

# An Inquiry-Based Laboratory Design for Microbial Ecology

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**Abstract:** There is a collective need to increase the use of inquiry-based instruction at the college level. This paper provides an example of how inquiry was successfully used in the laboratory component of an undergraduate course in microbial ecology. Students were offered a collection of field and laboratory methods to choose from, and they developed a research question that they tested through experimentation. Assessment was accomplished by evaluating authentic scientific meeting style presentations and a lab report in manuscript format. Students enjoyed the inquiry-based format, and the instructors found the experience to be valuable. An example such as this one hopefully will encourage more college faculty to use the inquiry method of instruction in their courses.

**Keywords:** inquiry, microbial ecology, laboratory, active learning, student research

## Introduction

College and university teachers are being encouraged to move away from the use of lecture and cookbook-style laboratories to active learning techniques including Problem-Based Learning, Cooperative Learning, and Inquiry-Based Instruction (Chickering and Gamson 1987; NRC 1996, 2000). Because science is at its core a process and not a list of facts (Schwab 1963), these forms of learning are in line with the cognitive processes that help students to develop as life-long learners (Norman and Schmidt 1992; Svinicki 1998).

Because of the impediments to adopting these strategies such as inadequate preparation of teachers (Supovitz et al. 2000; Colburn 2000; Roehring and Luft 2004), management issues (Colburn 2000; Roehring and Luft 2004), misunderstanding of how inquiry works (Colburn 2000), beliefs about teaching (Roehring and Luft 2004), and the need for change at the level of the classroom and administration (Drayton and Falk 2002), not enough college teachers are adopting Inquiry-Based Instruction (Colburn 2000; Straits and Wilke 2002; McComas 2005). Inquiry can be used successfully, however, as evidenced by its use at the elementary (Wittrock and Barrow 2000), middle school (Songer et al. 2002, 2003), high school (Kashmanian Oates 2002; Zion et al. 2004), and college levels (Mullen et al. 2003; DiPasquale et al. 2003; Sundberg et al. 2005). Inquiry is in use internationally (Abd-El-Khalick et al. 2004; Carber and Reis 2004) as well. Successes such as these should encourage more college teachers to use inquiry in their classrooms and laboratories.

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This paper offers an example of the successful use of inquiry in a laboratory setting. The objectives of this paper are to 1) provide an example of Inquiry-Based learning at the college level, 2) assess student and faculty impressions of the technique, and 3) encourage more college faculty to make use of inquiry in their teaching.

## Course Philosophy

The first offering of BIO 315, Microbial Ecology, at Central Connecticut State University in the fall of 2005 took the form of a course in two halves. The first half focused on the microbes of terrestrial soil environments and the second half focused on microbes in aquatic environments. The soils half was a sincere attempt to use active learning techniques in both the lecture and lab. The lecture portion made use of team-based learning (Michaelsen et al. 2004) and the laboratory portion, which is the basis of this paper, made use of Inquiry-Based Instruction.

We designed the laboratory to encourage students to see the topics through the process of science and to serve as an example of this teaching format for pre-service teachers who were taking the course (9 out of the 16 students who took the course were in the teacher preparation program). The goal was for all students to gain a new appreciation for the way that science is conducted and knowledge is acquired. Additionally, we wanted the pre-service teachers to see that a great deal of content can be learned in the inquiry format and to be less hesitant to use the technique in their own classrooms (Roehring and Luft 2004).

## Course Details

The 16 students who took the course had five weeks to work on their research project after spending the first week taking a walking tour of the forested park near campus that they used for their projects. We gave students a description of five common research techniques from which to choose in conducting their research (Table 1). During the five

weeks of their work, the students were required to come up with a question about soil microbes that interested them, write scientific hypotheses that could be tested using the available techniques, conduct the field and laboratory work, and complete the data analysis. The last week of the soils portion of the lab was used for groups to give a presentation of their work as if they were at a scientific conference.

TABLE 1. Research methodologies provided to students for use in their inquiry-based projects. All requisite materials were available in the lab room.

