# Effect of a Soil Microbial Activity Laboratory on Student Learning[1]

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## Abstract

Soil microbial activity contributes to soil and environmental quality. A detailed description of a laboratory exercise is provided that provides direct measures of soil microbial activity in 3 soil treatments: control (soil only), alfalfa (soil + alfalfa meal) and redwood (soil + redwood sawdust) is laid out and discussed. Students assess microbial activity in several ways: by comparing the odor generated, by observing the presence of fungal mycelia and by measuring $NO_3^-$ levels in the treated soil by two methods. Measuring $NO_3^-$ levels in two ways enables students to gain an understanding of how to compare benefits and deficits of different methods used to determine the same parameter. Measures of microbial activity are related to carbon and nitrogen cycles so that students may better understand how their experimental observations relate to the cycling of organic matter and nutrients. Understanding gained by students leads to a better appreciation of how organic matter and microorganisms affect the overall health of the ecosystem. Students earned a 60% average score on a pre-laboratory quiz. Students earned a 77.1% average score after completing the laboratory and writing a laboratory report. The gain in average student score was interpreted as significant as assessed by Cohen's D (1.27).

## Introduction

The purpose of this article is to clarify, within the context of Soil Science, how introductory college students develop their ability to collect and analyze scientific data in the completion of a scientific laboratory report assignment (Hattey and Patton 2009, Shukla and Sammis, 2012). Through this process, students produce a laboratory report in which they develop a hypothesis, test the hypothesis through data collection, then determine if the hypothesis is accepted or rejected.

Soil nutrient dynamics and organic residue decomposition are crucial processes contributing to soil and environmental quality. Organic matter (OM) decomposition and subsequent production of humus, release of nutrients to the soil environment, production of energy (heat), etc. provide the basis for many diverse ecological food-webs. The diversity of microbial communities as well as their abundance within soils results in soil being the most biologically diverse ecosystem on Earth (Yarwood and Sulzman, 2008). Moreover, nitrogen cycling plays a large role in soil fertility and environmental quality (Vitousek et al., 2009). Therefore, it is crucial that introductory-level undergraduate students be exposed to and begins to understand the role soil microbes play in organic matter and nitrogen cycling.

At California Polytechnic State University, San Luis Obispo (Cal Poly), Introductory Soil Science (SS 121) is a four unit (three hours of lecture and one three hour lab per week) course taught every Fall, Winter and Spring quarter and typically has between seven to nine laboratory sections per quarter. Each laboratory section has a maximum of 24 students from many majors within the College of Agriculture, Food and Environmental Sciences and a few majors outside the college (Table 1). Soil Science 121 is an introductory course designed for college freshmen and has no pre-requisites. The experiments performed in the laboratory are set-up to follow lecture topics. The experiments described in this article build on what has been done at Cal Poly for over a decade in the Soil Organic Matter, Humus and Microbial Activity laboratory experiment, which typically takes place toward the end of the quarter.

This article describes several simple procedures to assess microbial activity and nitrogen cycling in soil in a laboratory setting. The experiments are best suited to introductory-level environmental or soil science students. The experiment can be set-up by students or a technician in a short amount of time (15 min). After an incubation period (ideally 4 weeks when the laboratory temperature is ~ 25° C and the moisture content of the soil is maintained near field capacity), samples can be analyzed within a 3-hour laboratory period by the

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**Table 1. Typical characteristics of Cal Poly's laboratory portion of Introductory Soil Science course (SS 121).**

| Characteristic | Supporting Information |
|---|---|
| Typical class size (students) | 20-24 |
| College of Agriculture, Food, and Environmental Sciences (CAFES) majors taking course (represents 90% of all CAFES students) | Agricultural Business, Agricultural Science, Agricultural and Environmental Plant Sciences, Agricultural Science, Agricultural Systems Management, Bioresources and Agricultural Engineering, Environmental Earth Sciences, Environmental Management and Protection, Environmental Soil Science, Forestry and Natural Resources, and Wine and Viticulture |
| Majors outside the CAFES taking course (represents 10% of all students) | Biological Sciences, Chemistry, Civil Engineering, and Landscape Architecture |
| Year in school of students taking course | Freshman (50%), Sophomore (25%), Junior (15%), Senior (10%) |
| Total number of laboratory exercises students complete in course | Nine |

students. The day of analysis is when class discussion on organic matter and nitrogen cycling takes place.

