The Wonderful World of Microbes

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*Words bolded in dark blue are included in the glossary*

*The purpose of this zine is to reveal the Earth as a Microbial World!*
Land Acknowledgment

The UW-Madison Arboretum occupies Teejop (pronounced day-jope), the ancestral land of the Ho-Chunk Nation. In 1832, the Ho-Chunk peoples were forced to cede this territory and endured decades of genocide by the state and federal government. This history of colonization must be acknowledged. As you move through the Arboretum, please be respectful of any Indigenous spaces, such as the sacred effigy mounds.

Mission, Land Ethic, & Microbial Communities

**Mission:** The UW-Madison Arboretum’s mission is to conserve and restore Arboretum lands, advance restoration ecology, and foster the land ethic.

**The Land Ethic:** Simply, the land ethic is about caring for people, for land, and for strengthening the relationships between them. The land ethic expands the definition of “community” to include our human community with the other parts of Earth, including soils, waters, plants, and animals (the land).

“We abuse land because we regard it as a commodity belonging to us. When we see land as a community to which we belong, we may begin to use it with love and respect.”
- Aldo Leopold, A Sand County Almanac

**Microbes and Microbial Communities:** No organism lives in isolation, and that is especially true for microbes. *Microbes* are tiny living things that are too small to be seen by the naked eye. They are present in almost every environment on Earth. A *microbial community* is simply a group of microbes that share a common living space. Think of any ecosystem, animal, or even a piece of furniture; they all have microbial communities existing within and/or on them. So, the land ethic not only includes the natural systems we can see, but also microscopic organisms that can profoundly influence that environment! As humans continue to alter the Earth, we must consider our impact on these unseen organisms.

“We can love ourselves by loving the earth.”
- Wangari Maathai
Scientists classify organisms using taxonomic groupings. Domain is the broadest group. Two of the domains (Bacteria and Archaea) include microbial organisms with no nucleus. To distinguish the third domain, domain Eukarya, from the all-microbial domains Bacteria and Archaea, all we need to ask is:

**Does this organism’s cell(s) contain a nucleus?**

**Yes!**

Domain Eukarya contains a mix of microbial and non-microbial organisms, including animals, plants, fungi, and more. Typical microbes in this domain:

- Fungi grow in many shapes and sizes in diverse environments. Fungi are often multicellular organisms, such as mushrooms and molds, but they can also be single-cell yeasts, which help us make bread and beer!

- Other eukaryotic microbes include amoebas, protists, protozoa, plankton, tardigrades, slime molds (misnamed – these are not fungi!!), and many others.

**No!**

Domain Bacteria contains small single-celled organisms that are found in almost every explored environment (maybe even space!!).

Domain Archaea also contains small single-celled organisms, but they have different cell structures and metabolisms than bacteria. Originally, archaia were thought to only live in extreme environments (like hot springs), but now, we know they are found in a range of environments.

Viruses do not fall into any defined domain, and some scientists do not define viruses as living organisms. Viruses are tiny capsules containing genetic material (DNA or RNA) that require a living host in order to survive. Almost every organism on earth is a host of at least one virus. There are an estimated $10^{31}$ (10,000,000,000,000,000,000,000,000,000,000,000) viral particles on the planet at any one time!
Cyanobacteria and algae use sunlight, carbon, and hydrogen to produce energy while giving off oxygen.

At-Home Activity: Create Your Own Microbial Community!

To study microbes, scientists give the microbes a suitable place to live and reproduce. This is called culturing. There are two typical methods of culturing bacteria or fungi:

1) In pure cultures, the goal is to grow only a single species of bacteria or fungi.
2) In mixed cultures, the goal is to culture an entire microbial community by mimicking the natural environment as closely as possible.

While pure cultures often require unique ingredients and a controlled sterile environment, you can easily culture microbial communities from mud by creating a Winogradsky column, originally developed by the Russian microbiologist Sergei Winogradsky. You can also create mixed cultures from other sources by baking sourdough, pickling, and making wine, beer, and cheese.

