TOS PROTOCOL AND PROCEDURE: PLANT DIVERSITY SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/03/2011	ECO-00280	Draft release
B_DRAFT	01/13/2014	ECO-01140	Draft release. Will be finalized in next rev.
C	03/18/2014	ECO-01668	Production release, template change, and other changes as detailed in Appendix C (rev C only)
D	11/03/2014	ECO-02341	Migration to new protocol template
E	02/24/2015	ECO-02536	Naming convention for subplots was changed (see Figure 1). Enter 0.5 for estimates of cover <1%.
F	1/27/2016	ECO-03451	-Removed directive to record species height. -Changed directions for recording cover to include all vegetative cover under 300cm regardless of height of individual. -Added the Tower Base Plots to the description of which plot types to sample. -Updated sample timing and bout number information (Appendix E). -Changed definition of 'standing dead' to include woody species -More clearly defined 'wood'
G	11/07/2016	EECO-04366	-Clarified handling of unknown and morphologically difficult species -Removed directive to collect 'overstory' and to take pictures -Added further directions for collection of metadata with voucher specimens

H	05/31/2018	ECO-05490	-Added table to reflect expected time requirements -Updated morphospeciesID directions such that morphs can be shared across botanists or made unique if not shared within a site -Updated collection metadata requirement for voucher specimens -Integrated the SOP Collecting Plant Tissue for Archive Genetic Material into protocol

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1 OVERVIEW

1.1 Background

The purpose of plant diversity sampling is to describe inter- and intra-annual variation of the presence 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 data at multiple scales that provide an understanding of changes in composition, distribution, and abundance of native and non-native plant species. The data will be comparable within and across NEON sites and to other continental plant diversity 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.

NEON will collect and curate foliar material for analysis of plant genetic diversity over space and time. Plant tissue collections are integral to next generation phylogenetic and systematics studies including building morphological-genetic relationships, identifying species, and providing a foundation for population genetics and phylogenetic studies over the lifetime of the observatory. NEON will make plant tissue collected from select plant species available for analysis by the ecological community.

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

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. 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.

1.2.1 NEON Science Requirements and Data Products

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.

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 (RD[03]).

1.3 Acknowledgments

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, the Whittaker, and the Modified-Whittaker plot design. Special thanks belong to Ben Chemel, Tom Stohlgren, Geneva Chong, and Robert Peet.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHSS Policy, Program and Management Plan
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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000912	NEON Science Design for Plant Diversity
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001579	Datasheets for TOS Protocol and Procedure: Plant Diversity Sampling
RD[06]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass Per Area Measurements
RD[07]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
RD[08]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[09]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[10]	NEON.DOC.001237	NEON Theoretical Basis Document: TOS Plant Diversity – QA/QC of Raw Field And Lab Data
RD[11]	NEON.DOC.003564	NEON Standard Operating Procedure: Plant Pressing, Mounting, and Labeling (Herbarium Techniques)

2.3 Acronyms

All acronyms used in this document are defined in RD[01].

3 METHOD

This document describes the collection of plant diversity data designated to inform the objectives and meet the associated requirements of the National Ecological Observatory Network (NEON). Plant diversity sampling 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 $1m^2$ 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 unidentified 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. The samples must be of archival quality. Specimens should be collected when reproductive parts are present. Each year 20 species per year found in plots should be collected over the first several years of sampling. Vouchers to be housed at the Domain Support Facility should be dried, pressed and mounted. Vouchers to be sent to an archive facility must meet herbaria standards, and should be dried, pressed, and shipped. Plant tissue will also be collected and stored at an archive facility to enable the ecological community to conduct a variety of genetic investigations (RD[06]).

Archival plant tissue will be collected from the three 'Phase I' species selected for phenology observation at each site. This means archive tissue will be collected from the dominant species in the vicinity of the tower (see TOS Protocol and Procedure: Plant Phenology (RD[05]). Archived material, consisting of 30 samples per bout (10 replicates each from the three Phase I Phenology species) will be sampled from both the primary Phenology Plot and a subset of Distributed Base Plots. Samples will be dried with desiccant, stored at room temperature, and sent to a contracted archive facility.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC Plan (AD[06]).

In the interest of efficiency, make every effort to combine the plant diversity collection with other protocols when possible. Perhaps an herbaceous biomass bout can be completed by technicians not responsible for identifying plant species in the plot. Or, in agricultural systems where diversity is likely to be low, perhaps the protocol can be combined with a beetle bout at the same plot or mosquito sampling at the same site.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

The timing of field sampling will be determined annually by Field Operations based on criteria for sampling and approved annually by Science (Appendix E).

Complete sampling in approximately a 1-2 month period around peak flowering (Appendix E). Significant delays may change the detectability of species and influence the comparability of sampling bouts.

4.2 Criteria for Determining Onset and Cessation of Sampling

Plant diversity sampling: Sample bouts will be timed to maximize the number of plant species detected at a NEON site. Observations 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 climatic conditions. Sites with more than one peak may require multiple sampling bouts (Appendix E). The timing of sampling bouts will be further adjusted according to observations from the NEON phenology measurements (RD[07]), but the timing of sampling has generally been determined to support planning purposes (Appendix E).

Genetic archive tissue sampling: Sampling may begin and end any time during the growing season, as long as foliar tissues that are young and not approaching senescence. This is because genetic composition does not change with leaf canopy position or over the course of the growing season, but healthy, robust leaves are needed to enable high quality genetic analyses.

4.3 Timing for Laboratory Processing and Analysis

Plant diversity sampling: There are four conditions that require the collection of plant specimens that result in lab activities:

1. Identification at the Domain Support Facility. These species could not be efficiently identified in the field and were collected for identification by NEON staff. If possible, and to increase efficiency of field sampling and data quality, identify the species soon after the collection.
2. Inclusion at Domain Support Facility herbarium. These specimens should be pressed, dried, identified, mounted and labeled. A quality specimen might require the collection of two individuals should identification require destruction of the sample (e.g., flower and/or ovary dissection).
3. Identification by external expert. These specimens will be pressed and dried and prepared for shipment following the field season.
4. Archive at external facility. These specimens should be pressed, dried, identified if possible, and prepared for shipment following the field season.

Options one and two above are not mutually exclusive; a specimen collected for identification could be included in the reference herbarium. Similarly, numbers three and four above are not mutually exclusive. Some specimens will be sent for identification and then sent to an archive facility (RD [12]).

Ideally, specimens should not be left in the refrigerator for more than two days. They can be placed in a press, stored in a well-ventilated location, and identified at a later date. Any specimen destined for an archive should be placed in the -80°C freezer for two weeks after it is completely dried for decontamination (RD [12]).

Genetic archive tissue sampling: Upon collection, samples should be immediately placed in coin envelopes, then into resealable plastic bags filled with desiccant to begin the air-drying process. Desiccant should be changed as frequently as needed until samples have completely air-dried. This should take 1-3 days, depending on the local climate and vegetation type. Once dry, foliar samples for genetic archive should be shipped to the contracted archive facility according to the schedule provided by NEON CLA.

4.4 Sampling Timing Contingencies

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 during the same sampling bout (approximately 2 months) if the plot is to be considered complete. Delay of sampling should be recorded in data about the plot-sampling effort.
- Any changes that the plot undergoes should be noted in the data associated with the plot. For example, disturbance caused by a large tree falling in the plot, severe flood damage and erosion, or the disturbance from mammals – bison, cows, small mammals – during (between visits to a single plot) or between bouts should be noted in the system for documenting site disturbance.

- Deviations associated with the collection of data should not be made from this protocol. The number of people collecting data, the tools for defining the plot boundary, and the amount of material collected for the identification of unknown plant species may be altered to meet the needs of Operational constraints.

