

Teaching Quantitative Skills and Carbon Sequestration Concepts with the EREN Permanent Forest Plot Project

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The Ecological Research as Education Network (EREN) teaches students quantitative and conceptual skills for working with multi-site ecological data using collaborative research projects. The EREN Permanent Forest Plot Project (PFPP) is a network of 20 x 20 m plots in forested sites across the nation in which students collect tree diameter data using standardized methods. During two lab periods, students measure tree diameters and then use the PFPP database to explore research questions about forest carbon uptake. Students gain experience in tree identification, forestry techniques, allometric equations, statistical analysis with large datasets, and thinking across spatial scales.

Keywords: tree diameters, forest plots, collaborative research, allometric equation, spatial scale, carbon
Link to Supplemental Materials: http://www.ableweb.org/volumes/vol-40/1_Anderson

Introduction

Measuring carbon uptake by trees can provide insights into ecological processes happening at the scale of a single tree to the level of an entire forest, across a landscape, or across broad gradients of temperature and precipitation. Tree measurements can be done successfully by students after a short period of training, and the data present a wide range of options for exploration, analysis, and rich student learning experiences.

The Ecological Research as Education Network (EREN) aspires to teach students quantitative and conceptual skills for working with multi-site ecological data using collaborative

research projects. The EREN Permanent Forest Plot Project (PFPP) has organized about 60 undergraduate colleges to establish 20 x 20 m plots in forested sites across the nation in which students collect tree diameter data using standardized, coordinated methods. During two lab periods, students measure tree diameters in the field with the EREN protocols using basic and inexpensive forestry equipment, and then use the EREN PFPP database to explore a wide range of questions about carbon uptake by trees. Students gain experience in tree identification, diameter measurement techniques, allometric equations, working with large datasets, statistical analyses, and thinking about factors that affect tree growth at different spatial scales. Depending on plot placement, they can also explore questions such as carbon uptake differences in plots located on the forest edge as compared to the forest

interior. Extensive background on the PFPP is available here:

<http://erenweb.org/new-page/carbon-storage-project/>. The project is flexible and can be modified to focus on different kinds of experimental questions. There is a time investment involved in setting up an EREN plot (1-2 field hours per plot for two people) but then this plot can be re-used for many years.

This workshop introduces the basic technique for measuring tree diameters and then presents three options for working with carbon accumulation data from these trees:

1. an exercise at the scale of single trees that measures growth over time,
2. an exercise at the scale of a landscape where the amount of carbon accumulation at the plot scale is compared in different parts of a drainage basin, and
3. an exercise at a regional scale that uses multiple regression modelling to predict carbon accumulation in plots based on environmental variables such as temperature and precipitation.

These exercises are intended for a two lab module in an undergraduate biology class: the first lab involves field measurements of trees in the permanent forest plot, and the second focuses on data analysis. The data analysis phase can be adjusted to the level of the course, with the exercise on single trees being more appropriate in an introductory course and the exercise on multiple regression being more appropriate for an upper level course. The first two exercises use Microsoft Excel as an analysis tool, while the third exercise uses R. In the R exercise, some previous experience with R is assumed for the students, so it may be best to use this exercise as part of a class that uses R in other contexts as well. The exercises are presented as three separate modules so the instructor can choose the one that works best for their course learning goals.

Instructor preparation time for the field component is minimal once the permanent plot is established: diameter tapes, measuring sticks 1.37 m tall, clipboards, and field transportation are the main needs. Instructor preparation for the data analysis component involves posting datasets for students and making sure students have access to the appropriate software. Electronic resources are provided in this workshop to teach instructors how to do appropriate analyses in both Excel and R.

Student Outline

Field Exercise: Measuring the Diameters of Trees Using the EREN Permanent Forest Plot Protocol¹

Methods and Data Collection

Diameter at Breast Height

The abbreviation for “diameter at breast height” is dbh. This refers to a tree stem diameter measured at a standardized “breast height” of 1.37 meters above ground on the uphill side of the tree. Trees with irregularities or swelling, or other unusual situations may require special measurements (see figure below). A dbh can be measured using calipers, a special diameter tape (D-tape) that can be purchased from a forestry supply catalog, or the circumference can be measured with a regular measuring tape and the result divided by pi ($\pi = 3.14$) to calculate dbh.

Methods for Accurate dbh and dbh Increment Measurements

Measurement of biomass and carbon storage and accumulation rates in forested plots requires accurate, repeatable measurement of dbh. It is therefore very important to measure dbh at the exact same height on the tree each time it is measured. This may require physically marking the stem, especially when dbh is measured at a non-standard height due to one or more of the situations illustrated in the figure below. It is also important that repeat measurements of dbh are carried out during approximately the same time of year. It is generally best to measure dbh during the dormant season if growth increment over time will be determined. Annual growth increments are less accurate when measurements are taken during the active growth period, before diameter increment has ended for the year.

How to Use a Diameter Tape (D-tape)

The D-tape indirectly measures dbh. It measures the circumference of the tree, and has gradations in units of diameter (cm or in), obtained by dividing the circumference by 3.14 (pi, π). (If you do not have a diameter tape you can measure the circumference of the tree with an ordinary measuring tape and convert it to diameter by dividing by pi.)