To make a Winogradsky column, you’ll need mud, water, a clear jar or bottle, and a few additional supplies that provide nutrients to the microbes (like eggs and newspaper). I recommend the instructions on the Joyful Microbe blog (joyfulmicrobe.com/winogradsky-column). The Joyful Microbe blog also provides a workbook if you want to write down your observations or carry out other experiments. The results can be beautiful and last for years, as this microbial community is a self-sustaining ecosystem!

Purple non-sulfur bacteria come in a range of colors (despite their name). They prefer little to no oxygen and get their energy from sunlight and carbon.

Purple and green sulfur bacteria grow in no-oxygen conditions, and use light, carbon, and hydrogen for growth.

Sulfate-reducing bacteria consume the egg yolk and release hydrogen sulfide.
Microbes play a significant role in human health. We are most familiar with pathogens, or microbes that cause disease (aka germs). Here are some common pathogens (listed by scientific name) and the diseases they cause in humans.

- **Bacterial pathogens**
  - *Mycobacterium tuberculosis* - tuberculosis (aka consumption)
  - *Borrelia burgdorferi* - lyme disease
  - *Yersinia pestis* - bubonic plague

- **Viral pathogens**
  Viruses and the disease they cause often (but not always) share the same name.
  - SARS-CoV-2 (Coronavirus)
  - Influenzavirus
  - Ebolavirus

- **Fungal pathogens**
  - *Aspergillus fumigatus* causes a range of diseases that fall under the general term “aspergillosis.”
  - *Candida albicans* - candidiasis. This yeast is commonly found on implanted medical devices.

- **Eukaryotic microbial pathogens**
  - *Plasmodium* (multiple species) - malaria
  - *Leishmania* (multiple species) - leishmaniasis
  - *Toxoplasma gondii* - toxoplasmosis

Scientists estimate that human pathogens account for a tiny fraction - far less than 1% - of the total microbial species on Earth.

This zine focuses on a variety of microbes, including microbes that provide drugs to fend off disease, microbes that clean water and enrich soil, and microbes that support the lives of countless larger organisms that we see and appreciate every day, like trees and insects.
Antibiotic Discovery

Even before humans knew what microbes were, people treated illnesses by eating things that can hinder microbes, like garlic, herbs, and fungi. Now we develop antibiotics: drugs that kill microbes. This section focuses on antibiotics that target bacteria.

In the 1920s, Alexander Fleming accidentally discovered the first modern antibiotic, penicillin. After returning from a holiday, he found that one of his bacterial culture plates was contaminated with a mold, and the area around the mold contained a clearing with less bacterial growth. He eventually isolated penicillin from this mold, known now as *Penicillium rubens*.

Since this discovery, many more antibiotics have been isolated from microbial sources. In the past few decades, we have started to face two major problems:

1) **ANTIBIOTIC RESISTANCE**: Current drugs are increasingly less effective at killing bacteria. Bacteria are amazing at finding ways to evade or counteract things that can kill them.

2) **WE ARE NOT FINDING NEW DRUGS**: There have been no new clinical antibiotics since the early 2000s.

How do we get new drugs that can kill pathogens? Scientists have quite a few ideas. One of them is bioprospecting, or the exploration of environmental sources for new drugs. Turns out, microbes in any given environment are constantly trying to kill each other for food and space. As they battle, they evolve new ways to outdo competitors. One strategy involves producing chemicals to kill off other microbes. We can find these chemicals by going into different environments and isolating unique types of microbes (such as fungi that kill bacteria and bacteria that kill other bacteria).
Soil has been the conventional natural source for new antibiotics, and it is a magical place for microbiologists. While many people see dirt, scientists see a beautifully diverse community of microbes (fungi, bacteria, viruses, and more)! In a teaspoon of healthy soil, you can find more microbes than there are humans on Earth - about 10 billion microbes per gram of soil.

Soil is an arena for microbes to battle, adapt, and produce new chemicals. Potentially, we can identify these chemicals and use them to develop new antibiotic drugs to fight pathogens (germs). One group of soil bacteria in particular, the actinomycetes, have been a rich resource for antibiotic discovery. These microbes are responsible for producing 75% of the antibiotics widely used in medicine. Scientists continue global efforts to find microbes in soil that produce useful drugs. For example, the Tiny Earth initiative, headquartered at UW-Madison, is a global network dedicated to involving students in the search for new antibiotics from soil (learn more at the Tiny Earth website - tinyearth.wisc.edu).

