



<i>Title:</i> Field Protocol and Procedure: Plant Diversity	<i>Author:</i> D. Barnett	<i>Date:</i> 01/13/2014
<i>NEON Doc. #:</i> NEON.DOC.00014042		<i>Revision:</i> B_DRAFT

FIELD PROTOCOL AND PROCEDURE: PLANT DIVERSITY

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of NEON protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. Documentation of content changes (i.e. changes in particular tasks or safety practices) will 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
- site-specific safety practices
- general equipment maintenance

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

1.3 Acknowledgements

This protocol is based on a technique for sampling plant species diversity in a multi-scale plot that was created for use in The Carolina Vegetation Survey.

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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD [01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD [02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD [03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD [04]	NEON.DOC.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000912	Plant Diversity Science Design Document
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001025	Field Protocol and Procedure: Plot Establishment

2.3 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and 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, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

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3 BACKGROUND AND OBJECTIVES

3.1 Background

The purpose of plant diversity sampling is to describe inter- and intra-annual variation of the composition and abundance of plant species 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.

Plant species diversity will be measured once or twice annually in the field. The plot-based method yields plant species richness data at multiple scales that will provide an understanding of changes in species composition, changes in species distribution, and the introduction of non-native plant species. The data will be comparable within and across NEON sites and to other continental vegetation efforts to allow for a comprehensive understanding of the impacts of the drivers of change on the diversity of plant species and the functional role they play in ecological systems.

This document provides detailed guidance for assessing plant diversity in the Distributed Plots in the field, the collection and handling of unknown plant species, and the collection of voucher specimens for training and archiving purposes.

3.2 NEON Science Requirements

This protocol fulfills Observatory science requirements that reside in NEON’s Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON’s document repository, or upon request.

3.3 NEON Data Products

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog ([RD04]).

Table 1. Plant biodiversity data products that guide the study design for data collection.
 Many of these data products will be collected as part of the plant productivity and biogeochemistry protocols.

Data product	Units	Measurement resolution	Sampling frequency
Diversity			
Species composition/presence	list	per subplot, per plot, per year	1-2 bouts a year
Alpha diversity			
Species richness, Shannon and Simpson (diversity and entropy), Pielou's evenness	number	per subplot, per plot, per year	1-2 bouts a year
Observed Gamma diversity			
Species richness, Shannon and Simpson (diversity and entropy), Pielou's evenness	number	site	1-2 bouts a year

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Data product	Units	Measurement resolution	Sampling frequency
Estimated Gamma diversity (woody sp. count data)			
Rarefaction and extrapolation curves, asymptotic species richness, bias-controlled Shannon entropy, Simpson concentration	NA	site	1-2 bouts a year
Herbaceous species			
Abundance of herbaceous species, by species	% cover area ⁻¹	per subplot, per plot, per year	1-2 bouts a year
Average herbaceous height, by species	m	per subplot, per plot, per year	1-2 bouts a year
Density of stems (not collected in 2013)	Number area ⁻¹	per species, per subplot, per plot, per year	1-2 bouts a year
Other			
Species nativity	list	per species, per subplot, per plot, per year	annual

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4 PROTOCOL

This document describes the collection of plant diversity information designated to inform the goals and objectives of the National Ecological Observatory Network (NEON). Plant diversity shall occur according to a sample design – a statistically rigorous system that directs the spatial distribution of observations – at plots distributed across NEON sites. Plant species composition or presence and abundance data shall be collected in multi-scale plots, estimates of cover being limited to 1-m² subplots that shall be nested in larger plots where plant species composition will be recorded.

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 the collection and identification of difficult species are provided. 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. 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. 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.

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5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (RD[06]).

The plan will include:

- Hot checks in the field by HQ staff or contractor
- Cold checks in the field by a contractor or alternate NEON field crew
- Independent verification of unknown and voucher specimens by authorities at herbaria.

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

If the data collection cannot be completed due to safety or logistical reasons, sampling must resume at the plot later during the same sampling bout (approximately 45 days). Delay of sampling should be recorded in data about the plot-sampling effort.

Any changes that the plot undergoes during a particular sampling bout should be noted in the data associated with the plot.

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6 SAFETY

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). 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.

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7 PERSONNEL REQUIREMENTS

A minimum of two technicians are required for each plant diversity sampling team: a botanist and a technician. It is mandatory that one technician have experience with the identification of plants – preferably in the habitats found at the site where measures will be made, be able to use a dichotomous key, and have experience identifying plant specimens in the lab with a dissecting microscope and associated tools. At each site this technician must be able to identify most of the species in the field.

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8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (RD[04]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (RD[05]).

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9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

Sampling timing will be determined annually by Field Operations based on criteria for sampling and approved annually by Science.

9.1.1 Criteria for Determining Sampling Dates

Sample bouts will be timed to maximize the number of plant species detected at a NEON site. Samples will generally be made at peak phenology (when most species are flowering) to facilitate identification of individuals. Many NEON sites will not have a single peak in phenology due to plant adaptations that take advantage of different climate conditions. Sites with more than one peak may require multiple sampling bouts.

The specific timing of sampling bouts will be tied to observations from the NEON phenology measurements, but the timing of sampling has generally been determined to support planning purposes (Appendix A).

9.1.2 Sampling Frequency

Plant diversity will be sampled one or two times annually (Appendix A).

9.1.3 Sampling Timing Parameters

Complete sampling in approximately a 1 – 2 month period around peak phenology. Significant delays may change the detectability of species and influence the comparability of sampling bouts.

9.2 Equipment and Materials

Table 2 Materials and supplies required for one crew for the field Plant Sampling Procedure.

Maximo Item No.	Item Description	Quantity	Habitat Specific	Special Handling
	30 or 50 meter fiberglass tape measure	3		
	Global Positioning Unit	1		
	Plant press	3		
	Field notebook and pencil	1		
	Pin flags	At least 40		
	Nylon flagging	1		
	1-gallon zip-loc plastic bags	Many		
GN100001	Small carabiner and ring binder	1 ea.		
	Hand lens, 10x or 20x	1		
	Pruning sheers	1		
	Weeder	1		
MX100497	Meter stick	1		
	Subplot frame (assembly required)	1		

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	Digital camera	1		
	PDA or datasheets	1		
	Species list	1		
	Dichotomous keys	1		
	Hardware stakes and/or chaining pins	5		
	Adhesive labels	many		

9.3 Preparation

1) Subplot Frames

- a) The cover and identity of plant species will be recorded in 1-m² frames. The frames may be assembled from components prior to the sampling effort or purchased pre-fabricated (Appendix B).

2) Data Collection

- a) Prepare the PDA or data sheets for collecting data prior to leaving for the field. If the data is 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 have been downloaded and deleted. Be prepared to use provided datasheets if the device fails (dunked in a creek, lost, or crashes) or if data is to be collected on paper data sheets.

