**Climate Change Toolkit**

**The Carbon Cycle: Respiration**

**Standards**

- NGSS ESS2.E Biogeology
- NGSS ESS3.D Global climate change
- NGSS LS2.B Cycles of matter & energy transfer in ecosystems

**Grade Level:** High School/Middle School

**Equipment:**

- Outdoor Experiment: Appropriate field site - a bare soil or grassy space works well
- Indoor Experiment: Small flowerpots filled with biologically active soil (not sterile potting soil); 1 or 2 per group.
- Indoor Experiment (optional): a way to chill or warm the soil pots; bags of ice, a refrigerator, a hot plate or warming mat, etc.
- One-liter soil flux chambers (1 or 2 per group)
- Duct tape (or similar, to make an opaque chamber)
- Lab CO₂ probes and necessary hardware/software for probes
- Student lab handout (1 per student)
- Soil thermometers (optional)

**Introduction**

Students measure the flux of carbon dioxide from soil to the atmosphere using a lab CO₂ probe and home-made flux chambers. Simple graphing and applying a linear fit to the data incorporate numeracy skills in this activity.

**Why?**

Photosynthesis transfers carbon dioxide from the atmosphere to plants, releasing oxygen in the process. Respiration runs this reaction backwards, releasing CO₂ while consuming oxygen.

\[
C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O
\]

\[\text{glucose + oxygen = carbon dioxide + water}\]

Globally, photosynthesis removes 120 Pg (120 billion metric tons) of carbon every year from Earth’s atmosphere and turns it into the carbohydrates that make up living plants. Respiration and decay within ecosystems return that same amount of carbon to the atmosphere every year. We can quantitatively measure this flux in any outdoor environment, and compare it with anthropogenic carbon fluxes. Global climate change - especially rising temperature - can perturb the carbon cycle in both the short and long term.
**What?**
The movement of carbon dioxide from the atmosphere into biomass is part of the global carbon cycle (Figure 1).

- On very long timescales (thousands to millions of years) the geologic carbon cycle moves carbon from the Earth’s mantle and crustal rocks into the atmosphere through volcanic eruptions. Carbon is removed from the atmosphere by chemical reactions that weather and decompose rocks.
- On shorter timescales carbon moves from biomass to the atmosphere and back via photosynthesis and respiration.
- In the ocean carbon also cycles to the atmosphere and back through exchange with sea water.

Each of these three parts of the carbon cycle runs in a steady state, with all the inputs and outputs in balance. The fluxes can be very large, but as long as they are all balanced there is no overall accumulation in any one place. In contrast, the human contribution to the carbon cycle is one-way: humans burn fossil fuels and biomass releasing CO$_2$ to the atmosphere. There is no equivalent return process, thus humans upset the natural balance.

*Figure 1: This diagram of the fast (non-geologic) carbon cycle shows the movement of carbon between land, atmosphere, and oceans. Yellow numbers are natural fluxes, and red are human contributions in gigatons of carbon per year. White numbers indicate stored carbon.*

https://earthobservatory.nasa.gov/Features/CarbonCycle/images/carbon_cycle.jpg
Figure 2: The geologic carbon cycle. The terrestrial and marine carbon cycles from Figure 1, above, are placed within the plate tectonic setting of the geologic carbon cycle. Geologic processes are slower, but move many more gigatons tons of carbon (units are the same as above).

**How?**
We will measure the respiration and decomposition flux from the soil using a CO₂ probe. This can be done outdoors, or indoors in a terrarium or flowerpot. From these data we can estimate the contribution of our local environment to the global carbon cycle, or explore the change in respiration rate with changing temperature.

**PART 1 - BUILD THE EXPERIMENTAL COMPONENTS**
Make a plastic juice bottle flux chamber. The flux chamber will control the movement of CO₂ gas between the soil and probe, and allow us to make quantitative measurements. Choose a 2-quart (or similar) plastic juice bottle with a wide enough mouth to fit the CO₂ probe. Pour in one liter of water (that you've pre-measured in a beaker or graduated cylinder). With the cap on, turn the bottle upside down and draw a line at the one liter mark. Discard the water and cut off the bottom of the juice bottle evenly, just below the one liter line.
This will allow us to push the flux chamber a little bit into the soil to give it some stability during our measurements. Measure the cross-sectional area of the opening at the bottom of the bottle (length x width for a rectangular bottle; or πr² for circular). Measure in meters and record the value (or measure in cm and convert to meters: 1 cm = 0.01 m). Finally, cover the bottle with duct tape (or paint, or any other opaque material so that light does not pass through the bottle).

