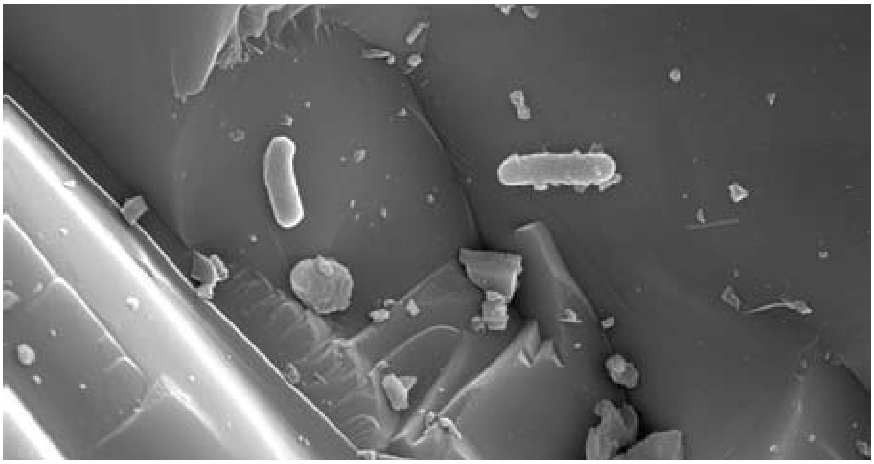
Teaching the Principles of Geomicrobiology and the Process of Experimental Research to Undergraduate and First-Year Graduate Students

By Rachel O'Brien and Jennifer A. Roberts

Geomicrobiology is an interdisciplinary course focused on experimental work. Our approach introduces students to scientific collaboration and peer review, produces significant gains in student knowledge, and promotes scientific curiosity and rigor.


Centers for Disease Control and Prevention

The bacterium Escherichia coli *on a quartz mineral surface.*

Geomicrobiology is an emergent subdiscipline of the geosciences, integrating concepts from microbiology, geology, chemistry, physics, mathematics, and engineering (Ehrlich 2002; Konhauser 2007). Microorganisms represent a living component of subsurface systems implicit within geologic and chemical processes. The subdiscipline covers a broad range of academic and practical research: from the origin and evolution of life to bioremediation schemes for toxic organic compounds and metals. By necessity, this interdisciplinary field combines the tools and field approach of the geologist and ecologist with the rigorous experimental approach of the chemist and biologist. Geomicrobiology affords students the opportunity to acquire basic and applied knowledge and skills, which facilitates their intellectual development and training as future scientists.

Geomicrobiology can be introduced at the undergraduate level using an Earth systems science approach (Ireton, Manduca, and Mogk 1996). Such an approach emphasizes the shallow subsurface as coupled subsystems (lithosphere, hydrosphere, atmosphere, and biosphere) where positive and negative feedbacks can be identified. A geomicrobiology course would be suitable for undergraduate majors in biology, chemistry, environmental science, environmental engineering, or geology. Our goal here is to present a framework for a geomicrobiology course design that has produced impressive gains in students' contextual knowledge (e.g., Felder and Brent 2004a). Our approach centers on the design and execution of a research experiment for students to (a) increase their understanding and application of the scientific method, (b) practice experimental analysis of a complex system, and (c) develop topical knowledge in geomicrobiology. This approach can be easily adapted to teach skills and topical material in other natural science and engineering programs.

Learning goals for our students

Thirteen students from two different institutions participated in the course. Each institution taught a concurrent section and had students communicate during the semester via e-mail and teleconference. Eight students from the University of Kansas (KU; a public research institution) enrolled, with 50% of students pursuing BS or MS degrees and 50% pursuing PhD degrees. Five undergraduates from Allegheny College (AC; a private liberal arts institution) participated. Both course sections had typical class sizes for an advanced topics seminar. Undergraduates in both courses were upper-level geology majors, and the graduate students were pursuing advanced degrees in geology or biology. All students came to the course with topical interest and intended to begin independent research within a year after taking the course.

