KONZA ENVIRONMENTAL KEEP EDUCATION PROGRAM

FACT SHEET:

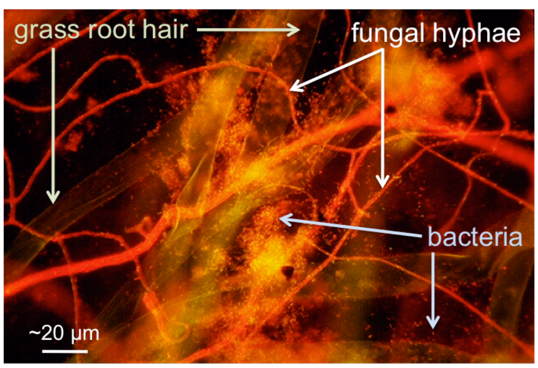
Microbial Life of the Prairie

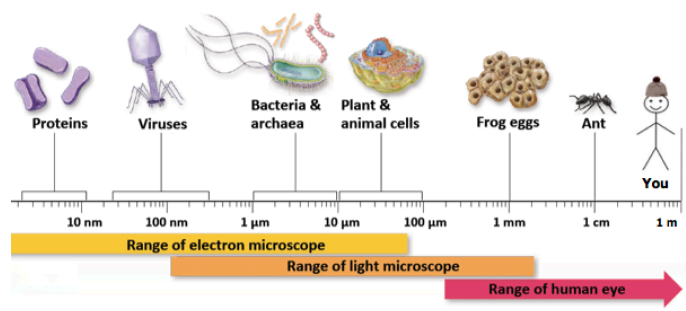
What is a microbe:

"micro" = microscopic – not viewable with the unaided eye. Broadly, any living organism that can only be seen with a microscope is a microbe.

The "µ" symbol is commonly used to indicate a micro measurement. For a micrometer – or 1 millionth of a meter – that measurement is indicated as "µm". Some scientists define microbe size as <200 µm (1/5 of a millimeter).

Most microorganisms are unicellular – one cell is one organism. Examples include bacteria, archaea, protists (including algae), and fungi. Certain types of microbe can live in visible multicellular filamentous colonies (some algae and bacteria) or form fruiting bodies (some fungi) during part of their life cycle.





Microbes live in prairie soil & water, in and on plants & animals:

Most of the diversity of life is microbial, and microorganisms are estimated to account for 17% of earth's total biomass[1], mostly found in soils and sediments. The vast majority of microbes are not pathogenic.

On the prairie, the growth and death of soil microbes helps to build soil organic matter, which holds nutrients and water in the soil. Microbial activity helps to decompose dead plant tissue and dissolve rocks, to release plant-available nutrients. Soil productivity and health is the foundation of a healthy ecosystem.

All microbes feed larger organisms and support food webs. In stream water, microbial algae and cyanobacteria act as tiny plants, and bacterial biofilms also feed invertebrates and fish. Microbial activity also helps to remove pollutants.

In animal guts, resident microbes help digest food. Harmless microbes living on plant and animal surfaces also help prevent invasion by pathogenic microbes.

*there are fun facts about prairie microbes throughout, with Konza research on p. 9!

Microbial life includes:

- **BACTERIA** – very small (0.5 – 10.0 μm), single-celled organisms with primitive cells (**prokaryotic** cells = no nucleus or other organelles). Bacteria make up an entire Domain of life: they are extremely diverse in both taxonomy and physiology. The bacterial importance in nutrient cycling cannot be understated. Some bacteria close the loop of nutrient cycling by converting compounds in the soil to gaseous counterparts, returning elements to the atmosphere. Others make compounds more easily available to other organisms, like plants, through byproducts of their metabolism. They also serve as food for other microbes and store carbon in their bodies. An important group of bacteria in soils is the Actintomycetes, including *Streptomyces* spp., many of which produce **antibiotics** to compete with other microbes in soil, and also produce the aromatic compound, **geosmin**, that we smell after a rainfall event. In native tallgrass prairie soils, we find an abundant type of bacteria in the Verrucomicrobia group whose function in the soil is still unknown, in part because it hasn't been captured in pure culture for laboratory study[2]. While well-known human-associated bacteria like *E. coli* can double in population size in hours under ideal conditions, most bacteria in soil are slower-growing and rarely find themselves living in ideal conditions: their populations take weeks to double, and often have very specific habitat and nutrient preferences that are a challenge to define and recreate in the lab.

