Investigating Freshwater Microbes and Their Role in the Carbon Cycle

Alignment to Essential Standards (grades 8-12)
8.P.1.1,1.3, 1.4
8E1.2, 1.3, 1.4
8L2.1, 8L3.1, 3.2, 3.3,
8.L.5.1
EEn2.4.2, 2.6.2, 2.6.4, 2.7.1
Bio 2.1.1, 4.2
Chm. 2.1.3,.2.2.2 ,.2.2.3

Summary

Recent studies show that the release of carbon dioxide into the atmosphere from freshwaters is more important in the global carbon cycle than previously recognized. The conventional model was that stream and rivers acted as "pipes" passively conveying terrestrial carbon (produced by decomposing plants) to the ocean. The new view is that freshwaters are "hotspots" for the transformation of terrestrial carbon while it makes its long journey from headwater streams to the ocean. Scientists now know that at least 100 million metric tons of CO_2 per year is released into the atmosphere from rivers and streams during terrestrial carbon's journey to the oceans. By comparison, motor vehicles currently emit over 900 million metric tons of CO_2 worldwide each year.

Climate change is a critical environmental issue of our time. Carbon dioxide is a greenhouse gas, and the new view of freshwaters as hotspots for carbon transformations has taught scientists more about how freshwater systems, such as rivers, may fit into the global carbon budget. Dissolved organic matter (DOM) is a critical intermediate in the global carbon cycle because it is the largest pool of reduced organic matter transferred from land to water that has the potential to be oxidized by bacteria and sunlight to CO_2.

Researchers at the UNC Department of Marine Science have conducted studies right here in North Carolina on the Neuse, Tar-Pamlico, Roanoke and Haw Rivers. They examine the source, quantity and availability of organic matter and the extent to which this organic matter is processed by freshwater microbial communities. Researchers at UNC Environmental Science and Engineering, Gillings School of Global Public Health, are studying the release of CO_2 from thawing soils in the Arctic. They examine the photochemical and biochemical interactions that affect DOM and, in turn, the transformation of DOM to atmospheric CO_2.

The following lessons offer classroom and field extensions of this research, providing opportunities for students to study freshwater microbes and gain an understanding of the role of organic matter, the carbon based compounds which come from once living things, in freshwater ecosystems. They are appropriate to incorporate with inquiries into carbon cycling, climate, microbiology, water chemistry, or ecology.

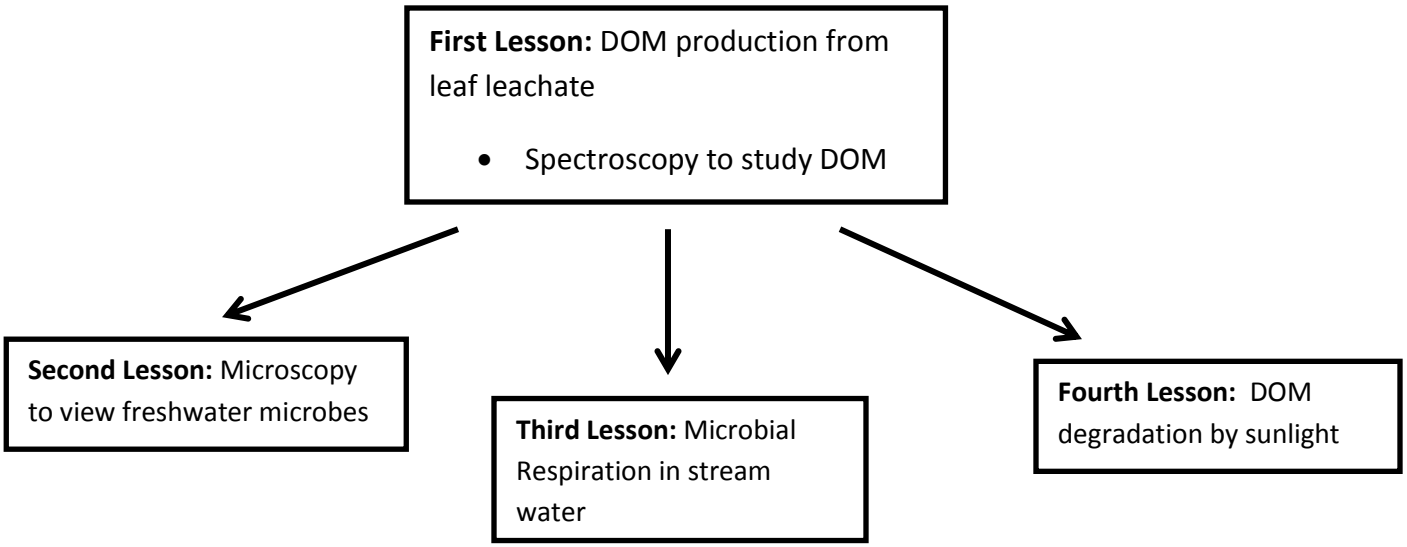
The first lesson introduces students to dissolved organic matter (DOM), and how to simulate and study DOM by creating a "tea" or leachate from leaves. An extension of this lesson involves using spectroscopy to study DOM, a technique that researchers use to quantify the

amount and identify the source of DOM.

