# TOS PROTOCOL AND PROCEDURE: DIV – Plant Diversity Sampling

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See configuration management system for approval history.

## 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.<br>-Changed directions for recording cover to include all vegetative cover under 300cm regardless of height of individual.<br>-Added the Tower Base Plots to the description of which plot types to sample.<br>-Updated sample timing and bout number information (Appendix E).<br>-Changed definition of ‘standing dead’ to include woody species<br>-More clearly defined ‘wood’ |
| G | 11/07/2016 | EECO-04366 | -Clarified handling of unknown and morphologically difficult species<br>-Removed directive to collect ‘overstory’ and to take pictures<br>-Added further directions for collection of metadata with voucher specimens |
| H | 12/22/2017 | ECO-05490 | -Added table to reflect expected time requirements<br>-Updated morphospeciesID directions such that morphs can be shared across botanists or made unique if not shared within a site<br>-Updated collection metadata requirement for voucher specimens<br>-Integrated the SOP Collecting Plant Tissue for Archive Genetic Material into protocol |
| J | 01/07/2019 | ECO-05874 | -Added information about barcodes in the Method section and specific instruction to use where appropriate |

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|---|---|---|---|
| | | | -Moved the 'Lumping' section to the unknown plant part of the document and shifted directive to allow determinations if possible as long as lumping list is maintained.<br>-Enter "No new taxa" when using the datasheet in the 10 and 100m$^2$ subplot sections when there are not species found in the nested subplots.<br>-Edits for clarity and simplification<br>-Added checklist |
| K | 02/13/2020 | ECO-06270 | -Updated to new template (NEON.DOC.050006vJ)<br>-Added guidelines for instances where sampling could not be completed<br>-Added guidance for data quality flags<br>-Enabled independent tracking of standing dead – herbaceous and standing dead - woody<br>-Added directions for processing and shipping of samples |

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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 is 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 are 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 (AD[07]).

A subset of observed plant species is collected and archived. Vouchers housed at NEON facilities – 'reference herbarium vouchers' - are for reference and to facilitate training and data quality. Vouchers archived at an external facility – 'archive vouchers' - provide a collection of species represented in the data to support research.

NEON collects and curates foliar material for analysis of plant genetic diversity over space and time. This 'genetic foliar tissue' is not to be confused with the foliar collections described in the Canopy Foliar Chemistry protocol (RD[07]). These genetic foliar 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 genetic foliar tissue samples 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 | 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 |
| AD[06] | NEON.DOC.004104 | NEON Science Data Quality Plan |
| AD[07] | NEON.DOC.000912 | NEON Science Design for Plant Diversity |

## 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: Manual Data Transcription |
| RD[05] | NEON.DOC.001579 | Datasheets for TOS Protocol and Procedure: Plant Diversity Sampling |
| RD[06] | NEON.DOC.003282 | NEON Protocol and Procedure: Site Management and Disturbance Data Collection |
| RD[07] | NEON.DOC.001024 | TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass Per Area Measurements |
| RD[08] | NEON.DOC.014040 | TOS Protocol and Procedure: Plant Phenology |
| RD[09] | NEON.DOC.000987 | TOS Protocol and Procedure: Measurement of Vegetation Structure |
| RD[10] | NEON.DOC.001025 | TOS Protocol and Procedure: Plot Establishment |
| RD[11] | NEON.DOC.001237 | NEON Theoretical Basis Document: TOS Plant Diversity – QA/QC of Raw Field And Lab Data |
| RD[12] | NEON.DOC.003564 | NEON Standard Operating Procedure: Plant Pressing, Mounting, and Labeling (Herbarium Techniques) |
| RD[13] | NEON.DOC.002984 | Standard Operating Procedure: Minimizing Site Disturbance During Aquatic and Terrestrial observation System Sampling |
| RD[14] | NEON.DOC.001716 | TOS Standard Operating Procedure: Toxicodendron Biomass and Handling |
| RD[15] | NEON.DOC.005224 | Shipping Ecological Samples, Sensors and Equipment |

## 2.3 Acronyms

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

## 2.4 Definitions

Service Now – The incident tracking software deployed by NEON to track and resolve field sampling issues.

Fulcrum – The software platform that provides the foundation for the collection of data in mobile computers.

Sampling Support Library – An NEON-specific intranet space to support protocols.

# 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 (Figure 1). 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 incidence are 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. Specimens are collected and stored in reference herbaria at Domain Support Facilities and for the ecological community at an external archive facility. Vouchers to be housed at the Domain Support Facility should be dried, pressed, mounted, and labeled. Vouchers to be sent to an archive facility are collected according to a specific design and resulting lists that specify specific species annually. These vouchers must meet herbaria standards, and should be dried, pressed, and shipped. A subset of vouchers for the external archive – that are included in the targeted voucher collection lists - provide a reference specimen for the genetic foliar tissue collections.

Archival genetic foliar tissue is collected from the three ‘Phase I’ species selected for phenology observation at each site. This means archive tissue is collected from the dominant species in the vicinity of the tower (see TOS Protocol and Procedure: Plant Phenology (RD[08]). Archived material, consisting of 30 samples per bout (10 replicates each from the three Phase I Phenology species) is sampled from both the primary Phenology Plot and a subset of Distributed Base Plots. Samples are dried with desiccant, stored at room temperature, and sent to a contracted archive facility. A voucher must be created from one individual of each species from which genetic foliar tissue is collected.

There are three situations that require the collection of plant specimens:

1. Morphospecies. These species could not be efficiently identified in the field and were collected for identification. If possible, and to increase efficiency of field sampling and data quality, identify the species soon after the collection. If NEON staff are not able to identify the specimen or require confirmation of a determination, specimens can be taken to an external local botanist or shipped to other external experts for determination.
2. Reference Herbarium Vouchers. These specimens should be pressed, dried, identified, mounted, labeled, and accessioned to the Domain Support Facility reference herbarium. The last three steps can be done after the field season. A quality specimen might require the collection of two individuals should identification require destruction of the sample (e.g., flower and/or ovary dissection). Use the voucher collection application and barcodes to track these specimens.
3. Archive Vouchers. These specimens are collected from a specific list that reflects the collection design and includes those vouchers of species that must be collected in support of the genetic foliar tissue collection. These vouchers should be pressed, dried, labeled, and shipped to the external archive following the field season. Use the voucher collection application and barcodes to track these specimens.

