RESEARCH ON LEARNING

Development of a Water-Quality Lab that Enhances Learning & Connects Students to the Land

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Abstract

A 3-week laboratory module was developed for an undergraduate microbiology course that would connect student learning to a real-life challenge, specifically a local water-quality project. The laboratory series included multiple field trips, sampling of soil and water, and subsequent analysis for bacteria and nitrate. Laboratory results confirmed the usefulness of comparing real environmental samples, and student survey and performance data supported the original hypothesis of this study in terms of student learning objectives.

Key Words: *Water quality; microbiology; agriculture; nitrate;* Escherichia coli.

Recent calls for change in undergraduate biology education have encouraged the development of curricula that focus on connecting science learning to real-world challenges (AAAS, 2009). The goal of such curricula would be not only to enhance science educational opportunities, but also to promote civic engagement. Assessment of such courses indicated that students made significant learning gains in multiple areas, including greater confidence and interest in science (Weston et al., 2006).

One of the major challenges facing humans worldwide is that of water quality. Recent assessments of rivers and streams in the United States indicated that 44% of them were impaired, the leading causes being pathogens, habitat alterations, and organic enrichment/oxygen depletion (U.S. Environmental Protection Agency [EPA], 2009). In Iowa, a major agricultural state, over 75% of assessed streams and rivers were impaired, including waterways in all 99 counties. Although one of the top sources of water impairments is agricultural activities, relatively few members of the nation's population have backgrounds that would provide them with substantial knowledge of agricultural practices (fewer than 1% claim farming as an occupation). The lack of familiarity with agricultural practices that affect water quality and the unique problems faced by agricultural producers poses challenges for developing an informed citizenry that will need to work cooperatively to address water-quality issues.

One of the major challenges facing humans worldwide is that of water quality.

The goal of this study was to develop a microbiology laboratory curriculum module that would connect student learning to a real-life challenge, specifically a local water-quality project. Inherent in this goal was to have students experience an impaired watershed in multiple ways – by physically touring and observing agricultural practices in the watershed, sampling and testing water and soil, and interacting with landowners. The watershed would also serve as the venue by which students could learn about the major impacts of microbes in water quality and natural environments, including nutrient cycling. The intent was to create a laboratory series capable of serving a large number of students, expanding on the positive outcomes of involving undergraduates in water-quality-related research projects or dedicated courses (James et al., 2006; Koosmann et al., 2011). Significantly, although the lab module was specifically developed for a college microbiology course, it could also be adapted for use in general biology or environmental science courses at the college or high school level.

The primary focus of the laboratory series was the Dry Run Creek Watershed, a 20,172-acre sub-watershed of the Upper Iowa River that drains into Decorah, Iowa (see Figure 1). Dry Run Creek has been designated as impaired by the State of Iowa because of high bacteria levels, and a collaborative partnership that includes Luther College, Iowa State Extension, the Iowa DNR, and local landowners was recently formed in order to improve water quality. Undergraduate student research projects were developed to monitor water at 13 sites in the watershed. The educational benefits that emerged from this work provided the motivation to expand it to a larger number of students. Several of the established sampling sites were thus chosen for the laboratory series, on the basis of their contrasting adjacent land use. Stream site S1, located downstream of woodlands and a natural spring, and site S2, located downstream of a large, active agricultural region, were used for examining turbidity and bacterial levels. Stream sites S3 and S4 were used for nitrate analysis. S3 is relatively unique in the watershed because it primarily drains a large farm in the conservation reserve

