NEON FSU Field and Lab Protocol for Ops CPER 2011: Plant Diversity

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1 INTRODUCTION

1.1 Purpose

The primary purpose of this document is to provide change controlled version of Observatory protocols for the 2011 Operations prototype at the CPER, and is the version used for external review by subject-matter experts. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

It does identify procedure-specific safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

1.3 Acknowledgements

If a protocol is based closely on the work of another program or author, note that here.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog
AD[04]	

2.2 Reference Documents

(If you want to reference other procedural documents (e.g. associated Protocol document), drawings, etc. then include filenames in the following sections.)

RD[01]	NEON.DOC.000008 NEON Acronym List
RD[02}	EHS Safety Policy and Program Manual
RD]03…]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
RD[06]	NEON.DOC.000243 NEON Glossary of Terms
	QA/PA Plan
	DOORS requirements
	ATBD

2.3 Acronyms

Insert table for definitions of acronyms used in this document.

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol
TBR	To Be Revised

3 BACKGROUND AND OBJECTIVES

3.1 Background

[TBR]

The purpose of this draft protocol is to inform the activities involved with planning and scheduling 2011 Plant Diversity Field Operations Prototype for CPER. This section outlines the field data collection and laboratory procedures to process unknown and voucher plant species.

The purpose of vegetation sampling is to describe inter- and intra-annual variation of plant diversity at NEON sites. In addition to providing habitat for local fauna, the flora at each site integrates a variety of biotic and abiotic factors that respond to environmental change.

The following sub-sections contain draft protocols that provide detailed guidance for assessing plant diversity in the Biodiversity Plots in the field, the collection and handling of unknown plant species, and the collection of voucher specimens for training and evaluation and verification purposes.

Plant species richness will be measured with a multi-scale circular plot. The plot-based method yields plant species richness data at multiple scales. This method is directly comparable to continental vegetation monitoring data collected in forested systems by the US Forest Service Forest Service Forest Inventory and Analysis Program.

Even experienced botanists will not know every species encountered in each plot. Typically it is not cost effective, and sometimes impossible, to spend time identifying a plant in the field. Therefore, instructions for identification of difficult species are provided. Voucher specimens provide a permanent record of the NEON naming convention, use of authorities, validation, and a means to track taxonomic naming conventions through time. In addition to the unknown species collected for identification, voucher specimens of twenty to forty of the common species found in the plots must be collected, pressed, mounted, and stored. The samples must be of archival quality. Specimens should be collected during peak phenology, and must be pressed, dried, and mounted according to herbaria standards such that species identity can be evaluated in the future.

3.2 Metadata Collection

[TBR]

Data about the samples will comply with the metadata fields outlined in a spreadsheet to be supplied by FSU. These metadata fields follow the standards outlined by the VegBank consortium.

This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.

Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page.

3.3 Science Requirements

This protocol fulfills the following Observatory science requirements:
List science requirements from DOORS that are met by this protocol.

3.4 Data Products

List Level 0 data products measured by protocol.

Table 1 A summary of field and related lab measurements and the associated NEON Data Products

Measurement	Data Product

4 PROTOCOL

4.1.1 Plot Location

Sampling will occur throughout the site. FSU is responsible for providing locations of each plot center.
Summarize the science rationale (e.g. experiment design), include key citations. Briefly summarize the procedure included in this document and variations in how NEON is implementing this protocol in different locations throughout Observatory (e.g. above ground tree biomass for temperate vs tropical zones). If the protocol is based on existing published procedures, reference those here (e.g. "These methods are based on the amazing work of John Updike (1964). No one has come up with anything better since then.").

A protocol is a formal summary description of a procedure and its related rational. A protocol includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result; can also be called a method.

Read more:
http://wiki.answers.com/Q/What_is_the_difference_between_a_method_and_a_procedure#ixzz1FInck5Na

Identify assumptions or known-unknowns of the chosen protocol.

Identify and summarize quantitative aspects of the procedure (timing, # plots, # samples, location of sensors).

Provide a simple timeline diagram or table if pre-field activities occur the day prior to the field day, or if the field procedure is a multi-day task.

5 QUALITY ASSURANCE AND QUALITY CONTROL

Summarize QA/QC plan and reference QA/QC document.