In the second lesson, students use microscopy to view freshwater microbes on glass cover slips. Students then determine how DOM influences microbe populations.

In the third lesson, students measure CO_2 production from stream water, which is a measure of microbial respiration. Students then determine how DOM influences respiration rates.

The fourth and final lesson focuses on DOM degradation by sunlight. Students will expose the DOM solutions created in the first lesson to sunlight. Changes in DOM color between light exposed samples and dark controls will be determined.



Investigating Freshwater Microbes and Their Role in the Carbon Cycle

Background

Microbes (or microscopic organisms) make up greater than half of the biomass of our planet. Freshwater ecosystems contain a great diversity of microbes, including bacteria, archaea, fungi and protists. They play critical roles in many biogeochemical processes, such as nitrogen fixation, oxygen production and cycling of nutrients and minerals. While it is know that freshwater microbes process organic matter in streams and rivers, many questions remain in the scientific community about the extent of this role, the species makeup of freshwater microbial communities, how these communities differ with changes in land use and water quality and the degree to which the microbes are active players in these changes. **Bacteria** make up the largest population of river microbes and will be the main focus of this study.

Leaves are a major nutrient source within streams, rivers and other freshwater systems. Bacteria play an important role in breaking down leaves into smaller, **dissolved organic matter (DOM)**. DOM is defined as any organic matter that can pass through a 0.2 micron filter. Anything larger than that is called particulate organic matter (POM). We can more closely examine how bacteria use the dissolved organic matter (DOM) from tree leaves by creating a leaf leachate and feeding this leachate to our bacteria.

Microbes break down large complex DOM into manageable bite sized pieces. DOM would not be available to higher trophic levels in the food web if it were not for the bacteria that use it as a food source and, in turn, become food for other organisms. A large amount of CO_2 is also released in the process. This processing of DOM to integrate it back into the classic food web is called the **microbial loop**.

The importance of organic matter on water quality is widely recognized, but challenges remain in quantifying fluxes of DOM in surface waters and understanding its composition and reactivity. In natural waters, the chemical character of dissolved organic matter depends on its sources, which include degrading plant and soil material delivered from the watershed and breakdown products of bacterial and algal matter in the water column. The quantity and quality of DOM in streams is dynamic, exhibiting short-term fluctuation due to diurnal influence from sunlight or from storm event driven inputs of DOM. Changing biogeochemical processes in the watershed over seasonal time scales are also reflected in DOM quality. Long term patterns of change in response to shifts in climate, land use or other perturbations in the watershed are also evident as changing quantity or quality of DOM, such as the recently reported increasing concentrations of DOM in North American and European freshwaters. Because DOM quantity and quality reflect a dynamic interplay between organic matter sources and biogeochemical processes, changes to water resources from climate, land use, and urbanization are expected to be evident as a shift in the concentration and chemical signatures of DOM.

Dissolved organic matter has long been recognized to be a critical water quality characteristic in forested catchments and is highly relevant to diverse environmental problems. DOM is a major component of the carbon cycle and energy balance in forested catchments and fractions of the DOM pool fuel the food web in aquatic ecosystems. Its key role in metabolism in streams has been demonstrated in environments ranging from the Arctic and temperate forests to southern blackwaters. Colored DOM acts as a sunscreen for aquatic ecosystems, absorbing visible and

ultraviolet radiation. DOM thus mediates photochemical processes, degrading in the presence of sunlight to form various degradation products including CO_2.

How can we study microorganisms and their interaction with DOM in the classroom?

One direct way of examining these microbes is by viewing them with a light microscope. Bacteria can be clearly distinguished from plant and animal cells by the lack of a nucleus. Bacteria are typically about 1-2 microns in size, on the order of magnitude of 10 times smaller than animal cells. However, there is a great variability in bacterial size, and a much broader range of sizes is found in freshwater than in typical laboratory cultures. By floating a glass cover slip in fresh stream water, bacteria will colonize the surface in just a few hours. With 400x magnification, many amazing microbes can be seen. Under laboratory conditions, this population will change over time as bacteria that are more sensitive to their immediate environment succumb to more vigorous species.

Another approach is to look at an action of the bacteria, which in this case is breaking down DOM and releasing CO_2. By measuring the concentration of CO_2 gas released by the water using a CO_2 meter, we have a measure of the *net* CO_2 production. Some CO_2 that is produced may be used in photosynthesis by some microbes in the water, so our measure does not represent the total CO_2 produced.