4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Plant diversity sampling will occur on the schedule described above at three Tower Base Plots and, at most sites, 30 Distributed Base Plots per site. Ideally, sampling will occur at these plots for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded or a stream moves after a flood and the location is no longer within the stream channel). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

Plant diversity should be collected at every plot each year. Criteria for moving a plot include:

- If 50% or more of each plot can't be sampled for two or more consecutive years
- Sampling at the plot becomes unsafe due to objective hazards
- Anthropogenic disturbances such as paved roads and buildings are constructed in the plot; this does not include management such as logging and agriculture the site was designed to measure.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

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 EHSS Policy, Program and Management Plan (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.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 1. Equipment list – Materials and supplies required for one crew for the plot-based plant diversity sampling procedure

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Ben Meadows Forestry Suppliers	100952 39167	R	Chaining pins or other suitable anchor	Anchor measuring tapes	4-6	N
		S	Cooler	Chill perishable plant vouchers in field	5	N
B&H	OLTG4B	R	Digital camera and SD card, 12 megapixel	Capture images of plants for species identification	1	N
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy	Navigate to sampling location	1	N
Fisher Scientific Grainer	19067113 3UZA9	R	Ice pack	Chill perishable plant vouchers in field	Many	N
Forestry Suppliers	61260	R	Magnifier hand-lens, 20X	Aid in species identification	Many	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Ben Meadows Forestry Suppliers	122732 39945	R	Measuring tape, minimum 50 m	Determine plot boundary	3	N
		R	Pruning shear	Collect voucher specimens	1 ea.	N
		R	Sampling frame, 1m^2	Delineate 1m^2 subplot	1	N
		S	Small carabiner and ring binder	Organize and carry unknown plant vouchers	1	N
		S	Weeder	Collect voucher specimens	1	N
		S	Meter stick	Evaluate plant height against 300cm	1	N
		R	Handheld computer	Data collection	1	N
Consumable items						
		S	AA battery	Spare battery for GPS receiver		
		S	Adhesive label	Label unknown and voucher specimens	1 sheet	N
		R	All weather copy paper	Print datasheets		
		S	Digital camera battery	Spare battery		
		R	Field notebook	Record field notes	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Grainger Forestry Suppliers	9WKP4 57880	S	Flagging tape	Delineate sampling area	1	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Organize and carry unknown plant vouchers and plant tissue	> 40	N
		R	Survey marking flag, PVC or fiberglass stake	Delineate sampling area	Many	N
Resources						
RD[05], RD[06]		R	Field datasheet	Record data	1	N
		S	Field guide, regional flora reference guide and/or key	Identify unknown species	1	N
		S	Field guide, species list	Identify unknown species	1	N

R/S=Required/Suggested

Table 2. Equipment list – Laboratory processing

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Forestry Suppliers	53872	R	Botany dissection kit	Identify unknown species	1	N
Forestry Suppliers Bioquip	53741 3127	R	Cardboard ventilator	Pressing plants		
		R	Microscope	Aid in species identification	1	N
Fisher Scientific	11350121	R	Paper blotters	Press collected individuals for identification	Many	N
Forestry Suppliers Bioquip	53674 3115	R	Plant press	Press collected individuals for identification	2	N
		R	Scissors or pruning shear	Prepare voucher specimen for mounting	1 ea.	N
Consumable items						
			Tabloid newspaper pages	Press collected individuals for identification		
Resources						
		R	Field guide, regional flora reference guide and/or key	Identify unknown species		N

R/S=Required/Suggested

Table 3. Equipment list: Sampling for genetic archive material at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy	Navigate to sampling locations	All	1	N
		R	Pruning shear (sharpened)	Obtain foliar tissue samples	All	1	N
		R	Tweezers	Obtain foliar tissue samples	All	1	
		R	Pole trimmer	Obtain foliar tissue samples	Tall or mixed-stature sites where foliage is out of reach	1	N
		R	Backpack	Transport field equipment	All	1	N
		S	Clipboard	Secure datasheets	All	1	N
Forestry Suppliers	61280 61260	S	Magnifier hand-lens, 10X/20X	Aid in species identification	Uncertain of species ID	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		S	Field guide, regional flora reference guide and/or key	Aid in species identification	Uncertain of species ID	1	N
Forestry Suppliers Bioquip	53674 3115	S	Plant press	Press collected individuals for identification	Uncertain of species ID	1	N
Consumable items							
		R	Field notebook	Record field notes	All	1	N
		R	AA battery	Spare battery for GPS receiver	All	2	N
		R	Nitrile gloves, powderless	Handle samples	All	1 box	
		R	Permanent marker	Label bags and envelopes	All	3	
		R	Adhesive barcode labels	Label sample containers with barcode-readable labels	Several sheets	1 per sample	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Store foliar samples	All	1 box	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
ULINE	S-14720	R	Coin envelope	Contain foliar tissue samples	All	1 box	N
		R	Color-change desiccant	Dry foliar tissue samples	All	1 bag	N
Resources							
RD [10]		R	Field Datasheets, Canopy Foliage Sampling	Back-up to record metadata	All	6	N

6.2 Training Requirements

All technicians must complete required safety training and protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[03]).

6.3 Specialized Skills

A minimum of two technicians is required for each plant diversity sampling team. It is mandatory that one technician have experience with the identification of plants – preferably in the habitats found at the site where observations 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.

6.4 Estimated Time

Plant diversity sampling: A plot should take 2-6 hours for a team of two to complete (Table 4). The time required will vary depending on a number of factors: species richness at the site, density of vegetation, taxonomic expertise, and environmental conditions. The timeframe is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress and see the section (B2) regarding searching the $10m^2$ and $100m^2$ subplots for more guidance. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Genetic archive tissue sampling: An experienced two-person team will require approximately 2-3 days to complete field sampling at one site. An additional 1-3 days may be needed to continue drying out tissues in the lab (e.g., changing desiccant).

Table 4. Estimated time required to complete field sampling and lab standard operating procedures.

SOP	Estimated total time	Suggested staff	Total person hours
A Preparing for sampling	1 hr	2	2 hrs.
B Field Sampling	2 – 6 hrs./plot	2	4 – 12 hrs./plot
C Laboratory processing and analyses	0.5 - 1 hr/plot	1 - 2	.5 - 1 hr/plot
D Collecting tissue for archive genetic material	20 – 30 mins/sample	1 - 2	20 – 60 mins/sample
E Data entry and verification	0.5 - 1 hr/plot	1	0.5 - 1 hr/plot
F Sample shipment	TBD	TBD	TBD

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

Assemble nested subplot frames if necessary.

- The cover and identity of plant species will be recorded in $1m^2$ frames.

Prepare data collection tools

- Prepare the mobile device for collecting data prior to leaving for the field. Be sure electronics are charged and applications and species lists are installed. Be prepared to use provided paper datasheets (RD[05]) if the electronic device fails (e.g. dunked in a creek, lost, or crashes).
- Plant species identified in the field will be recorded according to the NEON taxonID which uses codes from the USDA PLANTS database code. These codes will be downloaded onto the mobile device, but having a printed version of species found at the site during previous sampling years and the associated code is strongly recommended given the importance of tracking species by the correct codes. The Domain-specific plant lists and codes are also available on the internal NEON Sampling Support Library. The quality of the data depends on the correct species-code linkages.

Organize equipment and consumable items

- Plastic bags will be used to collect unknown plant species. Prior to going to the field be sure to have an ample number of loose bags. Adhesive labels or write in the rain will be needed and working permanent markers and pencils.

SOP B Field Sampling

B.1 Plot Establishment

Plant diversity sampling occurs in a square-shaped plot measuring 20m on a side and containing four $100m^2$ subplots (Figure 1). Each subplot contains nested subplots: a $1m^2$ subplot nested in a $10m^2$ subplot in each of two corners. For comparison of data across space and through time, it is important that the dimensions of these plots and subplots be consistent across plots and sites. This protocol assumes that plots will be marked by a center point and four corners. The permanent markers define the corners of the plot 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[09]). While delineating subplots, please take care to avoid trampling the plot – particularly the $1m^2$ subplots.

