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Soil Respiration

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This guided inquiry laboratory exercise is an excellent opportunity to introduce carbon cycling and some of the issues associated with climate change. Students in our major/minor programs generate a class hypothesis using summarized research on soil respiration and vote on a treatment option: soil type, litter type, water levels or temperature. Respiration chambers incubate for a week, and the amount of CO_2 produced over the seven days is determined the following week. This is an easy, low tech way to teach these concepts, but could be adapted to use probes to measure CO_2 directly. This activity is very adaptable to highlight different skills such as hypothesis testing, statistical analysis and experimental design. In institutions with more space resources this could be easily adapted for more inquiry-based approaches.

Keywords: climate change, carbon cycling, ecology, hypothesis testing

Introduction

The discipline of ecosystem ecology studies the relationship of the biological, chemical and physical processes at a location (Molles and Cahill 2011), and has biogeochemistry as a subdiscipline (the study of distribution and movement of elements in a specific geographical location between biotic and abiotic pools (Lawrence 2008)). The elements studied are usually those essential for life: examples are carbon, nitrogen, phosphorus and sulfur. The cycling of these nutrients combined with other environmental variables can determine the fundamental niches of organisms.

By the very nature of ecosystem ecology, it is a topic that is challenging to explore in the laboratory and in a hands-on way for students. This highly consistent lab uses an adaptation of the alkali trapping method for measuring CO_2, a common method for looking at CO_2 before the advent of cheap CO_2 probes (for critique on field measurements see Rochette and Hutchinson (2005) and Jensen et al. (1996)). While the formulae and math are visually intimidating, we have found that students are excited to bring their chemistry skills into ecology and are adept at both the titrations and the math. Additionally, this lab helps demonstrate to students that the non-biology courses required in their first and second year are important in their educational journey. It also introduces the use of statistics that look at differences between more than two treatments and has been used to look for interactions between factors.

Student Outline

Objectives

- Connect elements of the global carbon cycle to global climate change, especially soil respiration
- Use the alkali trapping method to evaluate different treatments that influence soil respiration
- Use the appropriate statistical analysis to look for differences in the treatments (ANOVA, Tukey's HSD post hoc test)

Introduction

The discipline of ecosystem ecology studies the relationship of the biological, chemical and physical processes at a location (Molles and Cahill 2011) and has biogeochemistry as a subdiscipline (the study of distribution and movement of elements in a specific geographical location between biotic and abiotic pools (Lawrence 2008)). The elements studied are usually those essential for life: examples are carbon, nitrogen, phosphorus and sulfur. The cycling of these nutrients combined with other environmental variables can determine the fundamental niches of organisms, as discussed in previous labs.

One of the most controversial and far reaching issues related to the environment today is that of global climate warming; September 2012 saw an entire *National Geographic* issue devoted to the weather and the potential links to warming climate, and October 2013 saw another issue focused on polar ice melting. Global warming is caused primarily through the action of atmospheric carbon dioxide (CO_2), water vapor, methane (CH_4), and nitrous oxide (N_2O); of these CO_2 is considered the most important anthropogenic greenhouse gas (Kellogg 1991; IPCC 2007). Increasing atmospheric concentrations of CO_2 are correlated with higher temperatures in geological times (Kump 2000) and are thought to be increasing annual global temperatures currently (Molles and Cahill 2011, IPCC 2007). Atmospheric CO_2 concentrations are part of the biogeochemical global carbon cycle, and changes in atmospheric CO_2 are usually studied at an ecosystem level (Smith et al. 2013).

As well as being present in the atmosphere, CO_2 is found in the ocean, freshwater, living or dead organisms, and the soil (Fig. 1). The majority of an ecosystem's carbon is temporarily trapped in sedimentary rock such as limestone, in fossil fuels such as coal and oil, or in the ocean waters (Molles and Cahill 2011). Temporary is a relative term, as it can be millions of years before natural weathering and combustion processes release this carbon. The movement, or flux, of carbon between the biotic and abiotic components of the ecosystem is cyclic, with the average residence time of carbon in organic molecules estimated to be between 20-50 years (Smith et al., 2013) and in the atmosphere to be between 3-4 years (Stiling 2012). Carbon cycling follows the law of conservation of matter: matter can neither be created nor destroyed, so like other nutrients, carbon changes form as it moves through the various pools (such as soil, organic matter, etc.) (Smith et al. 2013). Carbon dioxide is assimilated by plants during the process of photosynthesis and is converted to organic compounds - simple sugars such as glucose, or more complex carbohydrates such as cellulose and lignin. When plants are eaten, or die and decompose, the carbohydrates are broken down again to CO_2 and H_2O by the process of respiration:

$$C_6H_{12}O_6 + 6O_2 \leftrightarrow 6CO_2 + 6H_2O$$ **Eq. 1** (Molles and Cahill 2011)

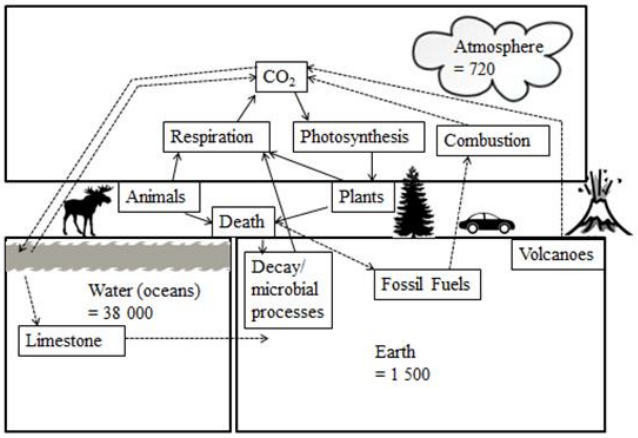


Figure 1. The carbon cycle. Numbers indicate the size of the carbon pools as 10^{15} g (Molles et al. 2017). Biotic exchanges/processes are solid lines, abiotic processes are dashed lines.