The loose end of the D-tape is “slung” around the tree by the right hand and caught by the left hand. The tape is pulled straight and taut around the tree. (Make certain that the tape is pulled straight and level, and does not angle or “wander” around the stem.) The right end of the tape is crossed over the left end, and the dbh of the tree is read along the bottom edge of the tape where they cross at the 0-line. Some D-tapes have a curved hook attached to the end, which can be fastened to the tree. This is especially helpful for VERY LARGE trees that are too big to easily wrap the tape around the stem.

Note: It is very important for biomass and carbon sequestration estimates that dbh is measured at a consistent height on the stem. Accurate height measurement may be facilitated by marking dbh on the stem with lead free paint (Sharpie brand oil based paint markers work very well) or survey crayons, or by using a physical guide. See “EREN PFPP Complete Protocol” (http://erenweb.org/wp-content/uploads/2014/08/EREN-PFPP_CompleteProtocols_30Aug2014_v1-06.pdf) for some methods of ensuring repeatable dbh measurements.

¹ For additional information about this exercise contact Jerald J. Dosch.

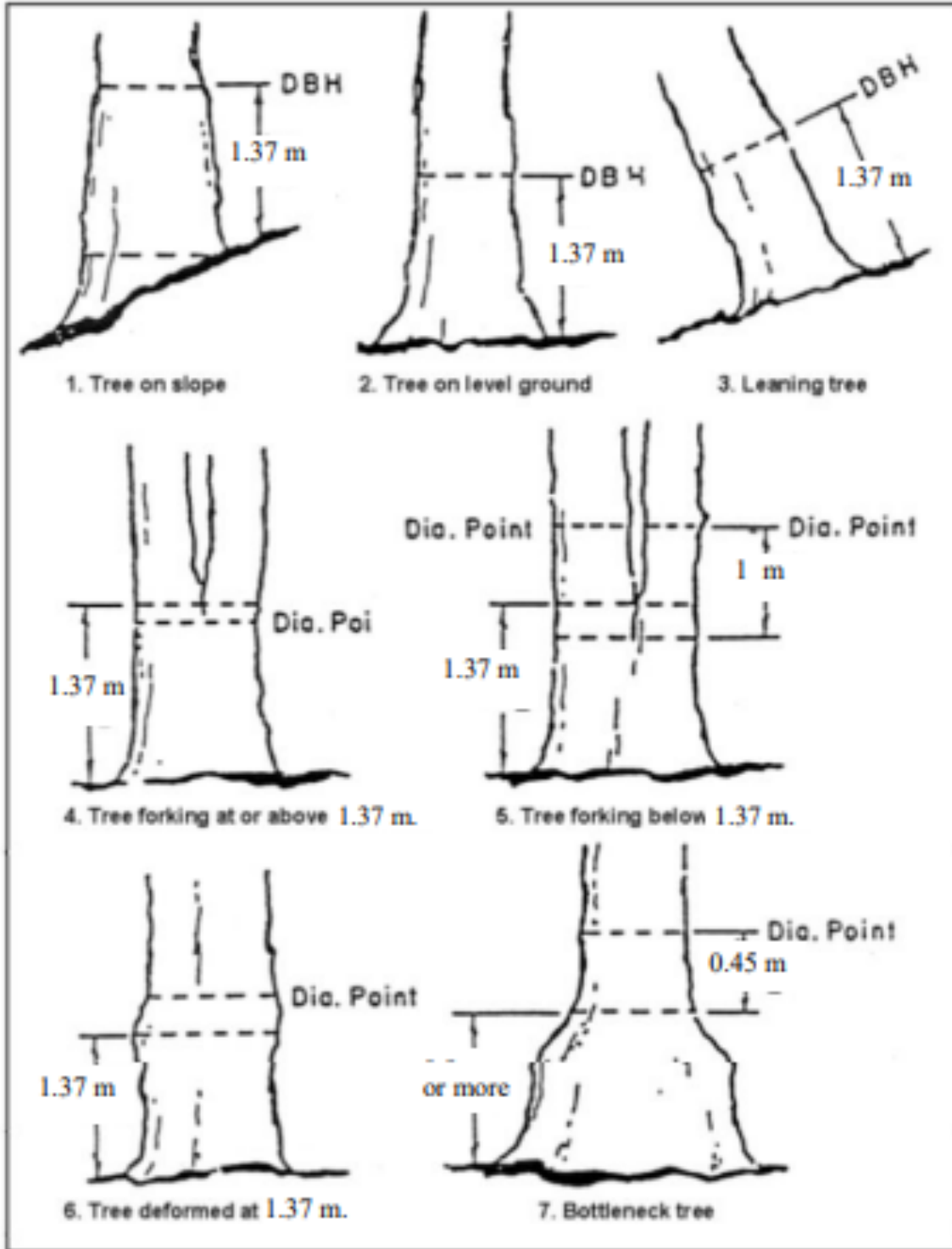


Figure 1. Location for measuring dbh in standard and irregular shaped stems. (Adapted from US Forest Service.)