1. Delineate the sides of the 100m^2 subplot, the 10m^2 nested subplot (3.16 m from the nearest permanent marker at the plot corners or center), and the 1m^2 nested subplot with flags or appropriate markers.
   a. Instructions in Appendix 0 assume the plot was established with precise square and exact 20m plot sides and that the tape can be stretched between corners with no obstacles.
   b. Instructions in Appendix B.2 recognize an inevitable lack of absolute precision of the established markers and obstacles that are likely to obstruct the tape when stretched between markers.
2. The 1m^2 nested subplot is delineated with a rigid frame anchored at the corner by a permanent plot marker, a secondary marker at most sites, or marked during setup (in the case of 40.1.1 and 32.4.1).

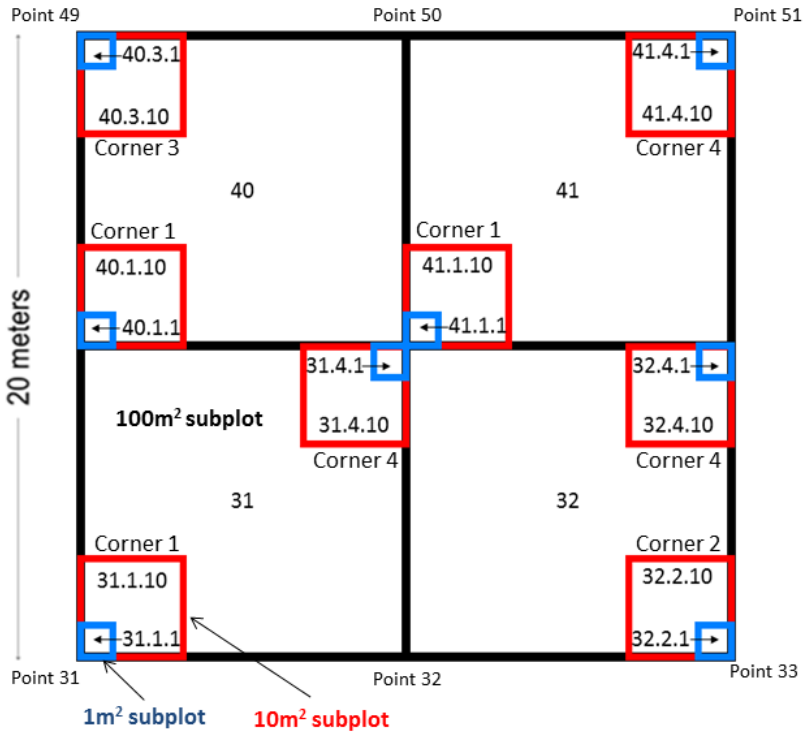

Figure 1. The square, multi-scale plot is used to record plant species composition and cover. The plot includes nested subplots at specific locations within the plot.

B.2 Observations

The plot-based collection requires observation of primarily abiotic elements – termed 'variables' – in 1m^2 nested subplots, and observations of vascular plant species at multiple spatial scales.

1. **Metadata.** Record plotID, boutNumber, the primary botanist (measuredBy), additional staff (recordedBy), and date which should reflect the day the sampling was completed (if working with paper datasheets; this information is captured automatically by the handheld).
2. **1m^2 Nested Subplot.** Record variable cover estimates, identify vascular plant species, and record cover by species and the presence of individuals greater than 300cm in height.
    a. Measure and record variables. Estimate and record the combined cover of each variable of abiotic (non-living) elements and non-vascular plant species in each 1m^2 nested subplot (Table 4). Other items such as bones, carcass, or trash should not be included in the cover estimates. Cover of any one variable shall not exceed 100 percent, but the total cover of multiple variables may be (but very rarely will be) greater than 100 percent. Observations should reflect those variables that cover the surface of the subplot (e.g. the moss growing on a rock, but not that part of the rock under the moss, or the litter on top of the soil but not the soil under the litter).

Table 5. Variables to be observed in the 1m^2 nested subplot.

Variable name	Description
Soil	Particles < 5mm diameter.
Rock	Inorganic particles ≥ 5mm diameter.
Wood	Woody organic material ≥ 5mm including living roots and material severed from the original source of growth and on the ground, including bark, fallen logs, other pieces of wood suspended in the air, and dead trees (either self-supported by roots, severed from roots, or uprooted) that are leaning > 45 degrees from vertical. Include the basal area and any woody, non-living organic material < 300cm in the cover estimate.
Litter	Unrooted organic material lying on the ground such as grass, leaves, pine needles, and twigs < 5 mm diameter.
Standing Dead	Standing dead woody material that is not severed from the original source of growth and not leaning > 45 degrees (please note 'wood' in the otherRemarks field). Also include desiccated herbaceous organic material from the previous calendar year or that cannot be identified (please note 'herbaceous' in the otherRemarks field). Species that might have been included had the sampling bout been longer or earlier in the year should be included in the plot species list.
Water	Standing or flowing water.
Lichen	Symbiotic fungus and alga.
Bryophytes/Moss	Typically small (1 – 10cm but up to 50cm), mosses, liverworts, and hornworts.
Other non-vascular	Algae, fungus, macrofungi, and biological soil crusts. Note in the comments what was observed.
Scat	Animal dung, make note of species it originated from if possible in comments.
Other	Trash, shells, bones, carcass, and other items that don't fit above.

b. Measure and record plant species data.

1) Record the presence of living vascular plant species with stems emerging from within the 1m^2 nested subplot by entering the NEON taxonID field for each species:

- Carry a site-specific species list that includes species lists from prior sampling efforts, a list of lumped genus or species, and a list of slash species (see B.3 Morphologically Challenging Species) to assist with determinations.
- Lists of species and NEON taxonID – one of previously observed species and a second regional species list - should be available on the mobile device.
- If a determination can't be made in the field see B.3 Morphologically Challenging Species, and B.4 Unknown Plant Species (morphospecies).
- If no species are found in the nested 1m^2 subplot, indicate that in the mobile device or in the taxonIDRemarks field of the first line of the datasheet.

If a species determination does not have a corresponding record in the species lists on the mobile device:

- Double check spelling and try entering both codes and scientific name.
- If the species is still not available, enter OTHE and put the scientific name and appropriate taxonID in the comments for that entry and please see the FAQ for entering plant data on the NEON intranet for more specifics on the use of OTHE.
  When back at the lab and prior to submitting the data, check synonyms in the NEON taxonomic table and the USDA PLANTS database and update the record if possible.

2) Estimate the combined cover of plant material < 300cm in height of all individuals by species in the nested 1m^2 subplot. Measure cover as the percentage of ground surface obscured by the vertical projection of all aboveground parts of each species (Figure 2).

- For all individuals and/or stems of each species < 300cm in height, include the combined cover of all living vegetation (woody, foliar, herbaceous) AND select 'N' for Plant Height Over 300cm
- For individuals or stems of each species > 300cm in height, record the combined cover of all plant material (the basal diameter, branches, foliage) < 300cm in height AND select 'Y' for Plant Height >300cm
- If there are individuals or stems of a single species both < 300cm and > 300cm in a single 1m^2 subplot, enter the combined cover of all vegetation < 300cm (as above), AND select 'Y' for Plant Height >300cm
- Only estimate cover of plants, or portions of plants, with stems that originate within or have some part of the stem inside the subplot frame. Epiphytes not actually rooted on the ground of the nested subplot, but that are rooted to trees in the space extending above the nested subplot should be included. Record cover of those individuals <300cm in height from the ground. For those individuals > 300cm, record the identity of the species and check the 'Plant Height Over 300cm'. It is understood that the identity and precise cover may be difficult to ascertain, in which case it might be necessary to identify to a higher taxonomic level.
- Estimate cover to nearest 1%.
- Enter 0.5 for estimates of cover <1%.