6 DECISION TREE

Delay	Action	Adverse Outcome	Outcome for Data Product
hours to several days	Compelete plots as possible given challenges, record date plot was started and completed and time required to finish for each effort.	may miss target sampling window	1. species richness due to changes in seasonal phenology could be influenced by significant changes in temporal sampling window. 2. not completing all plots impacts diversity metrics and target sample size.

Summarize for the field technician or manager any implementation decisions regarding the protocol.

7 SAFETY

[TBR]

Aside from general precautions associated with travel in a wildland setting, there are no procedure specific safety concerns associated with this protocol.

Personnel working at a NEON site should be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.
Any safety issues specific to the procedure should be detailed here, along with references to any pertinent safety standards.

8 PERSONNEL REQUIREMENTS

[TBR]

A minimum of two people are required for each plant diversity sampling team: a botanist and a technician. It is mandatory that one member of this team be a trained and experienced botanist who can identify most of the species at the CPER in the field. This person should have experience with identifying plants in the Shortgrass Steppe, be able to use a dichotomous key, and have experience identifying plant specimens in the lab with a dissecting microscope and associated tools. The technician and the botanist should also meet the following:

9 TRAINING REQUIREMENTS

Reference NEON Training Plan document relevant to this method.

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling Frequency and Timing

[TBR]

To capture plant diversity during different parts of the growing season, all plots will be sampled two times during the field season: June and August.

Based on sampling frequency information, list estimated dates that correspond with timing rules or estimated dates when timing rules are fulfilled for each NEON site where this procedure is implemented. Include range of scientifically acceptable sampling timing. If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.

Table 2 The approximate plant diversity sample dates for sampling at all NEON sites

Domain	Date	Frequency
1	June	Measure each plot once
2	August	Measure each plot once

10.2 Contingent decisions

[TBR]

Given any delay in plant biodiversity sampling, complete plots as possible, record date plot was started and completed and time required to complete each plot.

Summarize what-if decisions (how to accommodate site-specific or changing conditions).

Example: If it starts raining halfway through the microbe sampling, work must stop for the day. 24 hours after the rain stops, work can continue from the previous stopping point (i.e. work does not need to be repeated).

Example: If you are unable to begin checking small mammal traps prior to 9am, field technicians should open all traps to release animals and avoid heat-induced mortality. Work should start over that night by resetting the traps.

10.3 Field Procedure

[TBR]

Write the procedure as if a PDA were not available. This way, when the asteroid hits the earth and disrupts all electrical equipment, the field personnel can reference the "old school" procedure and collect data. These non-PDA procedures also help CI to define PDA and data ingest requirements, and provide sufficient information for an external reviewer to assess the procedure without the need of a PDA.

10.3.1 Equipment and Materials

Include all standard and unique equipment and capabilities required to execute the procedures in this document, including:

- *A detailed list of materials (e.g. equipment, sampling gear, sample containers, chemical preservatives) used in the field.*
- *Do not include materials used for separate but related activity; that will be included in the Procedure for that activity (i.e. lab vs. field materials).*
- *Describe the chemicals being used or as a preservative when samples are immediately returned to the field – exact chemical constituents and strength, and bottle size. Leave a place-marker in the draft if unknown.*
- *Illustrations of materials (e.g. sampling gear) – all in jpeg format, 3 inches wide*
- *Can be in bullet point or table format, but be consistent throughout the document.*

Table 3 Materials and supplies required for one crew for the field Plant Sampling Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
30 meter fiberglass tape measure	1	
Compass	1	
Global Positioning Unit	1	
Plant press	3	
Field notebook and pencil	1	
Pin flags	1	
Nylon flagging	1	
1-gallon zip-loc plastic bags	Many	
Small carabiner and ring binder	1 ea.	
Hand lens	1	
Scissors or pruning sheers	1	
Meter stick	1	
Subplot frame	1	
Digital camera	1	
PDA	1	
Species list for CPER	1	
Dichotomous keys	1	

10.3.2 Preparation

10.3.2.1 Plot Establishment

The center of each plot will be established by FSU prior to prototype field work. The field crew is responsible for establishing the rest of the sampling plot. Plant biodiversity sampling will happen at the center of the Biodiversity Plot in a circular plot with a 7.32 m radius (24 feet). There are three 1-m^2 square subplots. One edge of each subplot lies on each of three radii with azimuths of 30 degrees, 150 degrees, and 270 degrees from the plot center. The two corners of each subplot on each radius are at 4.57 m (15 ft) and 5.57 m from the plot center. The perimeter of the plot is 7.32 m (24 ft) from the plot center (Figure 1a). The proposed plot establishment dictates that the plot center, the two corners of each subplot that fall on the radii, and the plot perimeter at the end of each radii will be permanently marked. The perimeter of the plot should be temporarily marked as needed to define the 7.32 m circular plot boundary with wire pin flags (Figure 1b).