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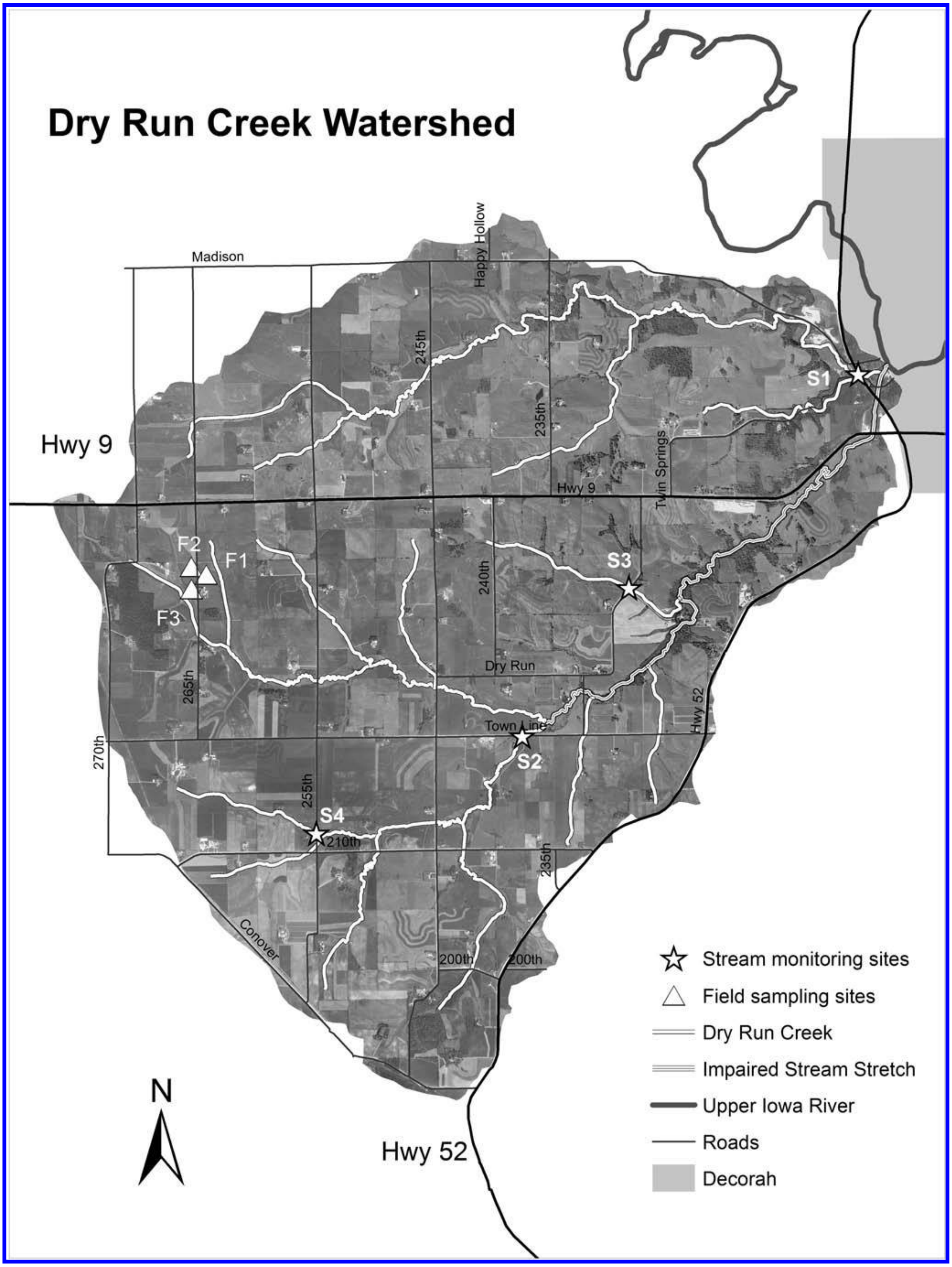

Figure 1. Dry Run Creek Watershed. Stars indicate stream monitoring sites (S1 and S2 for *E. coli* water samples, and S3 and S4 for nitrate water samples). Triangles (F1, F2, F3) indicate field soil sampling sites for soil nitrate analysis. (Figure generated by Chad Ingels, Iowa State Extension.)

program (CRP). This land is planted in long-term, resource-conserving cover crops (e.g., perennial grasses) instead of annual agricultural commodity crops; CRP ground is not tilled, nor will it receive fertilizer applications for 10–15 years. By contrast, stream site S4 drains active agricultural ground that is regularly fertilized and is located close to the origin of one of the streams high in the watershed, thereby minimizing dilution effects. Finally, field sites F1, F2, and F3 were chosen to obtain soil samples for nitrate analysis. F1 was planted in conventional corn, F2 in organic corn, and F3 in organic alfalfa.

