Biodiversity and Ecosystem Functioning

Prelabs

There is a Prelab quiz for each week of this two-part lab.

- **Week 1:** Study this Biodiversity and Ecosystem Functioning lab description; the textbook section "Biodiversity increases productivity" (starting on page 1185); the textbook sections on species interactions and the related lectures; and review the information about linear regression, *t*-tests, and chi-squared analysis in earlier labs. Then do the Lab 8 Prelab linked to the course website. The next week's prelab link is posted each Thursday afternoon. See "Submitting Catalyst Exercises" in this course manual; prelabs are due by Tuesday at 8:00 AM. (2 points)
- **Week 2:** Review the readings for Week 1, then do the Lab 9 Prelab linked to the course website. (2 points)

Learning Objectives

By the end of this two-part exercise, you should be able to:

- Collect and organize data efficiently and accurately
- State a hypothesis to explain why there is a relationship between productivity and species richness
- List one or more predictions made by that hypothesis
- Use statistical analysis and graphs to evaluate the prediction(s)
- Communicate results in a clear and organized way

Introduction

Current extinction rates are orders of magnitude higher than throughout much of earth's history. Should we care? You are probably familiar with many arguments for the protection of species. These range from utilitarian values (of use to humans: "That plant might contain the cure for cancer," "I like seeing whales.") to existence values ("Every species has a right to continue on the earth"). One argument of increasing interest to ecologists is the role of biological diversity in ecosystem functioning.

Biological diversity (or biodiversity) refers to differences among organic entities at genetic, species, and whole ecosystem levels. Here we focus on species diversity. Technically, species diversity refers to both the number and relative abundance of species. Species richness is simply the number of species.

Ecosystem functioning refers to the biophysical processes carried out by organisms in conjunction with their physical environment. Examples include biomass production, nutrient cycling, water purification, temperature moderation, pollination, erosion control, and decomposition. Biomass is the total mass of organisms in a population, created primarily through photosynthesis. In this lab, we will use plant biomass as an indicator of production.

Does a relationship exist between biodiversity, as measured by plant species richness, and productivity? If so, the relationship would be considered to be an **emergent property** of the community of organisms involved. The term emergent property refers to a relationship that is not visible at other levels of organization (e.g., when only individual species or organisms are considered), but which emerges when the overall community is analyzed. The key variable to consider is **community composition**, which refers to all the species found in a place, taking into account that some are common and others rare. For this lab, you should try to link data about particular species (Do they help or hinder other plant species? Do all species use light or nutrients in similar ways?

Is intraspecific competition strong or weak relative to interspecific competition?) to understanding why a relationship between species richness and biomass production emerges when all the species in a community are considered together.

This lab takes two weeks. In the first week, you will record data from an experiment started five to six weeks earlier and develop an hypothesis to test. In the second week, you and your lab partners will analyze the combined class data set, focusing on one species interaction that contributes to the overall results.

Week 1 – Build a Class Dataset

I. Species Richness, Layering, Percent Cover, and Biomass Production

1. About six weeks prior to this lab, seeds were planted in greenhouse "flats" (shallow plastic containers about 26 cm square). Seed density was kept constant at $0.12/cm^2$ by planting 80 seeds in each flat. Plants were chosen from a pool of 12 species. The characteristics of these species are described below.

