MEASURING SOIL MICROBIAL ACTIVITY

Overview: This activity examines how soil microbes, such as bacteria and fungi, are involved in carbon cycling. Students design experiments to explore the relationship between microbial respiration rates and soil variables such as temperature, habitat, soil type, and agricultural management choices. Four methods for measuring CO_2 released from soil are provided, one in the field (CO_2 probe), and three in the lab (CO_2 probe, bromothymol blue (BTB) and acid-base titration).

Levels
6 through undergraduate

Subjects
Science, Environmental Studies

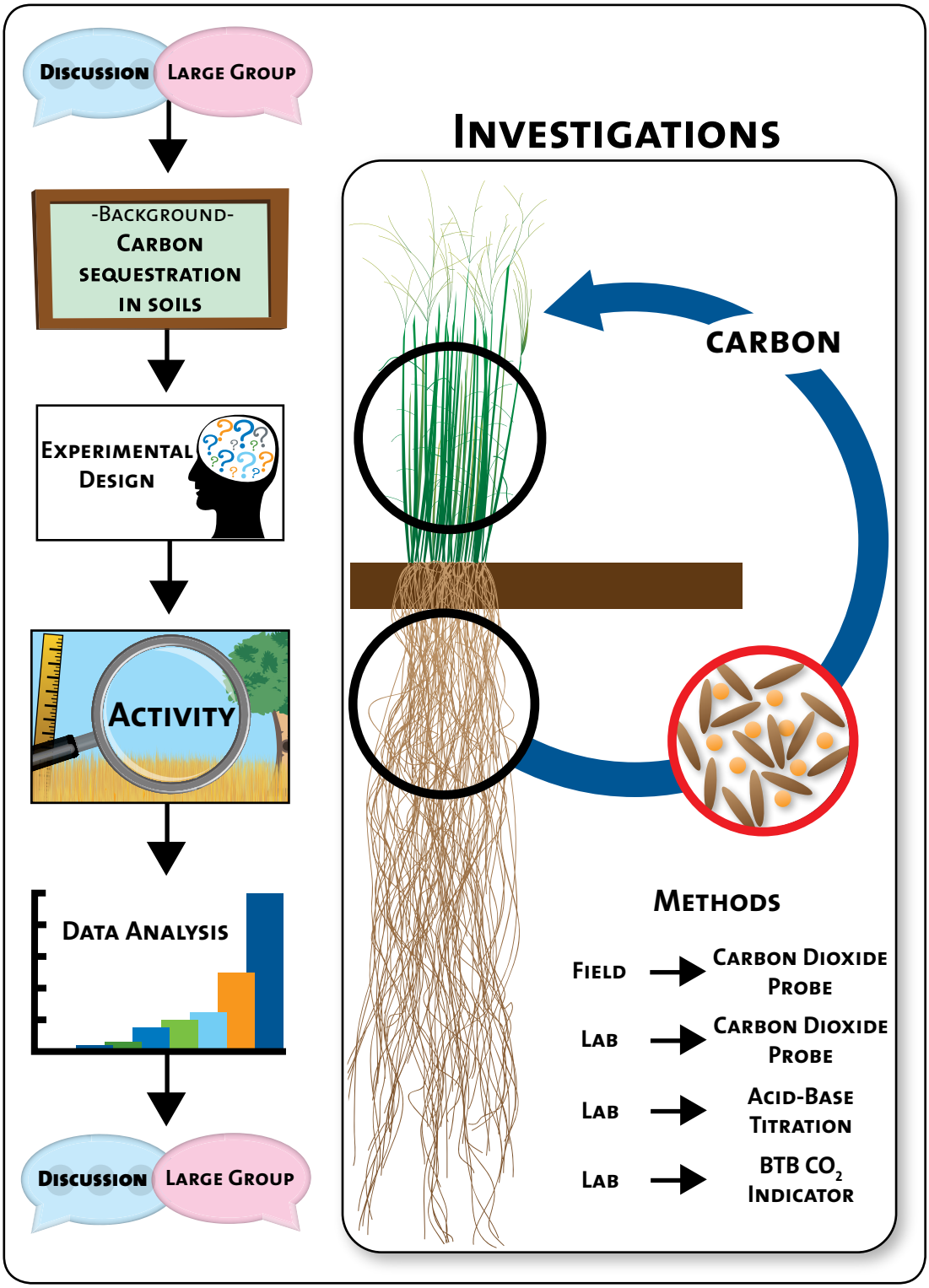
Objectives
Students will:
- Describe the role soil microbes play in the carbon cycle.
- Recognize and describe factors which affect the respiration rate of soil microbes
- Design, conduct and analyze an experiment to measure CO_2 emissions from soil
- Describe the movement of carbon through a field ecosystem, including above and below-ground components.

Materials
See instructions for detailed materials list.

Activity Time
Two-seven 50-minute class periods.