Microbiology (Bio243) is a midlevel, elective course in the biology curriculum that enrolls 90–100 students, consisting of biology (~70%) and nursing majors (~30%). Bio243 meets for three 60-minute lecture sessions and two 90-minute laboratory sessions per week. I hypothesized that this lab module would enhance student learning of microbiological, agricultural, and water-quality concepts. Specifically, after completion of the laboratory series, I hypothesized that students would

- Have a basic understanding of watersheds and global and local water-quality issues,
- Understand the role of microbes in water quality,
- Be able to identify how land use and agricultural practices have affected water quality,
- Be familiar with challenges faced by agricultural producers, and
- Be able to sample and analyze water and soil from the field.

○ Overview of the Laboratory Module

A 3-week, water-quality-focused laboratory series was developed for Bio243. The lab had four components, including an introduction and tour of the Dry Run Creek watershed, water monitoring from different sites for physical/chemical parameters and *Escherichia coli*, a field trip to the local wastewater treatment plant, and a return trip to the watershed for soil and water sampling and subsequent nitrate analysis. A more detailed outline of the schedule and topics is shown in Figure 2.

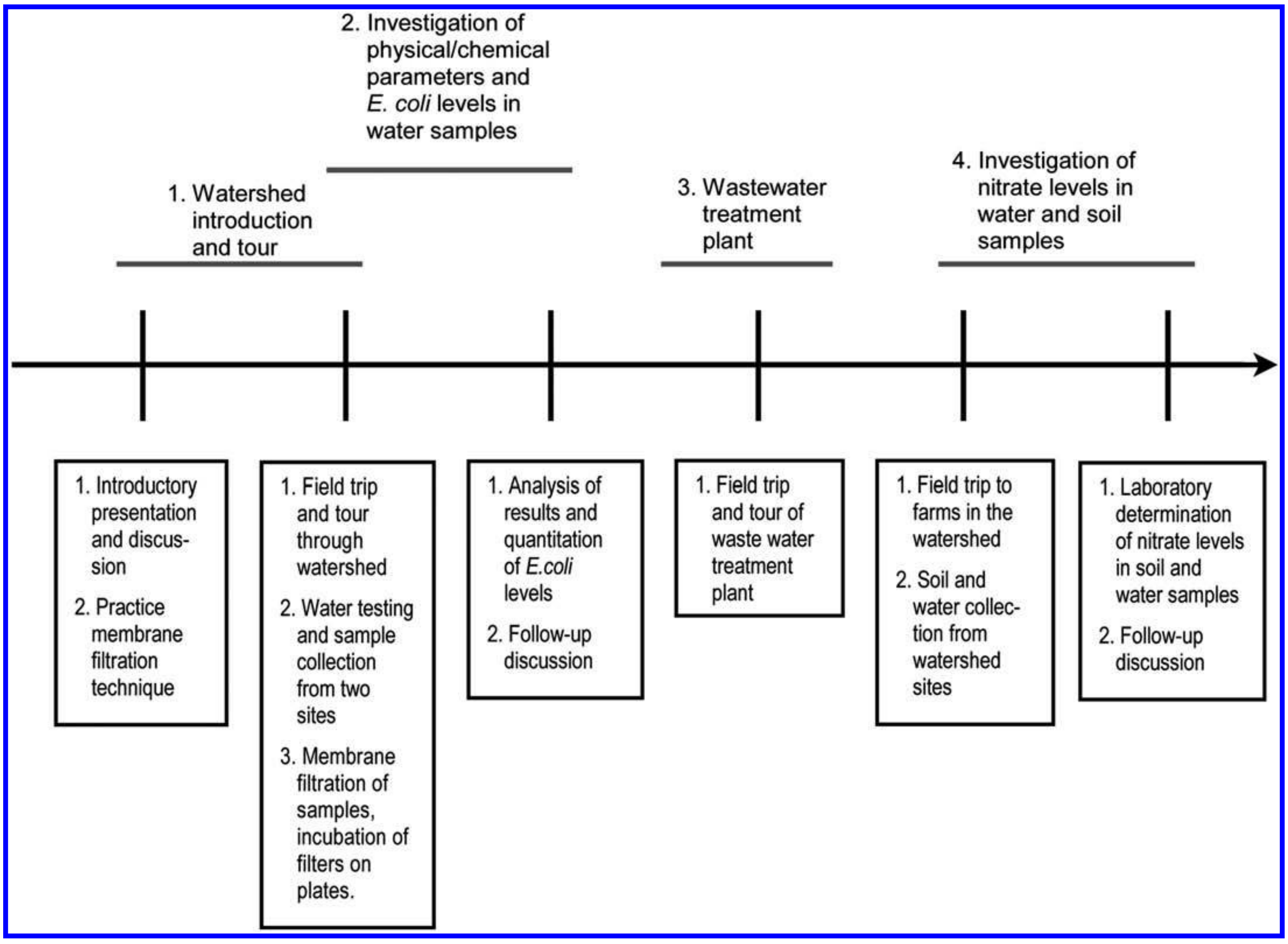

Figure 2. Laboratory schedule and events. Each vertical bar represents a laboratory session and is placed to indicate its position in the overall timeline of the laboratory series. Titles at the top of the figure represent distinct exercises, some of which required multiple lab sessions to complete (as indicated by the underlying line extending across multiple vertical bars). Detailed descriptions of each lab session are described below each vertical bar.

○ Results

Watershed Introduction & Tour

After field testing the water-quality lab module twice, it was clear that beginning the series with a thorough introduction to water-quality concepts was essential. In general, the students had very little knowledge of watersheds, surface and groundwater systems, the diversity of chemical and microbiological pollutants, or point and nonpoint sources of pollution. They were especially unfamiliar with agricultural practices and impacts on water quality. To address these issues, a PowerPoint presentation was performed to begin the lab series. The small lab sections were important in providing an environment conducive for discussion, as the students had many questions.

