Classroom Aquaponics: Exploring Nitrogen Cycling in a Closed System
Teacher's Guide

by Sean Mullen, Cornell University

Summary: Nutrient cycling is a fundamental concept in ecology and environmental science. However, many students have trouble visualizing how individual nutrients (e.g., nitrogen) cycle through complex natural ecosystems. To address this issue, this unit outlines an inquiry-based approach to investigating nutrient cycling in a simplified desktop ecosystem involving aquaria and hydroponically grown plants.

Subject: Ecology and Environmental Science

Audience: 9-12 ecology and general biology classes. However, this unit can be easily modified for younger students.

Timer Required: Initial setup takes approximately one 3 hour-lab period. Data collection takes approximately 30 minutes at regular intervals for 3-4 weeks.

Background

The growth of all organisms depends on the availability of mineral nutrients in the environment. Nitrogen is a particularly important component of the biosphere because it is required in large amounts as an essential component of proteins, nucleic acids and other cellular constituents. Nitrogen is abundant in the earth's atmosphere in the form of N_2 gas. However, in this form nitrogen is unavailable for use by most organisms because there is a triple bond between the two nitrogen atoms that makes the molecule almost inert. Nitrogen can be used for growth if it is first "fixed" (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions. Weathering of rocks releases small amounts of these ions but the process occurs so slowly that it has a negligible effect on the availability of fixed nitrogen. Thus, most available nitrogen is derived from other "fixation" pathways.

Nitrogen Fixation

Nitrogen fixation occurs in two major ways. The first is through high-energy fixation. Large amounts of energy produced by phenomena such as cosmic radiation, meteorite trails, and lightening strikes can drive the combination of nitrogen with the hydrogen and oxygen found in water. However, it is estimated that less than 8.9 kg N/ha is generated annually is this manner. The second, and largest, source of "fixed" nitrogen is through biological conversion. Atmospheric nitrogen can be fixed into biologically available forms, such as ammonia and nitrate, by 1) symbiotic bacteria commonly associated with legumes and with other plants having root nodules, 2) free-living aerobic bacteria, and 3) blue-green algae. Through biochemical pathways, these bacteria break the triple-bonded N_2 molecule into two nitrogen atoms, which then combine with hydrogen to form ammonia (NH_3). In this way, biological "fixation" generates approximately 90% of the fixed nitrogen contributed to the biotic environment each year…in other words, 100 to 200 kg N/ha!

Biological fixation of nitrogen is not easy, however. It takes approximately 10 grams of glucose to fix just 1 gram of nitrogen! . Given the limited availability of biologically useful nitrogen, it is not surprising that this nutrient is often a limiting factor for growth and biomass production in many environments where climate and water availability are not limiting.

The Nitrogen Cycle
In natural environments, nitrogen is frequently stored as decaying organic materials. It also is constantly being removed and added to both the biotic and abiotic environment in a complex biogeochemical cylce. "Fixed" nitrogen enters the biotic environment through the decay of organic materials (releasing amino acids, etc.), weathering, or from fixation by specialized bacteria (Figure 1). Generally, nitrogen is in the form of ammonium (NH_4^+) at this point and must undergo further modification to be more readily usable by plants. The process by which ammonia is converted to nitrites and nitrates is called **nitrification**. Two groups of microorganisms, *Nitrosomas* and *Nitrobacter*, drive this part of the nitrogen cycle. First, *Nitrosomas* bacteria utilize the ammonia available in soils as a source of energy. They metabolize the ammonia and promote its oxidation to nitrite ions and water. The nitrite-rich metabolic waste of these bacteria is then available as a food source for *Nitrobacter* bacteria, which oxidize the nitrite ions to nitrate . Plants then utilize nitrates for growth and respiration. Nitrogen taken up by plants is released as amino acids when the plant's tissue is eaten or the plant dies and begins to decay.

Nitrogen is also lost from the biotic portion of the cycle is two ways--denitrification and mineralization of organic materials. Mineralization of decaying organic matter binds the nitrogen in the abiotic environment until weathering releases it. **Denitrification**, or the reduction of nitrate to atmospheric nitrogen, is catalyzed under special, anaerobic conditions by denitrifying bacteria, such as *Pseudomonas*.