During respiration CO_2 is released either back to the atmosphere, in the case of terrestrial organisms, or into the water, in the case of aquatic organisms, to continue the carbon cycle.

Although the amount of CO_2 in the atmosphere remains relatively constant (global warming notwithstanding), there is an annual variation in CO_2 in the atmosphere, especially in the northern hemisphere (Smith et al. 2013). CO_2 in the atmosphere begins to decline each year in early spring, when leaves appear and photosynthesis begins, removing carbon from the atmosphere to be stored in plant material (Smith et al. 2013). The lowest CO_2 levels are reached in August, and by October CO_2 increases again, when leaves fall and decomposition exceeds photosynthesis (Subke et al. 2006).

If respiration is defined as the use of oxygen to break down organic compounds metabolically to release chemical energy, then soil respiration can be defined as the use of oxygen and/or the release of carbon dioxide by living organisms in the soil (Ryan and Law 2005). This oxidation of organic carbon in the soil, along with aerobic respiration at all trophic levels, is the primary means by which carbon is returned to the atmosphere (Smith et al. 2013). Soil respiration is the sum of autotrophic respiration from live plant roots and their partner mycorrhizae and of heterotrophic respiration by soil biota involved in decomposition (Fig. 2), each contributing about 50% of the CO2 emitted during the growing season (Ryan and Law 2005).

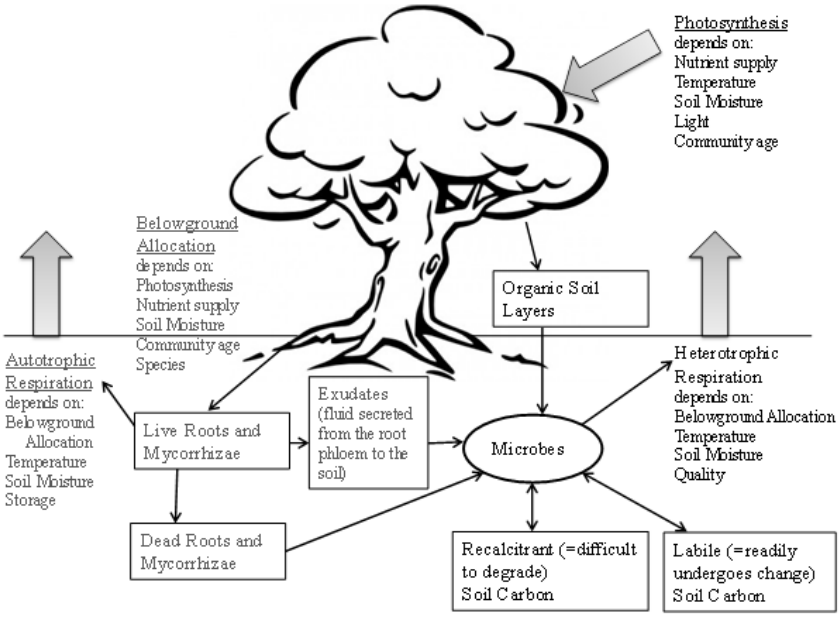


Figure 2. A conceptual model of carbon flow/ soil respiration between the atmosphere, plant biomass, and soil environment. Total soil respiration is the sum of autotrophic respiration from the plant roots with associated mycorrhizae and root zone (rhizosphere) bacteria and heterotrophic respiration from free-living bacteria and fungi (adapted from Ryan and Law 2005).

Decomposition of detritus (especially leaf litter) on the soil surface and resulting heterotrophic respiration is a significant source of CO_2 (Smith et al. 2013). Plant litter is decomposed in a sequential process that begins with leaching of water-soluble minerals and simple sugars, such as glucose, from the material (Stiling 2012). This is usually followed by fragmentation of the litter into smaller pieces, which occurs through both mechanical and biological means (Stiling 2012). The final stage of decomposition is mineralization, the conversion of large organic compounds to simpler inorganic forms by organisms known as decomposers or detritivores (Smith et al. 2013). The organisms involved in the process of decomposition function in a complex community with many trophic levels.

Detritivores, the organisms that feed on dead plant and animal matter, have variable impacts on decomposition rates, and are often separated by their physical size. The larger invertebrates (> 100 μm) are classified as meso, macro, and megafauna, while the smaller organisms (bacteria, fungi, nematodes, etc.) are classified as microflora/fauna or more often microbes (see Fig. 7.10 in Molles et al. 2017). The megafauna such as annelids (earthworms), arthropods (such as springtails, mites and millipedes), and molluscs (for example snails and slugs) fragment dead vegetation into smaller pieces, mix litter into the soil, and excrete partially digested plant matter (Molles and Cahill 2011, Smith et al. 2013). The microbes specialize in the mineralization of organic matter either through aerobic or anaerobic respiration (Smith et al. 2013). The interaction of the two groups of detritivores results in increased overall decomposition rates, as the actions of the larger invertebrates facilitate the mineralization by microbes (Stiling 2012).

