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## Recommended Citation

Johnson, Nancy C, V Bala Chaudhary, Jason D Hoeksema, John C Moore, Anne Pringle. "Mysterious mycorrhizae? A field trip and classroom experiment to demystify the symbioses formed between plants and fungi" in *The American Biology Teacher* 71(7), 2009.

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## INQUIRY & INVESTIGATION

### Mysterious Mycorrhizae? A Field Trip & Classroom Experiment To Demystify the Symbioses Formed Between Plants & Fungi

- NANCY C. JOHNSON, V. BALA CHAUDHARY, JASON D. HOEKSEMA, JOHN C. MOORE, ANNE PRINGLE, JAMES A. UMBANHOWAR, GAIL W.T. WILSON



Every day humans interact with fungi and their products in foods, medicines, and the environment. Yeasts are used to make bread and beer. Life saving antibiotics like penicillin and cephalosporins are fungal products, as are the enzymes added to detergents to boost their cleaning power. Fungi can cause problems when molds and mildews grow in our buildings, foods, or cultivated plants. Athlete's foot fungi cause discomfort, while Valley Fever fungus (*Coccidioides immitis*) and histoplasmosis (*Histoplasmosis capsulatum*) can cause life-threatening diseases. Despite their many roles in our daily lives, fungi often seem obscure and mysterious and are largely unknown and ignored by the American public.

Biology curricula cover fungi in units on bacteria, protists, and primitive plants, but fungi are more closely related to animals than to bacteria or plants (Wainwright et al., 1993). Like animals, fungi are heterotrophs and cannot create their own food; but, like plants, fungi have cell walls, and are for the most part immobile. Most species of fungi have a filamentous body with indeterminate growth; individual fungi can grow indefinitely until they are enormous and ancient. Students are surprised to learn that the largest and oldest organisms on Earth are fungi, not whales and trees (Smith et al., 1992). Fungi are extremely diverse but much of this biodiversity has yet to be described, as only 5% of an estimated 1.5 million fungal species have names (Hawksworth, 2001). Teachers can help make the fungal kingdom less mysterious and obscure by conducting classroom activities involving fungi. We encourage teachers to read Flannery (2004) and visit some excellent Web sites (Table 1) to gain more background about fungi. Here we describe a series of

field and laboratory activities to help teachers introduce students to the symbiotic fungi that are ubiquitous in most roots and soil. The primary objectives of these activities are as follows:

1. Gain an appreciation for the influence of invisible soil organisms on plant growth.
2. Learn about symbioses and their range of outcomes in nature.
3. Conduct an experiment, collect data over several weeks, analyze data, and learn about a method of scientific inquiry that fungal scientists commonly use.

#### ○ Learning Goals & Objectives

The purpose of this activity is to introduce students to fungus-root symbioses by comparing plants that are grown with or without mycorrhizal fungi and other soil inhabitants. Mycorrhizal fungi are associations between certain fungi and the roots of most plants. This exercise provides students with experience using the scientific method, spans several class periods requiring periodic monitoring, and takes advantage of the exciting visual connection that students make when watching plants develop over time. The topic, activities, and evaluation follow the stages of Engagement, Exploration, Explanation, Elaboration, and Evaluation of the 5E Learning Cycle Model developed by the BSCS (Bybee et al., 2006). The study of mycorrhizal symbioses fits well within current life science standards, and the classroom experiment promotes the general standards of inquiry-based science learning. Apart from the

**Table 1. Outstanding Web sites about fungi.**

Web address	Site content
<a href="http://www.fungi4schools.org">www.fungi4schools.org</a>	Maintained by the British Mycological Society. Offers a free book, <i>How the Mushroom Got Its Spots</i> , with activities targeted at elementary school children (search the site under "Key Stages 2 and 3").
<a href="http://www.Tomvolkfungi.net">www.Tomvolkfungi.net</a>	Offers a "fungus of the month" as well as classic pages on "holiday fungi."
<a href="http://www.mushroomexpert.com">www.mushroomexpert.com</a>	General information on collecting mushrooms from the wild
<a href="http://www.doctorfungus.org">www.doctorfungus.org</a>	General information, including advice on fungal infections and sick buildings
<a href="http://www.mycolog.com/chapter17.htm">www.mycolog.com/chapter17.htm</a>	Pictorial supplement to Chapter 17 of <i>The Fifth Kingdom</i> by Bryce Kendrick. Photographs and general information about mycorrhizal fungi. Notice that arbuscular mycorrhizae are referred to as "endomycorrhizae" in this Web site.

life science content, the study of mycorrhizae provides opportunities to integrate biological and mathematical problem-solving skills by applying basic mathematics within life science contexts, hone skills in scientific investigations, and develop communication skills to convey scientific concepts.

