ABSTRACT
Forests are excellent “outdoor classrooms” for active learning in ecology and evolution; however, in many areas trees have no leaves or visible animal activity for much of the year. Fallen leaves may preserve evidence of interactions such as herbivory and infectious diseases, although these can be difficult to differentiate from mechanical damage and decomposition in older fallen leaves. I developed an exercise in which students collect fallen leaves and observe several different types of damage to the leaves. I provide images and descriptions of different types of damage and practices to differentiate them. In addition, I provide a list of questions that can be answered by collecting fallen leaves and observations of damage. My students gained valuable quantitative literacy skills by entering data into an online worksheet and performing various calculations and data analysis techniques. This exercise provides many benefits and can be an engaging addition to a high school’s or college’s outdoor curriculum outside of the growing season.

Key Words: decomposition; herbivory; plant–animal interactions; plant diseases; quantitative literacy; woody plants.

Introduction
Education about the ecology of trees and forests is essential in a time of unprecedented global change. Historically, the United States has been a richly forested country, with approximately half of its land area (one billion acres) forested at the time of colonization by Europeans (Oswalt & Smith, 2014). During the 17th through 19th centuries, forests were extensively cleared (30–50% of forest area; Pimm & Askins, 1995); however, forest cover has been slowly increasing since the early 20th century as a result of concerted restoration efforts (Oswalt & Smith, 2014). Forestation has many benefits, as forests are important biomes that provide a higher value of ecosystem goods and services than any other terrestrial biomes (de Groot et al., 2012). Tree species diversity can increase the services provided by forests (Tekalign et al., 2017) and also increase the numbers of interactions between trees and population densities of organisms that would exploit trees, such as herbivores and pathogens (O’Brien et al., 2017).

Trees are the dominant macroscopic life-form on Earth (Bar-On et al., 2018) and interact with other organisms in myriad ways. Mutually beneficial interactions with pollinators and mycorrhizal fungi and commensal interactions with epiphytes and animals are abundant, as are interactions with herbivores and pathogens (Pautasso et al., 2005; Liebhold et al., 2017). Exploitative interactions, in which one species benefits at another’s expense, are particularly interesting from an ecological and evolutionary point of view, as herbivores and pathogens can influence tree population and community structure (Flower & Gonzalez-Meler, 2015) and act as strong selective agents (Bixenmann et al., 2016). Clearly, it is important to quantitatively survey these interactions.

Biology educators, researchers, and learners can gather evidence of interactions between trees and their natural enemies by observing damaged tree parts. Leaves are attacked in many ways, and observations of damaged leaves can be used to determine what organism damaged them (Labandiera et al., 2007). Evidence of damage can persist on fallen leaves of deciduous trees (Figure 1), which offer some advantages over leaves that are still attached to the tree. Fallen leaves are available in great numbers in the winter and spring, when deciduous trees possess no leaves in temperate regions. Fallen leaves can be more easily accessed than many leaves attached to high branches.

Here, I describe an activity in which students survey fallen leaves and record different types of damage to answer ecological questions and learn quantitative skills. Outdoor teaching exercises are engaging activities that expose students to the ecosystems and other content they are learning about in biology courses (Manner, 1995); however, activities must account for seasonal changes in regions with cold winter and springtime climates. This activity can introduce a strong quantitative component to an ecology, botany, or environmental science course, and provide an opportunity to collect data in the field during months in which actively growing plants are not readily accessible.
Learning Goals

By the conclusion of this activity, students should be able to

1. identify traces of plant interactions with herbivores and pathogens;
2. collect data in a structured manner and draw conclusions from those data;
3. interpret ecological interactions among plants, animals, and microbes using an evolutionary framework; and
4. connect ecological interactions to land use and global change.

Course Context

I developed this exercise for an ecology course at a public university in the southeastern United States, but it can also be used for high school biology and environmental science courses. The course is required of all biology majors, most of whom are pursuing a biomedical degree, and requires the introductory biology sequence as a prerequisite.