Figure 1. (a) A 168-m^2 circular plot will be used to record plant species richness and cover. The plot includes nested subplots at a specific distance and location from the center of the plot. (b) The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

10.3.2.2 Sampling Preparation

All necessary field equipment shall be assembled at least 2 days prior to field sampling to ensure datasheets are printed on water resistant paper and/or uploaded to the PDA, GPS waypoints for plot locations are uploaded, and all consumables are available in sufficient numbers to complete field work. See the official field equipment list in order to determine which equipment and consumables are required for a given set of tasks.

- *Describe all activities that must occur prior to arrival in the field, for example equipment calibration or preparation.*
- *A detailed list of tasks, using the numbering format shown below. Be consistent.*

10.3.2.2.1 Subplot Frames

The cover and identity of plant species will be recorded in 1-m^2 frames. The frames must be assembled prior to the sampling effort. Subplot frame (see http://www.nrel.colostate.edu/projects/fhm/equipment/SamplingFrame/VegSamplingFrameInstructions.htm)

10.3.2.2.2 Data Collection

Plant diversity data will likely be collected directly onto an electronic hand-held device. Be sure the unit is well charged by keeping it connected to the charger when not in the field. Prior to leaving for the field confirm that the software is loaded onto the device and the data from prior sampling efforts has been downloaded and deleted. Be prepared to use a paper backup if the device fails (dunked in a creek, lost, or crashes).

10.3.2.2.3 Consumable Items

Plastic bags will be used to collect unknown plant species. Assemble the collection rig (http://www.nrel.colostate.edu/projects/fhm/equipment/PlantCollecting/PlantCollectingRig.htm) prior to going to the field or be sure to have ample loose bags. Adhesive labels will be needed and working permanent markers and pencils.

- *Break each step down into actionable steps*
- *Described and list the tasks in chronological order*
- *If pre-field tasks occur over multiple days, break Section 7.1 down further (e.g.:*
  - *One week prior to sample collection, do this*
  - *Two days prior to sample collection, do this*
- *Be explicit and use language geared toward 3rd yr undergraduate student. Assume the user has no previous knowledge of the activity.*
- *Include a description of what sampling gear, equipment, etc. they need to verify are in working order and properly packaged for the field day.*
- *Define safe practices (e.g. use gloves to mix chemicals in the fume hood), where necessary at each step. Do not assume this information is written elsewhere – include all relevant safety procedures in every document. Each document should be written as a stand-alone document.*
- *Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3" wide.*

10.3.3 Sample Collection in the Field

10.3.3.1 General Collection

Vegetation richness and cover measurements is recorded at each 1-m^2 subplot, and richness is recorded across the entire plot as follows:

1. Locate the markers for the subplot on the 30 degree azimuth and place the subplot frame on the 'clockwise' side of the transect that is defined by the markers at 4.57 and 5.57 m.
2. Identify each species rooted in the subplot and estimate the combined aerial cover of all individuals to the nearest one percent (see next step for how to measure cover in the 1-m^2 subplots), and average height of all individuals. Record the species identity, corresponding standardized Natural Resource Conservation Service (NRCS) PLANTS database code, and cover for each species (see below for more information regarding estimating cover). If a species cannot be identified in the field, please refer to the next section "Unknown plant species".
3. Each 1-m^2 subplot frame should be calibrated (painted in 10 cm sections) to make cover estimates easier (Figure 4.2). Only estimate cover on plants, or portions of plant, that are rooted inside the subplot frame. Visually group species together into a percent cover. Fine tune that estimate by subtracting out any spaces or gaps. Familiarize yourself with what certain cover estimates (e.g., 1%, 10%, 15%, etc.) look like and use them as reference sizes. For example, if you know that 1% cover is about the same size as your fist, use your fist as a reference. There will often be overlap of plant species. Cover should be recorded as the total aerial coverage for each species; estimates should not exceed 100 percent for a single species, but total subplot cover may be greater than 100 percent.

