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Community Assembly in a Leaf-litter Invertebrate Community

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In this exercise, students design and carry out an experiment to test among competing hypotheses for community assembly, using a leaf-litter invertebrate community. By constructing mesh bags with leaf litter from which the invertebrates have been extracted, students can begin the experiment with an open community. By sampling a subset of leaf-litter bags at different time points over a 4-6 week time period, the degree to which community development is deterministic or random can be evaluated. This experiment could be used in introductory courses for majors or upper-level capstone courses, depending on the sophistication of statistical analysis.

Keywords: community development, succession, leaf litter, microinvertebrate

Introduction

This experiment is designed as a guided-inquiry exercise in which students pose experimental designs for testing models of community development and the instructor guides them to an acceptable experimental design. I do this as an iterative process. Each student answers the questions in the student handout as a pre-lab exercise. At the beginning of class, students work in their lab groups to develop consensus answers to the questions. Each group then presents their answers to the entire class. As a class, we then design an experiment that is implemented by the entire class.

Students place mesh bags with leaf litter from which invertebrates have been extracted in the field during the first lab period. Approximately 2-4 weeks later, they retrieve a subsample of leaf-litter bags and extract the invertebrates using a Berlese funnel. Another 2-4 weeks later, they retrieve the remaining leaf-litter bags and extract the invertebrates. During a final lab period, the students key out the invertebrates in their samples. This experiment could be used in introductory courses for majors or upper-level capstone courses, depending on the sophistication of statistical analysis.

I have included sample data from one iteration of the experiment. In addition, I provide details on data analysis of community sample related to testing the different hypotheses for community development. Additional references related to using leaf litter invertebrates for experiments in ecology courses or the ecology portion of introductory courses are also included.

Student Outline

Introduction

The structure of a community is determined by the populations of different species that share a common habitat. Many community-level processes, such as competition and predation, may be important in determining the structure of a community. However, in some communities, species interactions do not seem to drive the final structure of a community (Hubbell, 2001). Although what species occur in a given community and the interactions between them are of interest, ecologists are also interested in the change in species composition over time (i.e., succession or community development). In particular, community ecologists are trying to determine whether the history of invasion of new species into a community influences the final community structure (Chase, 2003).

Current competing hypotheses for community assembly are:

1. Community development is deterministic, such that the same initial species pool always results in the same community structure (single stable equilibrium). The equilibrium community depends on the local environment (Chase, 2003).
2. Community development is historical, such that the order in which species enter the community determines the community structure. Within a given environment, the final community structure might vary (multiple stable equilibria) (Chase, 2003).
3. Community development is random, and resulting community structure is unpredictable as species within a community are interchangeable (Hubbell, 2001).

In terrestrial ecosystems, soil and leaf litter invertebrates play an essential role in decomposition and nutrient cycling (Swift et al., 1979). Many of these invertebrates are detritivores (e.g., oribatid mites, springtails) (Gist and Crossley, 1975). However, others are predators of the detritivores or are top-level predators (e.g., mesostigmatid mites, araneidid spiders) (Gist and Crossley, 1975). These predatory species influence decomposition by directly or indirectly affecting the population sizes of the detritivores (USDA, 2010).

A variety of studies have shown that the quality of the leaf litter can have significant impacts on the rates of decomposition, as well as the structure of the soil and leaf-litter invertebrate community (Coleman and Crossley, 1996). Furthermore, the species diversity of the plant community can affect the soil and leaf litter invertebrate community by influencing the quality of the leaf litter (Siemann et al., 1998).

In this experiment, we will examine the development of a leaf-litter invertebrate community, because they can develop over relatively short periods of time (1-2 months). In addition, they can be sampled easily by extracting the invertebrates from the leaf litter using a Berlese funnel. A Berlese funnel uses a light bulb to dry out the leaves. The invertebrates move away from the light, exiting the leaf litter, falling into a funnel, and then are collected in a jar of ethanol beneath the funnel. The extraction process kills the invertebrates. So, we are unable to isolate particular species and introduce them into a community in a given order.

Questions

1. What data do you need to collect in order to distinguish among the three hypotheses above?
2. What experimental design would allow you to collect these data?
3. How can the results of the experiment be used to distinguish among the hypotheses?