Given nature of our students, we helped them practice basic scientific research skills and generate discipline-specific knowledge. We wanted students to develop a deeper approach to learning that would promote intellectual development (Felder and Brent 2004a). Our primary objective was to increase students' contextual knowledge of the physical, chemical, and microbiologic processes that interact in shallow, low-temperature groundwater systems. This involved the introduction of a range of topical material with the expectation that students would make the necessary connections to understand how subjects interrelated. The course also allowed students to practice conducting experimental work, from the design to the reporting phase. Given students' potential scientific career paths, we placed a particular teaching emphasis on how to design and propose professional research and how to interact with colleagues to accomplish this work. Lastly, we used a systems science approach to introduce topical principles and illustrate the strengths of investigating multiple phases (i.e., solid, liquid, gas) within a natural or experimental system.

Course design and assessment

The course was designed for problem-based learning, focused around a six-week research experiment. The overarching goal of the experiment was to examine mineral chemistry and its relationship to microbial mineral weathering reactions. Preparing, executing, and evaluating the experiment encompassed all the topical content and skills development we identified in our course goals. In addition, our pedagogical design was consistent with the maturity and capacity of our students, as well as with our respective teaching goals and skills. Research on problem-based learning has indicated that it facilitates skill development

TABLE 1

A summary of the course design, organized into three segments based on the course experiment. For each segment, we identify particular course tasks in the left column. Graded assignments to assess student learning and the grading practices are in the right column. The preparatory segment was about two weeks longer than planned in order to schedule a driller for field sampling; ideally this would have been one to two weeks shorter and the culminating segment correspondingly longer.

Preparation (initial six weeks of the semester)	
• Topical lectures: History of life on Earth, microbiology (cell structure, function, nutrition, metabolism, growth), redox, PH • Introduction to field site and previous research • Experimental-design lectures: Defining and testing a hypothesis; discussing the course experimental design • Tiered topical research and writing assignments: Students graded individually by their course instructor using common rubric • Initial proposal for experiment • Students graded individually by their course instructor using common rubric	• Tiered topical research and writing assignments (WAs) Students graded individually by their course instructor using common rubric. • Initial proposal for experiment Students graded individually by their course instructor using common rubric.
Experimental work (seven weeks)	
• Topical lectures: Chemical weathering, mineral solubility • Experimental-design lectures: Experimental error, sample replication • Field sampling: Groundwater, microbes, sediments • Preparation of experimental microcosms • Laboratory sampling and analytical techniques • Weekly data collection, reduction, and analysis • Initial data discussions (pH, alkalinity, CO^{2}) • Tiered topical research and writing assignments • Students graded individually by their course instructor using a common rubric	• Tiered topical research and writing assignments (WAs) Students graded individually by their course instructor using common rubric.
Culmination (four weeks—includes exam period)	
• Topical lectures: Microbially driven mineral weathering, layered microbial communities, characterizing populations and succession, carbonate chemistry of liquids and gases, Henry's law • Experimental design lectures: Elements of a research proposal • Final data discussions: microbial enumeration, silica, Fe(II), CH^{4}) • Group research presentations and discussion of results • Students graded as research groups by both instructors using a common rubric • Final project: Proposal for future experiment and predictions of microcosms after 6–12 months • Students graded individually by their course instructor using a common rubric	• Group research presentations and discussion of results Students graded individually by their course instructor using common rubric. • Final project: Proposal for future experiment and predictions of microcosms after 6-12 months Students graded individually by their course instructor using common rubric.

TABLE 2

Details of the experimental system used in the course. Each student constructed a set of microcosms for a particular Ni or Ni/Cu treatment. Each treatment was constructed and sampled in triplicate. The AC group had a total of 24 experimental bottles to sample during the aerobic experiment; the (larger) KU group had a total of 32 experimental bottles for the anaerobic experiment. The KU group had two additional glass treatments (Ni/Cu) and two additional analytes for the anaerobic system. Shaded region denotes work that we did not conduct on the solid phase but that would be useful to include in experimental work. The particular phases to characterize and the analyses to make could be tailored to each instructor's course-specific goals and analytical capability.