How can we study the interactions between sunlight and DOM in the classroom?

We can expose DOM to natural sunlight and monitor the loss of color or “fading” of DOM over time. One byproduct of this reaction is CO_2, therefore the more color loss the more CO_2 produced. We can control for dark processes that might cause “fading” by wrapping some DOM samples in opaque materials. We can then calculate “fading” as the difference in DOM color between the light exposed samples and the dark controls.

Lesson 1: Dissolved Organic Matter

Overview

The first lesson introduces students to dissolved organic matter (DOM), and how to simulate a major freshwater nutrient source by creating a "tea" or leachate from leaves. An extension of this lesson involves using spectroscopy to study DOM, a technique that researchers use to measure the quantity and identify the source of DOM.

Learning Objectives

- Describe freshwater organic sources and their potential fates.
- Know the difference between dissolved organic matter and particulate organic matter (POM) and how scientists measure DOM.

Materials

- Dry leaves from trees- ~1 quart of each type
- Stream/river water
- 0.2 micron filter
- filtration device
- Colorimeter
- Glass mason jars to collect samples
- Distilled water
- Sterile containers that can be sealed (mason jars work well for this too)

Activity 1 (2 separate class periods, not including filtration which can take a full day).

1. Through research, discussion and/or a walk along a stream or river, ask the students to list sources of organic matter in a stream or river. How might those sources increase with change in land use?
2. Define dissolved organic matter (DOM) and its importance to the aquatic food web. Look at *Measuring Dissolved and Particulate Organic Carbon* in the resources section at the end of the lesson.
3. Create DOM by collecting different types of dead leaves (oak, poplar, maple, etc. depending on availability) and place each type of leaves in clear glass mason jars. Pour distilled water over leaves, cap, and place in sun for 4-6 hours. It is fine to leave overnight. This will create a leaf leachate or "tea".
4. To remove particulate organic matter, allow the leaves to settle at the bottom of the jars. Then, filter the leachate liquid with a 0.2 micron filter into a sterile container and store sealed and out of direct sunlight. Anything that remains after filtering is considered DOM. The filter process will also remove any bacteria that were clinging to the leaves so that it is ready for the next activity.

Activity 2 (1 hour)

1. Have students characterize the DOM either visually or using a colorimeter or spectrophotometer. The worksheet provides directions for a colorimeter to look at absorbance of DOM at 430nm.
2. Have students research the difference between colorimeter and spectrophotometer and what it means to measure absorbance.
3. This activity is also the first step in lesson 4.

Assessment

Have the students draw a picture of sources of organic matter that may be found in a stream. Have them describe how these various types of organic matter (DOM and POM) may be processed biologically, chemically or mechanically.

Presentation: Have students present their results to the class. What observations have they made? What conclusions can be drawn? What new questions have arisen?

I. DOM Observations

Name:_____
Date:_____

Describe your DOM samples:

II. Absorbance at 430nm

Calibrate the colorimeter with distilled water by filling the cuvette with distilled water, placing it in the colorimeter, and zeroing. Then measure absorbance of each substance, making sure to rinse the cuvette with distilled water in between samples. Record your data below.

Substance	Absorbance at 430 nm

III. Questions

Which substance had the highest absorbance?

_____

Which substance had the highest concentration of DOM?

_____

Lesson 2: Microscopy: viewing freshwater microbes and investigating dissolved organic matter's effects on populations

Overview

In this lesson, students use microscopy to view freshwater microbes on glass cover slips. Students then determine how DOM influences microbe populations. In this study, we are looking at non-pathogenic heterotrophic bacteria. Common classroom studies of bacteria involve identifying cultured bacteria by shape and determining whether they are gram negative or gram positive. Comparing freshwater bacteria with familiar cultured bacteria may be a useful activity. Freshwater tend to be gram negative but have a much larger variety of shape and size than those found in laboratory cultures.

Learning Objectives

- Observe freshwater microbes under varying conditions.
- Identify different forms of bacteria.
- Assess microbe growth against different nutrient media.

Materials

- Compound microscope with 400x total magnification or better
- Glass slides
- Glass coverslips
- Fresh unfiltered river/stream water
- Sterile river/ stream water
- Leachate (DOM) from lesson 1
- Small dishes with loose fitting covers (i.e. petri dishes)
- 0.2 micron filter
- Filtration device

Pre-Activity

Students should become familiar with using a microscope to identify the three common distinct shapes of bacteria: cocci, bacilli and spirilli. (For convenience and review, pre- made slides of these bacteria can be purchased from educational science companies such as Microscope World.)