**PART 2 – SET UP AND RUN THE EXPERIMENT**

Decide if your students will work indoors or outdoors. The simplest version of this experiment - one flux chamber on a patch of bare soil - is the same in both indoor and outdoor environments. But each setting offers different opportunities to ask additional questions. For example, working indoors with small flowerpots allows students to manipulate some of the environmental parameters that control respiration, like temperature and moisture. Working outdoors, students can investigate the balance between photosynthesis and respiration. Instructions for both indoor/outdoor environments are given here.

If working outdoors, select a field site – any place with soil should work – a grassy field, forest, or park. An indoor experiment is best done in small pots of soil that fit well with your flux bottles (ex. Figure 6). Prep your CO₂ probe as you would for any experiment (set up computer interface, etc.). We will record CO₂ values at a time interval of 10 seconds over an experimental run of about 5-10 minutes.

- Remove the cap from the juice bottle flux chamber and insert the probe into the top of the bottle. The probe should fit loosely; make sure there is an air gap that will allow the CO₂ to flux through and out of the chamber. Allow a few minutes for the probe to equilibrate.
- Press the flux chamber into the soil so that the one-liter line is at ground level.
- Collect data for 5 – 15 minutes. Note that at the beginning of the experiment the CO₂ data may be noisy. If so, when analyzing the data ignore these first few minutes of measurements.

(Figure 3 caption below)
Figure 3: (Left) Sample data; a time series of carbon dioxide measurements. Note that data are noisy at the beginning of the experiment. For these data the slope is calculated only from 4-18 minutes. For any data set, look for a consistent increase early in the experiment; these will be the most characteristic data, unaffected by later CO₂ buildup in the chamber. (Right) Calculation of rate of change for CO₂ data. Students can measure the slope by hand or by using the curve fitting function in graphing software.

Note: protect the experiment from wind, as wind blowing across the top of the chamber disturbs the accumulation rate of CO₂.

PART 3 – ANALYZE THE DATA

The data collected by the CO₂ probe is in units of concentration – parts per million. It is often more useful to work in units of mass; grams, kilograms or metric tons of CO₂. If students compare their own measurements to those published in the literature they will certainly have to do some unit conversion. For example, in Figure 1 the fluxes are given as gigatons of carbon per m² per year. In the chart below we will convert the measured concentration flux into a mass flux of CO₂ – the mass of CO₂ per unit volume per unit time. We will use metric (mks) units, so we should end up with kg/s (although in this case its OK to use minutes if the probe was set up that way).

- Graph the data: time (min or sec) on the X-axis, and CO₂ (ppmv) on the Y-axis. To calculate the flux we will find the rate of change of the CO₂ concentration in the one-liter flask measured over a fixed interval of time (Figure 2).
- Draw a line – or use a computer to fit a straight line – through your data and calculate the slope. This is the flux rate in units of ppmv/time.

NOTE: For students who have not yet encountered moles as units of molecular mass, the simple rate calculation can substitute for the mass flux, and can be used to compare the behavior of different systems.

UNITS IN ATMOSPHERIC CHEMISTRY

Units and unit conversion are often tricky and always important. Also tricky is the fact that the units used in atmospheric chemistry might be unfamiliar to students.

Carbon dioxide is a trace gas in the atmosphere and its concentration is most commonly measured in parts per million by volume (ppmv). The current concentration of atmospheric CO₂ is 420 ppmv. Every million molecules of air contain 420 molecules of CO₂. In contrast, carbon dioxide emissions are usually reported in gigatons (Gt) = one billion metric tons (1 metric ton = 1000kg). A gigaton is also a petagram (Pg): 10¹⁵ grams. In order to convert units from concentration (ppmv) to mass (kg) students will first need to convert their measured volume to moles of CO₂ gas, and then convert moles to kilograms. -

An important note: atmospheric chemistry differs from aquatic chemistry, where a concentration of 1 ppm = 1 mg/liter. Because gases can easily expand in volume, this relationship is **not** true for gas concentrations in the atmosphere. This is why we specify ppmv, and why we have to first convert gases to moles in order to find mass.
Flux is an extensive property; one that changes with the size of the system. For example, the longer we observe, the more CO\textsubscript{2} moves through the flux chamber, or, the larger the chamber the more CO\textsubscript{2} will move through it. Therefore we need to normalize our measurement with respect to both time and the cross-sectional area of the flux chamber. The rate calculation already accounts for the time of observation. We still need to account for the basal area of the flux chamber.