3) Consumable Items

- a) Plastic bags will be used to collect unknown plant species. Prior to going to the field or be sure to have an ample number of loose bags and, if desired, assemble a system for collecting unknown species (Appendix C). Adhesive labels will be needed and working permanent markers and pencils.

9.4 Sample Collection in the Field

9.4.1 Plot Establishment

Plant diversity sampling occurs in a square-shaped plot measuring 20 m on a side and contains four 100-m² subplots (Figure 1). Each subplot contains nested subplots: a 1-m² subplot nested in a 10-m² subplot in each of two corners. This protocol assumes that plots will be marked by a center point and four corners. The permanent markers define the corners of the modules and should maintain comparability through time. If this is not the case, plots must be established during each sampling bout according to the Plot Establishment Protocol (RD[07]).

- 1) Establish 100-m² subplot and small, nested and subplots. For comparison of data across space and through time it is important that these be of consistent size.
 - a) Delineate the sides of the 100-m² subplot and 10-m² subplot 3.16 m from the nearest permanent marker at the plot corner or center with flags or appropriate markers.

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- i) Two options for delineating the plot are provided. Appendix D assumes the plot was established with precise square with exact 20 sides and that the tape can be stretched between corners with no obstacles. Appendix E recognizes an inevitable lack of absolute precision of the established markers and obstacles that are likely to obstruct the tape when stretched between markers.
- b) At the smallest scale, the 1-m² subplot, this will be achieved with a rigid subplot frame anchored at the corner by a permanent plot marker.

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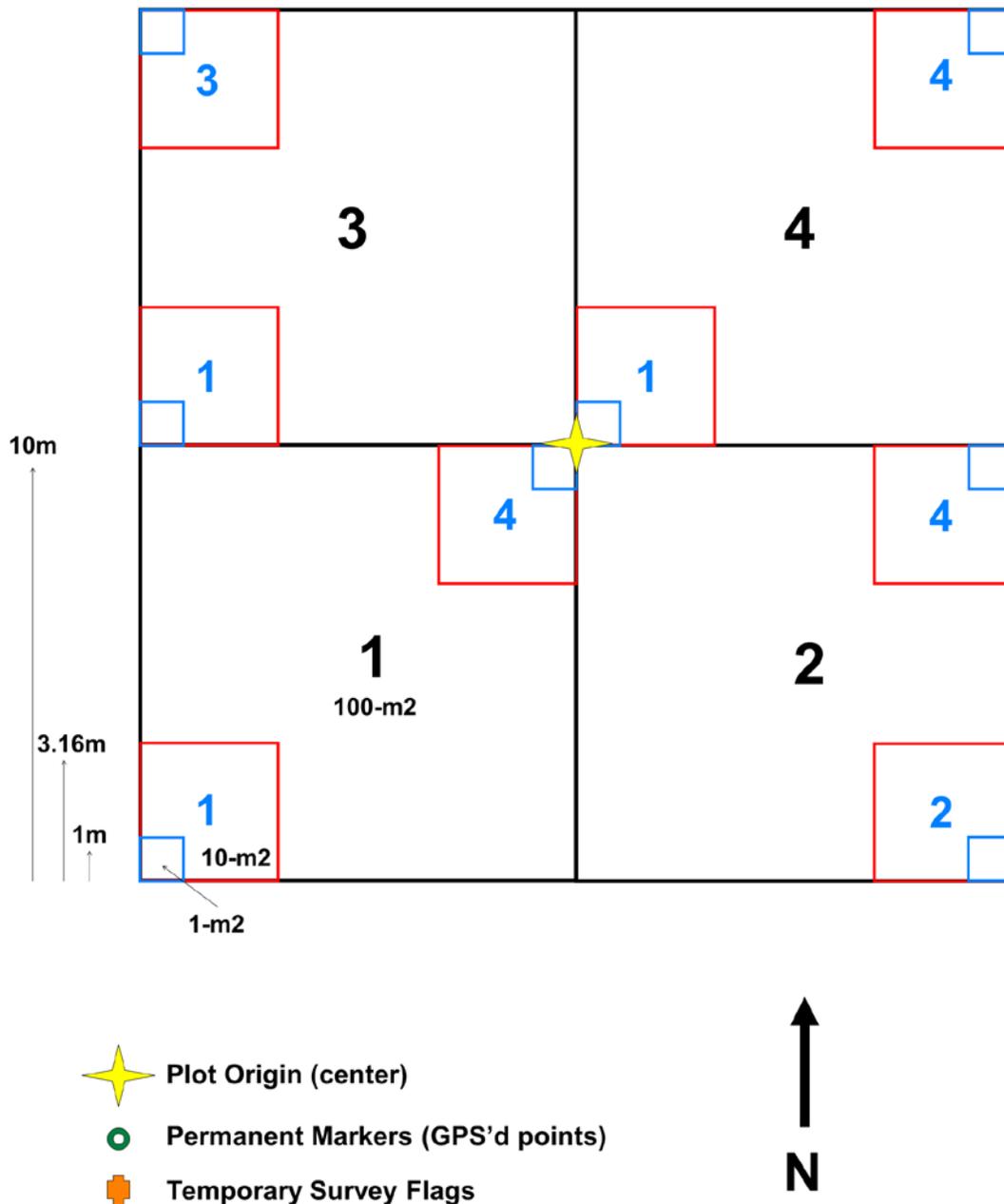


Figure 1. A square, multi-scale plot will be used to record plant species composition and cover. The plot includes nested subplots at specific locations within the plot.

9.4.2 General Collection

- 1) Measure vegetation composition/presence, cover and height measurement in each 1-m² subplot.
 - a) Locate the flags for the 1-m² subplots in the corners of the 100-m² subplots and place the subplot frame such that one corner touches the corner marker and two others touch the flags marked 1 m from the marker in both directions.

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- b) Estimate and record the combined cover of abiotic (non-living) elements and non-vascular plant species in each 1-m² subplot. 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.
- c) Identify each species rooted in the subplot and estimate the combined aerial cover of all individuals less than 3 m tall to the nearest one percent.
 - i) Each 1-m² subplot frame is calibrated in 10 cm sections to make cover estimates easier (Figure 2).
 - ii) Only estimate cover on plants, or portions of plant, that are rooted, or partially rooted, inside the subplot frame.
 - iii) Visually group species together into a percent cover.
 - iv) Fine tune that estimate by subtracting out any spaces or gaps.
 - (1) Familiarize yourself with what particular 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.
 - (2) Enter 't' for estimates of cover <1%.
 - v) 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.