Standards
Next Generation Science Standards (2013)
- Scientific and Engineering Practices: planning and carrying out investigations; analyzing & interpreting data; constructing explanations and designing solutions
- Disciplinary Core Ideas: from molecules to organisms; ecosystems
- Crosscutting Concepts: cause and effect; energy and matter
- Performance Expectations: See page 3 for details

NGSS Lead States. 2013. Next Generation Science Standards: For States by States. Washington DC: The National Academies Press



For Teachers - Measuring Soil Microbial Activity

Overview:

This activity examines how soil microbes, such as bacteria and fungi, are involved in carbon cycling. Students design experiments to explore the relationship between microbial respiration rates and soil variables such as temperature, habitat, soil type, and agricultural management choices. Four methods for measuring CO_2 released from soil are provided, one in the field (CO_2 probe), and three in the lab (CO_2 probe, bromothymol blue (BTB) and acid-base titration).

Activity time varies by technique and experimental design. The entire activity takes approximately three-seven 50-minute class periods spread out over a one to two week period.

Learning Outcomes: Students will...

- Describe the role soil microbes play in the carbon cycle.
- Recognize and describe factors which affect the respiration rate of soil microbes, including oxygen and water levels in the soil, temperature, and availability of digestible detritus.
- Design, conduct and analyze an experiment to measure CO_2 emissions from soil under different conditions.
- Describe the movement of carbon through a field ecosystem, including above and below-ground components.
- Explain how land management practices (tilling, fertilization, etc) and different plants (prairie, grass, etc) have an effect on carbon cycling.

This lesson assumes prior knowledge in the components of experimental design, data collection and basic statistics, cellular respiration, the carbon cycle, and the relationship between increased atmospheric CO_2 and climate change.

Standards

Next Generation Science Standards (2013)

Performance Expectations

Middle School:

- **MS-LS2-1.** Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.
- **MS-LS2-3.** Develop a model to describe the cycling of matter and flow of energy among living and nonliving parts of an ecosystem.
- **MS-LS2-4.** Construct an argument supported by empirical evidence that changes to physical or biological components of an ecosystem affect populations.

High School:

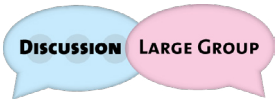
- **HS-LS1-7.** Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in new compounds are formed resulting in a net transfer of energy.
- **HS-LS2-3.** Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.
- **HS-LS2-5.** Develop a model to illustrate the role of photosynthesis and cellular respiration in the cycling of carbon among the biosphere, atmosphere, hydrosphere, and geosphere.

Next Generation Science Standards, continued

Scientific and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Planning and carrying out investigations Analyzing and interpreting data Constructing explanations and designing solutions	**LS1**: From molecules to organisms: Structures and processes **LS2**: Ecosystems: Interactions, energy and dynamics	Cause and effect: Mechanism and explanation Energy and matter: Flows, cycles, and conservation

See Appendix for alignment with other standards.

Sequence:



Part 1. Pre-Assessment & Discussion (pages 1-3) (one-two class periods)
Ask students to describe the difference between burning fossil fuels and biofuels (like ethanol) as it relates to the carbon cycle. How would you measure the ability of plants to take carbon dioxide out of the air? Where does the carbon go? Is it ever returned to the atmosphere? Use the soil community food web picture included in the student activities document as a talking point. Turn their attention to the role of soils in the cycle by reading *Carbon Sequestration in Soils*, found in this package to provide background information. Additional background material on soil microbes is provided in the supplementary materials folder in the package:

"Carbon Sequestration in Soils"
"Soil Microbes Presentation"
"Types of Soil Microbes and their Functions in Ecosystems"

- *Soil Microbes Presentation* provides photographs and descriptions of common soil microbes.
- *Types of Soil Microbes and their Functions in Ecosystems* provides a four page supplementary reading and worksheet to complement the presentation.

The background readings can be done as homework, but upon completion of the readings they should be able to answer the background questions on page 3 demonstrating that they have a basic understanding of what organisms might be respiring in a soil sample, the detritus-based food-web underground, and some factors that may influence the rate of soil microbial activity. Other sources of background material are suggested in the appendix.

Part 2. Experimental design (pages 4-17) (one-two class periods)
Decide which measurement technique you will be using of the four provided. Note that each activity requires different amounts of preparation, equipment, field time and follow-up analysis time. Data from the CO_2 probe techniques can be collected in one day. Most of the time is on the front end in those experiments. BTB will change color overnight and provides only a qualitative comparison. The BTB provides evidence of respiration for students to explain and can be used as a class demonstration and discussion. This method would work well for middle school students.

Titration takes longer at the end. Incubation time before titration is between 3 and 14 days; determine the incubation length based on your class schedule. See the answer key (p 17-18T) for additional hints on design.

Hand out the relevant *Methods* page and the *Experimental Design* questions. As a class, or in groups, read through the handouts and choose a question or write one to investigate. The basic procedures are described on the *Methods* pages, but the class should discuss where to sample, when to sample, what to compare, number of replicates, how to collect data, and if necessary, how to behave in the field to reduce disturbance and soil compaction. If you will be comparing results from year to year, it may be worth tracking weather patterns. A *Site Description* page is attached to this document as an option. Page 4 provides a guide for experimental design. Students should develop a hypothesis based on their understanding of how the system functions.

