Soil Respiration Partitioning with Stable Carbon Isotopic Probing Technique

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## A. Overview Information

**TITLE:** Soil Respiration Partitioning with Stable Carbon Isotopic Probing Technique

**ABSTRACT:** Students investigate how to partition soil respiration that is emitted from the mixed soil and litter materials using a Picarro G2131-*i* isotopic C analyzer in a lab experiment. Students in small groups design and conduct their own experiments to investigate the effects of soil warming on soil $CO_2$ emission and partition using stable carbon isotopic ($^{13}C$) mixing model. The projects contains two sessions. During the first session students learn the theory of the measurements and conduct soil respiration measurements. During the second session, students download and analyze data, and write a lab report as homework regarding the altered microbial substrate preference with soil warming.

**KEYWORD DESCRIPTORS**

- **Ecological Topic Keywords:** microbial respiration, substrate type, carbon dioxide, stable isotope, abiotic factors, biotic factors, carbon cycle, climate change, decomposition, greenhouse effect, global warming, microorganisms, soil temperature, soil moisture, soil respiration
- **Science Methodological Skills Keywords:** data collection and analysis, experimental design, field work, formulating hypotheses, graphing data, identify biotic-abiotic interactions, library research, scientific writing
- **Pedagogical Methods Keywords:** cooperative learning, group work assessment, guided inquiry, open-ended inquiry, peer evaluation, project-based teaching, rubric

**Class Time:** One 1-hour lecture and one 2-hour lab sessions
Outside of Class Time: Students will spend 5 to 6 hours, primarily writing up the associated draft and final lab reports.

**STUDENT PRODUCTS:** Group Experimental Design (1 page). Lab report (3-5 pages)

**SETTING:** This experiment can be used to partition different soils and litter types.

**COURSE CONTEXT:** This experiment can be used in a freshman-level introductory Environmental Science course and in an upper-level Ecology course.

**INSTITUTION:** Four-year, public, primarily undergraduate institution.

**TRANSFERABILITY:** This lab requires Piccaro G2131-*i* isotopic C analyzer equipment. With this equipment, this lab is very flexible and is easily translatable to larger or smaller class sizes and to non-majors classes. It can be adapted for measuring soils from cropland, garden and wetland, and litter materials from crop, tree and shrub. It can be used indoors or in a greenhouse by creating artificial soils in a plant tray or bin. The indoor setting gives experimenters greater control over environmental variables and allows them to manipulate the soil composition.

**ACKNOWLEDGEMENTS:** The original lab module was designed by Dr. Jeffrey A. Simmons using the soda-lime method to measure soil respiration. It was modified to use the Piccaro G2131-*i* isotopic C analyzer for soil respiration and stable isotopic measurements. Funding for development and testing of the original lab module was provided through an award from the NSF TUES/CCLI Program (#DUE-0410577) to J. Simmons.

## B. Synopsis of Lab Module

**Principal Ecological Question Addressed:** How does soil warming influence the partition of respiratory $CO_2$ derived from soil and amended litter material?

**What Happens:** Before the lab meets, students read about decomposition, the global carbon cycle, and how the Piccaro2131-*i* isotopic C analyzer can be used to measure soil respiration and $\delta^{13}C$ of $CO_2$. At the lab session, students will learn to operate the equipment and collaboratively design their own experiment that will examine the influence of a single environmental factor (i.e. temperature) on the partition of respiratory $CO_2$ emission derived from soil and litter. Based on the data collected, students use analysis of variance (ANOVA) to statistically analyze their results, and as homework write a lab report.

**Objectives:** At the end of this lab exercise students will be able to:

1. Explain how soil temperature can affect the partition of respiratory $CO_2$ emission derived from soil and amended litter material;
2. Use the scientific method appropriately to answer a question, including generating hypotheses, designing an experiment, and statistically analyzing data;
3. Clearly communicate scientific results in writing and in the appropriate format

**Equipment/ Logistics Required:**

- Piccaro G2131-*i* isotopic C analyzer
- Analyzer and jar connector (made in lab)
- Mason jars (10)
- Two incubators (Fisher Scientific) for laboratory warming experiments