How scientists grow microbes from soil

1. Scoop up soil in a sterile tube, add in salty liquid, mix
2. Grow microbes from soil mixture
3. Grow one microbe separately on another plate (see pure culture, page 3)
4. Grow the microbe with a set of human pathogens
   - Pathogen 1 grows
   - Pathogen 2 does not grow
5. If the microbe stops pathogen growth, isolate the chemicals responsible (many steps)
6. Possible drug discovery and clinical applications
7. Repeat steps 3-6 for all microbes that look different (yellow and blue)
Insect-Associated Microbes & Antibiotics

Microbes are everywhere, so any environment could be a source for antibiotic discovery. Sometimes insects and microbes form relationships (known as *symbioses*) that develop over millions of years. Symbioses can range from positive (beneficial to the insect) to negative (harmful to the insect). Microbial partners can produce chemicals to protect or harm their host, and scientists can try to isolate these chemicals for potential drug development.

One famous example of insect-microbe symbiosis is the leaf-cutter ant system.* The ants pick leaves but cannot eat them (1). Instead, they depend on a fungus, *Leucoagaricus gongylophorus*, to digest the leaf material (2). Then, the ants eat the fungus. The fungus grows in fungus gardens in the ant colony, and acts as the ants’ external gut!

The fungus garden is threatened by a specialized parasite, a fungus called *Escovopsis* (3). To protect the fungus garden, the ants formed a relationship with the bacteria *Pseudonocardia* (4), which produce antifungals that kill *Escovopsis*. Some of these antifungal compounds can fight pathogens.

This is just one example of an insect-microbe symbiosis that has yielded drugs. Another example is beewolf wasps that harbor *Streptomyces* in their antennae to fend off fungal infection.

*There are millions of other insects on Earth whose microbes have yet to be explored, meaning insects are a rich potential source for new drugs to counteract antibiotic resistance.*

*These ants live in South America, Central America, and the southern U.S. You can check out the Currie lab ant camera (currielab.wisc.edu), which streams the activity and fungus gardens of a leaf-cutter ant display colony! This colony can be found on the first floor of the Microbial Sciences Building on the UW-Madison campus.
Insect–Microbe Symbioses

Scientists estimate that there are around 5.5 million insect species, many of which depend on microbes to survive in unique environments. They rely on microbes for critical functions such as protection against pathogens and predators, supplying nutrients, detoxifying chemicals, and building body parts!

Aphids carry the bacteria *Buchnera aphidicola* in their bodies. The bacteria produce nutrients that aren’t found in the aphid’s diet of plant fluids. When a bacterial symbiont (symbiotic organism) lives inside an insect, it is known as endosymbiosis (endo = internal). Almost 20% of insect species engage in endosymbiosis with microbes!

Microbes can also help build body parts! Weevils house an endosymbiont, *Nardonella*, that produces components of the insect’s hard protective shell. Without *Nardonella*, the weevil cannot develop properly!

Gut microbes of honey and bumble bees are important for protecting the bees against pathogens. The herbicide glyphosate has been shown to alter the honey bee gut microbial community, increasing the bees’ susceptibility to bacterial pathogens.

As an example of parasitic symbiosis, *Ophiocordyceps unilateralis*, also known as the zombie–ant fungus, alters the behavior of their insect hosts. The fungus’ spores infect an ant (1), and then the ant travels to a spot with the right temperature and humidity for the fungus’ growth. The ant climbs up on a plant, bites down to secure its place, and then dies (2). The fungus consumes the ant and produces a fruiting body (3) that releases spores and starts the cycle anew (4).
Invasive species are organisms that are native to one area but arrive and begin to thrive in a new ecological environment. Invasive species are destructive and there are various approaches to reduce their harmful effects. One approach is called biocontrol, which uses microbes to suppress the invasive species’ growth. Viruses, bacteria, and fungi have all been used to control invasive organisms.

Applying the fungus *Beauvaria bassiana* on ash trees can reduce new colonization of the emerald ash borer by 41%. The fungus kills adult and larval stages of this beetle.

The bacterium *Bacillus thuringiensis* (Bt) produces a toxin that punches holes in the guts of invasive caterpillars, causing normal gut microbes to spill out and poison the bloodstream. Bt can be applied to the leaves of plants that the caterpillars eat.