Be aware that the acid-base titration method is more advanced and requires more up front explanation of how the NaOH trap works and the types of controls required. Page 14 asks students to demonstrate an understanding of these concepts before designing their experiment. Students may have a hard time grasping that one control must be a jar with a NaOH trap without soil and the other a jar with an NaOH trap with soil but no treatment to provide us with the baseline amount of CO_2 in the atmosphere of the jar, and then the soil alone to compare to their experimental results. Alternatively, have a class discussion before students design their experiments and talk through the following questions:

- What are we testing for? What respires in soil?
- What would amendments to the soil, such as fertilizer, sugar or water, do?
- Controls: How do we know this soil gives off any carbon dioxide under normal conditions? How would we measure that amount? Why do we need two types of control (no soil, just soil)?
- What does NaOH do in this experiment? (It may help to do a demonstration showing the pH of NaOH, distilled water, carbonated water and HCl)

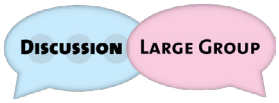
To save time when your are ready to do titrations, you might consider using one set of control jars that are shared between all experiments. You could use the control jars to demonstrate the titration method to the class and/or have groups that work faster titrate the control jars. Or, make sure there is repetition of experiments among groups so you have replicates of all jars types.

Part 3. Conduct experiment and analyze data (varies).

"Sample Data"

Note for Titration method: Titration of the NaOH trap can occur immediately at the end of the incubation period or the trap can be sealed tightly and stored at room temperature indefinitely for analysis later. Allow a full class period for titration of five jars/group. Set aside an additional 30-60 minutes to help students understand how to determine the amount of CO_2 produced. Make sure students understand the chemistry that occurred during the experiment and the math behind the calculations. Additional information is provided in the teacher answer key on pages 18-19T.

Note for BTB method: Results and data are based off extent of color change. The BTB solution will progress from blue to green to yellow as concentrations of carbon dioxide increase within the system. This will limit data analysis to qualitative observations (i.e. blue vs green vs yellow). However, the BTB provides useful evidence for students to explain the process of cellular respiration in soil, is inexpensive and can be done as a class demonstration or introduction to other more quantitative methods. See photographs from a sample experiment in the supplemental materials folder for a visual explanation. If students are not familiar with BTB as a pH indicator for carbon dioxide, demonstrate how BTB works by giving students petri dishes of BTB solution to exhale onto and/or blow into with straws and observe the color change. Included with the BTB Method Student Pages is a 1-page worksheet for students to predict and explain results of this small experiment. This worksheet on page 13 can be used to make this a shorter, simple lab activity.



Once students have averaged their own experimental data, set up a data collection system to easily share results for the whole class. Students should pool, average and graph data. Please refer to the "Sample data" Excel file in the supplementary materials folder for assistance with data analysis. Click on each of the tabs in the lower left of the screen to view sample data sets from different techniques.

Part 4. Discussion of results (page 19-20) (one 50-minute class periods)
Students should complete assessment question #1 before bringing the discussion to the entire class. Ask each group to report how the evidence they collected during their experiment supports or refutes their hypothesis. What have they learned from this investigation and what would they still like to know? Share and compare experimental results from the class. Are there any trends you see in soil microbial respiration rates? Are there further questions we could investigate? Changes to the experimental technique? Move on to the remaining assessment questions, which can be started in small groups and completed for homework.

Bring the discussion back to the idea of biofuels and the potential for crops to sequester carbon. They should be able to describe where carbon is stored in the field and how it moves from place to place. Discuss options farmers have to reduce emissions from their fields while retaining high yields.

A one page activity, "Making Predictions about Cellular Respiration by Soil Microbes" (page 20) could be used to assess student understanding of the whole activity. Also included with the BTB Student Pages is a 1-page worksheet following the "Predict, Explain, Observe, Explain" process for students to summarize and explain results in a more structured and simplified form.

Extensions

- Design a follow-up experiment that uses these results to make a prediction about a new set of variables.
- Measure biomass yield and root growth rates using the *Field Investigations* activity from GLBRC to close the carbon cycle loop.
- Explore the Smithsonian Institution's "Dig it! The Secrets of Soil, Greenhouse Gas Calculator" (*http://forces.si.edu/soils/index.html*) to examine how farming practices affect soil carbon sequestration.
- Use a Berlese funnel to collect soil organisms.

Master Materials List:

Field Method: Carbon Dioxide Probe

Safety warning: Please use proper precautions if students will use power tools, hand tools, sharp instruments, and strong adhesives. Also, please learn if any students are allergic to bee stings as fieldwork always presents possible exposure.

Note: Teachers may want to put these buckets in place in advance with or without students as they must be placed in the ground at least one week prior to sampling—they can remain in the ground indefinitely once in place.