- PVC made incubation vessel (10), diameter 2 inches and height 3 inches
- Glass balls (100)

**Summary of What is Due:**

- An Experimental Design written by student groups
- A formal, 3 to 5 page Lab Report written by individuals

## C. Description of the Lab Activity

### Introduction

Soil warming typically accelerates soil organic matter (SOM) decomposition and associated rates of $CO_2$ flux to the atmosphere. A 4 to 7°C increase in mean annual temperature (MAT) is expected this century in high-latitude ecosystems (IPCC 2013). Even a relatively small change in respiratory losses from the large soil organic carbon (SOC) stocks could potentially influence atmospheric $CO_2$ concentrations (Figure 1). Therefore, quantifying the potential vulnerability of this significant carbon pool to decomposition with warming is important for accurate predictions of SOM feedbacks to climate change.

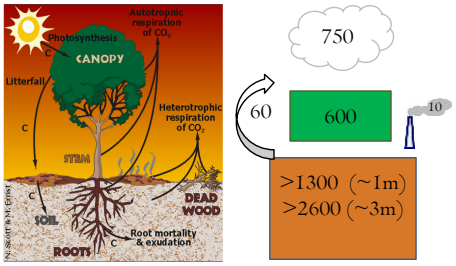


**Figure 1.** Terrestrial carbon cycling and the carbon stock (Pg) in each component.

Soil carbon decomposition is part of the global carbon cycle. Organic carbon in plants is transferred to the soil when plants shed their leaves or when they die. Decomposers then begin their work of breaking down the organic matter (Figure 2). Some of the organic carbon in the organic matter is converted into $CO_2$ which is ultimately released into the atmosphere. Most of organic carbon is transformed into soil organic matter pool ("humus"), dark in color and slow turnover. Over time, soil organic carbon is comprised of many different compounds exhibiting varying degrees of recalcitrance and turnover times (Trumbore 2009). Simply put, they can be categorized into two pools: a fast-cycling and a slow-cycling pool. An example for a fast-cycling compound is glucose and the example for a slow-cycling compound is lignin. The slow-cycling compounds usually contain longer and more complexed carbon chains than fast-cycling compounds, and are more resistant to microbial decomposition.

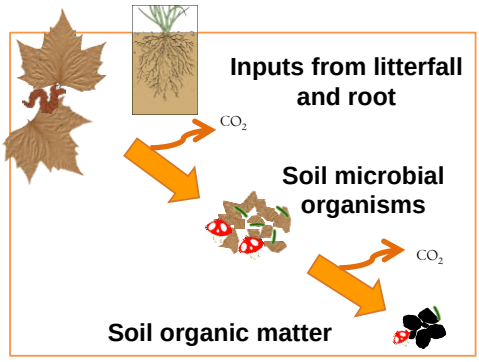


**Figure 2.** Soil organic carbon decomposition and $CO_2$ emission

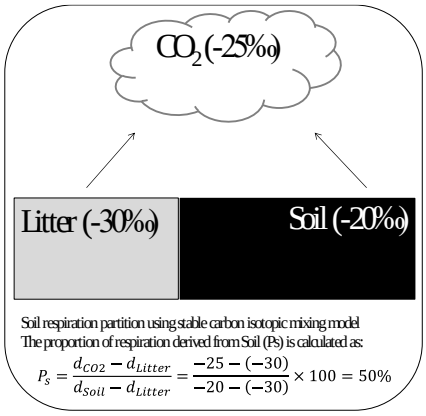


**Figure 3.** Stable carbon isotopic mixing model

The slow-cycling compounds are more $^{13}C$ enriched relative to the fast-cycling compounds because lighter C isotope ($^{12}C$) is more preferentially respired by microbes and released as $CO_2$ (Schlesinger and Bernhardt 2013). The Arrhenius equation supported that the slow-cycling and recalcitrant compounds are more sensitive to temperature than fast-cycling and more labile compounds (Davidson and Janssens 2006). However, if microbial substrate availability is hindered by dwindling supply or limited soil moisture content and associated diffusion of substrates to enzymatic reaction sites, heterotrophic respiration may not exhibit the temperature sensitivity. Given $^{13}C$ natural abundance in soil and litter materials, a stable isotopic mixing model (Figure 3) can be used to examine the temperature sensitivity of different compounds via partitioning the respiration derived from soil and added litter material (Li et al. 2012). This work and others alike helped achieve accurate predictions of SOM feedbacks to climate change. In this exercise, you will investigate the effects of temperature on partition of respiratory $CO_2$ emission derived from soil and litter material amended.