- **ARCHAEA** – these small single-celled organisms (0.5 – 10.0 μm) are the other Domain of simple prokaryotic cells, but they also share many characteristics with the more advanced cell type of humans and other Eukaryotes, making them unsusceptible to antibacterial compounds. Luckily there are no known pathogenic Archaea, which is also probably why most people haven't heard of this group! Many archaeans are capable of withstanding environmental conditions that other prokaryotes cannot, and can be very abundant in extremely salty or extremely hot habitats. In most temperate soils, Archaea are in the range of 0.1% to 1% the abundance of bacteria, but still do important work. The most common archaeans in prairie soil are from the group Crenarchaeota, and are important in the nitrogen cycle. Also, only archaea can make methane (natural gas). These microbes, referred to as **methanogens**, produce about one billion tons of methane each year globally[3]. Methanogens are known to be significant in soils that are waterlogged and often lack oxygen (like wetland soils). However, these archaea are found in all soils[4] and thus even prairie soils can produce methane. Soils also contain a bacterial group, the **methanotrophs** (that "eat" methane): The balance between soil being a methane source to the atmosphere, or a sink, depends on the relative activity of the microbial methanogens and methanotrophs.

- **FUNGI** – fungi (~2.0 – 50.0 µm+) are a unique and important group of microbes in the domain Eukarya (which also includes protists, plants and animals). Eukarya have larger and more complex cell structures than prokaryotes, and fungi are one of the most diverse kingdoms of eukaryotic life, with an estimated 1.5 to 5 million species. Fungi can be unicellular (a classic example is the baker's yeast *Saccharomyces cerevisiae*), but many in the soil typically grow as hyphae, which are fine filamentous strands normally no wider than 10 µm. Fungi, most prokaryotes, and animals, are **heterotrophs**, meaning we all have to eat food that we find in our environment; none of us can make our own sugars from carbon dioxide like the "self-feeding" **autotrophs**. Fungi in particular have evolved many strategies and enzymes to assist in the breakdown of complex carbohydrates, some of which can be developed for use in industrial paper or biofuel production. In soil, fungi exude these digestive enzymes to help decompose dead plant material into smaller sugars and nutrients that other organisms, including plants, can reuse. The most common fungi in prairie soils are in the Ascomycota group, and to our best understanding most are **saprophytes**, meaning they live on dead organic material. However, like the bacteria, the specific roles of most soil fungi are unknown. **Arbuscular mycorrhizal** fungi have a low total abundance in soil, but form important symbiotic relationships with plants, described more later.

- **PROTISTS** – organisms described as being "protists" encompass a wide and diverse group of taxonomic Phyla. What they all have in common is an advanced, **eukaryotic** cell rather than the primitive cells found in prokaryotes. Eukaryotic cells possess organelles, including the nucleus where DNA is enclosed. Some protists are pigmented, photosynthetic and autotrophic (including algae), while others are heterotrophs. Some protists are a single cell, like an amoeba, while other protists are formed by a large, organized collection of cells, like the giant kelp. The category of protist is really more a catch-all for any eukaryotic organism that is not a plant, animal, or fungus. Protists account for ~0.007% of global biomass[1]. This estimate may not sound like much, but algae are estimated to be responsible for 50% of the photosynthesis that takes place every year, making them very important players in the global carbon cycle[4]. Algae are most obviously important in prairie stream waters and biofilms growing on the rocks and sediments of the stream bed, which feed higher organisms. Algae are also present in soil in very low abundance, however they may bloom if conditions are right; if water ponds up in a wallow, for example. Most soil protists are heterotrophs that graze on bacteria and are then eaten by larger organisms (such as worms), contributing to soil food webs and nutrient recycling. The most common protists in prairie soil are in the Dinophyceae group of Dinoflagellates[5]. These are 50-100 µm heterotrophs that live in water-filled soil pores, where they can more easily move through the soil and capture their smaller unicellular prey.

