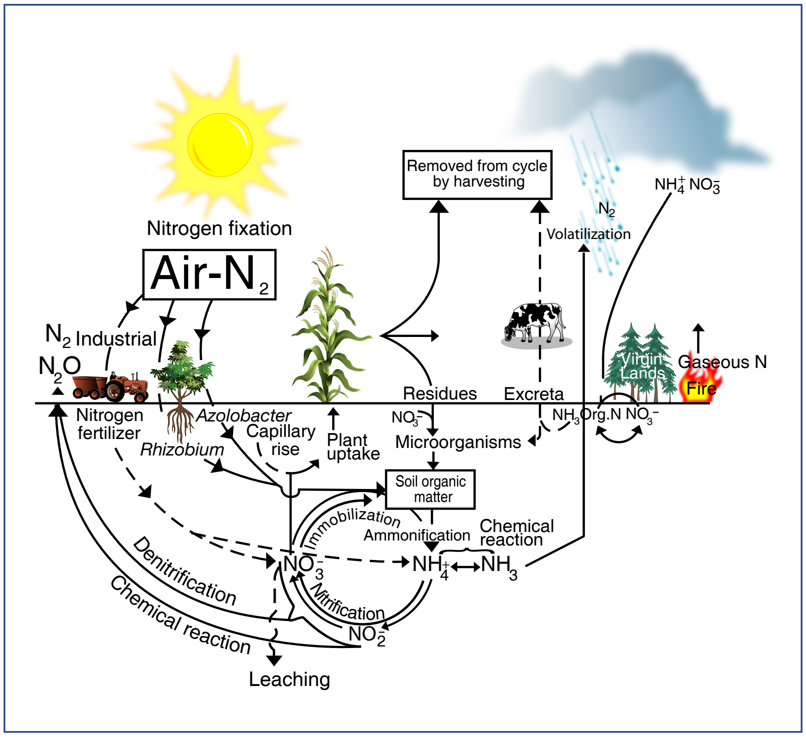
This activity was developed by SSSA for the 2014 AGI Earth Science Week Kit

Earth’s Interconnected Systems: Nitrogen



Credit: CFAES, The Ohio State University

Interconnected – this describes the nutrient cycles; the conversions, transformations, and movement of nutrients through the soil, abiotic and biotic components of the environment.

All biological organisms require certain nutrients in order to live. Plants require carbon, hydrogen, and oxygen from air and water, and nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, copper, zinc, nickel, chloride, boron, and molybdenum from soil. Animals require a few others. The conversions and transformations of nutrients in the environment result from chemical reactions, biological activity, or both. After water, nitrogen is the most limiting nutrient for the growth of most plants, especially the cereal grains that ultimately provide most of the calories humans consume.

Nitrogen has several valence states, and is present in the environment as dinitrogen (N_2), nitric oxide (NO), nitrous oxide (N_2O) and ammonia (NH_3) gasses, as the ions ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), and in organic forms such as proteins and enzymes. Nitric and nitrous oxides are greenhouse gasses that contribute to the brownish haze in smog. Nitrate and nitrite may contaminate surface or ground water. Most nitrogen in soil is present in organic forms, and most of the transformations are mediated by soil microorganisms.

Most soil-supplied nutrients are weathering products of rocks and minerals, but nitrogen is not found in any naturally-occurring minerals or rocks. And, although 78% of the atmosphere is dinitrogen, plants cannot use it. Plants take up nitrate and ammonium from the soil and use them to make amino acids, the building blocks of proteins. Nitrogen can be “fixed”, converted from dinitrogen into ammonium, through atmospheric, biological and industrial processes.

Nitrogen fixation requires a tremendous amount of energy in order to break the triple bond in dinitrogen. Lightning in the atmosphere generates ammonium which comes to the soil surface in precipitation. Microorganisms such as *Rhizobium*, *Frankia*, *Azotobacter*, *Anabeana*, and *Azolla* obtain energy from plant hosts, plant exudates, or photosynthesis. The Haber-Bosch process uses a catalyst, temperatures to 1200 C, and pressures to 1000 kPa. Without industrial nitrogen fixation for the production of fertilizer, world food production likely would be insufficient to support more than about 6 billion people.

This activity uses a qualitative nitrate test to observe results of some conversions and transformations in the nitrogen cycle. Qualitative nitrate test strips change color when exposed to nitrate; the color deepens as nitrate concentration increases. Ammonium and nitrate sources, water and sugar will be added to soil in various combinations. After incubation, nitrate will be measured with the test strips.

Materials Needed

- Computer with Internet connection
- Soil from yard or garden (may be repeated with sandbox sand)
- 200 to 250 ml containers with air-tight seals
- Ammonium-containing fertilizer† (check label), ammonia cleaning solution (if percent is listed), or reagent grade ammonium sulfate.
- Nitrate-containing fertilizer† (check label), e.g., Miracle- Gro® or Peter’s Professional®
- Sugar
- Nitrate water-quality test strips, e.g., Lamotte 2996®, EM Quant®, or Industrial Test Systems®
- Distilled water

† Fertilizer grades denote the guaranteed analysis (percentage) of elemental nitrogen, phosphate (reported as P_2O_5) and potash (reported as K_2O). The grade does not identify the nutrient source, such as ammonium nitrate or urea; that information will be reported with the guaranteed analysis.

