

Breaking It Down: What Factors Control Microbial Decomposition Rates?

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Abstract

Demonstrating and modeling changes in ecosystem processes in the laboratory classroom can be logistically difficult and expensive. This complexity often leaves little time for students to generate and test hypotheses. Yet, we must foster student understanding of how matter and energy move through ecosystems to develop an appreciation of how current ecosystems function and how human-mediated global change may alter ecosystem processes. In this lesson, we describe an adaptation of the Tea Bag Index (TBI) that provides students with an inexpensive, adaptable, and easily replicated method for testing how an ecosystem function (*i.e.*, decomposition by microorganisms) alters carbon flow between two carbon pools (*i.e.*, dead organic matter and the atmosphere). We outline the steps that small student research groups can take to develop testable research questions with an emphasis on how abiotic factors (*e.g.*, temperature, moisture availability) can influence the rate of biomass loss. We outline the equipment and methods that can be used for conceptual add-ons (*e.g.*, CO₂ gas analysis) and include exercises that work on teaching students principles of tidy data organizing and data analysis. Finally, we include rubrics for written and graph-based assignments and an example dataset to assist instructors in implementing the lab in their own courses. In post-lab evaluations, students reflected positively on this lab exercise in open-ended course evaluation prompts and we observed better quality data collection and analysis in subsequent experimental labs, likely motivated by the practice and guidelines provided in this lab module.

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Learning Goals

Students will:

- ◊ explore through experimentation the role of heterotrophic microbes in facilitating the flow of carbon through ecosystems.
- ◊ be able to explain the role decomposition plays in transforming organic carbon into organic and inorganic carbon compounds.
- ◊ understand how projected changes in Earth's climate are likely to affect ecosystem processes.
- ◊ know how to design an appropriately replicated experiment.
- ◊ From the Ecology Learning Framework:
 - » How does matter and energy move in an ecosystem?
 - » What impacts do humans have on ecosystems?
- ◊ From the Microbiology Learning Framework:
 - » How do microorganisms interact with their environment and modify each other?
- ◊ From the Science Process Skills Learning Framework:
 - » Plan, evaluate, and implement scientific investigations

Learning Objectives

Students will be able to:

- ◊ describe the transfer of solid (organic) matter to (inorganic) gas through decomposition and microbial respiration.
- ◊ predict how environmental conditions (*e.g.*, temperature, moisture availability) regulate ecosystem processes.
- ◊ design, evaluate, and communicate the results of an independent experiment.
- ◊ conduct and interpret statistical analyses on differences in responses between experimentally defined factors and their levels.
- ◊ conduct and interpret correlation analyses between two continuous environmental variables.

INTRODUCTION

Ecosystems are dynamic and are often defined by the pools and fluxes of energy that flow through the network of biotic communities that reside within a region. Atmospheric carbon is found in different forms (e.g., CO_2 , CO, CH_4), which are taken up by organisms and then later released by abiotic and biotic processes. This anabolism and catabolism of carbon-containing molecules facilitates energy transfer between different trophic levels. Consequently, the sources, fluxes, and states of carbon on a global scale are extremely important to living organisms. Broadly, carbon moves in two interconnected cycles: a slower geochemical cycle that takes place over millions of years and a more rapid biological carbon cycle that can turn over during only a few years. Biogeochemically speaking, the continental crust and upper mantle of the Earth contain the largest carbon pool on Earth; a significant portion of this pool is contained in sedimentary rocks. These substrates, when weathered, release carbon into the atmosphere and oceans. Oceanic carbon—primarily in the form of dissolved inorganic carbon (DIC)—is the next largest pool of carbon on the planet. In terrestrial systems, atmospheric carbon is transformed into organic carbon compounds via photosynthesis. These fixed organic carbon compounds accumulate in the soil or cycle back to the atmosphere via microbial respiration. Organic carbon compounds can also eventually leave the rapidly cycling pool and get stored as fossil carbon. Both organic and fossilized carbon stores may eventually cycle back to the atmosphere as CO_2 through processes like the burning of fossil fuels. Archer (1) provides a thorough review of the global carbon cycle and how these fluxes are likely to shift under future climate conditions.