- **ANIMALS** – an animal is a multicellular organism that is composed of organized collections of advanced eukaryotic cells. Soil animals include mammals, earthworms, arthropods of different sizes and life history stages, mites, collembolans and nematodes. The smallest (5-100 μm wide, 0.1-2 mm long) and most abundant animals in soil are nematodes. There are many types of these microscopic roundworms, but they are all heterotrophic: Some eat bacteria, others eat fungus, while still others eat the protists, plant root materials, or other nematodes. After nematodes and other animals feed and grow, they often release ammonium (NH_4^+) back to the soil because their food contains more protein than the animal needs and excess protein is broken down to ammonium, contributing to soil fertility. Mesofauna (medium-sized soil animals) like springtails eat fungal hyphae, then mites eat nematodes and springtails, and larger animals eat mites. Thus, a food chain emerges through which bacterial and fungal growth feeds large animals like invertebrates and insectivorous moles. This is called the **microbial loop**. While also not microbial, earthworms facilitate microbial diversity and activity in soil through functions like **communition** (breaking large pieces of plant litter into smaller pieces with higher surface area, increasing space for bacterial and fungal colonization), and aerating and mixing materials and nutrients between different soil layers.

- Is a **VIRUS** a microbe? – Yes, but a virus can't reproduce on its own, so it is not technically a living organism. A virus requires the functions of the host cell to reproduce, so it is a very small parasite. Viruses are highly diverse, range in size between 0.02 to 0.3 μm, and can infect every type of microorganism listed above in addition to plants and animals. Their broad importance includes helping to prevent any one population of microbe from growing very large, and sometimes moving useful genes from one cell to another.

Relative Diversity and Abundance of Soil Organisms
summarized from the literature and expert best estimates[6]
*"species" are only defined for animals and plants, "taxa" is a more generic term for smallest units of microbial diversity

	Diversity	**Abundance**
Prokaryotes	10-9,000 taxa*/cm^3	4-20 x $10^9/cm^3$
Fungi	200-235 taxa/g	100 meters/g
Protists	150-1,200 taxa/g	10^4 - $10^7/m^2$
Nematodes	10-100 species/m^2	2-90 x $10^5/m^2$
Oribatid Mites	100-150 species/m^2	1-10 x $10^4/m^2$
Earthworms	10-15 species/ha	300/m^2

How microbes fit into the prairie ecosystem:

Soil is chock full of microbes, and soil productivity and health is the foundation of a healthy ecosystem. Soil is composed of:

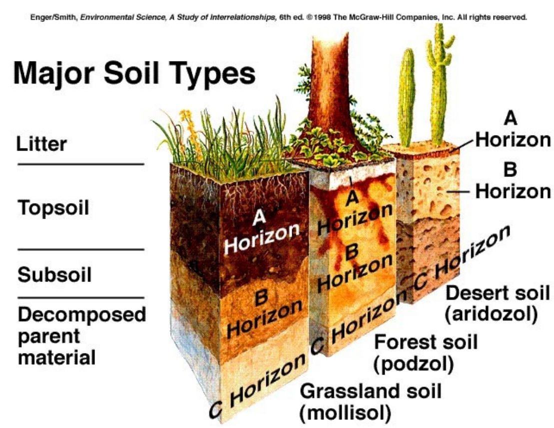
- small rocks and minerals (inorganic component)
- soil pore spaces: full of air and water
- organic matter – from dead and decomposing plants and other organisms
- living organisms – bacteria, archaea, fungi, protists, animals, plant roots

All of the element cycling processes that microbes contribute to healthy soil are also critical for supporting water quality in groundwaters, streams and lakes. One essential role that microbes play in an ecosystem is that of decomposer.

Decomposition = process in which organic substances are broken down into simpler molecules. Decomposition is part of the nutrient cycle and is essential for recycling the nutrients needed for life on Earth.

The three essential elements that are cycled through an ecosystem by decomposition are: Carbon, Nitrogen and Phosphorus.

Carbon (C)



Most prairie microbes are found in the soil and any discussion about microbes and carbon must include prairie soil. Many prairies are characterized by having thick, rich dark soil - termed **mollisols** - that are high in organic matter.