While measurement of tree dbh (diameter at breast height) is defined as the tree diameter measured at 1.37 m (4.5 ft) above ground line on the uphill side of the tree, this is not always as easy as it sounds. The diagram illustrates how to measure trees with irregularities:

- Examples 1 and 2 show dbh being measured using standard procedures.
- Example 3 shows how to measure a leaning tree.

- Example 4 shows how to measure a tree forked at or above dbh (1.37 m / 4.5 ft). If the fork is at dbh, measure just below the fork. If above dbh, measure at normal dbh. Although this is the official definition of dbh, for determination of volume when the fork is close to dbh, the two forks are sometimes considered as two separate trees and dbh and height are estimated for each fork.
- Example 5 shows how to measure a tree forked below dbh (1.37 m / 4.5 ft) as two separate trees: dbh is measured on each fork, 1 m above the base of the fork.
- Example 6 shows how to measure dbh of a tree with a deformed bole at dbh (1.37 m). A deformity may be caused by knots, turpentine scars, fire scars, etc. If the deformity is large and extends beyond your reach, estimate dbh at the nearest point above the deformity.
- Example 7 shows how to measure a bottleneck tree - for example, a tupelo or cypress that is growing in a wet area.

This field exercise, measuring the diameters for trees, was modified from “Measurement_of_Tree_Diameter” (New Mexico State University FFA Forestry) and “Condensed tree measurement for Ordway” (Macalester College). EREN PFPP Version 1.05 (30 July 2012) and from http://erenweb.org/wp-content/uploads/2014/08/EREN-PFPP_CompleteProtocols_30Aug2014v1-06.pdf and http://erenweb.org/wp-content/uploads/2012/08/EREN-PFPP_Appendix-VI_TreeDBHGuide_30July2012.pdf

Data Exercise 1: Biomass and Carbon Sequestration in Trees in Meredith Forest over a One-Year Period²

Note: Data files are available as electronic resources.

Methods and Data Collection

Part I. Site Description, Start Tree Measurements.

(a) We are participating in a national research network called EREN (Ecological Research as Education Network; www.erenweb.org). You will use, view, and download the following PFPP lab protocols and datasheets from the EREN website:

- EREN PFPP Protocol
- Tree Inventory Datasheet

The class will re-census trees and small stems in six 20 x 20 m plots (1 plot per study group) which are part of a larger 1 ha plot in the Meredith Forest. These six plots have been measured in the past and the data is available on the EREN database. You will take the diameter-at-breast-height (dbh) of each marked tree within your 20 x 20 m plot. We will use these measurements to determine the amount of biomass stored in the trees and how much is sequestered in the trees on an annual basis.

Part II. Proofreading and Uploading Data

(a) We will proofread the data entered in the Microsoft Excel template by having 1-2 group members read the original field data sheet out loud and the other 2 group members checking the Excel template for accuracy.

(b) Submit your proofread file to the Forest Lab Journal on Blackboard by next week's deadline.

(c) At a later date I will then upload the full plot data to the EREN database. The database is available at: <http://erenweb.org/data/c-storage/>

Part III: Calculating Carbon Sequestration

With your measurements, we can determine how much carbon accumulated in your trees and forest plot.

We can calculate the aboveground biomass of your forest plot (all the trees from your group combined) by using the Jenkins et al. (2003) allometric growth equation **for each stem of each tree (e.g. 240.1) and then summing each stem's biomass together for the entire 20 x 20 m plot.**

Jenkins et al. (2003) Biomass Equation:

$$bm = \text{Exp}(\beta_0 + \beta_1 \ln dbh)$$

bm = total aboveground biomass (kg)

dbh = diameter at breast height (cm);

Exp = exponential function

ln = natural log base "e"

Mixed hardwood	
B ₀	B ₁
-2.4800	2.4835

Using Excel:

1. Calculate the amount of biomass in your forest plot (20 x 20 m) using the Jenkins et al. (2003) equation above (type the equation into a cell in Excel as =EXP((-2.48)+(2.4835*ln(cell with dbh))). Remember you must use the equation for each stem and then sum all the stems together to get one value for total biomass for the plot. You

² For additional information about this exercise contact Erin S. Lindquist.

should do this in a new Excel file which is different than your raw data file. Your new file name should include the following in the specified order: 'YourLastName_BiomassCarbon_Version#_Date'

2. Now you are ready to calculate the amount of carbon that has been fixed (accumulated) into the forest plot from the atmosphere via photosynthesis. Typically we can estimate that 50% of the tree's biomass is carbon. Using that statement, calculate the amount of carbon that has been fixed in your forest plot (use this year's dbh measurements). Show your calculations in the submitted Excel tree biomass and carbon database.
3. Given your plot is 20 x 20 m (or 400 m²), how much carbon would one hectare (ha; 100 x 100 m or 10000 m²) contain in its trees? Show your calculations in the submitted Excel tree biomass and carbon file.
4. Now use the data provided from the Fall 2011 census (available online) of your plots to determine how much biomass each 20 x 20 plot accrued in two years. Use the total biomass of each plot, and calculate the total carbon sequestered in two years versus one year.
5. (a) Given that the average American emits around 20 tons (1 short ton= 907.185 kg) of carbon a year (The Encyclopedia of Earth 2013), calculate the **percentage** of your emitted carbon that the 20 x 20 m plot (or 1 ha) sequestered in one year.