System component	Initial composition	Measurements	Sampling frequency	Analytical techniques and equipment used	Concepts involved to interpret data
Gas (closed system)	Ambient air (aerobic) N_2 (anaerobic)	CO_2 CH_4 (anaerobic)	Weeks 1, 3, 5, 6	Gas chromatograph	Henry's law Microbial respiration Methanogenesis
Liquid	40 mL of groundwater from field site diluted 1:1 with deionized water	pH, total alkalinity	Beginning of experiment Weeks 2, 4, 6	pH meter Microburet titrations	Sources/sinks of H^+ Carbonate speciation
		Silica Ferrous iron (anaerobic)	Beginning of experiment Weeks 4, 5, 6	Colorimetric assays Spectrophotometer	Silicate/glass dissolution reactions and kinetics Iron reduction
Solid	1 mL of native consortia extracted from 30 g of sediment core and sterile/autoclaved control	Bacterial enumeration	Beginning and end of the experiment	Cell staining (DAPI) Optical microscope (100x magnification) with ultraviolet light	Microbial growth, toxicity, and cannibalism
	0.25 g of synthetic glass; Treatments: 0, 0.01, 0.05, 0.1, 1.0 and 2.0 mol % Ni (AC and KU); Ni/Cu: 0.01/0.01 and 0.1/0.01 (anaerobic)	Surface characterization	Beginning and end of the experiment	Scanning electron microscope	Surface reactions between bacteria and mineral surface

and deep conceptual understanding (see reviews in Malicky, Lord, and Huang 2007; Prince and Felder 2006). Such a design also included elements of quality undergraduate education as defined by Romer and others (1995): active learning, a collaborative investigation between faculty and students, multiple opportunities for assessment and feedback, expectations for student work outside of the classroom, and the opportunity for out-of-class contact with the faculty. Previous attempts (n = 3) by one of us to teach this topic as a subject-based lecture course yielded poor improvements in student skill and knowledge.

Collectively, we examined how increases in the nickel and copper content of silica glass (used as a proxy for silicate minerals) affected the extent of microbial-mediated chemical weathering. The semester was informally organized into three segments based on preparing, conducting, and interpreting the experimental work (Table 1). Experiments were conducted in 100 cm^3 microcosms filled with synthetic glass and groundwater and inoculated with native consortia of microorganisms collected from an aquifer. The initial gas phase was ambient air (aerobic system) or N_2 (anaerobic). This three-phase system helped students relate key concepts and use a suite of analytical skills. The laboratory resources needed to accomplish this task consist of affordable analytical equipment that should be found in most college science programs (Table 2).

Laboratory microcosms were designed to mimic field conditions by using materials from a petroleum-contaminated aquifer near Bemidji, Minnesota. A large oil spill in 1979 at this location led to the development of a contaminated shallow groundwater plume; the site was chosen based on extensive subsurface characterization and research (e.g., Bennett et al. 1993; Cozzarelli, Eganhouse, and Baedecker 1990; Baedecker et al. 1989; Hult 1984) and its relevance for basic and applied science (e.g., Hiebert and Bennett 1992; Bennett, Hiebert, and Rogers 2000; Bekins, Godsy, and Warren 1999; Amos et al. 2005).

Since the spill, distinct geochemical and microbiologic zonation has developed in the subsurface (Baedecker et al. 1993). We designed an experiment for this field site and applied it to different biogeochemical environments so that each course section had a unique, but related, experiment. Keeping the field site and experimental design similar between experiments provided a consistent framework for all students; differing geochemical and microbiologic conditions for each experiment allowed us to investigate both aerobic and anaerobic systems. Based on class size and student skills, we assigned the simpler aerobic system to the AC students and the more complex anaerobic system to KU students. Fieldwork at the Bemidji site allowed students to collect the materials necessary to construct the experiment and conceptually connect their experimental work to the field site, and it provided exposure to field sampling of sediment and preservation techniques to collect representative sediment, microbiological, and groundwater samples.

A tiered framework of course instruction and assignments provided multiple opportunities for students to practice using the information and skills we wanted them to develop (Felder and Brent 2004b). Common grading rubrics were developed and used by the instructors throughout the course. Repeated exposure throughout the course also allowed us to progressively increase the complexity of topics, assignments, and our expectations for student analysis. Traditional lectures, data acquisition and analysis, and class discussions were used to introduce and reinforce basic topical knowledge. We used various forms of proposal writing to take students' understanding and application of the scientific method to a level necessary for conducting interdisciplinary science.