Carbon bound in the biological carbon cycle tends to cycle quickly through terrestrial ecosystems. Autotrophs fix atmospheric CO_2 into organic compounds, which in turn are either (i) transferred to heterotrophs via consumption, (ii) used as energy stores and respired by the plants themselves, or (iii) deposited into the soil as dead organic matter. Once this organic matter is in the soil, decomposition—the breakdown of this organic material—proceeds in large part through the process of microbial heterotrophic respiration. The carbon-rich detritus is broken down and used to fuel ATP-synthesis for microorganisms. The carbon is then released as CO_2 gas back to the atmosphere or stored in another form within the soil. Importantly, decomposition is an **ecosystem process** which facilitates the cycling and movement of minerals, nutrients, and energy through a natural system. Like every other ecosystem process, microbial decomposition is a function of not just the microbes, but also the environments in which these microbes exist. State variables in an ecosystem model capture properties of the system at a particular point in time, such as total carbon content, while flux variables represent the flow or movement of things in time, like energy or nutrients (such as carbon), between different parts of the ecosystem. By examining how environmental factors can influence microbial respiration and thus rates of decomposition, we gain insights into what: (i) controls the amount of an element in any given pool (collection) within natural systems (**state** variables), (ii) dictates the rate of element movement between different pools in natural systems (**flux** variables), and (iii) impacts changing environmental conditions (e.g., global warming, droughts) may have on element flow through the biosphere.

This lab exercise explores how environmental conditions alter decomposition by experimentally evaluating factors that: (i) alter the rate of change in biomass (a potential proxy for available energy [2]) and (ii) drive rates of microbial respiration. Broadly, it considers how much and at what rate carbon bound up in biomass is transferred to the atmospheric pool of CO_2 . The standardized Tea Bag Index (TBI), developed by Keuskamp *et al.* (3), is a method for assessing decomposition rates in soil ecosystems. This method involves placing mesh bags with known qualities of decomposable plant material (i.e., tea leaves) into the environment (soil) and measuring their decomposition over time. This index offers a consistent way to compare decomposition rates across different ecosystems, and thus understand how different environmental factors influence organic matter breakdown. In this lab exercise, students use a modified version of the standardized Tea Bag Index (3) to design and conduct an experiment testing how one (or more) physical (i.e., non-living or abiotic) aspect of the environment contributes to the rate of microbial decomposition. The TBI approach is particularly useful in a classroom context because it standardizes the litter bag materials, leaf litter content, and litter bag dimensions, reducing variation in outcomes that may mask important trends. The TBI has been used in various modalities and research projects including:

- i. Citizen science research (4)
- ii. Intertidal zones (5)
- iii. Savanna and mountain forests (6)
- iv. Aquatic habitats (7)
- v. Agroecosystems (8)

Additional information about the TBI, associated field protocols, additional resources, and reports on global data collection efforts are available at the [TBI research group's website](#). Generally, this microcosm method provides a tractable experimental model of a large-scale ecosystem process with a protocol that is rapid, cheap, and ideal for hypothesis testing. There are, however, caveats to this system that—while not directly relevant to this teaching lab protocol—may influence conceptual extensions that science teachers may want to pursue with their students (9–11). For example, differences in tea bag material composition (e.g., Nylon vs. plant-based mesh, mesh size) can result in different losses of the hydrolysable fraction of tea biomass. However, given that this lesson protocol encourages the use of only one type of tea bag from one manufacturer, differences between manufacturers should not impede the ability of students to generate quality data from their experiments.

Intended Audience

This laboratory exercise is flexible with respect to the intended audience. The main goal of this exercise is to demonstrate the interconnectedness of abiotic parameters and biological processes. This exercise lends itself well to guided inquiry design for advanced/honors biology courses in high school or as a bounded inquiry design for first- or second-year college students in community college, liberal arts colleges, or large research universities. Specific college majors that may be best served by this Lesson include Biology, Environmental Studies, or similar majors unique to institutions that work towards developing student expertise in ecosystem processes; students pursuing a research concentration in their major

will particularly benefit from the experimental design, data analysis, and data visualization aspects of this lesson.

Required Learning Time

Timing for this lesson is flexible; we have conducted this lesson during four independent sessions over 2–3 weeks. The first session is 2–3 hours and covers a review of the project background, information on how to generate hypotheses, and setting up the experiment. The second session (one to seven days after the first session) takes 30 minutes to one hour and includes CO₂ gas measurements. The third session (14 to 19 days after the first session) involves removing tea bags from the decomposition units and placing the tea bags in a drying oven. The fourth session (48 hours following the third session) involves measuring the dry weight of the tea bag. Review and in-class instruction of tidy data structure and data analysis could occur during the fourth session of this laboratory. Depending on the prior knowledge base of the students this review could take 30 minutes to one hour (see Supporting File S1).