Methodology

Each student collects 50–100 fallen leaves. This collection can be easily accomplished during a single lab or class period, or as homework. Along with lab time, I allowed students to collect leaves at the location of their choosing on their own time. Students inspect each leaf for up to eight common types of damage caused by animals or microorganisms (Figure 2), as follows:

1. Edge feeding: tissue is removed from the leaf margin, commonly caused by beetles, caterpillars (including sawfly larvae), grasshoppers, or leafcutting bees
2. Hole feeding: circular or irregular perforations in leaf tissue, caused by beetles, caterpillars, or grasshoppers
3. Skeletonizing: leaf blade tissue is removed, leaving intact “lacelike” vascular tissue
4. Piercing-sucking feeding: small punctures, usually accompanied by circular discoloration or leaves shriveling, caused by true bugs
5. Surface feeding: abrasion of the leaf’s surface, which often produces a “window,” caused by small caterpillars or beetles
6. Leaf mining: mesophyll tissue removed between intact epidermal layers, leaving linear paths or blotches, caused by caterpillars and fly larvae
7. Galls: numerous swollen tissue growths, caused by many organisms, including fly larvae, wasp larvae, thrips, aphids, mites, and microbes
8. Microbial damage: numerous discolored spots or areas on the leaf

Removal of leaf tissue may be due to either mechanical damage or feeding. Removal by herbivores tends to form recognizable shapes and patterns on leaves, while removal due to mechanical damage tends to look more random (Figure 3). Many animals, especially invertebrates, cause specific types of damage that can be used to diagnose the animal group feeding on the leaf. For instance, beetles...
and grasshoppers both leave jagged or serrated edges when they feed on leaves, while caterpillars often leave smoother edges (Eiseman & Charney, 2010). Leafcutter bees cut round to crescent-shaped holes and do not feed on leaves, instead using leaf disks in nest construction (Pitts-Singer & Cane, 2011). Slugs leave jagged holes in both attached and fallen leaves, and if the feeding was recent, slime may be present. Thrips cause numerous small markings. Numerous species of caterpillars use silk to roll leaves (Eiseman & Charney, 2010). Each of these types of damage can be seen on fallen leaves.

There are two main confounding factors in the identification of damage to fallen leaves. First, animal detritivores such as slugs, millipedes, and annelids can physically remove tissue from dead leaves, and this may resemble the damage caused by herbivores feeding on live leaves. Students may be able to differentiate them by estimating the age of the damage, and by noting other signs such as mucus trails or damage by opportunistic microbes that colonized damage sites when the leaves were alive. Second, visually differentiating the signs of microbial decomposition of fallen leaves and microbial disease from when the leaves were alive can be difficult. Scrutiny may reveal active spore-producing structures, suggesting that the microbes are decomposing the leaves; however, microbes that attacked live leaves may also survive in fallen leaves (Wilson et al., 1999; Hyde & Jones, 2002). I generally did not attempt to identify whether the microbes colonized leaves before or after the leaves fell. For further information about defining types of damage, refer to Labandeira et al. (2007); although written for fossil leaf prints, this guide precisely defines and provides images of 10 different forms of leaf damage.

I trained my students to identify leaves by showing them leaves in the field, based on venation and shape alone. Identification of fallen leaves can be a challenge, as it can be difficult to determine whether leaves are simple or compound, and the leaf/leaflet arrangement on branches cannot be determined unless some foliage remains on the nearby tree. I also used an identification book (Watts, 1991) to assist students, and electronic resources such as iNaturalist (https://www.inaturalist.org) are readily available.

Online workbooks are advantageous, in that all invited students can enter data directly into a shared set that the class can use to answer questions. I used Microsoft Excel Online, with my institution’s Office 365 account. In this workbook, each row represents one leaf, and the columns are used for a continuously ascending identifying number (A), student name (B), leaf genus (C), locality information (D, E), and occurrence of each type of damage (F-M) (Figure 4). I recommend populating the first two columns in advance and populating a few rows with your own data to show students the format, facilitate consistent entries, and minimize errors. Typing comments into column headings with instructions may also ensure consistency (Figure 4). Another best practice is to have students download or copy the workbook into an offline version before doing any analysis.

I had my students enter data by counting the occurrence of each type of damage and typing the number into the online workbook. A single hole, skeletonized area, leaf mine, or discolored spot can take up substantial area on a leaf; depending on the questions posed, instructors may choose to replace the numbers with percent area. With this exercise, I was able to teach students how to copy cell contents and use the “=sum()” function of Excel (Figure 4).