(b) How much forest (hectares) would it take to sequester all of your annual carbon emissions?

(c) There is a net 6.5 Petagrams of carbon emitted into the atmosphere annually from the burning of fossil fuels. How much forest (in hectares) would it take to sequester all of this carbon?

(d) The Environmental Resources class at Meredith College did a greenhouse gas inventory for the entire campus and all its students, faculty, and staff in Fall 2009 (and again in Fall 2011). In 2009, they found that Meredith and its community of faculty, students, and staff emit 16,003,000 kg C yr⁻¹. How much forest (in hectares) would we need to offset (sequester) this much carbon annually?

Part IV. Analyzing dbh Measurements for Tree Growth

You will now calculate a two-tailed paired *t*-test to determine if the trees significantly grew in the past two years. Please show your work in Excel by making sure to enter the formulas and functions in Excel needed to make the necessary calculations and determine if you accept or reject the null hypothesis. Make sure to define your null and alternative hypotheses before you start the calculations.

Data Exercise 2: Comparison of Tree Biomass Accumulation on Two Landscape Positions in an Upland Oak-Hickory Watershed on the Cumberland Plateau, Sewanee, TN³

Note: Data files are available as electronic resources.

Instructor Notes

Objective: To calculate and compare aboveground biomass in the living, standing trees from three upland and the three drainage plots in the Split Creek Watershed on the Cumberland Plateau, Sewanee, TN, and analyze any changes that occurred from 2014 through 2018.

Background Information:

The Split Creek Watershed is located atop the Cumberland Plateau in central Tennessee. Fifty acres (20.23 hectares) in size, the forested watershed ranges in elevation from 564 to 594 m above sea level. The watershed contains two small streams (East and West Split Creek), which join just upstream of the flume. The flume is located at the northern edge of the Plateau. For more information go to the watershed website <http://www6.sewanee.edu/watershed/overview2.html> or look at the Split Creek site and plot data in the EREN PFPP database <http://forplot.erenweb.org/main.php>.

The Forest Community

The forest community is dominated by upland oaks and hickories. It is composed primarily of white oak (*Quercus alba*), chestnut oak (*Quercus prinus*), pale hickory (*Carya pallida*), and black oak (*Quercus velutina*). The canopy ranges from 60 to 75 ft in height (18.3 to 22.9 m), and the dominant trees range from 10 to 25 inches (25.4 to 63.5 cm) in diameter. Other common tree species include sourwood (*Oxydendrum arboreum*), red maple (*Acer rubrum*), blackgum (*Nyssa sylvatica*), and scarlet oak (*Quercus coccinea*).

EREN PFPP Plots

In winter 2012, six EREN PFPP plots were installed in the watershed by students in an upper level Forest Ecology class. Three plots were installed in a drainage, with the narrow intermittent creek running approximately through the middle of the plot. The plots were installed with a 20 meter gap between plots. The stream is narrow enough for students to step across it except in rare high water events. The trees form a closed canopy over the stream. The other three plots were installed approximately 160 meters from the drainage plots, at least 30 meters above the stream drainage. The average elevation of the drainage plots is 570 m and the upland plots is approximately 582 m. The data included in this activity were collected by students in winter 2014 and winter 2018.

Additional Logistical Notes:

Students collect the field data (plot and tree data) during one or two 3-hr labs, depending upon the number of students in the class. The dbh of every stem is measured at least 2 times until the dbh differs by less than 0.2 cm between measurements.

Students enter the data in a shared Google spreadsheet file that has been pre-formatted for them by the instructor, with a link located on Blackboard with the rest of the class files for the PFPP activity.

The instructor looks over the data entered by the students, identifies missing and questionable information, and either sends student groups back out to recollect the data prior to the next lab, or has work study students visit the field site and collect the needed data. The instructor then creates a “corrected” file that includes columns for all years of data needed in the analysis. This file is made available to all of the students in the class in either Google Drive or Blackboard.

In lecture or lab prior to working with the biomass data, the instructor has generally discussed the idea of biomass equations with students, and discussed the relationship between biomass and carbon content. The students have already covered in lecture the concepts of aboveground net primary production and biomass accumulation potential in different forest types. The students have already had one lab in which they were introduced to basic Microsoft Excel functions and the use of pivot tables using a different dataset.

³ For additional information about this exercise contact Karen Kuers.

The open ended questions at the end of the lab could be discussed in class or assigned as part of a lab report or research project.

Student Outline

Comparison of Tree Biomass Accumulation on Two Landscape Positions in an Upland Oak-Hickory Watershed on the Cumberland Plateau, Sewanee, TN

Objective: To calculate and compare aboveground biomass in the living, standing trees from three upland and the three drainage plots in the Split Creek Watershed on the Cumberland Plateau, Sewanee, TN and analyze any changes that occurred from 2014 through 2018.

