Soil Respiration at TSU Wetland

Table of Contents:

A. Overview Information

TITLE: Soil Respiration at TSU wetland

ABSTRACT: Students investigate the factors that control the rate at which CO_2 is emitted from soil using a Li-Cor 6400 Portable Photosynthesis and Respiration System at TSU wetland. Students in small groups design and conduct their own experiments to investigate the effects of soil and microclimate factors on soil respiration (or soil CO_2 emission). Some soil collars need to be set permanently at the TSU before the lab. Students need to read the lab manual before the class, lean the theory of the measurements and design their experiment, and conduct soil respiration measurements, download and analyze data, and write a lab report as homework.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** carbon dioxide, 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: Three-hour lab sessions (plus, possibly, one lecture period).

OUTSIDE OF CLASS TIME: Students will spend 4 to 6 hours, primarily writing up the associated draft and final lab reports.

STUDENT PRODUCTS: Lab report (3-5 pages)

SETTING: This experiment can be used in wetland and other ecosystems, such as grassland, forest, and croplands.

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

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

TRANSFERABILITY: This lab requires Li-Cor 6400 equipment, together with soil temperature and moisture sensors. 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 use in gardens, lawns, and construction sites. 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 by Dr. Dafeng Hui to use the Li-Cor 6400 Photosynthesis and Respiration System for soil respiration measurement. 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. Further modified by D. Hui to fit the ecology class at TSU with the financial support of NSF IUSE and TIP programs.

B. Synopsis of Lab Module

Principal Ecological Question Addressed: How do environmental factors influence the rate of CO_2 emission from soil at TSU wetland?

What Happens: Before the lab meets, students read about carbon decomposition, the global carbon cycle, and how the Li-Cor 6400 can be used to measure soil respiration. During the lab session, students will learn to operate the equipment and discuss experimental design that will examine the influence of a single environmental factor on the rate of CO_2 emission from soil. They will go to the field to measure soil respiration, and 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 environmental factors, such as soil characteristics and microclimate, can affect soil CO_2 emission
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:

- Li- 6400 Photosynthesis and Respiration System
- 6400-9 Soil CO_2 Flux Chamber
- Soil collars (15)

- Soil thermometers (connected to Li-6400)
- Soil moisture meter
- Soil pH meter (optional)

Summary of What is Due:

- A formal, 3 to 5 page Lab Report written by individuals

C. Description of the Lab Activity

Introduction

Soil organic carbon decomposition in soils is a key ecosystem function that in part determines the productivity and health of the plants growing there. Decomposers feed on dead organic matter and in the process break it down into its simplest components: carbon dioxide, water and nutrients (organic matter consists of material or molecules produced by living organisms). The process of decomposition releases large quantities of essential nutrients to the soil solution, thereby making them available to plant roots. In northern hardwood forests, for example, about 85% of a tree's nitrogen comes from decomposition (Bormann and Likens 1979). Thus, if decomposition of a forest is impaired by drought, acid rain or some other stress, the vegetation may experience nutrient deficiencies.

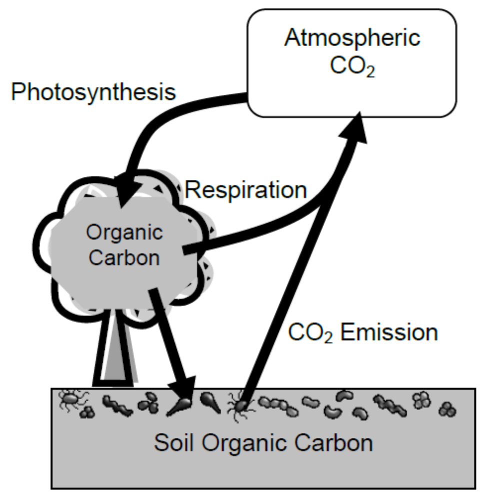

Figure 1. Box and arrow diagram of the terrestrial carbon cycle.