Prerequisite Student Knowledge

Students conducting this lab should have some experience with sterile technique, use of serological pipettes, experimental design, lab safety protocols, and using lab equipment (e.g., microbalances, drying ovens). Students should have background knowledge in the biophysical components of decomposition, respiration, microbial ecology, natural gradients in abiotic conditions, and carbon cycling; this laboratory can supplement class-based learning of these concepts or additional laboratory time can be devoted to reviewing these processes (Supporting File S1). Finally, students should have some familiarity with the basics of graph generation, generating averages and measures of variance, or data analysis in a spreadsheet program, such as Microsoft Excel or Google Sheets. However, this lesson and the proposed assessment tools can be modified to reflect the extent to which your students can use, and meaningfully understand, the different aspects of experimental design. For example, students without a solid grounding in statistical analysis could forego that component of this lesson and focus their attention on their observations and visually comparing average responses between treatment levels.

Prerequisite Teacher Knowledge

Teachers will best serve students in this lab if the teachers have a strong working knowledge of experimental design, the availability of resources to generate abiotic gradients (e.g., growth chambers, light sources, etc.), equipment used for attaining CO₂ gas measurements, and experience with tidy data organization (12), statistical analysis, data visualization, and knowledge of the core biological principles of microbial ecology and soil respiration.

SCIENTIFIC TEACHING THEMES

Active Learning

This laboratory module builds strongly on principles of team-based bounded inquiry design. Project-based learning modalities consistently generate strong student academic achievement (13, 14), stimulate student interest (15), and can enhance students' feelings of belonging in the STEM field (15, 16). This laboratory design provides a framework for

STEM students to generate and test their own hypotheses; the lesson timetable (Table 1) outlines student progression from brainstorming to hypothesis generation to hypothesis testing. More specifically, lab partners will discuss question prompts (see *Lesson Plan*), identify abiotic conditions they wish to evaluate, and generate concrete hypotheses and predictive plots estimating the outcomes of their work. Students will then work with instructors to design experiments to evaluate their hypotheses, reviewing concepts of experimental design and teamwork while organizing, collecting, analyzing, and visualizing data.

Assessment

Written reports are optimal for student knowledge retention and to facilitate evaluation of student content knowledge (17). To report on the outcome of this experiment, students follow prompts that help them generate a summative technical report (12 sentences total) that reports the outcome of their experiment (see *Lesson Plan* for guidelines). Communicating results in multiple modalities reinforces equitable access and engagement for all learners, consistent with [Universal Design for Learning \(UDL\) guidelines](#). Consequently, the technical abstract is paired with a data visualization (e.g., figure, table) that reinforces the results communicated in the abstract. Rubrics are provided for both assignments and the assessment of student work. Student perceptions of this lab were generally positive. In open-ended evaluations conducted at the term end ~14% of respondents (12/89 students) mention the “decomposition” lab by name as a positive aspect of the overall lab course, and only one student (~1%) mentioned they would replace this lab.

Inclusive Teaching

Students underrepresented in STEM fields report a stronger interest and commitment to STEM projects and exercises that are project or problem-based (15, 16). Consequently, the context for conducting this experiment and how the results could be applied will and should be a foundation of this lesson. This laboratory scaffolding used here has direct applications to how ecologists determine the ways in which climate change may modify CO₂ generation and carbon cycling processes. This real-world application and the modeling of a contemporary, global problem is an effective means for engaging student interest and enhancing students' perceptions that their results “mean something.” When introducing this lab, instructors can highlight the global effects of increased greenhouse gas emissions and their contribution to changes in the global carbon cycle. Additionally, instructors can use this opportunity to highlight how climate change effects modeled in the lab correspond to disparate consequences based on a population's geographic position and socio-economic status (18). By reinforcing **why** this experiment matters (e.g., applications, estimating future conditions), students underrepresented in STEM fields are likely to feel greater commitment to the lesson objectives and commit greater effort to lesson completion (15).