Methods and Data Collection

Part A: Selecting Your Treatment

Using the research summary provided (see Appendix), prepare to discuss with the class which variable you would like to see measured in this class experiment. As you will read, heterotrophic soil respiration rates depend on a variety of variables, including temperature, water availability, soil structure, and composition of the litter. You will need to decide which treatment your class will study and form a class hypothesis/prediction, as we can only test the effect of one variable (the treatment) on the amount of CO_2 released during decomposition of leaf litter. You should record the variable/treatment the class agrees on and the four conditions to be assessed (Table 1). You will then set up an experiment to determine the amount of CO_2 given off during soil respiration in 20 incubation chambers containing your treatment as outlined in Table 1. Your class will be divided into five groups, with each group responsible for one complete set of conditions for the selected treatment.

Table 1. Outline of conditions.

Water availability	
Low	____ mL
Medium-low	____ mL
Medium-high	____ mL
High	____ mL

Temperature	
Low	____ ºC
Medium-low	____ ºC
Medium-high	____ ºC
High	____ ºC

Soil type = 100mL	
Native	_____ g
Sand	_____ g
Clay	_____ g
Peat	_____ g

Litter type = 5g	
Litter #1	
Litter #2	
Litter #3	
Litter #4	

Part B: Set-up of Respiration Chambers

Your instructor will provide detailed instructions applicable to whichever treatment your class has chosen.

1. Label each of your four incubation chambers with lab section, group number or name and treatment/condition.
2. We are only varying one variable within our experiment (water availability, temperature, soil type or litter type); therefore the other variables will remain constant (Table 2). Record your conditions in Table 1.

Table 2. Outline of treatments and conditions.

Water availability	**Temperature**	**Soil type**	**Litter type**
Water (Table 2)	25.0 mL distilled water	25.0 mL distilled water	25.0 mL distilled water
20.0 °C	Temperature (Table 2)	20.0 °C	20.0 °C
95.0 g native topsoil	95.0 g native topsoil	Soil (Table 2)	95.0 g native topsoil
5.0 g deciduous forest litter	5.0 g deciduous forest litter	5.0 g deciduous forest litter	Litter (Table 2)

3. Add the appropriate amount and type of soil to each chamber (Table 2). For the soil type treatment, measure out 100.0 mL of each soil type, weigh the sample and record in Table 1, and add to each chamber.
4. Top with the appropriate amount and type of leaf litter (Table 2).
5. Add the appropriate amount of distilled water (Table 2).
6. Dispense 25.0 mL of 2.0 N sodium hydroxide (NaOH) into a small beaker. **Caution**: Sodium hydroxide is caustic. Wipe spills immediately. Avoid contact with skin or clothing. Wash thoroughly if base is spilled on skin or clothing.
7. Use long forceps to place the beaker in the incubation chamber, nestling it into the litter.
8. Screw the lid on the incubation chamber tightly, being careful not to spill the NaOH. Place the chambers in the appropriate location to incubate for one week (Table 2).

Part C: Alkali Trapping Method of CO_2 Determination

The alkali trapping method used to determine the amount of CO_2 generated during soil respiration takes advantage of the fact that CO_2 is weakly acidic. When CO_2 is absorbed by a basic solution, such as sodium hydroxide (NaOH), carbonate ions (CO_3^{-2}) are formed:

$$CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O \quad \textbf{Eq. 2}$$

The carbonate can be precipitated as barium carbonate ($BaCO_3$) by the addition of an excess amount of barium chloride ($BaCl_2$):

$$BaCl_2 + Na_2CO_3 \rightarrow BaCO_3 + 2\ NaCl \quad \textbf{Eq. 3}$$

We can then titrate the residual OH^- ions with hydrochloric acid (HCl) to determine how much of the initial base was not converted to carbonate, and by subtraction, calculate the amount of base that was used in the conversion to carbonate.

To calculate milligrams of CO_2 evolved:

$$\text{mg } CO_2 = ((\text{mL base} \times \text{N base}) - (\text{mL acid} \times \text{N acid})) \times \text{equivalent weight of } CO_2 \quad \textbf{Eq. 4}$$

In our case, we titrate 2.0 N NaOH with 1.0 N HCl, so to determine milligrams of CO_2 dissolved in our 5 mL sample this equation becomes:

$$\text{mg } CO_2 = ((5\text{ mL} \times 2.0\text{ N}) - (\text{mL HCl} \times 1.0\text{ N})) \times 22 \quad \textbf{Eq. 5}$$

To calculate total milligrams of CO_2 evolved per gram of soil/litter per day, we need to multiply this amount by 5 to determine how much CO_2 was absorbed by our entire 25 mL volume of NaOH, divide by the weight of the soil/litter sample, and divide by the number of incubation days:

$$\frac{\text{mg } CO_2}{\text{g/day}} = \frac{\text{mg } CO_2 \times 5}{\text{soil + litter weight (g)} \times 7\text{ days}} \quad \textbf{Eq. 6}$$