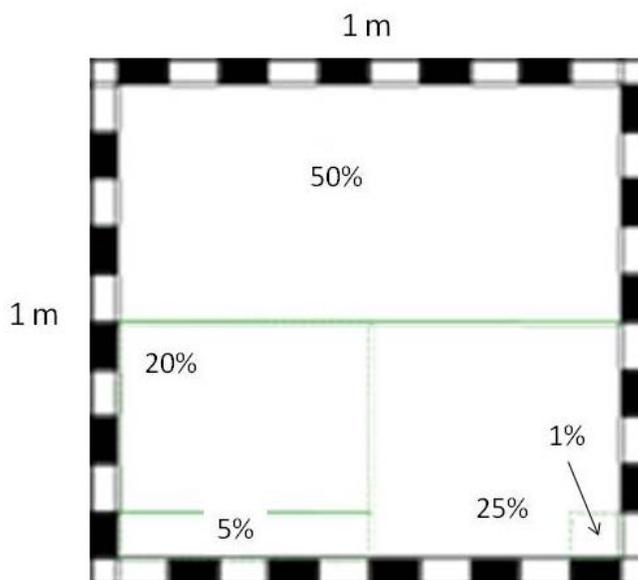


Figure 2. The 1-m² subplot is calibrated with black and white marks to make estimates of plant species cover more accurate and repeatable.

- a) Record the species identity, corresponding standardized Natural Resource Conservation Service (NRCS)/US Department of Agriculture (USDA) PLANTS database code, and cover for each species. If a species cannot be identified in the field, refer to section 9.4.3 Unknown Plant Species.

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- b) Estimate average height of all individuals less than 2 m tall.
 - i) Record height only for individuals less than 3 m.
 - ii) Estimate the average height of the tallest 1/3 of individuals in the subplot. For example, if there are several small sprouts of a shrub species, but the majority of the individuals of the species are approximately 2 m, height should be reflect an estimate of the average of these taller individuals.
 - (1) A meter stick can be helpful for estimating height of low-stature individuals such as herbaceous species
 - (2) Taller woody species will require estimation based on the height of known objects such as a technician working in the field.
 - (3) Enter 999 for estimates of average height > 3 m).
- c) Prior to removing the 1-m² subplot, take a picture of the nested 1-m² subplot such that the subplot frame fills the photograph while standing at the middle of one edge (outside the plot when possible). Record the photo information such that it can be attributed to the subplot in the database.
- 2) Record plant species composition/presence of all species rooted in each 10-m² subplot.
 - a) The subplot will be defined by the tape on the outside edge of the subplot. Other edges and corners will be defined by the flags inserted in the plot establishment phase. If there is question about the presence of a species in a plot, the third tape can be moved to better define the edge of the plot.
- 3) Record plant species composition in each 100-m² Module.
- 4) Repeat above for each of the four 100-m² subplots and nested subplots.

9.4.3 Unknown Plant Species

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

- 1) 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) Collected unknown specimens should be placed in sealable plastic bags (see Appendix C for optional system for organization and transport of specimens).
- 3) Collect representative parts of the entire individual, including the roots, flowers (if possible), and vegetative growth of grasses and forbs.
 - a) A piece of a branch is usually sufficient for trees and shrubs.
 - b) If a flower cannot be found, technicians can keep an eye open for an individual in flower for the rest of the sampling effort, but are not expected to return to a particular plot for the exclusive purpose of finding the individual in flower at a later date.
- 4) Label plant with a unique (to the technician) unknown name, number, description, botanist, date, and plot number.
- 5) 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.

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9.4.4 Morphologically Challenging Species

The identity of some species and sub-species will be difficult to separate at some sites. Because comparability of cover estimates must be consistent through time, it will be necessary to identify these groups of species (e.g. genus) and consistently lump them through time and across field sampling efforts.

It is difficult to know what this lumping might look like prior to the first field sampling year. A list of grouped species should be developed over the first year of sampling and based on conversations with botanists who work at the site or in the region if possible.

9.4.5 Voucher specimens

Plant species will be collected at NEON sites for the following purposes: 1) collected: fresh and/or dried samples to be used for identification, 2) to be pressed and incorporated into the domain support facility herbarium for training and quality assurance purposes, and 3) to be preserved as described and mandated by the archive program.

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.

9.5 Data Handling

All information from field data sheets must be entered or transferred from the electronic device and saved to the NEON server as directed by the Field Operations Manager.

9.6 Refreshing the Sampling Kit

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

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10 LABORATORY STANDARD OPERATING 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 be identified fresh (without pressing), within two days of collection. If the specimen was destroyed during identification or was not 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 at a NEON herbarium or other archive facility. These specimens should be treated with extra care to preserve diagnostic parts, pressed until dry, and mounted for preservation in a NEON herbarium or transferred to an archive as mandated by collection procedures.

10.1 Sample Processing 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. Specimens may remain in this state for months. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

10.2 Equipment and Materials

Table 3. Laboratory Equipment List

Maximo Item No.	Item Description	Quantity
	Dissecting microscope	1
	Plant keys	No more than 5 references
MX100316	Standard Plant Press, Wood Frame, 12 in. x 18 in., with Straps	2
	Botany dissection kit	1
MX100719	Glue Bottle, w/Brush Attached to Lid, Up to 12 oz Capacity	1
MX100679	Herbarium Mounting Glue	1
MX100680	Mounting Paper	1
306650000	Washer Flat .688 ID X 1.5 OD #5/8" SS	many

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	Herbarium glassine seed envelopes, 2" x 3.25"	1
	Herbarium labels	1
	Safety pins	1
	Tape, cloth, white gummed, 0.5", for herbarium mounting	1
	Wood, 2in x 2in x 8ft furring strip	1

10.3 Preparation

A clean and dry bench space is required.

10.4 Sample Processing in the Lab

10.4.1 Identification of Unknown Species

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 Wyoming, University of Florida, 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.

10.4.2 Plant Pressing and Drying

Press plants as soon as possible (and within 2 days of collection) in the lab at a clean and dry bench space. Plants are 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.
 - a) Open some flowers to show both the top and underside to illustrate the arrangement of flower parts.
 - b) Squash large fruits on the page or slice them in half.
 - c) Place dry, loose seeds or fruits in sealed packets.
 - d) Turn over some leaves or part of a single large leave to show underside.
- 3) Write the collection number, date, botanist, and initial identification on the inside of the newspaper and any seed packets.
- 4) 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.
- 5) 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.
- 6) 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.

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10.4.3 Sample Preservation: Processing Voucher Specimens

Herbarium specimens are catalogued with a standardized label. The label includes the family, genus, species, location description and coordinates, elevation, collector, collection date, and collection number.

Once specimens are dry, they must be mounted. 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 is 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.
- 11) Assign a standard accession number to each prepared sample.
- 12) Store the samples at -20 degrees C for forty-eight hours prior to permanent storage in herbarium cabinet.