Investigating Freshwater Microbes and Their Role in the Carbon Cycle

Activity 1 (1-2 hours)

1. Place fresh, unfiltered, river water in a dish on a stable surface (stream or pond water can also be used). Carefully float individual glass cover slips on top of the water overnight. Make sure to use more cover slips than you will need as they are difficult to manipulate and some may be lost in transfer.
2. Create a slide for microscopy by carefully removing a cover slip from the dish by picking it up with forceps or gloved fingers. If you accidentally get water on the back of the slip in this process, dap it off with a chem wipe while still holding the slip with forceps. Place the cover slip (wet side down) on a glass microscope slide, contacting one edge first and gently laying it down to avoid introducing air bubbles.
3. Examine with the microscope at 400x magnification. What do you see? Are bacilli, cocci and spirilli all present? What else is present? Sketch the different types. Roughly tally how many of each you see on each slip and look at these results for the whole class. Does each slip appear to have the same population?

Activity 2 (2 hours)

1. Using a sample of stream water that comes from the same place as the unfiltered fresh stream water (so it has the same osmolarity), sterilize the water using the 0.2 micron filter or any other method of sterilization. The purpose here is to remove any microbes in that sample so they do not get introduced into your experiment.
2. After floating coverslips in river water overnight to obtain microbes as you did in activity 1, remove those coverslips and float in new dishes, one containing 1:3 leachate (DOM) to sterile stream water and one with only sterile stream water.
3. After an overnight incubation, place slips on microscope slides and examine under 400X magnification. How do the slides differ? Are different types of bacteria represented? Does either set contain a larger number of microbes? Which coverslip appeared most to thrive? What happened to microbes without the enrichment of leaf leachate (i.e., the sterile stream water)?
4. Have the students fill out the Microbe Lab worksheet.

Extensions

- Repeat Activity 2 but experiment with different leachate to sterile water ratios. How do the results vary? Is there an 'optimum' concentration for each species? At some point, does the leachate become toxic to the bacteria? Why?

- Experiment with different incubation times. Which type of bacteria becomes the dominant species? What else do you notice?

- Try repeating Activity 2 using DOM created from different sources. How do populations differ?

- Try gram staining your stream bacteria. Do they test positive or negative? Is this typical?

Assessment

Microscopy test: Have students examine a slide. What different bacteria can they identify? What other features of the slide can they point out?

Presentation: Have students present their results to the class. What observations have they made? What conclusions can be drawn? What new questions have arisen?

Write-up: Have students write up this lab using standard scientific methods.

Microbes Lab

Name: _____
Date: _____

Identify 3 distinct types of bacteria on **pre-made slides**:

ID Notes	Drawing
Shape:	
Total magnification of image:	
ID Notes	**Drawing**
Shape:	
Total magnification of image:	
ID Notes	**Drawing**
Shape:	
Total magnification of image:	

Microbes Lab

Name: _____
Date: _____

Identify 2 types of aquatic bacteria on **stream water slides**:

ID Notes	Drawing
Shape:	
Total magnification of image:	
ID Notes	**Drawing**
Shape:	
Total magnification of image:	

Compare your stream microbes to the bacteria from the prepared slides. How are they similar? How are they different?

_____

_____

_____

How can you tell these organisms are bacteria?

_____

_____

Lesson 3: Microbe metabolism: measuring CO_2 levels from river water and how DOM influences microbe respiration rate.

Overview
Students measure CO_2 production from river water, which is a measure of microbe respiration. Students then determine how DOM influences respiration rates.

Learning Objectives
- Describe how microbes create CO_2 through the process of respiration.
- Understand that measuring CO_2 release is a net measure.
- Calculate and compare the rates of CO_2 release.

Materials
- CO_2 probes, incubation chambers, and data mate
- DOM (leachate) created in lesson 1
- River/stream water, unfiltered

Activity (1-2 hours)

1. Calibrate CO_2 sensor with outside air in chamber
2. Add 4 parts fresh, unfiltered river/stream water to one part DOM for each sample.
3. For a control sample, add one part sterile stream water instead of DOM for dilution effect.
4. Measure and graph CO_2 levels over time to view stream microbe respiration with different energy sources.
5. Have students fill out the Stream Microbe Respiration Lab worksheet and then graph the data and determine approximate rate of respiration.

Extension

What freshwater resources do you have on your campus? Collect water from these sources (may be stream, pond, or stormwater sources) and repeat the experiments above. How does the source of water affect what bacteria are present? Have the students think about where the sources of DOM in these local water sources. Are they natural? Natural but enhanced through human activity? Not naturally occurring?

Assessment

Have the students write up their results using standard scientific method.