Preparation (six weeks)

To prepare for the experiment, we presented topical material, introduced students to the field site and pertinent peer-reviewed literature, and developed the context for our research questions. Initial assignments involved student research and writing to independently augment a portion of the individual topical lectures. These short assignments required students to design a simple field- or lab-sampling event so we could gauge their comprehension of the factors involved in sampling and describing a complex system. For example, we asked students to design a sampling protocol for pH in a microbially active hot spring at Yellowstone National Park and requested details as to where, when, and how many times the measurement would be taken. We also asked them to address specific equipment they might need (bottles, pumps, etc.) and whether measurements would be done in the field or lab. These exercises helped students focus on the theoretical and practical aspects of hypothesis testing while giving us the opportunity to provide rapid feedback on their work.

Our preparations culminated in an assignment requiring students to propose a design for the class experiment. The research question was provided to them (How do increases in silicate-Ni concentration affect microbially mediated silicate weathering by a native, [an]aerobic microbial consortia?). The assignment prompted them to provide a guiding hypothesis, a list of variables to be tested, controls, replicates, materials used (cell type and abundance, solution composition, solid phase composition, reactor design), frequency and types of measurements made, and criteria to judge whether the results addressed the research question. Justification and support from scientific literature were expected. The assignment required ingenuity and critical thinking to execute the scientific method properly and allowed us to assess what students had learned of experimental design and the system we were going to study.

A common grading rubric was provided to each student two weeks before the assignment was due. Most students showed a basic understanding of the components to be tested. In many cases, proposed experiments did not properly address the research question, were unreasonable in their scope and budget, or lacked analysis of one or more components of the system. We provided students with written feedback on their proposals and then summarized the range of student ideas and designs in class. We then presented our experimental design to the class and discussed the choices we had made for specific decisions and techniques. The discussions set the stage for students investing in the experiment as we designed and gave them an understanding of the decision-making process involved. By examining the range of proposed work by their peers, students also realized that multiple approaches can successfully test the same hypothesis.

Experimental work (seven weeks)

KU students traveled to the field site to collect native microorganisms and groundwater for construction of the microcosms. They learned proper groundwater sampling protocol, field geochemistry, and aseptic sediment core collection techniques. These students processed groundwater and sediment samples for both KU and AC experiments; the samples were transported back to both labs and all students constructed the experimental microcosms and prepared sterile controls.

Each student was responsible for a specific experimental treatment and subsequent data interpretation. All students were involved in the six weeks of data collection; each student chose a measured variable and was responsible for creating and maintaining a spreadsheet for the particular analyte. A single lab notebook and spreadsheet were maintained throughout the experiment and served as a common repository for raw data. Data-collection techniques were presented in class, but students did weekly or bimonthly analyses

TABLE 3

Grading rubric for final, independent take-home project. Topics were assessed on a 5-point scale, in which 5 represented mastery and deep understanding of topics and 1 represented weak comprehension or mimicry of previous assignments.