Options above are not mutually exclusive; a specimen collected for identification could be included in the reference herbarium. Similarly, some specimens may require external determination prior to accession at the archive facility (RD [12]).

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 Service Now to report and 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 through Service Now.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

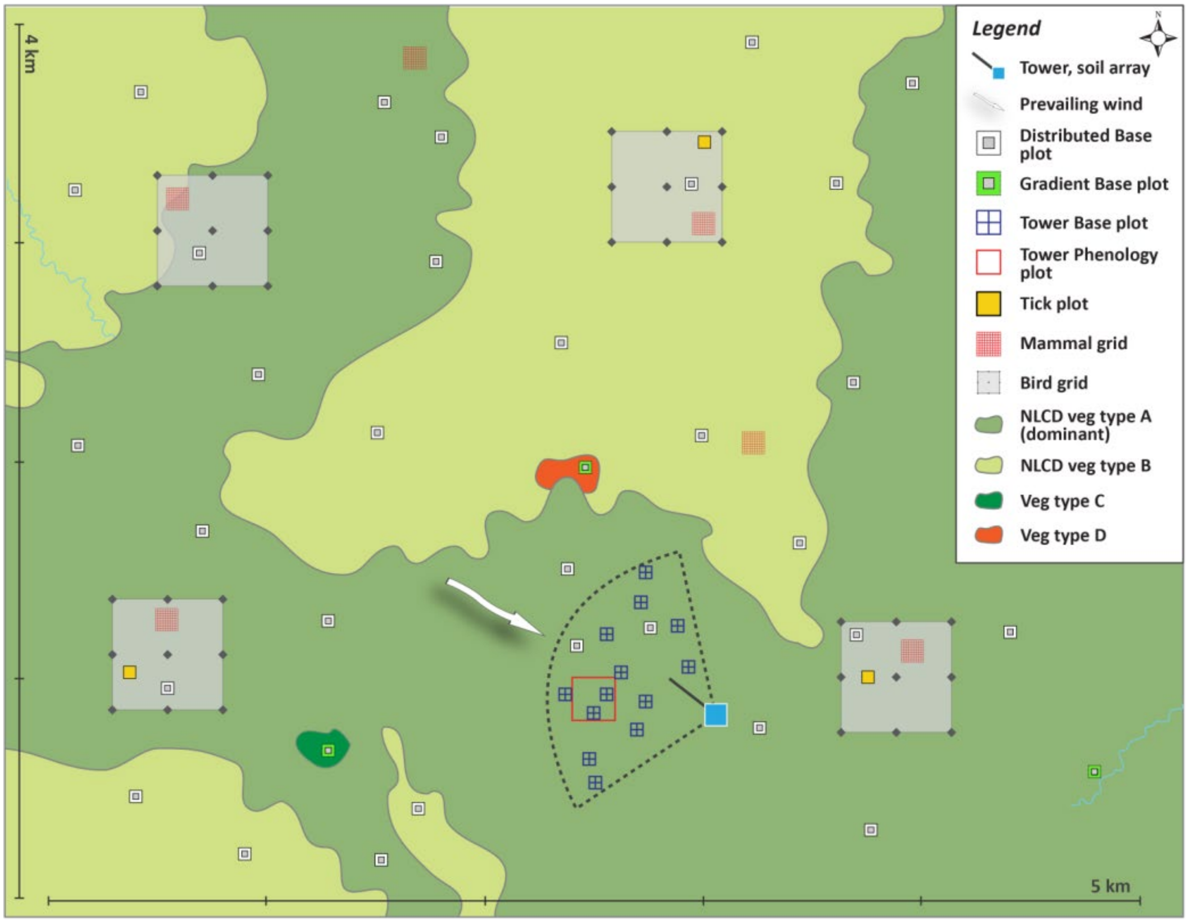

**Figure 1**. A schematic of sampling locations at a NEON site demonstrating the location of Distributed Base Plots and Tower Base Plots at which plant diversity protocol is implemented.

# 4 SAMPLING SCHEDULE

## 4.1 Sampling Frequency and Timing

Plant Diversity Sampling procedures are implemented according to criteria for sampling and resulting annual schedules. Plot-based sampling of plant diversity is completed one time per year at each plot at most sites and twice at select sites (Table 1, Appendix D). At each site, sample all the 1m$^2$ subplots annually. Every other year sample all subplots (the 10 and 100m$^2$ subplots that make the entire plot) at each site.

Plant vouchers for the external archive are collected annually (Table 1). Vouchers for the reference herbarium at the domain support facility should be collected opportunistically.

The collection of genetic foliar tissue for the archive is scheduled on an inter-annual basis at a given site (Table 1). Collections are made when newly emerged, young foliar tissue is available (but see Scheduling Considerations below).