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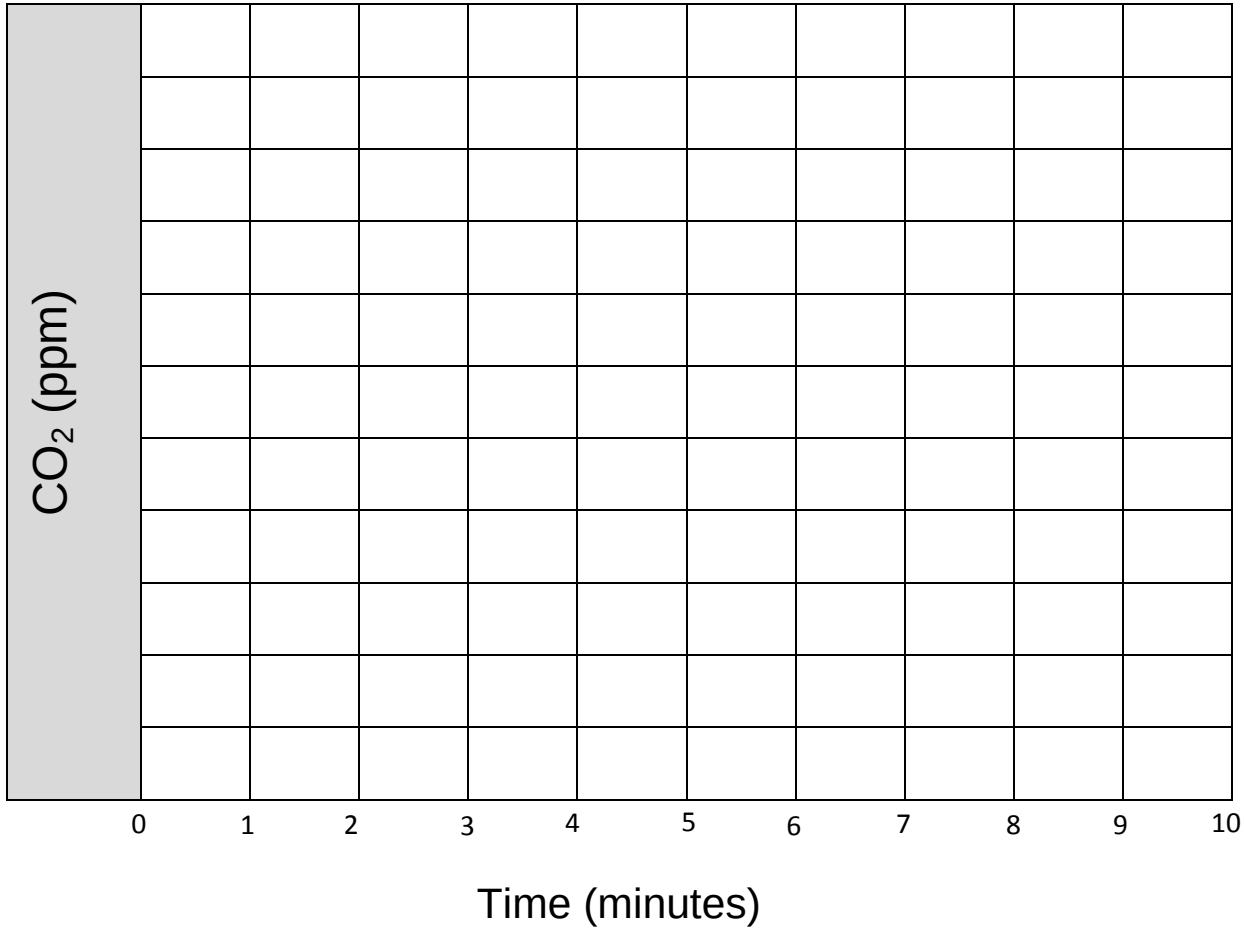
I. Stream Microbe Respiration Lab

Name:_____
Date:_____

A. Stream water

Add 40 mL of stream to bottle, making sure to keep probe dry. Monitor the concentration of CO_2 and record data below. Then graph data and determine approximate rate.