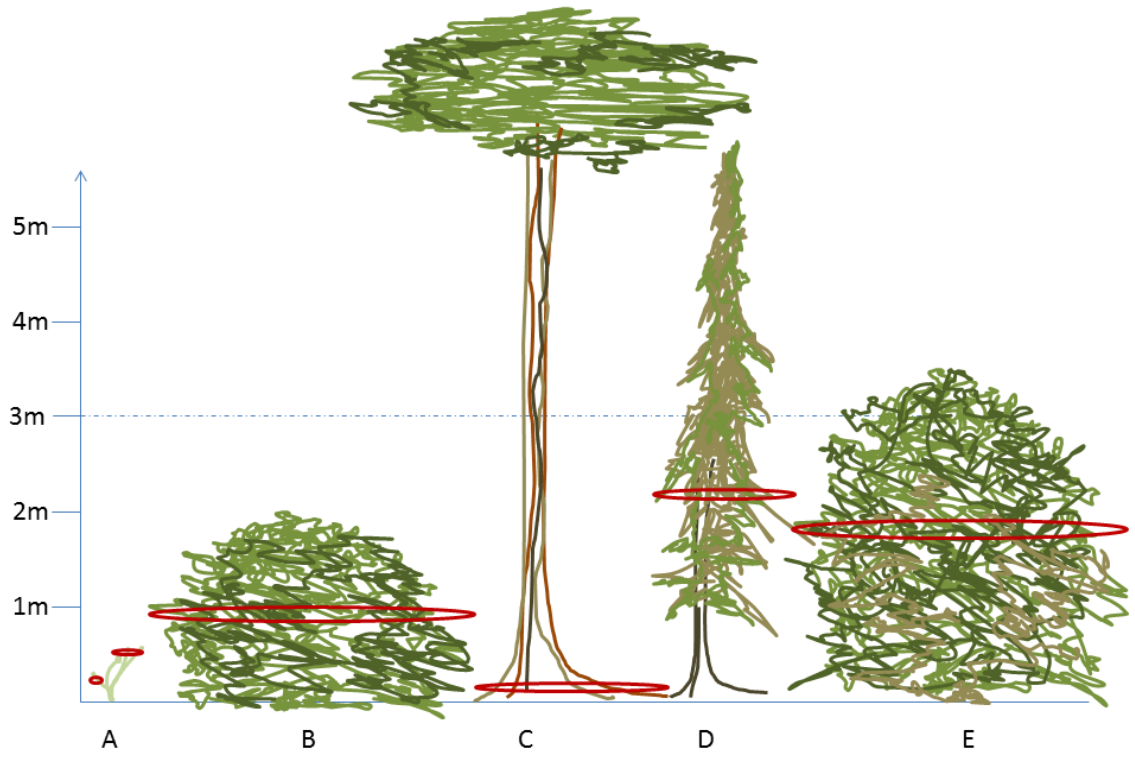

Figure 2. Estimates of cover should include all vegetative material < 300cm in height. For herbaceous growth (A), and shrubs (B) < 300cm, record the total combined cover by species; for tall trees with no woody branches or foliar growth < 300cm (C) record basal area and a height of > 300cm should be noted for that species; for trees (D) and shrubs (E) > 300cm that also have vegetative growth < 300cm, record the cover of vegetative growth < 300cm and indicate the presence of individuals > 300cm in height for that species. There will be instances when herbaceous growth <300cm (A) and trees >300cm (C) of the same species are found in the same $1m^2$ subplot, in these cases record the combined cover and indicate the presence of individuals by species > 300cm.

- There will often be spatial 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 the combined cover of multiple species may be greater than 100%.

  Cover estimates can be made more repeatable across observer, plots, and sites with calibration:

- 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.
- Each $1m^2$ nested subplot frame is calibrated in 10cm sections to make cover estimates easier (Figure 3).

- Visually group each species together into a percent cover.
- Fine tune estimate by subtracting out any spaces or gaps.
- Check that combined abiotic and select biotic variables (minus overstory) and your cover estimates for each taxonID sum to a total of at least 100%.

3. **10m^2 Nested Subplot.** Record the identity of all species with stems in each 10m^2 nested subplot as described for the 1m^2 nested subplot. It is not necessary to record all (but see below) species already documented in those 1m^2 nested subplots in each respective 10m^2 nested subplot.

   There is no specific time that should be spent looking for plant species during search efforts. The search is best thought of in terms of a species-accumulation curve. The rate at which new species are detected will decrease with time. A general guideline: if new species are being found, keep searching. If after five to ten minutes of gently moving dominant species to look for small and locally rare individuals – even crawling if necessary – no new species are found, then spend another five minutes and move on.

4. **100m^2 Subplot.** Record the identity of all plant species with stems in each 100m^2 subplot as described for the 10m^2 nested subplot. It is not necessary to record species already documented in nested subplots.

   As with searching the 10m^2 nested subplot, there is no specific time that should be spent looking for plant species during search efforts. The search is best thought of in terms of a species-accumulation curve. The rate at which new species are detected will decrease with time. A general guideline: if new species are being found, keep searching, covering the entire area in a systematic manner such as walking lines or a grid. If after ten minutes of gently moving dominant species to look for small and locally rare individuals – even crawling if necessary – while searching the entire subplot and no new species are found, then spend another ten to fifteen minutes and move on.

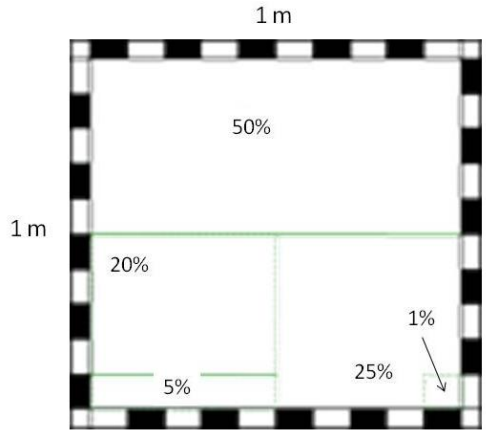

Figure 3. The 1 m² subplot is calibrated with black and white marks to make estimates of plant species cover more accurate and repeatable

B.3 Morphologically Challenging Species

- **Identification qualifiers.** In some cases there may be uncertainty regarding the identity of an individual. The lowest taxonomic rank that can be determined should be entered, and the appropriate identification qualifier code applied (Table 6). Codes should be applied to the finest level of taxonomy recorded. For example, CS ("Roughly equals but "not sure" about the species) should only be applied if both a genus and species are recorded, while CG ("Roughly equals but "not sure" about the genus) should only be entered if a species-level determination is not possible. These codes should also only be applied to the recorded observation if there is uncertainty regarding the determination. If a taxonomic definition is not possible at a particular resolution (e.g. NEVER enter cf. species code when Carex spp. – for example – is entered) or if a morphospeciesID is used for an unknown species (see below), identification qualifier codes should not be applied. Another example: If it is thought that an individual might belong to *Achnatherum* sp., enter 'cf.genus' in the identification qualifier field to indicate this uncertainty. However, if BROMU (*Bromus* sp.), is selected to indicate that the species is unknown (see section B.4 below), an identification qualifier of cf. species should NOT be entered.

- **Lumping** (also see 'Recording unknown species' below). Some species will be difficult to differentiate. Because comparability of cover estimates must be consistent through time, it is 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 lumped species – specific to each site - should be developed over the first year of sampling and, if possible, based on conversations with botanists who work at the site or in the region. These lumped species should be identified to genus, and the proper NEON taxonID followed by the spp. suffix should be recorded.

