An Exercise to Demonstrate Soil Microbial Diversity in Introductory Environmental Science Classrooms

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ABSTRACT High diversity of microorganisms in the soil matrix has been the focus of extensive research in the fields of soil biology and microbial ecology, and is a key concept that students in the environmental or biological sciences should understand. Two activities to demonstrate diversity and highlight the challenges faced in studying soil microbial diversity are presented here. Using a bag (soil sample) containing different candy types (soil microbes), students explore isolation and molecular techniques, two strategies used in studying microbial diversity, and are asked to consider the limitations these methods have in understanding complex microbial communities. In a second activity students use this collection of candy to construct mock-phylogenetic trees. These hands-on activities provide students with a fun way to learn about microbial diversity. Student assessment scores increased by 35% after participating in these activities and results from final exam questions show that a high percentage of studentsretained the major concepts demonstrated during these activities.

One gram of soil may contain 10 billion microorganisms and thousands of species (Torsvik et al., 1990), making soil the most biologically diverse ecosystem on Earth. Soil microorganisms are responsible for many critical processes including decomposition, bioremediation, and nitrification, and are also the source of novel biochemical pathways that may lead to development of new industrial processes, agricultural products, and antibiotics (Curtis and Sloan, 2005). Microbial-mediated processes and products are but two reasons why understanding microbial diversity is important for students in the environmental and biological fields. Understanding how microbial ecologists study this diversity also enhances student-training by exposing them to methods and skills that scientists use.

Despite its importance, microbial diversity can conceptually be difficult to teach, especially in classrooms composed primarily of undergraduates who have had minimal exposure to microbiology. In introductory environmental and soil science classes, laboratory and recitation activities are often paired with lecture-based material to reinforce key concepts. Traditional soil biology labs include dilution plate counts or microscope-based activities (Stromberger, 2005). These activities require considerable prep time and in the case of microscopes, a significant financial investment that may not always be feasible.

In this article we describe activities that accomplish two important teaching goals and can be carried out with little preparation or cost. First, these activities introduce some of the ideas and issues surrounding soil microbial diversity; second, they provide students with an opportunity to build science process skills (Wilke and Straits, 2005). Recently, science educators have emphasized a growing need for more inquiry-based learning in science classrooms, as a means to demonstrate that science is not a collection of facts in a textbook, but is instead a process of questioning, observing, and investigating (Handelsman, 2002). Activities such as those described here provide a framework for students to work in teams and to build their own knowledge of microbial diversity. Students are asked to go beyond memorizing the number of microbes in a gram of soil, and instead are challenged to build their own hypotheses about the nature and usefulness of this high soil microbial diversity.

Impact Statement

Students in introductory environmental science classrooms should not only be exposed to a variety of scientific topics, but should also be engaged in activities that require them to work in groups and to design hypotheses. An activity to demonstrate soil microbial diversity was used to teach students about factors leading to diversity and the methods used to study soil microbes. Students learned about microbial diversity while practicing the scientific method.

Learning Objectives

The activities described below are suited for an undergraduate introductory biology, soil science, or other environmental science class as part of a unit on soil biology. Learning objectives include identifying the roles of soil microbes, describing soil community food webs, and

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Abbreviations: DNA, deoxyribonucleic acid.