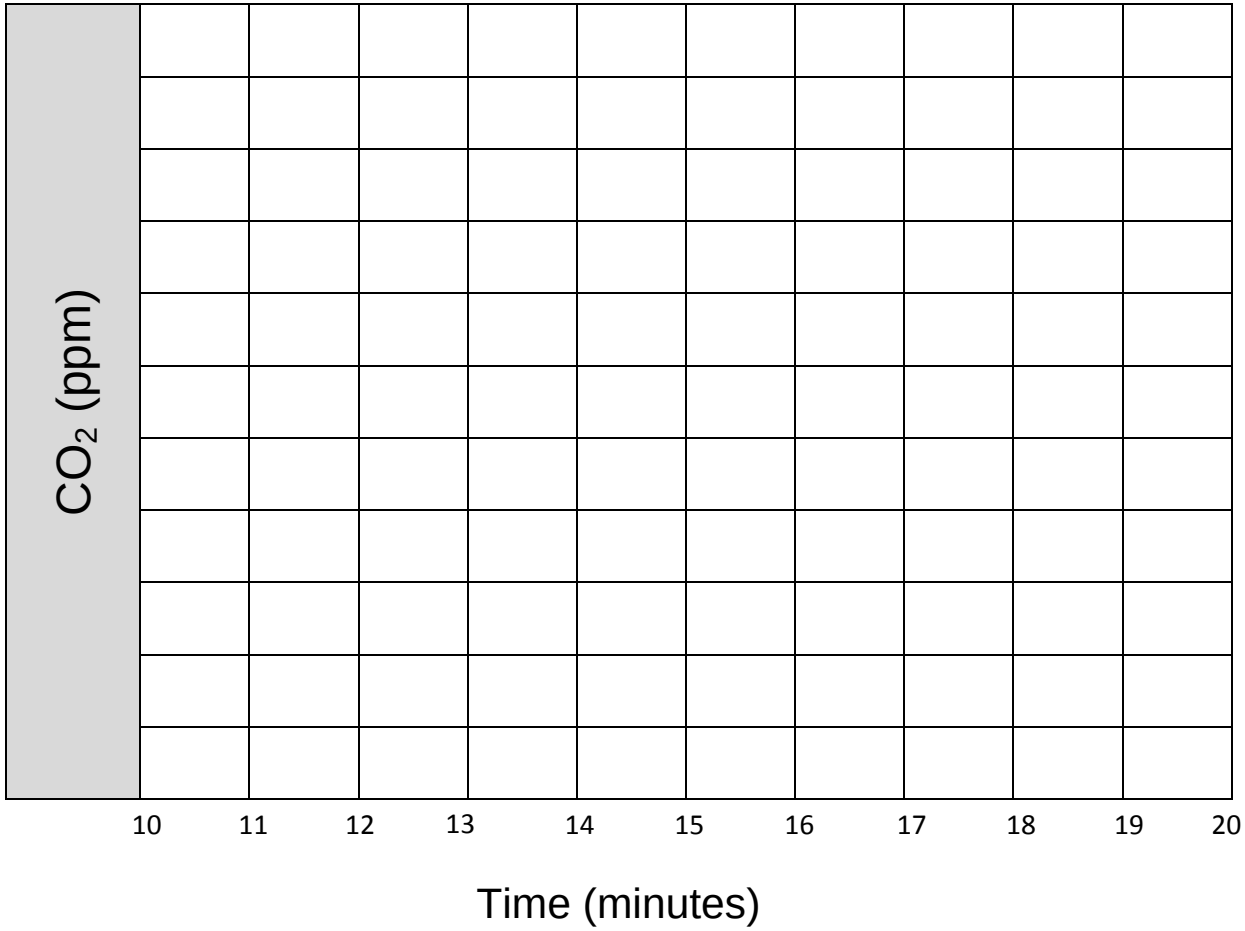
Time (minutes)	CO_2
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	



B. Stream water + DOM

Add 10 mL of DOM to stream water.

Time (minutes)	CO_2
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	



Rate of respiration by stream microbes: Δ [C02] / Δ time = _____

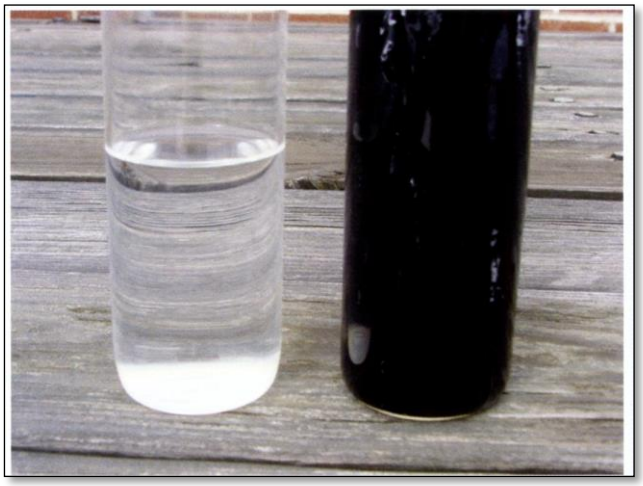
Rate of respiration with added DOM: Δ [C02] / Δ time = _____

Lesson 4: Investigating Sunlight's Role in Breaking Down Dissolved Organic Matter

Overview

Sunlight emits electromagnetic radiation, ranging from lower energy infrared light to higher energy ultra violet (UV) and visible light. The higher energy UV light emitted from the sun initiates a range of photochemical reactions in surface waters that have important implications for aquatic ecology and global carbon cycling. For example, naturally occurring dissolved organic matter (DOM) is the main light-absorbing constituent in natural waters, and acts as "sunscreen" for aquatic organisms by absorbing the harmful UV light. Given that DOM absorbs strongly in the visible portion of the electromagnetic spectrum, it is responsible for the typical light yellow to brown color of stream or lake water.

