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Teaching Issues and Experiments in Ecology - Volume 9, November 2013

EXPERIMENTS

Comparisons of Mycorrhizal Properties from Two Host Tree Species

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ABSTRACT

In this lab experiment, students learn about ectomycorrhizal (ECM) fungal properties associated with two host tree species to better under understand symbioses in general and gain experience using soil sampling and mycorrhizal field methods. Students will learn in more depth about terms and concepts related to symbioses (e.g. mutualisms, coevolution, host specificity) and about specific experimental methods. Two labs and 2-4 preceding lectures are required. In the first lab, students make field observations to form a hypothesis about ECM fungal colonization, record images of sporocarps, and extract roots. In the second, students process their roots and describe ECM fungi. They then analyze data, test their hypothesis, and summarize their findings and interpretations of lab content/concept questions in written and oral assessments.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** biodiversity, community ecology, forest ecology, fungal community, mutualism, mycorrhizae, Shannon Diversity Index, soil ecology, species diversity, species interactions, symbiosis

- **Science Methodological Skills Keywords:** collecting and presenting data, data analysis, experimental design, field observation skills, field work, hypothesis generation and testing, microscopy, oral presentation, quantitative data analysis, quantitative sampling, scientific writing

- **Pedagogical Methods Keywords:** background knowledge probe, cooperative learning groups, formative evaluation, group work assessment, lecture, muddiest point, problem based learning (PBL), questioning, rubric

CLASS TIME

Two lab and 2-4 preceding lecture sessions each are required. Students spend one hour in the field and two hours in the lab in the first lab session and three lab hours in the second. Note that the field location should be close enough to the location where the following lab is held to allow for the estimated two hour first day lab session. Two-four, one-hour, lecture sessions (or two longer ones) should be held prior to the labs in order to cover content related to symbioses and mycorrhizae as described in the abstract.

OUTSIDE OF CLASS TIME

Students need about 4-6 hours to identify sporocarps found, analyze and summarize data, and prepare their assessments.

STUDENT PRODUCTS

A written group Lab Data Analysis (Guidelines & Rubric Word file) and group PowerPoint based Lab Report Presentation (Guidelines & Rubric Word file).

SETTING

Field and lab. The field component can be conducted in any natural area where sporocarps and trees occur. Forests and wooded field edges are ideal, but single trees from campus grounds can also be used. The field component should ideally be conducted in the early fall or late spring, and within a week or so of a rain event, when soil conditions are most likely to support sporocarp production and ECM fungal root colonization. The lab component can be conducted in any lab with adequate sink, water, and dissecting microscope availability. Given that 18-24 students are best suited for the lab, with students working in groups of three, a maximum of 6-8 scopes are all that are needed.

COURSE CONTEXT

The lab could best be used in a mycology, fungal ecology, or other upper level ecology course that covers mycorrhizae and soil ecology topics on some level, regardless of the instructor's knowledge of mycology, and could be modified for partial use for lower level ones (see "Transferability" below). Inclusion of at least some lab portions in lower level courses is encouraged given that mycology and soil ecology topics are often not covered in introductory biology courses and that few programs require mycology in their curricula. The lab can also serve to guide undergraduate research projects. It has been used effectively in undergraduate and graduate content and research courses. A class of 18 students is ideal, but 24 can be facilitated.

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INSTITUTION

Public regional university with bachelors and masters programs.

TRANSFERABILITY

The lab is best fit for mycology and fungal ecology courses, and for upper level ecology elective courses (e.g., community or plant ecology), where students should have some basic knowledge of mycorrhizae and familiarity with field and microscopy methods. But it can be adapted for lower level majors courses. Adaptations could employ a number of approaches. One is to remove the entire root-sampling portion to focus on sporocarp identification and counts, but include an augmentation of it by showing colonized root samples retrieved by the instructor in the lab. Links between sporocarps and mycorrhizal roots could then be made to illustrate physical interaction between sporocarps, roots, and mycorrhizal fungi. In addition, a second approach might be to keep both the sporocarp and mycorrhizal description portions, but to remove most quantitative measurements. This would be similar to the first approach, but would allow students to still gain some experience with root sampling, prepping, and morphotyping to describe ECM morphotypes (i.e., unidentified species). Quantification of colonized and uncolonized root tips could be kept to give students at least some familiarity with measuring mycorrhizal colonization. The t-test could be conducted to test for differences in colonization as originally planned, or it too could be omitted. All other quantitative measures including percent colonization by morphotype (needed to construct community composition profiles), total colonization, and Shannon diversity could be omitted completely (or partially at the discretion of the instructor). Such modifications should not greatly alter the key experiment goals, which are to increase student knowledge of mycorrhizal ecology, to authenticate an understanding of symbioses, and give students exposure to ECM and soil ecology methods. Based on these and any other modifications, the homework, lab data analysis, and oral presentation would need modification to accommodate such changes. Instructors should be able to do that. Finally, whether modified or not, access to wooded habitats that are flat and accessible to ALL students is ideal, but any wooded area can be used.

Overall, whether intended for mycology or fungal ecology courses, or modified for use in related lower-level courses, what follows is a list of the essential background concepts and terms that instructors should know and be able to teach to students to ensure that they can answer questions related to explaining their results:

- Symbioses (e.g., mutualisms) as a key type of species interaction
- Community composition and diversity as ecological concepts

- Common measures of ECM fungal diversity (i.e., Shannon diversity index)
- ECM vegetative (i.e., root tip mantles) and reproductive (e.g., sporocarps) morphology, including some common representative taxa from the study region
- ECM root tip colonization as a proxy for abundance
- The influence of host specificity and size, and abiotic factors (e.g., soil moisture and nutrient availability) on ECM root tip and sporocarp abundance and diversity

ACKNOWLEDGEMENTS

I first learned how to use elements of this experiment as a middle school science teacher, but gained more formal guidance on mycorrhizal methods from my doctoral advisor, Dr. James Lewis, at Fordham University. I was encouraged to further develop the experiment by Drs. Bob Pohlad and Carolyn Thomas, Ferrum College, for use by the Collaboration through Appalachian Watershed Studies.

SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

Do ECM fungal colonization and community properties vary in association with different host tree species?