Soil carbon decomposition is also important because it is part of the global carbon cycle. The **carbon cycle** is the cyclical movement of carbon atoms from the atmosphere to the biosphere/lithosphere and back to the atmosphere (Figure 1). In the atmosphere, carbon is in the form of carbon dioxide gas. Through the process of photosynthesis, some of that carbon is converted into **organic carbon** which makes up organic matter or biomass. Plants and animals perform cellular **respiration** and convert a small percentage of that organic carbon back to CO_2.

A larger portion of that 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. Some of the organic carbon in the organic matter is converted into CO_2 which is released into the soil pore spaces leading to relatively high concentrations of CO_2 compared to the atmosphere. This difference in concentration causes CO_2 to diffuse from the soil to the atmosphere. This movement or **flux** of CO_2 is known as **CO_2 emission** (Figure 1).

Decomposition is not the only source of CO_2 in soil. In a forest or grassland ecosystem, plant roots are abundant in the soil and root cells perform cellular **respiration**, metabolizing carbohydrates that are sent down from the leaves. This CO_2 is released to the soil and can be responsible for anywhere between 0 and 60% of a soil's CO_2 emission. Note that CO_2 emission is the movement of CO_2 from soil to the atmosphere, whereas decomposition and root respiration are processes that produce CO_2 in the soil (Figure 2).

Release of CO_2 from soils has global implications because it occurs in ecosystems worldwide and its magnitude is such that it contributes significantly to the **greenhouse effect**. The greenhouse effect is a natural property of our atmosphere in which greenhouse gases prevent the transfer of heat from the earth's surface to outer space, thereby warming the atmosphere. Since the industrial revolution human activity (e.g., fossil fuel combustion and deforestation) has led to global increases in the concentrations of greenhouse gases (such as CO_2) in our atmosphere. This rapid increase will likely lead to a cascade of environmental impacts such as global warming, sea level rise, alteration of precipitation patterns, and increased storm severity (IPCC 2007).

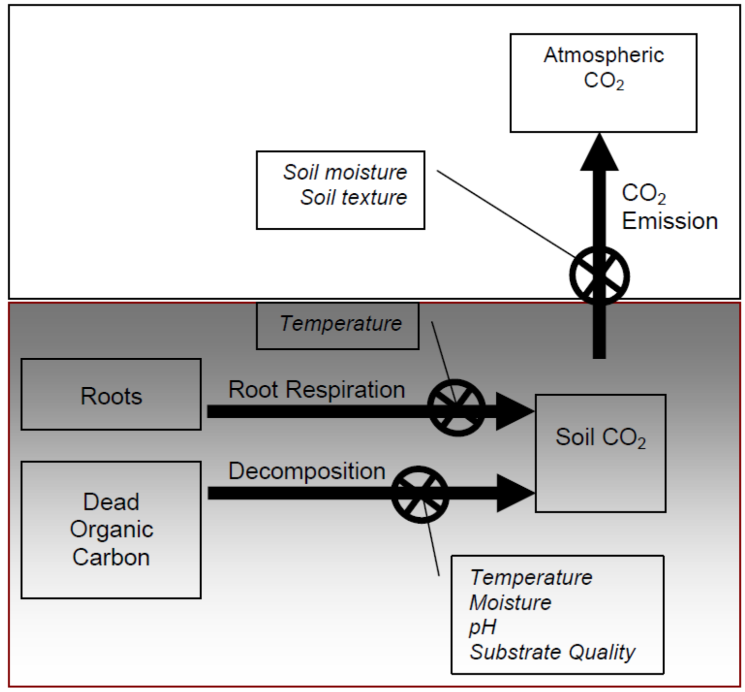

Figure 2. Flow diagram showing the pathway from organic carbon and roots in soil to atmospheric CO_2. Boxes represent amounts of carbon (mass) and arrows represent fluxes (mass per unit area per unit time). The italicized terms indicate environmental factors that control the fluxes.