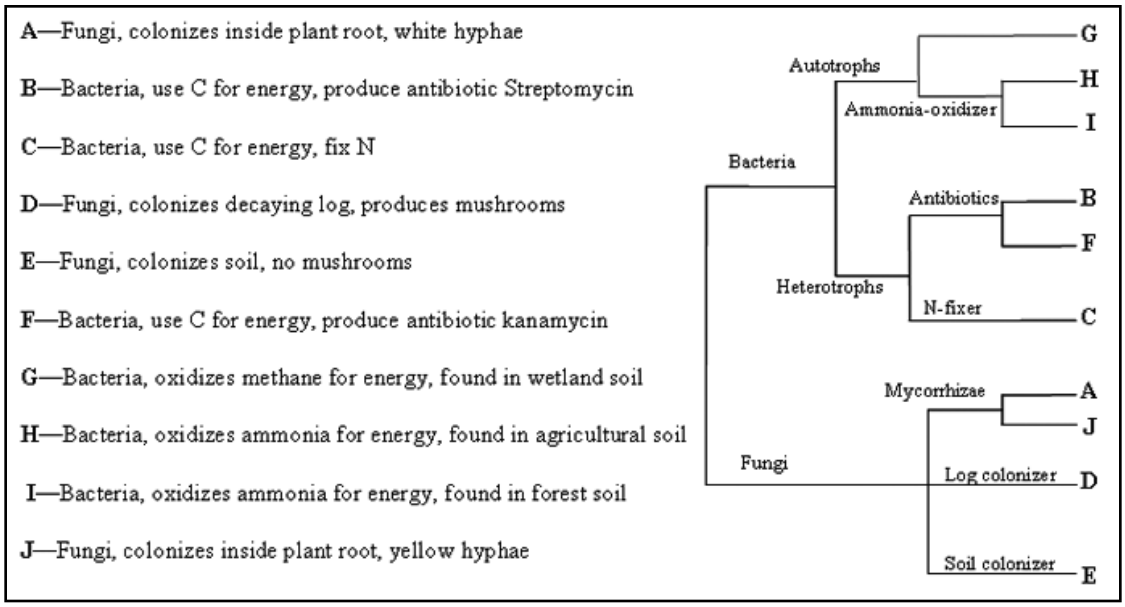

Fig. 1. Included in the background information, this figure gives descriptions of 10 example soil microorganisms and provides a phylogenetic tree that organizes these microorganisms based on physical and biochemical characteristics.

discussing beneficial functions of soil microbes in their ecosystem. Upon completion of the activities, students should be able to:

1. Describe the mechanisms that lead to high microbial diversity in soils and identify two techniques that microbial ecologists use to study soil microbes.
2. Categorize a mock microbial community composed of candy types by constructing graphs and phylogenetic trees.
3. Formulate a hypothesis describing how different sampling strategies (i.e., "culture-based" sampling vs. "DNA-based" sampling) might increase our understanding of the soil microbial community.

Background

Prior to the activities, a handout and a short presentation are used to introduce the concept of microbial diversity. The written and verbal material includes the following information:

"There are more than 10^{16} prokaryotes in a tonne of soil compared to a mere 10^{11} stars in our galaxy" (Curtis and Sloan, 2005). Not only are soil microbial communities large, but they are also extremely diverse. This diversity arises from many factors, but is in large part due to the environment in which they live: The soil matrix contains high surface area and micropores. Surface area provides microbes with attachment surfaces and micropores isolate soil microbes, allowing for their adaptation to unique micro-environments. The soil matrix contains billions of micropores, each of which may contain a unique microbial community. Torsvik et al. (2002) estimated that 100 cm^3 of soil contains 10,000 different microbial species.

Although we know that soil microbes are important and carry out many vital functions, understanding and studying microbial diversity in the soil matrix is challenging. Attachment to soil particles means that they cannot be easily extracted, and because a soil bacterium is roughly the same size as a clay particle, they can be hard to identify using microscopy. Furthermore, cell morphology gives little insight to a microbe's biochemical capability.

Historically, soil microbiologists lacked the tools needed to examine these communities. They relied on isolation and culture-based techniques, but to-date less than 1% of soil bacteria have been cultured (Amann et al., 1995). Developments of molecular-based methods (i.e., those based on DNA and other cell constituents) over the last 25 years have greatly improved our understanding of these complex communities by increasing the information we have about microbes living in the soil. In 1977, Martin Alexander identified nine groups of bacteria that he hypothesized to be the most dominant soil microbes. Today we know that Alexander's nine groups make up only 2.5 to 3.2% of soil bacteria (Janssen, 2006). A great deal of current research is focused on understanding this diversity and organizing the huge amount of molecular data that has been generated. Soil microbiologists use phylogenetic trees to organize the microbial communities that they study in the hopes of one day completing our picture of the soil microbes (Fig. 1; see Microbial Organization and Phylogenetic Trees, below).

Materials and Methods

An opaque bag filled with various candy pieces is presented to the class as a model soil microbial community. The composition of the bag can vary, but some general guidelines are presented below:

- Approximately 100 pieces of candy
- 15 to 20 unique types of candy (different shapes, colors, sizes)

- Some candy types should be rare species (2–3 per bag)
- Others types should be dominant in the soil system (i.e., a population of 25–30 Tootsie Rolls are a good option; see Supplementary Concepts, below)

Activity 1: Sampling Methods in Microbial Ecology

To demonstrate the limited view culture-based techniques provide, four students are each asked to remove one sample microbe (candy piece) from the bag. These samples represent the four culturable microbes in the soil community. The students are asked to describe these candy pieces; the candy types serve as an analogy for microbial characteristics (Table 1). These candy pieces are than replaced into the bag. A volunteer from the class is then asked to draw a pie chart that represents the community (Fig. 2).