10.5 Data Handling

After unknown specimens are identified, update the information in the data sheets, files or database.

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APPENDIX A ESTIMATED START TIME FOR SAMPLING.

Domain	Site	Number of Bouts	Bout Date	Bout Date
1	Harvard Forest	1	Late June – Early August	
3	Ordway-Swisher	tbd	tbd	
3	Disney	tbd	tbd	
10	CPER	2	May - June	August
10	RMNP	1	Late June – Early August	
10	Sterling	1	tbd	

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Item	How many you need	Comments	Click an image for larger view
PVC 90 degree elbows, half inch (nominal)	4	All this PVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 21 mm (7/8 inch).	
PVC straight couplings, half inch (nominal)	8		
PVC pipe, half inch (nominal), ten-foot sections	2	This kind of pipe usually comes in ten-foot lengths. You will need two of those to cut the 12 thirteen-inch sections you need.	
Drill and 5/16 inch bit	to use	If you are constructing a frame while on the road, many hardware stores will drill the holes for you.	

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Elastic shock cord, 1/4 inch (7 mm) diameter 12 feet



Black electrical tape (or black paint) one roll For marking calibration bands on the bars.



Drill a hole in the back of each 90 degree elbow, as shown.

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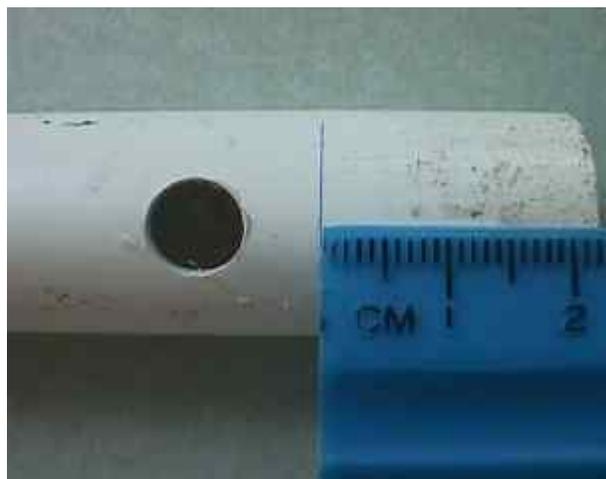
Cut 12 PVC sections 33 cm (thirteen inches) long. This size allows for the length added by the connectors, and the length that will slip into them. The finished frame fits almost exactly one square meter.

You can cut the PVC pipe any number of ways. If you are on the road, hacksaw blades are cheap and effective. You can wrap one end of the blade with tape to make it easier to grip.

There is no need to "bevel" the ends of the pipe sections. Beveling will spoil the stiffness of the finished frame.



Drill a hole in the side of three of the PVC sections (one for each bar).



Drill the hole about 33 mm (1-1/4 inch) from one end. This distance is not critical. It must be at least as far as the length that will slip into an elbow, 22 mm (7/8 inch), but it can be as much as 5 - 8 cm (2 - 3 inches).

If you are having the holes drilled at the time you buy the pipe, drill them in each end of the two ten-foot sections. Then you can cut the four needed 33 cm (13-inch) sections off of those ends.

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Push one end of the shockcord through the side hole. Easiest, from outside in, then down the inside of the pipe section.



When you have the shockcord through the pipe section and can get enough to work with, thread components together as shown:

- pipe section with side hole
- one straight coupling
- a pipe section with no side hole
- a second straight coupling
- another pipe section with no side hole
- a 90 degree elbow

Thread the shockcord out the hole in the back of the elbow.



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Tie a knot close to one end. Pull that end snug to the hole. Bring the PVC pieces together so the shockcord is just about the same length through them. Then, pull the shockcord out about a foot so it is stretched.



Tie a knot close to the second hole.

You can test it at this time to see if the tension feels right. You should be able to pull the PVC sections apart easily and fold up the bar. But when straightened out, the shockcord should hold the pieces tight enough together they don't slip apart by accident. There is no need to glue the PVC sections together.

When you are satisfied with the tension, you can tighten up the knot and cut the shockcord off to about an inch (few cm).

Marking the bars in 10 cm bands of alternating color will make it easier to visually estimate percent's of the enclosed 1 square meter area.

Electrical tape is a convenient way of marking the bands. Less messy than paint, plus it holds the ends of the shockcord from fraying.

If you use the wrapping pattern shown, all the cut ends of the shockcord will be covered.



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While wrapping the tape, simply skip up and over any couplings you come to.



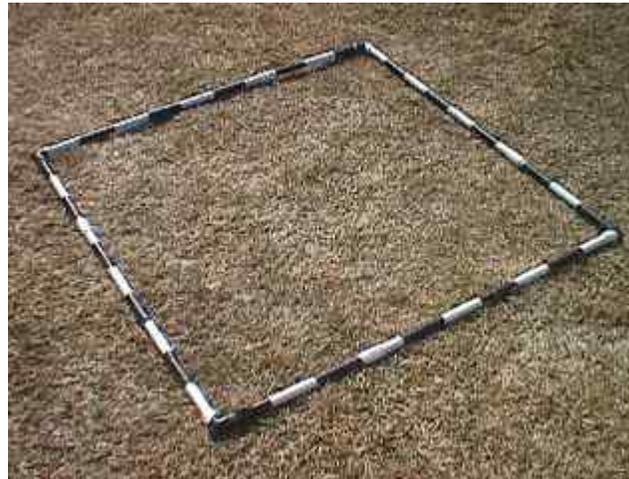
Note that tape is free of junctures, so segments can come apart

Make the other three bars the same way. It will be easier to get the shockcord threaded through the bars if you do not cut it first. When you are done, you will have a length of cord left over. You can tie this into a ring and use it like a big rubber band to hold the folded up frame all together in one bundle.



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To assemble the frame at a survey site, you push the "empty" end of a bar into the open side of the elbow on another bar. This usually holds together for the minor movements of adjusting the frame position on the ground, but comes apart easily to go around stems and brush.



Variant: Non-folding PVC frame:

To avoid the complications of the folding junctions, we offer this straight version. Some people prefer this design and use the bars as walking sticks.

Item	How many you need	Comments
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PVC pipe, half inch (nominal), ten-foot sections 2

PVC 90 degree elbows, half inch (nominal) 4

All this PVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 21 mm (7/8 inch).

PVC glue

Black electrical tape (or black paint) one roll For marking calibration bands.

Cut four sections of pipe, each 39-3/8 inches (about one meter) long. If you are being precise, make them 99.8 cm to allow for length added by the elbows, but this is usually negligible. You will be able to get three pieces out of a ten-foot length of pipe, but you will need another ten-foot section for the fourth bar.