Several environmental factors control the rates of decomposition and root respiration and therefore, the rate of CO_2 emission from soils. Since decomposition is an enzyme-mediated biological process carried out by bacteria and fungi, it is very sensitive to temperature. In most soils, the decomposition rate peaks at about 25°C and declines as temperature varies from this maximum. Soil moisture also affects the activity of microorganisms. Very dry or very wet (flooded) conditions tend to reduce decomposition rates (Hanson et al. 1993). A history of acid deposition can also lower the pH of soils thereby inhibiting decomposers.

Respiration rates will also depend on how fast CO_2 molecules can diffuse to the soil surface. Diffusion will be affected by soil moisture (how much of the pore space is filled with water) and soil texture (the size distribution of soil particles). Thus, it is likely that soil temperature, moisture, pH, density and texture will all influence soil respiration rates (Luo et al. 2001; Hui and Luo 2004). In this exercise, you will investigate the effects of these (and perhaps other) environmental factors on CO_2 emission (Figure 2).

One common method for measuring soil respiration is using the Li-6400 Photosynthesis and Respiration System connected with a soil CO_2 flux chamber. This closed dynamic chamber method involves placing a closed chamber over the soil surface. Tubes running from the top of the chamber will pass the air in the chamber through an infrared gas analyzer (IRGA), which continuously measures CO_2 concentration. The air will then be pumped back into the chamber. An initial CO_2 measurement is taken and subsequent measurements are taken at regular intervals over the next few minutes until a final CO_2 concentration is recorded at a predetermined end time. When these multiple data points are graphed, the points can be fitted with a linear regression equation, which will provide a slope. This slope can provide the rate of soil respiration. The 6400-09 Soil CO_2 Flux Chamber is an accessory that attaches directly to the sensor head of the LI-6400 – the mixing fan in the sensor head is used to move air through a perforated manifold in the 6400-09, thoroughly mixing the air in the chamber. OPEN instrument software includes a configuration for the 6400-09, soil respiration measurement.

Materials and Methods

Study Site:

With your Instructor, choose appropriate study locations at the TSU wetland that are relatively flat and are not extremely stony. You need to place five soil collars at each location on the ground where there are no living plants and no large stones. Depending on your experimental question you may want contrasting sites like water inlet location (more trees), middle (cattails) and the water outlet location (more bush and grasses).

Overview of Data Collection and Analysis Methods

Lab Session 1:

1. Learn to use the Li-6400 equipment for soil respiration measurements.
2. Bring the soil collars (already made in the lab) and go out to your field site. Take a few minutes to note the variations in microclimate and microtopography within the wetland.
3. In small groups design your experiment. You will be comparing the rate of soil respiration of three sites with different microclimates and/or soil characteristics. As a group, decide on the sites or the microclimates you would like to compare. Here are some suggestions but you are encouraged to think of your own:
   - Sites with different vegetation
   - Sun vs. shade
   - Ridgetop vs. valley bottom
   - With leaf layer vs. without leaf layer (i.e., the layer of dead leaves on the soil surface is removed)
4. As a group write out your Experimental Design. Show it to your Instructor for approval before proceeding. As homework type up your answers to the questions on the handout.
5. Place five soil collars at each location. Push a collar down on a relatively flat area of the soil. The entire rim of the collar must be inserted at least 1 cm into the soil so as to minimize gas exchange with the atmosphere. Carefully remove twigs and small rocks that lie under the rim without disturbing the leaves and soil surface under the chamber. Remove any green plants by pinching or cutting them at soil level.
6. Measure the depth of soil collars at four sides. Calculate the mean depth of each collar. This value will be used to correct the soil respiration measurement. You need to put the collars in the field at least 24 hours before measurements to minimize disturbance.