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Materials

- Duct tape to seal leaf-litter bags
- Wedding-veil fabric for leaf-litter bags (each bag is constructed from two 0.25 m^2 pieces of wedding veil with duct tape to seal the seams)
- Scissors for cutting fabric
- Meter sticks – 1 per group for measuring fabric
- Survey flags to mark location of leaf-litter bags in the field
- Extracted leaf litter (This can be prepared ahead of time or students can collect this during the first lab period).
- Top-loading balance to weigh leaf-litter samples
- Berlese funnels
  - Bucket Berlese funnel (**http://www.bioquip.com/search/DispProduct.asp?pid=2831**) $84.95 US
  - Collapsible Berlese funnel (**http://www.bioquip.com/search/DispProduct.asp?pid=2832**) $63.35 US
  - Carolina Biological (654148, $21.50 US)
  - See **Notes for the Instructor** for references on how to construct your own funnels
- Glass jars – 1 per sample – must hold at least 25 mL
- 70% ethanol for sample jars – approximately 25 mL per jar
- 70% ethanol in squirt bottles
- Petri dishes – 1 per student for keying out samples
- Probes – 1 per student for manipulating invertebrates
- Dissecting microscopes – 1 per student

Notes for the Instructor

Students typically are able to determine that they need to collect data on the species found in the community over time (question #1). However, they have more difficulty with questions #2 and #3. Students will often suggest taking leaf-litter samples from the field at different time points. However, this approach does not test for community development, as the community is already in place. This approach could be used to examine community structure. If samples are taken from the same area (assuming a constant environment in that area), they should have the same community structure under a deterministic model, but would have different structures under a historical or random model. To examine community development, the experiment needs to begin with patches in which a community of invertebrates is absent. In addition, students have some difficulty in determining how many time points are necessary. At a minimum, two time points are necessary to distinguish between deterministic and historical models. For question #3, students can often figure out how to determine whether community development is random or deterministic. However, the distinction between the historical and deterministic models is less clear to them (see predictions below).

To test the hypotheses for community development, leaf-litter bags filled with leaf litter from which the invertebrates have already been extracted with a Berlese funnel should be placed in the field. Leaf-litter bags are constructed from wedding-veil fabric and duct tape to seal the edges. Bags that are 25 cm x 25 cm with about 50 g of dry leaf litter work well. At least at total of 20 leaf-litter bags should be constructed. Half of the leaf-litter bags should be collected 2-3 weeks after placement. The remainder should be collected 4-6 weeks after placement. To fully test the historical model, the leaf-litter bags should be placed in pairs with one bag in the pair collected at the first sample and the other collected at the second sample.

To determine if the communities that have developed in the leaf-litter bags have reached some sort of equilibrium, the fully developed community (leaf litter collected from the forest) needs to be examined. This could be done with initial samples that are used to generate the leaf litter for the leaf-litter bags or from samples taken from the forest floor at the end of the experiment. The advantage of using the initial samples is that it reduces the number of samples that are necessary. However, students sometimes have difficulty understanding that samples taken at the beginning of the experiment actually represent an end point. Also, the invertebrate community on the forest floor could change over the course of the experiment.

Invertebrates are extracted from the leaf-litter bags using a Berlese funnel and then identified to order. For instructions on how to build a Berlese funnel, see Murray et al. (2002). An online key to leaf litter invertebrates can be found at **http://www.hope.edu/academic/biology/leaflitterarthropods/**. Another good dichotomous key can be found in Morgan and Carter (2008). If sample jars used to collect extracted invertebrates only contain about 25 mL of ethanol, the entire contents can be examined by pouring out subsamples into a Petri dish. If larger volumes are used, the coarse debris can be removed from the sample jars and the ethanol can be allowed to evaporate off to a lower sample volume prior to examination of the sample. In identifying the invertebrates in the sample, I have my students use a three-step approach. First, I have them use the key in Morgan and Carter (2008). Second, if they cannot find the taxa on the key, they compare their invertebrate to type specimens that have previously been collected and identified. Finally, if they are still unable to identify their invertebrate, they are allowed to consider it a new type, name it, and add it to the type-specimen collection. Because the students are comparing communities, exact taxonomic identification is not essential as long as the same taxonomy is used for all samples.