Using the Titration Equipment

1. Fill the burette with 1.0 N HCl by turning the stopcock so the channel to the reservoir flask is open while you raise the reservoir flask. Close the stopcock. If your reservoir flask is almost empty, fill it with HCl obtained from the dispenser on the side bench. If there are any air bubbles in the burette, raise and lower the reservoir flask, with the stopcock open to the reservoir, to raise and lower the level in the burette. This will encourage the bubbles to rise to the top. Do not tap the burette, as it is both expensive and fragile.
   Caution: Hydrochloric acid is caustic. Wipe spills immediately. Avoid contact with skin or clothing. Wash thoroughly if acid is spilled on skin or clothing.
2. Check the graduations on the burette for the calibration (Fig. 3). What is the total volume of your burette? Into what units is it graduated? Read the level of the HCl in the burette by reading the bottom of the meniscus. Have each member of your group verify the reading.
3. Open the stopcock slowly in the opposite direction (the channel to the dispensing tip is open) and allow a few mLs of HCl to drain from the burette into an empty 125 mL flask, then decrease the flow to a very slow drip. Practice this a few times until you learn to control the flow. Discard the "practice" HCl in the waste container provided.
4. Refill the burette with HCl and you are ready to titrate your samples.

Determine CO_2 Evolved

1. Carefully unscrew the lid of an incubation chamber and remove the container of NaOH.
2. Observe the contents of the incubation chamber. What do you see? What does it smell like?
3. Use a pipettor to transfer one 5.0 mL aliquot of NaOH into each of two 125 mL flasks.
4. Carefully unscrew the lid of an incubation chamber and remove the container of NaOH.
5. Observe the contents of the incubation chamber. What do you see? What does it smell like?
6. Use a pipettor to transfer one 5.0 mL aliquot of NaOH into each of two 125 mL flasks.
7. Use a syringe to dispense 10 mL of $BaCl_2$ into each flask.
8. Add seven drops of phenolphthalein indicator solution to one of the flasks. Swirl gently to mix.
9. Fill the burette with HCl and record the initial level in Table 4.

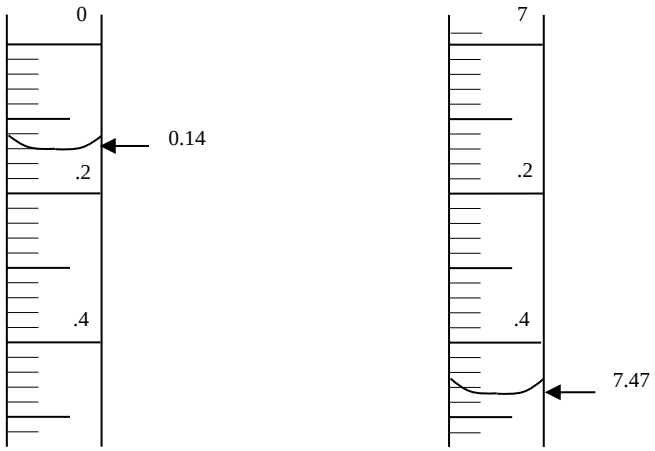


Figure 3. Read the level of HCl at the bottom of the meniscus, reading the numbers starting from the top of the burette. In this example, the initial reading (left burette) is 0.14. After titrating (right burette) the level is 7.47. The volume of HCl dispensed during the titration is: 7.47 – 0.14 = 7.33 mL

10. Titrate with HCl until the pink color just disappears. Don't release the acid too quickly, as some treatments may need only a few drops to neutralize the excess base. Swirl the flask continuously while titrating. Towards the end of the titration you will need to release HCl one drop at a time in order not to overshoot the end point.
11. Record the final level in the burette and subtract the initial reading to determine the volume of HCl used in the titration.
12. Repeat the titration (steps 5-8) with the sample in the second 125 mL flask.
13. Calculate the mean volume of HCl used in the titration of the two samples.
14. Calculate the amount of CO_2 evolved in a 5 mL sample.
15. Calculate total CO_2 generated per gram of soil/litter per day, and record in Table 3.
16. Discard the contents of the 125 mL flasks in the waste container provided and wash and rinse the flasks at the sink.
17. Repeat steps 1-12 for the remaining incubation chambers.

Dump all soil and litter into the garbage pail and rinse the incubation chambers at the sink.

Table 3. Group data. Titration volumes and calculation of soil respiration rates.

Treatment conditions	Initial burette level	Final burette level	Volume HCl (mL) (final - initial)	Mean HCl (mL)	CO_2 in 5 mL sample (mg) Eq. 5	Total CO_2/g/day (mg) Eq. 6
#1						
#2						
#3						
#4						

Part D: Statistical Primer

Single-factor Analysis of Variance (ANOVA)

We use a t-test if we want to test for differences between the means of two samples, but what if you have collected data from three or more samples? Instead of using multiple t-tests to compare two samples at a time, a simpler and more statistically rigorous way of testing the null hypothesis that two or more samples are drawn from the same population is with an **analysis of variance** (**ANOVA**) test. The ANOVA test is mathematically more complex than a t-test, and is generally done on a computer. With an ANOVA test, one determines the *F* statistic, which in this case is the ratio of the variation between a group of means relative to the variation within the groups:

$$F = \frac{MS_G}{MS_E} \qquad \textbf{Eq. 7}$$

Where MS_G (mean square the groups) is an estimate of the variance between groups, and MS_E (mean square the error) is an estimate of the within-group variance. These distinctions are not overly important for you to know, but since mean squares are calculated by dividing the sum of squares (the sum of the squared deviations) by the degrees of freedom, the value of *F* depends strongly on the degrees of freedom (so, sample size is important!).