- **Cryptic species** (slash species). Cryptic species issues arise when two species that are morphologically indistinguishable, but not necessarily of the same genus, in the field co-occur (or might co-occur) at a site. NEON intends to add these species pairs to the master taxon lists to account for this. If a cryptic species pair is not currently available in the master list, the proposed species pair must be entered in the crypticSpeciesGroups spreadsheet on the NEON SSL. In the case that it is and will remain difficult to differentiate between two species of a single genus, enter the NEON taxonID genus code followed by the sp. suffix (e.g., *Triticum* sp.) in the taxonID field, and enter the code for the two species in the taxonIDRemarks field (e.g. TRSA5/TRAE2) until the proposed "slash" pair is incorporated into the master list and is available on the data entry application.

Table 6. Identification qualifier codes (idQ) to designate unknown species or those species with uncertain identification in the field or after identification in the lab.

idqCode	identificationQualifier	Description
CS	cf. species	Roughly equals but "not sure" about the species
AS	aff. species	"Similar to, but is not" the species
CG	cf. genus	Roughly equals but "not sure" about the genus
AG	aff. genus	"Similar to, but is not" the genus
CB	cf. subspecies	Roughly equals but "not sure" about the subspecies
AB	aff. subspecies	"Similar to, but is not" the subspecies
CF	cf. family	Roughly equals but "not sure" about the family
AF	aff. family	"Similar to, but is not" the family
CV	cf. variety	Roughly equals but "not sure" about the variety
AV	aff. Variety	"Similar to, but is not" the variety

B.4 Unknown Plant Species

If a species determination cannot be made in the field, the presence of unknown species should be recorded or an individual should be collected for identification in the lab or with the assistance of expert botanists.

- **Individuals that can't be identified to species.** Species that can't be identified and do not possess sufficient parts to allow identification in the lab or with external help will likely be encountered (remember NEON has a process to solicit assistance with plant identification from expert botanists). The NEON master taxon lists include codes for instances when identification below a given taxonomic rank (e.g., family, genus) cannot be made. These are indicated by a 'sp.' or 'spp.' in the scientific name. **Use the 'sp.' designation when only one unknown species is present (e.g., single individual is found, or sufficient reproductive parts are present to assume all individuals are the same species). Spp. is used when the group of individuals in question might belong to more than one species (e.g., many individuals, morphologically distinct**

features not discernable). When one of these taxa is selected, an identification qualifier is not needed, unless the lowest taxonomic rank indicated (e.g., family, genus) is uncertain.

- If there is likely only one species (can be multiple individuals) within any particular plot/nested subplot, record the lowest taxon rank with the sp. suffix (e.g. *Triticum* sp.) even if multiple unknown species or a different unknown species of the same family/genus are found in a different plot/nested subplot.
- If there are multiple species within any particular plot/nested subplot, record the lowest taxon rank with the spp. suffix (e.g. *Triticum* spp.).
- If neither the genus nor the family can be determined, enter '2Plant Unknown Plant' in the taxonID field (datasheet or electronic device).
- For example, if you select BROMU (*Bromus* sp.), an identification qualifier of cf. species is unnecessary, as the 'sp,' indicates that the species is unknown. If, however, you think that the individual might belong to *Achnatherum* sp., you would enter cf. genus into the identification qualifier field to indicate this uncertainty.

- **Collecting and recording unknown species (morphospecies).** Tracking unknown species that can later be identified is expected during the course of this work. If domain staff or an external facility are likely able to subsequently identify the tracked individual or 'morphospecies' in a lab or herbarium, the known taxonomic information should be recorded, a morphospeciesID should be created to track the species, and a specimen should be collected or photographed. This morphospeciesID can be entered repeatedly as other individuals of this species are found while observing plots within a site. When the morphospecies is identified at a later date, a join between the morphospecies table and the plot data will update the taxonomy.
  - Create a morphospeciesID in the application on the mobile device
    a. **morphopeciesID**: Enter a descriptive name that will be memorable should the morphospecies be found in other plots. These morphospeciesID can be shared across staff within a site and a year. If shared, the lead botanist must provide direction, tools, and training to ensure all staff apply consistent morphospeciesID-species naming conventions. <u>If botanists work independently and morphospeciesIDs are not shared, add the botanist initials at the end of the morphospeciesID to protect against two botanists applying the same name to morphospecies that are different species.</u>
    b. **plotID**: the named location the individual was initially collected at or near.
    c. **morphospeciesIDRemarks:** Enter a description of the individual that might be useful when keying the plant in the lab (e.g. pubescent ligules, acidic moist habitat)
    d. **measuredBy:** The name of the person who observed the plot data and named the morphospecies.
    e. **date:** the date the morphospecies was created.
  - Enter the morphospeciesID in the data

a. Record the lowest taxon rank (family or genus) in the taxonID field.
b. Enter the morphospeciesID made available from the morphospecies application.
c. Enter other notes about the individual as needed in the morphospeciesIDRemarks field.

- Collecting a specimen
  a. Given NEON's long-term monitoring efforts, unknown species should be collected from outside the 20 m x 20 m plot (but can be collected outside this area in the 40 m x 40 m plot). Finding the same unknown species can sometimes take considerable time.
  b. Collect representative parts of the entire individual, including the roots, flowers (if possible), and vegetative growth of grasses and forbs. A piece of a branch is usually sufficient for trees and shrubs. 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.
  c. Place unknown specimens in sealable plastic bags (see **Error! Reference source not found.** for optional system for organization and transport of specimens). A cooler with an ice pack may also be used (optional) to prevent wilting of specimens, and may be particularly useful on hot days and/or when there is little shade available. Label plant with the unique (to the technician) morphospeciesID, measuredBy (botanist), date, GPS coordinates, elevation, and plot number (where species was initially found, if appropriate and if possible).
  d. If collection of an individual is not possible, take photograph(s) of the individual (including flowers and other parts crucial to identification) and record photographic information in the morphospeciesIDRemarks field. Once uploaded, the photograph should be labeled with morphospeciesID, plotID, and date as follows: alternatePappusHerb_CPER_001_20130812.
  e. 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. It is imperative that the label information remain associated with the specimen. Ideally, 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.
  f. If the unknown is to be sent to an external facility for identification, follow guidelines for drying and pressing the specimen (RD[11]).

B.5 Collections and Voucher Specimens

Plant species will be collected at NEON sites to: 1) facilitate the identification of species not identified in the field (see section B.4 above), 2) create an herbarium in the domain support facility for training and quality assurance purposes (RD[11]), 3) contribute to a long-term record of plant diversity observations as part of the NEON archive program (the exact number to be collected and archived is pending), and 4) to create an archival-quality voucher for plant genetic collections (RD[06]).

The following guidelines should be considered when collecting specimens:

1) Select specimens in good condition, free of damage from insects and/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.
4) Depending on the status of the collection and if the species needs to be identified, collect two or more vouchers: one for identification and one or more for archive.
5) Record pertinent label information in the voucher application for specimens destined for the Domain Support Facility herbarium, external archive, or external identification. Record:
    - **TaxonID.** The NEON taxonID to lowest possible taxonomic rank.
    - **MorphospeciesID** (if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.
    - **Identification qualifier** (if appropriate). The standardized term to qualify the identification of the organism when doubts about taxonomic identity exist.
    - **Collector name**. Name of the person responsible for recording original occurrence.
    - **Collection number**. An identifier given to the specimen at the time it was recorded; typically a collector-specific running number (sometimes called recordNumber).
    - **Voucher Sample ID**. This unique number is comprised of the prefix ‘pla’, site, date, time, collector initials, and collector number, e.g., pla.OAES.20151014.10:30.dtb.V123. The voucher application generates these sample IDs.
    - **Location – site and/or plot**. This should include the NEON site code (e.g., OAES) or the plot number (OAES_005) if the voucher is collected at or near (50m) of a plot.
    - **Location – if not at plot**. If the voucher is not collected in (or near) a plot, record coordinates, uncertainty (if available) and elevation.
    - **Locality**. Natural language description of the place where the organism was collected, e.g., Blue Mountains, 50m west of summit of Grandfather Mountain. This information must be included in the ‘remarks’ field in the voucher application.
    - **Date collected**. In the format yyyymmdd, e.g., 20161108.
    - **Habitat description**. A category or description of the habitat in which the specimen occurred.