This lab has been designed to be flexible and can easily be tailored to a learning institution's available finances and equipment. For example, measurements of CO₂ gas concentration in the bottle head space are supplementary and the costs associated with those pieces of equipment and

related supplies could be omitted if the lab instructor chooses to focus solely on measuring how the tea bag's weight changes over time in response to the variables the students choose. The juice bottles we used for this study are reusable with surface disinfection, reducing the need to repurchase new plastic bottles for each student cohort. There are many cost-effective options available for students to generate meaningful treatment levels to evaluate how physical conditions modify loss of biomass from tea bags (e.g., modifying substrate salinity with table salt, placing the bottles in dark versus sunlit locations). Finally, this lab may allow students with disabilities (that would preclude their ability to participate in field-based learning) to envisage and test how ecosystem processes are regulating nutrient flow under different conditions.

LESSON PLAN

Session One (2–3 Hours): Background, Hypothesis, and Experimental Setup

Part 1: Background (35–45 Minutes)

Instructor's Role: The instructor's role in session one of this laboratory is to **first** introduce the Big Ecological Question and the context of the project/problem the students will address with their hypotheses. This introduction (15 minutes) should refresh student understanding about major carbon cycle concepts, the role respiration plays in that cycling, and how current and future climate conditions may modify respiration. Next, the instructor will provide a series of questions for students to respond to individually before comparing their reflections with their lab partner and finally sharing their response with the whole laboratory class (i.e., Think-Pair-Share; Table 2, Supporting File S1). Questions can vary based on student experience and content knowledge; some example prompts are provided below. Instructors should not include more than four questions (two of which should have an applied focus) and limit student Think-Pair-Share to 20–30 minutes. Additional instructor resources for Think-Pair-Share are provided in Supporting File S1.

Part 2: Hypothesis Testing (30 Minutes)

Instructor's Role: Student pairs should take their reflections from the Think-Pair-Share and start thinking about the major research question they wish to test. At this point the instructor should demonstrate the experimental units the students will be using to test decomposition. The complexity of this research question is a function of what physical gradients the instructor can provide resources for; we provide a table below outlining some examples of different variables students could test with commonly available resources (Table 3). The instructor should have the students generate a specific, directional hypothesis regarding how one gradient in an environmental condition will modify a specific measure of decomposition. Each student group should propose at least one hypothesis framed around a substrate modification and at least one hypothesis pertaining to a climate modification.

Part 3: Experimental Unit Setup (60–90 Minutes)

The hypotheses students select should be experimentally tractable given the resources available to the class. Once student groups defined their hypothesis and the instructor agrees that the hypothesis is testable with the available resources, the students start construction of their experimental units, the decomposition bottle (Figure 1). A detailed description of the

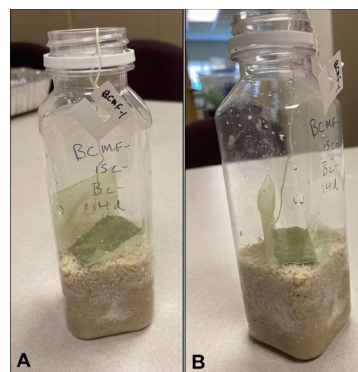


Figure 1. Decomposition bottle setup and labeling. (A) Optimal labeling (bottle and tea bag tag) and tea bag tag position for the experimental decomposition setup. (B) Side view of tea bag position on substrate surface to ensure continuous contact with the substrate in the decomposition bottle.

equipment and setup of a decomposition bottle are provided in Supporting File S2. Briefly, standardized weights of sterilized substrate are added aseptically to each bottle and the substrate is wetted to near saturation. Students then label and weigh the teabags that will be placed in their decomposition bottles. The teabag is put into the bottle, positioned such that the dried leaves contact the substrate. Finally, a small volume of sterile DI water (~3 mL) is added directly to each tea bag to catalyze decomposition. The decomposition bottles can now be moved to whatever environmental conditions are available and the student group wants to test. The steps outlined above can be modified if a student group's hypothesis would be better tested by modifying these starting conditions. For example, a group may want to add different amounts of water to the substrate to test how substrate moisture levels modify decomposition rates.