**Table 1**. Sampling frequency for TOS Protocol and Procedure: Plant Diversity Sampling procedures on a per SOP per plot type basis.

| SOP | Plot Type | Plot Number | Bout Duration | Bouts Per Year | Yearly Interval | Remarks |
|---|---|---|---|---|---|---|
| B, Plant Diversity | Tower | 3 | < 8 weeks | 1-2 bouts per sampling year | 1 y | Alternate between 1m$^2$ subplot and full plot sampling each year |
| | Distributed | All | < 8 weeks | 1-2 bouts per sampling year | 1 y | Alternate between 1m$^2$ subplot and full plot sampling each year |
| D, Voucher Specimens | NA | NA | ~10 weeks | 1 bout per sampling year | 1 y | Reference herbarium and archive vouchers |
| E, Genetic Foliar Tissue | Tower | 1 | ~8 weeks | 1 bout per sampling year | 5 y | Collect 3 samples/species on or near phenology plot |
| | NA | NA | ~8 weeks | 1 bout per sampling year | 5 y | Collect 7 samples/species at Distributed Base Plots or across the site |

***Scheduling Considerations***

1. Press all types of voucher specimens as soon as possible to preserve delicate plant parts.
    a. If possible press voucher specimens in the field.
    b. Placing specimens in a cooler in the field might be an appropriate alternative to prevent damage and wilting in hot environments.
    c. Specimens collected in plastic bags should be pressed upon return to the lab or refrigerator-preserved for no longer than two – five days prior to identification and/or pressing.
    d. Specimens collected for identification in the lab but not intended for either the reference herbarium or the external archive only need to be refrigerated and/or pressed if immediate determination is not possible.
2. A voucher must be made from one individual for each species from which genetic foliar tissue for the archive is collected. If it is not possible to simultaneously collect young tissue and voucher an individual with diagnostic plant parts, either collect young tissue and tag the

individual for voucher collection at a later date, or delay the collection of genetic foliar tissue for one of the 10 samples until requisite plant parts for the voucher are present.

3. If possible, combine the genetic foliar tissue collection effort with other field sampling efforts (e.g., plant phenology, herbaceous biomass, or plant diversity).

## 4.2 Criteria for Determining Onset and Cessation of Sampling

### 4.2.1 Plant diversity

Sample bouts are timed to maximize the number of plant species that can be detected at a NEON site. Observations are generally made during phenological peaks in diagnostic plant parts (primarily flower but also fruiting). The scheduled sampling start and end are generally informed by peaks in greenness according to 15-year MODIS averages and further refined by the NEON phenology observations (Appendix D, RD[08]). Additional details:

- Complete sampling bouts in approximately a 1-2 month period around peak flowering (Appendix D). Significant delays may change the detectability of species and influence the comparability of sampling bouts.
- Complete sampling prior to desiccation of species such that identifications and collection of comparable cover estimates are not possible.
- At agricultural sites the timing of sampling may require adjustment to coincide with the presence of primary crops. Sampling can be completed outside the two month sampling window at sites with plots in both wildland and agricultural cover types to capture the primary crop as well as the natural species composition.

### 4.2.2 Voucher specimens

The collection of all vouchers should generally correspond to the plot-based plant diversity sampling such that specimens display diagnostic plant parts. Additional notes on timing of voucher collections:

- Vouchers of most species should include flowers or fruit and roots, but these parts are not mandatory for all species (e.g., large coniferous species that can be determined by leaf structure).
- The reference herbarium vouchers can include specimens lacking reproductive parts if the species does not exhibit these parts during the scheduled sampling. Species collected for identification in the lab can be included in the herbarium, as well as species with a diversity of morphologies (e.g., burned *Quercus* sp. in Domain 3) and those that don't flower or fruit during the sampling window are useful for reference and training.

### 4.2.3 Genetic Foliar tissue

Sampling may begin and end any time during the growing season; target timing such that foliar tissues that are young and not approaching senescence are available for collection. One of the ten tissue collections per species must include a voucher of the same individual. If it is not possible to get young tissue when the diagnostic plant parts required for a herbarium-quality voucher are available, make one collection of older tissue when a quality voucher can be collected. The other nine tissue collections for that species should be made when young foliar tissue is available.

## 4.3 Timing for Laboratory Processing and Analysis

### 4.3.1 Plant diversity and voucher specimens

Specimens collected for identification, the reference herbarium, or the external archive that were not pressed in the field need to be preserved. They should be pressed the same day as collected or, if this is not possible, placed in a refrigerator for no more than two – five days prior to identification or pressing. Once placed in a plant press, specimens can be stored in a well-ventilated location and identified or prepared for shipping at a later date. Any specimen destined for the archive or for identification with an external botanist should be placed in the -80°C freezer for two weeks for decontamination after it is completely dried (RD [12]).

### 4.3.2 Genetic Foliar tissue

Drying the collected genetic foliar tissue is critical to a quality sample. Drying tissue with desiccant should take one to five days depending on local climate and vegetation type. Desiccant must be checked and changed as necessary for the duration of drying. Once dried, tissue can be stored until samples must be prepared for archive and shipping.

## 4.4 Sampling Timing Contingencies

Deviations from the criteria for sample timing and the approved schedule must result in specific action:

- Changes in schedule – sampling earlier or later than scheduled – must be approved.
- If vouchers or genetic foliar tissue are not collected according to the protocol, record the sample status in the data (see SOPs D and E).
- If plot-based plant diversity sampling can't be completed once a plot has been started, sampling must resume at the plot within the schedule of the same sampling bout for sampling to be considered complete.

## 4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather, or
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Fulcrum Site Management Application; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

**Missed or Incomplete Sampling Terms**

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (Appendix D).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (Figure 2).