- **Life stage**. The age class of the individual (e.g., 'fruiting', 'seedling'). In the voucher application this must be included in the 'remarks' field.
- **Associated taxa**. NEON taxonID of taxa and associations with the collected specimen. In the voucher application this must be included in the 'remarks' field.
- **Plant description**. A description of notable specimen characteristics e.g., Very small yellow flowers turning white with age, small lanceolate leaves. Flattened round fruit.

Follow the directions in the Plant Voucher Specimen Preparation (RD[11]) document for drying and mounting (if appropriate). Pass all samples that will go to the internal reference herbarium or that are destined for external archives through the -80°C freezer to kill any pests.

B.6 Refreshing Sampling Kit

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

SOP C Laboratory Processing and Analyses

The lab component of the plant biodiversity sampling includes three parts: plant pressing and drying (RD[11]), 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 for the particular specimen.

Some species will come from the field and be identified fresh (without pressing), ideally 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. Not every unknown plant species must be vouchered and submitted to the archive.

Some species will be collected and the botanist will not have time to identify them within two days, or will not be able to identify the specimen. These specimens should be pressed for identification at a later time (RD[11]), either by the botanist (with the help of an herbarium and/or books) or by sending them to an external expert.

Other specimens will be collected specifically for archive at the Domain Support Facility or an external archive facility. Specimens collected for external facilities should be treated with extra care to preserve diagnostic parts and pressed until dry but not mounted or labeled pending further directions from the archive. Those specimens destined for storage at the NEON Domain Support Facility should be mounted and labels should be prepared.

C.1 Sample Processing Timing

Specimens should not be left in the refrigerator for more than a few 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. Special care should be taken to ensure that specimens are not vulnerable to damage from insects.

C.2 Identification of Unknown Species

Ideally, a fresh (not pressed and dried) duplicate specimen – if the species is to be included in one of the archives described above - 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, a clean bench space in the lab, and a dissecting microscope and kit. If there is any doubt, a duplicate specimen should be submitted to a taxonomic expert for identification, or the specimen can be compared to online or local herbarium collections. Attempts should first be made to identify unknowns at the Domain Support Facility and possibly with the assistance of a herbarium. If identification is not possible, specimens can be sent to experts in the regional flora.

After unknown specimens are identified, update the information in the morphospecies table. In cases where the specimen was identified within two or three days of collection and the morphospecies was not frequently recorded in the data, the identity can be updated in the data. Updating in the data is not necessary, not recommended when a morphospecies was recorded frequently, and not possible after the data have been locked. Do not delete records from the morphospecies table.

SOP D Collecting Plant Tissue for Archive Genetic Material

The goal of this SOP is to collect fresh foliar tissue that is robust and not approaching senescence from 10 individuals from each of the three species selected for Phase I of the Phenology observations at a site. Three of those tissue samples should come from individuals tagged for observation in the Phenology Plot loop (see TOS Protocols Plant Phenology (RD[07]). The remaining seven tissue samples should be collected from individuals either within Distributed Base Plots or across the site if allowed by site-specific permit. When possible take samples from tagged individuals, and from the same individuals sampled for canopy chemistry and LMA where appropriate.

D.1 Timing

Sampling of archive genetic material takes place at each site every five years, the same year as the canopy foliage sampling for chemistry and LMA. The two collection efforts may be linked for logistical reasons (e.g., if it facilitates access to tall-statured vegetation that will otherwise be difficult to sample), but from a Science perspective the genetic collection may occur any time during the field season when fresh, young leaves can be collected. To ensure access to fresh, young tissue, it may be best to schedule sampling earlier in the growing season. Tissue for archive genetic material may be collected during

phenology or plant diversity sampling bouts, or in conjunction with other protocols as scheduling permits.

D.2 Field Collection

1. Locate and confirm the identity of individuals belonging to the species selected for Phase I of the Phenology sampling.
    a. Collect material from **3 individuals of each Phase I species from the Phenology Plot loop**. Sample phenology-tagged individuals unless these individuals are small stature annual or perennial species. In this case, collect from individuals of the same species in close proximity to tagged individuals or as available on the Phenology Plot loop.
    b. Collect material from **7 individuals of each Phase I species from Distributed Base Plots or across the site**. In the case of woody species, material should be preferentially collected from individuals tagged for Vegetation Structure.
2. With tweezers and while wearing nitrile gloves, collect approximately 10 cm^2 or 1 g fresh weight (about 0.2 g dried) of leaf material per individual. The leaf material should be collected from young, fresh leaves, but they do not need to be sun-lit (**Error! Reference source not found.**).

Figure 4. Collecting young green leaves from a single individual.

3. Before the sample is saved:
    a. Hard, leathery, or succulent leaf material should be cut into small strips.
    b. The surface or epidermis of pruniose or hairy leaves should be removed by scraping with a sharp knife or razor blade.
    c. If leaves are soft and juicy (or even succulent), more tissue, approximately 20 cm^2, should be collected and double the desiccant should be added.
    d. Avoid tissue that is host to parasites (e.g., mildew) or other potential contaminants.

4. Place the tissue in a coin envelope.
5. Label the envelope with a unique **geneticSampleID**. This will include the collection abbreviation (gen), **siteID** (e.g., OAES), **collectDate** (e.g., 20171014), and **collectTime** (e.g., 10.35), separate by periods.
    - Example label: gen.OAES.20171014.10:35
6. If available, affix an adhesive barcode label to the bag or envelope, without covering the human-readable label. When using a data entry application, scan this barcode label**.**
7. Using either a data entry application (strongly preferred) or paper datasheet (not recommended), record:
    - **Location – plotID**. The NEON plot number (OAES_005) if the sample is collected at or near (within 50 m) of a plot.
    - **Location – if not at plot**. If the sample is not collected in (or near) a plot, record coordinates, uncertainty (if available), and elevation.
    - **Tag ID** (if applicable). The NEON tag on the individual if one exists.
    - **TaxonID** (and associated information, e.g., idQualifier, if appropriate)**.** The NEON taxonID to lowest possible taxonomic rank.
    - **Morphospecies** (and associated information, if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.
    - **Plant condition.** The condition of the plant from which the material is collected.
    - **Start & End Date.** The date on which the collection started and ended.
    - **Collected By**. Name of the person responsible for recording original occurrence.
    - **Identified by** (if appropriate). The staff who identified the specimen.
    - **Recorded by.** Name of staff who recorded information.
8. Place sample in resealable 1-gallon plastic bag. *Multiple plant tissue samples stored in separate, labeled coin envelopes can be stored in one plastic bag*.
9. Color-change desiccant should be placed in the plastic bag, but outside the coin envelope. The desiccant should be 20-50 times the combined weight of all tissue samples in the bag.
10. Collect an archival-quality voucher specimen from one of the individuals of each species targeted for the genetic collection. The voucher should be from the same individual that the genetic sample was collected from where possible.
11. Using either a data entry application or the paper datasheets for voucher collection, record:
    - **TaxonID.** The NEON taxonID to lowest possible taxonomic rank.
    - **MorphospeciesID** (if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.