Methods and Data Collection

A. Calculation of Aboveground Biomass of Each Living Stem.

- 1) Open the Excel file with the Split Creek PFPP data from 2014 and 2018. Save a copy of the file as: SplitCreekData2014_2018_yourlastname
- 2) Highlight all of the data rows and columns. Sort the data by the species codes column in ascending alphabetical order. (Select “Data”, “Sort” and sort by “species_Code”, “A to Z”. If you included the header row in your selection make certain that the “my data has headers” box is checked.)
- 3) Add a new column immediately to the right of the 2014 dbh_cm column and label it “Amass14_kg” (aboveground tree biomass 2014).
- 4) Add another new column and place this immediately to the right of the 2018 dbh_cm column, and label it “Amass18_kg”.
- 5) Tree Biomass Calculations: Jenkins et al. (2004) developed equations to estimate total aboveground biomass (stem, branches, and foliage) for individual stems based upon stem dbh. They developed a set of 10 different equations that grouped species with similar form and wood specific gravity. The following table (Table 1) includes only the three equations that will be needed for the Split Creek dataset.

Table 1. Jenkins et al. (2004) equation coefficients for the species included in the Split Creek 2014 – 2018 dataset. The equation form is: $\ln y = \beta_0 + \beta_1 \ln x$ in which y = tree mass (kg), and x = dbh (cm).

Species_code	Scientific name	Common name	B ₀	B ₁
ACERUB	<i>Acer rubrum</i>	maple, red	-1.9123	2.3651
CORFLO	<i>Cornus florida</i>	dogwood	-2.4800	2.4835
LIRTUL	<i>Liriodendron tulipifera</i>	yellow-poplar		
NYSSYL	<i>Nyssa sylvatica</i>	blackgum		
OYXARB	<i>Oxydendrum arboretum</i>	sourwood		
PRUSER	<i>Prunus serotina</i>	cherry, black		
RHOSPP	<i>Rhododendron spp.</i>	rhododendron, deciduous		
ROBPSE	<i>Robinia pseudoacacia</i>	locust, black		
SASALB	<i>Sassafras albidum</i>	sassafras		
SNAG		all snags		
ACESAC	<i>Acer saccharum</i>	maple, sugar	-2.0127	2.4342
CARGLA	<i>Carya glabra</i>	hickory, pignut		
CAROVA	<i>Carya ovata</i>	hickory, shagbark		
CARPAL	<i>Carya pallida</i>	hickory, pale		
CARTOM	<i>Carya tomentosa</i>	hickory, mockernut		
QUEALB	<i>Quercus alba</i>	oak, white		
QUECOC	<i>Quercus coccinea</i>	oak, scarlet		
QUEMON	<i>Quercus montana</i>	oak, chestnut		

Note: The Jenkins et al. (2004) equations use natural logarithms ($e = 2.7182818$). In Excel use the function Ln (number) to get the natural logarithm of the dbh and use EXP (number) to obtain the antilog (i.e., convert the resulting logarithm back to its number). One way to do this in Excel is to set up a single formula that performs all of the log and antilog calculations.

Example: For the equation: $\ln y = \beta_0 + \beta_1 \ln x$ $y =$ Tree mass (kg); $x =$ dbh (cm)
if $\beta_0 = -1.9123$ and $\beta_1 = 2.3651$ and the dbh (cm) is in cell D4

The Excel equation in the “Amass14_kg” column would be: = EXP (-1.9123 + 2.3651 * Ln (D4))

- 6) Enter the appropriate equation into the “Amass14_kg” for the first individual of each tree species group. Copy the equation to each of the other members of that species group to calculate the aboveground biomass for each of the trees in 2014.
- 7) Once all of the equations are entered for Amass14_kg, copy the entire column and paste it into the Amass18_kg column to calculate the tree biomass for 2018. For this to work correctly you need to have properly positioned the columns in steps 3 and 4.
- 8) For any of the 2014 or 2018 calculations for which null was entered in dbh (all FS and MS trees) you will see #Value! in the output cell. Before continuing with data summary via pivot table, find each of those cells and delete the #Value!, leaving the cell blank.

B. Use Pivot Tables to Calculate 2014 and 2018 totals, and the Change from 2014 to 2018.

- 1) Highlight all of the data, including the headings, and create a pivot table to determine the total living “AMass14_kg” for the upland and drainage plots (contact the author for a handout with more complete instructions on creating Pivot Tables in Excel). Insert the Pivot table as a new worksheet. Insert “Landscape Position” and “Plot” both into the rows field, “InvStat 2014” in the columns field, and the values field should contain AMass14_kg. Make certain that the values field is set to “sum” and not to “count”.
Your output will look something like this:

2								
3	Sum of							
4	AMass14_kg							
5		DD	FS	IG	null RD	RL	Grand Total	
6	Drainage		177.456247	0.8151428	865.14314	29634.3391	30677.75358	
7	SCrk_51W		52.49527		407.226293	9190.01957	9649.741137	
8	SCrk_61W		37.8617339	0.8151428	265.998577	10464.624	10769.29945	
9	SCrk_71W		87.0992432		191.91827	9979.69549	10258.713	
10	Upland	43.7981334	333.313534		1293.04696	20347.7415	22017.90013	
11	SCrk_112E	43.7981334	153.009206		474.54607	6474.29597	7145.649381	
12	SCrk_113E		83.4648513		243.595778	7429.2759	7756.336529	
13	SCrk_114E		96.8394769		574.905115	6444.16963	7115.91422	
14	Grand Total	43.7981334	510.769781	0.8151428	2158.1901	49982.0806	52695.65371	

- 2) Create a second pivot table to determine the total “AMass_kg18” for your upland plot and your drainage plot. Again highlight all of the data, including the headings, and create a pivot table to determine the total living “AMass18_kg” for the upland and drainage plots. Insert the Pivot table as a new worksheet. Insert “Landscape Position” and “Plot” both into the rows field, “InvStat 2018” in the columns field, and the values field should contain AMass18_kg. Make certain that the values field is set to “sum” and not to “count”.