DOM is a complex mixture of thousands of molecules produced through the breakdown of microorganisms (e.g., algae and bacteria) and terrestrial organic matter (e.g., plants and leaves). DOM derived from the breakdown of microorganisms is generally less complex, smaller, less colored, more susceptible to microbial respiration, and absorbs less sunlight compared to DOM derived from the breakdown of terrestrial organic matter.



The absorption of sunlight by colored DOM results in photochemical reactions that convert DOM to CO_2, a greenhouse gas. This important photochemical reaction between sunlight and DOM can be observed as "fading" or loss of color. For instance, the picture above shows DOM exposed to sunlight (left) and DOM kept in the dark (right).

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The indirect effects of sunlight on biological processes in aquatic environments are not as well recognized as the "sunscreen" properties of DOM. Researchers are now looking at how abiotic photo alterations of organic matter change bacterial function and activity. For instance, sunlight can break down large terrestrially derived molecules of DOM into smaller molecules that are more readily respired by bacteria to CO_2. Alternatively, sunlight and bacteria can "compete" to oxidize the same DOM molecule to CO_2, which can effectively slow or limit bacterial respiration.

In this lesson, the students will make leaf leachate and filter it to create DOM. DOM is defined as any organic matter that can pass through a 0.2 micron filter. The DOM will be exposed to sunlight. The amount of color in both sunlight exposed samples and dark controls will be determined using a colorimeter. Loss of color will be quantified as the difference in color between light exposed samples and dark controls.

Learning Objectives

- Understand the characteristics of DOM, including source and optical properties.
- Understand how sunlight can convert DOM to CO_2.
- Describe how sunlight can influence microbial processing of DOM.
- Design and execute an experiment to quantify sunlight's role in breaking down DOM.
- Critically think about ways human activities may influence microbial and photochemical processing of DOM.

Materials

- Fine mesh if filtration device and filter are not available
- 6 clear glass jars or gallon plastic bags
- Foil
- Colorimeter
- DOM (Leachate from lesson 1)

Note

You will use DOM (leaf leachate) that you made during lesson 1. However, if you were unable to do lesson 1 because you did not have a filtration system, this lesson can be used with leachate made by filtering the leaf water with the finest mesh you can find, such as cheese cloth or coffee filters.

Activity (2 class sessions, 1 week apart)

1. Pour filtered leachate into 6 clear plastic bags or clear glass jars (called containers below).
2. Number each container 1- 6.

3. Wrap 3 of the containers in foil or place in black trash bag to block out light. Leave the other 3 uncovered.
4. Set out all 6 samples in the sun.
5. After 1 week, remove the containers from the sun.
6. Measure a sample from each container using a colorimeter. Set absorbance at 430 nm. This can also be done visually if a colorimeter is not available.
7. Record data and compare light and dark treatments.

Extension

Depending on classroom time, the samples can be measured once after a given number of weeks or can be measured each week, to track the changes over time. The longer the samples are exposed to sunlight, the more change will be noted. Noticeable changes should be expected after one week of sunlight exposure.

Assessment

Write-up: Have students write up this lab using standard scientific methods. This includes answering the Discussion and Critical Thinking questions listed below.

Discussion

Did you see a noticeable change in absorbance over time? If so, how many weeks did it take and explain why you saw a change in absorbance?

Was there any change with the dark controls? If so, what do you think could explain this change?

Critical Thinking

Imagine the city you live in is planning on clear-cutting a local forested watershed to build a shopping center. A stream runs right through the heart of this forested watershed. Using the concepts introduced in this lesson, along with any outside resources, answer the following questions:

1) How would the source, complexity, size, and sunlight absorbing properties of DOM change within the stream?

2) How would the changes in DOM chemistry listed in question 1 influence microbial processing of DOM within the stream?

3) How would the changes in DOM chemistry listed in question 1 influence photochemical processing of DOM within the stream?

4) What if the forested watershed were converted to agricultural fields. Would your answers to questions 1, 2, and 3 change? If so, how?

Remember to cite any outside resources used.

DOM Observation Worksheet

Name_____
Date: _____

Calibrate the colorimeter by filling the cuvette with distilled water, placing it in the colorimeter and making sure the reading is zero. Then measure the absorbance of a sample from each of the six containers. Make sure to rinse the cuvette with distilled water between samples. Record data below. Make additional copies of table for additional weeks of data collection.