What Happens

Prior to conducting the lab, students should have been exposed to background readings on terms and concepts related to species interactions (e.g. symbioses) and community properties (e.g. composition and diversity) in 1-2 lecture session(s), and on fungal and mycorrhizal biology and ecology (see Section 4, Introducing the Experiment to Your Students for recommended readings for those with little mycological knowledge) in 1-2 lecture session(s) (or cover both in fewer but longer sessions) taught by the instructor. During lecture, the instructor should engage students in a discussion based on questions related to content, and write a Muddiest Question (as before, see Section 4 for a list of potential questions). They also read the Lab Overview (Word file) to become familiar with the material so that they will better understand the lab's goals and methods. Students then conduct the field component one week and the indoor lab the following week in which the instructor facilitates. Outside of class, groups analyze data, test a hypothesis, interpret results, and summarize findings in a written Lab Data Analysis and oral group Lab Report Presentation that is given in a subsequent class in which the instructor moderates.

Experiment Objectives

1. This exercise introduces students to leaf morphology, species identification, data synthesis, and prediction. Apply the scientific method by making predictions and collecting, analyzing, and interpreting data, and writing a summary of their data analysis.
2. Use fundamental ectomycorrhizal quantification and description methods.
3. Prepare and deliver a presentation of lab results and conclusions.
4. Authenticate lecture material to ECM communities and their host plants. Specifically, students will be able to describe:
    a. the vegetative and reproductive characteristics of ECM fungi (e.g. hyphae, mycelium, and sporocarps)
    b. the morphological "interface" of ECM roots (e.g. hyphal mantles, emanating hyphae, and Hartig nets)
    c. mechanisms by which ECM fungi interact with hosts including the role of host "specificity" and size in influencing ECM colonization, and morphotype community composition and diversity as shown by mantles or sporocarps
    d. abiotic factors (e.g. soil moisture and nutrient levels) that influence ECM colonization and diversity.

Equipment/ Logistics Required

Based on a class size of 18, with six groups of three students:
Backpack (6; 1 per group)
Beakers (36; 6, 500 ml & 6, 100 ml, per group)
Digital/cell phone camera (6; 1 per group)
DBH tape (6; 1 per group)
Dissecting microscope (10-40x) & light source (6; 1 per group each)
Hand clicker (6; 1 per group)
Cotton gloves (18 pairs; 3 per group)
Laptops (6; 1 per group)
Mushroom ID guides (6; 1 per group); *Apps/ web guides are also useful (see Overview)*
Paper bags (72; 12 per group)
Petri dishes (6; 1 per group)
Soil knives or spades (6; 1 per group)
Soil sieves (6; 1 per group)
Scissors (6; 1 per group)
Tree chalk (6; 1 per group)
Tweezers (6; 1 per group)
Wax pencils (6; 1 per group)

Summary of What is Due

A group Lab Data Analysis and PowerPoint-based Lab Report Presentation summarizing results, conclusions, and interpretations are due. A first draft of the data analysis will be turned in for instructor feedback and then a final draft will be submitted within one week after receiving and addressing any feedback.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

Mycorrhizal fungi are key components of biotic communities, affecting plant composition and productivity through effects on their growth and survival (Smith & Read 1997). They vary spatially from habitats to ecosystems (Kranabetter et al. 1999), and include many globally and locally endemic species (Kendricks 1992, Dahlberg 2001). Most occur as ectomycorrhizal (ECM) or endomycorrhizal (VAM) types, and as fewer types that associate with ericaceous plants (e.g. blueberries) and orchids. ECM fungi associate with fewer plant species than do VAM fungi, but are equally important due to their disproportionate occurrence in a few terrestrial ecosystems (Dahlberg 2001). For example, ECM fungi are common in boreal and temperate forests, which cover more than 15% of global land area and account for nearly 20% of NPP in these biomes (Schlesinger 1997).

ECM fungi primarily associate with woody plants (Allen 1991), enhancing plant (i.e. “host”) nutrient and water access, while gaining access to host carbohydrates (Smith & Read 1997). Enhanced resource access is vital for temperate trees because nutrients, like N and P, and water can be scarce in temperate forest soils (Termorshuizen & Ket 1991). ECM fungi access these resources via **mycelia**, thin thread-like **hyphal** assemblages that grow in soil and organic matter, increasing host root surface area. In return, host photosynthates are transferred to the fungal partners and serve as their main C source (Smith & Read 1997), accounting for 25-40% of tree C production (Lewis & Strain 1996). Thus, ECM fungi greatly influence forest productivity (Dahlberg 2001).

ECM fungi have been identified in most temperate forests (Smith & Read 1997) with up to 8000 species associating with about 2000 host tree species (Kendricks 1992, Dahlberg 2001). Reflecting the greater diversity of fungi compared to hosts, most ECM fungal communities are more speciose than are those of their hosts. Estimates of **species richness** ranging from 6-9 fungal species per tree to that 10-100 x greater than that of hosts have been found in North American conifer forests (Kranabetter et al., 1999). ECM fungal communities are often

dominated by a few highly abundant species, while most show intermediate to low abundances (Gehring et al., 1998). Studies have described ECM fungal communities in which nine of 69 species accounted for 67% of host root colonization (Dahlberg et al., 1997), one where 2% of species did for 40% (Gehring et al., 1998), and one where 50 species did for less than 1% (Goodman & Trofymow 1998).

Recognition of ECM fungal diversity began in the nineteenth century with Robert Hartig, who illustrated mycorrhizae from trees (Kelly 1950, Molina 1985). Later, Albert Bernhard Frank coined the term *mykorhiza* to describe mutualisms between truffles and trees, and suggested that they benefit tree growth (Allen 1991). In 1900 M. Stahl contributed to this work by reporting associations between specific mycorrhizae and plants. Most past assessments of ECM fungal diversity relied on identification and counts of **sporocarps**, like mushrooms (i.e. Dahlberg 2001), until such surveys were found to be poor sole indicators of ECM diversity given that many ECM fungi are inconspicuous and fruit infrequently (Gardes & Bruns 1996, Jonsson 1998). One study, for example, found that sporocarps accounted for just 5% of all ECM fungal species in one forest (Danielson 1984). More recently, **morphotyping**, a method used in this lab, improved ECM identification by using descriptions of ECM root structures. Morphotyping uses macro- and microscopic mycelial descriptions that form around and within host roots to describe **morphotypes**. Morphotyping is also beneficial because it can be used to link individual ECM fungi to hosts (Dahlberg 2001).