4. Estimate and record the combined cover of abiotic (non-living) elements in each subplot. Similar to the cover of plant species, abiotic cover of any one element shall not exceed 100 percent, but the total cover of multiple elements may be greater than 100 percent. Abiotic elements include, litter, wood, rock, soil, and water.
5. Move subplot frame to the 150 degree radius and repeat steps 1 – 3.
6. Move subplot frame to the 270 degree radius and repeat steps 1 – 3.
7. Record species richness in the entire 168-m^2 plot. After completing the three subplots, the botanist completes a walking survey of the entire plot (within the 7.32 m radius) looking for and recording any species that were not previously found on any of the 1-m^2 subplots. Occasionally there will be a previously undetected species near the plot perimeter. The botanist can stretch a tape measure from the plot center to the perimeter of the circular plot to evaluate inclusion (rooted in the plot) of specific individuals. Cover measurements are not made outside the 1-m^2 subplots.
8. Photograph the plot by taking a picture from the center of the plot in the direction of each of the three transects.
9. Record photo numbers, camera, and date.

10.3.3.2 Measuring Cover in the 1-m^2 subplots

Each 1-m^2 subplot frame should be calibrated (painted in 10 cm sections) to make cover estimates easier (Figure 4.2). Only estimate cover on plants, or portions of plant, that are rooted inside the subplot frame. Visually group species together into a percent cover. Fine tune that estimate by subtracting out any spaces or gaps. Familiarize yourself with what certain cover estimates (e.g., 1%, 10%, 15%, etc.) look like and use them as reference sizes. For example, if you know that 1% cover is about the same size as your fist, use your fist as a reference. There will often be overlap of plant species. Cover should be recorded as the total aerial coverage for each species; estimates should not exceed 100 percent for a single species, but total subplot cover may be greater than 100 percent.

10.3.3.3 Unknown plant species

In the field, perform the following steps for individuals that cannot be identified:

1. Collected unknown specimens should be placed in one gallon plastic bags. The entire individual, including the roots and vegetative growth of grasses and forbs should be collected. A piece of a branch is usually sufficient for trees and shrubs. Given NEON's long-term monitoring efforts, unknown species should be collected from outside the plot. Finding the same unknown species can sometimes take considerable time.
2. Label plant with a unique unknown name, number, description, botanist, date, and plot number.
3. At the end of the field day, place plastic bags in a refrigerator until they are identified and/or placed in a plant press and dried for identification at a later date. Specimens should not be left in the refrigerator for more than two days. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

10.3.3.4 Voucher Specimens

While samples are being collected for biodiversity identification, two specimens of each species should be collected: one fresh to be used for identification, and one to be pressed and incorporated into the herbarium. Occasionally a third specimen needs to be collected if pressing of the specimen is going to be difficult.

If possible, all parts of a plant should be collected, the roots, stems, flowers, fruits, and seeds as follows:

1. Select specimens in good condition, free of insect damage, rust, or disease.
2. If possible, all parts of a plant should be collected, the roots, stems, flowers, fruits, and seeds. Collect at least stems, leaves, and flowers or fruit of herbaceous plants, and twigs, leaves, and flowers or catkins of trees and shrubs.
3. Place all specimens of a single species from one locality into one collection bag.

- As each specimen is collected, assign a unique collection number and record date, name of collector, location (GPS), elevation (GPS), habitat information, slope, aspect, and soil information in a field notebook.*A detailed list of tasks:*
- *Break each step down*
- *Describe and list the tasks in chronological order*
- *List decision criteria used to implement procedure based on plot/sampling location conditions (i.e. sampling plot composition, stream width, etc.).*
- *If field tasks occur over multiple days or are complex, break this section down further (as shown in previous Section)*
- *Be explicit and use language geared toward 3rd yr undergraduate student*
- *Include detailed instructions on assembly of sampling gear, if gear is assembled in the field. If gear is assembled/disassembled in the Domain Office (e.g. not in the field), include that detail in the Pre- or Post-Field Task sections*
- *Include safety issues and practices*
- *Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3" wide.*