Glue one elbow onto one end of each bar. It is necessary to glue them or the frame falls apart too easily in use, and the elbows get lost.

Wrap with black tape, or paint bands, to mark the tenths of meters.

Variant: Lightweight folding PVC frame:

This is the same as the regular folding frame except you use (nominal) half inch CPVC pipe and fittings. This type of pipe is usually cream yellow in color, and much thinner and lighter. Even though both these kinds of pipe are called "half inch" the CPVC is about half the size of the "regular" PVC pipe. You can use thinner shock cord or even flat elastic (from a fabric store) in a pinch.

Item	How many you need	Comments	Click an image for larger view
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CPVC half inch (nominal), 90 degree elbows 4

All this CPVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 16 mm (5/8 inch).

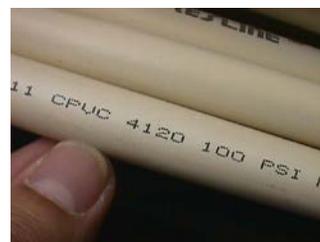


CPVC half inch (nominal), straight couplings 8



CPVC half inch (nominal) pipe, ten foot lengths 2

This kind of pipe usually comes in ten-foot lengths. You will need two of those to cut the 12 33-cm (thirteen-inch) sections you need.



Shock cord, 4 mm (3/16 inch) diameter, or flat elastic 18 mm (3/4 inch) wide. 12 feet



Drill and 3/16 inch bit to use

If you are constructing a frame while on the road, many hardware stores will drill the holes for you.

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Black electrical tape

one roll

For marking calibration bands on the bars.



Construction is the same as the folding frame above except that holes are 3/16 inch, and must be at least 14 mm (1/2 inch) from the end of a bar section.

This design is a welcome saving in weight on long hikes. However, the frame is "floppier", and more fragile. It is hard to avoid cracking the straight couplings.

For general use, we recommend a frame made of half inch (nominal) "regular" PVC; or if the greater expense is no problem, the tent-pole version below.



Left: Ultralight frame made from CPVC, about 670 g (1.5 pounds)

Right: Standard frame made from "regular" PVC, about 900 g (2 pounds) if using "SDR 13.5" pipe about 1.3 kg (2.9 pounds) if from "Schedule 40" pipe

Variant: Ultralight folding tent-pole frame:

This design has proved as serviceable as the sturdy PVC, though at a higher cost in materials. It is a great saving in size and weight: about 0.7 pounds or 11 ounces (300g).

Materials for one bar are: Three tent pole sections, two grommet tips, a length of elastic shock cord, and materials for marking and connection. Here is the parts list for the four bars that make up a

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complete sampling frame.

You can order starred (*) items from the address given below.

Item	How many you need	Comments	Click an image for larger view
Standard 13-inch tent pole sections, with end insert*	8	<p>The length is 13 inches, not including the insert.</p> <p>The diameter you need for all sections is "three four three", which means 0.343 inches.</p>	
Standard 13-inch tent pole sections, without end insert*	4		
Elastic shock cord, 1/8 inch (3 mm) diameter*	12 feet (about 4m). This is sold by the foot.	This is a standard diameter to fit the tent pole sections.	
Tent pole grommet tips*	8		

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Adhesive backed velcro, two contrasting colors

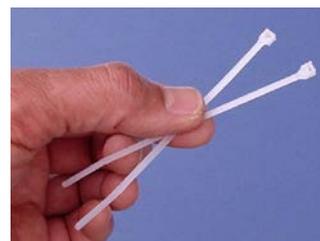
4 inches (10 cm) of each color. This is typically sold by the half foot, so 6 inches is probably the minimum you can buy.

You will only use the hook of one color and the pile of the other color, but the hook and pile are usually sold together.



Nylon cable ties. 8

Get the smallest ones available. If possible, match the velcro colors, 4 of each color.



PVC "electrical tape", two different colors. one roll of each color

For marking the calibration bands on the bars. One color should **match** one of the velcro colors, the other should **contrast with** the bar color (see below).



* Tent pole sections, and related items, are available from:

Tentpole	Technologies,	LLC
8212	NE	Circle
Vancouver,	WA	98662-1300
Phone:	360-260-9527	or 800-266-9527
Fax:		360-260-9937
E-Mail:		tentpoles@comcast.net
Website:	http://www.polesforyou.com/	

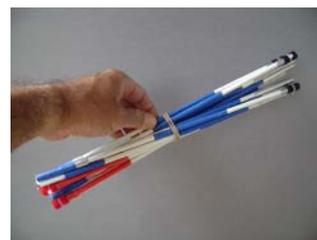
If you explain what you need, Tentpole Technologies will now pre-assemble the one-meter "legs". This is a great convenience.

In this case, you can skip the assembly instructions below, down to the section on marking with colored tape.

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Tent pole sections made of fiberglass are becoming available. These are lighter and cheaper.

Assembly is slightly different than shown below, but the principle is the same. The finished frame weighs only about 240g, compared to 300g for aluminum poles.



Before you begin to assemble the tent pole sections, note the following:

Some of the tent pole sections have an "insert", a narrow extension on the end, which slips into the adjoining section.

Each bar is made up of two "insert" sections and one "plain" section.

You should assemble the bar with the two "insert" sections in series, and then the "plain" section. Then, the completed bar will fold up to minimum length. Minimum length is preferred, to carry the equipment in the smallest possible space.



shortest folding pattern -- **preferred**



schematic -- **preferred**

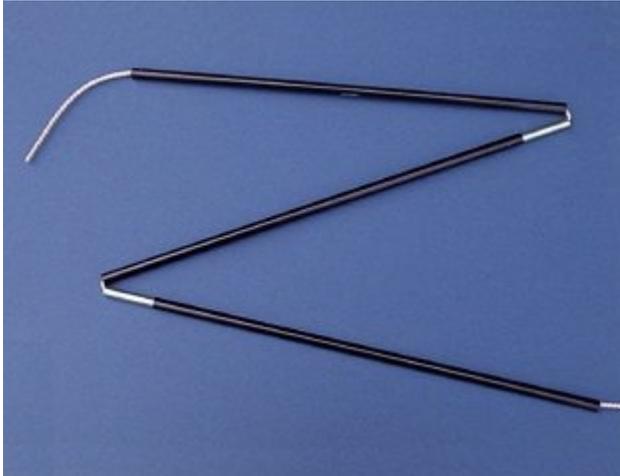


longer -- avoid



schematic -- avoid

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Thread the shock cord through the tent pole sections as shown.

Tie the free end of the cord to a grommet tip. A double half-hitch knot is good. Pull the knot tight to make it as small as possible. It will be a close fit inside the tube.