Functional group	Species	Seed wt. (mg)	Description
Leafy herbaceous	Mustard greens	2.6	Leaves emerge near soil from fairly short stem Blades and petioles light green, long and thin Blades deeply serrated and pointed
Leafy herbaceous	Sweet basil	1.7	Leaves broad, almond-shaped, opposite on stem Petiole much shorter than blade Strong, sweet-spicy odor
Leafy herbaceous	Arugula	1.7	Leaves emerge near soil from fairly short stem Often hints of purple in petiole Length of petiole approx = blade Some blades tear-shaped, others deeply lobed
Leafy herbaceous	Parsley	2.3	Leaves flat with long green petioles Blade divided nearly into three Blades deeply serrated and pointy
Grass-like	Millet	6.6	Long wide hairy blades emerge directly from soil
Grass-like	Bunching onion	2.2	Long thin rounded blades emerge directly from soil
Grass-like	Annual ryegrass	2.7	Long thin flat blades emerge directly from soil
Grass-like	Flax	5.4	Long stem, many very small narrow leaves with blades attached directly to stem
<u>Enlarged taproot</u>	Rutabaga	2.4	Thick stem/root near soil surface Rounded, lobed leaves attached just above the soil Leaves often with purple and/or yellow tinge Leaves can be hairy
<u>Enlarged taproot</u>	Carrot	1.1	Long petiole with distinct ridges at edges Petioles appear to emerge directly from soil Blades very finely serrated, almost feathery
Nitrogen-fixing	Crimson clover	3.0	Long petiole Leaf consists of 3 small, nearly round leaflets Blades usually hairy
Nitrogen-fixing	Alfalfa	2.1	Long petiole Leaves consist of 3 small, nearly round leaflets Blades without hairs

Species richness per flat is 1, 2, 4, or 8. There are only 12 possible combinations single-species treatments (monocultures) of these 12 species, but there are 66 different combinations of two species (e.g., AB, AC, … EH, EI, … JK, JL, KL), and many possible combinations 4 or 8 species.

There is insufficient greenhouse space to grow all of those combinations (and not enough students to harvest all of them!), so only some combinations were set up to represent each level of species richness.

2. Students at each lab bench will describe and harvest the plants in one of the plant sets A-N in the table below. Students in other lab sections will examine additional replicates of the same set, and other students will harvest multiple replicates of the other sets. In the second week of this lab, you will have access to the entire class dataset, all replicates of all sets.

   The following table lists the species in the 12 different sets. Species in the four functional groups appear in different typestyles.

	Species Richness					
Set	Single species 1a	Single species 1b	Two species	Four species	Eight species	
A	Basil	Parsley	Basil Parsley	Basil Parsley Mustard Arugula	Basil Parsley Mustard Arugula	Carrot **Clover** *Millet* Rutabaga
B	Basil	*Onion*	Basil *Onion*	Basil *Onion* Arugula *Millet*	Basil *Onion* Arugula *Millet*	Mustard Parsley *Ryegrass* *Flax*
C	Parsley	Carrot	Parsley Carrot	Parsley Carrot Basil Rutabaga	Parsley Carrot Basil Rutabaga	*Onion* Arugula *Ryegrass* *Millet*
D	*Millet*	**Alfalfa**	*Millet* **Alfalfa**	*Millet* **Alfalfa** *Flax* **Clover**	*Millet* **Alfalfa** *Flax* **Clover**	Arugula *Onion* Basil Parsley
E	Mustard	*Flax*	Mustard *Flax*	Mustard *Flax* Carrot **Clover**	Mustard *Flax* Carrot **Clover**	Basil **Alfalfa** Parsley *Onion*
F	Rutabaga	Carrot	Rutabaga Carrot	Rutabaga Carrot Basil **Alfalfa**	Rutabaga Carrot Basil **Alfalfa**	Mustard Arugula *Ryegrass* **Clover**
G	*Onion*	*Millet*	*Onion* *Millet*	*Onion* *Millet* Carrot **Clover**	*Onion* *Millet* Carrot **Clover**	Basil Rutabaga *Ryegrass* **Alfalfa**
H	**Clover**	Mustard	**Clover** Mustard	**Clover** Mustard **Alfalfa** Arugula	**Clover** Mustard **Alfalfa** Arugula	Carrot *Flax* Rutabaga *Onion*
I	Arugula	Carrot	Arugula Carrot	Arugula Carrot *Onion* Parsley	Arugula Carrot *Onion* Parsley	Mustard *Ryegrass* *Flax* Rutabaga
J	*Rygrass*	**Alfalfa**	*Rygrass* **Alfalfa**	*Rygrass* **Alfalfa** *Flax* Mustard	*Rygrass* **Alfalfa** *Flax* Mustard	Arugula *Millet* Parsley **Clover**
K	Mustard	Arugula	Mustard Arugula	Mustard Arugula *Onion* Rutabaga	Mustard Arugula *Onion* Rutabaga	*Millet* *Flax* **Clover** **Alfalfa**
L	*Ryegrass*	*Flax*	*Ryegrass* *Flax*	*Ryegrass* *Flax* *Onion* *Millet*	*Ryegrass* *Flax* *Onion* *Millet*	Parsley Carrot **Alfalfa** Rutabaga
M	*Millet*	Rutabaga	*Millet* Rutabaga	*Millet* Rutabaga Parsley **Alfalfa**	*Millet* Rutabaga Parsley **Alfalfa**	*Flax* *Ryegrass* Mustard Basil
N	Rutabaga	**Clover**	Rutabaga **Clover**	Rutabaga **Clover** *Ryegrass* Basil	Rutabaga **Clover** *Ryegrass* Basil	Mustard Arugula Carrot **Alfalfa**