If our data fails to reject the null hypothesis, we would expect that the variances between groups and within groups would be similar. However, if our data does not support the null hypothesis, and assuming we have appropriately sampled the data, the *F* ratio will be significant, as the variance between groups would be greater than the variance within groups. The Microsoft Excel ANOVA test also gives the P-value, and calls it the significance level – the probability that a random variable is greater than or equal to the calculated value of the *F* statistic.

Let's say we performed a study where we counted the number of chironomid larvae (a type of aquatic insect) in 15 one-meter x one-meter quadrats in three different ponds located in central Alberta (Table 4). We can perform an analysis of variance (single factor ANOVA) to test the null hypothesis that the mean number of chironomid larvae in the three ponds are equal. A general alternative hypothesis is that at least one of the means is different.

Table 4. Number of chironomid larvae in three ponds located in central Alberta.

Quadrat #	Pond #1	Pond #2	Pond #3
1	8	6	8
2	9	9	6
3	5	9	4
4	7	6	7
5	8	9	7
6	9	6	7
7	11	7	5
8	6	6	8
9	8	10	5
10	10	9	4
11	8	8	5
12	6	7	6
13	9	5	8
14	10	9	5
15	7	9	7

After entering the data from Table 4 into Microsoft Excel and performing a single factor ANOVA test, the program would generate the results in a table that looks similar to that of Table 5. The *F* statistic generated by this ANOVA test is 6.43 which is greater than the critical *F* value of 3.22. The probability of obtaining this result when the statistical population means are equal is very small, P = 0.0037, therefore, we can reject the null hypothesis and conclude location does have an effect the abundance of chironomid larvae ($F_{2,42} = 6.43$, $P < 0.05$).

Now that we know that one (or more) of the groups is/are different, we would need further tests (called post-hoc) to determine which groups differ (see below), and further research to explore possible explanations for those differences. Differences are usually the result of simultaneous effects of multiple variables. In our chironomid larvae example, we may wish to collect data on biotic and abiotic variables such as temperature, day length, and food availability, and perform more sophisticated statistical analyses to establish which variables, or combination of variables, may be responsible for the different chironomid abundances.

Table 5. Results of a single-factor ANOVA statistical test (MS Excel) performed on chironomid larvae data from three central Alberta ponds.

Source of Variation	SS	df	MS	*F*	P-value	F_{crit} $\alpha = 0.05$
Between Groups	31.24	2	15.62	6.43	0.0037	3.22
Within Groups	102.00	42	2.43	-	-	-
Total	133.34	44	-	-	-	-

Post-hoc Tests

Post-hoc tests are used when a statistical test, such as ANOVA, has been performed, but additional information is needed to determine which means are significantly different from one another. Tukey's HSD test is often used in conjunction with ANOVA. It is a single step multiple comparison procedure that compares all possible pairs of means and identifies any differences between two means that is greater than the standard error. Tukey's HSD test has two assumptions:

1. Data is independent
2. Variance is homogeneous

And the statistical hypotheses for this test are:

H_o: $\mu_1 = \mu_2 = \mu_3 = \mu_n$
H_A: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_n$

The basic equation calculating the test statistic (q-value) for the test is:

$$q_s = \frac{Ya - Yb}{SE} \qquad \textbf{Eq. 8}$$

Where:
Y_a = larger mean (of the two means being compared)
Y_b = smaller mean (of the two means being compared)
SE = standard error

If $q_s > q_{critical}$, (a q-value obtained from the studentized range distribution), we can conclude that the two means being compared are significantly different from each other. As this test is computationally very difficult, it is generally done using computer software. Your instructor will provide further details on how to perform this test using statistical software and your class data.