Ultimately, the study of mycorrhizae is an important ecological endeavor as it incorporates both above- and belowground biota and their interactions. The purpose of this lab is to introduce you to methods used to describe and assess ECM fungi and their communities in association with given host species. Lab benefits include giving you experience with common ECM and soil field and lab methods, in collecting and analyzing data, and having you construct community profiles. While the lab's focus is on ECM fungal identification methods, data collection, analysis, and interpretations, you can apply what you learn to many biotic systems with which you are interested in and conduct research.

Materials and Methods

Study Site(s): Any tree assemblage. Ideally, forest habitats should be used, but woodlots or campus trees that are accessible to ALL students work. Sites should also have loose soil free of excessive organic detritus and rocks. A late successional beech-oak forest located at the university natural area is ideal

because it has many host tree species (e.g. beech, hickory, oak, and white pine) useful for root comparisons that usually harbor mushrooms in spring and fall. On that note, you should conduct the field component in the early fall or late spring, ideally after a rain event, when soil conditions are better for sporocarp production and ECM colonization. This site is largely free of undergrowth and lacks thorny plants and vines. In addition, there are flat trails running through the forest located near a campus bus stop and parking lot. You can also use a campus quad, which usually has many tree species, but fewer sporocarps.

Overview of Data Collection and Analysis Methods:

Before Lab Session 1:

1. In the week before the lab, form groups of three with the approval of your instructor and carefully pay attention when prepped about the field site.

2. Read the Lab Overview and become familiar with it and pay attention to the experimental design and methods to be used in the field component. Peruse online mushroom and tree identification guides or Apps to familiarize yourself with common sporocarps and trees they you may encounter. Helpful for mushrooms (first three) and trees (last):

- http://www.rogersmushrooms.com
- http://www.mycokey.com/newMycoKeySite/MycoKeyIdentQuick.html
- http://www.audubonguides.com/field-guides/mushroom-identification-app.html
- https://itunes.apple.com/us/app/audubon-trees/id334843956?mt=8

Day 1, Lab Session 1 – Sporocarp Hunting & Coring (1 hour):

In order to observe, characterize, and test a hypothesis related to ECM fungal colonization, you have to first find them. To do so you will extract soil cores near the trunks of selected trees, but only after designing your own experiment to test the hypothesis you form. Before you do this, first search for sporocarps, which can serve as indicators that ECM fungi are present in the soils near your selected trees. *What to do?*

Figure 1: Bolete sporocarp, New Jersey Pine Barrens.

1. Walk around a chosen field site making observations that allow you to choose two tree species to sample. You might choose a hardwood

and a conifer species (e.g. *red oak* and *white pine*), or two species of the same genus (e.g. *red* and *white oak*), knowing that some species (e.g. *maples*) are not ECM. Once you choose these, form a hypothesis about ECM root tip colonization (e.g. Host 1 colonization = Host 2 colonization). It should be based on prior class discussions about tree abundance and size, and associated environmental conditions. For example, you might hypothesize that colonization will be greater on red oak than white pine because oak diameters are larger or because pine soils are drier. *Clarify your hypothesis and have it approved by your instructor before proceeding.*

2. Once your hypothesis is approved, choose six trees of each species and measure each with a DBH tape, making sure that all are > 8 cm. These trees will serve as ECM root sources. Also, make sure that each tree is at least 3 m from any other. Number trees of each species 1-6 with tree chalk. Now, begin your search for sporocarps (Figure 1).

3. Look in a circular area about 3 m around each tree. Take pictures of any sporocarps found. Try to get pictures of caps (tops & undersides), gills or pores, and stipes. Note that finding a sporocarp does not mean that it is ECM, but it may be if close to a tree. Also, count the number of sporocarps of each type. You are now ready to extract ECM fungi.

4. At each tree find a point that is about 1 meter from its base, and free of surface roots, detritus, and rocks. Clear detritus from each point, taking care not to disturb any sub-surface roots or soil around each tree.

5. Use a soil knife to cut a 13 x 13 cm and 10 cm deep core at each point. Each should contain ECM colonized roots. Place the cores in paper bags marked with the tree number and species, and store in a backpack. Once all are extracted, return to the lab.

Day 1, Lab Session 1 – Root Prep (2 hours):

Once in the lab it is time to prep the cores to examine ECM fungi in them. This requires **CAREFUL** and **PATIENT** work.

1. Remove cores from bags one at a time, placing each in a 500 ml beaker labeled with the tree number and species. Gently fill each beaker (so as to not damage the fungi) with water until each core is immersed. Soak for 1/2 hour while comparing your sporocarp pictures to those in guidebooks (e.g., Miller, Jr. & Miller 2006) or the web sources or Apps such as those

provided earlier. Try to guess the identity of each species knowing that visual imagery alone is not a guarantee that you will correctly ID them. Use cap size, gills or pores, stipe rings, colors, etc. to help. Record possible species (or genus) and common names in Table 1 (Excel file). Periodically check your cores to ensure that they are still fully immersed.

2. After soaking, drain and remove any detritus from each core while looking for roots, placing all that you find in a 100 ml beaker (similarly labeled) filled with water. Finding roots can be time consuming if cores contain a lot of detritus. If so, gently loosen the roots and rinse them **VERY** gently with water over a 500 µm **sieve** to prevent drain clogs. After rinsing, ECM fungi should be more visible. Once roots are separated from most detritus, soak them another 1/2 hour, again using the time to ID sporocarps.

3. After the second soaking, again gently rinse roots to remove any remaining detritus. ECM fungi should now be much more visible. Place them back in beakers filled with water until all are rinsed. Depending on how many roots there are, remove a subsample of about 25% of all (if there are only a few roots, use them all). An ideal sample will have ≈ 4-5 branched root fragments = 30 cm in length laid end-to-end. **APPROXIMATE**! Discard all other roots in a compost bin.

4. Using scissors, carefully cut the fragments into smaller ones and place them back into the marked, but now rinsed and refilled, beakers. Once done, place them in a cool location designated by your instructor. This completes the first lab session.

Lab Session 1 Homework:

1. Continue as before identifying sporocarps. Note that some of the more common ECM fungi found in North America are *Amanita*, *Boletus*, *Lactarius* and *Russula* species. Record findings in Table 1 (Excel file).

2. Review Lab Overview Session 2 paying attention to the microscopy methods and morphotype characters to be used. Also, peruse the ECM fungal morphology websites: http://www.mykoweb.com/articles/Mycorrhizas_2.html and http://mycorrhizas.info/ecm.html for more familiarization with mycorrhizal methods.

Day 2, Lab Session 2 – Morphotyping (3 hours):

In order to describe and count ECM morphotypes, use a **dissecting microscope** with 10-40x magnifications. It's now time to describe and count.