This activity involves a field trip to a local park to collect natural soil, and an experiment followed by an extended observation period. Each student or group should sow and monitor two plants, one with living soil and one with dead soil. The cumulative data collected by the entire class can be used to determine whether or not plants grow differently when inoculated with soil organisms including mycorrhizal fungi. At least two different plant species should be grown in this exercise including a plant species that generally benefits from mycorrhizae and one that does not benefit from the symbiosis. This activity can be conducted by students at all grade levels. Younger students might record their predictions and observations using pictures, reporting their results aloud, while older students could collect more quantitative data and write detailed laboratory reports.

## ○ Mutualism, Symbiosis & Mycorrhizae

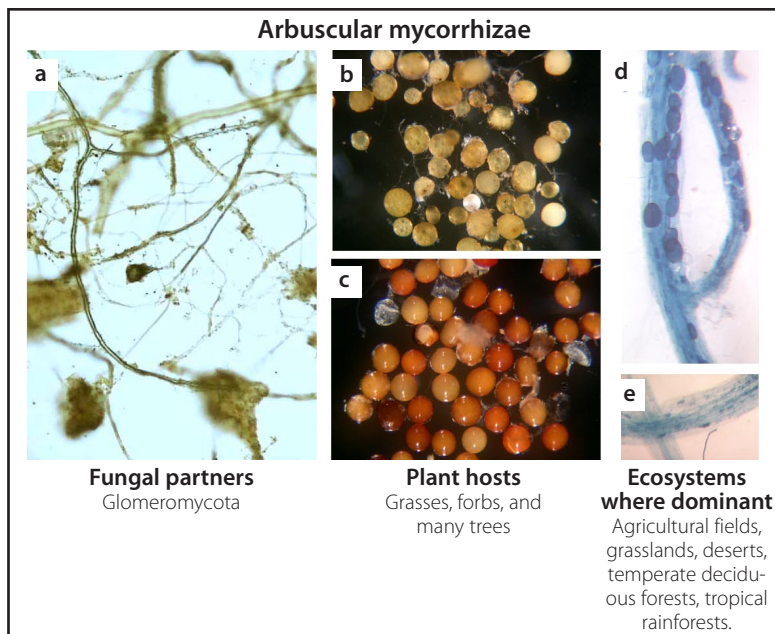
Symbiosis occurs when individuals of different species live in a close physical association (Boucher et al., 1982). The word mycorrhiza is Latin for “fungus-root.” Mycorrhizal associations involving soil fungi and plant roots are among the most common symbioses on Earth. There are many different types of mycorrhizal symbioses involving many different groups of fungi and almost every species of plant. Fossil evidence shows the earliest land plants formed mycorrhizal symbioses virtually identical to present day arbuscular mycorrhizae (Pirozynski & Malloch, 1975; Redecker et al., 2000). These primitive mycorrhizal fungi are in the phylum Glomeromycota (Figure 1). Subsequently, different types of mycorrhizal symbioses evolved including ectomycorrhizae. These associations involve fungal species within the phyla Basidiomycota, Ascomycota, and Zygomycota (Figure 2). Today, arbuscular mycorrhizal symbioses are abundant in agricultural crops, grasslands, deserts, temperate deciduous forests, and tropical rainforests. Ectomycorrhizae are abundant in trees and other woody plant species in temperate and boreal forests.