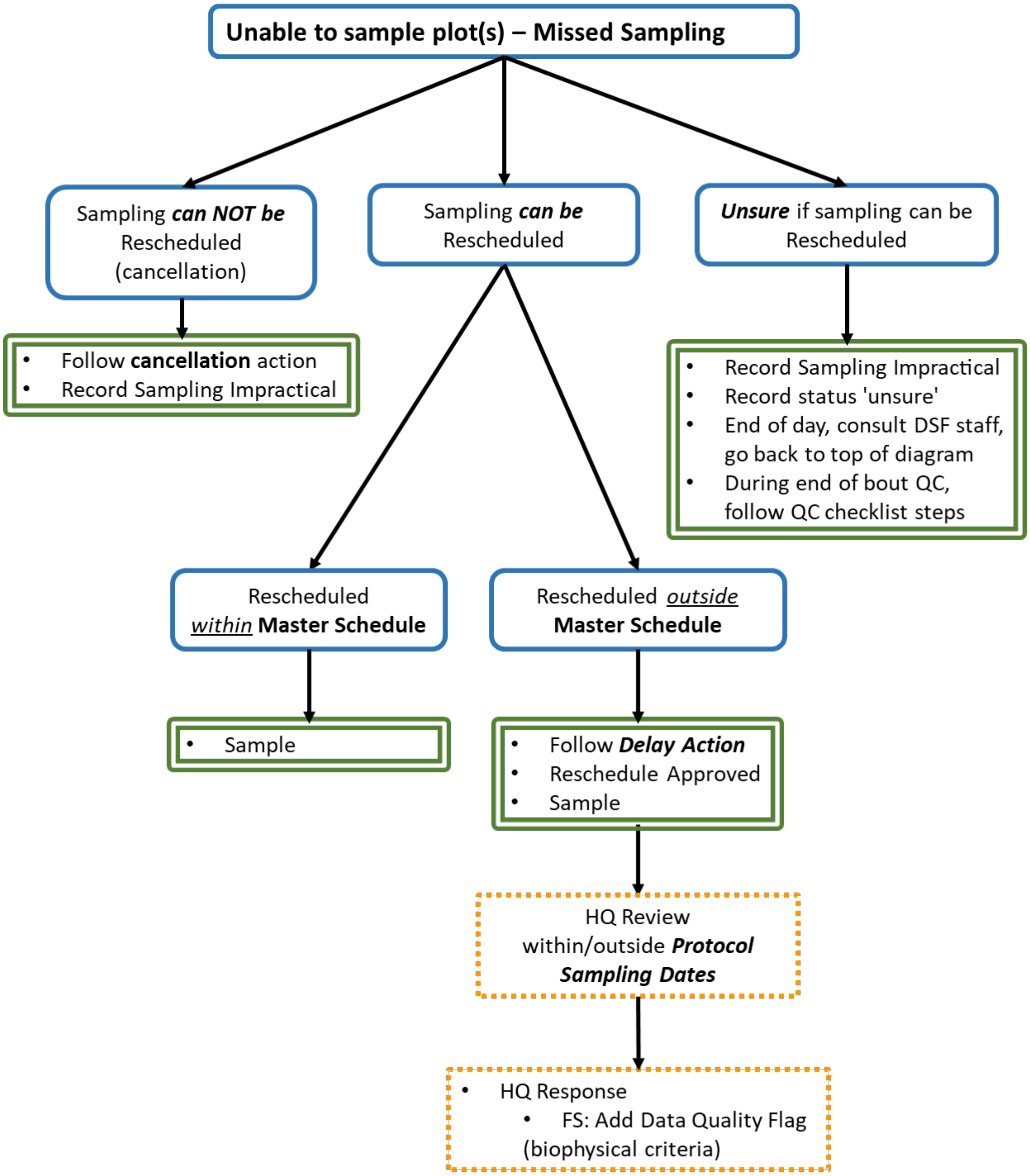

**Figure 2**. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Blue rounded boxes represent contingencies, green double line boxes describe the required actions, and orange dotted boxes indicate HQ actions. Required delay and cancellation actions are outlined for each protocol in the 'Scheduled Field Activities – Delays and Cancellations' spreadsheet available on the SSL. Missed Sampling events may also require a Data Quality flag and/or creation of a Site Management record.

**To Report Missed or Incomplete Sampling:**

1. Missed or Incomplete Sampling must be communicated to Science by a Service Now Incident.

   a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (Figure 2).
   b. The lead Field Ecologist should consult the Delayed or Cancelled Activities table to best determine when reporting is required.
2. Create a Fulcrum record for each Missed Sampling event in the field. That is, if data are recorded in the field at the plot level, a record must be made for each plot missed.
   a. For plant diversity, record each plot not sampled in each bout in the Plant Diversity Application; it could be all plots, a subset of plots, or a subplot within a plot. For example, if the plot ONAQ_004 could not be sampled a record would need to be created for subplots. If part of ONAQ_004 was not sampled, a sampling impractical record would be recorded for those subplots not sampled.
   b. For both the voucher and genetic foliar tissue collections, create a record for each sample that could not be collected such that the total number of records matches the number of prescribed samples in the respective Fulcrum applications.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (Table 2).
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Data Quality Flag, **Biophysical Criteria**, must also be recorded (Table 3).

**Table 2**. Sampling Impractical reasons entered in the application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

| Sampling Impractical reason | Description |
|---|---|
| Other | Sampling location inaccessible due to other ecological reason described in the remarks |
| Location flooded | Standing or flowing water too deep to complete sampling |
| Logistical | Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field) |
| Management | Management activities such as controlled burn, pesticide applications, etc. |
| Extreme weather | Events (e.g., thunderstorms, hurricanes) that compromise safety and access |

## 4.6 Biophysical Criteria

The Protocol Sampling Dates and the resulting Scheduled Sampling Dates are based on historical data. While schedules can and should be refined to optimize the criteria for sample timing (i.e., when many species contain reproductive parts, when young foliar tissue is available), it may not always be possible to optimize sample timing. Staff may not be available if species flower early on a hot and dry year, for example, or delays in suboptimal sampling of desiccated herbaceous and grass species. Conversely, as described above, sampling outside the Protocol and Scheduled Sampling Dates might actually satisfy the biophysical criteria for sampling. The Biophysical Criteria field is intended to communicate such instances to users of the data. If sampling takes place according to schedule and sampling criteria, no value is entered. If criteria are not met, enter one of the pre-defined sampling values (Table 3).

Please use these data quality flags only when truly appropriate any remark might cause users of the data to remove the data from consideration when working with the data.