The instructor-led visual and audio tour (made possible by using handheld radios) through the Dry Run Creek watershed was an eye-opening experience for the students. Even though most of them travel regularly in the Midwest, few admitted to ever closely examining the land as they passed. During the tour, we traveled mostly on rural gravel roads, observing, defining, and comparing different types of land use, including active agricultural fields (e.g., corn, soybeans, alfalfa), land in the CRP, pastures being actively grazed by livestock, and woodlands. Special attention was paid to how water flowed through the watershed and the effect of this flow on nonpoint-source pollutants (e.g., animal waste, fertilizers). Many types of agricultural conservation practices were also identified (e.g., no-till farming, crop rotation, contour farming, terraces, grassed waterways, and filter strips). The critical role of fertilizers, in particular nitrogen fertilizers, on agricultural production was shared, along with potential impacts on water quality. As we passed by fields of alfalfa and soybeans, the important role of symbiotic nitrogen-fixing bacteria for these legume plants was emphasized, and contrasted with the lack of this activity (and subsequent need for increased artificial fertilization) for corn. Microbial involvement in the generation and depletion of nitrate in the soil was also communicated. Finally, as we drove past farms and observed land, outbuildings, and visible equipment (some of which was usually operating), the tremendous capital investment required for farming was shared, including specific examples of equipment costs, crop inputs, and price per acre of land in our area.

Testing Water for Chemical/Physical Parameters & *E. coli*

We stopped at two stream sites (S1 and S2) during the tour for the class to get out of the van and into the water to perform testing and sampling (time constraints prevented sampling from more sites). Student groups first measured physical/chemical parameters and then collected water samples for subsequent analysis of *E. coli* levels using a standard EPA protocol (EPA, 2006). Because the normal habitat of *E. coli* is the gastrointestinal tract of warm-blooded animals, this bacterium serves as an indicator of fecal contamination and implies that gut-associated microbial pathogens may be present. The results shown in Table 1 indicate that the two sites exhibited reproducible differences in almost every parameter. The most dramatic differences between S1 and S2 were in turbidity and *E. coli*, which each exhibited over 15-fold higher levels at S2, surpassing state averages and standards (Table 1). The students could easily observe the cloudiness of the water and the density of purple-colored *E. coli* colonies on plates (see Figure 3). During the follow-up discussion to this lab, the students hypothesized about what could be responsible for the differences observed, including land use, run-off, livestock and wildlife access to the stream, and potentially faulty septic-tank inputs.