Mycorrhizae are trading partnerships between plant hosts and fungal symbionts. In these symbioses, carbohydrate from the plant is provided to the fungus in return for soil nutrients. The fine, thread-like hyphae of mycorrhizal fungi more thoroughly explore tiny pores in soil compared to much thicker plant roots. Thus, host plants gain absorptive capability when they form mycorrhizal partnerships. Hyphae of mycorrhizal fungi are unbelievably abundant in most soils. A single handful of soil from a typical forest, grassland, or agricultural field may contain many kilometers of invisible mycorrhizal hyphae that are ultimately linked to plant hosts.

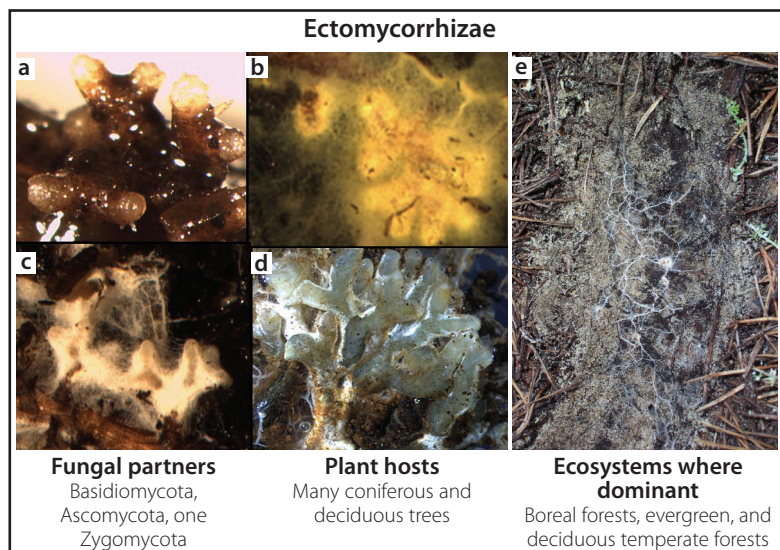
Mutualism is defined as a mutually-beneficial association between individuals of different species. The words symbiosis and mutualism are often treated as synonyms in modern language; however originally the term symbiosis was defined with no reference to the rela-

tive benefits gained by each partner, and symbiosis can describe either mutualism or parasitism (deBary, 1879). Mycorrhizae can function as either mutualisms or parasitisms, and the impact of a mycorrhizal symbiosis on plant health often depends upon environmental conditions (Johnson et al., 1997; Egger & Hibbett, 2004). Mycorrhizae are generally most mutualistic in low-fertility soil. Adding mineral fertilizer, particularly phosphorus, will reduce

**Figure 1.** Arbuscular mycorrhizal fungi form large quantities of tangled hyphal webs (a) in the soil. These fungi reproduce with microscopic spores. Micrographs show spores produced by the species *Glomus mosseae* (b) and *Acaulospora laevis* (c) enlarged 10 times. Arbuscular mycorrhizal fungal vesicles (d) and arbuscules (e) living inside plant roots are stained blue and enlarged 100 times.



**Figure 2.** Four different species of ectomycorrhizal fungi (a-d) growing on the roots of pine seedlings share characteristic dichotomous branching pattern, but differ in color and texture. These photographs were taken using a dissecting microscope and are magnified approximately 20 times. Ectomycorrhizal fungal networks are easily seen by the naked eye on a pine forest floor (e).



mycorrhizal benefits to host plants and can shift the symbiosis toward a parasitism. Also, plant species vary in the degree to which they depend upon a fungal partner. Pine trees cannot survive without ectomycorrhizae, while members of the mustard family are often parasitized by mycorrhizal fungi. To measure the effects that mycorrhizae and other soil organisms have on plant growth, scientists often inoculate plants with mycorrhizal fungi and other soil organisms, and compare the growth of inoculated plants with that of plants grown in sterile soil.