A novel method for measuring soil respiration and simultaneously $\delta^{13}C$ of respiratory $CO_2$ is using the Piccaro G2131-*i* carbon analyzer. The sophisticated instrument applied the Cavity Ring-Down Spectroscopy (CRDS, Figure 4). Nearly every small gas-phase molecule (e.g., $CO_2$, $H_2O$, $H_2S$, $NH_3$) has a unique near-infrared absorption spectrum. At sub-atmospheric pressure, this consists of a series of narrow, well-resolved, sharp lines, each at a characteristic wavelength. Because these lines are well-spaced and their wavelength is well-known, the concentration of any species can be determined by measuring the strength of this absorption, i.e. the height of a specific absorption peak. But, in conventional infrared spectrometers, trace gases provide far too little absorption to measure, typically limiting sensitivity to the parts per million at best. CRDS avoids this sensitivity limitation by using an effective pathlength of many kilometers (Figure 3). It enables gases to be monitored in seconds or less at the parts per billion level, and some gases at the parts per trillion level. The data was recorded and can be processed by Excel for deriving the $CO_2$ concentration and $\delta^{13}C$ of $CO_2$ based on a large volume of high-frequency measurements.

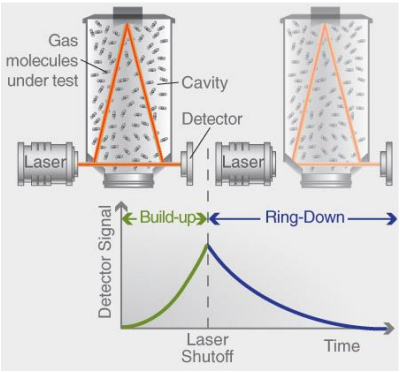

**Figure 4.** Cavity Ring-Down Spectroscopy (CRDS)

## Materials and Methods

### Research Sites:

There are three different types of soil samples and one litter material that were collected from two research sites, one at the TSU campus farm and another at the Cheatham TSU farm. At the TSU campus farm, soil samples were collected from the switchgrass and gamagrass research plots and the litter material was collected from a nearby switchgrass plot by compositing the aboveground tissues and stems of three individual plants. The $\delta^{13}C$ of the three soil samples and litter material have been analyzed

outside of TSU and were already known and available for the exercise. With your Instructor, choose one soil type and the litter material. You need to obtain three soil samples from the soil type that you selected. Depending on your experimental question you may want contrasting sites with different plant type or different location.

**Overview of Data Collection and Analysis Methods**

**Lab Session I:**

1. Learn to use the Piccaro G2131-*i* Carbon Analyzer equipment for soil respiration and isotopic measurements. (The equipment will be turned on at least one hour ago prior to the class.)
2. Choose to join one group. Each group has incubation jars to measure under one incubation temperature (15 ℃ or 20 ℃).
3. Bring the mason jar from one incubator (already incubated for a few days) and connect with the analyzer.
4. In small groups design your experiment. You will be comparing the rate of soil respiration of jars collected from two incubators set at different temperatures.
5. Each student will pick his/her jar and record the jar number.
6. Place the mason jar in connection with the analyzer and screw tight until no move of the lid. Put the jar positioned vertically with proper arrangement of jar head and analyzer connector;
7. Due to limited time, each group can only measure one jar during lab session (~45 min per jar). The instructor's research assistant will measure the rest jars and send data to students.
8. Fill up the Measurement Log attached below during the lab session so that the information on the log will be used for data analysis.
9. Connect the jar with instrument and start the measurement. Each measurement lasts about 45 minutes. Observe the real-time $CO_2$ and $\delta^{13}C$ curve during the measurement.
10. Disconnect the jar after measurement is complete.

**Homework:**

Write a lab report using the proper format. Your Instructor will assign a due date for the lab report.