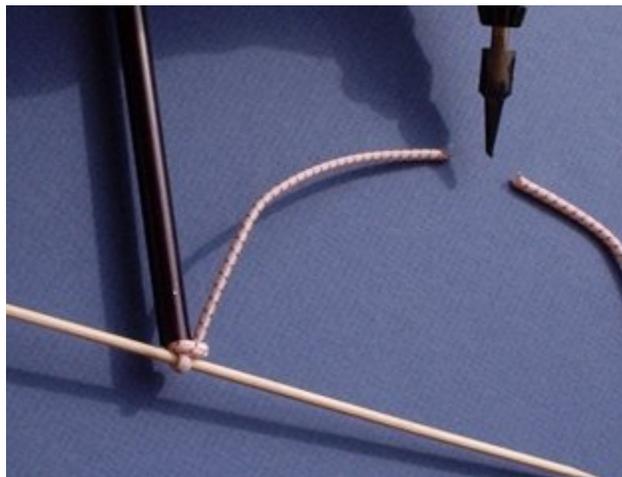
Push the grommet tip down into the tube.



Pull the other end of the shock cord out about 4 inches (10 cm) beyond slack and mark it to cut.

While working on this end, you can keep the cord from pulling down into the pole section tube by tying a slipknot and putting a small stick through the loop of the knot.

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You can cut the cord with a soldering iron, which will melt the ends and prevent any fraying. However, just cutting with scissors works acceptably well.

After tying on the other grommet tip and assembling the bar, note that the 1-meter length goes slightly beyond the ends of the pole sections. This 1-meter point is where you want the poles to connect, though a little variation is not critical.

You will use velcro to "connect" the bars, hook velcro on one bar end and pile velcro on the other. The connection does not have to be rigid. In fact it's useful to have the junction flexible, with the ability to easily disconnect and reconnect.

Visual cues help you quickly see which points will stick to each other, so we will make the two ends different colors.



We'll refer to the ends of the bars as the "red end" and the "black end", though of course they can be any contrasting colors. Also, the hook and pile velcro can be switched from how we describe them here.

Cut a strip of red pile velcro about an inch (few cm) long and wrap it around the grommet tip on what will be the red end of a bar. Also, wrap the end of the bar, for 10cm, with tape of the same color.

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The adhesive back of the Velcro makes it easy to apply. However in the field it will tend to come loose with dirt and moisture. So use a cable tie to hold it on securely.

You can even hold the Velcro on with a cable tie alone, if you can't get adhesive back Velcro.

Wrap the rest of the bar in alternating bands of the contrasting color tape. These 10cm bands make it easy to visually estimate percent's within the enclosed one square meter area.

Use the brightest colors you can find, to make the frame visually evident in the field and avoid losing it in the brush.



Assemble the black end the same as you did the red end, except use hook velcro.

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Assemble the other bars.

The red end of one bar will stick to the black end of another bar.



The bars will stay together strongly enough to pick up the frame as a piece.

In practice, in the field, you will often assemble the frame in place by threading the bars between stems and branches. The ends will stick when you bring them in contact.



Folded up, the frame makes a small bundle weighing only 300g (aluminum poles) or 240g (fiberglass poles).

You can use a left over piece of the shock cord to make a band to hold the bundle together. If you buy the poles pre-assembled, a rubber band will serve as well.

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APPENDIX C BUILDING A SYSTEM FOR COLLECTING UNKNOWN PLANTS IN THE FIELD.

There are many methods for handling plant samples you collect. Here is a "recipe" for equipment that costs little to assemble. Many botanists have found this useful.

As with any recipe, you will want to vary it to your own taste.



The basic ingredients are few:

- large plastic ziplock bags, available at any grocery store.
- a miniature "caribeen" keyclip.
- a large binder ring (**not** a keyring), available from most any office supply store.

(Depiction of a certain brand name does not imply endorsement of that brand)

You may want to choose a brightly colored minibiner, to spot easier in the brush.



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Bags *without* the slider work best. The sliders get caught on things.



Many botanists are familiar with using plastic bags to hold plants. This idea makes everything more hands-free. Simply punch the binder ring through a corner of each bag.



You may want to punch through the bottom corners of all bags except one. That way, most openings will hang down.

The one bag pointing up serves as a pouch for equipment such as specimen labels, pens, and collecting tools.

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A handy tool is a table knife, available for about ten cents at any second hand store. You can use it to both dig and cut.

Choose knives constructed in one solid piece.

Mark knives with colored flagging ribbon held on with duct tape. Otherwise, a dropped knife is very hard to see in the brush.



To complete the setup, close the binder ring and clip the mini-biner through.



The unit can hang from a belt loop. The slick plastic seldom gets tangled and torn as you walk through brush. When bags do wear out, just replace them.

When you collect a plant, simply swing one of the bags up, drop in the sample, and zip the bag shut.

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The one bag with opening at the top stands out as the holder for equipment.

(Some people prefer all the bags up. They are concerned specimens will fall out, though this does not happen if the bags are zipped shut.)

Some collectors pre-load each bag with a label, ready for the specimen.



Plants don't tend to "cook" in the bags unless left lying directly in the sun. However, this can happen quickly. One case to watch out for is sunlight coming through vehicle windows, even while driving with air conditioning on.

You can keep a few un-punched zip lock bags in the equipment pouch for crispy-dry specimens. Carefully insert the plant, add a squirt of water, blow up the bag like a pillow, and zip it shut. By the time you are ready to press, the dry specimen will have softened enough to flatten without crumbling.

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APPENDIX D DELINEATING A PRECISE PLOT WITH LITTLE TO OBSTRUCT THE TAPE ON THE PERIMETER.

The perimeter of the plot and subplots shall be delineated by tape measures and subplot frames as follows (Figure 3):

- 1) Record date and plot number.
- 2) Begin in the south-west corner of the plot, at most sites this permanent marker will be labeled with information about the plot.
- 3) Anchor a 50 m tape and extend it towards the south-east corner.
 - a) Walk on the south side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
 - b) While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m, 16.84 m, and 19 m.
- 4) Anchor the tape at the 20 m with a tent stake (or chaining pin) at the south-east corner of the plot and pull it towards the marker at the north-east corner of the plot.
 - a) Walk on the east side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
 - b) While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 21 m, 23.16 m, 30 m, 36.84 m, and 39 m.
- 5) Move to the south-west corner of the plot.
- 6) Anchor the second 50 m tape and extend it towards the north-west corner.
 - a) Walk on the west side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
 - b) While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m, 16.84 m, and 19 m.
- 7) Anchor the tape at the 20 m with a tent stake (or chaining pin) at the north-west corner of the plot and pull it towards the marker at the north-east corner of the plot.
 - a) Walk on the north side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
 - b) While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at, 21 m, 23.16 m, 30 m, 36.84 m, and 39 m.
- 8) Anchor a third tape at the center of the plot and extend it south toward the flag that at 10 m.
 - a) Insert pin flags into the ground at 1 m and 3.16 m.
- 9) Return to the center and extend the tape east toward the flag that at 30 m.
 - a) Insert pin flags into the ground at 1 m and 3.16 m.
- 10) Return to the center and extend the tape north toward the flag at 30 m.
 - a) Insert pin flags into the ground at 1 m and 3.16 m.
- 11) Return to the center and extend the tape west toward the flag at 10 m. Leave the tape in this place to facilitate sampling Module 1.
 - a) Insert pin flags into the ground at 1 m and 3.16 m.