Protocol variation: If you plan to do long-term monitoring in the bucket, only cut the plants down to the height of the rim to protect the plant community around the bucket. Be aware, however, that your CO2 measurements may include dark respiration of plants, not only soil microbes. If you cut plants to the ground, move the chamber for your next sample date.

Materials list to construct and install eight monitoring stations

- Eight white 5-gallon pails
- Eight white 5-gallon pail tops
- Hacksaw or heavy duty utility knife (more than one if possible)
- Drill and bits (to match gasket size)
- Eight #8 rubber stoppers
- E6000 Adhesive or like product (E6000 can be bought at larger craft stores)
- Up to 8 Trowels
- Eight lavatory pop-up drain gaskets (1 7/16" internal diameter for Vernier CO_2 brand probe)
- Spare lumber that spans top of pail
- 1-3 Mallets
- Eight-24 scissors or plant shears
- At least one Vernier Lab Quest and CO2 Probe, or equivalent
- Optional: Soil temperature probe
- Optional: Garden gloves

Lab Method: Carbon Dioxide Probe

Materials needed for each student team:

- Soil, at least 500 ml (200 mL per treatment / 100 mL per container)
  - -A good topsoil with loamy characteristics (not very sandy or clayey) will be best. Clean of rocks, leaves, roots, etc
  - -Should be somewhat moist, but not saturated/muddy
- 2 or more CO_2 probes (Vernier, or equivalent--probe-computer interface, software, etc) (Note: in a pinch you could use only one probe and do several runs in series.)
- 2 or more 250 ml Nalgene bottles with openings to fit probes
- 250 ml graduated cylinder
- 10 ml graduated cylinder or teaspoon
- 4 or more 9 oz (~250 ml) cups
- 2 or more spoons, for mixing solutions into soils
- Water (tap water or distilled)

Lab Method: Bromothymol Blue

Materials needed for each student team:

- Soil, at least 600 ml per treatment (300 mL in each container)
  - -A good topsoil with loamy characteristics (not very sandy or clayey) will be best. Clean of rocks, leaves, roots, etc
  - -Should be somewhat moist, but not saturated/muddy
- Bromothymol blue stock solution
- 2 or more petri dishes
- 2 or more large, clear containers with air tight covers*
- 250 ml graduated cylinder
- 10 ml graduated cylinder or teaspoon
- 2 or more spoons, for mixing solutions into soils
- Distilled water

*A variety of containers can be used efficiently. A 2000 ml polycarbonate container works well and can be purchased from http://www.usplastic.com/catalog/item.aspx?itemid=84337&catid=574.

Preparing BTB: When CO_2 dissolves in water, it forms a weak acid (carbonic acid) which makes the pH of pure water as low as 5.5. In solutions with pH > 7.1 BTB is blue. In solutions with pH < 6.4 BTB is yellow. Most tap water has too many ions in it that buffer the pH of the water, making it slow to acidify with added CO_2, so distilled water is necessary. BTB solution can be diluted in a 10:1 solution with water to conserve your stock. It will work the same way when it is more diluted, but the color will appear lighter. If your solution is too green, try adding a dilute strong base to increase the pH. A few drops of 0.1 M NaOH can be added and mixed in to create the desired blue color.

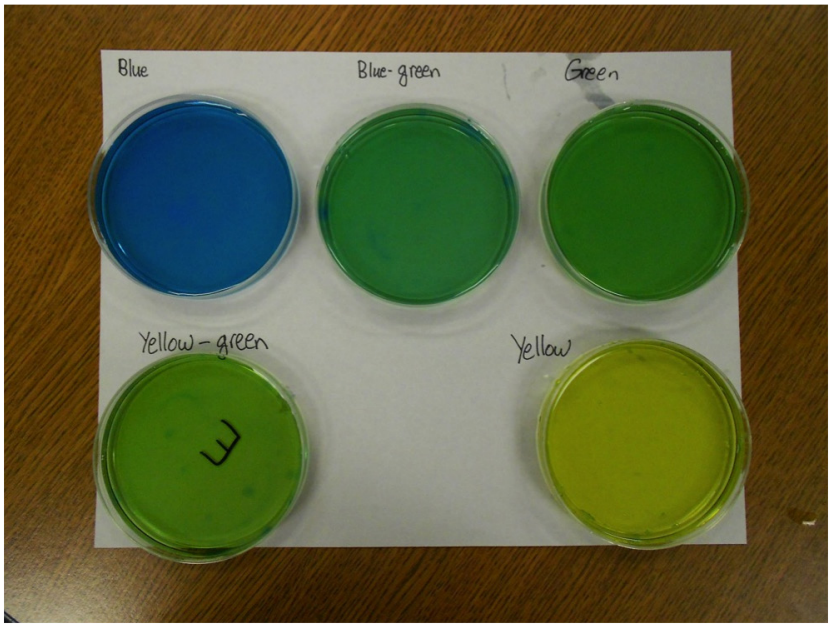
Carbon dioxide changes the pH of water by dissolving to form the weak acid H_2CO_3, called carbonic acid according to the following reaction:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

After that, carbonic acid reacts slightly and reversibly in water to form a hydronium cation, H_3O^+, and the bicarbonate ion, HCO^{3-}, according to the following reaction:

$$H_2CO_3 + H_2O \longrightarrow HCO_3^- + H_3O^+$$

This chemical behavior explains why distilled water, which normally has a neutral pH of 7 has an acidic pH of approximately 5.5 when it has been exposed to air (and ambient CO_2).