## Learning Objectives

Upon completion of this exercise, students should be able to:

1. Construct relevant graphs that visually display the results of the experiments.
2. Understand the difference between qualitative data vs. quantitative data.
3. Define humus and organic matter and understand what makes them different.
4. Measure microbial transformations of organic matter and nitrogen in one soil by several methods.
5. Compare methods of determining microbial activity in soil.
6. Know the products of organic matter decomposition.
7. Understand how the carbon to nitrogen ratio (C/N) of organic materials applied to soil influences nitrogen availability.
8. Learn the carbon and nitrogen cycles.

## Materials and Methods

### Experimental Set-up (by technician)

This part of the experiment is performed by the instructor or a technician a few days prior to when the students begin the experimental set-up. We have found the ideal soil texture to use for these experiments is loamy sand with ~ 1 % OM. We have experimented with soils having more clay; however, experimental results were less consistent than when using a loamy sand textured soil. Air-dried soil was passed through a No. 10 sieve and all particles coarser than 2 mm were removed. The sieved soil was divided into 3 treatments: a control treatment (soil only), an alfalfa treatment and a redwood treatment. The alfalfa and redwood were added to the soil at a rate of 2.5 %m. Deionized water was added to all 3 treatments to bring the soil water content to field capacity (~ 20 %m).

### Experimental Set-up (15 minutes): Week 1

Students, in groups of 3 to 4, prepare 2 control, 2 alfalfa and 2 redwood soil samples for a 4 week incubation period by adding an amount of 25±3 g (experimentation has demonstrated that a relatively large range in starting sample masses produces similar data) of each treatment to appropriately labeled, 100 mL wide-mouth plastic containers with lids. Initial measurement should include the mass of the soil + plastic container and the temperature of the laboratory.

### Incubation Period (5 minutes/week): Weeks 2 – 4 Laboratories

The student groups are reminded to check the moisture status of their treatments at some point during each of the laboratory periods of weeks 2 to 4 and record the ambient temperature of the laboratory.

### Analysis and Discussion (2.5 hours): Week 5 Laboratory

*Organic matter cycling*

The laboratory period begins with a discussion of the carbon cycle using Equation [1] and a diagram of this cycle from Brady and Weil (2008).

organic substances + microorganisms + suitable environment ------>

$CO_2$ + $H_2O$ + energy + humus + new microbial cells + inorganic plant nutrients [1]

Through the lens of Equation [1], students and instructors reflect on the results of the experiment that has been going on for four weeks [1]. The discussion focuses on organic nutrient cycling, microorganisms, what constitutes a suitable environment for microorganisms and benefits of humus to ecosystem function, an interactive critical thinking exercise. The students are shown examples of alfalfa meal and redwood sawdust and are asked probing questions to ensure they are aware of the differences between them. A brainstorming discussion on microorganisms follows where the students are asked where they think the microorganisms originated from in the experiment. Next, a list of common soil microorganisms is generated. The students and instructor jointly develop a list of factors to define a suitable environment for microorganisms and decomposition. Finally, students are asked to define the benefits of having humus in the soil and the role of soil microbes in nutrient cycling in ecological systems.

*Teacher Instructions for Guiding Student Observation of Soil Samples*

The laboratory class session during the fifth week of the quarter is dedicated to data collection and analysis. Students are instructed to collect the samples they have been monitoring over the past four weeks. At this point

in the experiment it is particularly important to remind students not to open the lids of the plastic containers. Prior to opening the containers, the students are instructed to formalize a hypothesis based on expected physical and chemical characteristics of various treatments. It is also explained that one indicator of abundant microbial activity is the presence of an earthy-musty odor. Students are asked to develop a hypothesis about which of their treatments should have had the greatest microbial activity during the incubation period. This activity can then be used to facilitate a discussion considering the desirable "qualities" of various soil types. This initial class discussion should ensure that prior to proceeding; students understand that they should expect different results from the various experimental treatments.