- **Identification qualifier** (if appropriate). The standardized term to qualify the identification of the organism when doubts about taxonomic identity exist.
- **Collector name**. Name of the person responsible for recording original occurrence.
- **Collection number**. An identifier given to the specimen at the time it was recorded; typically a collector-specific running number (sometimes called recordNumber).
- **Voucher Sample ID**. This unique number is comprised of the prefix 'pla', site, date, time, collector initials, and collector number, e.g., pla.OAES.20151014.10:30.dtb.V123. The voucher application generates these sample IDs.
- **Location – site and/or plot**. This should include the NEON site code (e.g., OAES) or the plot number (OAES_005) if the voucher is collected at or near (50m) of a plot.
- **Location – if not at plot**. If the voucher is not collected in (or near) a plot, record coordinates, uncertainty (if available) and elevation.
- **Locality**. Natural language description of the place where the organism was collected, e.g., Blue Mountains, 50m west of summit of Grandfather Mountain. This information must be included in the 'remarks' field in the voucher application.
- **Date collected**. In the format yyyymmdd, e.g., 20161108.
- **Habitat description**. A category or description of the habitat in which the specimen occurred.
- **Life stage**. The age class of the individual (e.g., 'fruiting', 'seedling'). In the voucher application this must be included in the 'remarks' field.
- **Associated taxa**. NEON taxonID of taxa and associations with the collected specimen. In the voucher application this must be included in the 'remarks' field.
- **Plant description**. A description of notable specimen characteristics e.g., Very small yellow flowers turning white with age, small lanceolate leaves. Flattened round fruit.

12. Additionally:

    a. The voucher itself must be labeled such that voucher and tissue can be unambiguously linked. Record the **voucherSampleID** on a label with the specimen.

    b. If available, affix an adhesive barcode label to the voucher, without covering the human-readable label. When using a data entry application, scan this barcode label**.**

    c. If no reproductive parts are present, it is acceptable to voucher a target individual at a later date when reproductive parts are available; this may be possible with individuals from the Phenology Plot, given frequent sampling throughout the growing season. However, if collecting a voucher at a later date will not be possible, voucher when the genetic archive tissue is collected.

    d. Do not collect vouchers from *tagged* forb or grass species in the Phenology Plot, but do collect them from tagged trees and shrubs as long as tagged individual are not harmed (see Plant Phenology (RD[05]) and Vegetation Structure (RD[09]) protocols). For herbaceous

plants, vouchers should be collected from non-tagged individuals in the Phenology Plot loop or the destructive sampling area of Distributed Base plots.

13. Make sure vouchers of these species are represented in the herbarium collection at the Domain Support Facility.

D.3 Sample Handling

1. Desiccant drying capacity (e.g., color change indicator) must be checked frequently – initially every 6 to 12 hours, less frequently thereafter, to ensure rapid drying. Desiccant may need to be replaced 1-3 times (for succulent or very wet leaves) to fully desiccate the tissue. At particularly humid sites, it may be appropriate to store samples in a desiccant chamber if space is available.
2. Store samples in a cool (ambient), dry location until they can be shipped to the designated archive facility. Bags should be well-sealed to exclude external moisture.
3. Dry and press the voucher specimens for each of the three species sampled. Do not mount the vouchers as they will be shipped to an external facility for archive.

D.4 Shipping Instructions

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the "Shipping Information for External Facilities" document on CLA's NEON intranet site.

More information regarding the shipment of the desiccated plant tissue and voucher specimens will become available after institutions and facilities are identified. Currently, desiccated samples should be held in cool, dry locations at domain facilities until further instruction is provided.

SOP E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout

(where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Protocol-specific instructions and the associated data ingest workbook for entering data can be found on the NEON intranet in the FSU-FOPs folder. Prior to entering data please be sure to check the codes of each species to be sure that that appropriate NEON taxonID is attributed to the species detected. Due to the volume of plant species in the US reflected in the USDA PLANTS database and adopted for the NEON taxonomic table, the codes are often a bit more cryptic than four letters corresponding to genus and species. If the data is collected on a paper datasheet, it is preferable that the person who collected the data also enters the data or is at least familiar with the flora at the site to reduce the possibility of errors in the data entry process. If the wrong code was used on the paper datasheet, the correct NEON taxonID must be annotated on the sheet.

E.1 Sample Labels & Identifiers

By default each sample, subsample or mixture produced by this protocol is assigned a human-readable sample identifier which contains information about the location, date, and/or taxonomy of the collected sample. Each sample may also be associated with a scannable barcode, which will not contain information specific to sample provenance, but will reduce transcription errors associated with writing sample identifiers by hand.

If available, adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, it is encouraged to make these up in advance. Use the appropriate barcode label type with each container (i.e., cryo-safe barcode labels only used for samples that are stored at -80°C, etc).

Barcodes are scanned into the mobile application when the sample is placed into the container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. If multiple vials or containers are required to contain a sample from one trap, place the barcode on the outer container that will hold all vials *associated* with just that sample (i.e., if a catch cup from collection fills ten 50-mL falcon tubes, the single barcode is applied to the outer Ziploc container not each vial containing 1/10 the sample; the database cannot handle 10 barcodes mapping to the same sample).

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.

SOP F Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not label-specific or logistical demands. For that information, reference the NEON CLA Intranet site (available through the sampling support library) and the Domain Chemical and Hygiene Plan and Biosafety Manual.

Shipping details are TBD and will be included in a future revision of this protocol (as of rev F).

F.1 Handling Hazardous Material

N/A

F.2 Supplies/Containers

TBD

F.3 Timelines

TBD

F.4 Conditions

TBD

F.5 Grouping/Splitting Samples

TBD

F.6 Return of Materials or Containers

TBD

F.7 Shipping Inventory

TBD

F.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 7. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001579	Datasheets for TOS Protocol and Procedure: Plant Diversity Sampling

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

Tape measures and flags shall delineate and anchor the subplots. In relatively flat terrain with few obstacles such as trees, logs, and rocks, stretching tape around multiple sides of the plot results in precise placement of subplots (see B.1). In most cases obstacles will often result in a tape not reading exactly 40 m after stretching from point 31 past point 33 (south side of plot) to point 51 (east side of plot). In these cases (see B.2), the 1 m2 and 10 m2 subplots should be 1 m and 3.16 m in linear distance from the closest corner (typically a primary or secondary marker) – subplots should be anchored at the proximal plot corners and plot center (points 31, 33, 41, 49, 51). For example, 1 m and 3.16 m should be subtracted from whatever reading is displayed on the tape at point 33 to define the south side of subplots 32.2.1 and 32.2.10. Because there is not typically a marker at point 40 or point 42, these markers should be placed as close to 10 m north of points 31 and 33.

If permitted by the site host, and time and material allow, there is no reason from a NEON Science perspective not to increase the number of markers left at a plot. Placing more secondary markers could reduce plot establishment required for plant diversity sampling and increase repeatability of data.

B.1 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 5):

1) Begin in the south-west corner of the plot (point 31), at most sites this permanent marker will be labeled with information about the plot.

2) Anchor a 50 m tape and extend it towards the south-east corner (point 33).
    a. Walk on the south side of the tape to avoid trampling plants 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.

3) Anchor the tape at the 20 m at the south-east corner of the plot (point 33) and pull it towards the marker at the north-east corner (point 51) of the plot.
    a. Walk on the east side of the tape to avoid trampling plants 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.
4) Return to the south-west corner (point 31) of the plot.

5) Anchor the second 50 m tape and extend it towards the north-west corner (point 49).
    a. Walk on the west side of the tape to avoid trampling plants 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.

6) Anchor the tape at the 20 m at the north-west corner (point 49) of the plot and pull it towards the marker at the north-east corner (point 51) of the plot.
   a. Walk on the north side of the tape to avoid trampling plants 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.

7) Anchor a third tape at the center of the plot (point 41) and extend it south toward the flag that at 10m.
   a. Insert pin flags into the ground at 1 m and 3.16 m.