Lab Session 2:

1. Bring the Li-6400 to the field site with instructor.
2. Warm up the equipment and follow the instruction for soil respiration measurements.
3. Sampling sites. You may measure any or all of the following. Your Instructor may have additional parameters for you to measure.
   - Soil moisture
   - Soil pH

4. Download data to computer and use the soil collar depths to correct the respiration measurements.

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

Questions for Further Thought and Discussion

1. How did your sampling sites differ in terms of temperature, moisture, pH or other characteristics? Could these differences explain the differences you observed in soil respiration rate?
2. The soil under your collars probably contained plant roots. How might these plant roots have affected your soil respiration rates? Explain. Design an experiment using certain collars that would allow you to determine what proportion of the CO_2 emitted came from roots and what proportion came from decomposition.
3. Explain how decomposition in soils is linked to the greenhouse effect.
4. Calculate the average CO_2 emission rate and standard deviations for each sample location (or perform a statistical test). Put these values in a table. Then write two to three paragraphs describing and interpreting the results of your experiment.
5. The temperature and moisture data you collected represent point-in-time measurements. Do you think the temperatures and soil moisture values are representative of the microclimate during the entire incubation period? What would be a more accurate way to quantify the microclimate during the incubation period?
6. Are there other environmental or site factors that you did not measure that could explain the differing rates of CO_2 emission between your sampling locations? Explain how they would affect the emission rate.
7. Because decomposition is a temperature-dependent process, it is expected to be affected by global warming. Write down one or two predictions about how decomposition in soil will change and how those changes will affect plants. Then conduct a literature search to find out what the experts are predicting. Were your predictions correct? If not, why not? What other predictions have the experts made? Some search phrases that will aid you in your search are: soil CO_2, CO_2 emissions, soil respiration, global warming soil carbon, tundra soils, global warming positive feedback, soil respiration temperature, decomposition temperature.

References

Bormann, F.H. and G.E. Likens. 1979. *Pattern and Process in a Forested Ecosystem*. Springer-Verlag, New York, NY.

Buchman, N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. *Soil Biology and Biochemistry* 32:1625-1635.

Hanson, P.J., S.D. Wullschleger, S.A. Bohlman, and D.E. Todd. 1993. Seasonal and topographic patterns of forest floor CO2 efflux from an upland oak forest. *Tree Physiology* 13:1-15.

Hui, D. and Y. Luo. 2004. Evaluation of soil CO2 production and transport in Duke Forest using a process-based modeling approach. Global Biogeochemical Cycles, 18, GB4029, doi:10.1029/2004GB002297.

IPCC, 2007: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

Luo, Y., S. Wan, D. Hui, L. Wallace. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413: 622-625.

Raich, J.W., and W.H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44B:81-99.

Rustad, L.E., T.G. Huntington, R.D. Boone. 2000. Controls on soil respiration: Implications for climate change. *Biogeochemistry* 48:1-6.

Schlesinger, W.H., and J.A. Andrews. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry*. 48:7-20.

Simmons, J.A., I.J. Fernandez, R.D. Briggs and M.D. Delaney. 1996. Forest floor carbon pools and fluxes along a regional climate gradient in Maine, USA. *Forest Ecology and Management* 84:81-95.

Simmons., J.A. 2009. Decomposition and Soil CO_2 Emission. *Teaching Issues and Experiments in Ecology*, Vol. 6: Experiment #2 [online]. http://tiee.ecoed.net/vol/v6/experiments/soil_respiration/abstract.html

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 atmospheric CO_2 into organic C in plants is_________.
A. *Photosynthesis*

Q. If global warming were to lead to warmer soil temperatures and therefore faster decomposition worldwide, what would you expect to happen to the levels of CO_2 in the atmosphere (all else being equal)? Explain.
A. *Faster decomposition would lead to greater CO_2 emission rates which would lead to an increase in atmospheric CO_2 concentration.*

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 Li-6400 for soil respiration is relatively simple compared to leaf photosynthesis measurement. 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 environmental factors affect soil respiration.

Once students get to the TSU wetland, they usually need some guidance in knowing what to look for. You may point out differences in soil type, leaf litter types and amounts, sunlight, slope, and topography and ask them how the microclimate might differ in each case. Give them some time (15 – 20 minutes) to walk around, observe, and as a group come up with an idea for an experiment. They write out their experimental design while outside and then type it up as homework before turning it in.

