# Exploration of Beneficial Species Interactions between Legumes & Rhizobia:

## What does a plant need to survive & thrive?

High School and Middle School  Biology Lesson developed with support from the American Society of Plant Biologists (aspb.org)

*Lesson plan template adapted from https://bscs.org*

Written by:

Kerrie Rovito, NBCT, Waters Elementary School, Chicago Public Schools, Chicago, USA

Lara Smetana, Ph.D. School of Education, Loyola University Chicago, Chicago, USA

Michael Grillo, Ph.D. College of Arts & Sciences, Loyola University Chicago, Chicago, USA

Alyssa Hoffman, North Grand High School, Chicago Public Schools, Chicago, USA

Sydney DelGhingaro, Marie Curie High School, Chicago Public Schools, Chicago, USA

# Exploration of Beneficial Species Interactions between Legumes & Rhizobia:

### What does a plant need to survive & thrive?

## Inquiry Overview

**Species Interactions (Mutualism):** *What is the interaction between legumes & rhizobia?*
The classic mutualism between plants in the legume family and nitrogen-fixing bacteria (termed rhizobia) is one of the economically and ecologically important species interactions on Earth. Through this beneficial species interaction nodules are formed on legume roots which house the rhizobia. Rhizobia convert atmospheric nitrogen into a form that is available to the plant (ammonia). In return the plant provides the rhizobia with a source of organic carbon through sugars produced via photosynthesis.

**Prior Knowledge:**
**Photosynthesis:** *How do plants get their energy to grow?*
Photosynthesis is a process plants use to store energy from the sun by converting atmospheric carbon dioxide into sugars. This lesson will build on student prior knowledge of the inputs and outputs of photosynthesis to understand the mutualistic relationship between rhizobia and legumes and bacteria's role in converting atmospheric nitrogen into a form that plants can use.

**Extension:**
**Energy cycling:** How does this help them survive and thrive?
Biological nitrogen fixation is one of the most important processes for life on Earth. Moreover nitrogen is the primary nutrient that limits plant growth and development. Rhizobia fix available nitrogen ($N_2$) into a form that plants can use ($NH_3$). After having students see part of the nitrogen cycle occurring, students can compare this cycle to other energy cycles (ex: carbon cycle).

# Learning Objectives

**Students will do the following:**

- Make predictions about the effect of rhizobia bacteria on grass and clover plants.
- Plan and carry out an investigation to test the effect of soil with and without rhizobium bacteria on grass and clover plant growth.
- Make observations & record qualitative and quantitative data from the growing plants.
- Analyze and interpret data to make claims about the effects of rhizobia on clover and grass plant growth.
- Develop, revise, and use a model based on evidence to illustrate and explain the mutualistic relationship between legumes and rhizobia bacteria.

**Next Generation Science Standards Disciplinary Core Ideas for High School Life Science that could be addressed with this lesson:**

- LS2.A: Interdependent Relationships in Ecosystems
- LS2B Cycles of Matter and Energy Transfer in Ecosystem
- LS2.C: Ecosystem Dynamics, Functioning, and Resilience
- LS4D Biodiversity and Humans

**Next Generation Science Standards Disciplinary Core Ideas for Middle School Life Science that could be addressed with this lesson:**

- LS1-6 Matter and Energy in Organisms and Ecosystems
- LS2-3 Matter and Energy in Organisms and Ecosystems
- LS1-5 Growth, Development, and Reproduction of Organisms

**Common Core Math Standards that could be addressed with this lesson:**

- **MP.4** Model with mathematics.
- **6.RP.A.3** Use ratio and rate reasoning to solve real-world and mathematical problems.
- **6.EE.C.9** Use variables to represent two quantities in a real-world problem that change in relationship to one another; write an equation to express one quantity, thought of as the dependent variable, in terms of the other quantity, thought of as the independent variable. Analyze the relationship between the dependent and independent variables using graphs and tables, and relate these to the equation.
- **6.SP.B.5** Summarize numerical data sets in relation to their context.