In general terms, the nitrogen cycle is typical of the cycle of many nutrients that are important to all forms of life. It is multi-faceted and fairly complex, incorporating numerous pathways and time scales. Given this complexity, it can be difficult for students to fully grasp how nitrogen and other nutrients flow through natural ecosystems. Fortunately, closed, artificial systems, such as aquaponics, can provide a simplified version of natural systems that can aid in student learning. In addition, these classroom systems can be easily replicated and manipulated, allowing the students to develop a much more sophisticated understanding of how nutrient cycling occurs and how it impacts the biotic and abiotic components of a model ecosystem.

Figure 1. Nitrogen Cycle (for example, see the figure at http://www.geog.ouc.bc.ca/physgeog/contents/9s.html**)**

Aquaponics
Aquaponics is the combination of aquaculture (fish farming) and hydroponics (growing plants in media other than soil). While commercial aquaponics is a multi-million dollar/year industry, the basic principles and equipment remain easily transferable to the classroom. The central problem in traditional aquaculture is water quality. Raising commercial fish at high density leads to quickly escalating levels of ammonia and nitrite in the water. These compounds are produced naturally in the fish effluent and must be laboriously removed through complex filtration systems in commercial setups. An analogous situation occurs in your own fish tank at home. Fish produce large volumes of nitrogenous wastes that must be removed by appropriate filters or frequent water changes. Conversely, hydroponic technologies strive to provide plants with a nitrogen-rich source of growing media and a perfect balance of other macro- and micro-nutrients. In this case, the difficulty is simply the high cost of constantly adding soluble nutrients to hydroponic setups. Aquaponics offers a solution to both industries by combining a recirculating system that incorporates three main components: fish, plants, and bacteria! The fish provide ammonia rich effluent that bacteria quickly convert to nitrite and nitrate. While ammonia and nitrite are toxic to fish at very low levels (several ppm), the fish are able to tolerate several hundred ppm of nitrate. The plants benefit from the nitrogen and nutrient rich effluent and remove large amounts of nitrogenous wastes (in the form of nitrates) from the water, which is then returned to the fish tanks. This can be done in aquaria or in bigger, more elaborate classroom setups such as the 400-gallon unit constructed by students at Beall High School in Maryland (see http://www.geocities.com/kenbaxter2/Aquaponics/Aqua1.jpg).

Learning Objectives
Student will:
1) Learn to identify components of the biotic and abiotic environment
2) Learn to differentiate between heterotrophic and autotrophic organisms
3) Understand nutrient cycling (nitrogen cycling in particular)
4) Measure levels of ammonia, nitrite, and nitrate
5) Measure plant growth
6) Construct graphs of nutrient levels and plant growth rates
7) Generate hypotheses about the impact of nutrients on plant growth

National Science Education Standards
Content Standard A - Science as Inquiry
- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard B - Physical Sciences
- Chemical Reactions
- Interactions of Energy and Matter

Content Standard C - Life science
- Interdependence of organisms
- Matter, energy, and organization in living systems

Behavior of organisms

Content Standard D - Earth and Space Science
Energy in the earth system
Geochemical cycles

Content Standard E - Science and Technology
Abilities of technological design
Understandings about science and technology

Content Standard F - Science in Personal and Social Perspectives
Population growth
Natural resources
Environmental quality
Natural and human-induced hazards
Science and technology in local, national, and global challenges

Content Standard G - History and Nature of Science
Science as a human endeavor
Nature of scientific knowledge

Equipment and Materials

- 10-gallon aquarium
- under-gravel filter
- small water pump
- fine/coarse gravel
- bacterial culture starter solution -- available in all pet shops
- standard water conditioner -- available at pet supply stores
- Styrofoam board - or other floating substrate
- lettuce seedlings in rockwool -- can be grown by students or purchased
- lights
- ornamental aquarium fish (~5)
- aquarium test kits for ammonia, nitrite, and nitrate (~$10/kit x 50-75 tests/kit)

Procedure

The initial setups can be constructed in advance by the teacher or can be incorporated as part of the lab to add a hands-on element and engage students who learn well in active situations. The steps regarding aquarium setup are standard. Any healthy aquarium needs to be set up for at least a few days to a week to be conditioned for fish. In addition, this will help give the bacteria time to become established in the gravel bed. However, these steps can be done by the students with the caveat that data collection be postponed for several days to a week.