Session Two (45–60 Minutes): Prepping Tea Bags and Optional Additional Data

Part 4: Pulling, Cleaning and Drying Tea Bags (15–20 Minutes)

Tea bags must be dried prior to final weight measurements to make sure that residual water in the tea bag is not providing an over-estimation of remaining biomass in each bag. Students should remove their tea bags from their decomposition bottles using the protocols outlined in Supporting File S2 and dry the tea bags at a low temperature (~60–65 °C) for 48 hours prior to making final tea bag weight measurements and analysis (session three).

Instructor's Role: For some student groups it may be unreasonable to ask students to make an additional trip to the laboratory between lab sessions. For these students, one of the instructors could complete this task for the students so that all teabag samples are dried and ready for analysis by the start of session three.

Part 5 (Optional): Experimental Add-Ons: A Case Study with CO₂ Measurements (30–40 Minutes)

The experimental unit setup described above is a scaffold for providing inexpensive visualizations and measurements of biomass loss and estimates of microbial decomposition in a hypothesis testing framework. The experimental unit structure and design is flexible enough, however, to permit additional measurements that could be paired with estimates of biomass loss. For example, access to an elemental analyzer would

allow students to estimate how carbon, nitrogen, and hydrogen concentrations change over time as decomposition progresses. A modified protocol outlining how to alter the setup to permit estimates of CO₂ concentration in the headspace of each decomposition unit is provided in Supporting File S2 using a modified decomposition bottle setup.

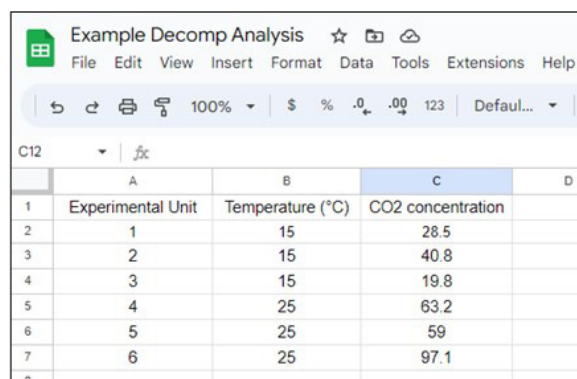
Session Three (2–3 Hours): Data Collection, Organization, Visualization, and Analysis

Part 6: Data Collection and Organization: Tidying Up (35–50 Minutes)

During the second lab section, students start by weighing the dried tea bags from their decomposition units. This is a great lab for discussing how preparation and organization can lead to efficient data analysis and visualization and teach the formatting associated with tidy data sets (*i.e.*, “a standard way of mapping the meaning of a dataset to its structure” [12]). The method of collection and how collected data are organized in a spreadsheet can vary based on student experiences in research and their understanding of the methods being used to analyze the data. For example, in a decomposition study that examined how incubation temperature influenced CO₂ generation within a decomposition unit, student data may be collected in formats that require modification to “tidy” the data structure in order to proceed with analysis (Tables 4 and 5).

To analyze and visualize the data collected in this study, it is now necessary to convert these data structures into tidy data format. In brief, data sets collected for one study (*i.e.*, one type of experimental unit) can apply tidy data structure following two standard guidelines (modified from Wickham [12]):

1. All the variables are represented by different columns. For example, in a decomposition study that examined how temperature influenced CO₂ generation within a decomposition unit there would (at a minimum) be columns with the headers identifying experimental unit number, the temperature treatment, and the amount of CO₂ measured.
2. Every unique observation unit is a row. Because this decomposition lab applies to treatments typically at the level of the decomposition bottle, the conditions/treatments imposed on the tea bag in that bottle and responses are measured from each bottle, each decomposition bottle is an experimental unit and gets its own row in the data set.



	A	B	C	D
1	Experimental Unit	Temperature (°C)	CO2 concentration	
2	1	15	28.5	
3	2	15	40.8	
4	3	15	19.8	
5	4	25	63.2	
6	5	25	59	
7	6	25	97.1	

Figure 2. Tidy data structure in Google Sheets. A screenshot of example data entered into Google sheets with the data in tidy structure.

We can convert the information collected by students in example Table 4 and Table 5 into a data structure that is readily visualized and analyzed in Google Sheets, Microsoft Excel, or R (Table 6, Figure 2). A list of spreadsheet programs for data organization is provided in Supporting File S1.