In chemistry, it's important to know that any molecule that is referred to as **organic** is a carbon-based molecule. Therefore, a soil that is dark and organic, has a high amount of carbon in it. The **microbial life** in these soils help break down large complex carbohydrates in this organic matter, like cellulose, into smaller molecules like sugars.

The breakdown of organic matter (= dead plants, animals, fungus, protists, etc…) by microbes serves two functions:

1) Providing energy for the life functions of microbes
2) Supplying carbon for the formation of new microbe cells

Not all organic matter is the same. Recently deceased organisms (plants, animals, fungus, protists) are readily decomposed because of the relatively high moisture, sugar and nutrient content. Organic matter is a general term, and is in reality quite chemically variable, including carbohydrates, proteins, fats and nucleic acids; carbon, nitrogen, phosphorus and micronutrients.

Organic matter includes simple sugars, which are used by microbes very quickly as an energy source. The process by which all cells, including microbes, plants and animals, turn sugars into energy is called **respiration**. The product of respiration is carbon dioxide (CO_2). So, microbial decomposition and respiration turns organic carbon into CO_2. After the most sugar- and nutrient-rich organic matter has been decomposed, the process slows dramatically. Organic matter that is resistant to decomposition often contains high **lignin** levels. Lignin is a complex organic molecule that makes plant cell walls rigid and decay-resistant. Organic matter that is high in lignin might persist for centuries or longer in a soil.

The amount of carbon that is stored (**sequestered**) in a soil may vary depending on how the soil has been used. The amount of tilling, or plowing, a soil experiences directly influences the amount of microbial biomass and organic matter that stays in the soil. It is well established that **an untilled soil holds more carbon than a tilled soil**. When soil is tilled, the physical disruption breaks up plant root and fungal hyphal networks, and crumbles large soil aggregates into small particles. These networks and soil aggregates contain and protect a large amount of microbial biomass and organic matter. With tilling, many of these organisms die, and cannot recover if soil is repeatedly tilled. Also, the organic matter released from disrupted soil aggregates can leach out of the soil, and will be more quickly respired to CO_2. Also, smaller particles, which are also covered in microbes and organic matter, are more easily lost though **erosion**. Less aggregation means less organic matter, fewer soil microbes, lower decomposition and lower available soil nutrients.

Nitrogen (N)

All living cells are protein factories. Any living cell uses its DNA as a blueprint to dictate the construction of a new protein. Important molecules such as enzymes and muscle tissue, hair fibers and cell walls are constructed using proteins.

Microbes are no different from other cells in their ability to construct proteins, and just like any other cell, microbes need the element **nitrogen** to form a new protein. As soil microbes decompose dead materials (which often contain lots of nitrogen), they release nitrogen as amino acids and ammonium that can be used for plant growth as well as microbial growth. This nitrogen that microbes have freed from the soil organic matter serves to help ensure life on our planet.

To understand all of this we must first understand the nitrogen cycle.

> Background: Our atmosphere is composed mostly of nitrogen – which surprises many people who assume that the most abundant gas in our air is oxygen.
>
> Air composition: Nitrogen-77% (as N_2, nitrogen gas), Oxygen- 21%, Argon-1%, Carbon dioxide (CO_2)- 0.04%, Trace elements and water vapor- 0.96%.

All organisms require nitrogen, specifically to make proteins, and nitrogen often limits plant growth. So that begs the question, **if there's so much nitrogen in the air, why is there a problem with having enough nitrogen for plant growth**? Answer: because most organisms, including plants, can't use nitrogen directly from the air. They need certain microbes to alter the form of atmospheric nitrogen from N_2 to NH_4^+ (ammonium), a process called nitrogen fixation.