**Table 2. Suggested plants species to use for the experiment.**

Mycorrhizal hosts	Non-mycorrhizal plants
Corn	Mustard
Marigold	Broccoli
Sunflower	Radish

- Horticultural sand or playground sand, available from nurseries or hardware stores
- Small (pea-sized) gravel to place at the bottom of each pot
- Phosphorus-free nitrogen fertilizer
- Rulers (12 inch)

- Balance
- Optional: dissecting microscope

## ○ Field Trip

### Supplies

- Shovels or hand trowels
- Buckets
- Inquiry exercise derived from the questions and answers in Appendix 1. Worksheets may be prepared for each student. Alternatively, teachers may wish to have a less formal exchange of questions and answers.
- Hand lenses (optional)

Take students to an area with natural or semi-natural vegetation where it is permissible to dig small holes in the ground. Areas with both grasses and trees, especially pines, are preferable because they will contain both arbuscular mycorrhizal and ectomycorrhizal symbioses. Brush away the leaf litter beneath trees and look for thread-like networks of fungal mycelium in the area between the organic and mineral soil layers. White mycelium may be especially obvious (Figure 2e), although fungal mycelium can be many colors, including yellow, orange, black, and brown. The mycelium that you find may belong to either mycorrhizal fungi or saprobic fungi that live on dead organic matter. If possible, use a shovel or trowel to dig into the soil. Arbuscular mycorrhizal fungi are too small to see without a microscope, but if you dig up some tree roots (pines are best) and are fortunate, you may be able to find ectomycorrhizal root tips that can be examined with the naked eye or hand lenses (Figure 2). Don't worry if you see no sign of fungi; this is to be expected, and can be used to demonstrate the "present but invisible" nature of most fungi. Before leaving the site, use the shovel to collect three to four liters of soil from the rooting zone of grasses or broadleaf plants growing in full sunlight, far away from trees. These areas will have the highest populations of root-inhabiting organisms, including arbuscular mycorrhizal fungi. Keep the soil cool during the trip back to school.

## ○ Classroom Activities

### Supplies

- Fresh field soil, collected as described above
- Baking trays and a 400 °F oven, or a microwave oven
- Small pots or 500 ml plastic beverage bottles that can be made into pots. Have two pots for each student or group, with a minimum of 24 total pots.
- Organic seeds (not treated with fungicides). These are available from many nurseries and seed catalogues. At least two plant species should be used, including one that benefits from mycorrhizae and one that does not (Table 2).

## Teacher Preparation

Create dead and living inoculum by dividing the soil into two portions; store the living portion in a cool place, and kill the organisms in the dead inoculum through heating in either a conventional or a microwave oven. If a conventional oven is used, spread the soil on a cookie sheet and bake at 400 °F for one hour. If a microwave oven is used, place approximately one quart of soil in a covered two-quart casserole dish. If needed, add enough water so that it is moist to the touch and microwave it at high power for six minutes, keeping it covered as it cools.

If pots are not available, cut tops off of bottles, as shown in Figure 3 and punch several drainage holes in the bottom of each bottle using a nail or awl. Prepare a Data Collection Table as shown in Appendix 2.

## Student Activities

### Plant Establishment, Maintenance & Monitoring

Place approximately 1 cm of gravel in the bottom of each pot for drainage. Fill the pot two-thirds full with horticultural sand, and place approximately 100 ml of inoculum soil on top of the horticultural sand. Then add a top layer of horticultural sand so that the pot is filled to within 2 cm of top. Sow four or five seeds in each pot and thin to one plant each by the third week.

Write experimental treatments (plant species and live or dead soil inoculum) and student names on the pots. The treatment description should be the same as on the Data Chart. At planting, add a dilute solution of phosphorus-free nitrogen fertilizer. Add more fertilizer later if plants begin to exhibit yellow leaves or other signs of nutrient deficiency. **Do not** over-fertilize or the mycorrhizae will not form properly!

Keep plants in a sunny, warm place. If natural sunlight is not available, cool white fluorescent bulbs, or a combination of fluorescent and incandescent bulbs can be used. Water plants as necessary to keep soil moist but not too wet.

## ○ Data Collection

Monitor plant development weekly by recording shoot height on the Data Chart (Appendix 2). Older students may also record observations in individual laboratory notebooks. Drawings or photographs of the plants are also helpful for recording plant development and comparing the treatment effects (Figure 3).

After eight to 12 weeks, gently remove plants by tipping the pots on their sides over a newspaper. If bottles were used, cut the bottle in half vertically using scissors. Gently shake soil from the roots and gently wash them in clean tap water. Have students observe and draw the root structure and size of their plants grown with living or dead soil inoculum. If possible, have the students examine the roots through a dissecting microscope.