Invasive species can also impact microbial communities. For example, invasive jumping worms can alter the microbial community in soil. We don’t yet understand what the ecological impact of this might be.

What about invasive microbes? While difficult to detect, invasive microbes alter ecosystems significantly. In the United States, white nose syndrome in bats is caused by a non-native fungus, *Pseudogymnoascus destructans*, and deformed wings in monarch butterflies can be caused by infection with a non-native protozoan, *Ophryocystis elektroscirrha* (OE). Both invasive microbes have devastating effects on these animals.

On the flip side, non-harmful microbes can also spread. In fact, sometimes scientists intentionally spread microbes in an attempt to stimulate positive change. Some examples: the microbial insecticides described above can help restore invaded ecosystems; scientists have found bacteria that counteract *P. destructans* and can treat bats with it; and some microbes can even clean polluted ecosystems!
Microbes & Wastewater Treatment

In healthy lakes, phosphorus and nitrogen are important nutrients to support life. However, large influxes from wastewater and stormwater runoff can imbalance microbial communities and cause algae blooms (see page 12). Microbes are an integral part of cleaning city and industry wastewaters.

When you flush your toilet or take a shower, the wastewater flows through sewage pipes to a water treatment center, which uses physical and biological processes that mimic the natural ways that wetlands, streams and lakes purify water. The first two stages are entirely physical and remove large objects and grease/oil that end up in waterways.

The third stage is the biological treatment. The most common process is activated sludge. The organic waste (sludge) is “activated” by a community of bacteria and other microbes that are really good at eating carbon, nitrogen, and phosphorus. This occurs in basins built to stimulate microbial growth. The treated water is all pumped into another basin, where the solids and microbes settle to the bottom by gravity. A small portion of the sludge is recycled back into the activated sludge basin to “activate” microbial growth on new incoming waste material. The rest is prepared as fertilizer, which is often applied to farm fields to improve crop growth.

Biological nutrient removal with microbes is a more sustainable, effective, and economical process than chemical treatment methods.

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Microbes within Arboretum Ecosystems

UW-Madison Arboretum ecosystems include:
- Prairies and Oak Savannas
- Forests (Coniferous and Deciduous)
- Wetlands

In the next few sections, we will explore the unique and shared microbial processes that are critical for ecosystems to function.

Microbes in Wetlands

Wetlands are areas where water covers (or nearly covers) the soil for at least part of the year. At the Arboretum, about one-third of the 1,200 acres are wetlands. Six types of wetlands (sedge meadow, wet prairie, marsh, ponds, springs and groundwater seeps, and fens) can be found here, plus some constructed ponds to treat stormwater runoff.

Wetlands are critical in improving water quality, reducing flooding, providing habitat, and enabling the transfer of carbon, nitrogen, phosphorus, sulfur, and iron from the soil and air to wetland organisms. These components are then recycled back to the soil through decomposition. This process is called nutrient cycling. Microbes are responsible for many of these wetland services!

Nutrient cycling happens through the food web, where interconnected interactions and feeding relationships determine ecosystem health. Unsurprisingly, microbes make up an important part of the food web.

While plants also play a role, microbial transformations provide the majority of pollutant removal. As in wastewater treatment, aquatic ecosystems depend on microbes to use and recycle carbon, nitrogen, and phosphorus. They are also involved in removing heavy metals, such as copper and lead!
Microbes that use the sun to produce energy, such as algae and cyanobacteria, are crucial for a healthy aquatic ecosystem. However, when phosphorus and nitrogen levels get too high (usually due to fertilizer runoff and leaf inputs), a growth of algae or cyanobacteria occurs, known as an algae bloom. A bloom lowers oxygen levels because oxygen is consumed as algae and cyanobacteria decompose. This causes a dead zone where many organisms, like fish and plants, can no longer survive. The death of these organisms also consumes oxygen, worsening the conditions. Also, toxins are produced by some algae or cyanobacteria that can harm humans and dogs that go into the water near blooms. Shellfish from these affected areas are dangerous to eat, as the toxins can be concentrated in their bodies.