Activity 1: Molecular-Based Techniques and the Species Concept

Students are reminded that molecular-based techniques have improved our understanding of the soil community by providing information about microbes that cannot be easily cultured. To further reinforce this concept, groups of four to five students develop hypotheses about the differences in culture and DNA based sampling. Additionally, these groups are asked to predict how a more comprehensive sampling method, such as those based on DNA, may increase our understanding of microbial community composition. One student from each of the groups shares these hypotheses with the class.

Twenty students are each asked to remove one sample microbe from the bag. These microbes are counted and recorded (Table 1). To record numbers, a selected student is asked to hold up his/her organism and give a brief description; other students with the same type of microbe are asked to raise their hands. The count for each species is recorded (Table 1). During the recording process, the question of group assignment typically arises. For example two students might have drawn red gummy bears, but one of these bears is cinnamon flavored and the other is cherry (see Supplementary Concepts, below). As a group, the students should decide if these represent different species. There is no right answer; it is the discussion about the definition of microbial species that is important.

Once the DNA-based sampling (20 candy pieces) has been recorded, three to four students are asked to come to the front of the classroom to sort and count all the candy pieces in the bag. In the students' lab write-ups, they are responsible for drawing pie charts (Fig. 2) of the DNA and total community compositions and for answering synthesis questions. Examples of synthesis questions include:

Table 1. A sample set of data collected during Activity 1 by students in Introductory Soil Science. Data was used to construct pie charts shown in Fig. 2.

Species	Description	Culturing	DNA	Total no. in sample
1	green lollipop	1	1	1
2	yellow gummy bear	1	1	3
3	caramel	2	4	7
4	bubble gum		1	11
5	red gummy bear		2	4
6	cinnamon Jolly Rancher		1	2
7	Tootsie Roll		1	31
8	butterscotch		2	9
9	purple gummy bear		3	4
10	raspberry Jolly Rancher		2	4
11	connimon gummy bear		1	5
12	sour-apple Jolly Rancher		1	3
13	banana taffy			8
14	cherry taffy			4
15	Smarties			1
16	orange drop			1
17	chocolate mint			2
Grand total				100

Were some microbes better represented in the culture-based or DNA sampling than others? Was there a microbe in the bag that we didn't describe in either procedure? Give two reasons why we might not be able to describe a microbe in soil.

While selected students count and organize candy, the instructor leads a class discussion about the mechanisms that lead to high diversity in soils. The students are asked to imagine that the classroom represents the space inside a soil aggregate and the candy pieces represent bacteria and single-celled fungi. Again working in groups and assisted by the background information provided, students discuss the mechanisms that lead to microbial diversity. The groups are

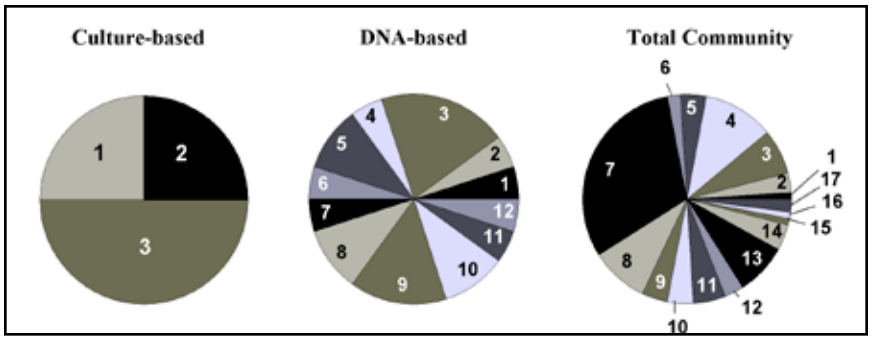

Fig. 2. Pie charts representing the sample data from Activity 1. Numbers represent the species number as recorded in Table 1. Students construct pie charts such as these, as part of their homework assignments.

Table 2. Questions used in pre- and post-assessments to determine if microbial diversity activities increased student understanding of soil microbial diversity and the factors contributing to this diversity.