**Common Core Language Arts Standards that could be addressed with this lesson:**

- **RST.6-8.1** Cite specific textual evidence to support analysis of science and technical texts.
- **RST.6-8.7** Integrate quantitative or technical information expressed in words in a text with a version of that information expressed visually (e.g., in a flowchart, diagram, model, graph, or table).
- **RST.6-8.8** Distinguish among facts, reasoned judgment based on research findings, and speculation in a text.
- **RI.8.8** Trace and evaluate the argument and specific claims in a text, assessing whether the reasoning is sound and the evidence is relevant and sufficient to support the claims.
- **WHST.6-8.1** Write arguments to support claims with clear reasons and relevant evidence.
- **WHST.6-8.2** Write informative/explanatory texts to examine a topic and convey ideas, concepts, and information through the selection, organization, and analysis of relevant content.
- **WHST.6-8.9** Draw evidence from literary or informational texts to support analysis, reflection, and research.
- **SL.8.1** Engage effectively in a range of collaborative discussions (one-on-one, in groups, and teacher-led) with diverse partners on grade 8 topics, texts, and issues, building on

others' ideas and expressing their own clearly.
- **SL.8.4** Present claims and findings, emphasizing salient points in a focused, coherent manner with relevant evidence, sound valid reasoning, and well-chosen details; use appropriate eye contact, adequate volume, and clear pronunciation.
- **SL.8.5** Integrate multimedia and visual displays into presentations to clarify information, strengthen claims and evidence, and add interest.

### Next Generation Science Standards Cross-Cutting Concepts* and Science & Engineering Practices+ that could be addressed with this lesson:

- Cause & Effect*
- Energy & Matter*
- Structure & Function*
- Stability & Change*
- Asking Questions+
- Developing & Using Models+
- Planning & Carrying Out Investigations+
- Analyzing & Interpreting Data+
- Constructing Explanations+
- Obtaining, Evaluating & Communicating Information+

### ASPB Principles of Plant Biology that could be addressed with this lesson:

**Principle 1**. Plants use the same biological processes and biochemistry as microbes and animals. Yet plants are unique because they mix the sunlight's energy with chemicals for growth. This process of photosynthesis makes the world's supply of food and energy.
**Principle 2**. Plants require certain inorganic elements from soil for growth. Plants play an essential role in the circulation of these nutrients within the biosphere
**Principle 5.** Plants, like animals & many microbes, respire & utilize energy to grow & reproduce.
**Principle 7.** Plants exhibit diversity in size and shape ranging from single cells to giant trees.
**Principle 11**. Plant growth and development are under the control of hormones and can be affected by external signals such as light, gravity, touch or environmental stresses.
**Principle 12**. Plants live in and adapt to a wide variety of environments. Plants provide diverse habitats for birds, beneficial insects and other wildlife in ecosystems.

### Safety Considerations:

Safety precautions include wearing protective eyewear when using chemicals for testing, closed-toe shoes, and other protective clothing and sunscreen; and having a first aid kit. The soil may contain organisms that could be an irritant to skin, so students should wash their hands thoroughly after touching the plants and soil.

# Materials Preparation

Review the At-A-Glance to see pacing and slides presentation. This lesson can be taught with students working individually and in groups. Consider how you will group students prior to starting the lesson based on the number of containers, seeds and grow lights you have.

Slides Presentation for High School and Middle School Classrooms

# Teacher Background

### About Legumes

The mutually beneficial species interaction between plants in the legume family (e.g., beans, peas, lentils, clover, etc.) and symbiotic nitrogen fixing bacteria (collectively termed rhizobia) is one of the most economically and ecologically important interactions on Earth. Nitrogen is the primary nutrient that limits plant growth and is therefore the main component of synthetic fertilizer used for agriculture. Ironically our atmosphere is predominantly composed of nitrogen gas, but it is not in a form that is accessible to plants. However, through this mutualism legume plants receive an organic form of nitrogen that they can use from symbiotic rhizobia. Legumes develop specialized root organs called nodules, which house the rhizobia and are the site of nitrogen fixation. Nitrogen fixation is a process where atmospheric nitrogen is converted to ammonia, which is a usable form of nitrogen for plants. In return plants provide sugars, a source of carbon, to the rhizobia that are produced by the plants through photosynthesis. This symbiosis is why farmers often practice crop rotations where legumes (i.e., soybeans) are grown one year followed by a non-legume, typically corn which requires a large amount of nitrogen, the following year. The mutualism with rhizobia allows legumes to inhabit very nutrient poor soils where other plants are unable to thrive.