10.3.4 Sample Preservation

Specimens should not be left in the refrigerator for more than two days. They can be placed in the press, stored in a well ventilated location, and identified at a later date. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

- *Detail the task in order from start to finish*
- *Include how long (time/days/yrs/indefinitely) sample can be preserved in this mode.*
- *Only include activities that relate to the Field Task.*
- *If a sample is processed at the Domain Lab, that work is a written in the next Section or as a separate document if complex.*

10.3.5 Sample Shipping (may not be applicable for all Field SOPs)

- *Provide detail on shipping specifics (e.g. wrap the sample containers in a plastic bag, seal the top. Place containers upright in cooler labeled "Elephant Samples", include four reusable ice packs. Seal the container.*
- *In addition to the shipping label, the following hazmat labels are required:*
- *Check with EHS for label requirements*

10.3.6 Data Handling (may not applicable for all Field SOPs)

At the end of each field day, all information from field data sheets must be typed up or transferred from the PDAs and saved to the NEON server as directed by the Field Manager.

If plant diversity data is collected using the palm PDA:

1. Connect the palm to the computer with the supplied cable
2. 'HotSynced' the palm
3. Open the appropriate Micro Soft Access database and follow the on-screen instructions for uploading electronic data to the database
4. Make a copy of the database with the current date and store the dated and working version on the NEON network drive or multiple hard drives

Briefly explain data upload steps (e.g. enter data into excel file name "XXXXX", plug in PDA, etc., etc.).

10.3.7 Refreshing the Field Sampling Kit

Be sure to have sufficient plastic bags, adhesive labels and permanent markers for the next field sampling effort.

- *Provide detail on how to restock the sampling kit with non-perishable items. Best practice is to restock the sampling kit after the sample event, with a check at the start of each sample event that the kit is appropriately stocked*
- *Reference the materials list, above*
- *Be explicit and ensure information does not overlap or refute early info (ex. If preservatives used in the field have to remain cold, then the 'refreshment' of the preservative is a detailed in Section 7.2 and not here.)*

10.3.8 Equipment Maintenance, Cleaning, and Storage

- *Include maintenance of sampling gear as they relate to this Procedure, such as battery recharge.*
- *Do <u>not</u> include vehicle maintenance or maintenance of gear commonly used in the field such as a mosquito net or boots. Maintenance of common field items will be a different document.*
- *Include relevant safety issues and practices*

- *Be explicit*
- *Illustrations or photographs of tasks that would benefit from an illustration, jpeg 3" wide.*

11 LAB STANDARD OPERATING PROCEDURE

[TBR]

Write the lab procedures as if a PDA were not available. Use the same sections as the field protocol, above.

Separate the lab procedures into multiple sections and add on to the above title. For example:
11 Lab Standard Operating Procedure - Plant identification and drying

11.1 *12 Lab Standard Operating Procedure - Plant mounting*Timing

Specimens should not be left in the refrigerator for more than two days. They can be placed in the press, stored in a well ventilated location, and identified at a later date. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

Provide details on preferred timing of sample processing at the domain labs AND the maximum time between field collection and lab processing If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.

11.2 Lab Procedure

The lab component of the plant biodiversity sampling includes three parts, plant pressing and drying, identification, and processing of a subset of collected species for voucher specimens. The exact order and need for each step with each specimen will depend on scheduling and time, and the objectives of the particular specimen.

Some species will come from the field and identified fresh (without pressing), within two days time of collection. If the specimen was destroyed during identification or was never intended for vouchering, there is no need to save and press the specimen.

Some species will be collected and the botanist will not have time to identify it within two days, or will not be able to identify the specimen. These specimens should be pressed for identification at a later time, either by the botanist with the help of an herbarium, or sent to an expert.

Other specimens will be collected specifically for vouchering. These specimens should be treated with extra care to preserve diagnostic parts, pressed until dry, and mounted for preservation in a NEON herbarium.