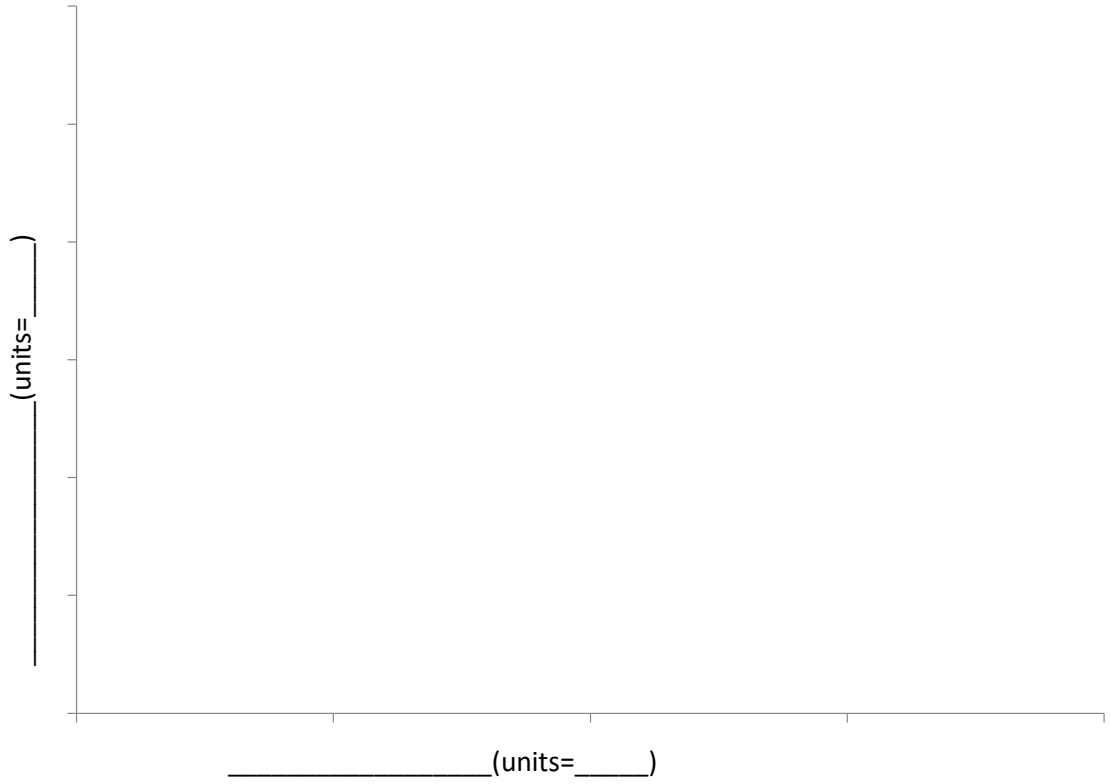
Part E: Data Analysis

1. Combine your results with those from the other groups in your class in Table 6.
2. Use a **single-factor ANOVA** analysis to analyze the effect of your treatments on the rate of soil respiration. Do your results support your hypotheses? If you have a significant difference in the means of your treatments, conduct a **Post-hoc test** to find which treatment(s) is/are significantly different.
3. Construct a bar graph with mean respiration rate on the y-axis and your treatment levels on the x-axis to illustrate the effect your treatments on soil respiration.
4. Did your treatments affect the rate of soil respiration?
5. What variables not specifically tested for in your experiment may have affected your results?

Table 6. Class data. Soil respiration rates (mg CO_2/gram/day).

Treatment conditions	Group #				
	1	2	3	4	5
#1					
#2					
#3					
#4					

Sketch the averages for the different conditions on the graph below, remembering you will want to include a measure of variation and which conditions were different from others.



Figure_____

_____

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_____

What do you conclude about the evolution of CO_2 in your treatments?

Part F: Review Questions

Nutrient dynamics in various ecosystems are often studied to assess the anthropogenic impacts of pollution. Temperate systems experience leaf fall in the autumn, and this is known to be significant input of carbon and associated nutrients that enters the ecosystems in a short time. A study is proposed to look at the effect of increasing biofilms, differing environmental conditions and macroinvertebrate biomass might play a role in the rate of decomposition in a wetland gradient.

a) Using Table 8 from the Soil respiration research summary (Appendix A), come up with a hypothesis for the decomposition rate of various plant matter present in the wetland after leaf fall. You would expect that there would be fallen leaves, sedge plants and cattail present. Your hypothesis should follow the tips for creating a good hypothesis. (4 points)

b) Following the study, the decomposition rate of fallen leaves and macroinvertebrate biomass was compared independently at three sites in the wetland gradient. Using the table following, indicate which (if any) of the variables had significant differences across the three sites. Justify your answer. (1 point)

Variable	*F*	*p*
Decomposition rate (fallen leaves)	0.67	0.526
Macroinvertebrate biomass	32.1	0.0001

c) What would you need to do to determine which sites are different from each other? (1 point)

d) The comparison of algal biomass with respect to decomposition revealed the following Tukey's pair-wise comparisons. Treatments joined with underlining are not significantly different (Tukey's test, $p<0.05$). Which treatment(s) were different for which variables? (2 points)

Variable	*F*	*Treatments:*		
Decomposition rate	0.67	Ridge	Transition	Valley
Algae (leaf)	14.78**	Ridge	Transition	Valley
Algae(tile)	6.22*	Ridge	Transition	Valley

* $p<0.05$; ***$p<0.001$

Please check your answers with your Instructor.

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Materials

This lab does require a fair amount of technical time, as there are several chemicals used and a variety of glassware including the respiration chambers (mason jars). The concentrations of chemicals are given in normality, and the calculations included to determine the weight or volume required to make the solution.

Part B: Set up of respiration chambers

- Glass mason jars – 1 L (20/ 25 students)
- Soil types (2000 mL/ 25 students)
  - Native topsoil
  - Sand
  - Peat
  - Clay
- Litter types (1000 g/25 students)
  - Deciduous leaves
  - Pine/spruce needles
  - Alfalfa
  - Cattails/sedges
- R.O. Water (up to 2 L/ 25 students)
  - In wash bottles (5)
- 2N NaOH (2 L/ 25 students) made in bulk in a carboy
  - In labelled wash bottles (5)
- Glass vials to hold 30-40 mL (20/ 25 students)
- Graduated cylinders – 25 mL (10 minimum)
- Beakers – 250 mL (5-10)
- Long forceps (5)
- Electronic balances (2 minimum)
  - 1 each for soil and litter types. More is better to avoid bottlenecks.
  - Large weigh boats (~20)
- Safety glasses – enough for 1/student

Chemicals required:

2N NaOH = 79.99 g/L (at least 2L/ 25 students)

Part C: Alkali trapping method of CO_2 determination

- Burette setups (10 / 25 students) consisting of
  - 50mL burette
  - Burette stand w/ clamp
- 125mL Erlenmeyer flasks (20/ 25 students)
- Plastic pipettes – 10 mL (20/25 students)
- Long forceps (5/ 25 students)
- Syringes – 20 mL size (5/ 25 students)
- 3N $BaCl_2$ (~400 mL/ 25 students)
- 1N HCl (titrant) (~600 mL minimum /25 students) ma
- Phenolphthalein indicator (~300 mL/ 25 students)
- Waste containers
  Lots. Generally 2 x 4 L jugs / 25 students.
- Safety glasses (1 pair/student)