Wastewater Treatment Plant

In order for the students to experience an example of a point-source pollutant, as well as the positive role that microbes can play in water treatment, a second field trip was taken to the local wastewater treatment facility. The students received an extensive tour of the facility by the plant manager. The Bio243 instructor provided supplementary information that connected the processes in the plant to bacterial metabolism. The tour included observation of primary treatment (physical removal of solids), secondary treatment (microbiological removal of organic pollutants), anaerobic digestion by microbes, final clarification, and UV sterilization of the treated water leaving the plant. In addition, the students were introduced to the laboratory analyses performed on the water at different stages. The students learned that the primary danger in raw sewage is not the microbes, but rather the organic waste, which provides otherwise limiting nutrients to stream microbes whose subsequent growth depletes the stream of oxygen. The students also learned about the dramatic effects of these anoxic conditions for aquatic macroorganisms, ranging from local streams to the "dead zone" in the Gulf of Mexico.

Investigation of Nitrate Levels in Water & Soil Samples

The final portion of the water-quality lab sequence involved a second visit to the Dry Run Creek watershed, this time to collect both soil

Table 1. Sample water-quality testing results from two sites in the Dry Run Creek Watershed.[a]

Site Number	Site Description (upstream land use)	Temperature[b] (°C)	Dissolved oxygen[b] (mg/L)	pH[c]	Conductivity[b] (μS/cm)	Turbidity[d] (NTU)	*E. coli* (CFU/mL)
Site 1	Woodlands, natural spring	10.3 ± 0.2	10.32 ± 0.26	7.90 ± 0.01	647 ± 11	1.18 ± 0.09	69 ± 34
Site 2	Active agricultural	13.4 ± 1.9	9.38 ± 0.69	8.27 ± 0.05	712 ± 3	20.5 ± 3.2	1063 ± 198
Statewide averages/standards		Variable	Cold water std = 7.0	8.2 (statewide average)	610 (statewide average)	18 (statewide average)	630[e] (geometric mean std)

[a]Data represent class averages ± SD from Fall 2011.
[b]Measured using a YSI handheld oxygen/conductivity/temperature meter.
[c]Measured using a YSI Ecosense pH pen.
[d]Measured using a Hach 2100 turbidimeter.
[e]Iowa standard for class A2 waterways (secondary contact recreational use).

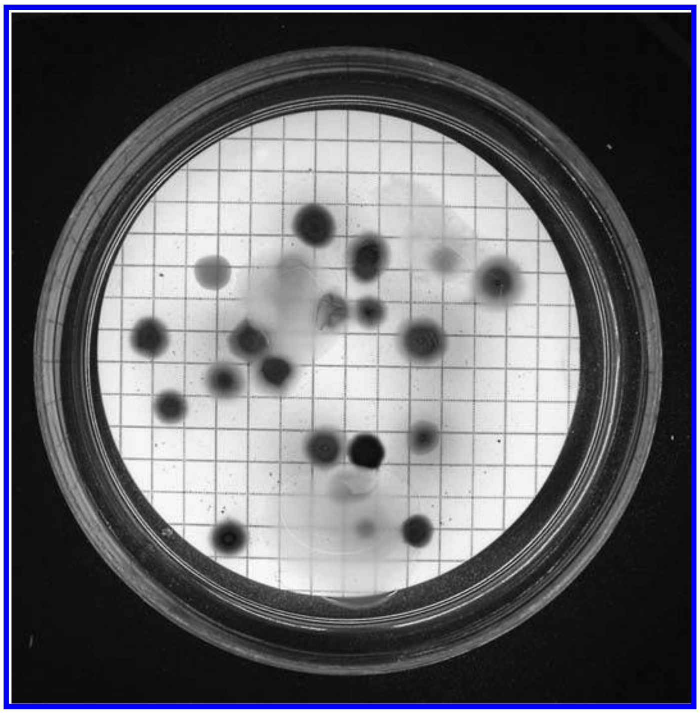

Figure 3. Sample plate results. Purple colonies (dark circles) indicate *E. coli*. (Imaging performed by Robert Fitton.)