(Note: It is also possible to highlight and copy the output of the first pivot table, and then paste it several rows below the original output. If you click inside this output you will have the option to change the row, columns and values just for this output. As described above, insert “Landscape Position” and “Plot” both into the rows field, “InvStat 2018” in the columns field, and the values field should contain AMass18_kg. Make certain that the values box is set to “sum” and not to “count”.)

- 3) Calculate living biomass totals per plot: Next you will extract the information from the AMass14_kg pivot table that you will need to calculate the living biomass values per hectare and copy it to another location on your spreadsheet (it is not easy to manipulate the data within a pivot table output). The pivot tables currently include data for both living and dead stems. For this analysis you only need the information for the living stems. Living stems include “IG” - ingrowth since the last measurement, and “RL” - repeat measurements of living stems present both sample years. Copy the column in the pivot table with the plot names/locations, and paste the data in another, nearby location in your Excel file. Next copy the individual data for the IG and RL columns and paste that data next to the column with the plot names. (It is best not to include the Drainage and Upland totals at this point.) Add a column to the right and call it “Living14_kg”. In that column calculate the sum of IG plus RL for each individual plot.

Amass14_kg	IG	RL	Living14_kg	ALMass14_Mgha
Drainage				
SCrk_51W		9190.02		
SCrk_61W	0.815143	10464.62		
SCrk_71W		9979.695		
Upland				
SCrk_112E		6474.296		
SCrk_113E		7429.276		
SCrk_114E		6444.17		

- 4) Convert plot living biomass totals (kg) to totals per hectare (Mg/ha): Add another column to the right of “Living14_kg” and label it “ALMass14_Mgha”. The “Living14_kg” data is in kg per plot (kg plot⁻¹). We want to have the data in Mg per ha to more easily compare with published values and data from other locations. To make this conversion we need to know the size of the plot (each plot is 20m x 20m, or 400 m²), the number of m² in 1 ha (10,000 m² = 1 ha.), and the relationship between kg and Mg (1000 kg = 1 Mg.) Use these conversions to compute the ALMass14_Mgha values for each of the plots in 2014.

It is best to set up these kinds of conversions as a series of ratios to reduce the chance of multiplying when you should divide. For example:

$$\frac{9190.02 \text{ kg}}{1 \text{ plot}} \times \frac{1 \text{ plot}}{400 \text{ m}^2} \times \frac{10,000 \text{ m}^2}{1 \text{ ha}} \times \frac{1 \text{ Mg}}{1000 \text{ kg}} =$$

$$\frac{9190.02 \text{ kg}}{1 \text{ plot}} \times \frac{1 \text{ plot}}{400 \text{ m}^2} \times \frac{10,000 \text{ m}^2}{1 \text{ ha}} \times \frac{1 \text{ Mg}}{1000 \text{ kg}} = 228.8 \frac{\text{Mg}}{\text{ha}}$$

See that all of the units except Mg and ha cancel, giving the answer: 228.8 Mg ha⁻¹

Note: In Excel this formula would be: = 9190.01/1*1/400*10000/1*1/1000. While you can omit all of the 1's in the equation, it can be useful to include them to allow you to later recheck your calculation.

- 5) Repeat steps 3 and 4 for the 2018 data, naming the new columns “Living18_kg” and “ALMass18_Mgha”.
- 6) Combine the 2014 and 2018 ALMass_Mgha data into a single table to make the overall analysis easier. Add an additional column to the right of ALMass18_Mgha, and label it “ALMassChg_Mgha”.

The table should look something like this:

Site	Plot	ALMass14_Mgha	ALMass18_Mgha	ALMassChg_Mgha
Drainage	SCrk_51W			
Drainage	SCrk_61W			
Drainage	SCrk_71W			
Upland	SCrk_112E			
Upland	SCrk_113E			
Upland	SCrk_114E			

7) In the “ALMassChg_Mgha” column, calculate the change from 2014 to 2018 by subtracting the 2014 biomass values from the value in 2018. Positive values will indicate an increase and negative values will indicate a decrease in living mass.

C. Data Analysis: Perform the statistical analyses, using alpha = 0.05, to answer the questions below (contact the author for the handout on performing statistical analyses in Excel.)

-Are there significant differences in the average living biomass (Mg ha^{-1}) between the drainage and upland plot for 2014? For 2018?

-Are there significant differences in the average living biomass (Mg ha^{-1}) between 2014 and 2018 for the upland? The drainage?