**UNIT CONVERSION FROM CONCENTRATION TO MASS FLUX OF CO\textsubscript{2}**

<table>
<thead>
<tr>
<th>Steps in Unit Conversion</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Calculate the cross-sectional area of the flux chamber in units of square meters.</td>
<td>m\textsuperscript{2}</td>
</tr>
<tr>
<td>2. Recall that at standard temperature and pressure (STP) one mole of gas occupies a volume of 22.4 liters. Invert this quantity to find the number of moles in a single liter of gas (e.g. the size of our flux chamber).</td>
<td>mol</td>
</tr>
<tr>
<td>3. From the graphed data, calculate the rate of change of CO\textsubscript{2} in the flask in ppmv/second.</td>
<td>ppmv/s</td>
</tr>
<tr>
<td>4. Convert this rate of change from ppmv to its decimal equivalent (to do this, divide the rate by 1,000,000). This allows us to use the rate to calculate mass.</td>
<td></td>
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<tr>
<td>5. Multiply the rate from Step 4 by the number of moles from Step 2, then divide by the cross-sectional area of the flux chamber from Step 1. The result is the flux of CO\textsubscript{2} in units of mol/m\textsuperscript{2}/s.</td>
<td>mol/m\textsuperscript{2}/s</td>
</tr>
<tr>
<td>6. The mass of CO\textsubscript{2} is 44 g/mol (C=12, O=16). Multiply the result from Step 5 by 44 g/mol to convert the flux to units of g/m\textsuperscript{2}/s.</td>
<td>gCO\textsubscript{2}/m\textsuperscript{2}/s</td>
</tr>
<tr>
<td>7. This result is what we were looking for: the mass flux of CO\textsubscript{2} per unit area per unit time. In order to compare our result with published data, there are most likely more unit conversions to do – but simpler ones – converting g to kg or minutes to years. See below.</td>
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PART 4: ADDITIONAL EXPERIMENTS

The simple experiment allows students to measure the respiration flux of CO₂ from a small patch of soil. This result is much more interesting when considered in context. For example, students who worked outdoors might want to compare their measured flux to globally mapped respiration fluxes (Figure 5), or consider a land surface where both photosynthesis and respiration are important (see below). Students working indoors have the opportunity to manipulate the environment of their soil experiments - for example by changing the temperature or moisture content of their soil pots. Two sets of additional experiments are described below, (1) a paired photosynthesis experiment in an outdoor environment, and (2) a heating/cooling experiment for an indoor setting.

4A: OUTDOORS: A PAIRED RESPIRATION/PHOTOSYNTHESIS EXPERIMENT

On a grassy outdoor site both photosynthesis and respiration contribute to the CO₂ flux. Here students can set up a paired experiment with an opaque chamber and a clear chamber. The darkness of the opaque chamber suppresses the photosynthesis of the grass, allowing students to measure the respiration flux and then compare it to the result from the clear chamber. This experiment will work on a cloudy day, but the results are more dramatic when the sun is out.

- Set up and run the experiment as before, this time placing the clear and opaque chambers side-by-side in the grass, pressing them down firmly so that the lower edges of the chambers are in contact with the soil, not just sitting loosely on the grass. Note: if there are not enough CO₂ probes to measure CO₂ simultaneously the experiments can be run sequentially.
- Compare the respiration rates measured in the two chambers.

The respiration flux in the opaque chamber is the soil respiration flux. In the clear chamber the same soil respiration occurs, but the measured concentration will be impacted by the rate of photosynthesis of the grass. This may be high or low, depending on the health and condition of the grass, thus the slope of the CO₂ curve can be quite variable. The clear chamber measurements record the net sum of two competing processes (in Figure 4 below, they are exactly balanced).

Figure 4 caption below
Figure 4: A paired respiration/photosynthesis experiment. Clear and opaque flux chambers are placed side-by-side in a grassy lawn. Data is collected simultaneously in both chambers. The CO$_2$ concentration in the opaque chamber is shown on the left-hand graph, in orange; data from the clear chamber is shown on the right-hand graph, in green. The photosynthesis data (center) was collected separately.

For additional context, student data collected in an outdoor environment can be compared with maps of regional or global respiration CO$_2$ flux (Figure 5). These data are the average annual soil fluxes in units of megagrams of carbon (not CO$_2$) per square kilometer per year, so for direct comparison students will need to do additional unit conversion:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Formula</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.</td>
<td>First change grams of CO$_2$ to grams of carbon. The fraction of C in CO$_2$ is 12/44 (mass of CO$_2$=44, mass of C=12). Multiply your result in Step 6 by $(12/44)$.</td>
<td>gC/m$^2$/s</td>
<td></td>
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<tr>
<td>9.</td>
<td>Convert the time from seconds to years. $1\text{yr} = (365\text{days})(24\text{hrs})(60\text{min})(60\text{sec})$.</td>
<td>gC/m$^2$/yr</td>
<td></td>
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<tr>
<td>10.</td>
<td>Convert m$^2$ to km$^2$ ($1\text{km}=1000\text{m}$)</td>
<td>gC/km$^2$/yr</td>
<td></td>
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<tr>
<td>11.</td>
<td>Convert g to Mg ($1\text{Mg}=1,000,000g$)</td>
<td>MgC/km$^2$/yr</td>
<td></td>
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</tbody>
</table>

Figure 5: Global mean annual soil carbon flux (MgC/km$^2$/yr), Raich et al., 2003 (https://cdiac.ess-dive.lbl.gov/epubs/ndp/ndp081/map_yrmn.html)
Questions: Locate the place you made your measurement on the map (approximately).