Chemicals required:

3N $BaCl_2$:

$$3N = \frac{1\ Mol}{2\ eq} X\ \frac{3\ eq}{1\ L} = \frac{1.5\ Mol}{L}$$

$$1.5M\ BaCl_2 = \frac{1.5\ Mol}{L}\ X\ \frac{208.23\ g}{Mol} = \frac{312.35\ \text{g}}{\text{L}}$$

1% Phenolphthalein:

For 100mL, dissolve 1g Phenolphthalein in 50mL 95% EtOH. Transfer to 100mL volumetric flask and dilute to final volume with distilled water.

1N HCl:

Concentrated HCl (38%) = 12Mol/L = 12N. Dilute to appropriate volume, i.e. to make 6L, add 500mL of concentrated HCl to 4L RO water. Dilute up to 6L. We use a small carboy with spigot to dispense into squirt bottles.

Notes for the Instructor

The lab exercise generally takes 1-1.5 hours for Part A and B and about the same time for Part C, D and E. In our 3-hour labs it is generally combined with another activity, but this would be excellent in a 2-hour lab time as well. The lab exercise as described does not include methodology controls due to space and time constraints (we are using native topsoil with deciduous litter and 25 mL of water as our control). However, additional controls could easily be added that looked at no soil, no water, no litter, and/or an empty jar with an alkali trap. This lab could presumably also be adapted to use CO_2 probes or compare with them.

We have set this up to run a single factor ANOVA with 5 replicates as we generally have ~20 students to share the work, but you could add more replicates or factors. You could run 4 replicates, or this can be adapted to a two-way ANOVA as was done in the past at the University of Alberta:

Respiration setup for a two way ANOVA has been done with 16 incubation chambers containing combinations of two types of leaf litter and two levels of one other factor (moisture, oxygen – lids sealed vs. opened briefly, temperature and particle size – crushed or uncrushed) as outlined in Table 7 below. The class was divided into four groups, with each group responsible for one replicate of the experiment.

Table 7. Outline of treatments combining two types of leaf litter with two levels of one other factor.

	Litter type 1	**Litter type 2**
Low treatment factor	Treatment 1	Treatment 2
High treatment factor	Treatment 3	Treatment 4

This is a lab that has never let us down as a single factor experiment run for one week. We have experienced saturation issues when run as two factor experiment - there was more CO_2 produced than could be absorbed by 25 mL of sodium hydroxide (NaOH) (legumes are generally high producers and combined with high temperature or high moisture were guilty of this; likewise running for more than a week can cause a similar issue). This results in an inability to calculate the amount of CO_2 produced (all you can say is it was greater than 1.654 mg CO_2/g/day for native topsoil – the theoretical limit of absorption for 25 mL of NaOH). You could try using more NaOH and changing the formulas if you want to avoid this issue or want to run the incubation longer. We presume that this would not be a problem with the use of CO_2 probes.

Although this lab generates a fair amount of chemical waste, we have found that our chemistry department is happy to combine our alkaline waste with their generally acidic waste for disposal.

Technical Considerations

During set up, we try to arrange materials such that we avoid "traffic jams" at specific stations. Soil types are on one side bench, litter types on another, with balances at each at both locations (2-4 balances/location). Jars and vials are placed to be easily accessible to all students.

Note that you can modify the soil and litter choices to reflect your region. We have discovered that you really don't want to buy the bagged stuff from a greenhouse/ hardware store, as it is usually amended with organics and/or fertilizer. We tend to go to a garden center and buy local topsoil that they have in bulk (or soil from someone's yard would work). If the soil treatment is being tested it is important for students to weigh the soil (we have them use 100 mL to keep the volume consistent, but the weight needs to be added to the litter weight to calculate the proper mg/g/day result).

It is important to be accurate in the preparation of the NaOH and HCl chemical solutions. You can test the concentrations by titrating your NaOH solution + indicator with the HCl solution, and for 5 mL of NaOH you should use 10 mL of HCl. We have found it best to make a bulk solution in carboys so that the solutions are consistent across sections. We keep our carboys/chemical wash bottles in the fumehood with bench coat covering the work surface and graduated cylinders for measuring NaOH during Part B. It is good to have RO water at a station with graduated cylinders to avoid bottlenecks.

For the analysis week (Part C) we use one burette setup per pair of students on the benches. A burette setup consists of:

- Burette
- Burette stand and clamp
- 125mL Erlenmeyer flask

For each group of 4 students / 2 buretttes, we include on the bench one 500mL bottle of 3N $BaCl_2$, two 20mL syringes labeled $BaCl_2$ (as syringes made a cheap/easy measuring device for students), and one bottle of Phenolphthalein (with dropper). Pipettes and pipette aids are on the front bench in our labs but could be included in the burette setup.