Week #	Sample from Container Number #	Absorbance at 430nm
Week #	**Sample from Container Number #**	**Absorbance at 430nm**

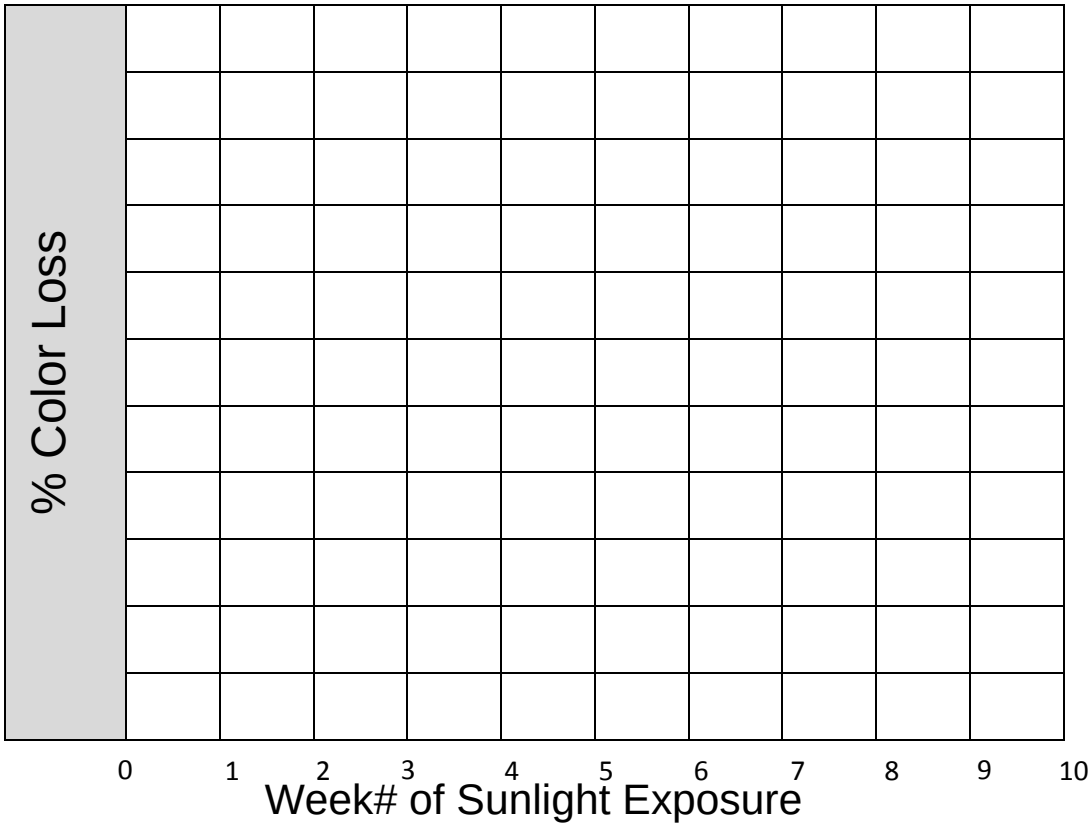
Name: _____

Date: _____

Sunlight Exposure Worksheet

If you took multiple measurements over time, plot time of sunlight exposure on the x-axis and percent color loss on the y-axis. Percent color loss is equal to:

100 ***** ((absorbance of dark control - absorbance of sunlight exposed sample) ***/*** absorbance of dark control)). What sort of relationship do you see?



Resources

Microbial Life Educational Resources: Measuring Dissolved and Particulate Organic Carbon (DOC and POC)
http://serc.carleton.edu/microbelife/research_methods/biogeochemical/organic_carbon.html

Microscope viewing of bacteria:
http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/wimsmall/smal1.html

Techniques for identifying bacteria:
http://en.wikipedia.org/wiki/Bacterial_taxonomy
http://webcache.googleusercontent.com/search?q=cache:OxxCt6PFQFoJ:www.mona.uwi.edu/biochem/courses/bc34m/documents/microbe_identification.ppt+microbe+identification+powerpoint&cd=2&hl=en&ct=clnk&gl=us&client=safari&source=www.google.com

Understanding roles of bacteria in the environment:
http://en.wikipedia.org/wiki/Environmental_microbiology

Leaves as a nutrient source for rivers:
http://www.waterencyclopedia.com/La-Mi/Microbes-in-Lakes-and-Streams.html

Small Freshwater Organisms
http://www.funsci.com/fun3_en/guide/guide1/micro1_en.htm

Ecology, 81(12), 2000, pp. 3445–3463 Organic Matter Flow in Stream Food Webs with Reduced Detrital Resource Base. Robert O. Hall, Jr. et. al.
http://www.uwyo.edu/bhall/reprints/hall%20et%20al.%202000.pdf

Nature Geoscience, 4, 2011 pp. 839-842. Significant efflux of carbon dioxide from streams and rivers in the United States, Butman and RaymondJournal name:
http://www.nature.com/ngeo/journal/v4/n12/full/ngeo1294.html

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