1. Using the root fragments collected before, remove a batch with tweezers and lay them flat in a grid-lined Petri dish. Fill with **COLD** water (has fewer air bubbles than warm), using a pipette bottle until all are immersed. Gently place the dish under a scope and adjust the light so that the roots are clearly visible. Using 10x and the grid, move the dish from top to bottom and right to left to observe any colonized root tip **mantles**, which should appear as colorful thickened structures protruding from longer roots like those shown in Figure 2.

Figure 2: Slightly bent and grainy copper morphotype with a few protruding hyphae.

Refer to the provided web sources for help in describing the following macroscopic mantle characters you will likely see:

a. Mantle **COLOR**: Most colors are variations of brown, white, or yellow while some can be colorful (Figures 1 and 2). One easily described and identifiable species you will likely see is the charcoal black *Cenococcum geophilum* (see the third image down under C. Structure and Developmental Stages, 2. Soil Hyphae, from http://mycorrhizas.info/ecm.html). Also see images under, 4. Mycorrhizal Roots for other color examples.

b. Root tip **SHAPE**: Beaded, bent, and straight are common. See http://forestrydev.org/biodiversity/bcern/glossary/glossary_system-tips_e.html for examples.
c. Mantle surface **TEXTURE**: This is subjective and many ECM biologists use fabric analogies like *cottony*, *felty*, *silky*, or *smooth* as descriptors. Use terms that are meaningful to you, but help distinguish morphotypes. See http://forestrydev.org/biodiversity/bcern/glossary/glossary_system-tips_e.html for examples.
d. Presence of **HYPHAE** protruding from mantles is another key descriptor. Such hyphae can be long or short, thick or thin, and bottle brush or whisker-like. They range from profuse (think cotton candy) to sparse or absent. See examples from http://www.for.gov.bc.ca/rni/research/Date_Creek/Mycorrhizae_Table/Mycorrhizae.htm. Simply note whether hyphae are present or not, and their general appearance as just described.

Note that you will NOT be identifying morphotypes taxonomically – only DESCRIBING them, with the exception of *C. geophilum*, which, as stated earlier, is easy to ID. Record your descriptions for each morphotype or species in Table 2 (Excel file). Once done, you are ready to count **colonized** (i.e. tips covered by ECM mantles) and **uncolonized** (i.e. tips not covered by mantles) root tip numbers.

2. Count the number of colonized and uncolonized tips. For visuals of uncolonized mantles see the third image down under B. Root Systems, 1. Root System Diversity, from http://mycorrhizas.info/root.html. Uncolonized tips will “stick out” as they are usually much thinner than colonized mantles. Once you can distinguish between the two, count them using a clicker for help! Also, again use dish gridlines to help you systematically move left to right and up and down to make your counts. There may be few or many colonized and uncolonized tips, but you will find them. Count all samples from every core of each host species and record the data in a lab notebook or spreadsheet as directed by your instructor. Once done, the lab work is complete.

Lab Session 2 Data Analysis & Homework:

After describing, counting, and recording your data, it is time to quantify ECM colonization and diversity, and characterize community composition, from each host. You will also conduct a t-test to determine if there are differences in total ECM colonization between them. Calculate: (1) **total colonized** and

uncolonized root tip numbers, (2) **percent colonization per morphotype**, (3) **total percent colonization**, and (4) **Shannon diversity** for roots from each host. Record all results in a notebook or spreadsheet.

1. **Total colonized root tip number** is a summative measure of the total number of root tips covered by each ECM morphotype from every core per host. To determine, count the total number of colonized tips for each morphotype and then sum them collectively. Conversely, determine the total number of root tips NOT colonized (i.e. **total uncolonized root tip number**) from every core per host. When counting colonized tips, be sure to record how many there are for each morphotype and for each host species, since this data will be used later to calculate other variables.

2. **Percent colonization per morphotype** is a measure of the relative abundance of each morphotype. Some might be highly abundant (i.e. dominant), some less so, and others much less so (i.e. rare). To determine the percent colonization for each type, divide the summative total colonized root tip number for each type (which you determined in step 1) across all cores by the total colonized root tip number by all types pooled across cores of each host species which you also determined before. These percent colonization per morphotype values can now be used to characterize the composition for each host species by recording and comparing them in Table 3 (Excel file).

3. **Total percent colonization** is a measure of the percentage of root tips colonized across all root tips, including uncolonized tips. To determine this, sum the total colonized root tip number and total uncolonized root tip number (which you determined in step 1) for each core of each host to get a value for each. Next divide the total colonized root tip number per host by this value just determined to get a **total percent colonization** value for each host (e.g. 58% and 64%). Note that the greater the percent colonization, the greater the degree of interaction between the ECM fungi you described and the two host species.

4. **Shannon Diversity Index** (**H**) can give you a measure of ECM fungal community diversity. The greater the index value is, the greater the contribution by more morphotypes to overall community structure. The index is calculated as:

$$\mathbf{H} = -\sum_{i=1}^{s} P_i \ln P_i$$

where **H** = the index value and P_i = the proportion of individual colonized root tips per morphotype. The index ranges from 0 to > 1.0 with diversity increasing with the value.

5. Conduct a **Student's t-test** to test the hypothesis you formed earlier regarding differences in total root tip colonization between hosts. Do this using the values per core for each host (n = 6 for each) you determined in step 1 before. Though t-tests have been used in class before, you may want to go over an example or two during office hours for refreshing.

6. Answer the following questions related to the calculations and t-test:

    a. With which host were there more sporocarp types? What might this say about mushroom bearing ECM fungal diversity in the vicinity of the trees you sampled? How might sporocarp numbers be used to estimate the abundance of ECM fungi colonizing host trees in the vicinity of them?

    b. How did total colonized root tip number compare between hosts based on the t-test results? Further, what types of ECM properties might explain the results? For help, think of class discussions about the nature of mycorrhizal symbioses (e.g. tree size and carbohydrate supply, host specificity, etc.).

    c. How did total percent colonization compare between hosts and what might explain any differences, or lack thereof? How does this variable differ from total root tip colonization and what value might the difference provide in understanding mycorrhizal associations between fungi and host plants?

    d. Generally describe the ECM morphotype composition found for each host. To do so, distinguish which types were unique to and common between them. And, decide which types should be considered specialists or generalists, and explain why. Did any type(s) dominate composition on either host? If so, which? Could any be designated as "rare" on either host? If so, which? What might explain why some types are more or less abundant than others? As a hint, think again about discussions in class about the mechanisms of mycorrhizal symbioses (e.g. host specificity and their ability to produce and share carbohydrates).

e. Did Shannon diversity differ between hosts? If so, first explain why it differed quantitatively and then provide a biological explanation for these differences based on discussions held in class on how abundance and species numbers influence the index. Perhaps more important, and like the prior question, think about how specificity and tree size may influence this measure as well. Likewise, if diversity did not differ greatly between hosts, then try to explain why it did not.

f. Thinking about the lab that you have now conducted and gotten results for, explain how it has helped you better understand the concept of mycorrhizal symbioses. In addition, explain how the variables you measured have helped you to better understand concepts such as abundance, community composition, dominant and rare species, and community diversity since these concepts can be applied to any biological organisms (symbiotic or not).