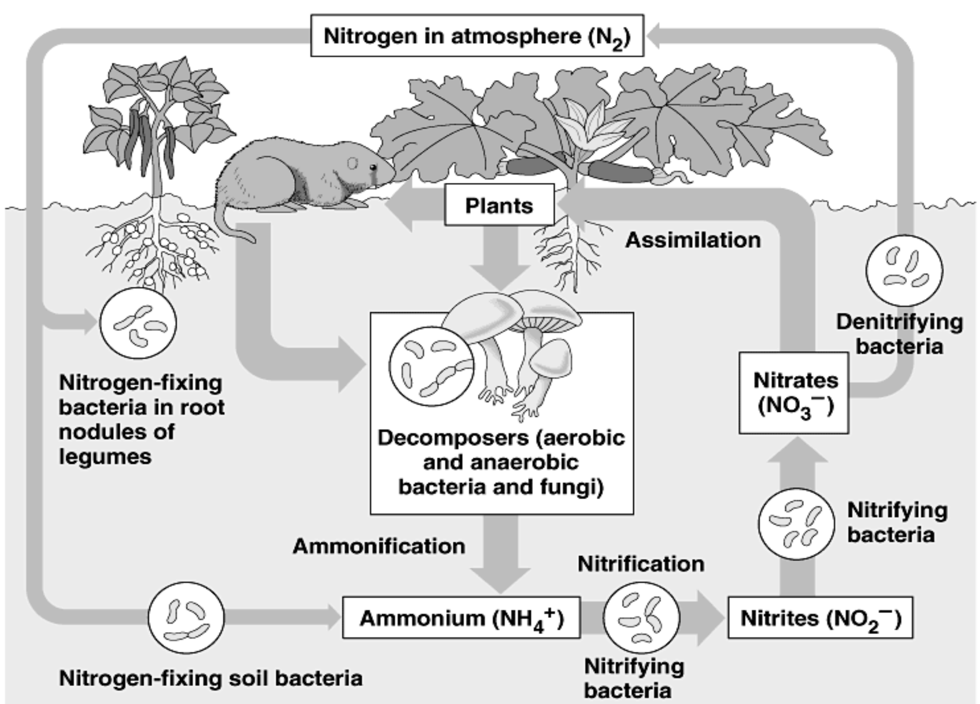
Different types of microbes have different roles in the nitrogen cycle – here is a list of some of them based on their jobs:

- **Decomposing fungi and bacteria** break down proteins and other molecules in dead organic material to release ammonium (NH_4^+). There are many individual decomposition steps in this process, but with ammonium as the end product, this is often referred to as **ammonification**. This may also be called **nitrogen mineralization,** since it is the process by which N in organic matter is released to "mineral" (non-organic) form. Many different types of bacteria and fungi make enzymes that contribute to the decomposition and ammonification processes. Prairie soil has a high diversity and abundance of microbes that participate in these processes, because almost all of the prairie soil N is in organic matter, and it takes a lot of microbial activity, with many types of microbes working together, to mineralize this N. Where bison are present, they help mineralize N through their own digestive systems, so microbes in the soil have to work less hard to release it, and ammonification (and plant-available N) is higher.

- **Nitrifying bacteria and archaea** can take NH_4^+ and convert it to NO_2^- (nitrite) and NO_3^- (nitrate). While N as nitrate can be used by plants, NH_4^+ is the preferred form because less energy is needed to take it up. Also, nitrate dissolves in water more easily, so can be quickly leached, or lost from soil as water moves through. Nitrification is an essential step in the N cycle, but high nitrification in the soil is often not desirable because it removes the most plant-available type of N, and promotes N losses from soil and subsequent pollution of groundwaters, streams and lakes. Nitrifying microbes are found in all soils and waters, but because the process provides very little energy for the cell, these microbes grow slowly and are generally very low in abundance. Not very many types of bacteria and archaea can perform nitrification. The low-N Konza Prairie soil has a relatively high amount of nitrifying archaea (from a group named Crenarchaeota), in contrast to highly fertilized cropped soils which often have fast-nitrifying bacteria (like *Nitrosomonas* spp.).

- **Denitrifying bacteria** take NO_3^- from the soil and convert it through several steps of work to N_2O (nitrous oxide gas) and N_2 (dinitrogen gas), which then move into the atmosphere. This happens faster when there is more NO_3^- available from nitrification. Many different types of bacteria can perform denitrification, because it provides a lot of energy, but most of these bacteria only denitrify when oxygen is less available, for example when soil is waterlogged after a rainfall. Denitrification represents a loss of plant-available N, which can be less desirable in soil; however in polluted waters, denitrification is more desirable because it removes the excess N which can help cause algal blooms. Also, different types of bacteria perform the different steps in denitrification, and some only produce N_2O, not N_2. N_2O is a greenhouse gas that traps almost 300 times more heat in the atmosphere than carbon dioxide, while N_2 has no impact on global warming. There are also certain types of bacteria, like some *Anaeromyxobacter* spp., that specialize in turning N_2O into N_2 gas. Konza prairie soil has many bacteria that can contribute to the denitrification process, including *Anaeromyxobacter* spp..