<p><b>Soil Macrofauna Methods</b></p> <ol style="list-style-type: none"><li>1. Collect soil samples.</li><li>2. Place soil on top of screen in bottom of funnel.</li><li>3. Add 1 cm of ethanol to the bottom of the collection vessel.</li><li>4. Position the funnel on top of the collection vessel with the neck inserted in the collecting vessel and turn on the lights above the funnel.</li><li>5. Allow apparatus to set for a week.</li><li>6. Spread collection onto a petri dish and identify the collection under a dissecting microscope.</li></ol>
<p><b>Bacterial and Fungal Morphospecies</b></p> <ol style="list-style-type: none"><li>1. Mix agar using directions on label. Make glucose agar, R2A agar, and Rose Bengal agar (500 mL is sufficient for 15 – 18 plates).</li><li>2. Autoclave agar and allow to cool a bit</li><li>3. Pour agar into sterile petri dishes and allow to cool.</li><li>4. Collect soil samples.</li><li>5. Weigh 5 g of soil into a 50 mL centrifuge tube and fill to the 50 mL mark with DI water.</li><li>6. Centrifuge at 1500 RPM for 15 minutes.</li><li>7. Remove 5 mL and add those 5 mL to another 50 mL centrifuge tube and fill that tube to the 50 mL mark with DI water.</li><li>8. Centrifuge at 3500 RPM for 10 minutes.</li><li>9. Create a dilution series by pipetting 1 mL from the second centrifugation into a beaker and adding 9 mL of DI water. Repeat this process two more times. This dilution creates concentrations of <math>10^{-1}</math>, <math>10^{-2}</math>, and <math>10^{-3}</math>.</li><li>10. Sterilize an inoculation loop using a flame and inoculate two petri dishes of each medium for each dilution level.</li><li>11. Place petri dishes in 35° C incubator and allow colonies to form.</li><li>12. Assess the communities for morphospecies.</li></ol>
<p><b>Nitrification Rates</b></p> <ol style="list-style-type: none"><li>1. Collect fresh soil sample.</li><li>2. Rebury half of the sample within a Ziploc bag.</li><li>3. Return for the buried sample in two weeks.</li><li>4. Mix 20 g soil with 100 mL 1M potassium sulfate.</li><li>5. Shake every minute or two for 30 minutes.</li><li>6. Filter the liquid through filter paper in a funnel into 100 mL volumetric flask.</li><li>7. Bring to volume with the 1M potassium sulfate.</li><li>8. Dilute to ¼ strength for nitrate analysis.</li><li>9. Create 50 and 100 µg/L <math>\text{NO}_3^-</math> N standards.</li><li>10. For each sample and standard, place 30 mL liquid in a flask and add one packet of the Hach 6 nitrate reagent and stir sample continuously for three minutes.</li><li>11. Allow sample to set for two minutes.</li><li>12. Decant 25 mL of sample into another flask and add one packet of the Hach 3 nitrite reagent. Shake to dissolve.</li><li>13. Allow sample to set for 10 minutes.</li><li>14. Zero Hach DR 2000 spectrophotometer with potassium sulfate at 507 nm.</li><li>15. Read absorbance of untreated, diluted extract in the cuvette.</li><li>16. Transfer the sample to the cuvette and read absorbance.</li><li>17. Subtract the absorbance of the untreated sample from the absorbance of the treated sample to get the corrected absorbance.</li><li>18. Create a regression of absorbance vs. <math>\text{NO}_3^-</math> N of standards.</li><li>19. Use this regression to determine the <math>\text{NO}_3^-</math> N concentrations of your samples.</li></ol>

TABLE 1  
(Continued)