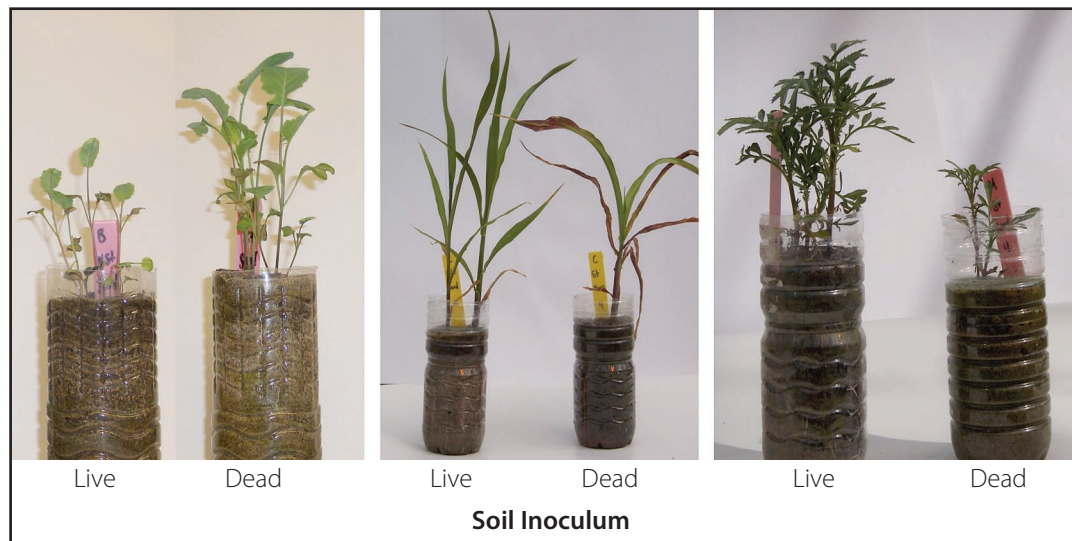
Plants can be weighed fresh, or for a more accurate measurement, they can be dried in a warm oven (60 °C/ 150 °F) for 48 hours before they are weighed. Plant weights and final plant heights should be recorded on the Data Chart.

## ○ Data Analysis & Synthesis

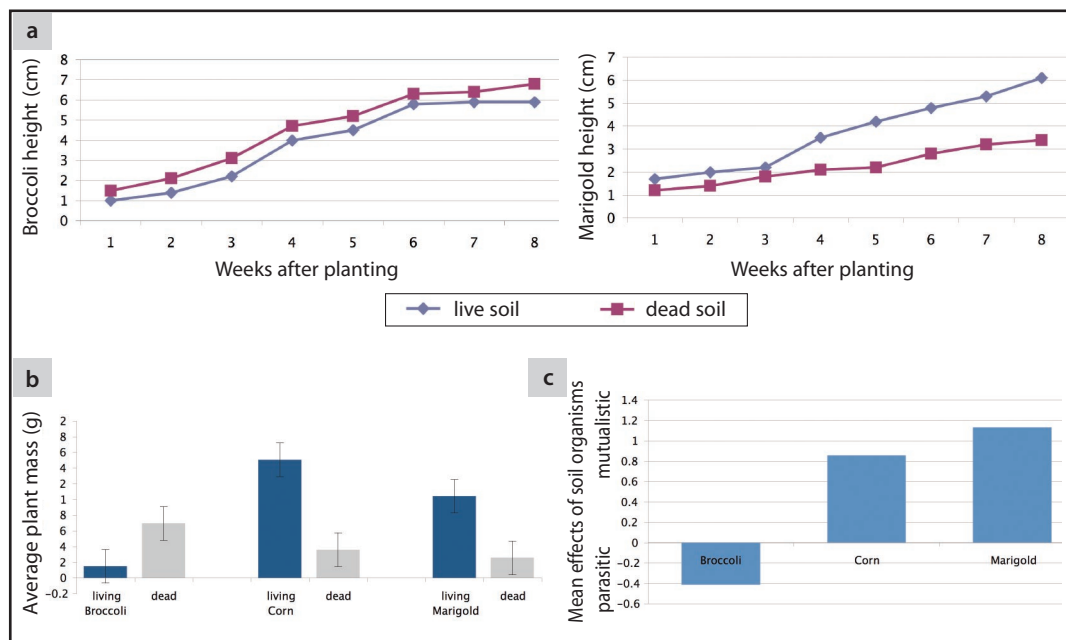
The class data set can be analyzed in several ways. Students of all ages may benefit from getting experience plotting their height data over time (Figure 4a). More advanced courses may calculate means and standard deviations of the final plant weights and heights and make bar graphs to compare the treatment effects across plant species (Figure 4b). The effect of soil organisms on plant growth can be determined by each student by simply subtracting the weight of his/her plant inoculated with the dead soil from that of his/her plant inoculated with live inoculum (plant mass<sub>live inoc.</sub> - plant mass<sub>dead inoc.</sub>). If the calculated number is negative, then the soil organisms were behaving like parasites; if it is positive, they were behaving like mutualists. Average soil organism effect for each plant species can be calculated and plotted on a graph with a y-axis that has both positive and negative values (Figure 4c).