After the initial discussion of the scientific method and hypothesis testing, students are told to open their samples and pay particular attention to how they smell and look. The students and instructor then discuss that odor is a qualitative variable and can be converted to a quantitative variable by creating an odor scoring system. Another topic for discussion is the cause of odor is primarily due to actinobacterial and cyanobacterial activity within the soil. The two volatile metabolites primarily responsible for producing this odor are geosmin and 2-methylisoborneol (Stahl and Parkin, 1994).

The students use a datasheet (Table 1) to rank (higher order thinking skills) their samples in terms of the earthy-musty odor. The instructor helps students establish a 10 point scale where 0 = no earthy-musty odor and 10 = very strong, earthy musty odor. In establishing the measurement criteria, students may also be introduced to the concept of scientific practice and the need to develop numerical criteria with which to evaluate differences among treatments in an experiment. The instructor may also points out that this quantitative approach is only truly valid for comparing treatments within an experiment that have undergone similar procedures. The instructor should also stress the importance of doing things exactly the same between the three treatments and procedurally within a treatment will greatly reduce errors in measurement.

The next task for students is to visually observe similarities and differences among treatments, before, after, or at the same time as they are assessing the odor differences among treatments. Ideally, each group of students will have access to a microscope to check for the presence of fungal mycelia. The presence of mycelium is an important indicator of microbial activity and is easy to observe with a microscope. A datasheet can be developed to convert the mycelia data into a quantitative variable by developing a scale for measurement. For example of a 10 point scale, 0 = no observable mycelia and 10 = mycelia are visible without the aid of the microscope.

The typical results for this experiment find the earthy-musty odor and presence of mycelia follow similar trends. The alfalfa treatment has the greatest earthy-musty odor and greatest quantity of fungal mycelia, followed by the control and then the redwood has the lowest amount of odor and mycelia. It is important to note that through oratory replication over many years, the differences in student opinion about the ranking of the treatments in terms of intensity of earthy-musty odor and amount of fungal mycelia is slight.

*Nitrate Extractions*

The duplicate treatments are separated and one set of samples undergoes a simple extraction with 1 M $NH_4C_2H_3O_2$ pH 4 and the other set of samples is brought to saturation with DI (deionized) $H_2O$. Both of these procedures are for determination of $NO_3^-$. The 1 M $NH_4C_2H_3O_2$ pH 4 extraction ($NO_3^-$ Red method) is similar to a procedure outlined by Singh (1988). The 1 M $NH_4C_2H_3O_2$ pH 4 is made by placing ~ 400 mL DI $H_2O$ in a 1 L volumetric flask and slowly adding 40 mL glacial $HC_2H_3O_2$ and 10 mL $NH_4OH$. The solution is then made up to 1 L by adding additional DI $H_2O$. The final solution pH is typically 4 and does not require pH adjustment. The extraction is performed by adding 40 mL of the 1 M $NH_4C_2H_3O_2$ pH 4 to each plastic container and stirring the samples with glass stir rods for 2 min. The samples are set aside to allow the suspended soil to settle out prior to filtering the liquid supernatant. The other set of samples is brought to saturation by adding ~ 8 g DI $H_2O$ ($NO_3^-$ Strip method). This extraction is similar to a procedure described by NRCS (2012). The samples are stirred to form a saturated paste and then a Whatman No. 1 filter paper, folded into a cone, is inserted into the saturated paste, cone tip pointed down and into the saturated paste until the tip of the filter paper contacts the bottom of the container. The sample is then set aside to allow time for water from the saturated soil paste to filter through the sides of the filter cone and accumulate.

*Nitrogen Cycle*

The nitrogen cycle is introduced in greater detail than before and discussed while the extracts are incubating. A diagram of the nitrogen cycle is discussed and related to the carbon cycle. The students are reminded that we will be determining the $NO_3^-$ levels in their samples by two different methods. To check for student understanding, they are asked what processes of the nitrogen cycle had to occur in order for us to be able to determine $NO_3^-$ levels. The fates and forms of nitrogen are also discussed at this time to provide, for example, an opportunity for students to understand why and how $NO_3^-$ leaching takes place and how this process can lead to environmental quality problems.