### About the Nitrogen Cycle

The nitrogen cycle is a biogeochemical process that describes how Nitrogen moves and changes in Earth's ecosystems. Nitrogen is one of the  essential elements for the growth and development of living organisms, including plants, animals, and microorganisms. Even though the air is primarily composed of nitrogen (roughly 78%), it cannot be taken in through breathing and  it can only be used after it has been fixed by bacteria and taken up through plant roots and transferred to plants.

The nitrogen cycle consists of many steps that change nitrogen from its atmospheric form ($N_2$) into different forms that can be utilized by plants and animals.

1. **Nitrogen Fixation:** The cycle begins with nitrogen fixation, where atmospheric nitrogen ($N_2$) is converted into a different form, either ammonia ($NH_3$) or nitrate ($NO_3$-). This process is completed by nitrogen-fixing bacteria (called **rhizobia**), which can be found in soil on its own or when they form symbiotic relationships with certain plants, such as legumes (e.g., soybeans, clover) or some trees. These bacteria have the ability to convert atmospheric nitrogen into ammonia through biological processes.

2. **Nitrification:** Once ammonia is produced, it undergoes nitrification, which involves two steps. First, ammonia is converted into nitrite ($NO_2$-) by nitrifying bacteria and adding oxygen. Ammonia is toxic to many organisms, so this first step is especially important. Next, nitrite is further oxidized to nitrate ($NO_3$-). Nitrate is a more readily available form of nitrogen for plants and is used by plants to promote growth. Many plants that are not growing tall are found to have nitrogen deficiencies.

3. **Assimilation:** In the assimilation process, plants and microorganisms take up nitrate and ammonia from the soil and convert them into organic nitrogen compounds, such as amino acids and proteins.

4. **Ammonification:** When organisms die, or when their waste decomposes, organic nitrogen compounds are broken down by decomposers such as bacteria and fungi. This process is known as ammonification, where the organic nitrogen is converted back into ammonia.

5. **Denitrification**: Denitrification is the final step of the nitrogen cycle, where some types of bacteria change nitrate back into atmospheric nitrogen. They convert nitrate into nitrogen gas ($N_2$), which is released back into the atmosphere, completing the cycle.

The other elements that plants rely on are potassium and phosphorus, and people who are testing soil quality to determine if plants will grow successfully normally look at the NPK (nitrogen, phosphorus, potassium) levels to determine if it needs to be amended with compost or synthetic fertilizers.

## Vocabulary used in this inquiry

**Nitrogen Cycle**
**Photosynthesis**
**Rhizobia**
**Mutualism**
**Bacteria**
**Photosynthesis**
**Legume**
**Grass**
**Glucose**
**Carbon Dioxide**
**Oxygen**
**Sunlight**
**Nodules**
**Nitrogen Fixation**

### Materials you will need

- Cups (2 per group). Cups should have drainage holes added to the bottom to allow excess water to drain out.
- Inoculated Clover Seeds ~15 per group
- Non-inoculated Clover Seeds ~15 per group
- Rye Grass Seeds ~ 15 per group
- 1 small tray per group
- 1 large tray for each class to store plants
- Grow lights (If you do not have a sunny window)
- Light timer for grow lights
- 1 ziplock bag per group to mix soil (reuse for each class)
- Potting Soil ~1/2 cup per group
- Sand ~1 cup per group
- *Optional: Pinto Bean Seeds

## At-A-Glance: Species Interactions (Mutualism)

| Day 1 | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 5 min | 1. Hook interest in plants using seeds.<br>Pass out clover and grass seeds to each lab group. Have students start discussing each prompt as a group then move to a class discussion to collect everyone's ideas.<br>Have students make observations of the seeds. Before you tell students what type of seeds they are, have them make predictions. Use questioning to see what prior knowledge they have. Have you seen seeds before? Do they look like seeds you have planted before? What do you think will grow from these seeds?<br>After giving them time to make predictions about the type of seeds, introduce the seed types. Start having them make comparisons using a question like:<br>***How are the grass and clover seeds similar and different?*** | - Containers of grass & legume seeds for each table/group.<br>- Magnifying glasses<br>Slide 2 |
| 15 min | What do plants need to grow, survive, and thrive?<br><br>First ask students: What does a plant need to grow?<br>Individually think about the answer to the question<br>Pair with one other person & discuss your answers to the question.<br>Share your ideas with the class. Record all the initial ideas on a large sheet of paper to look at during this lesson to help revise original ideas.<br>First ask students: What does a plant need to thrive & survive?<br>Individually think about the answer to the question<br>Pair with one other person & discuss your answers to the question.<br>Share your ideas with the class. Record all the initial ideas on a large sheet of paper to look at during this lesson to help revise original ideas. | - Large paper to document initial student ideas<br>Slides 3 & 4 |
| 15 min | Plant and experiment introduction - Planting Procedure<br><br>*Teacher Note: A mixture of soil & sand is used to limit the nutrients available to the clover to promote better nodule formation.* | ***Student Handout:*** *Procedure, Pre-Lab Questions & Hypotheses.*<br><br>Slide 5 |