**Table 3.** Protocol-specific Biophysical Criteria indicators entered in the Plant Diversity, Terrestrial Voucher, and Genetic Foliar Tissue Fulcrum applications.

| Biophysical Criteria | Description |
|---|---|
| Other | Sampling biophysical criteria due to other ecological reason described in the remarks |
| OK<br>– Measurements outside intended sampling window but biophysical criteria met | Sampling not within the Protocol or Scheduling Sampling Dates but the criteria for sampling was appropriate |
| Most plants not yet flowering | Sampling occurred prior to the target when most of the plant species at the site are flowering |
| Most plants senesced | Sampling occurred after the target when most of the plant species at the site are flowering |

## 4.7 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, site-specific species diversity, environmental conditions, and distance between sample plots. The timeframe provided below 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. 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.

### 4.7.1 Plant diversity

The time required will vary depending on a number of factors: species richness at the site, density of vegetation, taxonomic expertise, and environmental conditions (Table 4). The timeframe is an estimate based on completion of the 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.

### 4.7.2 Voucher specimens

Vouchers should be collected while in the field making the plot-based plant diversity observations or collecting genetic foliar tissue. For efficiency, specimens should be collected during sampling, near the plot, or while walking to plots. Collection in the field should take 10-15 minutes including data collection. Times associated with lab processing are documented elsewhere (RD[12]).

### 4.7.3 Genetic foliar tissue

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

**Table 4**. Estimated staff and labor hours required for implementation of SOPs.

| SOP | Estimated total time | Suggested staff | Total person hours |
|---|---|---|---|
| A, Preparing for sampling | 1 hr | 2 | 2 hrs. |
| B, Plant Diversity - Field | 2 – 6 hrs./plot | 2 | 4 – 12 hrs./plot |
| B, Plant Diversity - Lab | 1 - 2 | 1 - 2 | 2 - 4 |
| C.4, Lab: Identification of Unknown Plant Species | 0.5 - 1 hr/plot | 1 - 2 | .5 - 1 hr/plot |
| D.1, Field: Voucher Specimens | 20 – 30 mins/sample | 1 - 2 | 20 – 60 mins/sample |
| D.2, Lab: Voucher Handling | 20 mins – 2 hrs/sample | 1 | |
| E.1, Field: Genetic Foliar Tissue | 20 – 30 mins/sample | 1 - 2 | 20 – 60 mins/sample |
| E.2, Lab: Genetic Foliar Tissue | 30 mins/ssample | 1 | 30 mins/sample |
| G, Data entry and verification | 0.5 - 1 hr/plot | 1 | 0.5 - 1 hr/plot |

## 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 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. Field staff should wear protective equipment for toxicodendron appropriate (AD[02], RD[14]).

# 6 PERSONNEL

## 6.1 Training Requirements

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

## 6.2 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 are 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 quickly identify most of the species in the field.

# 7 STANDARD OPERATING PROCEDURES

## SOP Overview

The protocol is comprised of multiple standard operating procedures.

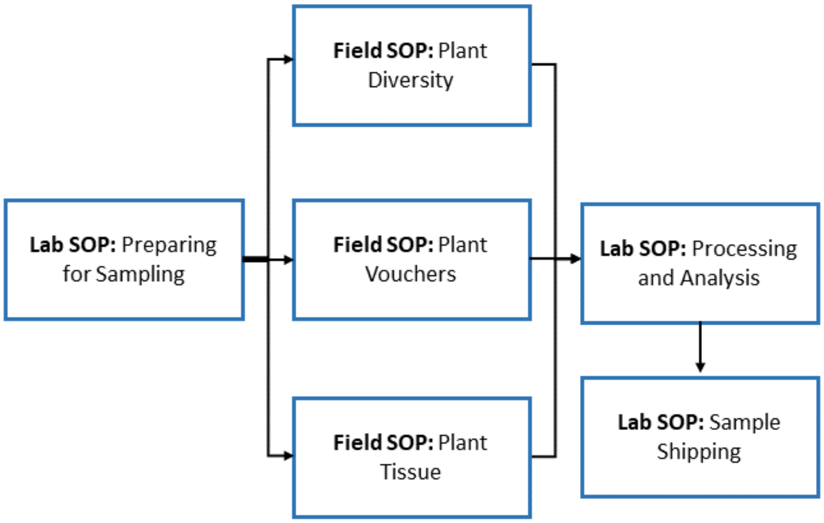

**Figure 3**. Overview of the SOPs in the plant diversity protocol.

## SOP A Preparing for Sampling

### A.1 Preparing for Data Capture

Collecting data with Fulcrum applications is the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

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.

### A.2 Labels and Identifiers

The SOPs in this protocol rely on Type II and (optionally) Type I barcodes. Type II or Type I barcodes – 'field barcodes' - are applied to plant vouchers and genetic foliar tissue collected in the field. Type II barcodes – 'archive barcodes' must be applied to vouchers and genetic foliar tissue cryo vials which will be transferred to the NEON bioarchive (Figure 3).

Type I (prefix A, plus 11 numbers) are for all field samples and any non-cryo applications; they have a tolerance from 4C to 105C and still scan. Type II (prefix B, plus 11 numbers) are the large size cryo safe barcodes usable on most cryo samples (rated for liquid nitrogen).

For field sampling prepare sample containers by affixing one Type II or Type I adhesive barcode label to bag (voucher) or coin envelope (genetic foliar tissue sample) used to contain each sample collected in the field. 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).

Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

**Figure 4**. An example of a Type I and Type II barcodes. The large-size, field-tolerant Type I barcodes have a prefix of 'A' followed by 11 numbers. The cryo safe Type II barcodes have a prefix of 'B' followed by 11 numbers.

The Type II, barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode lengthwise along a vial, *not* horizontally wrapping around a vial (see SOP E).

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. A reference to the types of samples that require barcodes is provided (Table 5). The rule of thumb is that the primary field sample (identified as 'Plant voucher; field sample' and 'Genetic foliar tissue; field sample' in Table 5) should get a primary barcode due to its importance in generating future samples. Likewise, vouchers and genetic foliar tissue in the final sample stage (identified as 'Plant voucher; herbarium specimen' and 'Genetic foliar tissue, archive sample' in Table 5) must have a Type II barcode affixed to assist in management and tracking the shipping and receipt of samples destined for the archive facility, and vouchers in reference herbaria.