Prairies are dominated by grasses and wildflowers. Most of the prairie microbial processes occur underground or within plants and animals. As in aquatic environments, microbes recycle nutrients in terrestrial ecosystems by decomposing material (dead animals, plants, other microbes) and making it available to living organisms. The most well-studied soil microbes are bacteria and fungi.

One major microbial process in prairie soils is nitrogen fixation. Essentially, microbes transform an unusable form of nitrogen ($N_2$) into fixed nitrogen ($NH_3$) that microbes, plants, and other organisms can use to live. Nitrogen is a core component in many building blocks of life, such as DNA and amino acids.
Bacteria are largely responsible for nitrogen fixation, and in prairies they often reside in the root nodules of legumes (for example, pea and bean plants). This group of bacteria are known as rhizobia, which are naturally found in soil. In this symbiotic relationship, the usable nitrogen converted by bacteria is used for plant growth, while the bacteria receive sugars produced by the plant, as well as protection from pathogens and predators. When the plant dies, the rhizobia are released back into the soil where they can form new associations with other plant roots.

Prescribed Burns & Microbial Communities

Prairies and oak savannas evolved with naturally occurring fires and still depend on fire to remove old plant material and stimulate new growth. With the suppression of naturally occurring fires, land managers now use prescribed fire, or carefully controlled fires set under specific conditions. Prior to European colonization, the Ho-Chunk people used this technique to maintain ecosystems in the current day Arboretum lands.

The complex response of microbial communities to fire depends on a variety of factors including fire severity, moisture levels, and season. Generally, soil microbes are key to ecosystem regeneration and recovery. Stress-tolerant microbes do better in these areas and fire-warmed soil often increases microbial activity, which releases nutrients from decaying material that help native plants thrive. However, the results from many scientific studies indicate that microbial responses to fire are as diverse and complex as the microbial communities themselves, meaning a lot more investigation in this area is needed!
Microbes in Forests

While exploring a forest, you might notice plants but not the hidden microbial life in the soil that the plants need to survive. More than 90% of all plants depend on what are known as mycorrhizal symbioses (myco = fungi, rhiza = roots, symbioses = partnerships). Through these symbioses, plants and fungi have access to resources they could otherwise not get.

Plants provide the energy-rich carbon compounds (sugars and fats) produced during photosynthesis to the fungi, and fungi transfer water and nutrients essential for life from the soil to the plant. Specifically, structures known as fungal mycelium absorb nutrients from the soil. Mycelium consist of bundles of fine tubular structures (hyphae) that branch out and search for food. When the hyphae find food, they digest it, and absorb it in their “bodies,” and share it with the mycelial network and connected plants. Some fungi exist only as mycelium over their entire lifetime, while others produce fruiting bodies when the conditions are right. The most common type of fruiting body we see are mushrooms, which occur when certain types of fungi fuse their hyphae together. The hyphae rapidly inflate with water absorbed from their surroundings. This is why there are more mushrooms after it rains.

Mycorrhizal networks form when multiple plants and fungi associate with each other, often exchanging nutrients. Mycorrhizal networks can be huge, connecting miles of plants and fungi to each other. Globally, mycorrhizal fungi are so abundant in soil that the estimated total length of mycorrhizal hyphae in the top four inches of soil is around half the width of our galaxy (~300,000,000,000,000,000,000 or 3x10^{17} miles)!!!

Mycorrhizal symbioses

Plants provide

Fungal hyphae searching for food

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Some plants have “hacked” into mycorrhizal networks to cheat the system. These plants are known as *mycoheterotrophs* — they get 100% of their energy from the mycorrhizal networks, including the carbon produced by other plants. Mycoheterotrophs can be identified by their lack of green since they don’t photosynthesize. In the Arboretum, look for ghost plants (*Monotropa uniflora*). They are completely white and depend entirely on mycorrhizal networks.

If plant life (and by extension everything else) depends on mycorrhizal networks, what will happen as environmental change threatens these interactions? We all depend on the ability of plants and fungi to adapt to changing conditions, such as pollution, deforestation, and climate change. But plants do not carry the mycorrhizal fungi in their seeds; fungi and plants are constantly reforming their relationships in the soil. So what happens if we wipe out a critical mycorrhizal species in a certain environment?