Part 7: Data Visualization and Analysis (60–90 Minutes)

Measuring green tea leaf decomposition in these juice bottle units generates an experimental system that lends itself well towards building complexity in visualization and analysis. The visualizations and analysis of data depend on the hypotheses being tested, but with tidy data organization, spreadsheet programs should allow rapid visualization of student data to visualize trends. Microsoft Excel and Google Sheets both work well to get basic visualizations of student data (Figure 3).

Analysis of student decomposition data should closely follow their experimental design. Some statistical tests (*e.g.*, paired *t* test, independent *t* tests, and Pearson coefficients) can be conducted within spreadsheet programs and add-ons may be available to include other types of analyses in these spreadsheet programs (*e.g.*, XLMiner Analysis ToolPak in Google Sheets). Other free web-based interfaces allow students to copy their data from their spreadsheets into pre-organized input forms and generate statistical results. For example, [VassarStats](#) (authored and moderated by Richard Lowry) provides an excellent resource for free and accessible statistical computation and provides [an informative web-based companion text-book](#). See Supporting File S1 for additional references for data visualization and analysis.

Below is a list of commonly used statistical tests with examples of decomposition research questions and data that could be analyzed by that test.

1. ***t* test:** Comparison of a continuous response variable between two discrete levels within an experimental factor.

Example Question: When soils are fully saturated with water, is the amount of biomass remaining after 14 days greater in decomposition units held at a constant 15 °C versus decomposition units held at a constant 25 °C?

2. **One-Way ANOVA:** Comparison of a continuous response variable between more than two levels within an experimental factor.

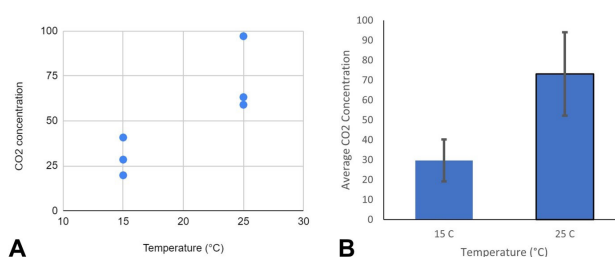


Figure 3. Example graphs generated from tidy data. (A) Google Sheets scatterplot depicting raw data values from example data. (B) Microsoft Excel column plot depicting average CO₂ concentrations ± standard deviations for example temperature treatments.

Example Question: When fully-saturated soils are held at a constant 25 °C, does the amount of biomass remaining after 14 days differ between experimental units that are exposed to one of three light-dark photoperiods levels: 8-16, 12-12, and 16-8?

3. **Linear Regression:** Testing the accuracy of a continuous variable to estimate the value of a continuous response variable.

Example Question: When experimental units have fully-saturated soils and are held at a constant 25 °C, does the initial dry biomass of the tea bag correlate with the amount of biomass remaining after 14 days?

Part 8 (Optional): Analysis Add-On: Data Visualization, Organization, and Analysis in R

In our labs, we successfully introduced statistical programs designed for education (*i.e.*, [DataClassroom U](#)) in the R programming language as an interface to analyze experimental data, but using R is not necessary to achieve the learning outcomes described above.

Part 9: Student Research Product: Technical Abstract and Figure Guidelines (15–20 Minutes)

This lab's assessment entails two required products from each student: (i) a short technical report, similar to an abstract, describing the project's conceptual background, the research question, a brief summary of the approach taken, a results statement, and concluding thoughts; and (ii) a publication-quality figure and caption depicting the trends relevant to the student's primary question. Pedagogically, the technical report portion of the assignment engages the "Writing-to-Learn" paradigm (17), reinforcing STEM student learning by requiring knowledge recall, summarization, and application. Assessments that have greater perceived "utility value" by students increase equitability with STEM courses (19, 20); the figure and caption generation portion of the assessment provides students additional practical, professional practice in results visualization and communication. Student-facing assignment guidelines are provided in Supporting File S3. Examples of rubrics for the technical report and figure assignment are provided in Supporting Files S4 and S5, respectively.