1. Roughly what percentage of soil bacteria have been characterized to date?
   a. 0.5–1% b. 5–10% c. 10–25% d. 25–50% e. 50–75%
2. Traditionally soil biologists studied microbes in the soil by:
   a. Looking at soil under a microscope
   b. Separating microbes from soil in a centrifuge
   c. Growing soil microbes on a Petri dish
   d. Examining plant roots
3. The relationship between soil microbes is often shown using:
   a. Bar graphs
   b. Electron micrographs
   c. Phylogenetic trees
   d. X-ray crystallography
4. Which of these functions do soil microbes carry out?
   a. Degradation of harmful pesticides
   b. Nitrogen fixation
   c. Predation on other microorganisms
   d. All of the above
5. About how many bacteria are there in a gram of soil?
   a. 1000 b. 10,000 c. 1 million d. 10 billion
6. The diversity of soil microbes in part is due to:
   a. Soil texture
   b. Isolated micropores (myriad habitats)
   c. Water movement
   d. No one knows
7. The biggest challenge to understanding soil microbial diversity is:
   a. Developing tools to investigate microbial diversity
   b. Convincing people microbial diversity is important
   c. Figuring out what to study
   d. Locating soil types with high diversity
8. As of the late 1990s and into the 21st century, the tools commonly used to study soil microbes utilize soil:
   a. DNA b. energy c. clay content d. carbon content
9. Bacterial cell size is:
   a. The same as fungal cell
   b. The same size as a clay particle
   c. Bigger than a sand grain
   d. Smaller than anything else in soil
10. It is important to study soil microbial diversity, because it may:
    a. Help in drug development
    b. Provide insights about pesticide degradation
    c. Provide better agricultural methods
    d. All of the above
    e. None of the above

given approximately 5 minutes to complete this task and then students share their ideas with the class. There are a variety of mechanisms that students cite including physical separation by micropores, the small size of organisms in relation to their environment, and the ability of microorganisms to fill different ecological niches.

Activity 2: Microbial Organization and Phylogenetic Trees

The total community (all the candy pieces) is used in a second activity to expose students to phylogenetic trees and demonstrate the importance of this concept in understanding the relationships among microorganisms. The background handout includes a sample phylogenetic tree (Fig. 1), and this model soil community is discussed in class. The example microbes include functional groups that the students have been learning about, such as ammonia-oxidizing bacteria and antibiotic synthesizing bacteria. The instructor explains how microbial ecologists have used phylogenetic trees to organize microbes, and the students are asked, as a class, to list reasons why these trees are important. The instructor supplements this list with information not raised by the students, such as the use of phylogenetic trees to characterize as yet unclassified organisms (Hugenholtz et al., 1998).

After the class discussion, students work in groups (four or five students per group) to construct phylogenetic trees representing the candy community. Their trees will also be included in the lab report, along with questions related to phylogenetic trees used in microbial ecology. Examples of questions include: What were the characteristics that you used in organizing the microbial community? How would this tree be different if it had only been constructed based on the culturing method? In microbial ecology phylogenetic trees are the primary method used to organize microorganisms—what implications would this have for defining taxonomic groups?

Supplementary Concepts

During these activities, student's questions may lead to a number of supplementary concepts. For instance, questions concerning group organization allow the instructor to introduce the spe-

cies concept of prokaryotes (Cohan, 2001). Students are asked to compare the species concepts of eukaryotes and prokaryotes and to consider ways that microbial ecologists might determine group assignments.

Another result of candy selection has often been low representation of Tootsie Rolls drawn during the culturing and DNA based sampling. Perhaps the Tootsie Rolls' small size leads to these results even though they are a large fraction of the population. When this occurs, Tootsie Rolls become the candy equivalent to Acidobacteria (Quaiser et al., 2003). Students are told that the discovery of high acidobacterial populations within soils (often 30–50% of 16S sequences obtained) is one of the most striking discoveries made using DNA-based techniques.

Although the activities outlined here are designed for an introductory level class, they could be expanded in more advanced classes. For instance, a data set like this may be used in teaching upper-level undergraduates or beginning graduate students about diversity calculations (Martin, 2002). In this case, more material on the estimates of microbial community size and numbers of species could be provided.

Results and Discussion

These activities were performed during two terms of Introductory Soil Science (a 300-level course for science majors at Oregon State University). During Activity 1, students were asked to formulate a hypothesis describing how different sampling strategies (i.e., "culture-based" sampling vs. "DNA-based" sampling) might increase our understanding of the soil microbial community. Most of the student groups hypothesized that the microbial community composition would be more accurate using "DNA-based" sampling. Some of the groups also suggested that more rare species would be identified using "DNA-based" sampling.