11.2.1 Equipment and Materials

Table 4 Materials and supplies required for the Plant Biodiversity Lab Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Plant presses	3	
Dissecting microscope	1	
Dissecting kit	1	
Acid-free mounting paper	many	
Acid-free envelopes	many	
Glue in hand-held dispensers	1	
Finished acid-free herbarium labels	many	
Wooden spacer blocks	several	
Weights, metal washers	many	
Scissors	1	
Damp cloth for wiping glue spills	several	
Cardboard sheets	several	
Plant dichotomous keys	2	

11.2.2 Preparation

A clean and dry bench space is required.

11.2.3 Identification of Unknown Species

Specimens need to be correctly identified. Ideally, the fresh (not pressed and dried) duplicate specimen that is not to be included in the herbarium should be used for identification. Identification requires basic knowledge of morphological characteristics of different plant families, plant keys, and access to a herbarium (University of Colorado, Colorado State University, etc.), a clean bench space in the lab, a dissecting microscope, and dissecting kit. If there is any doubt, a duplicate specimen should be submitted to a taxonomic expert for identification.

After unknown specimens are identified, the information should be entered into the Micro Soft Access database in the 'Enter Unknowns' form.

11.2.4 Plant Pressing and Drying

Plants should be pressed as soon as possible in the lab at a clean and dry bench space. Plants will be pressed and dried in standard plant presses and newspaper as follows:

1. Tabloid newspaper is ideal for pressing plants since it is the same size as the plant press and the herbarium paper to which the specimens will be mounted.
2. Within the fold of one sheet of newspaper, arrange plant parts of a single specimen carefully with minimum overlap.

3. Open some flowers to show both the top and underside to illustrate the arrangement of flower parts.
4. Squash large fruits on the page or slice them in half.

5. Place dry, loose seeds or fruits in sealed packets.
6. Turn over some leaves or part of a single large leave to show underside.
7. Write the collection number, date, botanist, and initial identification on the inside of the newspaper and any seed packets.
8. Close newspaper and separate individual newspaper folds with blotting paper. Up to three newspaper folds, separated by blotting paper, can be included between cardboard ventilation separators.
9. Place the stack of specimens, blotting paper, and cardboard between the wooden plant press panels and tighten the straps as much as possible. It can be helpful to kneel on the press to tighten. Make sure the press is even.
10. Dry specimens in press. Grass and shrub specimens will dry in well ventilated part of the lab. Fleshy or aquatic plant specimens will require a dryer.

11.2.5 Processing Voucher Specimens

Herbarium specimens will be catalogued with a standardized label. The label should include the family, genus, species, location description and coordinates, elevation, collector, collection date, and collection number.

Once specimens are dry, they must be mounted. Mounting can occur at any time, but is required prior to level 0 data collection to be complete. Specimen mounting requires skill and patience. All supplies should be of museum quality since the longevity of the specimens is directly related to the substances they contact. Mounting should be done at a large and clean bench space in the lab and completed as follows:

1. Glue acid free label to acid free herbarium mounting paper.
2. Leave space on the sheet for seed and fragment packets.
3. Remove any soil clinging to the roots and stems.
4. Use scissors or pruning shears to trim large specimens to fit the sheets.
5. Place a sheet of mounting paper on a cardboard sheet.
6. Arrange the plants on the mounting paper. Avoid placing any material at the edge of the mounting sheet.
7. Hold the specimen down with weights such as plastic-coated lead bars or metal washers until the glue dries.
8. Attach the specimen to the mounting paper with thin ribbons of glue running from the paper across the plant part to the paper. The glue should not cover any parts necessary for identification.
9. Small drops of glue should be applied to the underside of large leaves and flower heads, and multi-stemmed specimens (some grasses) require long glue straps to catch all the stems.
10. When the sheet is finished, dry mounted specimens by separating cardboard supports with wooden blocks.

A standard accession number will be given to each prepared sample. The samples should be stored at -20 degrees C for forty-eight hours prior to permanent storage in herbarium cabinet.

11.2.6 Sample Preservation

11.2.7 Sample Shipping

11.2.8 Data Handling

At the end of each field day, all information from lab data sheets must be typed up or transferred from the PDAs and saved to the NEON server as directed by the Field Manager.

11.2.9 Refreshing the Laboratory Supplies

11.2.10 Laboratory Maintenance, Cleaning, Storage

12 DEFINITIONS

Define all protocol specific technical terms in alphabetical format.