**Table 5**. Sample types and barcodes used.

| **Sample Type** | **Description** | **Example Identifier** | **Fulcrum App** | **Container Type** | **Barcode Used** | **Barcode Required?** | **Barcode Qty** |
|---|---|---|---|---|---|---|---|
| Plant voucher; field samples | Field-collect plant specimens for the reference herbarium or external archive | pla.OAES.20151014.10:30.dtb.V123 *(pla.site.date.time.initials.voucherNumber)* | DIV: Voucher Collection | Plastic bag or plant press | Type I | Strongly recommended | 1 per plant specimen; 20/site/year |
| Plant vouchers; herbarium specimen | Plant voucher herbarium or archive samples | pla.OAES.20151014.10:30.dtb.V123 *(pla.site.date.time.initials.voucherNumber)* | DIV: Voucher Collection | Mounted or dried and pressed | Type I | **Always Required** | 1 per plant specimen; 20/site/year |
| Genetic foliar tissue; field sample | Foliar tissue in coin envelope | gen.OAES.20171014.10:35 *(gen.siteID.date.time)* | DIV: Gen Archive | Coin envelope | Type I | Strongly recommended | 1 per Sample; 30/site/year |
| Genetic foliar tissue; archive sample | Foliar tissue in cryo vial | gen.OAES.20171014.10:35 *(gen.siteID.date.time)* | DIV: Gen Archive | 10 mL cryo vial | Type II | **Always Required** | 1 per Sample; 30/site/year |

## A.3 Preparing for the Field

A successful field campaign requires preparation and organization of equipment and logistical planning.

1. The cover and identity of plant species will be recorded in 1m$^2$ frames. Assemble nested subplot frames if necessary.
2. Prepare data collection tools:
    a. 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).
    b. Plant species identified in the field are recorded according to the NEON taxonID which uses codes from the USDA PLANTS database code. These codes must be downloaded onto the mobile device, but having a printed version of species found at the site during previous sampling years and the associated plant code is strongly recommended given the importance of tracking species by the correct codes. The Domain-specific plant lists and codes are available on the internal NEON Sampling Support Library. The quality of the data depends on the correct species-code linkages.
3. Organize equipment and consumable items. Plastic bags are used to collect unknown plant species. Prior to going to the field be sure to have an ample number of loose bags. Adhesive labels and/or Rite in the Rain paper is needed along with working permanent markers and pencils.
4. Review site maps and plans for driving and hiking to plots. Care should be taken to adhere to efficient travel that also minimizes impacts to sites and plots.

## SOP B Plant Diversity

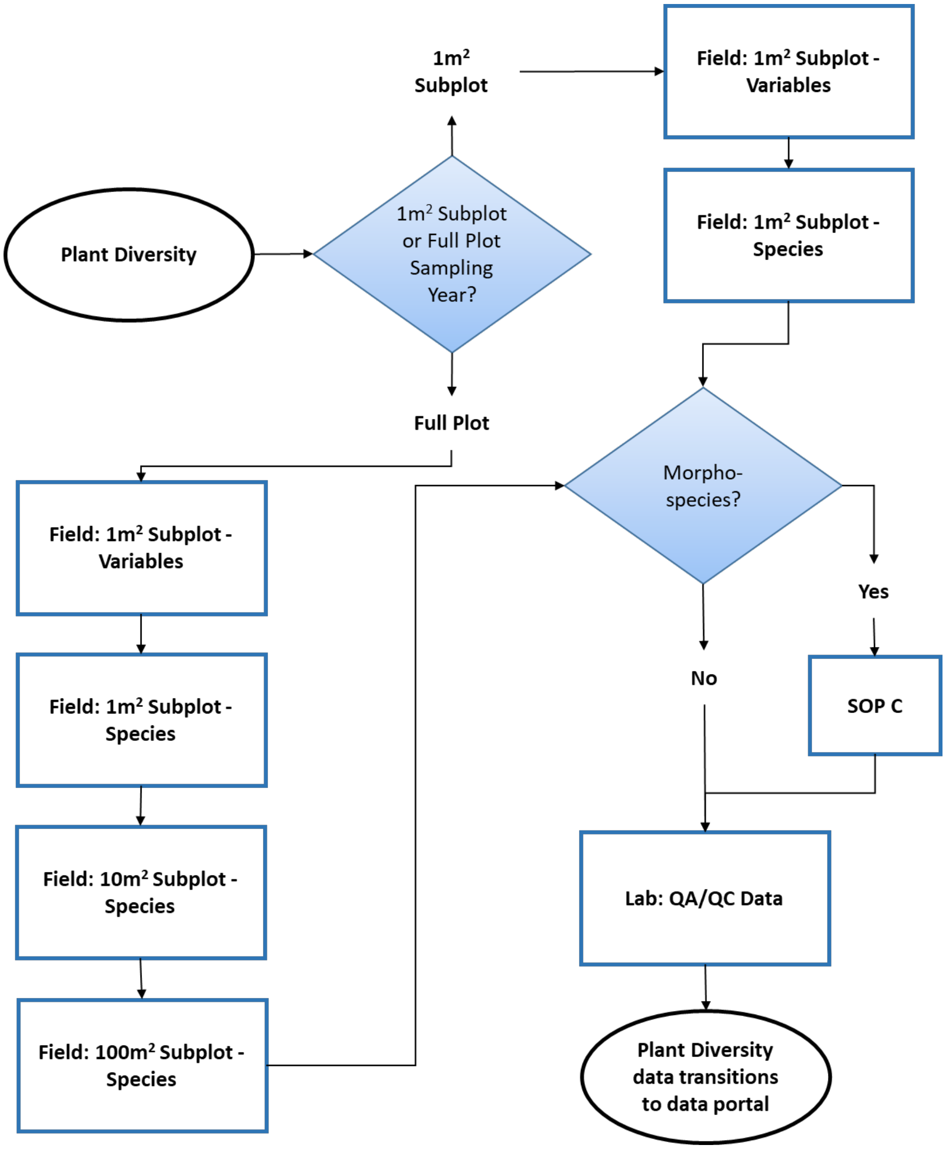

**Figure 5**. Schematic of plant diversity sampling.

### B.1 Spatially Linked Protocols

Other protocols are completed at the plots at which plant diversity sampling occurs and, in many cases, at plots in close proximity. Be aware of other sampling activities and make every effort to mitigate impacts by staying out of plots unless necessary.