Figure 3. Quercus leaves in an arboretum in Jackson Country, Georgia, October 2018, found damaged by living organisms (left) and with mechanical damage (right).

Figure 4. Screen capture of online worksheet, showing worksheet setup, instructions in column header comments (cell F1), and column totals (row 1002) calculated using the “=sum()” function. Student names are fabricated.
Questions

The data collected in this project can answer a number of questions, for example those listed below. Novice questions can be answered by students who have novice identification, quantification, and spreadsheet proficiency. Intermediate questions can be answered by students experienced in data collection and analysis. Advanced questions can be answered by students who have advanced identification skills and access to historical resources.

1. Novice: What tree genus is (most/least) susceptible to (herbivory/infectious disease)?
   How to answer: Compare the percentage of damaged leaves among genera.

2. Novice: Do trees in urban systems experience more stress and susceptibility to infectious disease?
   How to answer: Compare the number of leaves damaged by pathogens between residential or urban trees and preserves or natural forests.

3. Intermediate: Are native trees associated with more arthropod diversity?
   How to answer: Identify the number of different types of damage by arthropods, and compare this on both native and exotic tree species in your particular area. A native/exotic column can be added to the spreadsheet.

4. Intermediate: Are trees in urban systems associated with fewer arthropods?
   How to answer: Compare the number of different damage types between residential or urban trees and preserves or natural forests.

5. Intermediate: Is damage by free-living (whole, edge, skeletonizing) or parasitic (mines, galls) herbivores more common? Does this vary by type of forest?
   How to answer: Group damage is that caused by free-living herbivores and that caused by parasitic herbivores. Piercing-sucking damage can be caused by either, so it can complicate this question. Compare the percentage of trees with damage by each group.

6. Intermediate: Do fallen leaves accurately indicate the associations occurring on the trees themselves?
   How to answer: Record the damage on equivalent numbers of fallen leaves, living leaves, and, if possible, dead leaves still attached to trees. Compare the amounts and types of damage.

7. Advanced: How have associations between trees, animals, and microbes changed because of anthropogenic global change?
   How to answer: Compare the damage found on fallen leaves to that found on leaves in herbarium collections and photographs.

8. Advanced: How often does herbivory facilitate transmission of or invasion by pathogenic microorganisms?
   How to answer: Closely examine leaves for signs of punctures and chewing damage caused by herbivores, and sample and culture microbes from the damaged parts. Identify the types of microbes and how the presence of each is associated with physical damage.

9. Advanced: Is there any evidence of a coevolutionary relationship between particular plant clades and types of microbial damage observed?
   How to answer: Culture microbial swabs in lab on nutrient media, identify microbial colonies, and statistically determine whether they are associated with plant taxa at any given level (order, family, genus) of resolution. Habitat type (xeric, hydric, edge, forest type, etc.) could also be used as variables.

These questions can be answered statistically with (1) t-tests or analysis of variance (ANOVA), two parametric models that identify significant differences in group means based on the amount of error among/within groups (e.g., tree genera, or type of forest); or (2) chi-square tests of independence, which determine a relationship between two categorical variables. For example, ANOVA can be used to compare the mean number of associations among five tree genera (question 3), or a chi-square test can be used to determine if one tree genus is more likely to have galls than another (question 5).

Student Data

I received data from 36 students in February–March 2018. After discarding 50 observations of ferns, data from 3550 individual tree leaves were collected. Approximately one-third were collected from the forested “outdoor classroom” on our campus, another one-third were collected from the students’ residences, and most of the rest were collected from two nearby forest preserves and a botanical garden. Twenty-one different tree genera were represented, including Ginkgo, a deciduous gymnosperm. Almost all leaves were damaged; this was expected, since most had fallen at least two months before.