7. Finally, write your group Lab Data Analysis and craft your Lab Report Presentation. Note that first drafts should be turned into your instructor at least three days before due. *See* Word *files for guidelines and rubrics for each assessment.*

Questions for Further Thought and Discussion:

1. Did you find any differences in sporocarp species composition between hosts? What might explain any differences or lack thereof?

2. Did you find any differences in the abundance of any particular sporocarps between individual trees of the same host or between hosts? What might explain any differences or lack thereof?

3. How might differences in total colonized root tip number from each host be explained by host properties (e.g. size or host specificity) and concepts discussed in class regarding mycorrhizal symbioses?

4. What do the total percent colonization findings tell you about how well roots are colonized by ECM fungi on each host and why? Think again about host specificity and host size for starters, or any other potential factors discussed in class.

5. Describe the ECM fungal community composition found on each host using any terms and variables you like.

6. How would you describe Shannon diversity from each host species? In particular, was it high, moderate, or low given the index relies on morphotype/species abundance and types that was discussed in class?

7. Does the number of sporocarp types found associated with each host qualitatively correlate with Shannon diversity of ECM types found on the sampled roots? If so or not, any ideas as to why or why not?

8. Which host might be affected more by its ECM associations in terms of the potential amount of photosynthate it may be sharing with its fungal symbionts? Think about the carbon costs that can be associated with different levels of ECM colonization.

9. Now that you are more familiar with some of the field and lab methods used to study mycorrhizae, can you devise an example of a study in which these methods could be used to examine other questions related to host tree roots or other soil organisms (e.g. insects, worms, etc.), albeit with modification? To do so, simply describe a study question, which methods you'd use generally, and for which soil organisms. For example, you might ask the question: Do conifer roots differ from those of broadleaved roots in terms of their diameter in soils with variable moisture levels?

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Tools for Assessment of Student Learning Outcomes:

Assessments

Two assessments allow the instructor to determine whether student outcomes are met: the Lab Data Analysis (Guidelines & Rubric Word file) and the Lab Report Presentation (Guidelines & Rubric Word file). As stated before as the experiment's objectives, students will:

1. Apply the scientific method by making predictions and collecting, analyzing, and interpreting data, and by writing a data analysis summary.
2. Use fundamental ectomycorrhizal quantification and description methods.
3. Prepare and deliver a presentation of lab results and conclusions.
4. Authenticate lecture material to ECM communities and their host plants.

The data analysis will ensure that students understand experimental design elements (e.g. sampling approaches and replicates) and methods used, can apply the methods learned to other studies, can apply the scientific method through observations and hypothesis formation, data collection, and analysis, and can summarize findings in written and oral presentation formats. It is encouraged that students be allowed to turn in a first draft of the analysis for instructor feedback, and be given time to prepare and turn in a final draft to address any feedback. The presentation ensures that students can craft a logical and organized PowerPoint presentation that summarizes all lab criteria. Students are directed to use Lab Data Analysis and Lab Report Presentation Guidelines & Rubrics for assistance with each assessment.

Example Take Home Exam Questions

Following the lab, students will receive questions on a summative exam requiring them to address related higher order questions and to use knowledge learned to solve a lab-related problem. Example questions include:

1. For the mycorrhizal lab, you were required to explain why some morphotypes colonized only one host tree species, while others did so on both. Most of you correctly answered that such associations reflect ECM host specificity. Given that we only introduced this concept, we didn't discuss what regulates it. Thus, using any resources (that are cited), explain some regulatory processes that drive ECM symbioses and, more specifically, defend or refute the following two theories: (a) plants initiate ECM symbioses or (b) fungi initiate them via "benign parasitism".

2. As you learned in the mycorrhizal lab, the Shannon Diversity Index can be used to quantify diversity in any biological community. Given your use of the index, you now have an opportunity to use it in your new job as a field biologist at the state Department of Conservation. The department intends to selectively harvest trees in game lands as part of a management plan and then sell them. So, you have been asked to participate on a plan development team. Specifically, you have been asked to recommend 3-4 tree species that should not be fully harvested in the game lands so that they can serve as "seeders" to help with forest regeneration. Given what you have learned about ECM fungi in the mycorrhizal lab, especially the role of host specificity and how it influences ECM fungal diversity, explain how you would (a) articulate the need to use a study of ECM diversity to help determine which tree species should be used as seeders, (b) why this matters in terms of long-term tree diversity, and (c) how you would do this via a pre-harvest study.

3. In the mycorrhizal lab you discovered that some ECM fungi colonize more than one host, while others only colonize one. List and explain how key abiotic and biotic factors contribute to these patterns and then explain, from a fungal perspective, the benefits and limitations of being a "specialist" and "generalist". Use the literature to support for your views.

NOTES TO FACULTY

Challenges to Anticipate and Solve

Challenge #1: *Lack of familiarity with content and field organisms* is a common issue, but not insurmountable. This is simply due to the fact that fungal biology and field mycology are rarely taught in K-12 or college biology courses. In addition, besides mushrooms, most students know little about common sporocarps (e.g. boletes, chanterelles, morels, etc.). You can get around this by having lectures that introduce the concepts of species interactions, especially symbioses, community properties (e.g. composition and diversity), and some basics of fungal and mycorrhizal biology. In addition, familiarizing students with a few common local mushroom taxa and showing them specimens and images of common sporocarps, root systems, and mycorrhizal fungal types will help a lot.

Challenge #2: *Study organism availability is unpredictable,* especially for sporocarps. Students may find just a few (or no) sporocarps when conducting the lab. To address this possibility, it is suggested that you (a) hold the lab in early fall or late spring, especially after a rain event when sporocarp abundance in northern latitudes is often highest, (b) visit study sites a week before the lab to assess sporocarp numbers, or (c) omit the sporocarp component as the ECM section alone is still valuable.