Part 10: Limitations of this Lab Protocol and Design

1. This protocol only tests decomposition by the bacteria and fungi already present in the tea bag itself. Microbial communities may differ between bags necessitating adequate replication to account for variance in the communities of microbes that are present.
2. The diversity of microbes in different ecosystems is not accounted for using this approach. This can be remedied by conducting the assay using unsterilized soil from different ecosystems, or by inoculating the sterilized soil with specific microbes, and measuring respiration and weight loss in these systems relative to uninoculated systems.
3. This assay may not fully capture the complexity of natural decomposition processes, as it uses a simplified substrate (tea) that may not represent all types of organic matter. This can however be remedied by certain strategies. For example, by comparing the results of two assays—one with less processed tea that contains

relatively higher labile carbon compounds; and another with more processed tea that contains more recalcitrant compounds—one could make inferences about how different substrates could alter the outcome.

4. Sense-sensitive students are likely to be less than enthusiastic about collecting and cleaning the tea bags after 12 or more days. Decomposition is not often a clean, pleasant smelling biological process and this aspect of the experiment may dissuade student engagement. For scent-sensitive students, it may be necessary to partition duties such that they are not exposed directly to the decomposed tea bags or additional PPE supplies can be provided to minimize adverse stimuli (*e.g.*, nose plugs).

TEACHING DISCUSSION

This lesson was trialed at Gonzaga University during Spring term 2023 in seven lab sections that included a total of 115 sophomore biology majors taking the co-requisite courses Bio 206 Ecology and Bio 206L Laboratory in Ecology. Anecdotally, students enjoyed the practice of hypothesis testing and working through the progression of analysis and graphics generation. Although some students were deterred from the lab given the smell and texture of the tea bags after 14+ days decomposing, other students mentioned the lab as one of their favorites, stating consistently that it was fun to see the process of decomposition take place and pair it with quantitative analysis of their results to evaluate their hypotheses.

While measurements of CO₂ concentrations are a good add-on to this lab, CO₂ measurements are **not** essential for the lab to complete the hypothesis generating and testing learning objectives. CO₂ sensors can be expensive, and we found that CO₂ generation tracks closely with biomass loss with this design (Figure 4), suggesting that the inexpensive metric of weight loss reasonably predicts the amount of CO₂ generated in each bottle. As a resource for the instructors implementing this lab, it may be helpful to have a sample data from other previous runs of this experiment. We have included a set of raw data in tidy format and an example of graphical output from this data as an instructor reference (Supporting File S6).

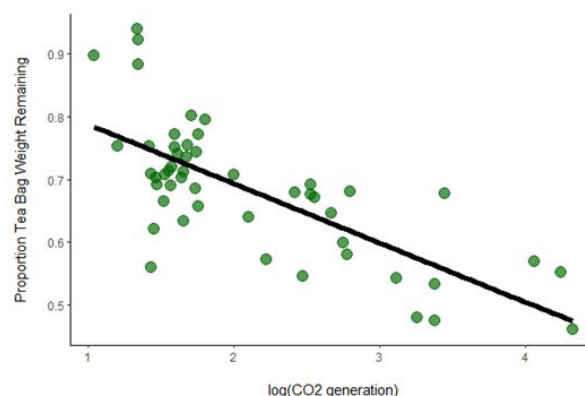


Figure 4. Relationship between CO₂ generation and tea bag weight loss. A linear regression demonstrating how CO₂ concentration measured 24 hours after project start correlates with amount of tea biomass lost after nearly two weeks. The strong negative relationship we observed suggests that loss of biomass from the tea bags is a good approximation for CO₂ generated by microbes in these decomposition units. If equipment needed to estimate CO₂ concentrations in the students' experimental units is too costly, estimates of biomass loss are still a strong means of estimating how CO₂ generation was affected by the student's experimental condition.

SUPPORTING MATERIALS

- S1. Exploring Decomposition Rates – Background materials guidelines
- S2. Exploring Decomposition Rates – Details of decomposition unit setup
- S3. Exploring Decomposition Rates – Example student-facing assignment
- S4. Exploring Decomposition Rates – Example technical report rubric
- S5. Exploring Decomposition Rates – Example figure and caption rubric
- S6. Exploring Decomposition Rates – Example data set and figure

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Table 1. Lesson plan timetable. A proposed timetable for covering three independent meetings that cover hypothesis generation, experimental setup, data collection, and data organization and analysis.