3. Examine the flats on your lab bench and familiarize your self with the appearance of the different species. Use the species descriptions and set list above, and the labeled "reference collection" in your lab room, as guides. Be able to identify both mature and very young plants. For the high-diversity treatments, have each lab group member identify individual plants separately, then make sure you all agree on which species you are actually seeing.
4. Determine the number of layers. Observe each flat from the side. How many layers of plant growth do you see? A "layer" occurs where leaves are relatively dense. Think, for example, of tree canopies, shrubs, and herbaceous ground cover in a forest. Each student at your end of the bench should estimate the number of layers individually. Then average the four observations, round to the nearest integer, and record the average in the table below.
5. Determine the percent cover. Place the flats **on the floor** with space between them, and observe from directly above. Can you see any bare soil, or do plant leaves obscure all of it? Each student at your end of the bench should estimate the percentage of soil covered by leaves when viewed from above; do not count growth that overhangs the sides. Use the pictures as a guide. Average the four observations, round to the nearest integer, and record.
6. Harvest and count individuals.

   For each flat, harvest the above-ground biomass by cutting plants at the soil line. Observe the following special instructions:

   <u>Ryegrass</u>: One plant often has multiple stems from a single set of roots. Pull the plants up a centimeter or so before counting individuals, then remove the roots. If you cut before counting, you won't be able to determine accurately the number of individuals.

   <u>Carrot, rutabaga</u>: Include the white part of the stem and the taproot swelling, even if below ground, that will become the rutabaga or carrot. Include the white part of the rutabaga stem.

   <u>Onion</u>: Include the bulb.

   <u>Leaves that have dropped off</u>: Do not count as separate individuals, but do identify them and include with the biomass for that species.

   <u>Plants should be clean</u>: Free of soil and surface water.

   Keep the harvested plants in piles separated by species and richness; for example, basil in the 1-, 2-, 4-, and 8-species treatments should be counted and weighed separately. Place each pile on a damp paper towel labeled with the species and richness; cover with another damp towel. Count the number of individuals in each pile and record in the data table.
7. Determine the "fresh weight" of plants in each pile. To do this, tare the balance by zeroing it with a weigh boat on the pan. Then transfer a pile of plants from the paper to the weigh boat. Wait for the numbers on the balance to stabilize, then record the biomass to two decimal places (hundredths of a gram). Transfer the pile back to the paper towel and cover. Be sure that the weigh boat is clean and dry, then tare the balance and weigh the next pile.

   Be sure to **SAVE ALL OF YOUR PLANTS** on and under damp paper towels until your TA has entered your data into the computer and says that you may discard the plants.
8. Report your data. Read your data to your TA, who will enter it into a computer spreadsheet. When all of the data has been entered, your TA will display the data for the entire lab section.