Analyzing BTB Color Change Results: BTB will provide qualitative evidence of CO_2. Possible colors include blue, blue-green, green, yellow-green and yellow. To make the degree of color change semi-quantitative, you can assign numbers to each color (0-4) where higher numbers equal a greater degree of color change from blue to yellow so that numbers would be assigned as follows: blue = 0, blue-green = 1, green = 2, yellow-green = 3, yellow = 4. Subtracting initial from final color values can give a numerical representation of the degree of color change and CO_2 emissions in the system. As an example the results of the sample experiment in the accompanying Power Point presentation are summarized below.

Treatment	Initial Color	Final Color (24 hours)	Final - Initial
Soil + Sugar	Blue (0)	Yellow (4)	4
Soil Only	Blue (0)	Blue-Green (1)	1
No Soil	Blue (0)	Blue (0)	0

Use either the CO_2 probe or titration method to measure and compare continuous changes in carbon dioxide concentrations over time.

Lab Method: Acid-base Titration

Materials needed for each student team:

- Fresh soil from sample site(s)
- Pint size wide-mouth mason-type jars with lids (at least 4)
- 30 ml centrifuge tubes (at least 4)*
- 1M NaOH
- 1 M HCl
- BaCl2, 50% solution
- Phenolphthalein
- 1 ml pipettes
- 25 ml titration burette and stand

- 50 ml beaker
- Graduated cylinder
- Scale

*30 ml tubes available at *http://www.evergreensci.com*

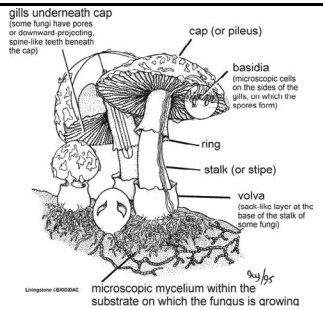
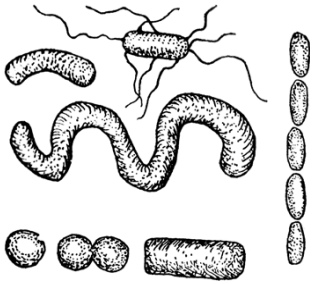
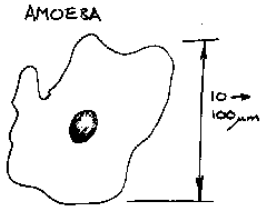
Preparing solutions:

50% $BaCl_2$ = Put 50 g $BaCl_2$ in a beaker, and add enough distilled water to bring the final volume to 100 ml (this will be oversaturated)

1M HCl (assume starting with concentrated 12.2 M) = 82 ml HCl per liter of water (add acid to the water)

1M NaOH = Dissolve 40 g NaOH in enough water to dissolve, then bring volume up to 1 liter

Description of Soil Microbe Community

	How they look	Abundance (tons/hectare)	Habitat characteristics	Potential role in food chain and ecosystem
Fungi		*2-5*	*Aerobic only* *Tolerant of acidic soil* *Moderately dr* *Forest, compost, garden/field, prairie*	*Considerable variability by species* *Decomposers - consume lignin and soil humic acids, among other materials* *Nitrogen fixation*
Bacteria		*1-4*	*Widely variable air, moisture, temperature, acidity tolerance* *Found in all ecosystems*	*Considerable variability by species* *Decomposers - consume chitin and cellulose, among other materials* *Autotrophs* *Parasites* *Nitrogen fixation and release*
Protozoans		*0-0.5*	*Aerobic only* *Need water* *Forest, compost, garden/field, prairie; aerated portions of marsh and wetland*	*Predators of bacteria and fungi* *Decomposers*
Nematodes		*0-0.2*	*Aerobic only* *Tolerant of acidic soil* *Forest, compost, garden/field, prairie; aerated portion of marsh and wetland*	*Parasites of plants and soil microbes* *Predators*

adapted from Killham 1999

BACKGROUND

Background questions

1. How does carbon get into the soil? In what forms?

   Carbon enters the soil in the form of materials from dead plant and animals. The C is stored in sugars, starches, proteins, lignin, nucleic acids, and other molecules.

2. How is carbon in the soil returned to the atmosphere? Is it all returned at the same rate? Explain.

   As decomposing soil microbes such as bacteria, fungi, and protists break down organic matter in the process of cellular respiration, they release gaseous carbon dioxide into the atmosphere. Some materials are more readily decomposed by microbes than others. Fast decomposition recycles C quickly, other items may be buried and decompose very slowly or not at all. Microbes respire just like other organisms as part of forming usable chemical energy in the form of ATP.

