health. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 377, 20210155.
- Pozo, M. I., Lievens, B., & Jacquemyn, H. (2015). “Impact of microorganisms on nectar chemistry, pollinator attraction and plant fitness.” In: Peck, R. L. (ed.) *Nectar: production, chemical composition and benefits to animals and plants*. New York, NY, USA: Nova Science, 1–40.
- Quevedo-Caraballo, S., & Álvarez-Pérez, S. (2025). “The role of phenotypic plasticity and within-environment trait variability in the assembly of the nectar microbiome and plant-microbe-animal interactions.” *Ecology and Evolution*, 15(3), e71059.
- Quevedo-Caraballo, S., de Vega, C., Lievens, B., Fukami, T., & Álvarez-Pérez, S. (2025). “Tiny but mighty? Overview of a decade of research on nectar bacteria.” *New Phytologist*, 245(5), 1897-1910.
- Steffan, S. A., Dharampal, P. S., Kueneman, J. G., Keller, A., Argueta-Guzmán,

A learner-centric microbiology education framework

- M. P., McFrederick, Q. S., Buchmann, S. L., Vannette, R. L., Edlund, A. F., Mezera, C. C., Amon, N., & Danforth, B. N. (2024). “Microbes, the ‘silent third partners’ of bee-angiosperm mutualisms.” *Trends in Ecology and Evolution*, 3, 65-77.
- Vannette, R. L. (2020). “The floral microbiome: plant, pollinator, and microbial perspectives.” *Annual Review of Ecology, Evolution, and Systematics*, 51, 363-386.
2. *Use of nectar microbes in sustainable agriculture and biotechnology*
- Álvarez-Pérez, S., Lievens, B., & de Vega, C. (2024). “Floral nectar and honeydew microbial diversity and their role in biocontrol of insect pests and pollination.” *Current Opinion in Insect Science*, 61, 101138.
 - Cusumano, A., & Lievens, B. (2023). “Microbe-mediated alterations in floral nectar: consequences for insect parasitoids.” *Current Opinion in Insect Science*, 60, 101116.
 - Fenner, E. D., Scapini, T., da Costa Diniz, M., Giehl, A., Treichel, H., Álvarez-Pérez, S., & Alves, S. L. Jr. (2022). “Nature’s most fruitful threesome: the relationship between yeasts, insects, and angiosperms.” *Journal of Fungi*, 8, 894.

Teaching Aids

1. *Papers:*

- Fukami, T. (2013). “Integrating inquiry-based teaching with faculty research.” *Science*, 339, 1536-1537.
- Kloser, M. J., Brownell, S. E., Chiariello, N. R., & Fukami, T. (2011). “Integrating teaching and research in undergraduate biology laboratory education.” *PLOS Biology*, 9, e1001174.
- Kloser, M. J., Brownell, S. E., Shavelson, R. J., & Fukami, T. (2013). “Effects of a research-based ecology lab course: a study of non-volunteer achievement, self-confidence and perception of lab course purpose.” *Journal of College Science Teaching*, 42, 90-99.

2. *Videos (in English):*

- Chappell, C. R. (2017). “*Historical Contingency in the Fukami Lab*”: 2-min video on the use of floral nectar microbes to address historical contingency and other ecological questions. YouTube link: https://youtu.be/_3YXz5-MOkg?feature=shared (last accessed: Jul. 22, 2025).
- Hebert T., & Fukami, T. (2023). “*Anna’s hummingbird visiting artificial flowers at Jasper Ridge Biological Preserve*”: 20-second video of Anna’s hummingbird (*Calypte anna*) visiting artificial flowers filled with artificial nectar at Stanford University’s Jasper Ridge Biological Preserve (‘Ootchamin ‘Ooyakma) (Stanford, CA, USA). YouTube link: <https://youtu.be/LbD2r43dvnQ?feature=shared> (last accessed: Jul. 22, 2025).