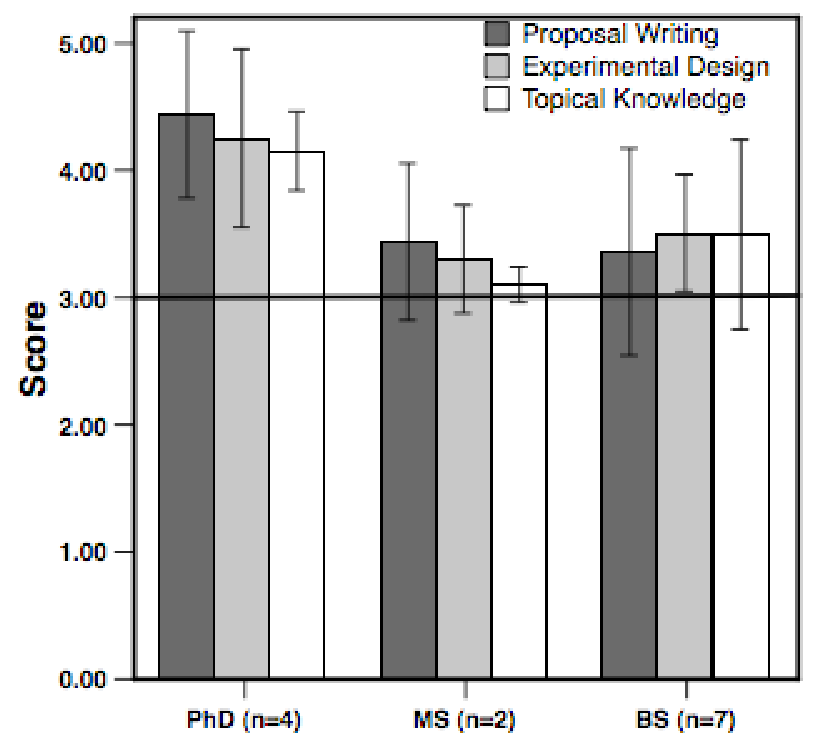
Possible score	Assessment of comprehension and performance	Topics graded
1	No comprehension of topic or did not perform task	Proposal for future work (proposal writing and experimental design)
		Research question
		Hypothesis
		Predictions
		Variables to be tested
		Control(s)
2	Identifies topic but shows no understanding, performs task but in perfunctory manner (copies original proposal for experimental design)	Replicates
		Cell type and abundance
		Solution composition
		Silicate composition
		Reactor design
		Measured variables (units, expected concentrations, all phases, analytical equipment)
3	Shows adequate but not deep understanding of topic, performs task but shows no original thought	Sampling procedure
		Frequency of measurement (will there be a lag phase?)
		Length of experiment
		Figures/tables (experimental design, results, others?)
		Support from data and literature
		What would the devil's advocate say?
4	Shows deep understanding of topic but hazy on some details, performs task well with some original thought	Prediction of long-term biogeochemical changes (topical knowledge)
		Biomass abundance
		Population succession
		Geochemical indicators
		Redox
		pH
		Glass weathering
		Secondary mineral formation
5	Shows mastery of topic, performs task very well with original thought and support for ideas	Support from data and literature
		General components
		Problem statement
		Graphs and tables
		Clarity/style of written description of design
		Format of proposal (title, page numbers, organization, references, and length)

outside of class (Table 2). Students gained technical experience in wet chemistry (pipetting, pH measurement, basic instrumentation, and calibration curves), microbiology (sterile technique, cell enumeration, statistical relevancy of measurements), data reduction, and data analysis.

The most intellectually stimulating aspect of the experimental phase involved dedicated class meetings—known as data discussions—when the group critically examined a particular data set. Students prepared pertinent figures and tables for each discussion. As a group, we analyzed the data (and its presentation) and looked for trends, outliers, or indications of analytical error. As the data set became more complex, we began discussing possible mechanisms and processes to describe observed patterns. We strove to have students explicitly refer back to course lecture notes and readings during these discussions to reinforce the application of theory to the interpretation of experimental results. Student initiative and participation were not formally graded; our experience indicated that students were both eager and prepared to discuss the experimental data in class and, as analysts, were invested in its quality and significance.

FIGURE 1

Mean student performance on final project as a function of degree program. Three evaluative criteria were scored and summed for the final project score. Bars indicate mean scores and whiskers indicate the standard deviation about the mean. Horizontal line (score of 3) indicates adequate understanding of topic (see Table 3 for more description).



Culmination (four weeks)