| | | |
|---|---|---|
| | As a class, read the lab introduction.<br>In small groups, read procedure & answer the pre-lab questions.<br>After reading both the introduction & procedure, have students discuss these questions with their group:<br>-What is bacteria?<br>-How will the bacteria affect the plants?<br>-Is bacteria good or bad?<br>-What does bacteria do?<br><br>After discussing questions, students make hypotheses on their own. (Handout Pg 1) | |
| 10 min | Preview the lab materials and demonstrate how to plant the seeds. After, have students label all their containers to be ready to plant on Day 2.<br><br>Make sure you do NOT pack in the dirt, it should be loosely covered!<br><br>Explain over watering and demonstrate how to water<br><br>Students pre-label their containers to be ready for planting on Day 2 | Slide 6 |
| **Suggested End of Day 1** | | |

| **Day 2** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 30 min | Follow the procedure and plant all plants. Take initial observations of the seeds and soil.<br><br>-Have each group collect the materials<br>-Have students plant and ensure that students do not over-water their plants<br><br>Decide as a class what information should be recorded on the observation sheet.<br><br>For the next 3-5 weeks have students take observations daily and water the plants when the soil is beginning to dry out. | **Student Handouts:**<br>1) Plant Observation Log<br>2) Lab Procedure<br><br>Example Observations on Slide 9<br><br>***Teacher's Note:***<br><br>*Plants take 3-5 weeks to grow.*<br><br>*Once the clover has full clover leaves, you can harvest. The longer you wait to harvest, the better the nodules will be when harvested.* |

| | | |
|---|---|---|
| | **Note:** Students have also enjoyed planting pinto beans along with the clover because they didn't have to harvest the pinto beans. Students were able to take home the pinto beans if they wanted. For students who do not want their pinto beans, you can harvest these beans to see the nodules and compare to what they saw on the clover. These nodules are a little easier to count/see so this could be helpful for some diverse learners. | *Have students take daily observations on their worksheet. Decide as a class what information should be recorded on their observation sheets. (For instance: color, height, leave number, etc.) See an example on slide number 9.*<br><br>*Students should water the plants when they notice the soil is beginning to dry out. Record what days the plants were watered on the observation sheet.*<br>*Note: If the weather forecast for the weekend is extreme and your building is not temperature controlled have a plan!* |
| 20 min | Discuss - What is the nitrogen cycle? Review the process of nitrogen fixation. | **Video Introduction:**<br>The Nitrogen Cycle (5.44 minutes)<br>Amoeba Sisters - Nitrogen Cycle (begin at 3:57) |
| **Suggested End of Day 2** | | |

| **Day 3** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 30 min | Start by showing the video - How does the nitrogen cycle work? As a review of yesterday's lab.<br><br>Next, students will work in small groups to read the website information on the nitrogen cycle. Divide students into different groups to read the section and design a poster telling the class about their portion of the cycle. They should cover:<br><br>-What is the nitrogen cycle?<br>-Nitrogen fixation<br>-Mineralization<br>-Nitrification<br>-Immobilization<br>-Denitrification<br>-Nitrogen is crucial for life<br><br>*As an option, students could make a small skit or act out each of their stages of the cycle to present to the class. They could also come up with a movement that represents their portion of the process that students have to do in order as they learn about each of the steps as a class.* | **Video:** How does the nitrogen cycle work? (2.36 minutes)<br><br>**Reading:** What is the nitrogen cycle and why is it the key to life? |
| 20 min | Students work together to create scientific models of the nitrogen cycle in their science notebooks or on posters. Students should work together to draw the important factors in the nitrogen cycle, arrows showing how nitrogen travels, and how nitrogen is | **NSTA:** How to create a scientific model |