Challenge #3: *Students are not familiar with soil methods or morphotyping.* Simply put, few to none of your students will have conducted a lab using belowground organisms, especially of mycorrhizae. Thus, expect a learning curve and some squeamishness regarding working with soil and detritus. Still, few worries as students can be brought up to speed on extracting cores, picking detritus from roots, and learning morphotyping. To reduce learning time, however, it is recommended that you walk students through each procedure by showing them images at the end of a lecture preceding the lab or by demonstrating the procedures for them in the field and lab. One other suggestion for morphotyping is to show them morphotype images in the lab and ask them to take a crack at describing them. This makes them aware as a group of what is expected and allows you to facilitate the lab more easily. Finally, have students work in groups since “many hands and minds” reduces “hands up” during a lab.

Challenge #4: *Data calculations and analysis can be problematic.* This will almost certainly occur for some students, so expect it. However, as with the prior challenge, taking time to go over measured variables, showing an example data table, and conducting a practice t-test in a preceding lab can prevent issues from

arising later. My experience has been that if a lab is conducted in a semester when students have not used t-tests, they will need a refresher or tutorial beforehand. However, if a lab is conducted at semesters end, you may only need to help with the calculations. On that note, calculating Shannon diversity can be tough for some students, but I have found that there are always students who can readily calculate them and they can help those who cannot. For those who still struggle, use your office hours to go over the index with them.

Comments on Introducing the Experiment to Your Students:

Before the lab, I will have lectured on key lab related conceptual topics (e.g. symbioses [including mycorrhizae], and community composition and diversity) and on basic fungal characteristics and taxa (especially of mycorrhizal Basidiomycetes like boletes and mushrooms common in my area) and ecological importance (e.g. decomposers and mycorrhizae). Two-four PowerPoint based lectures, consecutive or not, will have been held to encourage discussions based on textbook and web source readings (see below) to provide the instructor with adequate background to teach the topics, and to provide discussion questions for the students (see below). Once sufficient background coverage has been made, I then tell my students how lucky they are to be able to apply these concepts in a mycorrhizal lab, since few biology majors will. This usually garners some laughs, but a case for why the lab is important can then be made, especially in terms of how it can authenticate the concept of symbioses and introduce them to soil methods. I then go over the experimental design and methods to be used. Most important, I spend time "prepping" students on basic morphotyping techniques by showing them root samples and fungal mantles. When time permits, I also have them describe sample mantles with the characters they will use. Experience has it that these approaches reduce their learning curve once conducting the lab. Finally, I take them outside at the beginning of another lab and show them where they will conduct the field segment of the lab and go over the design and methods to be used there. I also ask for volunteers to take root cores, which I sift through on site to show them ECM root tips visible to the naked eye. Lastly, I have them look for sporocarps on site.

Supplemental Reading Sources for Instructors

(1) Biology. 2011. Solomon, E., Berg, L., and D.W. Martin. Brooks/Cole, 9th edition. Read the chapter on Fungi, focusing on their characteristics and morphology (e.g. hyphae and mycelium). (2) Biology of Plants. 2013. Evert, R.H. and S.E. Eichhorn. W.H. Freeman, 8th edition. Read Chapter 15 on Fungi and Chapter 32 on The Dynamics of Communities and Ecosystems. (3) Fundamentals of the Fungi. 1996. Moore-Landecker, E. Prentice Hall, 4th edition.

Read chapter 16: Fungi as Mutualist Symbionts (pp. 482-518). (4) Mycorrhizal Symbiosis. Smith, S.E. and D.J. Read. Academic Press, 3rd edition. Read the Introduction (pp. 1-9) and skim Chapter 6: Structure and Development of Ectomycorrhizal Roots, reading closely the section titled "Specificity in Ectomycorrhizal Symbioses" (pp. 209-211). Note that, depending on the course, students will also have been assigned reading on the abovementioned topics in a textbook they are using for the class in which the lab is conducted. If there is no relevant reading material, then the above sources (especially 1-3) could be provided by the instructor as a supplement.

Example Discussion Questions for Students

1. What is a sporocarp and what are some common types associated with ECM fungi?
2. What are some common ECM boletes and mushrooms found in this region?
3. Define hyphae and mycelium, and describe where are they generally found in soil.
4. Describe the general root system of a typical host tree from whole to cellular levels (e.g. lateral roots, simple or fine roots, epidermal root cells, cortical root cells, etc.).
5. Describe how ECM fungal mycelium interacts with host roots at the cellular level (e.g. in forming intercellular "Hartig nets").
6. Define host specificity and distinguish between two specificity types (i.e. generalists and specialists).
7. Explain how host specificity can influence ECM fungal diversity.
8. Explain, using comparative examples, how tree size may influence ECM colonization on hosts. Leading question: Does tree size correlate with photosynthate production?

Comments on the Data Collection and Analysis Methods:

This lab is best conducted in wooded habitats that are flat and accessible to ALL students. If such sites are not available, try using campus woodlots, wooded edges near parking lots, or campus trees as a last resort! Note, however, that if using campus trees, sporocarps are less likely to be found. Also, find sites that have an abundance of at least two dominant tree species, and enough for sampling. Ideally, more than one group should sample no single tree in order to reduce soil disturbances. Some trampling is unavoidable, and root extractions will expose soil and roots. Thus, have students stay on trails as much as possible, being careful not to trample herbaceous plants and seedlings, and to look for sporocarps since they can be inconspicuous and easily crushed. As for

core holes, have students fill them with loose rock, soil, and leaves after sampling. If campus trees are used, it is advisable to take smaller samples. One last important point regards sporocarp **TOXICITY**. Since many are poisonous, tell students to **NOT** touch them. If they do, have them wash their hands thoroughly with soap back in the lab.

Depending on sporocarp availability, you may have to omit this part of the lab, or provide students with presence and abundance data collected for chosen hosts from prior labs. Boletes and mushrooms are notoriously inconsistent in fruiting. Thus, do not expect that a site that yielded many sporocarps one semester will do so in subsequent ones. Also be aware that weather conditions, especially rain, influence sporocarp abundance. If there has been a dry spell before the lab, you may find none. Roots, however, are more forgiving, but they too are generally more abundant in moist soil, and easier to analyze.