Discuss the results with the students, including possible explanations for the findings, and an assessment of whether the results were surprising or conformed to their expectations. Assure the students that it is not a problem if their findings do not conform to their predictions. Students need to learn that during the normal scientific process, there can be many reasons for deviations from expected results; it is a frequent occurrence in scientific inquiry.

**Figure 3.** Results of the experiment can be illustrated with photographs. These pictures show that after eight weeks in a warm greenhouse, broccoli (left) grew better without mycorrhizal fungi and other organisms in the living soil, while corn and marigolds (middle and right) grew better with these soil organisms.



**Figure 4.** Line graphs (a) show how plant height changes with time, broccoli on the left, marigold on the right. Bar graphs (b) show how living or dead soil organisms can affect mean plant weight at the end of the experiment. Comparing standard deviations (small lines on the bars) indicates the magnitude of the variability within the means. Graphs of the mean effects of soil organisms (plant mass<sub>live inoc.</sub> - plant mass<sub>dead inoc.</sub>) further illustrate this with a y-axis that includes negative and positive values (c). Positive values indicate that the soil organisms are mutualistic and negative values indicate that the soil organisms are parasitic to the plants.



## ○ Recommendations & Conclusions

The proposed activities align well with the learning cycle concepts that have been developed over the years (Karplus, 1975; Osborne & Wittrock, 1983; Barman, 1989; Ramsey, 1993). The recent incarnation of the 5E Learning Cycle has been adopted by

the BSCS science programs (Bybee et al., 2006). This model includes the stages of Engagement, Exploration, Explanation, Elaboration, and Evaluation. The topic of mycorrhizal symbiosis offers an excellent topic of **engagement** in that it connects relatively unknown subjects (mycorrhizae and symbiosis) with topics that students are more familiar with (plants and soil). The hands-on activities and field trips provide a means of **exploration** that are content rich yet attainable from a logistical and budgetary standpoint. The experiments, analysis, and reporting present a forum for students to demonstrate (**explanation**) their understanding of important science content and process skills. The content and process skill sets are germane to a wide range of science topics and allow for students to apply (**elaboration**) their newly-acquired concepts and skills to related science topics. The exercises provide multiple points of **evaluation** allowing for the assessment of student development in science content, process, and communication skills, and the effectiveness of the lesson.

## ○ Acknowledgments

This work was conducted as a part of the Narrowing the Gap Between Theory and Practice in Mycorrhizal Management Working Group supported by the National Center for Ecological Analysis and Synthesis, a center supported by the National Science Foundation (Grant # DEB-0553768); the University of California, Santa Barbara; and the state of California. We gratefully acknowledge financial support from the Radcliffe Institute for Advanced Study to AP and a National Science Foundation grant DEB-03116136 to NCJ. We thank Mindy Bell and the 9th grade students at Flagstaff Arts and Leadership Academy for help in developing and testing protocols. Crystal Sinn, a biology education major at Kansas State University, conducted a pilot study of the described experiment and provided photographs (Figure 3) and data (Figure 4) for this manuscript. •

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- Wainright, P.O., Hinkle, G., Sogin, M.L. & Stickel S.K. (1993). Monophyletic origins of the metazoa: an evolutionary link with fungi. *Science*, 260, 340-34. Table 1. Outstanding Web sites about fungi.