### B.2 Field: Plot Establishment

Plant diversity sampling occurs in a square-shaped plot measuring 20m on a side and containing four 100m$^2$ subplots (Figure 5). Two of the 100m$^2$ subplots (32 and 40) contain a 1m$^2$ subplot nested within a

10m$^2$ subplot in each of two corners. The remaining two 100m$^2$ subplots (31 and 41) contain a single 1m$^2$ subplot nested within a 10m$^2$ subplot (Figure 5). 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 are 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[10]). While delineating subplots, please take care to avoid trampling the plot – particularly the 1m$^2$ subplots.

1. On years when sampling just the 1m$^2$ subplot, the subplots can be placed according to the corner markers. If markers are not present, see Appendix F for plot establishment directions.
2. On years when all subplots are to be sampled, 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 F 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 F.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.
3. 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).

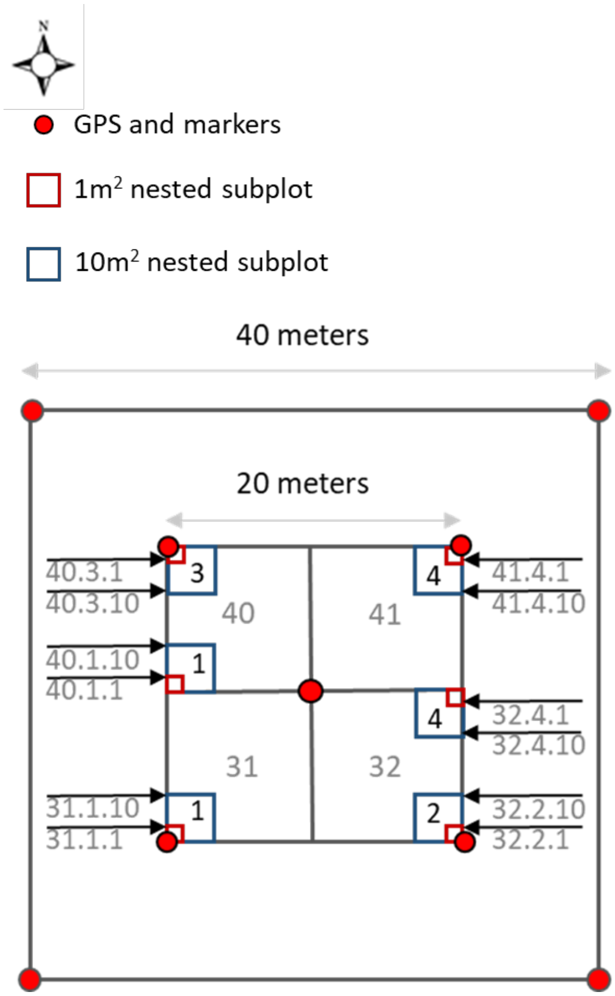

**Figure 6**. The square, multi-scale plot used to record plant species composition and cover. The plot includes nested subplots at specific locations within the plot. The 100m$^2$ subplot naming (e.g., 31, 32, 40, 41) corresponds to the point identification (see for point identification logic and description) in the southwest corner of the subplot. The 100m$^2$ subplot corners are numbered counter clockwise starting in the southwest corner. The 100m$^2$ subplot identifier, the subplot corner, and the scale of observation name the 1 and 10m$^2$ subplots. Subplot 32.2.1, for example, is the 1m$^2$ subplot in corner 2 of 100m$^2$ subplot 32.

## B.3 Field: Metadata

Record Domain ID, Site ID, Plot ID, 100m$^2$ Subplot ID, Bout Number, the primary botanist (Measured By), additional staff (Recorded By), and Date, which should reflect the day the sampling was completed (if working with paper datasheets; this information is captured automatically by the handheld device).

## B.4 Field: 1m$^2$ Subplot – Variables

The plot-based collection requires observation of primarily abiotic elements – termed 'Variables' – in 1m$^2$ nested subplots. 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 5).

- Items such as bones, carcass, or trash should be included in the cover estimates as "other".
- 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 6**. Variables to be observed in the 1m$^2$ nested subplot.

| Variable name | Description |
|---|---|
| Soil | Organic or inorganic particles < 5 mm diameter. |
| Rock | Inorganic particles ≥ 5 mm diameter. |
| Wood | Woody, organic material ≥ 5 mm 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 < 300 cm in the cover estimate. |
| Litter | Unrooted organic material lying on the ground such as grass, leaves, pine needles, and twigs (wood) < 5 mm diameter. |
| Standing Dead, Woody | Standing, dead, woody material that is not severed from the original source of growth and not leaning > 45 degrees. Similar to estimating the cover of living material (see B.5), record only largest cover under 300cm if individuals exceed that height. However, unlike directions in B.2, do not record information about height. |
| Standing Dead, Herbaceous | Desiccated herbaceous organic material from the previous calendar year or that cannot be identified. Species that might have been included had the sampling bout been longer or occurred 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 – 10 cm 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.5 Field: 1m$^2$ Subplot – Species

Cover abundance data of plant species diversity is documented in the 1m$^2$ subplots. Record percent cover by vascular plant species, and the presence of individual plants greater than 300cm in height.

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
    - If a determination can't be made in the field see SOP C Morphologically Challenging Species.
    - If no species are found in the nested 1m$^2$ subplot, select Target taxa present=No in the mobile device, or write in the taxonIDRemarks field of the first line of the datasheet No target taxa present.
    - 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.