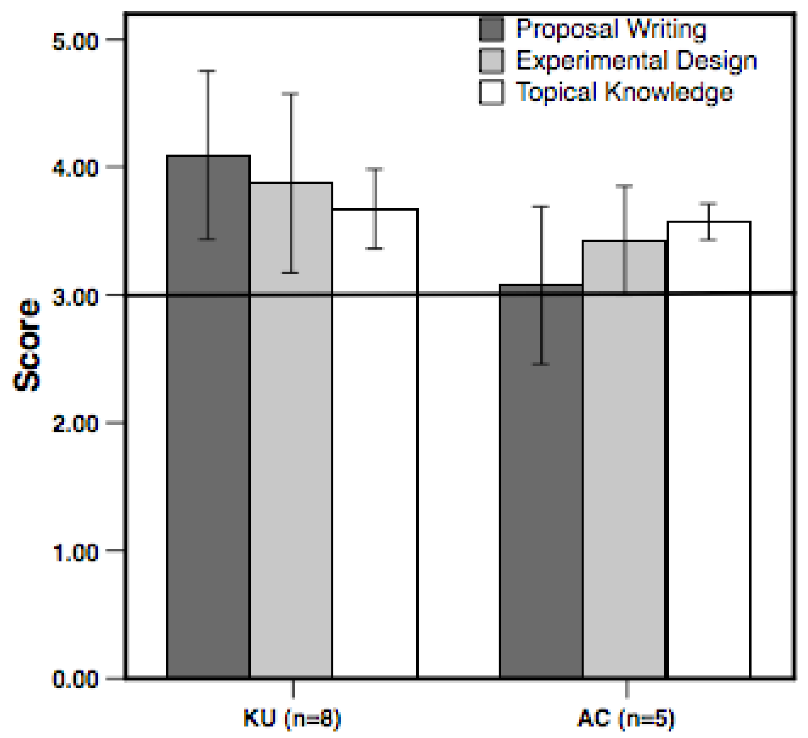
Following the experiment, students at each institution prepared a group presentation to communicate their respective results and interpretations. (A grading rubric was provided to each group in advance.) Each group presentation was delivered during a teleconference session. To introduce the culture of scientific presentation and peer review, we simulated the conditions of a professional meeting (e.g., timed 15-minute formal presentations followed by a question-and-discussion period). We chose not to time the question-and-discussion periods so as to maximize group interactions and exploration of the material. While only a few students gave the oral presentations, each research team was held responsible for preparing the presentation and posing salient questions to the other group. Both instructors graded both presentations independently and agreed upon a single group grade for each research team.

The culminating assessment tool for the course was an independent take-home project. Students submitted a 10- to 15-page professional report in response to two distinct tasks. The first task required students to write a proposal for future experiments based on results from the class experiment. This assessed their performance in writing scientific proposals that exemplify critical thinking and evaluation of scientific questions and hypotheses. Additionally, we used the first task to assess whether students understood the fundamentals of experimental design in a systems-based experiment. The second task asked students to make predictions about the class experiment if it were to continue running for 6 to 12 months. This allowed us to assess whether students had achieved a deep understanding of topical material. The grading rubric for the final project (shown in Table 3) was provided to students several weeks before the deadline.

Students at all levels (BS, MS, and PhD) and at both institutions scored, on average, above 3 (on a 5-point scale) in all three knowledge areas (Figure 1). As we expected, doctoral students performed better than MS and BS students in all areas. MS and BS students showed little difference in performance; we attribute this to the fact that the MS students were in their first semester as graduate students and came directly from their BS degrees without additional experience. The data illustrate a different trend when analyzed by institution (Figure 2). All KU students performed highest in proposal writing, followed by experimental design, and finally topical knowledge; this trend was reversed for all AC students. It is unclear whether personal teaching bias or specialty drove this trend, or if instructors responded

FIGURE 2

Mean student performance on final project as a function of institution. Three evaluative criteria were scored and summed for the final project score. Bars indicate mean scores and whiskers indicate standard deviation about the mean. Horizontal line (score of 3) indicates adequate understanding of topic (see Table 3 for more description).



to the needs of each student population. Another possible explanation is that graduate students, who have more practice writing proposals, are stronger in this particular skill relative to undergraduate students. Nonetheless, our final assessment data speak to an overall student mastery of the three major learning goals for the course.

Student assessment and anecdotal evidence

Student evaluations of their learning support our formal assessment results. Table 4 shows data collected from students rating their personal improvement in specific research skills pertaining to experimental design and topical knowledge by the end of the course. All students self-reported improvement in every evaluation category. Graduate students reported less significant improvement in individual topics, while undergraduates reported more significant improvement. Most students had little knowledge of geomicrobiology a priori and felt that the hands-on approach was valuable in gaining significant knowledge in the field. Two of the thirteen students commented that it was one of the best classes they had ever taken. Both KU and AC undergraduate students felt the pace of the course was too fast, and a majority of all KU and AC students felt that the workload was too heavy for at least part of the semester. Students also had concerns about the amount of group work and felt that groups within the class should be small (no more than four students per group). Additional in-class time for data collection and discussion, coupled with assigning grades for activities that are more time intensive, might alleviate stress over workload and pace in future classes.