Data Analysis

The exact predictions for each of the models of community development depend on how the data are analyzed. Two approaches are possible. One is to just examine species diversity (e.g., species richness, Simpson diversity, Shannon-Weaver diversity; see formulas below). This is the simpler approach and more appropriate at the introductory level. The second approach is to calculate some estimate of community similarity (or dissimilarity) between samples. Similarity

can be calculated using standard Pearson's correlation coefficients or indices of similarity, such as Jaccard's Index, Bray-Curtis Index, or Percentage Similarity (see formulas below). Dissimilarity is just (1 – similarity). At the introductory level, similarity could be examined qualitatively by determining the identity of the most-abundant species in the samples. For all analyses, students could analyze the data for the most-abundant species rather than all of the species in the samples. The Bray-Curtis Index has the advantage over other similarity indices that it is not biased by the rare taxa within a particular community.

Below are the predictions of the three models for the two different approaches to analysis.

Deterministic Model

Species diversity should be the same or similar for all samples within each time point. All sample communities should be the same or similar within each time point. Within a particular time point, students could determine if average species similarity across all sample pairs is significantly greater than zero with a single sample t-test. The model makes no predictions about how diversity should change over time or similarity between community samples across time.

Historical Model

Species diversity and community similarity might differ among samples at the initial time point. However, samples that have similar diversity or are similar at the first time point should have similar diversity or be similar in the paired samples at the second time point. To test this prediction, students could plot pairwise similarities at time period 1 versus pairwise similarities at time period 2. The model predicts a significant positive relationship in this regression analysis.

Random Model

Species diversity and community similarity should be variable among samples at each time point.

Krebs (1999) is a good resource for information on diversity and similarity indices.

Diversity Indices

Species richness: a direct count of the number of species. Does not take into account species evenness

Simpson index: $D = 1/\Sigma p_i^2$ where p_i is the proportion of individuals of species i in the entire sample. D ranges from 1 to the number of species in the sample. The more even the sample, the closer D is to the total number of species in the sample

Shannon-Weaver index: $H = -\Sigma p_i \ln p_i$ where p_i is the proportion of individuals of species i in the entire sample. H is roughly proportional to the logarithm of the number of species. As a result, the index is sometimes expressed as e^H.

Similarity indices: All similarity indices are calculated between pairs of samples. Dissimilarity can be calculated as (1-similarity).

Jaccard's index = $a/(a+b+c)$

where a is the number of taxa common to both samples, b is the number of taxa unique to sample 1, and c is the number of taxa unique to sample 2. Values closer to 1 indicate communities that are more similar.

Bray-Curtis index = $1 - \frac{\Sigma_{i=1}^{n} |X_{ij} - X_{ik}|}{\Sigma_{i=1}^{n} (X_{ij} + X_{ik})}$

where X_{ij}, X_{ik} is the number of individuals of species i in each sample (j, k)

Percentage similarity (Renkonen index) = $\sum_i \min(p_{1i}, p_{2i})$

where p_{1i} is the percentage of species i in sample 1 and p_{2i} is the percentage of species i in sample 2.

All of the diversity and similarity indices can be calculated in Excel using formulas. However, for the similarity indices, this can be quite tedious as you need to do a large number of pairwise comparisons. One possibility, especially for upper-level classes, is to use R (open-source statistical software) to calculate these indices. Below is a brief tutorial on installing and using R for calculating diversity and similarity indices.

You can download and install R from **http://www.r-project.org/**. R runs on any operating system. After installing R, you need to install the "vegan" package for R. To do this, open R and then click "Packages" -> "Install Packages" and select a CRAN site and then "Vegan". Once you have installed "vegan," you need to load it by clicking "Packages" -> "Load Package" and clicking "vegan."

Your data need to be set up such that each row represents a different community sample and each column represents a different taxonomic group. Save your file as a comma-delimited file.

To read the data into R, use the following syntax:

dataset_name<-read.table("*filename.csv*", header = TRUE, row.names = 1, sep = ",")

(Note that you can change the default directory by clicking "File" -> "Change dir". This allows you to just use the file name without the entire directory structure in the read.table command.)

To calculate different measures of species diversity for each community sample, use the following syntax:

Species richness: specnumber(*dataset_name*)

Shannon-Weaver:diversity (*dataset_name*, index="shannon")

Simpson: diversity(*dataset_name*, index="simpson") (This returns 1-D, where D = 3*pi2*.)

Simpson: diversity(*dataset_name*, index="invsimpson") (This returns the formula given above.)

To calculate different measures of community similarity for pairwise comparisons of community samples, use the following syntax:

Jaccard's index: vegdist(*dataset_name*, method="jaccard")
Bray-Curtis index: vegdist(*dataset_name*, method="bray")

Percent similarity: Interestingly, this is not available in R, but could be calculated relatively easily with formulas in Excel.