<p><b>Soil Respiration Assay</b></p> <ol style="list-style-type: none"> <li>1. Collect soil samples.</li> <li>2. Place 10 g soil in the biometer flask and stopper.</li> <li>3. Add 20 mL 2M NaOH to the opposite side of the biometer flask and stopper.</li> <li>4. Incubate for 24 h.</li> <li>5. Unstopper and add 5 mL 1M BaCl<sub>2</sub> to the NaOH and allow precipitation of carbonate to stop.</li> <li>6. Decant NaOH solution into a beaker and add 5 mL thymolphthalein indicator to produce blue coloration.</li> <li>7. Titrate with 2N HCl to the thymolphthalein endpoint (clear).</li> <li>8. CO<sub>2</sub> evolution is equal to <math>(V - B) * NE</math>, where B is HCl needed for a control setup, V is the HCl needed for the soil setup, N = 2 (HCl normality), and E is the equivalent weight (22 for CO<sub>2</sub>). Correct the result to grams of CO<sub>2</sub> evolved per gram of soil per hour.</li> </ol>
<p><b>Mychorrhizae Assessment</b></p> <ol style="list-style-type: none"> <li>1. Collect root samples.</li> <li>2. Wash to remove soil particles.</li> <li>3. Trim and fit in the cassettes. Pack loosely.</li> <li>4. Preboil sufficient 10% KOH to cover cassettes, then soak cassettes in KOH for 10 – 20 minutes to clear the roots.</li> <li>5. Wash roots with DI water 5 times.</li> <li>6. Immerse cassettes in 2% HCl for 15 – 20 minutes.</li> <li>7. Preboil sufficient stain solution (trypan blue and acid fuschin should each be used) to cover the cassettes, then soak cassettes for 5 minutes.</li> <li>8. Rinse roots with DI water 5 times.</li> <li>9. Store roots in DI water at 4° C for one week.</li> <li>10. Assess degree of mycorrhizal infection under a microscope.</li> </ol>

Students worked in the same three groups that they used for the team-based learning (Michaelsen et al. 2004) that they were experiencing in the lecture portion of the course. As the students worked, the faculty were available for answering any questions that the students had and helped the students to organize materials and techniques. The faculty also provided instruction regarding the methods that the students chose to use if the students had not used a similar method in any previous course. The research projects that students chose included structural and functional comparisons of soil microbes at an increasing distance from a stream, between intact forest and areas that were logged, and beneath invasive species and beneath native species.

This design provided an authentic research experience for the students (McComas 2005), and helped them to appreciate the challenges of conducting the research that professional scientists perform to provide the knowledge that that is incorporated into science textbooks. Along with an authentic research experience, Inquiry-Based Instruction needs to include appropriate and authentic

assessment of student learning (NRC 1996; Straits and Wilke 2002; Colburn 2004; McComas 2005). We accomplished this assessment in two forms. First, student groups gave a scientific presentation describing their questions, hypotheses, methods, and results (Table 2). Second, students individually completed lab reports in the standard scientific format (Table 3). The faculty provided time during lab for students to ask any questions they had about how to present and write in a scientific format. Given that this was a 300 level course, most students had already been exposed to primary literature. Performing these assessment activities required students to act as scientists. (When scientists do research, they present the results of their research at scientific meetings and also submit their work for publication.) In addition, students gave each other peer evaluation grades after the presentations based on group contracts written at the beginning of the lab. A student's average peer evaluation grade (as a percentage) was multiplied by their group's presentation grade to determine the student's grade on the presentation.

**TABLE 2. Rubric used for grading the group scientific presentations of the students' research.**

Title Grade	15 Title is brief but descriptive of the study	10 Title is excessively long or does not indicate the subject of the study	0 Title is missing
Introduction Grade	15 Introduction brings the intelligent, lay-audience up to speed, indicates the importance of the topic studied, and ends with the objectives of the study	10 Introduction is lacking in 1 of the required components	5 Introduction is lacking in 2 or more of the required components
Methods Grade	15 Methods section provides a general background of how the study was done	10 Methods were minimal and left the audience wondering what was actually done	0 Methods were absent
Results and Discussion Grade	15 Results & Discussion gives figures and/or tables to lucidly display the data, oral description fairly interprets the data, provides caution regarding the limitations of the study, and provides direction for future research	10 Results & Discussion lack 1 or 2 of the required components	5 Results & Discussion lack 3 or more of the required components
Conclusion Grade	15 Conclusion wraps up the findings of the study and offers direction for the future	10 Conclusion is lacking in 1 of the required components	5 Conclusion is lacking in both of the required components
General Grade	15 No spelling errors, presentation is easy to follow, and PowerPoint slides are easy to see and understand	10 Presentation is lacking in 1 general requirement	5 Presentation is lacking in 2 or more general requirements
	Final Grade (including 10 free points)		Average Peer Evaluation (multiplied by group's grade to calculate student's Final Grade)