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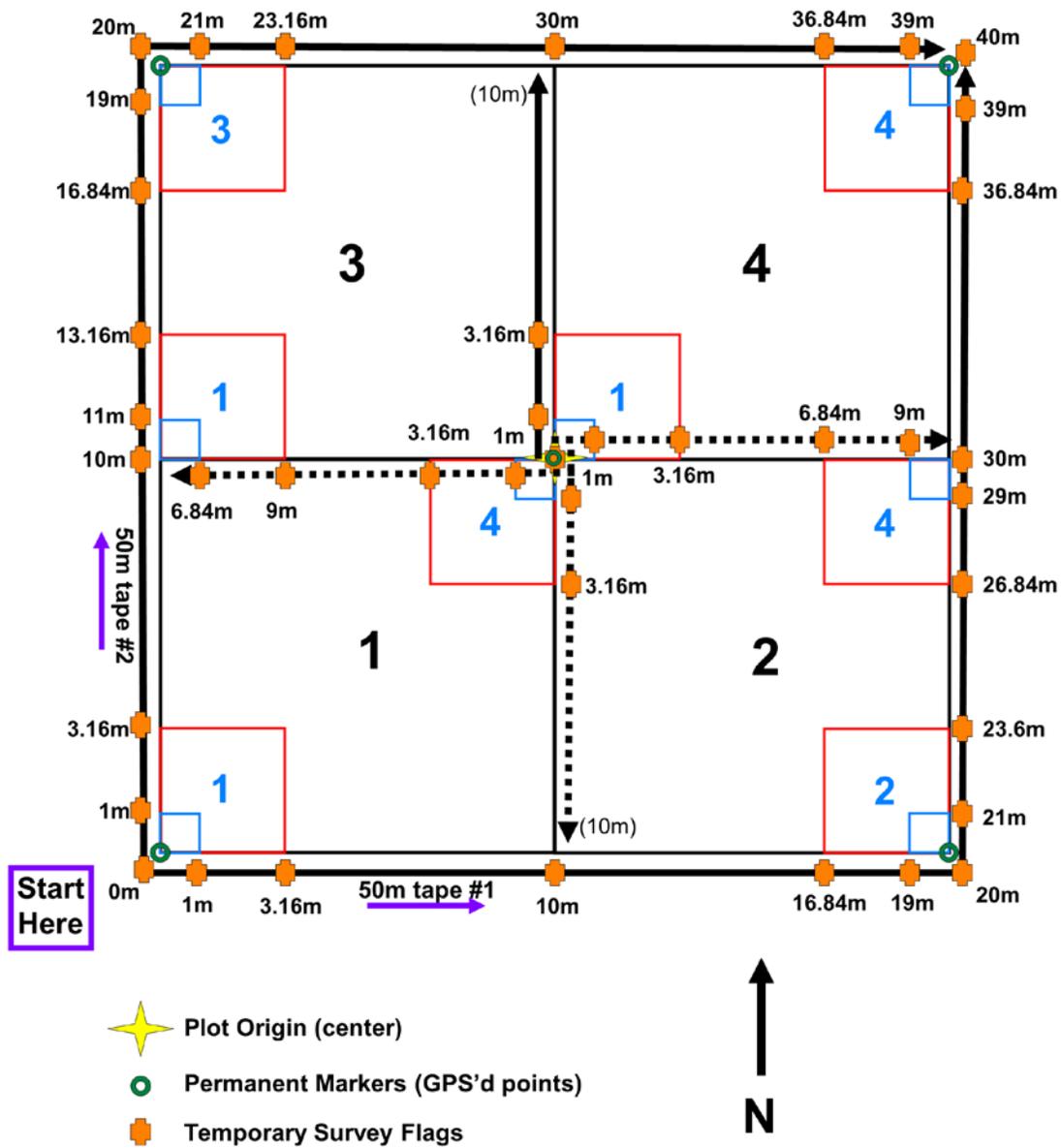


Figure 3. The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

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APPENDIX E PLOT DELINEATION WITH SOME LACK OF PRECISION IN PLOT AND SOME OBSTACLES

This method is very similar to Appendix D, but it recognizes that deviations in the distance between markers and obstacles in the tape may prevent the measures from working as described in Appendix D (e.g. if the tape must go around a tree between the southwest corner and the south-east corner the tape may not intersect the permanent marker at 20 m). The important difference is that subplots will be established from the nearest permanent marker. The idea is to delineate the plot boundary by connecting the permanent markers with the tape measure. The tape should be kept as close as possible to the ground, be forced through shrubs, and around trees to maintain the straightest line possible between markers. With two people, one person can anchor the tape at the south-west corner and pull the tape towards a person standing at the destination marker, or one person can hold the tape at the south-west corner and a second person can pull the tape towards the target marker. A compass might be helpful for establishing the direction the tape should be pulled.

After the tape is extended the subplot and 10 m markers can be established by pulling the tape tight from the nearest permanent marker and accounting for trees and other obstacles as needed. A string or equivalent material that measures 3.16 m is likely easier to use for establishing sides of the 10-m² subplot. The perimeter of the plot and subplots can be delineated by tape measures and subplot frames as follows (Figure 3):

- 1) Record date and plot number.
- 2) Begin in the south-west corner of the plot, at most sites this permanent marker will be labeled with information about the plot.
- 3) Anchor a 50 m tape and extend it towards the south-east corner, walking on the south side of the tape and following a path that creates the straightest possible line towards the marker in the south-east corner.
- 4) Wrap the tape at the south-east corner/permanent marker and extend it to the north-east corner at approximately 40 m on the tape.
- 5) Return to the south-west corner and while pulling the tape tight towards the south-east corner, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m.
- 6) Proceed to the south-east corner and pull the tape tight (either wrapped around the marker or/with a second person holding) from the south-east corner back towards the south-west corner and insert flags at a distance of 1 m and 3.16 m from the south-east corner on the south edge of the plot.
- 7) With the tape anchored at the south-east corner, pull it tight towards the north-east corner of the plot and insert pin flags at 1 m, 3.16 m, and 10 m along the east side of the plot.
- 8) From this 10 m mark on the east edge of the plot, pull the tape tight back towards the south-east corner and insert flags at a distance of 1 m and 3.16 m from the 10 m mark towards the south-east corner.