We simply do not know enough about these amazing networks to understand the damage we may be doing and how we can conserve these microbes. However, scientists around the world are tackling these questions. Scientists are also exploring how fungi can counteract pollution and climate change. Fungi can break down many things, including toxins produced by wildfires, cigarette butts, and plastics! So they are promising candidates for cleaning contaminated lands. Fungi can even decompose radioactive material and have been observed thriving around the Chernobyl Nuclear Power Plant!!
Microbes are by definition only visible using a microscope, but you can still see evidence of microbial life - known as **microbial markers**. As you walk around the Arboretum or other outdoor spaces, look for these microbial markers!

### Wetlands

- **Methanogens** are archaea that produce the gas methane, deep in the sediments of wetlands/marshes where there is no oxygen. Microbial markers of methanogens include **bubbles** around aquatic plants and **popping noises** as the gas travels through plant stems. The release of methane and other gases can also produce **marsh gas** – and even light! This phenomenon is called “will o’ wisps” and “ghost lights,” among other names.

- **Metal oxidizers** are a group of bacteria that use metals like manganese, copper, or iron for energy. If you see a **rainbow sheen** on water that looks like an oil spill, this is evidence of bacteria using manganese. To check if it’s a result of bacteria or oil, poke the film with a stick. If it fragments like breaking glass, it’s caused by bacteria. For bacteria that use iron, the wetland or river will often have a **red-orange tinge**.

### Forests & Prairies

- Some microbial markers are smells! You know that **earthy smell** that’s characteristic after a rain? That is due to a compound called geosmin, produced by soil-dwelling bacteria! They produce geosmin to attract insects that will carry them to new environments.

- **Lumps on trees**, called **tree galls**, can be caused by viruses, bacteria, or fungi. One bacterium, *Agrobacterium tumefaciens*, injects its own DNA into plant cells, triggering uncontrolled plant cell growth. Galls usually do not harm plants.

- **Mushrooms** are the reproductive stage of fungi and often appear after rain. There are so many variations of mushrooms throughout the year! **Wood rot fungi** break down complex plant structures, often attacking at lines of weakness, and leave behind a distinct look known as **cubic rot**.
Glossary

Activated sludge - a common biological process used in wastewater treatment
Algae bloom - a rapid increase in the population of algae or cyanobacteria in aquatic ecosystems
Antibiotic - a molecule known to kill or inhibit the growth of microbes
Biocontrol - the reduction of pest populations through the use of natural enemies (like microbes)
Bioprospecting - exploring natural environments for useful products
Endosymbiosis - a type of symbiosis where one organism lives inside the other
Mycoheterotroph - a plant that completely depends on a fungal partner to provide nutrients and minerals
Mycorrhiza - fungi in symbiotic association with plant roots
Nitrogen fixation - process of converting nitrogen gas ($N_2$) into ammonia ($NH_3$)
Nucleus - compartment that holds the DNA of a eukaryotic cell
Nutrient cycle - the movement of organic and inorganic matter through an ecosystem to be used for living things
Pathogens - microbial species that cause disease (e.g. human pathogens)
Rhizobia - nitrogen-fixing bacteria found in soil or plant root nodules
Symbiosis - an intimate association between two or more dissimilar organisms
Taxonomy - a system of classification for scientists to catalog all organisms on earth. The broadest group is domain and the most specific group is species.

Resources for further exploration

Books:
- A Field Guide to Bacteria by Betsey Dexter Dyer
- Life at the Edge of Sight by Scott Chimileski and Roberto Kolter
- The Wondrous Workings of Planet Earth by Rachel Ignotofsky
- I Contain Multitudes by Ed Yong
- Entangled Life: How Fungi Make our Worlds, Change our Minds, and Shape our Futures by Merlin Sheldrake

Blogs & Websites:
- Joyful Microbe (joyfulmicrobe.com)
- Small Things Considered (schaechter.asmblog.org)
- Mycologos (mycologos.world)

Societies & Community Science:
- Madison Mycological Society (madisonmycologicalsociety.com)
- Wisconsin Mycological Society (wisconsinmycologicalsociety.org)
- iNaturalist (inaturalist.org)
- Earth Microbiome Project (earthmicrobiome.org)
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