Students may have trouble formulating a colonization hypothesis since they will likely have just learned about how host specificity and host size can affect ECM colonization. Thus, you may need to use hints to help them. In addition, students may have difficulty conceptualizing what ECM colonization is, given that mycelium, like clonal plants, may not have "distinct individuals" to count. Use analogies like "leaves on a branch" to help them conceptualize what they are trying to hypothesize.

Students are probably well versed in developing and giving PowerPoint presentations. However, they may not have given a "lab report presentation" before. Thus, review carefully the Lab Report Presentation Guidelines and Rubric and show them some example presentations given in previous classes, critiquing them for suggestions and emphases.

Comments on Questions for Further Thought:

1. *Did you find any differences in sporocarp species composition between hosts? What might explain any differences or lack thereof?*
   Let students think for themselves about this question, but if they struggle, remind them of class discussions on symbioses, coevolution, and host specificity. Also, have them consider possible differences in soil conditions unique to each host species.

2. *Did you find any differences in the abundance of any particular sporocarps between individual trees of the same host or between hosts? What might explain any differences or lack thereof?*

Students might struggle with this question given that it may appear to be similar to the prior. However, have them carefully consider it again to see how it differs. You may hint that sporocarp abundance can reflect the nutritional status of ECM fungal counterparts or host light environment, resulting in support for more or fewer sporocarps, and to consider whether the sporocarps belong to mycorrhizal or saprophytic fungi, or both.

3. *How might differences in total colonized root tip number from each host be explained by host properties (e.g. size or host specificity) and concepts discussed in class regarding mycorrhizal symbioses?*
   Similar to the prior question, the answer(s) to this one relates to the ability of hosts to support more or fewer ECM fungi, which can reflect host photosynthetic status, and nutrient and water access. Thus, instructors might use hints or guiding questions to lead students to answers.

4. *What do the total percent colonization findings tell you about how well roots are colonized by ECM fungi on each host and why? Think again about host specificity and host size for starters, or any other potential factors discussed in class.*
   Handle as you did for the prior question, but acknowledge that there may be a contradictory explanation as to why more or fewer roots are colonized. The instructor may use a "devil's advocate" approach if, for example, a student feels that low percent colonization equates to the inability of the host to support many ECM fungi. Instead, you might ask, "Is it possible that this host doesn't NEED any ECM associations?" Then, ask them why that may be the case.

5. *Describe the ECM fungal community composition found on each host using any terms and variables you like.*
   For this question, there is no one correct answer. Instead, the point is to have students apply their understanding of what community composition is, which can be abstract. In particular, the question allows you to gauge how well students can incorporate ideas of morphotype richness and "species variety", how individual species abundances can "paint" composition via dominance or rareness, and how specificity drives composition. I suggest that instructors use guiding questions to get students to feel comfortable defining composition in their own way. This can be tough, but it can effectively facilitate both abstract and critical thinking. Further, to better answer this question, students are directed to search and read relevant peer-reviewed ESA journal articles from, for example, *Ecology* and *Frontiers in Ecology and the Environment*, or from relevant non-ESA journals (e.g. *Mycorrhiza* or *New Phytologist*) to gain

information and perspective to better understand ECM composition and apply it to better answer this question. If students struggle to find articles, they might be provided with 1-2 articles that nicely cover the concept. Gehring et al. 1998 and Dahlberg 2001 are excellent examples.

6. *How would you describe Shannon diversity from each host species? In particular, was it high, moderate, or low given the index value's reliance on morphotype/species abundance and types discussed in class?*
   For this question, instructors may have students articulate why Shannon diversity is low or high, and how it compares between hosts. Also, understanding the variables that influence the index is important. Ask them, for example, how ECM abundance and richness affect the index to help them formulate good answers. Bottom line – they should be prepared to answer more than just, "diversity is high for one species and low for another". If they do, pick at them to articulate why this is the case. In addition, the question is valuable in that it forces students to understand how an index is constructed and calculated, and used both qualitatively and quantitatively to describe ecological communities. To better answer this question, as with the prior question, students should be directed to search and read articles from ESA journals such as *Ecology* or *Frontiers in Ecology and the Environment* or from relevant non-ESA journals to gain information and perspective to better understand the topic and apply that information to better answer the question. Good examples include Hill 1973, Peet 1974, and Dahlberg, A. 2001.

7. *Does the number of sporocarp types found associated with each host qualitatively correlate with Shannon diversity of ECM types found on the sampled roots? If so or not, any ideas as to why or why not?*
   This is a challenge question and is really meant to engender questions regarding whether sporocarp numbers are accurate representations of belowground mycelial counterparts. Have students ponder and attempt to answer, using guiding questions like, "Why would more sporocarps be associated with high Shannon diversity?" or "If you have low sporocarp richness, but high ECM fungal diversity, might the sporocarps be saprophytic and not mycorrhizal?" Run with it, making it a higher order "question generator" to further explore ECM concepts.

8. *Which host might be affected more by its ECM associations in terms of the potential amount of photosynthate it may be sharing with its fungal symbionts? Think about the carbon costs that can be associated with different levels of ECM colonization.*

This is also a challenge question and like the prior can be used to foster class discussion. The answer relates to ECM host-fungal physiological interactions, and so may not be appropriate for lower level courses or upper ones besides mycology. If used, however, lead students to think about photosynthate costs and benefits, carbon sinks, etc.

9. *Now that you more familiar with some of the field and lab methods used to study mycorrhizae, can you devise an example of a study in which these methods could be used to examine other questions related to host tree roots or other soil organisms (e.g. insects, worms, etc.), albeit with modification? To do so, simply describe a study question, some basic methods you might use, and for which soil organisms. For example, you might ask the question: Do conifer roots differ from broadleaved roots in terms of their diameter in soils with variable moisture levels?*
This is perhaps the least difficult question for students to answer as it allows them to run with ideas. But, the hard part is having them try and determine how the methods they used might be modified or used as are to address other study questions. Note that this question could also be rewritten as a problem solving (PBL) type.

Comments on the Assessment of Student Learning Outcomes:

As a reminder, there are two assessments for the lab, the Lab Data Analysis and Lab Report Presentation. Each has guidelines and rubrics, but, like any assessment instrument, are open to differences in interpretation, can lack clarity, and can be subjective. Thus, there are learning curves that students and instructors face. The following are pointers on how to best use them.