3. Experiments such as this one measuring soil microbe respiration in a jar or bucket, extrapolate small-scale research results to entire ecosystems such as a forest or prairie.
   a. How is this model useful?

      Lab/field (varies): We have taken actual soil from the field. The type and density of microbes in our soil sample approximates their actual occurrence; we are measuring an activity that microbes will carry out regardless of where they are in order to acquire the matter and energy necessary for their survival.

      Lab: This experiment allows us to focus on a single variable such as temperature or moisture and speculate about its effect on the process of gas exchange. A field study has other benefits, but it also has confounding variables that make it difficult to determine causal factors. Combining field and lab studies is the approach often taken by ecologists to address the benefits and drawbacks to each.

b. What are the limitations of this model?

Field: This is not a uniform environment, answers will vary by location and timing. We disturb the soil by setting up the experiment. Some respiration may come from the plants, not the soil organisms and there is no way to tease out the difference.

Lab: Once we take microbes out of their natural habitat, conditions no longer match their habitat. Living in a glass jar is very different from living in a forest or field – indoor environments are far less variable than soil outdoors. Resource inputs change as well – no new inputs (e.g., rain, animal defecation, etc). Oxygen may also become a limiting factor in a sealed container.

Experimental Design Hints

Hypotheses will vary. One thing to be aware of is whether students are comparing different field sites (different "samples"), or taking soil from one site and amending it in the lab (different "treatments"). Each approach can be useful, but comparing different sites may introduce other variables that students should consider and account for in their explanations and experimental design. Do the different sites have the same or different soil types? Often times there will be different soil types. How might soil type affect what kinds of soil microbes live there? If the sites have the same soil type, are they managed differently? Is one fertilized and the other not?

Three factors affect soil respiration: amount of organic material available underground for microbes to consume; quality of organic material—high in simple sugars vs high in "indigestible" lignin; soil conditions (moisture levels, temperature, and oxygen levels).

Prediction for the relationship between temperature and carbon dioxide production: Increased temperatures leads to increased respiration, up to a certain point. Heat stress above ~40°C should slow down respiration –

students may have trouble predicting this but should have some concept of impacts of excessive heat. Minimum temperature for respiration is surprisingly low – could be subject of follow-up experiments.

Prediction for the relationship between incubation time and carbon dioxide production: Increased incubation time leads to increased respiration up to a certain point. For example, once available food resources are used up, or conditions become too dry, respiration will no longer increase.

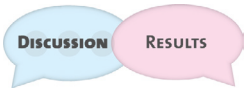
Each treatment should have at least 2 jars so that an average can be calculated. Most researchers strive for 3 or more replicates, up to hundreds, depending on feasibility; in this case the number of replicates depends on available resources, a constraint that any researcher must consider.

Without a control, it is not possible to distinguish between ambient levels of CO_2 and CO_2 produced by soil microbe respiration. With the control, we can subtract out ambient levels.

Chemistry details of titration activity

The equations associated with this the NaOH trap are:

$$CO_{2(g)} + H_2O_{(l)} \rightleftharpoons H_2CO_{3\,(aq)}$$



(carbon dioxide combining with water to form carbonic acid)

and

$$H_2CO_{3(aq)} + 2\,NaOH_{(aq)} \rightleftharpoons 2\,H_2O_{(l)} + 2\,Na^+_{(aq)} + CO_3^{2-}{}_{(aq)}$$

(acid and base react to form water)

Discuss how the OH^- in the trap are neutralized in a 2:1 ratio by the hydrogen ions from carbonic acid (2 OH^-s used for each molecule of H_2CO_3 which has two H^+). This lowers the pH in the trap. By the end of the incubation period in the jar, some OH^- remains in the trap leaving the solution basic, but not as basic as before. The HCl will be used to neutralize the remaining

base in the trap. The amount of HCl needed will vary based on the amount of CO_2 produced by soil microbes. (It may be helpful to draw a pH scale and use pH strips to measure pH of the original HCl and NaOH solutions, the NaOH solution after incubation with soil, and again after completing titration.)

*The following conversions are used to calculate the amount of CO_2 produced by soil microbes (mg CO_2 = (B-V) * NE see full equation explanation on student page). Note the 2:1 ratio of NaOH to CO_2 which accounts for E=22 in the student explanation. The same ratio applies for calculating mg C.*

$$\text{ml HCl solution used}\left(\frac{1\text{ L HCl}}{1000\text{ ml HCl}}\right)\left(\frac{1\text{ mol HCl}}{1\text{ L HCl}}\right)\left(\frac{1\text{ mol NaOH}}{1\text{ mol HCl}}\right)\left(\frac{1\text{ mol } CO_2}{2\text{ mol NaOH}}\right)\left(\frac{44\text{ g } CO_2}{1\text{ mol } CO_2}\right)\left(\frac{1000\text{ mg}}{\text{g}}\right)=\text{mg } CO_2 \text{ released}$$

Analysis and Discussion Questions

1. After a class discussion on graphing and statistics, construct a graph to represent your data.
   a. Assess whether your data support your hypothesis.
   b. Using scientific reasoning, discuss possible reasons for the relationships you see in average CO_2 production between treatments.
   c. Discuss any sources of error in your experiment and explain how you would redesign your experiment to account for these.