*C/N ratio*

The concept of C/N ratio is constructed and this concept is related to the C/N ratios of the treatments as well as the processes of mineralization and immobilization. It is explained that optimal microbial decomposition/mineralization of organic materials occurs when

the C/N ratio of these materials ≤ 20 and that when C/N ratios are higher than this the nitrogen ($NO_3^-$) is rendered unavailable to plants and is immobilized by microorganisms. The alfalfa meal has a C/N ~ 13 whereas the C/N of the redwood sawdust > 600 (Brady and Weil, 2008). Based on this discussion, the students are asked to generate a hypothesis about how their samples will rank relative to each other in terms of measured $NO_3^-$ levels.

*$NO_3^-$ Measurement – $NO_3^-$ Red Method*

The students are instructed to obtain glass observation vials (~ 15 mL capacity) as well as their $NO_3^-$ Red filtered extracts and measure 10 mL of solution from each treatment. A ¼ tablespoon of nitrate colorizing powder is added to the glass observation vials. The nitrate colorizing powder is prepared in advance by grinding each of the following components with a mortar and pestle, mixing them one-by-one and then grinding them together into a fine, homogeneous powder, 37 g citric acid, 5 g manganese sulfate monohydrate, 2 g sulfanilamide, 1 g N-1-naphthylethelenediamine dihydrochloride and 1 g finely powdered zinc (Singh, 1988). These chemicals may all be purchased through Fisher Scientific (Fairlawn, NJ). It is recommended to prepare this powder fresh on a weekly basis; however, we have been able to store this material in a freezer (0° C) for up to 1 year without it losing its efficacy. The 10 mL extracts are poured into the glass observation vials containing the nitrate colorizing powder. Upon contact of the liquid extract with the powder, the mixture will turn a reddish-pink color with intensity proportional to the quantity of nitrate in the extract. The tops of the glass vials should be covered with parafilm and inverted several times for mixing. Between 1 and 5 min after mixing the extract with the powder, the treatments should be compared to each other for measurement of $NO_3^-$. After a period of about 5 min, the intensity of the reddish-pink color dissipates.

The reddish-pink color may be interpreted qualitatively or it may be easily measured quantitatively. One way to obtain an accurate numeric measure for the quantity of $NO_3^-$ in the extract is to prepare several standard $NO_3^-$ solutions from 0 – 200 (or higher if necessary) ppm and develop the reddish-pink color in the same way as the experimental samples. A color photo of the reddish-pink color developed in the standards can be used to make a comparison with the experimental samples.

*$NO_3^-$ Measurement – $NO_3^-$ Strip Method*

The students are instructed to obtain their $NO_3^-$ Strip method samples. At this point, there should be clear solution that has filtered into the cone tip of the filter paper. Students should use an eye-dropper to transfer 2 drops of this solution onto the reaction zones of a nitrate test strip (we suggest EM Quant 10020 Nitrate Test Strips, Gallade Chemical, Santa Ana, CA as these strips have a dynamic range of 10 – 500 ppm) so as to thoroughly moisten the reaction zones of the test strip. Excess solution should be shaken off the reaction zones and the color of reaction zones should be compared to the color scale on the test strip container, after a period of 60 s, to obtain a value for $NO_3^-$. The EM Quant 10020 test strips measure both $NO_3^-$ and $NO_2^-$; however, we generally disregard the values for $NO_2^-$ as they are generally very low.

*$NO_3^-$ Measurement – Comparison of Methods and Data Analysis*

A table may be used by the students to organize, compare and contrast their $NO_3^-$ measurements. The students should be reminded that though they obtained numerical values for $NO_3^-$, their data are somewhat subjective based on their interpretation of the intensity of the reddish-pink color (in the case of the $NO_3^-$ Red method) and the interpretation of the color of the reaction zones of the test strips (in the case of the $NO_3^-$ Strip method). The data may also be graphed in order for the students to visually compare the $NO_3^-$ measurements by each method. A discussion about potential sources of experimental error can follow and may include such considerations as importance of standardized data collection procedures, perceived accuracy, time of analysis, cost, generation of waste, etc.