**TABLE 3. Rubric used for grading the individual scientific reports of the students' research.**

Title Grade	10 Title is brief but descriptive of the study	5 Title is excessively long or does not indicate the subject of the study	0 <input type="checkbox"/> Title is missing
Introduction Grade	10 Introduction brings the intelligent, lay-reader up to speed, cites background literature, indicates the importance of the topic studied, and ends with the objectives of the study	5 Introduction is lacking in 1 or 2 of the required components	0 Introduction is lacking in 3 or more of the required components
Methods Grade	10 Methods section details the materials used and the methods employed in enough detail to repeat the study	5 Materials <u>or</u> sufficient detail are lacking	0 Materials <u>and</u> sufficient detail are lacking
Results Grade	10 Results section gives figures and/or tables to lucidly display the data, factual representation of the results are given in text format, and interpretation of the data is NOT present.	5 Results lack 1 of the required components	0 Results lack 2 or more of the required components
Discussion Grade	10 Discussion interprets the data fairly, indicates the relevance of the current findings to other literature, provides caution regarding the limitations of the study, and provides direction for future research	5 Discussion is lacking in 1 or 2 of the required components	0 Discussion is lacking in 3 or more of the required components
Literature Citation Grade	10 Literature citations include at least 5 primary literature sources, are cited accurately, and are referenced in the paper.	5 Literature Cited is lacking in 1 of the required components	0 Literature Cited is lacking in 2 or more of the required components
General Grade	10 No spelling errors, all statements are easy to read and understand, and pages are numbered	5 Paper is lacking in 1 general requirement	0 Paper is lacking in 2 or more general requirements
	← Final Grade (including 30 free points)		

## Outcomes

The need for information regarding student and faculty perceptions of Inquiry-Based Instruction (Keys and Bryan 2001) encouraged us to conduct a voluntary survey of the students regarding their opinions about the lab. They were asked to comment on class activities that encouraged them to learn, class activities that made learning difficult, and

whether they would have preferred a cookbook style lab in place of the Inquiry-Based lab experience.

Fourteen students completed the voluntary survey. Students wrote that picking an experimental idea for lab, group responsibilities, and group work in general encouraged them to learn. One student wrote that the groups were too large and this made it difficult to learn. Eleven of the students stated that they preferred the Inquiry-Based style of lab and

three indicated that they would have preferred a cookbook style lab. Students wrote that the Inquiry-Based lab was a “great learning experience” and that “predetermined labs can be boring.” Students liked the Inquiry-Based lab because it “allows for independent learning” and noted that they “teach more than the cookbook labs.” One student reported that s/he “enjoyed this course more than any other biology course I’ve had so far (not kidding).” The one complaint that was raised was a need for more time to complete the research. Clearly students were excited about the work and wanted more time to conduct the project. Based upon these comments we are convinced that the students enjoyed the Inquiry-Based experience and appreciated the flexibility and education it provided them.

The experience to be rewarding and informative for the instructors. Students were engaged throughout the experience and were keenly interested in their results. These are the responses that faculty hope to get from their students. The design of the lab required a front-loading of the effort by the faculty. Each method had to be tested before the semester and all requisite materials needed to be acquired and made available by the start of the semester. Once the lab began, however, we were free to concentrate on the process that the students were

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following and encourage them to develop interesting hypotheses and research. This experience is in contrast to formulaic labs, where the faculty must prepare the lab each week and spend time simply making sure that the lab is working. In this inquiry format, students performed problem solving and the faculty could serve as guides (King 1993). Collectively, these experiences were rewarding for both students and faculty as the students took ownership of their projects and worked diligently toward their successful completion.

### Conclusions

This paper serves as an example of the use of inquiry in a college laboratory. It is hoped that the report of successful implementation of Inquiry-Based Instruction in this lab will encourage more college teachers to use Inquiry-Based Instruction since this method is engaging for the students, rewarding for the faculty, and in line with science teaching standards. Students found the experience to be rewarding, educational, and enjoyable. We were encouraged to continue using the technique because of the success in this first offering of the course.

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