Students will have to decide whether to construct a bar graph (for discrete independent variable data – e.g., habitat type or incubation temperature) or line graph/scatter plot (for continuous data – e.g., incubation time). For examples, see the Sample Data file in supplementary materials (check all tabs). If you have over five replicates per treatment standard deviation can be calculated and added to averages portrayed on the graphs. Advanced students may want to do ANOVA or T-test calculations.

"Sample Data"

Possible sources of error: problems in the protocol (e.g., methodology for sampling soil may not have been representative), or problems

in technique (e.g., students may have had trouble titrating in 0.5 ml increments

2. Organisms above- and below-ground respire and produce CO_2, and they are assembled into complex ecosystems.
   a. How do organisms above- and below-ground function similarly in terms of respiration and metabolism?

   Organisms underground are undergoing cellular respiration just like those above-ground; however underground environments may be limited by oxygen and food availability more than for above-ground organisms. Below ground organisms have anaerobic alternatives.

   b. Draw a diagram which demonstrates how carbon moves through your study site. Include the plants, roots, soil organisms with linking verbs describing the processes that are occurring.

Drawings should demonstrate photosynthesis, carbon storage in leaf, stem, and roots, leaf and root death, decomposition of matter by microbes, and plant respiration.

3. How do you think climate (especially temperature and moisture levels) affects the rate of soil carbon cycling in each of the following states: Alaska, Minnesota, New Mexico and Florida?

Alaskan soil is dominated by permafrost, which is frozen year round. Therefore, biological processes such as cellular respiration cannot take place except in a thin upper layer of soil that does thaw during warmer months.

Climate in a state like Minnesota is characterized by more variability between the cold winter months and the warm, rainy summer months. Bacteria and other microbes must be adapted to these fluctuations to survive. During the warm, moist summer months, there are high levels of

respiration by soil microbes; during the winter months microbes become dormant in the freezing conditions and no respiration takes place.

New Mexico is characterized by warmer, more stable weather conditions but moisture is a limiting factor. Some bacteria are capable of surviving its dry conditions, but not in as high of a density. Given the warmer weather year-round, however, overall levels of microbial respiration may be comparable to that of Minnesota.

Florida is characterized by warmer weather year-round with fairly regular rainfall; therefore, levels of respiration by soil microbes would be highest in this location.

4. CO_2 production by soil microbial communities is often discussed in relationship to large-scale trends in atmospheric CO_2 concentrations and global climate change. Think about your answer to the previous question. The Arctic, including the permafrost in Alaska, is currently experiencing a significant rise in overall temperatures. How might this affect the rate of soil respiration in this region? Would you expect this change to lead to a global change in the atmospheric CO_2 levels?

Microbes release CO_2 in large amounts (due to their abundance); changes in climate as a result of fossil fuel combustion may lead to a positive feedback loop in which more CO_2 is released – in this case by soil microbe activity as a result of overall warming of earth's climate (e.g., thawing of permafrost exposes once frozen plant matter. If microbes colonize this biomass, they may release stored carbon and lead to increased atmospheric CO_2 levels). So soil microbial activity may be tied in to atmospheric CO_2 concentrations and therefore global climate change. Since this is a relatively new research area, many questions remain about the role of microbial respiration in regulating climate.

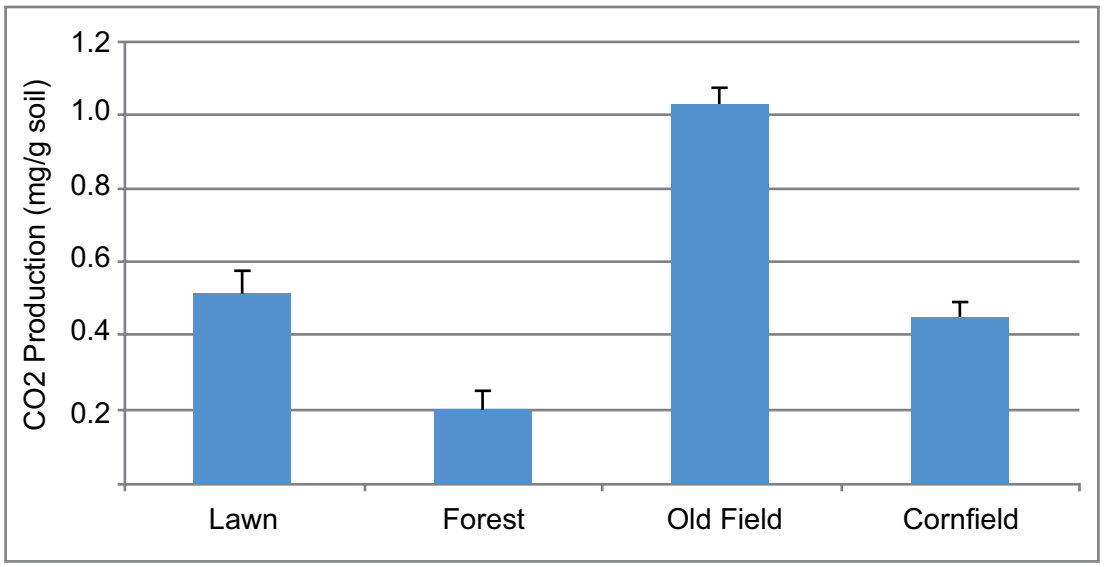
5. In terms of greenhouse gas emissions from soils, what might be the advantage of growing perennial prairie grass plots for biofuels instead of annual crops, such as corn? Provide one additional piece of advice for a farmer who is trying to decide what to grow or how to grow crops to be used for biofuels if they want to reduce overall emissions from their field.