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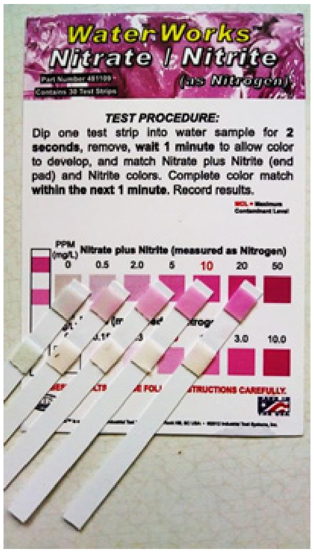

Nitrate/Nitrite test strips.

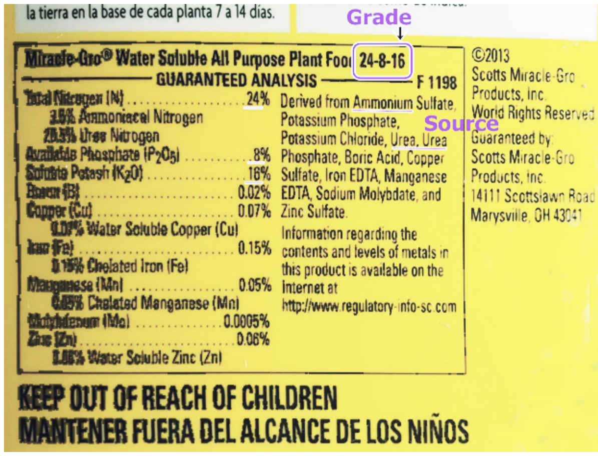

Figure 1 shows a fertilizer with a grade of 24-8-16. The nitrogen is derived from ammonium sulfate, urea, and urea phosphate. Urea forms ammonium as it dissolves in water. This fertilizer should not test positive for nitrate.

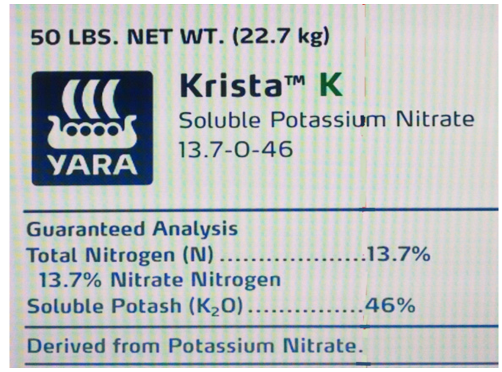

Figure 2 shows a fertilizer with a grade of 13.7-0-46. The nitrogen is derived from potassium nitrate. This fertilizer should test positive for nitrate.

Procedure

The target nitrogen concentration in the soil is 40 ppm.

The amount of water added to the soil will be approximately 50% of saturation (about 50% of the pores in the soil will be filled with water).

For each nitrogen source, prepare 1 liter of nitrogen solution to add to the soil using the calculations demonstrated.

[N] = nitrogen concentration in soil, 40 ppm in this experiment

W = water added to soil (g = ml), 20 g for soils in this experiment

X = % nitrogen in fertilizer expressed as a decimal, e.g., 10% N = 0.10 N

Y = g fertilizer in 100 g soil

Z = g fertilizer in 1000 ml water

Formula 1: Conversion of nitrogen concentration (ppm) to fertilizer (g) in 100 g soil

$$Y = \frac{[N]}{X \cdot 10000} = \frac{40}{X \cdot 10000}$$

Formula 2: Conversion of fertilizer in 100 g soil to fertilizer in 1000 ml water

$$Z = \left(\frac{Y}{W}\right) \cdot 1000 = \left(\frac{Y}{20}\right) \cdot 1000$$

Calculation 1: Use Formula 1 with a fertilizer that is 30% N to find the amount of fertilizer needed in 100 g soil.

$$Y = \frac{40}{0.30 \cdot 10000} = 0.0133\ g\ fertilizer/100\ g\ soil$$

Calculation 2: Use Formula 2 and result of Calculation 1 to find the amount of fertilizer to add to 1000 ml water.

$$Z = \left(\frac{0.0133}{20}\right) \cdot 1000 = 0.67\ g\ fertilizer/1000\ ml\ water$$

Calculation 3: The nitrogen content of a fertilizer may be estimated using the molecular weights when the chemical formula is known. This example uses urea: $CO(NH_2)_2$.

$$\% \ N = \left(\frac{2 \cdot 14}{12 + 16 + 2 \cdot (14 + 2)} \right) \cdot 100 = \frac{28}{60} \cdot 100 = 0.467 \cdot 100 = 46.7\%$$

If urea were pure, it would be 46.7% N, however, some impurities are introduced in the synthesis, so the N content of urea is typically 44% to 45%.

The result of Calculation 2 requires the addition of 0.67 g fertilizer to 1000 ml (1 liter) of distilled water. Some may not have a balance with 0.01 g precision. In that case, add 6.7 g fertilizer to 100 ml distilled water, then dilute the solution to 1000 ml (add 900 ml distilled water).