- **Nitrogen-fixing bacteria** can take N_2 and convert it to NH_4^+. These N-fixers, such as some common *Bradyrhizobium* spp., are often found in nodules within the roots of legume plants. There are also "free-living N-fixers" in the prairie soil, including *Azospirillum* spp. It takes a lot of energy to break the very stable N_2 molecule and turn it into two biologically reactive NH_4^+ molecules. This is why many N-fixers work directly in symbiosis with plants: The plant delivers energy as sugars directly to bacteria in the root nodule, and the nodule protects the bacterial colony from air, both of which are essential for N fixation activity. After N is fixed and incorporated into plant tissue, it supplements all processes of the N cycle, including decompostion, ammonification, nitrification and denitrification. Besides bacteria, humans are the only other type of organism that can fix nitrogen. This happens in industrial fertilizer production plants, which also use a lot of energy.



Phosphorus (P)

Phosphorus is essential for all living organisms, because it is part of the molecule that carries energy to fuel all cellular work. In plants, P supports photosynthesis, respiration, energy storage and transfer, cell division and growth, and more.

For phosphorus to be available for plant or microbial use, it needs to be in soluble phosphate form that roots and cells can access. But, most phosphorus is **immobile in the soil** – in part because phosphorus stays put as a solid precipitate in soils – **especially soils with calcium**. Therefore, phosphorus is limiting in areas with soil that is relatively high in calcium carbonate, and low in acidity that helps dissolve minerals. The limestone-based soil at Konza Prairie and much of the Flint Hills has low P availability.

Phosphorus is also held in organic matter, like nitrogen, and the decomposition process also helps release available phosphate into the soil. Most plants and microorganisms produce the enzymes that drive decomposition for P. Acidity produced through all biological activity also helps to mobilize P, and some plants and microbes produce special acid compounds to enhance this process.

One category of fungi, the **arbuscular mycorrhizae (AM),** including *Glomus* spp. and *Archaeosporales* spp. in prairie soils, play a major role in improving the access plants have to P. AM fungi live partly within roots of a plant, while filaments called hyphae grow out into the soil. These hyphae are smaller than even the finest plant root hair, and extend the reach for phosphorus into the smallest pores in the soil. AM scavenge more phosphate than a plant can on its own, and the plant feeds the fungus sugars to fuel its foraging growth activity. Without this **symbiosis**, an interaction in which both partners benefit, plant production in the tallgrass prairie ecosystem would be significantly lower.

<u>Konza Prairie</u> – soil microbial research observations:

- AM fungi also release proteins into the soil that promote aggregation by helping soil particles stick together. These proteins are collectively called **glomalins**. The more P-limited the plant, the more carbon it feeds its AM fungal partners, and as a consequence both hyphal growth and soil aggregation increase[7]. This creates more habitat for other microbes and allows more accumulation of organic matter.

- While the plant-AM fungi relationship is generally a positive one for both partners, there are conditions under which the symbotic relationship can turn into a **parasitic** relationship (an interaction in which one partner has a negative impact on the other). This can happen if a plant isn't able to photosynthesize enough sugar in excess of its own growth needs to support the fungus. In that case, the fungal maintenance demands actually decrease total plant growth. This is more likely to happen when a plant is growing in the shade, or when N (not P) is the primary element limiting plant growth[8].

- Native tallgrass prairie soil has a large and diverse community of bacteria, archaea, and fungi. The seasonality of these groups shows similarities and differences: Most of these soil microbe populations survive through the winter, but bacteria show a "bloom" of growth during the height of the plant growing season (June - August)[9]. The total soil microbial biomass also increases as the summer progresses[10]. The activity of plants and soil microbes is strongly linked in all ecosystems, but grassland plants have particularly high levels of root growth and associated microbial activity. The **rhizosphere** is a term for the microhabitat near (a few millimeters adjacent to) plant roots, where microbial growth is highest due to plant root exudation of sugars.