| | | |
|---|---|---|
| | transformed. | |
| 10 min | Fill out plant observation guide | **Student Handouts:**<br>1) Plant Observation Log |
| **Suggested End of Day 3** | | |

| **Day 4** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 50 min | Students work in groups to compare the nitrogen cycle to something else in their life. Examples of their comparisons could be a movie, a book, a sport, or a game.<br><br>They should determine how their chosen item compares to the rhizoba's role in nitrogen cycle in terms of:<br><br>● **Specialization**<br>● **Nourishment**<br>● **Symbiotic Relationships**<br>● **Impact on their Environment**<br>● **Success Factors** | **Poster Paper, Markers**<br><br>**Poster Grading Rubric**<br><br>*Example: The nitrogen cycle and a restaurant can be compared in several ways:*<br><br>*1. Specialization: Just like a restaurant specializes in a specific cuisine or type of food, rhizobia-fixing bacteria specialize in a specific symbiotic relationship with leguminous plants. They have the unique ability to form nodules on the roots of these plants and convert atmospheric nitrogen into a form that the plants can utilize.*<br><br>*2. Nourishment: A restaurant provides nourishment to its customers through the food it serves. Similarly, rhizobia-fixing bacteria play a crucial role in providing nitrogen nutrition to leguminous plants. By fixing nitrogen from the air, they help these plants grow and thrive, enhancing soil fertility.*<br><br>*3. Symbiotic relationship: A successful restaurant often relies on a symbiotic relationship with its customers. The customers provide the patronage and support that keep the restaurant running. Similarly, leguminous plants provide a home for rhizobia-fixing bacteria by forming nodules on their roots, while the bacteria provide essential nitrogen to the plants. It's a mutually beneficial partnership.*<br><br>*4. Environmental impact: Restaurants can have an impact on the environment through their sourcing practices, waste management, and sustainability efforts. Similarly, rhizobia-fixing bacteria contribute positively to the environment by reducing the need for synthetic fertilizers. This reduces the potential for water pollution and greenhouse gas emissions associated with nitrogen-based fertilizers.*<br><br>*6. Success factors: A successful restaurant often depends on factors like the quality of its food, service, ambiance,* |

| | | |
|---|---|---|
| | | *and location. Similarly, the success of rhizobia-fixing bacteria depends on factors such as their compatibility with specific legume plants, the efficiency of nitrogen fixation, and the presence of suitable environmental conditions.* |
| 10 min | Fill out plant observation guide | **Student Handouts:**<br>1) Plant Observation Log |
| **Suggested End of Day 4** | | |

| **Day 5** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 30 min | Discuss - Besides nitrogen, what do plants need to grow? What other nutrients are important for plant growth?<br><br>Let students know that two other ways gardeners and scientists can measure what is in our soil is with pH and NPK balance. NPK levels are referring to the nitrogen, phosphorus, and potassium levels in the soil. All are important for plant growth.<br><br>Have students go outside and find a small cup of soil. They will add ~ 1 tablespoon of soil and mix it with half a cup of water. Let the soil material settle overnight for testing. | **Cups (clear if possible) - 1 per group**<br>**Marker to label cup location and group number**<br>**Small shovels to collect soil sample** |
| 20 min | In their science notebooks, have students watch the following videos. Take notes for each of the different elements–what happens if they are missing in any of the plants?<br><br>Discuss as a class - What do they think they could be added to the soil to help balance these elements? | **Videos:**<br><br>**Nitrogen Importance:** Understanding Our Soil (4 minutes)<br><br>**Phosphorus Importance:** Soil Nutrients and Phosphorus (3 minutes)<br><br>**Potassium Importance:** Potassium Requirements for your plants (3 minutes) |
| 10 min | Fill out plant observation guide | **Student Handouts:**<br>1) Plant Observation Log |
| **Suggested End of Day 5** | | |