An option for an upper level class is to give them less information about the environmental factors (i.e., edit the Introduction section to omit these), let them generate their hypotheses "from scratch" and run their experiments. This option will likely require more time for discussion.

Data Collection and Analysis Methods Used in the Experiment

A forest setting works best because the herbaceous vegetation is sparse and the soil organic matter content is high. Any green plant in the chamber may remove CO_2 through photosynthesis, so it is important to remove or avoid all plants (unless you want to measure photosynthesis).

Weather is an important consideration. When soil temperatures are low, soil respiration slows down. It takes longer time for each measurement. Avoid rainy days. Excessive moisture as during a rain storm or flooded conditions inhibits CO_2 diffusion from soil.

Questions for Further Thought

1. Instructors may need to help students summarize their environmental measurements and to figure out exactly what they mean. For example, students may not immediately grasp the idea that a south-facing slope gets more direct sunlight than a north-facing slope and that this could effect soil temperature and moisture.
2. It is important for students to recognize that plant roots can contribute to soil respiration as well as microorganisms.

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 indoors when the weather does not cooperate. Plant trays or plastic bins containing 2 to 4 cm of soil material set up in a lab or greenhouse make suitable substrates. Students can compare CO_2 emission rates among contrasting soil types, amounts of organic matter, soil depths, soil temperatures (using a plant germination heating mat), or moisture levels. The indoor setting would also be more suitable for students with physical disabilities.

The Li-6400 Photosynthesis and Respiration System can be used in different ecosystems. For long term field soil respiration measurements, herbs should be removed when present. They can be removed by pinching or cutting them off at ground level. In a lawn or grassland you can treat small patches with herbicide several days ahead of time. Note that when a plant is killed the rapid decomposition of its roots will create a spike in decomposition between 2 and 5 days later. So soil respiration must be measured immediately after cutting the plant or after 5 days have elapsed.

E. Appendix

Data collected in the experiment.

Example raw data

Obs	EFFLUX	Tsoil_C	Tsch_C
Mid			
12	3.179116	20.68756	31.3728
24	3.146781	20.72096	31.3309
36	3.242657	20.78589	31.25249
45	4.174765	20.85595	31.0539
54	4.203438	20.90987	31.04225
63	4.207072	20.94398	30.99305
71	5.609813	21.13337	30.84875
79	5.317935	21.16612	30.85812
87	5.151033	21.17044	30.83782
Lower			
98	3.709507	22.62482	31.55846
109	3.387169	22.68491	31.6538
120	3.435496	22.576	31.73737
128	5.623061	21.69645	31.71256
136	4.899517	21.73264	31.71213
144	4.573259	21.74524	31.71168
163	2.060814	19.66632	31.27159
183	1.988183	19.61368	31.00879
203	1.934515	19.73821	30.71653
Upper			
217	2.692942	22.60286	31.43043
231	2.710422	22.45089	31.50981
246	2.649676	22.27291	31.61602
260	2.759553	22.49298	31.93816
275	2.648223	22.37635	32.21785
283	25.76958	22.55898	32.4167

296	2.882185	20.86976	32.54871
310	2.768243	20.71835	32.67701
324	2.750149	20.61065	32.77332
343	2.087795	21.11235	33.08505
362	2.010044	20.98128	33.18765
381	2.048227	21.01626	33.19784

Table 1. Soil respiration rates and key environmental variables measured in TSU wetland in Nashville on 5/1/2015 (hypothetical data).

Location	Soil Respiration Rate* (μ mol CO_2 m^{-2} s^{-1})	St. Deviation	N	Initial Soil Temp (C)	Initial Soil Water Content (% vol)
Water inlet	13.4 b	1.09	5	17.4	4.01
Middle	12.5 b	0.83	5	19.4	1.24
Water outlet	8.7 a	1.46	5	18.9	0.79

Notes: * Treatment effect was significant according to a one-way ANOVA ($p < 0.05$). Means followed by different letters were significantly different according to a LSD multiple comparison test.

.