- Prairie soil bacteria are tough. In addition to surviving the winter, with its repeated periods of soil freeze/thaw, most Konza prairie soil bacteria survive or thrive through **drought**. When comparing treatment vs. control at the Rainfall Manipulation Plots (RaMPs), after 15 years of increased field drought conditions the soil contains more bacteria that is capable of surviving and growing after drying and rewetting than the control[11,12]. There is also a high abundance of dry-down/re-wet "survivors" to begin with, possibly related to prevalence of drought in the history of the Great Plains[13].

- Decades of nitrogen **fertilization** affects which types of archaea, bacteria and fungi are most abundant in the soil, and their activity. After long-term fertilization, there are more nitrifyers and different types of denitrifyers, and the soil is more likely to lose nitrogen through denitrification[14]. Many other bacteria and fungi also change in abundance, and the microbes in chronically fertilized soil demonstrate a loss of innate fertility, seen as a decline in their organic matter decomposition activity. Most surprisingly, leaving the prairie unburned for decades caused these responses to fertilization to be stronger[9]: In other words, the soil microbes are more vulnerable to the effects of fertilization when prairie is not burned.

- Finally, the presence of **bison** on the prairie definitely affects which types of microbes are more abundant in the soil. There are more similar types of microbes at longer distances apart within the bison enclosure, which implies that the bison help move microorganisms around as they roam[15]. Whether this is because of redistribution of microbes in dung, tracking soil on hooves, or just keeping the tall grass down and letting more wind through, we don't know yet. Across the uplands, we have measured the highest soil bacterial diversity in the annually burned and ungrazed watersheds, which is not what we expected!

<u>Konza Prairie</u> – aquatic microbial research observations:

- Surface water and **groundwater** are often thought of as separate reservoirs of water but in reality, the two are intimately linked. Water moves down through (**infiltrates**) the soil, then through the subsurface, where it recharges aquifers and eventually discharges again at the surface in springs, which feed the streams. This water movement can happen over short or long distances and time periods. Groundwaters carry nutrients and microbes from the soil into the stream, so the land use and microbial activity in surface soil (for example, prairie versus row-crop) affects the amounts of different nutrients, and the types of microbes, that are found in the underlying groundwater[16].

- Surface waters and groundwaters within native prairies are largely unimpacted by nutrient contamination issues that are detected on highly agricultural landscapes. However, groundwater chemistry monitoring at Konza indicates about a 20% increase in groundwater CO_2 from 1991 to 2005[17]. The main source of CO_2 to groundwater is soil respiration, suggesting that cellular **respiration** by plants and soil microbes has increased over time. This added CO_2 also affects the water pH, and thus the levels of some nutrients and minerals dissolved in the water.

- Bacteria isolated from stream waters high in the prairie, where there is only grass along the banks, grow well in the lab when fed a grass **leachate** (water soaked with grass leaves, like a tea), but not with oak leaf leachate[18]. In contrast, bacteria isolated from stream waters in the gallery forest can grow on either grass or oak "tea". Also, cutting down the woody vegetation along streams changes the types of bacteria and fungi living in the **riparian** (near-stream) soil[19]. This is why we wonder whether increases in woody cover might affect the types of microbes living in streams.

- The amount of light reaching the stream water definitely affects the types of microbes living in stream **biofilms**, the slimy growth on the surface of rocks and sediments, which include a high abundance of algae, bacteria and other protists[20]. These biofilms are scoured by flood and lost during drought, but regrow very quickly, within days, when water reaches a stable base flow again, and maintain high growth rates for weeks. This rapid growth supports the recovery of stream food webs after these common prairie disturbances.

- In addition to microbes affecting water chemistry, the presence of **human pathogens** can also degrade water quality. As noted above, pathogens make up only a very small fraction of microbial life. Most (for example, the bacterium *E. coli.,* the protist *Giardia* sp.) are dispersed through the guts and waste of warm-blooded animals. So, surface water and groundwater is only likely to contain significant quantities of pathogens if it is contaminated with feces from infected animals. To our knowledge, such impacts have not been observed at Konza.

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