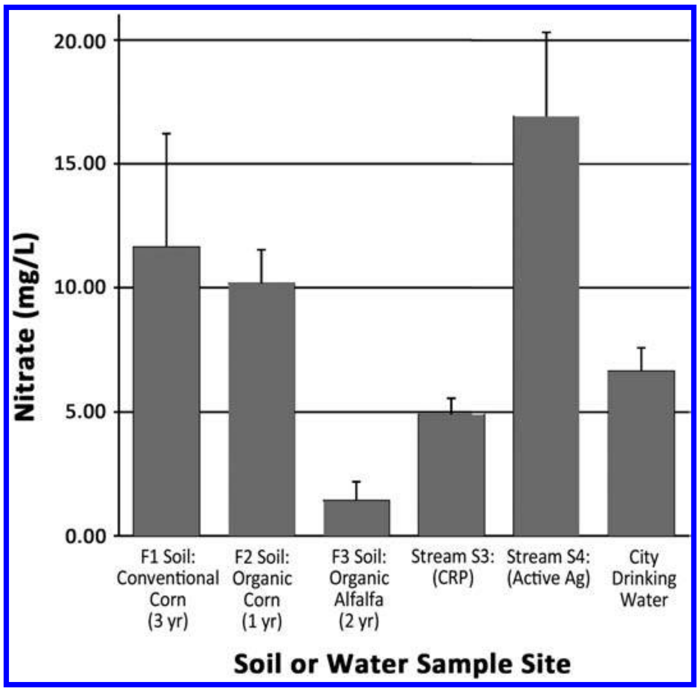

Figure 4. Nitrate levels of Dry Run Creek soil and water samples. Samples were analyzed by the Fall 2011 class. Error bars represent +1 standard deviation.

and water samples for nitrate analysis and to interact with a farmer. To prepare for this trip, the students completed questions from a required reading that provided information about nutrient cycling, the nitrogen cycle, the role of microbes in this cycle, the consequences of nitrate pollution in water, and the reasons for testing nitrate in soil and water from both an environmental and farmer's perspective. This preparation was essential for subsequent activities. During the field trip, brief stops were made to collect water from stream sites S3 and S4. The majority of the time was spent at two farms, where the students used soil corers to collect 12-inch-deep soil samples from three fields with different crop and fertilization histories. These included a field planted in conventional corn for 3 years in a row, a field planted in organically grown corn (following a rotation from alfalfa the previous year), and a field of organically grown alfalfa. A farmer also joined our group and shared some information about his specific agricultural practices and rationale. The students asked numerous questions about agricultural production; many commented afterward how meaningful it was to put a human face with the land.

During the following lab period, students extracted nitrate from dried soil samples with 0.01 M $CaCl_2$ and then used a standard procedure to measure nitrate levels in both the water and soil samples (NitraVer5, Hach Co., Loveland, Colorado). The class data shown in Figure 4 indicated that nitrate levels differed substantially among the samples. Soil samples from corn fields, which received fertilizer in the form of anhydrous ammonia (F1) or cover crop and manure (F2), had 7- to 8-fold higher average nitrate levels than the nonfertilized alfalfa field (F3). The class discussed these interpretations, as well as hypotheses for the significant difference between alfalfa and corn, and provided this data to the interested landowners to assist them with their nitrogen application management. Results from the water samples also indicated significant differences, with S4, which drains active agricultural ground, having the highest average levels (17.92 mg/L) and S3, which drains mostly CRP ground, having the lowest levels (4.90 mg/L). Interestingly, city tap water had an average level of 6.64 mg/L. Because the safe drinking-water standard for nitrate is 10 mg/L, these results provoked active student discussion in terms of possible explanations for the data. The students were especially attentive to how this project had connected the Dry Run Creek Watershed with their own drinking water, because Dry Run Creek empties into the river that runs through the City of Decorah. Decorah's relatively shallow wells are in close proximity to the river, and state geological surveys have indicated that a portion of the water produced by the wells originates in the river.

○ Assessment of Student Learning

The hypotheses of this work were tested in several ways. The students completed a Likert-style survey at the end of the course, designed to evaluate progress on learning objectives and the extent to which different laboratory activities contributed to learning. The students also provided qualitative responses on the survey. The results suggested that the five learning objectives were achieved for most students, with 64–78% of the students reporting that their knowledge had increased a tremendous or significant amount (Table 2). Objectives focused on threats to water quality, the nitrogen cycle and involvement of microbes, and the connections between agricultural practices and water quality received the highest average scores. The last question concerning the impacts of chloride on water quality served as an internal control, because chloride impacts on water quality were mentioned only briefly and were not a focus of the module. The altered response profile of this question helps to validate the prior student responses.

Additional survey questions were focused on determining which specific aspects of the module contributed to student learning.

Table 2. Student evaluation of progress on learning objectives.