Sample Student Data

The following data were collected by students in the upper-level ecology course at Emory University during Fall 2008. Fifteen samples of the mature leaf-litter community were analyzed. In addition, community structure was determined for five pairs of leaf-litter bags.

The mature leaf-litter community samples were quite variable in community structure, as represented by the relative abundance of the four most abundant species in each sample (Figure 1). In addition, community structure was quite variable across leaf-litter bags within and between time points (Figure 2). Average similarity, determined using a Bray-Curtis Index, was low for both initial (0.21 on a range from 0 to 1 with 1 being identical communities) and final samples (0.37). Furthermore, pairwise similarities between initial samples were unrelated to pairwise similarities between final samples from the same sites ($F_{1,8}$=0.189, P=0.68). As a result, the data are consistent with a random model of community development.

Additional exercises using leaf-litter invertebrates

A variety of other published laboratory exercises use leaf-litter and soil invertebrates as model systems. Edgar (1992) and Boyce (2005) describe leaf-litter and soil invertebrate communities in general and describe a variety of ways in which they can be used in laboratory teaching. Murray et al. (2002) discuss using leaf-litter invertebrates to test island biogeography theory. Finally, Winnett-Murray and Hertel (2007) describe using leaf-litter invertebrates in the context of examining the effects of invasive plant species on ecological communities.

Acknowledgements

This experiment was originally inspired by a similar experiment using perforated PVC pipes as artificial logs used by Dr. Bruce Milne at the University of New Mexico. I modified the experiment to use leaf litter, due to exposure to Dr. Kathy Winnett-Murray's labs using leaf-litter invertebrates. I thank the participants in the workshop for their comments that have allowed me to clarify (I hope) the predictions of the three models and how they might be tested with the data collected. I especially thank Dr. Timothy Menzel for the suggestion to use a regression analysis to test the historical model.

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About the Author

Christopher Beck earned his B.S. in biology from the College of William and Mary and his Ph.D. in ecology from the Institute of Ecology at the University of Georgia. He is a senior lecturer at Emory University in Atlanta, where he teaches organismal biology, ecology, and ecology lab. He serves on the website committee of ABLE, and is currently the lead editor for Teaching Issues and Experiments in Ecology (**tiee.esa.org**), a peer-reviewed ecology education publication of the Ecological Society of America.