A learner-centric microbiology education framework

- Fukami, T. (2021). “*Flowers as islands*”: 30-min talk providing an overview of ecological research using flowers as a natural microcosm. YouTube link: <https://youtu.be/vSfbKTmr2PU?feature=shared> (last accessed: Jul. 22, 2025).

Glossary:

- ***Antibiosis:*** Interaction between organisms in which at least one of them is adversely affected by the release of substances from the other.
- ***Biofuels:*** Renewable alternatives to fossil fuels, derived from biomass such as plants and their secretions (including floral nectar), waste, and agricultural by-products.
- ***Biorreactor:*** Device or system designed to maintain a biologically active environment where microorganisms or biochemically active substances can grow and carry out chemical processes in a controlled manner. These devices are used in industries such as pharmaceuticals, food, and agricultural products to cultivate microorganisms under optimal conditions, controlling variables such as temperature, pH, and oxygen.
- ***Competitive exclusion:*** A state in which two species competing for the same resources cannot stably coexist. One will outcompete and exclude the other.
- ***Cross-feeding:*** Exchange of nutrients and/or energy between different microbial species. This process is crucial for the stability and function of some microbial communities, such as those found in the gut of diverse animal species.
- ***Dispersal:*** Movement of microorganisms or their propagules (e.g., spores) across space. This movement can be active, driven by the microorganisms themselves (e.g., through motility), or passive, relying on external factors like wind, water, or animals.
- ***Environmental filter:*** The set of environmental conditions that determines the establishment of a group of species in a given habitat.
- ***Fitness:*** An organism’s ability to survive and reproduce in its environment. It encompasses various traits and abilities that enhance an organism’s capacity to thrive, adapt to changes, and effectively utilize resources.
- ***Generation time (or doubling time):*** Refers to the time it takes for a microbial population to double in size through cell division.
- ***Habitat:*** The natural environment in which an organism usually lives.
- ***Habitat generalists:*** Microbes that originate from different environmental sources and usually show no specific adaptations to floral nectar.
- ***Hierarchical structure:*** Organization of living things from the simplest to the most complex, where each level builds upon the previous one. It includes atoms, molecules, organelles, cells, tissues, organs, organ systems, organisms, populations, communities, ecosystems, and the biosphere. This hierarchy helps to manage the complexity of life by breaking it down into manageable levels.
- ***Historical contingency:*** The idea that the state of an ecosystem is significantly influenced by its past events and conditions, rather than being solely determined

A learner-centric microbiology education framework

by its current state.

- ***Island-like habitats:*** Areas of suitable habitat isolated by unsuitable environments, similar to how islands are isolated by water.
- ***Metacommunity dynamics:*** The study of how local communities interact and change over time, influenced by both local factors (e.g., environmental conditions and species interactions) and regional factors (e.g., dispersal). Essentially, it is about understanding how the interconnectedness of these communities shapes their overall structure and diversity.
- ***Multi-scale:*** This term refers to something involving many levels or sizes.
- ***Mutualism:*** Type of ecological interaction where each species experiences a net positive effect from the association.
- ***Nectar robbers:*** Animals who feed on nectar through holes bitten in flowers, rather than by entering through the flowers' natural openings, thus avoiding contact with the floral reproductive structures and not contributing to pollination.
- ***Nectar specialists:*** Microbes that are mainly dispersed from flower to flower by animal visitors and typically show much higher levels of specialization and are highly adapted to survive in nectar.
- ***Priority effects:*** Effects that the arrival order and initial abundance of species have on the development of assembling communities at a local site (e.g., a flower).
- ***Rhizosphere:*** Narrow soil zone influenced by plant root growth, characterized by distinct physical, chemical, and biological properties compared to bulk soil, and which typically hosts highly diverse microbial communities.
- ***Synergy:*** Interaction or cooperation between living organisms giving rise to a whole that is greater than the simple sum of its parts.
- ***Volatile organic compounds (VOCs):*** diverse group of carbon-containing chemicals that easily evaporate and are released into the air from various sources, including flowers.