Statement: As a result of this laboratory, rate the extent to which your knowledge has increased in the following areas:	Tremendous Amount (5)	Significant Amount (4)	Moderate Amount (3)	Slight Amount (2)	Did Not Increase at All (1)	Average Score[a]	Percentage Answering "Tremendous" or "Significant" Amount
Threats to water quality (e.g., chemical, bacterial)	25	37	18	7	0	3.92 ± 0.91	71%
The processes that occur in the nitrogen cycle and the specific contributions of microbes in these processes	34	34	15	3	1	4.11 ± 0.89	78%
Connections between farming practices and soil/water quality	28	33	18	6	2	3.91 ± 1.01	70%
Challenges faced by farmers (e.g., financial, environmental, scale)	24	33	14	13	3	3.71 ± 1.13	66%
Procedures for soil and water testing and why this information is useful	25	31	25	6	0	3.86 ± 0.92	64%
The causes and impacts of excess chloride on water quality (control question)	6	12	18	18	33	2.31 ± 1.30	21%

[a]Mean score of the entire class (n = 87) ± SD. Data are from fall 2011.

Students reported that field trips (that included direct observation of land-use patterns and sampling of soil/water), being able to measure real environmental samples in the lab, and the knowledge that the lab tied into an ongoing local watershed project all made significant contributions, with over two-thirds of the students indicating a tremendous or significant increase in knowledge for each category (data not shown).

A final assessment of student learning was obtained by determining whole-class performance on specific lab exam questions. Eight water-quality/nitrogen-cycle questions from the final lab exam were assessed (Table 3). Students performed well on these questions. The percentage of the class that answered them completely correctly averaged 90% (range: 81–100%). When partial credit was taken into account, the average increased to 93%. The strong class performance provided evidence that significant student learning occurred in these topic areas.

○ Discussion

The goal of this study was to develop a laboratory module that would enhance student learning by connecting the educational experience to a real-life challenge. Students were introduced to water-quality concepts through multiple field trips and laboratory analyses of environmental samples. Results from the water and soil testing of watershed sites indicated that there were substantial differences in multiple parameters, including turbidity, *E. coli*, and nitrate levels. These differences, in combination with students' direct observations of watershed site locations, allowed them to generate useful hypotheses to explain the data and compare them to other published reports linking water quality to land use (Schilling & Spooner, 2006). The use of sites that exhibited dramatic differences in water-quality parameters was a key component of this educational experience, and this study suggests that adjacent land use can aid in the identification of such sites. Student evaluation of progress indicated a substantial knowledge increase in each of the five major objectives, with a collective average score of 3.90 (= significant amount), supporting the original hypotheses of the study. Whole-class performance on pertinent exam questions served as an independent measure for student learning and provided further evidence for learning gains.

Results from this study add to the growing body of work that indicate the value of water-quality projects for science education (James et al., 2006; AAAS, 2009; Koosmann et al., 2011). Significantly, because of the large number of polluted streams, rivers, lakes, and oceans, project sites for these types of educational experiences are widely (and unfortunately) available. Appropriate sites can be identified by contacting local municipalities, state extension agencies, and natural resource agencies. Significantly, laboratory analyses of water and soil are technically doable (and tangible) for a wide range of students, and a large variety of water and soil testing kits and reagents are commercially available, appropriate for different budgets and levels of expertise. For example, although we measured nitrate levels in the laboratory using a method that involved spectrophotometry and generation of a nitrate standard curve, simpler methods are also available that are quantitative, visual, and low-cost, and can be performed in the field (e.g., Nitrate Test Strips or the Nitrate Color Disc Test Kit; Hach Co.). Finally, water-quality projects are often of mutual interest to educators, students, and community members because they center on an issue of public concern. Thus, these projects have the potential

Table 3. Student performance on relevant laboratory exam questions.

Exam Question	Percent of Class Answering[a]		
	Incorrect	Partially Correct	Completely Correct
1. Describe one way that farmers can lose their nitrogen input, including the forms of nitrogen involved and the conditions that promote loss. Include relevant biological processes.	7%	3%	90%
2. Describe a second way that farmers can lose their nitrogen input and explain as above.	11%	6%	83%
3. Why would a farmer be interested in testing his soil for nitrate levels? Explain.	0%	0%	100%
4. Thoroughly describe and explain one significant problem that can arise from having excess nitrate in the soil (either from an environmental or human health perspective).	0%	0%	100%
5. Why do farmers spend significant dollars to tile their fields? Explain.	10%	9%	81%
6. Why might it be valuable for college students to have an understanding of Midwest farming practices and challenges? (whether or not they are involved in agriculture)	0%	1%	99%
7. What rather unique characteristics do alfalfa and soybean plants share?	8%	6%	86%
8. Based on your knowledge of the nitrogen cycle and current farming practices, would you expect a multiyear alfalfa or a multiyear corn field to have higher [NO_3^-] levels? Thoroughly explain why.	17%	2%	81%

[a](n = 87). Data are from fall 2011.