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- 9) Proceed to the north-east corner of the plot and pull the tape tight from the north-east corner back towards the south-east corner and insert flags at a distance of 1 m and 3.16 m from the north-east corner on the east edge of the plot.
- 10) Return to the south-west corner of the plot. Anchor the second 50 m tape and extend it towards the north-west corner, walking on the west side of the tape and following a path that creates the straightest possible line towards the marker at the north-west corner.
- 11) Wrap the tape at the north-west corner/permanent marker and extend it to the north-east corner at approximately 40 m on the tape.
- 12) Return to the south-west corner and while pulling the tape tight towards the north-west corner, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m on the west side of the plot.
- 13) From this 10 m mark on the west edge of the plot, pull the tape tight towards the north-west corner and place flags towards the north-west corner at a distance of 1 m and 3.16 m from the 10 m mark on the west edge of the plot.
- 14) Proceed to the north-west corner and pull the tape tight (either wrapped around the marker or/or with a second person holding) from the north-west corner back towards the south-west corner and insert flags at a distance of 1 m and 3.16 m from the north-west corner on the west edge of the plot.
- 15) With the tape anchored at the north-west corner, pull it tight towards the north-east corner of the plot and insert pin flags at 1 m, 3.16 m, and 10 m along the north side of the plot.
- 16) Proceed to the north-east corner of the plot and pull the tape tight from the north-east corner back towards the north-west corner and insert flags at a distance of 1 m and 3.16 m from the north-east corner on the north edge of the plot.
- 17) Proceed to the center of the plot.
- 18) Extend the third tape from the middle of the plot towards the 10 m mark on the north edge of the plot and while pulling the tape tight from the center, insert flags at a distance of 1 m and 3.16 m from the center.
- 19) Repeat the previous step in each direction from the plot center.

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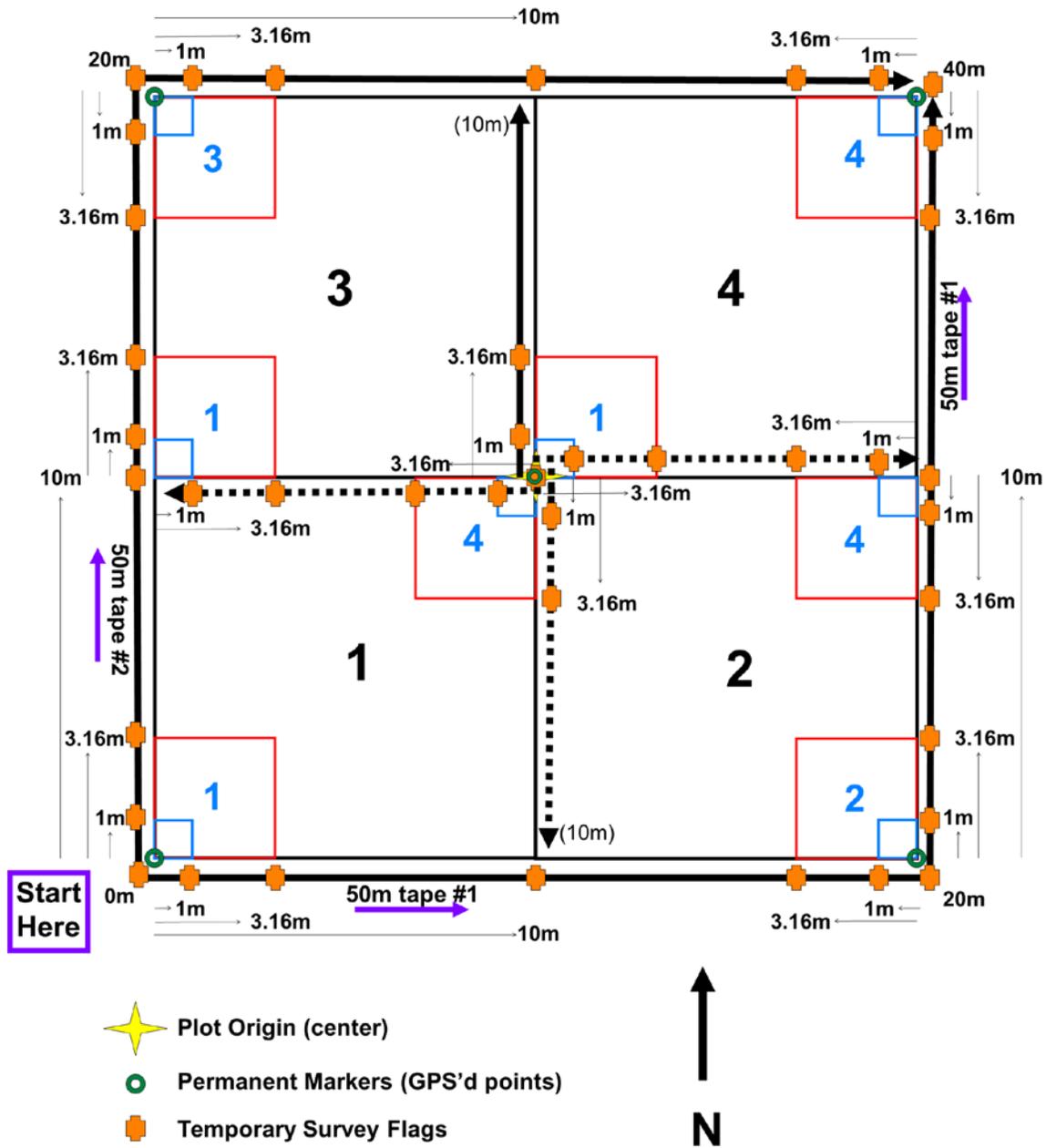


Figure 4. The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

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APPENDIX F DATASHEET

Plot ID: _____ **PLANT DIVERSITY DATA SHEET**

page ___ of ___

Botanist: _____

Notes: _____

Technician: _____

Start Date (YYYYMMDD)/Time (24 hr): _____

% cover less than 1%, enter 't' for trace
height greater than 200 cm (2 m), enter 999

Complete Date (YYYYMMDD)/Time (24 hr): _____

Module 1

corner 1, 1 m ²				corner 1, 10 m ²				corner 4, 1 m ²				corner 4, 10 m ²		100 m ² , module	
Variables			%cover							Variables			%cover		
soil				no data			soil				no data		no data		
rock							rock								
litter							litter								
water							water								
wood							wood								
lichen							lichen								
moss							moss								
duff							duff								
overstory							overstory								
ScientificName	idQ	%cover	ht _{cm}				ScientificName	idQ	ScientificName	idQ					%cover