## Results and Discussion

The experiments described above build on what has been done at Cal Poly for over a decade in the Soil Organic Matter, Humus and Microbial Activity laboratory experiment, which typically takes place toward the middle to end of the 10 week term. We assessed the effectiveness of the experiments to meet the learning objectives (see Introduction) by conducting a pre- and post-laboratory 10 question multiple-choice quiz (Figure 1) aligned with the learning objectives (see Introduction section) in two laboratory sections (19 and 23 students, respectfully) with 42 students. Our goal was to gain a better understanding of how student involvement and participation in the exercise effected attainment of learning objectives related to organic matter and nutrient cycling in the soil environment.

One week prior to analysis and discussion of the experiments (week 4), students were given the complete laboratory procedures as well as background information related to the exercise and instructed to read these materials prior to coming to lab the following week. They were also given an informed consent form, indicating their agreement to participate in the identical pre and post laboratory assessment process.

Students had access, time and were encouraged to review the material and information covered on both the pre-exercise and post-exercise quiz. Students answered an average of 6.00 questions out of 10 correctly on the pre-exercise quiz, whereas the average on the post-exercise quiz was 7.71 (Table 2). Approximately 67% of the students were able to identify several ways microbial activity may be assessed on the pre-exercise quiz while

**Figure 1. Pre and Post Exercise Quiz Questions**

**1.** Soil organic matter includes:
a. Plant and animal residues at various stages of decomposition.
b. Cells and tissues of living soil organisms.
c. Substances synthesized by soil organisms.
d. All of the above

**2.** The products of organic matter decomposition include all of the following except:
a. Water b. Charcoal c. Nutrients d. Heat e. Humus

**3.** All of the following are examples of quantitative data except:
a. The amount of nitrate in units of mg/L.
b. The intensity of color represented on a scale from 1 to 10.
c. The intensity of smell represented as slight, medium, strong.
d. The amount of carbon dioxide in the atmosphere represented as a percentage.
e. The measurement of ocean wave height in feet.

**4.** The following factors are important when assessing soil microbial activity:
a. Soil temperature
b. Soil pH
c. Nutrient supply
d. Water content
e. All of the above

**5.** Cultivated soils lose humus by biological decomposition at a rate of:
a. Less than 1% per year.
b. 1-2% per year.
c. 2-5% per year.
d. 5-10% per year.
e. Up to 50% per year.

**6.** Microbial activity in soil may be determined by:
a. Observing the soil and looking for mycelia.
b. Assessing the degree of musty smell in soil.
c. Measuring the quantity of soil nitrate.
d. Determining the amount of soil humus.
e. All of the above.

**7.** Management such as ____ can be used to sustain humus levels and productivity.
a. Crop residue management
b. Conservation tillage
c. Crop rotations
d. None of the above
e. All of the above

**8.** Organic material having a high carbon to nitrogen ratio (C/N), such as barley straw (450/1)…
a. Will decay rapidly in the soil.
b. Will decay slowly in the soil.

**9.** Identify the process labeled #9 on the diagram† of the nitrogen cycle (diagram not shown here).
a. Immobilization b. Denitrification c. Nitrification d. Mineralization
e. Decomposition

**10.** Identify the process labeled #10 on the diagram† of the nitrogen cycle (diagram not shown here).
a. Immobilization b. Denitrification c. Nitrification d. Mineralization
e. Decomposition

†We used a simplified diagram of the nitrogen cycle similar to what is found in most introductory soil science text books.

91% of the students were able to do so on the post-exercise quiz. Moreover, the students had a much improved grasp of how the quality of organic matter sources, especially in regards to C/N ratios, affects decomposition after completing the exercise.

Most students (31 of 42; 78.6%) gained at least one point from pre quiz to post quiz. Half of the students (21 of 42; 50%) gained at least two points from pre quiz to post quiz. Table 3 delineates how students gained between the two points of measurement.