| Day 6 | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 30 min | Using the NPK and pH test strips, determine if there is an element we would need to add to our garden to enrich the soil, or if they are all at healthy levels. Some of the materials that they could use to organically help balance the soil could include:<br>• **Nitrogen:** coffee grounds, manure, fresh cut greens, cover crops<br>• **Potassium:** Banana peels, citrus rinds, wood ash<br>• **Phosphorus:** Fresh compost, bone meal | **NPK Test Strips**<br><br>**Worksheet -** NPK Testing<br><br>*Examples of some of the materials that they could use to organically help balance the soil could include:*<br>• ***Nitrogen:*** *coffee grounds, manure, fresh cut greens, cover crops*<br>• ***Potassium:*** *Banana peels, citrus rinds, wood ash*<br>• ***Phosphorus:*** *Fresh compost, bone meal* |
| 10 min | Discuss as a class: What NPK and pH levels were the soil samples? Did everyone's soil type have the same results? Are there any trends in what levels were found in different areas? What could lead to this? | |
| 10 mi | Fill out plant observation guide | **Student Handouts:**<br>1) Plant Observation Log |
| **Suggested End of Day 6** | | |

| Day 7 | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 10 mins | Discuss as a class: Yesterday they may have seen that NPK levels were depleted in some of their soil samples. While we are able to add things like coffee grounds, banana peels, and compost on a small scale, how would this look in larger agriculture settings?<br><br>What are some methods that farmers currently use to increase their crop yields? | |
| 30 mins | Assign students one of the three articles (jigsaw w/group) & class discussion about the inoculating legume plants with nitrogen and the role of mycorrhizal fungus in plant growth. | **Article**:<br><br>1. Inoculating legume plants with nitrogen<br>2. Introduction to Mycorrhizal Symbiosis<br>3. What Are the Benefits of Growing Multiple Crop Species Together? |

| | | |
|---|---|---|
| | | What is a jigsaw?  Jigsaw Template |
| 10 min | Time for students to apply what they have learned - have them take the Nitrogen Cycle challenge. They are able to choose where they would like to look at the nitrogen cycle as it is used in agriculture, and answer questions based on how nitrogen is fixed through the process. If students answer incorrectly, the challenge will tell them the correct answer and why. | Nitrogen Cycle Challenge |
| 10 min | Fill out plant observation guide | **Student Handouts:**<br>1) Plant Observation Log |
| **Suggested end of Day 7** | | |

| **Day 8** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 30 min | Students will analyze a DataNugget showing the scientific research featuring clover and rhizobia growth when fertilizer is added as variable to the soil.<br><br>Begin by reading the background information about Iniyan's research and discuss as a class the connections students have to the lab they are currently completing. How do they think Iniyan's research will be similar to theirs? How is it different? What do they think is going to be the outcome of the experiment?<br><br>Looking at the initial graph, students should discuss with their groups the independent and dependent variables for this data. | **Data Nugget Link:** Cheaters in Nature<br><br>*There is a teacher's guide to accompany this lesson with answers, tips, and additional information you can use in your class. In order to access that material so it is not widely distributed online, you can submit a request located at the top of the page. You should be approved within 3 days.*<br><br>***Note, the difference in student versions refers to the graphs supplied in the DataNugget. Graph type A contains a graph already completed for students to analyze, Graph type B has students graph some of the data, and Graph type C has students graph all of the data. It is recommended for this lab that you use Graph type A.* |
| 20 min | Discuss the data Iniyan found as a class. What do they notice about the rhizobia levels in the soil that had fertilizer vs soil that did not have fertilizer? Students should mark the graphs with as many statements about the data as they can see.<br><br>For example - the hybrid clover had an average of 9.04 cms as a stem height, and the white clover had an average stem height of 9.98 cm in the low nitrogen environment | |

| | | |
|---|---|---|
| | End DataNugget for the day, complete the second portion in the next class period. | |
| 10 mi | Fill out plant observation guide | Student Handouts:<br>1) Plant Observation Log |
| **Suggested End of Day 8** | | |

| **Day 9** | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 50 min | Students will analyze a DataNugget showing the scientific research featuring clover and rhizobia growth when fertilizer is added as variable to the soil.<br><br>Discuss the data Iniyan found as a class. Using the data that students found yesterday, they will work with their groups to make a scientific claim, explain the evidence that was important throughout the lab to answer Iniyan's question, and write their reasoning using the data. If this is the first time your class has written a CER response, more time may need to be spent on this process.<br><br>Students will work together in their groups to complete the second portion of the DataNugget lab.<br><br>If you have extra time, students can write their CER response on posters and the class can complete a gallery walk to see the CER response other groups concluded from the data. | **Video to share:** Plant Microbe Interactions<br>**Data Nugget Link:** Cheaters in Nature<br><br><br>**Link to teachers guide -** click here<br><br>CER Explanation - High School<br>CER Explanation - Middle School<br>(although these are suggested grade levels, please use the video explanation that you feel works best for your classroom)<br><br>CER Template |
| 10 mi | Fill out plant observation guide | Student Handouts:<br>1) Plant Observation Log |
| **Suggested End of Day 9** | | |