-What is the average aboveground biomass increase per year ($\text{Mg ha}^{-1} \text{ yr}^{-1}$) between 2014 and 2018 for the drainage and the upland? (The data represents 4 growing seasons.)

- What was the average carbon (C) accumulation per year for the two locations?

(Note: There are various methods of converting biomass to C. Because of the large amount of cellulose that makes up plant biomass, the carbon content of plants is sometimes estimated from the C% in cellulose. The chemical formula for cellulose is $\text{C}_6 \text{H}_{10} \text{O}_5$). Using the atomic mass of each element (12.0107; 1.00794; and 15.9994 respectively) gives a carbon % of 44.5% for cellulose. (Measured C concentrations in trees generally range between 44 and 50%.) Use the value calculated from cellulose to estimate the average C mass per hectare of the living stems in the upland and drainage plots, and use this to determine the C change per year over the 4 years.)

Data Exercise 3: Multiple Regression Analyses Using PFPP Data⁴

Note: Data files and R code are available as electronic resources.

Learning Goals

After completing this lab exercise, students should (1) improve their confidence with exploring quantitative patterns in larger datasets, (2) be able to propose reasonable hypotheses to explain differences in tree carbon storage across sites, and (3) gain experience using multiple regression techniques to construct a model to predict the amount of carbon stored in forest plots based on various environmental variables.

Background

We know that the natural environment varies across both time and space. For example, we can expect longer, colder winters at more northern latitudes and drier conditions called “rain shadows” on the leeward sides of mountain ranges. We can expect changes in temperature and precipitation as the seasons vary at a single site. We also know that the bedrock, soils, and organisms differ across sites. The science of ecology strives to identify patterns and guiding principles in the midst of this variation. One example of a question we can ask to help us to explain ecological variation across sites and the effects of this variation on living things is:

Which of the many environmental factors that vary across sites are most important in driving the growth of trees? Is one having a much stronger effect than others?

This exercise uses tree diameter data from the Permanent Forest Plot Project of the Ecological Research as Education Network (EREN). Trees in 20 x 20 m plots distributed across the United States were measured by undergraduate students with the goal of monitoring carbon accumulation in forest plots. We have selected a subset of these data for you to explore in order to learn more about environmental variation that may affect carbon accumulation in forests at regional scales. We are also introducing you to a statistical technique called **multiple regression** which allows one to identify which of several possible independent predictor variables (such as temperature or precipitation) is having the strongest effect on a dependent variable (in this case, carbon accumulation by trees).

Instructions

Work with a partner to complete this electronic worksheet by the end of lab – you may turn in one worksheet for both students.

Make sure you have R and R Studio installed on your computer before you begin this exercise. Some portions of these R instructions are modified from the Ohio Wesleyan University Biology 122 Lab Manual (Anderson et al. Spring 2018).

1. Access your file online as directed by your instructor. An allometric equation for relating tree diameter to total aboveground biomass (Jenkins et al. 2003) has been added to one column of the file to calculate biomass (in kg) from dbh for trees from forest sites across the country. More recently updated allometric equations can be found in Chojnacky et al. (2014). This biomass value has been multiplied by 0.5 to estimate the amount of carbon stored in each tree. First examine the file to get a sense of what the columns mean. Open R Studio and set up a new project with a new working directory. Open a new script file by clicking on “File” and choosing “New File” and “R Script”. This script file is a convenient place to run all your command scripts separately from your output. Activate the appropriate packages by inputting these commands to the script window, highlighting them, and selecting “Run”.

```
require(mosaic)
require(datasets)
require(lattice)
```

2. Download your data as directed by your instructor and save it to your desktop or other convenient location. Import the data file into R (we have called this file “MultiSiteDataPFPP” in this example). In the top right window of R Studio, click on the “Environment” tab to bring it forward. Click on “Import Dataset”. You

⁴For additional information about this exercise, contact Laurel J. Anderson and Jose-Luis Machado

may have some options based on the file extension. The file may end with a .csv extension, e.g., “pfpp.csv”. The extension “csv” stands for “comma separated value” and it is the file type used for spreadsheet data when saved as plain text. Data entry, while not literally impossible in R, is definitely not recommended. Data is most easily entered by hand using a spreadsheet program (e.g. Excel, Sheets) and then saved as .csv for import by R. R Studio also allows you to import directly from an Excel file (.xls). Choose the “From Text (base)” option if your file ends in “csv” or “From Excel” if it is a standard Excel file. An import window will pop up. “Browse” for your file and the click “Import”. At this point, in your “Environment” window you should have an entry under Data telling you how many observations and variables are in the data set. There will be a tab in your upper left (script) window for the data set. You can view the spreadsheet and verify it imported correctly.