1. How does your result compare? Is it the same/higher/lower?
2. Since the map shows annual values, how might your result be different if you could make measurements all year long?
3. Examine the map. Where are C fluxes high (generally)? Where are they low?
4. What conclusions can you draw about how environmental conditions control the rates of soil respiration and decomposition?
5. If climate is warming globally, how might that change these results several decades into the future?
6. Look again at the global carbon cycle in Figure 1. Consider the three terrestrial reservoirs for carbon:
   - Atmosphere 800 Gt
   - Biosphere 550 Gt
   - Soil 2300 Gt
   Make a hypothesis that predicts how the rate of respiration will change in a global warming scenario, and how that will impact the size of the Atmosphere and Soil reservoirs for carbon.

4B: Indoors: The relationship between temperature and respiration

Using the pots of soil in the indoor experiment, students can manipulate the temperature of the soil to examine the temperature control on respiration. This can be done two ways:

1. A side-by-side experiment with one heated pot and one chilled pot (ex, use a water bath to control temperature, making sure that the pots are sealed and cannot become water-logged)
2. A sequential experiment with one pot that begins chilled and is then warmed (as would happen during the course of a day, or seasonally)

The second protocol is described here. Store the soil pots in a refrigerator until ready for use. Have all the experimental apparatus ready to go, as the soil samples will begin to warm as soon as they are removed from the fridge. If soil thermometers are available (or any metal-stemmed thermometer) they can be placed in the pots - outside the flux chamber - to monitor the soil temperature throughout the experiment (Figure 6).

- Remove pots from fridge and immediately measure the CO2 flux, ca. 10 minutes (pots can be wrapped in a dish towel or other insulator to slow the initial rate of warming).
- After 10 minutes place the pots on a hot plate (or in a hot water bath; make sure pots are sealed so water cannot enter) and gently heat for 10 minutes, measuring the CO2 flux while heating.
- Optional; heat for an additional 10 minutes while continuing to measure CO2.
- Graph the data. This can be done as two separate graphs for heating and chilling, or as a single time series for the entire length of the experiment.
- Find the slope of the change in CO2 concentration as a function of time for the chilled soil, and separately find the slope for the warmed soil.
Figure 6: A 30-minute experiment where a chilled soil sample is removed from the refrigerator and allowed to sit at room temp for 10 minutes while CO₂ concentration is measured. A soil thermometer in the pot records the change in temperature over this interval, from 5°C to 8°C. The sample is then warmed on a hot plate for 20 minutes, increasing the soil temperature to 40°C. The slope is calculated in 5-minute increments, showing the increasing respiration rate with increasing temperature. In this experiment the first 5 minutes of CO₂ data are noisy, and thus not used in the rate comparison.

PART 5: DISCUSSION

1. Are there any differences in respiration rate when the soil is warm or cold? Describe.
2. Look at the map of global soil respiration (Figure X). Is there a relationship between your heating/chilling experiments and the pattern on the map? Describe.
3. If climate is warming globally, how might that change these results several decades into the future?
4. Look again at the global carbon cycle in Figure 1. Consider the three terrestrial reservoirs for carbon:
   - Atmosphere 800 Gt
   - Biosphere 550 Gt
   - Soil 2300 Gt
Make a hypothesis that predicts how the rate of respiration will change in a global warming scenario, and how that will impact the size of the Atmosphere and Soil reservoirs for carbon.
There are a variety of conclusions that students can draw from their respiration experiments. The simplest might be to think about why we store perishable food in the fridge?

Beyond that, these experiments have implications for the global carbon cycle, and particularly for our climate as the Earth warms. The terrestrial soil carbon reservoir is much larger than the carbon reservoir of terrestrial biomass and that atmospheric carbon reservoir. Increased global temperatures lead to increased soil respiration rates that will transfer carbon from soil to the atmosphere. This is an example of a "positive" or reinforcing feedback. Warmer temperatures lead to faster transfer of CO₂ from soil to the atmosphere, where it causes increased warming, which reinforces the faster respiration, etc. The fact that the soil reservoir is almost three times as large as the atmosphere means that the effect of this feedback is potentially very large.

If this conclusion is a source of anxiety for students, encourage them to think about photosynthesis (especially if they have done the paired outdoor experiment) and how simple actions like planting trees actually can have a real impact on climate.