1. The Lab Data Analysis Guidelines & Rubric are helpful and few questions will arise over specific calculations or questions since these are either easy to understand or students will ask questions for clarification before turning in their analyses. However, instructors should look over the guidelines and rubric and modify them to their own liking if they find that some instructions need clarity. In addition, it is encouraged to let students turn in a first draft for instructor feedback, which can help identify potential issues stated above. The overall assessment ensures that students meet learning goals 1, 2, and 4 either directly or indirectly. In particular, the rubric clearly requires that students meet all four sub-components of goal 4, which requires them to authenticate lecture materials through descriptive and quantitative lab components and results interpretations (e.g., Criteria 2 and 3 under “Specific results include:”).

2. The analysis rubric is fairly straightforward and students generally do not quibble with it as long as the scales are explained and they are given example answers for some questions. The rubric ensures that students address learning goals 1, 2, and 4.

3. Instructors should emphasize the need to write well, use correct grammar, and write in a logical and organized manner. It is helpful to tell students that good writing is as relevant to science as it is to "writing intensive" subjects. So, practice, practice, and practice. This portion of the rubric ensures that students address aspects of learning goal 1.

4. The Lab Report Presentation Guidelines & Rubric are straightforward and helpful to students preparing for this, especially if prior presentation examples are shown. Use prior examples (if you can) to point out organization, graphs (as examples to use), and how to reduce slide wordiness via bullets. The overall assessment ensures that students meet learning goal 3, as well as some aspects of 4 (i.e., the criteria under "Lab Content & Experiment").

5. Instructors are encouraged to go over the rubric and to not just post it on a website. Also, emphasize to students that they will be assessed not only on their experimental design, analyses, and interpretations, but also on their ability to craft an organized PowerPoint and deliver it professionally. Point out the need for equal participation by all group members, clear speaking and pacing, graph and table explanations, and professional speaking behavior (e.g., no hands in pockets, gum chewing, etc.) when presenting. The rubric ensures that students meet learning objective 3.

Comments on Formative Evaluation of this Experiment:

This experiment employs a number of formative evaluation approaches recognized by TIEE that are meant to help ensure that students are successful in meeting the lab's objectives:

1. In lecture before the lab, use a **Muddiest Point** to find any weaknesses in student understanding about related concepts (e.g., coevolution). At the end of class prior to the lab, have students form groups and ask them to answer the question: "Of the concepts covered this week, which was most difficult for you to understand"? Then ask them to discuss and choose a concept that they struggled with the most, and explain why. Then discuss it and have your students follow up with suggestions on how to better teach the concept. This has two benefits. First, it allows students to work

together to identify difficult conceptual material and to articulate why it may be difficult to understand. My experience here is that it gives students a better understanding of such concepts and allows them to see other students struggling like them in open discussion. The Muddiest Point follow up is also beneficial because it can provide you with a clear opportunity to assess student understanding of difficult concepts, which you can improve upon. The Muddiest Point is not graded, but allows you to formatively assess your students. This addresses the fourth learning objective.

2. The Lab Data Analysis is a traditional formative assessment. It employs a rubric containing a standard lab format that the instructor uses to grade student analyses. However, instead of grading it in the usual manner of receiving it from students with no feedback before grading, groups should be allowed to turn in a first draft for feedback. Such feedback doesn't take anything away from a student's ability to learn the material, because it allows them to see any errors they may have made and to revisit questions that they may not have fully thought about. In addition, they can have a week to turn in a revision, which is similar to how they will operate in future careers. This assessment benefits instructors too, as student weaknesses can be viewed as a reflection on instructor design and use of the analysis. By allowing students to turn in a first draft, instructors can identify weaknesses in it, allowing for changes to be made that can reduce future weaknesses. This addresses the first learning objective.

3. The main assessment for the experiment is the group Lab Report Presentation which is assessed using a standard rubric by the instructor, but also includes student input on their performance. Thus, it has a **Self-Evaluation**, which is one of the TIEE formative assessment techniques. This evaluation is really a reflection that allows each group member to make regarding their contribution to addressing presentation criteria, their work ethic, and their level of cooperation with group members. It makes up a small portion of the full grade but gives students some ownership over it, while giving the instructor an opportunity to reflect on the value of this assessment component. This addresses the third learning objective.

4. A summative exam following the lab can include applied "problem-solving" questions that incorporate the **Transfer and Apply** technique, helping the instructor address the fourth learning objective. This is an important formative assessment method because it allows the instructor to determine whether students can synthesize lecture and lab material and

methods in order to apply them to real-world situations, including problem solving or consultation needed for use in their future careers.

Comments on Translating the Activity to Other Institutional Scales or Locations:

1. This lab can be used for standard class sizes (i.e., 16-24 students) not too different from those found at medium-large institutions, so scaling up may not be needed. However, smaller institutions that may have smaller class sizes can still use it. Smaller sizes can maintain the group format, but groups might be made smaller, or fewer groups the size of those used in my lab could be used. In addition, it may be helpful for instructors at smaller institutions to review the translation of the experiment to lower level courses as described in the “Transferability” section, given that many upper level or mycology courses may not be offered at them.

2. This lab can be used in any plant habitat that has ECM associations, so it can be used in most geographic areas. Instructors should, however, time the lab to coincide with peak ECM growing seasons, which usually occur during early fall and late spring in North America. Instructors should be aware of adverse soil conditions (e.g., drought) that may render the lab undoable. The lab also can be used as a template for studying other soil organisms, though not necessarily those which interact with plants symbiotically. Soil invertebrates that can be captured, or cultured microbes, for example, could be quantified using the same lab design for entomology, microbial, and other related courses.

3. As stated before, efforts should (and almost certainly can) be made to find sites that ALL students can access for the field component. As for the lab component, institutions must adhere to the ADA and have labs that are accessible to all students.

This lab CAN be used (with modification) at the secondary level. I know because I conducted sporocarp surveys and simple core analyses when I taught middle school. However, the level of topic coverage for mutualisms and mycorrhizal biology should certainly be lower for typical 7-12 classes, so expectations regarding calculations, hypothesis testing, and higher order class discussions will have to be modified or omitted, except perhaps for AP or other advanced biology courses. Still, 7-12 teachers should NOT shy away from the lab. As for K-6, some greatly modified lab components might be used such as showing soil cores,

colonized roots, sporocarps, etc. However, given sporocarp toxicity, NO child should handle any.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

See example data tables (Excel file), which provides examples of how the experiment's data can be organized, how calculations can be made, and the results of a t-test using the data. Calculations of Shannon diversity are not provided, but can be requested from the author if needed.

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