Preparation

1. Add 20 ml water to a container and set aside. Label water.
2. Add 20 ml of the nitrate solution to a container. Label nitrate.
3. Add 20 ml of the ammonium solution to a container. Label ammonium.

Test water and solutions for nitrate:

4. Write water, nitrate, and ammonium on test strips. Immerse the test strips into the appropriate solution in container.
5. Allow color to develop per test strip instructions, 1 minute for most test strips.
6. Compare color on test strips to standard in test strip package. Record results; photos are useful to compare the colors among the treatments with the color standard.
7. Place lids loosely on containers and set them aside.

Add fertilizer solutions to soils:

8. Add 7.5 g sugar to a beaker with 90 ml water. Mix until dissolved.
9. Add 100 g soil to 6 containers (200 to 250 ml).
10. Add 20 ml of distilled water to the soil in a container. Place lid loosely on container.
    Label Soil + water.
11. Add 20 ml of distilled water and 30 ml of the sugar-water solution to the soil in a container. Seal the container (air-tight). Label Soil + sugar water.
12. Add 20 ml of the nitrate solution to the soil in a container. Place lid loosely on container.
    Label Soil + nitrate.
13. Add 20 ml of the nitrate solution and 30 ml of the sugar-water solution to the soil in a container. Seal the container (air-tight). Label Soil + nitrate + sugar water.
14. Add 20 ml of the ammonium solution to the soil in a container. Place lid loosely on container. Label Soil + ammonium.
15. Add 20 ml of the ammonium solution and 30 ml of the sugar-water solution to the soil in a container. Seal the container (air-tight). Label Soil + ammonium + sugar water.
16. For replication, repeat Steps 8 through 15. May be repeated with playbox sand. For sand, reduce water, nitrate solution, and ammonium solutions to 15 ml, but add 30 ml of the sugar-water solution, as before.
17. Place containers in a 20 to 30 ˚C room for 2 weeks.

Analysis

18. Open containers, waft and smell; record differences.
19. Add 100-ml water to soil in loosely-sealed containers that received only water, nitrate solution, or ammonium solution.
20. Add 50-ml water to the soil in the sealed containers that also received the sugar-water solution.
21. Stir all containers for 1 minute. Wait 5 minutes for soil to settle.
22. Prepare a test strip for each container, labeling it as the container is labeled.
23. Immerse a test strip in the solution in each container. (Do not dip test strips into soil.)
24. Allow color to develop per test strip instructions, 1 minute for most test strips.
25. Compare color on test strips to standard in test strip package. Record results. Photos are useful.

The results are the relative amount of water-soluble nitrate. Some strips also test for nitrite.

What to expect:

The control soil provides the background nitrate concentration for comparison.

Unsealed containers should maintain aerobic conditions. The same concentration of nitrogen was added in both solutions, the difference was the form of nitrogen. The nitrogen in these containers may be lost to the atmosphere, or transformed into another form.

If the ammonium treatment has no more nitrate than the control, ammonia likely was lost to the atmosphere as a gas. If so, there may be an odor when the container is opened that is similar to ammonia cleaning solution. If the ammonium treatment has more nitrate than the control, ammonium was converted to nitrite by *Nitrosomonas*, which was then converted to nitrate by *Nitrobacter*. This process is called nitrification. Compare the amount of nitrate between the soils with nitrate or ammonium solutions added. In natural systems, nitrate is soluble and will leach out of a soil profile into groundwater.

Sealed containers had more water and an energy source (sugar) to encourage microbial activity, potentially to develop anaerobic conditions.

In these treatments, aerobic microorganisms should use all available oxygen for respiration as the sugar is consumed for energy, resulting in development of anaerobic conditions. As oxygen becomes more limited, the active organisms shift from aerobic to facultative to anaerobic. Facultative and anaerobic microorganisms may begin to "breathe" nitrogen, using it as a terminal electron acceptor in place of oxygen. In this process, called denitrification, nitrate is converted to nitric or nitrous oxides which are lost to the atmosphere as gases. If so, there may be a noticeable odor when the containers are opened and the nitrate levels may be less. These gases are important greenhouse gasses, and contribute to the brown haze in smog.

Variations:

Replication (2 or 3)
Use sand (sieved sand is available at many stores for play, landscaping, or construction) or another soil
Vary the incubation time, for example, 1 week, 3 weeks, 5 weeks (denitrification may increase with incubation time.
Vary the temperature, for example 10 and 30 ˚C. Within certain limits, biological activity increases with temperature.

Discussion:

The control soil measures the background nitrate concentration for comparison. If the ammonium treatment has no more nitrate than the control, ammonia was lost to the atmosphere as a gas. If the ammonium treatment has more nitrate than the control, ammonium was converted to nitrate by *Nitrosomonas* and *Nitrobacter*. In anaerobic conditions, denitrification may occur and nitric or nitrous oxides lost to the atmosphere. If so, there may be a noticeable odor when the containers are opened, and the nitrate levels may be less. What did you find?