Overall, the leaves most commonly sampled were Quercus (oak) leaves (Table 1), and the most common damage types identified were holes in the leaves followed by discoloration (spots). The students were not trained to determine whether discoloration was caused by pathogens when the leaves were alive or by decomposition in the litter. Leaf mines were the rarest. These trends were largely consistent regardless of genus, although some genera had more discolored leaves than leaves with holes. It is likely that fewer leaves will appear damaged if sampled closer to the time they fall, because decomposers will have had less time to break them down. More fallen leaves may appear more damaged later in fall or winter, when mechanical breakdown and biological decomposition will have had more time to occur. Although the number of damaged leaves was high, there was still variation in the number of damaged leaves among tree genera (Table 1). This may be due to physical properties such as toughness or water content, or to chemical properties such as phenolics or terpenes (Hartmann, 2007). Both physical and chemical properties can vary among trees and influence the durability of fallen leaves. The environment the trees grow in may also influence damage and decomposition. Students should be informed that these factors may influence their findings, causing leaves of some trees to be more or less represented.
Student Learning, Responses & Feedback

My students used the shared Excel workbook to summarize damage rates and types for the entire class and were prompted to identify trees that were most and least susceptible to damage. I mainly assessed their ability to summarize large data sets and answer question 1. The students learned how to use keys to identify trees to genus accurately. The students varied in their ability to accurately identify some types of damage, particularly in distinguishing damage that occurred before or after the leaf fell; however, I did not assess grades on the basis of accurate damage identification. Most students were able to answer question 1 correctly on the basis of the class data set and also use literature to list some reasons why certain trees were more or less susceptible to damage.

I prompted the students to indicate the most difficult component of the exercise. Overwhelmingly, the most frequent response was that identifying different types of damage was difficult, followed by identifying leaves to genus. Further training in identification can be provided in advance if that is the goal of the instructor. I also found that some students were recalcitrant in correctly entering data in an online workbook, to the point that four of my 40 students never provided data of any kind. Although data entry was a challenge, data entry precedes the data analysis that is a vital component of science. Data entry is a skill students need to learn, and as online workbooks become more ubiquitous, students must learn how to complete them and share findings. Partially populating the spreadsheet, especially the columns for ID and student name, can help persuade students to participate.

I asked my students to state what they had learned, to compare their feedback with my assessment. The most common responses were that they had learned about plant identification, taxonomy, and the ecology of plant–animal interactions. Several admitted they did not know how to identify any tree genera in advance, and many did not know to italicize genus names. They were surprised about the extent of damage, especially physical damage by herbivores. Some students were also interested to learn about the opportunistic microbes that colonized holes made by herbivores. Some indicated that the exercise demonstrated plant–herbivore coevolution in a “hands-on” way. Overall, the students enjoyed the exercise.

Conclusions

I found that this activity was a success in meeting its learning goals. Students with limited ecological background and little quantitative experience were able to generate a large data set from fallen leaves and use that data set to answer questions about forest ecology and evolution. The main challenge or limitation I encountered was that students had a difficult time accurately identifying the organisms that caused damage to fallen leaves. This challenge can be overcome by devoting additional time to identification, potentially providing students with physical examples of each type along with images. The main advantage of the activity is that great numbers of fallen leaves are available outside of the growing season, when leaves attached to trees are not available for educational activities.

There are numerous ways to modify the activity for adoption at different learning levels. Children that are in middle school or younger can learn about the importance of trees and how trees fit into their surroundings and complete the activity without using large worksheets. High school students can use this activity to learn about the scientific method and hypothesis testing, or be introduced to different ecological interactions as a “scavenger hunt.” Some quantitative skills can be introduced by having middle school or high school students simply count damaged leaves and calculate damage rates that can be compared among different tree genera or species.

This activity can be an engaging and hands-on addition to a biology curriculum outside of a region’s growing season for trees. Trees dominate many terrestrial landscapes and interact in many ways with other organisms. This activity can help students learn

| Genus           | Number of Leaves | Percent Damaged |
|-----------------|------------------|-----------------|
| Acer (maples)   | 313              | 85              |
| Aesculus (buckeyes) | 143          | 75.6            |
| Carya (hickories) | 141            | 78              |
| Fagus (beech)   | 350              | 88              |
| Ilex (hollies)  | 99               | 77              |
| Liquidambar (sweetgums) | 182     | 85.7            |
| Liriodendron (tulip trees) | 56      | 94.6            |
| Magnolia (magnolia) | 280          | 86              |
| Platanus (sycamores) | 84           | 95.2            |
| Quercus (oaks)  | 1706             | 85.1            |
botany, ecology, evolution, and how to answer questions using the scientific method.

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