During the second term, the effectiveness of the activity to meet learning outcomes was evaluated using a pre- and post-assessment. Just before receiving the microbial diversity handouts and 1 week before the in-class activity, students were asked to answer 10 multiple-choice questions related to microbial diversity (Table 2). After completing the pre-assessment they were given the background information and encouraged to read this material for the lab session the following week. After students completed the activity, they were given the same 10 questions in a post assessment. Eighty-three students took the two exams; the mean number of questions answered correctly on the pre-assessment was 4.9 out of 10, whereas the mean on the post assessment was 8.4 (Fig. 3). When asked about mechanisms that lead to high microbial diversity in soils, only 20% of students identified micropores on the pre-assessment, but 75% were able to identify this factor on the post-assessments. The majority of students were also able to identify two techniques that microbial ecologists use to study soil microbes.

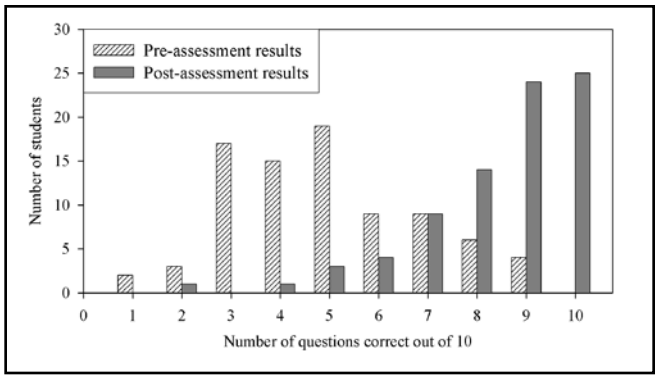

Fig. 3. Pre- and post-assessment results from Introductory Soil Science, a 300-level course taught winter term 2006. Scores improved from a mean of 4.9 correct answers on the pre-assessment to 8.4 correct on the post-assessment.

Two questions related to these activities were placed on the course final exam (Table 3). The students were again asked a question about the mechanisms that lead to high diversity and 73% of students answered this question correctly. Students were also asked to categorize microbes by completing a phylogenetic tree. Ninety-nine percent of students correctly categorized the given microbes.

This set of activities appears to be an effective means to introduce students to microbial diversity. Students appeared to have fun organizing the candy into groups, and several students commented that they spent extra time on their lab reports, making clean and detailed phylogenetic trees. Both assessment and final exam results showed that students learned about the mechanisms that lead to high microbial diversity in soil and about the construction of phylogenetic trees.

Overall these activities fit well in an introductory course where students have little prior knowledge of microbial diversity. Ausubel's theory of meaningful learning states that learners increase their knowledge base when they relate new information to prior knowledge (Wallace et al., 2003). Pairing the model candy-community to functional and phylogenetic groups of soil microbes provides a

Table 3. Two questions related to microbial diversity activities were included on the Introductory Soil Science final exam to determine if students retained the information they learned during these activities.

Question	No. correct responses	No. incorrect responses
The diversity of soil microbes in part is caused by:		
a. soil texture	61	22
b. isolated micropores (myriad habitats)		
c. water movement		
d. no one knows		
A soil microbiologist interested in a local soil community isolates and characterizes the following soil microbes. Using the diagram provided below, place the missing letters (A, F, H, I) in the appropriate places (lines provided)	82	1

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bridge from the knowledge students already possess to the concepts pertinent to understanding soil microbes. They are given a concrete example that helps them to grasp the abstract concept of microbial diversity (Byers and Fitzgerald, 2002). Educators in both science and agricultural fields have emphasized the importance of activities that utilize students' information-processing skills (Thirteen Ed Online, 2004) and point out that skill building in this area is not only important for future scientists, but also provides better understanding of the scientific process to the general public (Gilbert, 2000). The community sampling activity demonstrates how scientists' perceptions of the world can change and emphasizes the constructive nature of scientific knowledge.

Conclusions

The activities described here are a useful teaching tool that can be employed in introductory science classrooms at the undergraduate level with little preparation or monetary expense. The benefits of incorporating these activities include stimulating student thinking about an area of science that they may have not considered before and exposing them to a contemporary scientific endeavor. Assessments help demonstrate that students learned about soil microbial communities. Most importantly students are given an opportunity for increased interaction in the classroom and a chance to practice scientific process skills.

Dedication

Elizabeth Sulzman died on 10 June 2007. Elizabeth lived life fully. She was a radiant person who touched the lives of all whom she met. She was recognized as a master teacher by her peers locally, regionally, and nationally. She inspired students in her classes to think of soils as part of their everyday lives and, for some, as a possible career. She inspired the graduate students with whom she taught to make teaching a significant part of their professional lives. This article is dedicated to Elizabeth and the enthusiasm and spirit she brought to the soil science teaching profession.

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