1) Set up aquarium with 10 gallons of water at least a week before the lab is planned. (Treat the water with any standard water conditioner available at pet supply stores.)
2) Place under-gravel filter in bottom of tank and cover with 5-10 lbs. of gravel.

3) Add approximately 1ml of bacterial culture starter (the farther in advance of the lab this is done the better). The bacteria need several days to become established. Any normal fish tank will have a healthy stock of bacteria in the gravel bed if it has been set up for any significant length of time. (In this case, you do not need the starter culture.)
4) Add the fish to the pre-conditioned tank.
5) Place the water pump in the tank to circulate water and plug in. Cut small (~1 inch) round holes in the Styrofoam for the lettuce seedlings. Place the rockwool seedlings in the Styrofoam and float it on the top of the aquarium. Don't use too many plants or crowding will affect their growth.
6) Turn on the lights above the plants!
7) Take initial measurements of the plants (height, length, number of leaves, etc.).
8) Take initial water samples and test for ammonia, nitrite, and nitrate.
9) Feed fish regularly and measure plant and water quality (ammonia, nitrite, nitrate) variables at regular intervals for 1-2 weeks.
10) Plot the data and discuss the shift in ammonia, nitrite, and nitrate levels in the context of the nitrogen cycle.
11) Generate hypotheses about the role of the plants and the effects of varying concentrations of each nutrient on their growth.

Variations

This lab can be made much more hands-on and open-ended with a few simple steps. First, consider letting the students design the setup. Second, use replicates and adjust the variables. For instance, what happens if you use more plants, less plants, none? What about the number of fish? Do plants grow better in normal hydroponic solution or on the fish effluent? How does photoperiod affect plant growth rate? How does pH change over time in each tank? Does that impact plant growth? Bacterial conversion rates? Fish growth? This project can be easily modified to fit whatever goal you have for your classroom, it is relatively inexpensive, and it incorporates large amounts of potential content and plenty of opportunity for inquiry.

Assessment Strategy

Standard written assessments of students' knowledge regarding nutrient cycling should be given prior to starting this unit. Normal lectures will impart practical background knowledge and, hopefully, the hands-on lab will solidify these concepts in the students' minds. Individual projects and presentations are excellent ways to assess how engaged each student was and how deeply they understood their own setups. Post-tests also can be used to assess improvement in students' understanding of concepts related to nutrient cycling.

Teaching Tips

The protocol outline above is a bare bones conceptual map. You need to flesh out the project and mold it to your own personal needs. This project was implemented at an alternative middle and high school this year with a group of 10 students who became very involved. The students built recirculating tanks and hydroponic setups and completed both group and independent projects. Goals varied from understanding nutrient cycling to design challenges aimed at producing sustainable, closed systems. Students experimented with pH, bacterial population size (measured by surface area), fish and plant number, photoperiod, and other environmental variables. Overall, it proved a flexible and innovative system to explore a large variety of concepts and provided many synergistic activities.

Potential Problems
Fish and plants are living organisms that will die if not properly cared for. Giving students responsibility for maintaining aquaponic setups will help engage them but should be overseen by a responsible adult. Another potential problem is the potential for lack of clear results. Different treatments will usually provide clear differences in plant growth. For instance, plants may grow faster on fish effluent than on tanks with no fish. However, each setup will be different, and results may be hard to predict. Unclear results are part of science and usually lead to interesting and testable hypotheses. I encourage teachers to allow projects to be as open-ended as possible.

References
Background information about nitrogen cycling was adapted from:
Smith, R.L. 1992. Elements of Ecology, 3rd ed. Harper Collins Publishers, Inc. New York, New York. 617pp.