Table 4. Summary of microbiology concepts included in laboratory series.

Microbiology Concept	Laboratory Activity
Microbial metabolism (aerobic/anaerobic respiration, fermentation, methanogenesis)	Waste water treatment plant: Process of microbial degradation of sewage
Quantitation of microbes from environmental samples; use of differential/selective media	Investigation of *E. coli* levels: Membrane filtration
Microbial pathogenesis	Investigation of *E. coli* levels: Use of *E. coli* as indicator for possible microbial pathogens in water; risks and impacts of infections
Environmental microbiology	Wastewater treatment plant and Investigation of nitrate levels: Microbial-based ecological changes due to increased nutrient levels (e.g., Dead Zone)
Microbial nutrient cycling	Watershed tour and Investigation of nitrate levels in soil and water: Major roles of microbes in the nitrogen cycle (e.g., nitrogen fixation, production and depletion of nitrates in soil, importance for agricultural production)

to be expanded into classroom service-learning projects, which have multiple educational benefits (Leege & Cawthorn, 2008).

A project-based water-quality laboratory can also serve as an attractive, interdisciplinary approach for teaching multiple microbiology concepts. This lab module presented students with opportunities to learn a variety of concepts, many of which are considered central to the field. Table 4 summarizes the microbiology concepts offered by the different lab activities.

An important aspect of this study was the intentional focus on local land use, allowing students to observe and learn about agricultural practices that affect water quality, and to meet producers. The objective was to offer balanced exposure to water-quality challenges, incorporating both environmental and agricultural perspectives. This approach was motivated by the reality that designing solutions to complex problems such as water quality will ultimately require an understanding of, and cooperation between, all stakeholders. Identifying willing agricultural producers can be accomplished through local agencies, including county agricultural service, soil and water conservation, and extension agencies. Significantly, in response to the open-ended, qualitative survey question "What was the most valuable thing you learned from the water quality/nitrogen cycle laboratories," 76% of student responses mentioned agricultural practices, challenges, and/or connections to water quality. Many student comments reflected their prior lack of familiarity with agriculture and the significant impact this laboratory had on their understanding. Several specific comments are below:

- "Before enrolling in Micro, I was unclear of the whole nitrogen impact in farming. Now, I can't drive past a corn, bean, or hayfield without thinking about it!"

- “I really enjoyed learning about how farmers use the land, and how their decisions can have such a great effect on the environment. I didn’t have any idea how interconnected those were.”
- “The most valuable thing I learned from the water quality/nitrogen cycle lab was about water quality and how we, in the Midwest, have a direct effect on these environmental issues. I am from a rural farming community in northwest Iowa. Lots of my relatives are farmers. I know that conventional farming and anhydrous ammonia nitrogen fertilization can have detrimental effects. However, I had never really considered how much I cared about it or stopped to think about drinking water quality or the Dead Zone in the Gulf of Mexico.”

Performing multiple, related water-quality exercises was important to the educational experience of this lab sequence, as it provided opportunities to make connections between and reinforce concepts. Students learned that agricultural practices can result in both bacterial and chemical pollution of streams, and that there are local and global impacts of this pollution. Students also learned that nutrients themselves are a major pollutant, whether the source was human or animal waste or fertilizer run-off. Finally, at the wastewater treatment plant, students learned that there were ways to dramatically reduce both nutrient-based and bacterial pollution, and that bacteria played a major role in this process. Significantly, when students were asked “What aspect of the lab was most responsible (for your learning),” 77% of respondents indicated field trips or an activity that was performed during a field trip. There are many challenges to designing effective field experiences, including teachers’ lack of familiarity and confidence in outdoor learning. Hopefully, this study can serve as a resource to overcome some of these barriers for water-quality-related projects.

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