9. CLEAN UP (-2 points, or more, for messes left behind!) Do this while other students finish harvesting and read data to TA.
   - **The sinks are not trash cans; dispose of nothing in the sink!**
   - Cut plants – **Save** until your data is reviewed by your TA, then put plants in the white bucket on the rear counter.
   - Paper towels – flatten, put in the bucket with plants.
   - Plant labels – put in the plastic beaker on the rear counter.
   - Black trays and flats with soil – stack neatly on the cart at the front of the lab.
   - White trays – wipe out soil and plant parts, leave trays on your bench.
   - Bench top – wipe up spills and soil, organize the supplies.
   - Scissors (4) – put in the weigh boat.
   - Balance – leave nothing on the balance pan, not even the weigh boat.
10. After everyone's data has been recorded, there will be a general discussion, led by your TA, of possible reasons for your overall lab section results. It's easy to see why adding individuals (more seeds per flat) would result in more biomass, at least up to a point. (What do you suppose eventually limits plant growth, so that even carpeting a pot with seeds results in no more total biomass than a gardener's best-spaced planting?) It's a bit trickier to figure out why adding species would result in more production (assuming that it does, as has been shown in many other studies).

    Some hints: Are interactions between members of the same species equivalent to interactions between members of different species? Which interactions might be more negative? Are there possibilities for positive interactions, such that plants stimulate each other's growth? What is more important, species richness or the number of functional groups?
11. In groups of four students, review the "Week 2" portion of the lab below, then develop a hypothesis for next week. Explain how you will test that hypothesis by completing "Design for Experiment to be done Next Week," and give it to your TA by the end of lab.

Data Table

Lab Section ____________ TA __________________________ Plant Set Letter ___________

Species names (**list in the same order as in the sets table**)	% cover (nearest integer)	# layers (nearest integer)	Number of individuals	Biomass (two decimal places)
ONE SPECIES				
1a.				
1b.				
TWO SPECIES				
1.				
2.				
TOTAL				
FOUR SPECIES				
1.				
2.				
3.				
4.				
TOTAL				
EIGHT SPECIES				
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
TOTAL				

Be sure that your TA enters these data into the lab computers before you leave!
We will combine the data from all lab sections for next week.

Week 1: Design for Experiment to be done Next Week

Student Names (4) ______________________________ ______________________________

______________________________ ______________________________

Question that you wish to explore:

Hypothesis:

Reasoning: How does your hypothesis relate to the natural history of these plants/communities? Which mechanism (resource use efficiency, facilitation, sampling effect, or some other) is involved?

Prediction of your hypothesis:

What data (which plant treatments) will you use?

Which statistical test will you use, and why is that test appropriate?
(Discuss this with your TA; be sure that the analysis you plan to do will work for the data available.)

In the space below, sketch what your graph will look like if the hypothesis is supported. Be sure to use an informative title and label the axes.

Week 2 – Analyze the Class Dataset

II. Testing Hypotheses About Plant Species Richness And Biomass Production: The Roles And Interactions Of Particular Species

In week 2 of this lab, groups of four students will test a hypothesis, write a report, and present their results to the class with a whiteboard poster.

Your TA will demonstrate how to download the class data file and explain how the data is organized. The file contains data from all lab sections combined into one Excel spreadsheet. There will be four to seven replicates of each plant set (more, if several quarters are combined). The sample below shows only a portion of such a file; the full file will have data for all twelve species. In addition to the raw data, the combined file includes six summary columns.

Your hypothesis can use all or only a subset of the class data, but you must use all of the data that is relevant to your hypothesis. Select the relevant treatments and use them to test your hypothesis. You will have about an hour during the lab session to organize the data and analyze it graphically and statistically. Then each lab group will have 2-3 minutes for a whiteboard poster presentation.