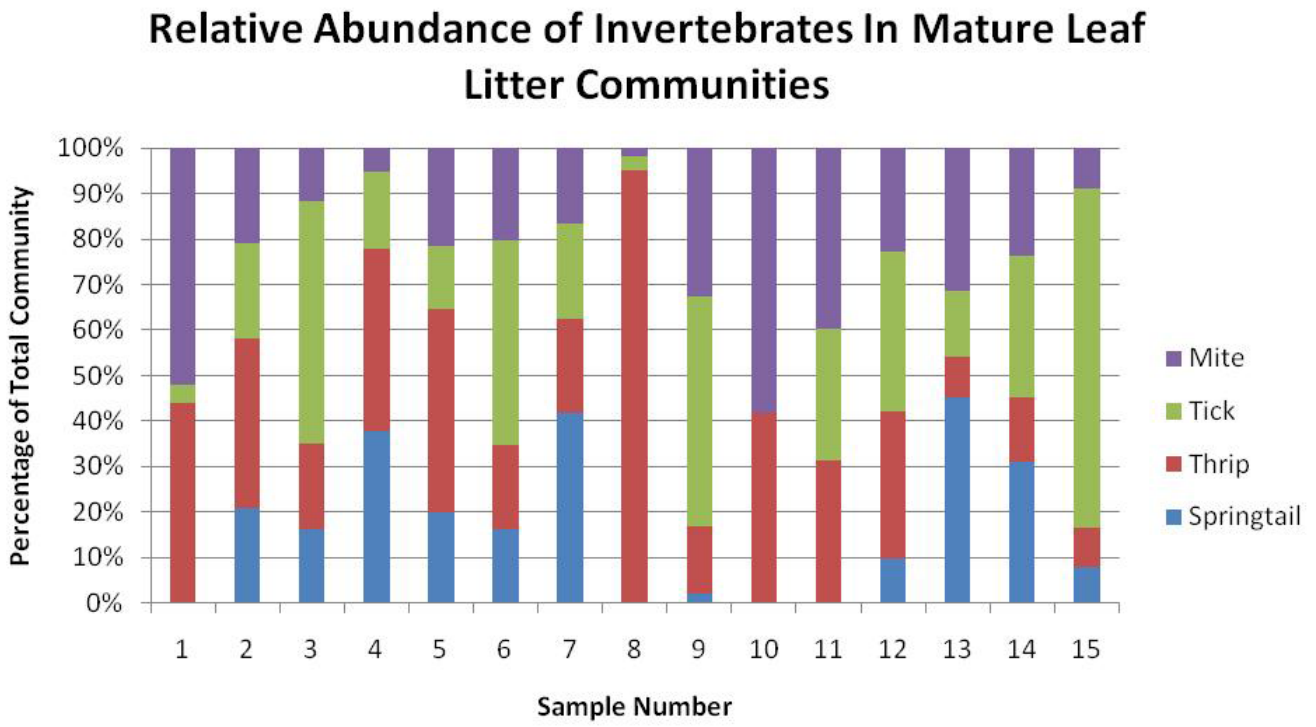

Figure 1. The relative abundance of the four most abundant taxa in mature leaf-litter invertebrate communities.

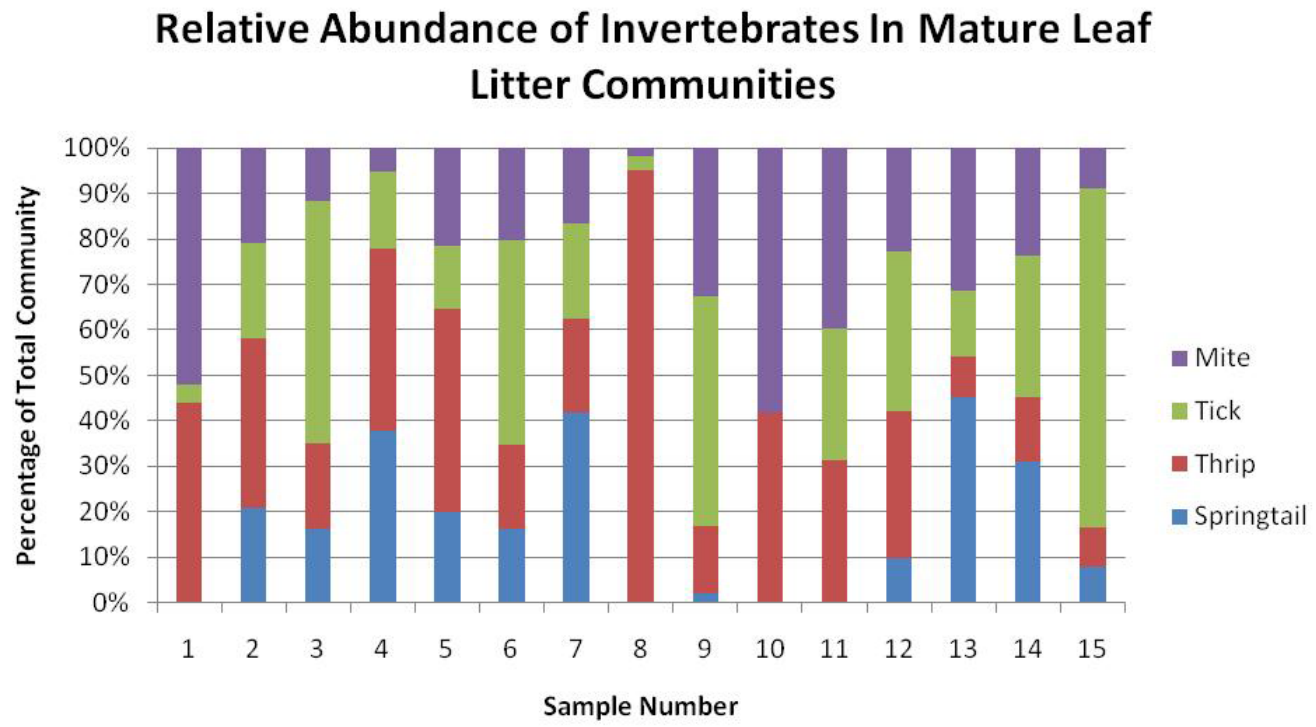

Figure 2. Similarity of communities in paired leaf-litter bags over time.

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