## 3-4 weeks later for harvesting

| **Harvesting Day 1** |
|---|

| Timing | Activity | Materials needed |
|---|---|---|
| 5 mins | Ask students "What do you expect to see when we harvest the plants?"<br><br>If students do not mention anything about the roots, prompt them to also look under the soil. | **Slides -** Page 7 |
| 5 mins | Revisit initial hypothesis<br>Have students look back on their initial hypothesis worksheet.<br><br>Next, take a poll by raising hands to the following questions:<br><br>What group did you think would grow best?<br>What group did you think would not grow the best?<br><br>Share within groups:<br><br>Why did you make these hypotheses? | **Slides -** Page 8 |
| 20 min | Following the harvesting procedure, have each group harvest their 2 plants. | **Slides -** Page 9, 10 and 11 |
| 10 min | Measure & Record Observations of the harvested plants. Next, discuss as a class what information should be recorded. | **Student Handout:** Plant Harvest Data Collection<br><br>*On this worksheet:*<br>*Record observations, both qualitative and quantitative. Decide as a class what information should be recorded on their observation sheets. (For instance, height, nodule #s, mass, color, etc.)*<br>*Students make a drawing of their plant. Explain this can be the entire plant and a zoomed in section if you see something that looks interesting or you feel should be documented.* |
| 5 mins | Instruct students to find at least one other group to compare plants with & discuss if any data trends they found were shared between the groups. | **Slides –** Page 13 |
| 10 mins | Individually, students should reflect on their hypotheses and determine if their guesses were supported or unsupported using the data. They should stop when they get to the post lab questions. | **Student Handout:** Reflecting on your hypothesis |
| **Suggested end of Harvesting Day 1** | | |

| Harvesting Day 2 | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 10 mins | Generate and record new questions and ideas that students have about the results from their lab. Examples: Why do the legumes have bumps and the grass does not? Why do some plants grow better with bacteria?<br><br>Prompt students to move away from "What" questions to "How" & "Why" questions<br><br>*Ex: "How do the bumps form?" "Why don't the bumps kill the clover?"*<br><br>Have students share their questions in small groups and write two questions on a post and stick them on the board. | **Slides -** 13 and 14 |
| 25 mins | Read article (jigsaw w/group) & class discussion about the bacteria impact on the clover plants<br><br>Students can work independently, read as a class or do a jigsaw with their group.<br><br>*Note: The questions on the document are optional but could be a good supplement.*<br><br>Go over student questions:<br>-Which questions were answered?<br>-What questions do we still have? | **Article**: What makes some species successful Invaders?<br><br>What is a jigsaw? Jigsaw Template |
| 15 mins | Using information from the lab and the jigsaw reading, students should complete their student handout and post lab questions. | **Student Handout:** Post-Lab Questions |
| **Suggested end of Harvesting Day 2** | | |

| Harvesting Day 3 - Assessment | | |
|---|---|---|
| **Timing** | **Activity** | **Materials needed** |
| 15 mins | Review concept mapping and modeling of interactions. | How to create a scientific model in your classroom |
| 35 mins | Using provided images for the processes that take | Scientific Model template and grading rubric |

| | | |
|---|---|---|
| | place in the clover, students create a concept map to act as their summative assessment to illustrate and explain the beneficial species Interactions that take place between legumes and rhizobia. | |
| **Suggested end of Harvesting Day 3** | | |

## Additional Resources

**Extension:** Energy Cycling Clover Lab

**Extension:** Effects of Rhizobium Bacteria on Nitrogen Availability

**Extension:** Long-term nitrogen fertilizer use disrupts plant-microbe mutualisms

**Extension:** How do bacteria help plants?

**Extension:** How do nutrients change flowering in prairies?

**EdPuzzle: Nitrogen Cycle -** Could be used for formative assessment to check for student understanding

**Newsela:** Super Herbivore Leafcutter Ants are Super Busy

**Newsela:** How the Nitrogen Cycle Works

**Newsela:** The Nitrogen Cycle

**DataNugget:** Fair Traders or Freeloaders?

**DataNugget:** Does a Partner In Crime Make it Easier to Invade?