Lab	Plant set	Treatment	Clover No.	Clover biomass	Alfalfa No.	Alfalfa biomass	Total No. plants	No. Fxnl Grps	No. Species	Total biomass	% Cover	No. Layers
AA	D	2			26	12.18	57	2	2	38.47	70	3
AC	D	2			27	9.83	56	2	2	48.42	70	2
AE	D	2			15	5.45	39	2	2	19.67	30	2
AB	D	2			28	12.70	51	2	2	46.95	50	3
AF	D	2			31	9.61	64	2	2	28.08	50	2
AA	D	4	18	19.93	11	2.73	44	2	4	32.54	50	3
AC	D	4	19	24.19	8	1.99	50	2	4	45.84	70	3
AE	D	4	18	25.58	16	4.35	63	2	4	45.35	75	3
AB	D	4	21	25.10	12	3.20	59	2	4	56.48	60	4
AF	D	4	19	16.84	7	3.04	54	2	4	41.19	60	3
AA	D	8	8	12.60	15	4.07	47	3	8	58.86	80	4
AC	D	8	11	15.65	4	0.88	52	3	8	69.19	90	3
AE	D	8	13	14.09	4	0.95	46	3	7	68.48	75	2
AB	D	8	10	14.35	8	1.81	61	3	8	74.59	85	4
AF	D	8	10	14.29	8	3.05	58	3	8	41.91	60	3
AA	D	1a					34	1	1	36.14	60	2
AC	D	1a					57	1	1	72.84	60	1
AE	D	1a					36	1	1	29.95	30	1
AB	D	1a					54	1	1	75.90	60	1
AF	D	1a					57	1	1	22.10	40	1
AA	D	1b			46	16.47	46	1	1	16.47	70	1
AC	D	1b			51	19.13	51	1	1	19.13	60	1
AE	D	1b			49	20.33	49	1	1	20.33	50	1
AB	D	1b			54	24.99	54	1	1	24.99	45	3
AF	D	1b			61	23.97	61	1	1	23.97	70	1

III. Analyzing the Data

Your hypothesis probably falls into one of two basic categories.

1) You may be interested the relationship between two continuous variables, such as species richness or functional group richness; percent cover; number of layers; density per species or total density; biomass per individual, per species, or total biomass. The data for such hypotheses can be analyzed using simple linear regression.

2) You may be interested in comparing different species combinations or numbers of species, for example all treatments that include a particular species, all treatments with a given number of species, or a species alone vs. in combination with another species. The data for such hypotheses can be analyzed using *t*-tests.

   Note that if there are three or more categories to be compared (e.g., species X alone, species X with species A, species X with species B), an analysis of variance (ANOVA) should be performed. However, since we haven't taught the use of ANOVA this quarter, you may perform several pair-wise *t*-tests instead; your TA will explain how to do this.

There are some hypotheses that might be amenable to chi-squared analysis, if you have a rationale for predicting the "expected" results. Your TA can help you decide if that is appropriate.

IV. Suggestions for your Poster Presentation

For your presentation, write your hypothesis on the whiteboard, sketch your graph, and include other relevant information such as statistical test results. Your TA may suggest a specific format.

The time available for your presentation will be limited, so focus on key points. You can save time by omitting information common to all student groups: the basic setup for this experiment (various combinations of plants grown in the greenhouse; estimation of percent cover and layers; harvest of above ground biomass, etc.) and conceptual background (resource use efficiency, facilitation, etc.).

Consider including the following:

- Your hypothesis and reasoning (why did you think it would be correct?)
- The mechanism involved: resource use efficiency, facilitation, sampling effect, or some other
- The plant treatments used (which plant set(s), which species richness(es))
- The statistical analysis used, why it was appropriate for your data, and how the statistical results informed your interpretation
- Results (graph and stats) and interpretation

Week 2: Biodiversity and Ecosystem Functioning Experimental Report

Student Names (4) ____

Question that you explored:
Hypothesis:
Prediction of your hypothesis:
Data used (which plant set(s), richness(es)):
Which statistical test did you use, and why was that test appropriate?
Results: Describe your results and **attach** a copy of your **graph** and **statistical analysis**. Include p-values, R^2 values, and n (sample sizes) as appropriate.
Interpretation of results: 1. What do the results tell you about species interactions? 2. How do they bear on mechanisms for a positive relationship between biodiversity and production? 3. How do your results compare and contrast with the results of other student groups?