### BIO

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**Appendix 1.** The following questions will help direct class inquiry during the field trip and classroom phases of this exercise and encourage students to learn more by taking an active role in the experimental method. Although the experimental design has been pre-determined in this exercise, students will be empowered if they take an active role in posing the hypotheses and making predictions that can be tested by the experiment.

FIELD TRIP	
Questions	Answers
Do you think that there may be invisible fungus in the soil?	A: Yes, many kilometers of hyphae from mycorrhizal fungi can be found in a handful of soil.
What kinds of fungi can we expect to see?	A: Both saprobic and mycorrhizal fungi can be expected. Saprobes grow on nonliving plant parts like dead leaves and wood. Ectomycorrhizal fungi grow on living roots of trees, and arbuscular mycorrhizal fungi grow in living roots of grasses, broadleaf plants, and some trees.
Which types of mycorrhizal fungi form mushrooms?	A: Arbuscular mycorrhizal fungi do not form mushrooms; but many ectomycorrhizal fungi form mushrooms as part of their sexual reproductive life cycle. Keep in mind that other fungi, including saprobes, also form mushrooms.
Are mycorrhizal fungi poisonous?	A: Fungi are chemically diverse. Some of them, including ectomycorrhizal fungi, contain chemicals that can kill human beings or make them very sick. Many others have chemicals that are beneficial to human beings.

CLASSROOM	
If mycorrhizae are important for nutrient uptake by some plant species (corn, marigold, sunflower), but not others (mustard, broccoli, radish), then predict how the growth of these plants will differ if they are grown with or without mycorrhizal fungi.	A: Expect corn, marigold, and sunflower to grow best with living soil inoculum. Mustard, broccoli, and radish should have no preference or perhaps even grow best with the dead soil inoculum.
How might soil fertility influence this response?	A: When soil fertility is low, plant hosts depend upon mycorrhizal fungi to acquire soil nutrients. We should expect the symbiosis to be a mutualism, and mycorrhizal plants (grown in living soil) should be larger than non-mycorrhizal plants (grown in baked soil). In contrast, non-host species, like mustard, will not grow larger in the living soil.
How can plant growth responses to living or dead soil organisms be measured over a two- to three-month period?	Scientists often make graphs of their measurements; both line graphs and bar graphs are commonly used. Line graphs are best for showing changes over time, and bar graphs are used to show differences in the mean values of measurements.
There is a great deal of variability in the sizes of the plants, even within the same treatment. How can you tell if the differences in means are meaningful or just the result of random chance?	Scientists often use statistics to determine the significance of the differences they observe. One of the simplest approaches is to calculate a standard deviation shown as lines on each of the bars (averages) in Figure 4b.

**Appendix 2.** Example of a worksheet to record plant heights each week and plant weights at the end of the experiment (after six to 12 weeks).

Students (Names)	Plant Species	Inoculum Treatment	Week 1 (cm)	Week 2 (cm)	Week 3 (cm)	... Week z Height (cm)	Week z Weight (g)	Difference
Clarence	Broccoli	live soil					X g	X - Y
Clarence	Broccoli	baked soil					Y g	
Tonya	Broccoli	live soil						
Tonya	Broccoli	baked soil						
Sandra	Broccoli	live soil						
Sandra	Broccoli	baked soil						
Oliver	Broccoli	live soil						
Oliver	Broccoli	baked soil						
Raj	Broccoli	live soil						
Raj	Broccoli	baked soil						
Sam	Broccoli	live soil						
Sam	Broccoli	baked soil						
Penelope	Marigold	live soil						
Penelope	Marigold	baked soil						
Ben	Marigold	live soil						
Ben	Marigold	baked soil						
LaToya	Marigold	live soil						
LaToya	Marigold	baked soil						
Oskar	Marigold	live soil						
Oskar	Marigold	baked soil						
Pearl	Marigold	live soil						
Pearl	Marigold	baked soil						
Tony	Marigold	live soil						
Tony	Marigold	baked soil						



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