The assessment data suggest students had an improved understanding of organic matter decomposition as well as carbon and nitrogen cycling in soil after completing the experiments described above. The results supported our hypothesis of enhanced learning

**Table 2. Pre & Post Quiz Scores (N=42).**

| Ten Question Quiz | Pre Quiz Mean Scores *(S.D.)* | Post Quiz Mean Scores *(S.D.)* | Difference | Cohen's D |
|---|---|---|---|---|
| | 6.00 *(1.34)* | 7.71 (1.33) | +1.71 | 1.27 |

**Table 3. Gain by Student Frequency and Mode per Amount of Gain. (N = 42)**

| Pre to Post Gain | F | % | Range (Min. to Max.) |
|---|---|---|---|
| +5 | 2 | 4.7 | 3-9 |
| +4 | 3 | 7.1 | 4-10 |
| +3 | 8 | 19.0 | 4-9 |
| +2 | 8 | 19.0 | 5-10 |
| +1 | 12 | 28.6 | 3-9 |
| +0 | 8 | 19.0 | 6-9 |
| -1 | 0 | 0.0 | 0-0 |
| -2 | 1 | 2.4 | 6-4 |
| Overall | 42 | 100.0 | 3-10 |

of key concepts of soil microbial activity. We attribute this to the fact that students were provided with opportunities to study, think about and evaluate carbon and nitrogen flux in soil not just as discrete elements from the periodic table, but in terms of their cycling in the soil environment. Using cycles and systems-thinking has been shown to be an effective means by which students develop higher-order cognitive skills (Zoller, 2012) and is necessary for them to understand soil's pivotal role in cycling nutrients (especially nitrogen) and driving ecosystem processes (Brady and Weil, 2008).

We also attribute the improvement in scores to the fact that students were provided with "learn-by-doing" or kinesthetic modes of knowledge acquisition. There is much educational research suggesting this is a beneficial way for students to learn and supplements other learning styles (e.g. visual, aural, read/write; Breckler et al., 2009; Eudoxie, 2011; Murphy et al., 2004).

The third factor to improved student understanding is the requirement that the students generate and test two hypotheses before they begin the analysis. Within their teams, students had to come to a consensus about the hypotheses prior to assessing and analyzing their samples (e.g. hypothesis testing). The first hypothesis was related to ascertaining which treatment (control, alfalfa, or redwood) should have had the greatest amount of microbial activity over the incubation period. The second hypothesis considered the same treatments and their relative levels of $NO_3^-$ after the incubation period. Hypothesis development and testing are important components of the scientific method and allow/require students to critically evaluate their conceptions about a given set of experimental conditions (Burgh and Nichols, 2012; Vick et al., 2012). We noticed that this component of the exercise facilitated the students working together productively as well as take more ownership over their experiments.

## Conclusions

This laboratory exercise allowed students to assess both physical and chemical factors of different organic materials and soils amended with those materials. The instructor-led discussions that take place during these experiments focus on three important concepts. First, we discuss the role of microorganisms in the carbon and nitrogen cycles. Second, we discuss differences in "quality" among organic materials that are commonly added to soils and how this

affects microbial activity. This leads to a dialogue about the influence of C/N ratios of organic materials on mineralization and immobilization. Lastly, the importance of the interconnectedness of microorganisms and their environmental conditions in nutrient and organic matter cycling in the larger ecosystem is conveyed.

This laboratory exercise has been most meaningful for students when the students have had prior exposure to some of the concepts found in the carbon and nitrogen cycles. It is particularly helpful when the students understand nutrient forms and how these forms (cations, anions and neutral species) move through the soil. It is also helpful when the students consider the three treatments (control, alfalfa and redwood) along with their nutrient contents (specifically C/N ratios). This reinforces the need for a control as a baseline treatment, which allows for meaningful comparisons of the other two treatments.

Assessments demonstrated that students' understanding of nutrient and organic matter cycling in soils increased as a result of completing this exercise. Students answered an average of 6.00 questions out of 10 correctly on the pre-exercise quiz and 7.71 out of 10 questions on the post-exercise quiz covering the exercise's learning objectives. This was most likely a result of the experiments and instruction focusing on systems-thinking, conducting the experiments in a "learn-by-doing" environment, as well as providing students with opportunities to generate and test hypotheses, which helped them take ownership and interest in what they were doing. To further validate the enhanced learning attributed to the laboratory experience, it would be interesting to test a group of students that don't do the pre-test to compare the results.

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