**Data Analysis:**

1. Open the data file in excel. Then find out the time period of measurement (measurement start time to measurement end time) and copy data of measurement period with their column names to a new sheet.
2. Insert two new columns at the right end of the table and name them as "$CO_2$ total concentration" and "1/ total $CO_2$", respectively. Calculate these two columns as followings, $CO_2$ total concentration = X12CO2_dry (Column T) + X13CO2_dry (Column V). 1/ total $CO_2$=1/ $CO_2$ total concentration.
3. Draw a scatter plot using "Time" (column B) as x-axis and "$CO_2$ total concentration" as y-axis. Then find the calculation period in this plot when there is a straight line (as long as possible, Figure 5).
4. Remove the data beyond the calculation period and plot another scatter plot using "$CO_2$ total concentration" as y-axis and "FRAC_HRS_SINCE_JAN1" (column D) as x-axis. Derive the slope of the line by right click the line and click "Add trendline" then select "Display equation on chart". The slope is the value before x. The respiration rate (mg $CO_2 \cdot h^{-1} \cdot g\ soil^{-1}$) = slope*0.000127

5. Plot a second scatter plot using "Delta_Raw " (Column Y) as y-axis and "1/total $CO_2$" as x-axis. Derive the equation of the line with same method described above. Record the intercept of the equation and it is the wanted value of $\delta^{13}C$.
6. Compare your result with other group members. Calculate the average respiration rate and $\delta^{13}C$ of each group. Compare the averages of your group with another group.

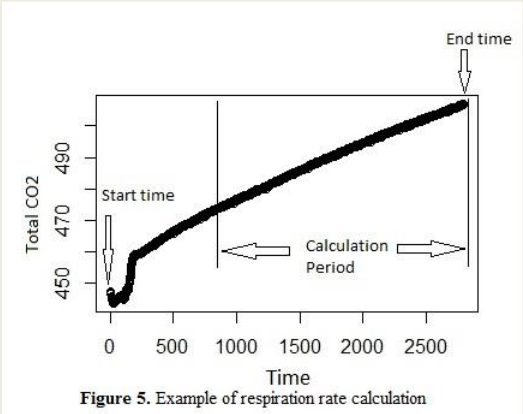

**Figure 5.** Example of respiration rate calculation

**Questions:**

1. Where is the carbon in soil from? Can you list the source of soil C. And where will soil C go besides into atmosphere as $CO_2$? List some examples of fast- and slow-cycling organic compounds in soils.
2. By comparing your group's result with another group, how does temperature affect respiration rate? And what are possible explanations?
3. What is the average $\delta^{13}C$ of your measurement? Is it lower or higher than the soil average (i.e. -20‰)? And what are possible explanations?
4. How do you convert the reading of $CO_2$ concentration (i.e. ppm) to the respiration rate (i.e. mg $CO_2 \cdot h^{-1} \cdot g\ soil^{-1}$)?
5. The $\delta^{13}C$ of fast-cycling and slow-cycling soil organic carbon are assumed to be -30‰ and -15‰, what type of SOC fraction has been preferentially acquired by microbes by comparing the average $\delta^{13}C$ of your measurement and the derived attribution of soil respiration at low and high temperatures?

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**Measurement Log**

**Group: _____ Incubation temperature:_____ Date:_____**

| Name | Jar # | Measurement start time | Measurement end time | $CO_2$ concentration at start time | $\delta^{13}C$ at start time |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**Measurement Log**

**Group: _____ Date:_____**

| Name | Jar # | Measurement start time | Measurement end time | $CO_2$ concentration at start time | δ13 C at start time |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
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**Tools for Assessment of Student Learning Outcomes:**

**Assessment**

You will be assessed on two aspects of this project - the experimental design and the written lab report. The experimental design will be used to assess your ability to use the scientific method appropriately to answer a question. The lab report will be used to test your comprehension of the principles behind soil respiration and your ability to communicate in writing in proper scientific format.