13 REFERENCES

Use Ecology style

APPENDIX A Field Data Sheets

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

Plot Name/Number:									
Date:									
Botanist:									
Technician:									
Start Date/Time:									
Complete Date/Time:									
30 subplot:			**150 subplot:**			**270 subplot**			**Circular Plot Search:**
Abiotic Variables	**% Cover**		**Abiotic Variables**	**% Cover**		**Abiotic Variables**	**% Cover**		**Species**
soil			soil			soil			
rock			rock			rock			
litter			litter			litter			
water			water			water			
Species	**% Cover**		**Species**	**% Cover**		**Species**	**% Cover**		

Include copies of all data sheets – jpg format
(data sheets are useful for CI to define PDA and data ingest requirements)

APPENDIX B Lab Data Sheets

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

Botanist:							
Date:							
Unknown Name	**Family**	**Genus**	**Species**	**Authority**	**Identification Method**	**Vouchered (y/n)**	**Number**

Include copies of all data sheets – jpg format
(data sheets are useful for CI to define PDA and data ingest requirements)

APPENDIX C Considerations for implementation

Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.

Describe any component of the process that may interfere with the accuracy of the final product.

Discuss how to avoid common errors in sampling or common ways samples can be contaminated.

Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results")... i.e. call out weird issues and folklore explicitly. See: http://en.wikipedia.org/wiki/Phantom_of_Heilbronn

APPENDIX D Procedure Checklist
APPENDIX E Tables
APPENDIX F Figures

a. b.

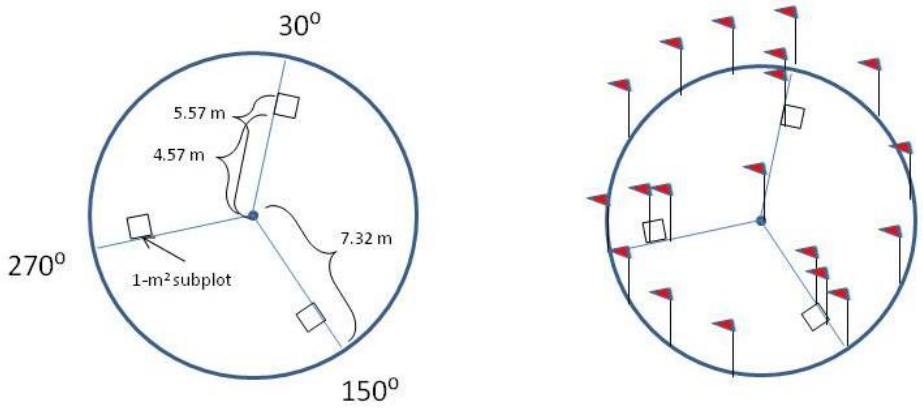

Figure 1 (a) A 168-m^2 circular plot will be used to record plant species richness and cover. The plot includes nested subplots at a specific distance and location from the center of the plot. (b) The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

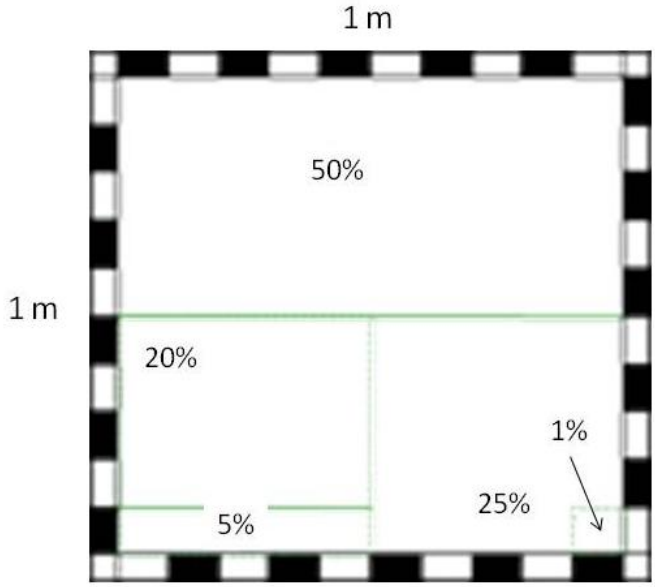

Figure 2 The 1-m^2 subplot is calibrated with black and white marks to make estimates of plant species cover more accurate and repeatable.