3. First, get a sense of the range of climate conditions for the sites in this dataset. Use the favstats command to generate some descriptive statistics for mean air temperature, precipitation, and growing season length by state, as shown in the example command below. Note that you will need a separate favstats command for each variable. A command for mean air temperature is shown below. Modify it for the other variables and the name of your dataset as needed.

```
favstats(meanairtemp~state, data = MultiSiteDataPFPP)
```

4. Make a bar plot of the mean climatic conditions for the different states using the command below. You first have to use the command “tapply” to calculate means and standard deviations manually to make a bar plot. Once you tell R how to calculate means and standard deviations, making a simple bar plot is easy. Show your graph to your instructor and copy and paste it into this document before moving on. Based on these climate descriptions of your sites, which sites do you think will have the greatest carbon accumulation and why? Stated another way, which environmental factor will be the strongest predictor of carbon accumulation and why?

```
mean_temp <-tapply(MultiSiteDataPFPP$meanairtemp,
list(MultiSiteDataPFPP $state), mean, na.rm = TRUE)
mean_temp

sd_temp<-tapply(MultiSiteDataPFPP$meanairtemp, list(MultiSiteDataPFPP
$state), sd, na.rm = TRUE)
sd_temp

barplot(mean_temp)
```

Now we need to add error bars, axis labels, etc. Here is code that will add these items:

'mids' is the code telling R to put error bars in the 'middle' of the bars.

'Arrows' is a command that tells R to draw a line (arrow) that is placed in the middle of the bars.

Once we tell R to put an arrow or a line in the middle of the bars, the next step is to tell R how long to make the arrows. We want the length to be 1 sd above the bar, and one sd below the bar.

'xlab' and 'ylab' is where you tell R what to label each axis. 'ylim' tells R what scale to use on the y-axis.

```
barplot(mean_temp, xlab = "State", ylab = "Mean annual temperature in
degrees C", ylim=c(0,800))
```

```
mids<-barplot(mean_temp, xlab = "State", ylab = "Mean annual
temperature in degrees C", ylim=c(0,800))
mids

arrows(mids, mean_temp + sd_temp, mids, mean_temp - sd_temp,
angle=90,code=3,length=0.1)
```

5. Which species in the data set, across all sites, stores the most carbon (is the largest) on average? Examine the means of each species individually using the following command that will calculate the means of the all species and give you a barplot without error bars. There are so many names that not all will display on the x-axis, but you can see the range of tree sizes:

```
mean_by_species <-mean(carbonkg~species, data = MultiSiteDataPFPP,
na.rm = TRUE)

mean_by_species

barplot(mean_by_species)
```

6. Which species stores the most carbon on average according to your graph? Record your answer here: _____ . Suppose we have sites along a precipitation gradient that are dominated by different species. How would this affect our attempts to show that tree growth/carbon accumulation is related to precipitation?
7. What about the total carbon per plot at each site? As directed by your instructor, import a dataset into R that contains plot level carbon sums in Mg per ha (we have called this file "PlotSummaryDataPFPP" in this example). Use multiple linear regression to build a model that shows which variables best predict the total carbon for each plot, using the commands below. However, first we should check for correlations among the predictor variables because we don't want to put redundant variables into the model. For example, it is likely that mean annual temperature and growing season length will be correlated, and we don't need both in the model. Make a scatterplot and run a correlation analysis using the commands below to check how correlated they are. What is the outcome?

```
xyplot(MeanAirTemp~GrowSeason, PlotSummaryDataPFPP, xlab = "Growing
Season Length", ylab = "MeanAirTemp")
```

```
cor(PlotSummaryDataPFPP$MeanAirTemp, PlotSummaryDataPFPP$GrowSeason)
```

```
cor.test(PlotSummaryDataPFPP$MeanAirTemp,
PlotSummaryDataPFPP$GrowSeason)
```

8. We should select either growing season length or mean annual temperature as a predictor variable, since they are strongly correlated. Below is a model that uses growing season length and precipitation as predictor variables. You should also choose the variable tree density as one of your predictors to control for the number of trees in each plot. Run an analysis for carbon sum as a dependent variable. Also, make scatter plots showing the relationship between carbon sum and growing season length, average precipitation, and tree density. Discuss your results with your instructor and record the major outcomes of your analysis below. Think about the species data above. How might this affect your analysis?

```
ERENPFPP.lm = lm(CarbonSum~PrecipAvg+GrowSeason+Density, data=
PlotSummaryDataPFPP, na.rm = TRUE)
summary(ERENPFPP.lm)
```



```
xyplot(CarbonSum~PrecipAvg, PlotSummaryDataPFPP, xlab =  
"Precipitation", ylab = "Carbon Sum")
```

```
xyplot(CarbonSum~GrowSeason, PlotSummaryDataPFPP, xlab = "Growing  
Season Length", ylab = "Carbon Sum")
```

```
xyplot(CarbonSum~Density, PlotSummaryDataPFPP, xlab = "Growing Season  
Length", ylab = "Carbon Sum")
```

These commands complete the multiple linear regression analysis and scatter plots for the carbon sum analysis.

9. Send this completed Word document with your graphs (name it using your last names and the date in the title) to your instructor. You are done!

Materials

Field Measurements of Tree Diameter in a Plot Already Established by the Instructor:

- 8 clipboards
- Copies of the EREN datasheet for recording diameters
- Methods handout with figure of “odd” trees
- 8 diameter tapes (available from several online suppliers)
- 8 sticks cut to 1.37 m height for rapid measurement of “breast height” in the field

Data Analysis Lab:

- Students bring their own laptop for R or Excel - R will run on either Apple or Windows platforms
- Instructions for using Excel or R and performing data analyses - suggest these be provided as electronic documents.
- Online data files

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