Experimental Design Guidelines
Lab Report Guidelines
Rubrics
    Experimental Design Rubric
    Lab Report Prime Trait Assessment (EXCEL file and WORD file)

**Sample Exam Questions**

**Q.** The process that converts soil organic C in plants into atmospheric $CO_2$ is_____.
**A.** *Decomposition*

**Q.** If global warming were to lead to warmer soil temperatures, what would you expect the decomposition of soil organic carbon? What type of soil organic carbon components (i.e. fast cycling vs. slow cycling) will respond more pronouncedly to warming? Explain.
**A.** *Faster decomposition would lead to greater $CO_2$ emission rates which would be driven by more pronounced decomposition of slow cycling soil organic carbon.*

**D. Comments to Faculty Users**

**Experiment Description**

**Introducing the Experiment to Your Students**

Typically the introductory material (such as decomposition and the carbon cycle) can be covered in lecture before the lab activity, so students are somewhat familiar with it. In lab we begin indoors where the concepts can be reviewed briefly. The operation of the Piccaro G2131-*i* carbon analyzer for soil respiration and stable isotopic signature is taught indoor. Students need to understand how the equipment works before starting the measurements. After they learn to use the machine, they can work as groups to design their own experiment to determine how temperature affects microbial preference for different substrates.

**Data Collection and Analysis Methods Used in the Experiment**

Choosing two substrates that differ more pronouncedly from each other in $\delta^{13}C$, as a larger difference between the two sources is favorable for applying the two-source stable isotopic mixing model method.

Soil moisture is an important control factor in regulating soil respiration. When soil moisture are low, soil respiration slows down. It takes longer time and likely less accuracy for each measurement. Avoid choosing dry soil samples. In the condition that dry soils are only choice, add the equivalent amount of water to each sample prior to the incubation.

**Questions for Further Thought**

1. Instructors may need to help students understand the concept of natural abundance of stable carbon isotope of a material and to clarify why microbes prefer for a substrate than another. For example,
2. It is important for students to understand the assumption that was made by applying the stable isotopic mixing model. That is, the stable carbon isotopic signature of substrate and the respired $CO_2$ derived from it are equivalent.

**Comments on Formative Evaluation of this Experiment**

Two types of formative evaluation can be used in this exercise. The first type of formative evaluation is already imbedded in the exercise and that is the Experimental Design assignment. This assignment evaluates two aspects of learning objectives 1 and 3: writing hypotheses and writing a methods section. During Session 2 we go over this assignment and that gives students a chance to correct mistakes and ask questions.

The second formative evaluation is a Quiz/Survey given at the beginning of Lab Session 2. It is intended to assess the degree to which they have achieved learning objective 2 and also to identify any problem areas. The quiz portion contains five objective questions to assess content knowledge. The survey portion contains two questions asking students about 1) anything that is not clear, 2) the hardest part of the activity so far. No grade was associated with the quiz in my courses but an instructor could use it as a graded assignment.

**Comments on Translating the Activity to Other Institutional Scales or Locations:**

The experiment can be adapted for respiration partitioning between any two type of soil or plant substrates that are subject to microbial decomposition. The litter materials can be replaced by high $^{12}C$ atomic ratio materials that are commercially available. The purchased material has a much more negative $\delta^{13}C$ value relative to soil samples so it turns out to be favorable to separate the two sources and evaluate how microbial substrate preferences are altered with temperature or other environmental factors. Students can compare $CO_2$ emission rates and microbial substrate preference among contrasting soil types, litter types, nitrogen availability or moisture levels. The indoor setting would also be more suitable for students with physical disabilities.

The Piccaro G2131-*i* carbon analyzer can be used in different experimental context. For soil with live roots or plants, a special container that can accommodate the soil-plant system can be connected with the machine. For measurement in the field, a closed chamber should be used to cover soil surface and connect with the machines. Note that when the moisture interference seems serious from the reading, a drying material should be implemented to secure a relatively dry air into the machine.

**E. Appendix**

Data collected in the experiment.

| Sample type | $\delta^{13}C$ (‰) | St. Deviation | N |
|---|---|---|---|
| Soil I | -20.8 | 1.1 | 5 |
| Soil II | -20.9 | 1.2 | 5 |
| Soil III | -21.2 | 0.8 | 10 |
| Pine needles | -30.0* | 0.9 | 5 |

Notes: * Treatment effect was significant according to a one-way ANOVA ($p < 0.05$).