Activity	Description	Estimated Time	Notes
Session 1			
Background discussion	Instructor-led discussion of carbon cycling, the role of decomposition, and the role of the environment in decomposition	15 minutes	We emphasized the visual elements of the cycle and a few examples of how abiotic factors, such as temperature, influence the rate of processes
Think-Pair-Share	Student pairs work through Think-Pair-Share questions	20–30 minutes	Circling the room and visiting groups during this session helped catalyze conversation
Hypothesis/ Research question generation	Student pairs examine available equipment and formalize a research question to evaluate. Identify the experimental design and statistical tests that will evaluate their question	30 minutes	Meeting with groups individually to make sure that hypotheses are testable was productive, but may add a few minutes to the duration of this step
Experimental setup	Student pairs will setup their experimental units following the lesson protocol outlined above	60–90 minutes	
Session 2 (Any Time 1 Day to 7 Days After Session 1) — OPTIONAL			
CO ₂ generation measurement	Using CO ₂ sensing probes to estimate respiration within each decomposition bottle	30–40 minutes	Working with one or two groups individually to teach them the technique and then letting these groups teach subsequent ones divided instructor time. It may be worthwhile to setup a sign-up schedule where groups come in collectively to make these measurements
Session 3a (12 Days After Session 1)			
Pulling, surface, cleaning, and drying tea bags	Tea bags should be removed from bottles 48 hours prior to lab time, surface cleaned, and dried for 48 hours at ~60°C	15–20 minutes	This part can smell bad, and the tea bag texture with fungus and bacteria on the outside can be off-putting. Make sure students are aware of these components of the collection
Session 3b (14 Days After Session 1)			
Weighing tea bags	Tea bags should be removed from drying oven and promptly weighed. Students will use starting and ending dry weight to estimate proportion biomass lost	15–20 minutes	Using scale that measure to the nearest 1.0 milligram (0.001 g) is helpful, but we have also picked up differences using scales that measure to the nearest 10 milligrams (0.01 g)
Data tidying	Students will take their collected data and get it into tidy format	20–30 minutes	
Data visualization and analysis	Students will generate graphs and conduct statistical tests on their experimental data	60–90 minutes	
Assignment review	Students will review what is required of them regarding assessment for this lab protocol	15–20 minutes	This part can also be assigned remotely if you would prefer your students to focus on analysis and visualization during lab time

Table 2. Think-Pair-Share questions. Examples of questions that will help guide and catalyze discussion between students in a Think-Pair-Share format.

Section Content Knowledge Refresher Questions	
1.	Why do microbes respire?
2.	What are the inputs and outputs of microbial respiration?
3.	How does respiration differ between anaerobic and aerobic microbes?
4.	Draw a concept map that depicts the process of decomposition and include in the diagram how microbial respiration relates to carbon cycling.
5.	What happens to an ecosystem if the microbes stop respiring?
6.	How might climate change influence decomposition of dead organic matter?
Applied Questions	
1.	How is the climate in your region expected to change in the next 100 years?
2.	Under what abiotic conditions would you expect the decomposition of dead organic matter to increase? To decrease?
3.	How would you measure decomposition?
4.	How would you design an experiment to evaluate how one (or more) abiotic factors influenced decomposition rate?
5.	Generate a predictive plot of how one (or more) abiotic conditions will affect decomposition.

Table 3. Example experimental factors. Examples of physical factors that could be manipulated in student experiments modified to test their effects on decomposition rates. Students could consider manipulating the substrate, the climate, or both in their experimental units.

Substrate Modification	Climate Modification
Soil texture	Temperature
Soil particle size	Wind speed
Soil pH	Photoperiod
Soil saturation	Light quality
Soil salinity	Light intensity
Organic soil pollutants	Relative humidity
Inorganic soil pollutants	Fluctuations in any of the above variables

Table 4. “Untidy” data collection example one. First example of how a student may collect data for this experiment that will require transformation to tidy structure.

15 °C	25 °C
28.5	63.2
40.8	59.0
19.8	97.1

Table 5. “Untidy” data collection example two. Second example of how a student may collect data for this experiment that will require transformation to tidy structure.

Temperature (°C)	Unit 1	Unit 2	Unit 3
15 °C	28.5	40.8	19.8
25 °C	63.0	59.0	97.1

Table 6. Tidy data collection example. Fictional data set demonstrating the structure of column headers and rows in a tidy data structure ready for conversion to a datasheet (e.g., Google Sheets) for analysis.

Experimental Unit	Temperature (°C)	CO ₂ Concentration
1	15	28.5
2	15	40.8
3	15	19.8
4	25	63.2
5	25	59.0
6	25	97.1

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