We continue to interact with many students after the completion the course and have observed their progress in research endeavors. In individual cases, proposal writing has vastly improved. Graduate students at KU must submit short research proposals to the faculty for review before embarking on data collection. All AC juniors write a graded proposal for mandatory independent research they conduct as seniors. Three students from our course prepared exemplary proposals unaided by their faculty mentors. We believe these performances were partially attributable to skills developed in the course.

Another tangible product from the course has been continued research collaboration between the KU instructor and students in their independent research projects. Three students have continued to collaborate with the instructor on their research. Of note is one MS student's addition of experimental laboratory work to his project to explain field trends, and he has implemented this new component of research into his MS thesis under the supervision of another faculty member (Schillig et al. 2007). One undergraduate applied techniques she learned in the course to her senior thesis research. Both students took ownership of their research and implemented their knowledge into ongoing research. Furthermore, it is impressive that the growth in scientific maturity gained in the course prompted students to interact with faculty mentors as collaborators far earlier than most undergraduate and graduate students. These observations of student performance and behavior suggest meaningful advances in students' intellectual development.

Summary

Our course design is one example of how to bring an authentic, collaborative research problem into the undergraduate or early graduate curriculum. The teacher/scholar model can be applied successfully in the classroom, given careful course design and a sustained teaching effort that sets high expectations while supporting students as they work. Classroom research exposes

TABLE 4

Student assessment of personal improvement acquired in the course in three areas: basic scientific skills, the design of experiments, and topical knowledge. Values indicate percentage of student response. Graduate students (G), *n* = 5; undergraduate students (U), *n* = 5. The student survey included a "No improvement" category for each question, but no responses of this type were made. Shaded area delineates aspects of understanding and application of the scientific method from experimental analysis (above) and topical knowledge (below).

Topic		Some improvement	Significant improvement
Collecting experimental data and documenting laboratory work	G	80%	20%
	U		100%
Data reduction/analysis	G	60%	40%
	U	20%	80%
Experience with analytical methods and instrumentation	G	20%	80%
	U	20%	80%
Interpreting data/information	G	60%	40%
	U	20%	80%
Designing and conducting a professional oral presentation	G	100%	
	U	20%	80%
Confidence working with real data sets	G	60%	40%
	U	40%	60%
Understanding details of experimental work reported in the literature	G	40%	60%
	U	40%	60%
Identifying underlying assumptions within an experiment	G	80%	20%
	U	20%	80%
Creating a research question, hypothesis, and prediction	G	100%	
	U	60%	40%
Considering all the details involved in designing an experiment	G	40%	60%
	U	40%	60%
Evolution of life and role of eukaryotes in geologic processes	G	40%	60%
	U		100%
Microbial physiology and metabolism	G	40%	60%
	U	40%	60%
Geochemistry of silicate mineral weathering	G	40%	60%
	U	20%	80%
Redox chemistry	G	60%	40%
	U	60%	40%
Systems science: Relationships among solid, liquid, and gas phases	G	40%	60%
	U	20%	80%

all students (versus a select few) to the process of science and prepares them for future independent work. Students, especially in a liberal arts setting, can also begin to appreciate their teachers as scholars.

All of the skills and knowledge we wanted our students to learn were presented, practiced, and reiterated throughout the semester in a variety of ways (writing, reading and discussing papers, evaluating data, collecting data, and interpreting data). This helped facilitate deep learning and introduced students to the intellectual stamina required for research.

There are two central elements to our design: the use of a laboratory (or field) research project as the locus for learning and the choice of particular experimental work to develop a set of skills and topical knowledge. Formal and informal assessment data suggest that this approach was successful for teaching an advanced, interdisciplinary course. As such, our course design can be applied to a broad range of topics across the natural sciences. ■

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Rachel O'Brien (robrien@allegheny.edu) *is an associate professor in the Department of Geology at Allegheny College in Meadville, Pennsylvania.* ***Jennifer A. Roberts*** *is an associate professor in the Department of Geology at the University of Kansas in Lawrence, Kansas.*