Except for the initial planting, perennial crop soils would not be disturbed from year to year, allowing carbon from plant material to build up in the soil. Decomposition rates in undisturbed soils is expected to be slower than in disturbed soils (from tilling or planting annual crops). There is more available biomass in a prairie both above and below-ground, however it may not decompose if it is not disturbed.

Farmers could consider planting perennial crops, which take carbon and sequester it in extensive root systems that remain underground. They can practice no-till farming, which reduces microbial activity. They may also choose not to convert established perennials or forests to annual crops; a conversion process which released a tremendous amount of greenhouse gases.

Making Predictions about Cellular Respiration by Soil Microbes

The data below show measured microbial activity from four soil samples from different habitats – corn field, old field (overgrown farmland), lawn, and forest.



1. Examine the data above for each soil type. Label each bar (1-4) with the corresponding habitat. Provide a rationale for your predictions. Feel free to use the back of this page if you need more space.

   Explanation: These data are somewhat counterintuitive. Why would microbial activity in a forest be so low? Most likely this is because woody vegetation is rich in lignin, which is more difficult to break down than the cellulose found in grasses and flowers characteristic of lawns and fields. Less respiration would occur there than in the other habitats. Comparing an old field, a cornfield, and a lawn: an old field would have a richer supply of organic matter than a cornfield or lawn. There may, however, be other factors for a lawn or cornfield such as time of year and time since last application of fertilizer (organic or synthetic) that may lead to different results depending on the sampling date. An old field would also have a less disturbed soil microbe community than a cornfield, which could be another reason why soil microbes in the old field released more CO2 after being disturbed, allowing additional oxygen input and mixing of microbes and plant material.

2. Were your predictions correct? Provide logical explanations for any discrepancies.

   It is less important to focus on "right answers" than on the quality of their rationale in #1. These are complex ecosystems, which are not well understood and difficult to describe. Students will need to explain if/why their predictions differed from their predictions in #1. This question will help teachers understand any preconceptions students hold about soil carbon cycling. Consider following up with a third question, asking students what further sampling they might do to make sense of this data. They could sample the same sites at different times of year, compare soil types at each site, sample additional lawns, forests, etc to see if the results are consistent, etc. For reference purposes, this data was collected in June 2009 near Kalamazoo, Michigan.

Appendix

Suggested References

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Images Courtesy of: Ivy Livingstone, Biodidac Project (fungus); Educational Technology Clearinghouse, University of South Florida (bacteria); National Science Foundation "simscience" (Amoeba), United Nations Food and Agriculture Organization (Nematode)

Standards:

WI MODEL ACADEMIC STANDARDS:

E.8.4 – Using the science themes, analyze the influence living organisms have had on the Earth's systems, including their impact on the composition of the atmosphere and the weathering of rocks.

E.12.2 – Analyze the geochemical and physical cycles of the earth and use them to describe movements of matter

F.8.8 – Show through investigations how organisms both depend on and contribute to the balance or imbalance of populations and/or ecosystems, which in turn contribute to the total system of life on the planet.

C.8.3 – Design and safely conduct investigations that provide reliable quantitative or qualitative data, as appropriate, to answer their questions.

AAAS PROJECT 2061 (1993):

1B The Nature of Science: Scientific Inquiry
5E The Living Environment: Flow of Matter and Energy
8A The Designed World: Agriculture
11A Systems

See page 3 for Next Generation Science Standards (2013) alignment.

Titration activity developed by Stephen Laubach, University of Wisconsin-Madison Department of Curriculum and Instruction and Kevin Budsberg, UW-Madison Department of Soil Science, in Dr. Teri Balser's lab at the University of Wisconsin-Madison. Field procedure developed by Jake Eaton, Madison Country Day School, Waunakee WI, while working with Dr. Randy Jackson's field team at the University of Wisconsin-Madison.
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