As previously mentioned, 1N HCl is stored in a carboy in a fumehood, with wash bottles used to transfer HCl to burettes and for filling burettes between titrations. We also have extra bottles of $BaCl_2$ as this can take time to dissolve (and/or light heating) but this solution does not require special storage. The $BaCl_2$ keeps from term to term, and as it can take time to make up we prefer to have extra on hand. This solution is close to or at saturation and is added in excess, so if some precipitates out or students add more than indicated it does not affect the experiment. To make up the $BaCl_2$ solution, dissolve 312.35 g/L of $BaCl_2$ in distilled water on stirrer plate overnight (this is very close to saturation for this chemical so it takes a while, or you can cheat by heating it slightly). Transfer to 500mL media bottles for storage.

We use clearly labelled waste containers that are also in the fumehood, with funnels to minimize spillage. We find that they need to be checked after each lab, depending on the size of the container. Inorganic waste is disposed of as per University guidelines.

Clean up can be messy for this lab with the amount of dirt and glassware. Soil and litter are scraped into the garbage (we use the disposable pipettes for scrapping), and jars are rinsed by students. To prevent soil from going down the drain we use sieves in the sinks and a cart as a drying rack at the back of the lab for mason jars. To remove the precipitate from the titration glassware we rinse with 5% HCl solution (or leftover 1N HCl).

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About the Authors

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Donna Wakeford is a retired biology technician and Lab Coordinator from the University of Alberta in Edmonton, where she was involved with a variety of courses in plant and animal diversity, ecology and physiology. She currently enjoys reading, solitaire, and cat walking.

Appendix A

Research Summary Provided to Generate Class Hypothesis:

The rate of decomposition and therefore the amount of heterotrophic soil respiration can vary widely depending on the abiotic and biotic conditions. Abiotic factors such as oxygen, temperature, moisture, soil structure and the chemical composition of litter directly affect respiration rates (Subke et al. 2006). Oxygen is a necessary molecule in the respiration equation (Equation 1 for this lab), so if oxygen is present then decomposition can proceed faster than under anaerobic conditions (Smith et al. 2013). Temperature and moisture have been positively correlated with soil respiration rates, suggesting that future increases in global temperature and precipitation could increase CO_2 emitted from soils (Colman and Shimel 2013, Ryan and Law 2005).

Soil texture can have a significant impact on the microbial community and therefore on soil respiration through the relative amounts of clay, sand, and silt (Coleman et al. 2004). Clay content has been shown to have a negative relationship with microbial respiration (Colman and Schimel 2013). Sand offers less organic matter and larger pore sizes so that CO_2 is immediately released into the atmosphere and not held in the soil (Bouma and Bryla, 2000). Peat soils have a higher rate of respiration than clay soils, and both support higher respiration rates than sandy soils (Koizumi et al. 1999). The nature of the organic matter in the soil can also influence respiration: soils in broadleaf or mixedwood forests support increased decomposition rates compared to conifer forests (Prescott et al. 2000).

The composition of the plant litter or other materials has also been shown to influence the rate of soil respiration, as different types of carbon compounds break down at different rates. Simple sugars such as glucose are small, soluble in water, and are quickly lost by leaching or are immediately consumed by microbes (Smith et al. 2013). **Cellulose**, a major plant carbohydrate, is a larger, more complex molecule, and is more difficult to decompose (Smith et al. 2013). Breakdown of the cellulose molecule occurs extracellularly, when enzymes are secreted which depolymerize the cellulose molecule. An even larger and more complex plant polymer is **lignin**, which provides structural support in woody plants. Lignin is usually only broken down by fungi, again by extracellular decomposition, and in leaf litter with a high lignin content decomposition can be relatively slow (Smith et al. 2013, Molles and Cahill 2011). Broadleaf plant litter has been shown to decompose at a faster rate than needle litter in British Columbia forests (Prescott et al. 2000), potentially related to amount of lignin.

The rate of soil respiration also varies according to the ratio of carbohydrates to other compounds present in the leaf is also related to the plant litter quality. The primary nutrient influencing initial decomposition and the soil respiration rate is nitrogen, and this is usually expressed as the **C:N ratio** (Prescott 2006). Nitrogen is a necessary source of nutrients for decomposers, so leaves with a low C:N ratio tend to be both higher in nutrients and have less cellulose/lignin, making them faster to decompose, while those with a high C:N ratio tend to be high in cellulose/lignin and slow to break down (Stiling 2012). For example, fresh leaves off the tree have an average C:N ratio of 14:1, whereas leaves that have fallen from the tree have an average C:N ratio of 31:1 (Table 8).

Table 8. Initial average C:N ratios of selected litter types reported in the literature.

Litter type	*Average C:N ratio*	*Source:*
Fresh leaves	14:1	Seneviratne (2000)
Litter (fallen leaves)	31:1	"
Legume plants	16:1	"
Trembling aspen	70:1	Moore *et al*. (2011)
White birch	67:1	"
Carex (sedge)	26:1	Bayley and Mewhort (2004)
Typha (cattail)	35.5:1	Steinbachová-Vojiskova *et al*. (2006)
Jack pine	39:1	Moore *et al*. (2011)
Black spruce	68:1	"

Heterotrophic soil respiration rates are used in many areas of ecology, agriculture and industry. They can provide information about the ability of specific soil microbes to decompose organic matter; they can be used to study the biodegradability of organic compounds, especially synthetic organic chemicals; they quantify the effect of potentially toxic chemicals in agriculture, especially relating to soil damage; they are used to evaluate the ability of damaged soils to recover from contamination by mining activities, chemical waste disposal, or other industrial practices; and they are used to determine the ability of different soil types to support agriculture.

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