8) 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,3.16 m, 6.84 m, and 9 m.

9) 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.

10) Return to the center and extend the tape west toward the flag at 10 m. Insert pin flags into the ground at 1 m, 3.16 m, 6.84 m, and 9 m.

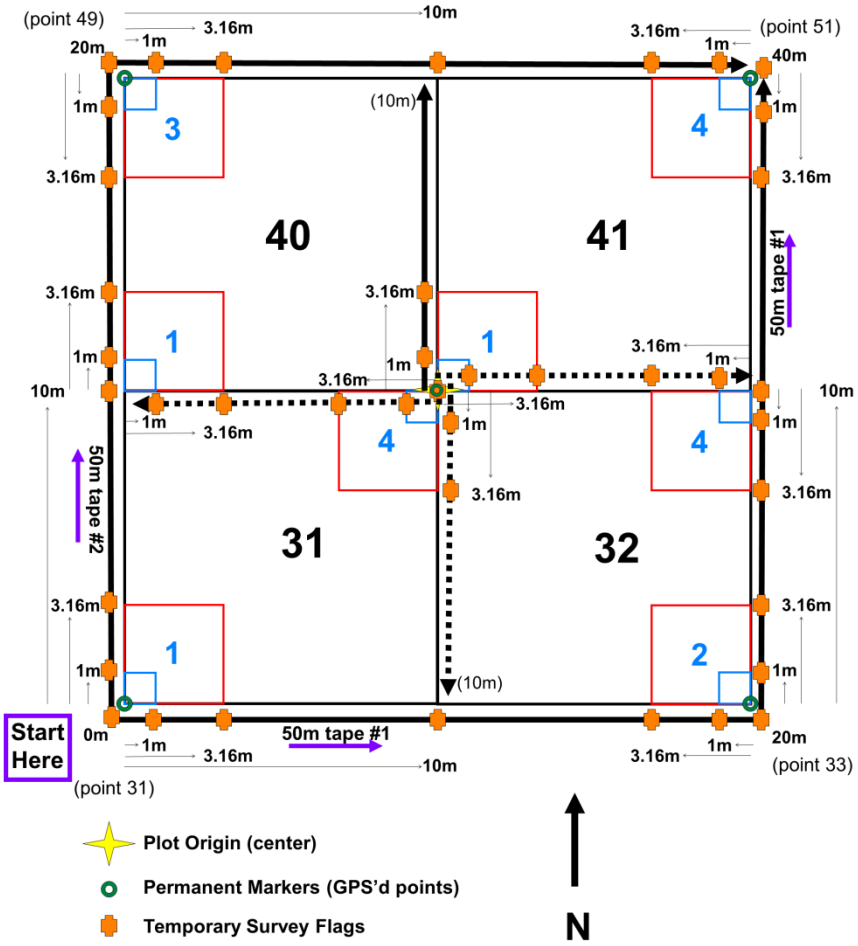

Figure 5. The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

B.2 Plot delineation with some lack of precision in plot and some obstacles (this will be most cases).

This method is very similar to the previously described, 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 0 (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 $10m^2$ subplot. The perimeter of the plot and subplots can be delineated by tape measures and subplot frames as follows (Figure 5):

1) Record date and plot number.

2) Begin in the south-west corner of the plot (point 31), 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 (point 33), 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 (point 33) and extend it to the north-east corner (point 51) at approximately 40 m on the tape.

5) Return to the south-west corner (point 31) and while pulling the tape tight towards the south-east corner (point 33), 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 (point 33) and pull the tape tight (either wrapped around the marker or/or with a second person holding) from the south-east corner back towards the south-west corner (point 31) 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 (point 33), pull it tight towards the north-east corner (point 51) of the plot and insert pin flags at 1 m, 3.16 m, and 10 m from the south-east corner 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 (point 33) and insert flags at a distance of 1 m and 3.16 m from the 10 m mark towards the south-east corner.

9) Proceed to the north-east corner (point 51) of the plot and pull the tape tight from the north-east corner back towards the south-east corner (point 33) 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 (point 31) of the plot. Anchor the second 50 m tape and extend it towards the north-west corner (point 49), 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 (point 49).

11) Wrap the tape at the north-west corner (point 49)/permanent marker and extend it to the north-east corner (point 51) at approximately 40 m on the tape.

12) Return to the south-west corner (point 31) and while pulling the tape tight towards the north-west corner (point 49), 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 (point 49) and place flags towards the north-west corner (point 49) 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 (point 49) and pull the tape tight (either wrapped around the marker or/or with a second person holding) from the north-west corner (point 49) back towards the south-west corner (point 31) and insert flags at a distance of 1 m and 3.16 m from the north-west corner (point 49) on the west edge of the plot.

15) With the tape anchored at the north-west corner (point 49), pull it tight towards the north-east corner (point 51) 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 (point 51) of the plot and pull the tape tight from the north-east corner (point 51) back towards the north-west corner (point 49) and insert flags at a distance of 1 m and 3.16 m from the north-east corner (point 51) on the north edge of the plot.

17) Proceed to the center of the plot (point 41).

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.

20) The boundary of the 10m^2 nested subplots can be defined by tape measures and pin flags. For 10m^2 nested subplots on the perimeter, a tape can be extended from a previously inserted survey or pin flag that is 3.16m from the corner where subplots are nested. To maintain a square nested subplot, this tape can target a pin flag that is 3.16m from a corner or center on the perimeter of an opposite side of the 100m^2 subplot (10 m away). Locating and aiming this targeted flag may require the help of a second person in dense vegetation. For example, the edge of the 10m^2 nested subplot in corner 1 of subplot 31 can be defined by stretching a tape from the flag at 3.16m on the south edge of the subplot toward the flag 3.16 m towards the center of the plot from the west edge. Delineating the boundary of the 10m^2 nested subplots anchored at the center of the plot requires that the target flag be added 3.16m from the flag at the middle of the a 20m edge of the plot. For example to defining the edge of the 10m^2 nested subplot in corner 1 of subplot 41 would require a flag 3.16m from the flag that is 10m between point 49 and 51 or the between point 33 and 51.

APPENDIX C CHECKLISTS

N/A

APPENDIX D REMINDERS

N/A

APPENDIX E ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are based on historic records and are estimates for the start and stop dates of sampling. It is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling, as described in Section 4 of this protocol. Dates for earliest onset of greenness and onset of minimum greenness that define the growing season can be found in the Plant Phenology protocol (RD[07]). The floristic peak – that time when the greatest number of species can possess parts required for identification – will occur inside that time period. The dates listed below reflect approximate start and end dates based on these greenness data. Please adjust this timing based on experience, expert opinion, and data from the Phenology sampling. If a site might require a second bout, please contact NEON TOS Science.

Table 8. Estimated dates of historical temperature thresholds

Domain	Site	# of Bouts	Approx. Start Date 1	Approx. End Date 1	Approx. Start Date 2	Approx. End Date 2
01	all	1	June	August		
02	all	1	April/May	August		
03	all	1	July	November		
04	all	1	May	November		
05	all	1	June	August		
06	all	1	April/May	September		
07	all	1	May	September		
08	all	1	April	September		
09	all	1	May/June	August		
10	CPER	2	May	June	August	September
	RMNP	1	June	August		
	STER	1	May	August		
11	CLBJ	TBD	April	June		
	OAES	2	April	June	July	September

12	YELL	1	June	August		
13	NIWO	1	June	August		
	MOAB	1	March	July		
14	all	2	Feb	April	July	October
15	ONAQ	1	March	July		
16	all	1	May	August		
17	SJER	1	February	April		
	SOAP	1	May	August		
	TEAK	1	June	August		
18	all	1	June	August		
19	all	1	June	August		
20	PUUM	1	January	March		

APPENDIX F SITE-SPECIFIC INFORMATION

N/A