TIPS & TRICKS

**Use Existing Species Lists**

- Carry site- and plot- specific lists from prior efforts
- Lists of frequently observed species common taxa list on the handheld device

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; estimates should not exceed 100 percent for a single species, but the combined cover of multiple species – even just the biotic component of the observation - may be greater than 100% (Figure 6).
    - If there are any individuals present in the 1m$^2$ subplot > 300cm in height, select 'Yes' for 'Are any plant heights Greater Than 300 cm?'
    - 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 'No' for Plant Height Over 300cm (this option is not available or needed if 'No' was selected above).
    - 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 'Yes' 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 'Yes' for Plant Height >300cm.

- Estimate and record only the cover of plants, or portions of plants, with stems or parts of stems that originate within 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%.
- There will often be spatial overlap of plant species.

**Use visual aids to estimate cover**

- Be familiar with cover estimates (e.g., 1%, 25%) in the sampling frame
- Use 10cm delineations on 1m2 frames to guide cover estimates
- Visually group species into a section of the sampling frame
- Fine tune estimates by removing gaps
- Check that combined plants and variables sum to at least 100%

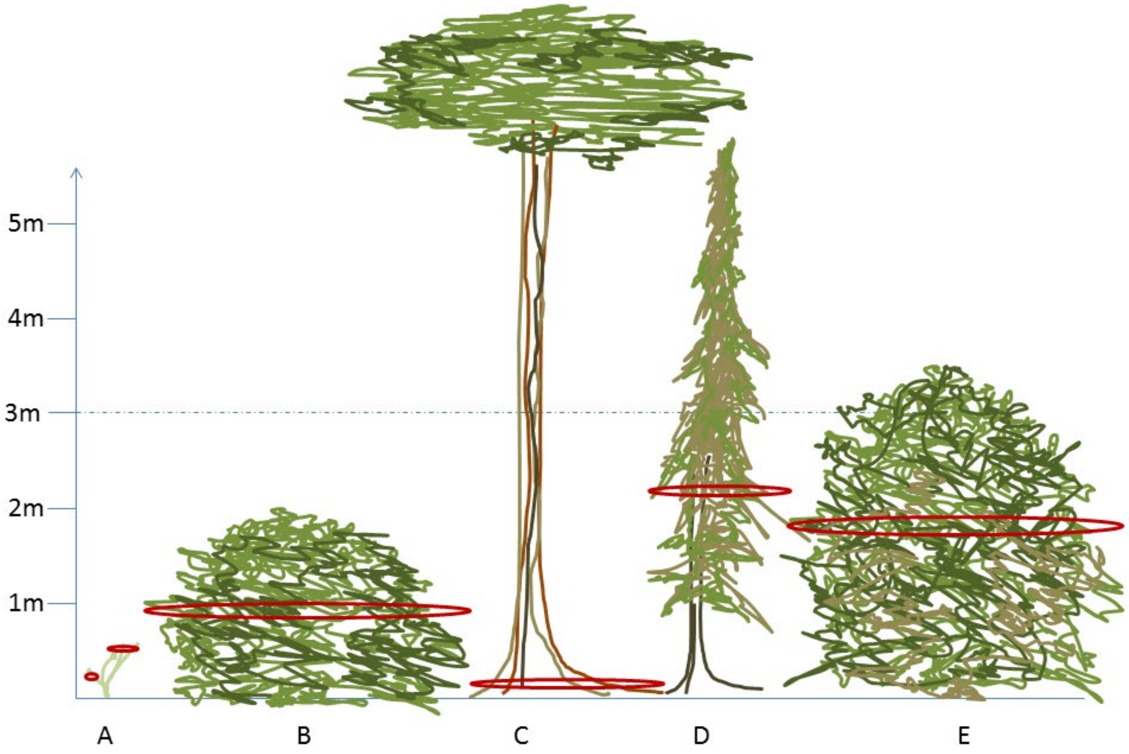

**Figure 7**. Estimates of cover should include all vegetative material < 300cm in height. For herbaceous growth (A), and shrubs (B) < 300cm, record the total combined cover by species; for tall trees with no woody branches or foliar growth < 300cm (C) record basal area (not covered by moss, litter etc.) 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 are 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.

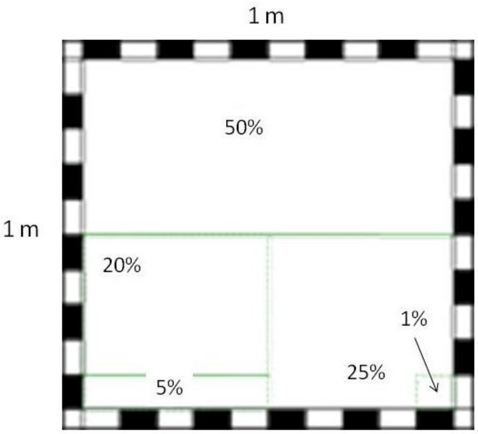

**Figure 8**. The 1 m$^2$ subplot is calibrated with black and white marks to make estimates of plant species cover more accurate and repeatable.

### B.6 Field: 10m$^2$ Subplot - Species

Record the identity of all species that emerge from within (or epiphytic) each 10m$^2$ nested subplot. It is not necessary to record species already documented in the 1m$^2$ nested subplots in each respective 10m$^2$ nested subplot. If there are no new taxa present select ‘No’ in the handheld computer for the question, ‘Are There Any New Taxa Here?’, or write “No new taxa” in the appropriate 10m$^2$ space on the datasheet.

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 decreases 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.

### B.7 Field: 100m$^2$ Subplot - Species

Record the identity of all plant species that emerge from within (or epiphytic) each 100m$^2$ subplot. It is not necessary to record species already documented in nested subplots. If there are no new taxa present select ‘No’ in the handheld computer for the question, ‘Are There Any New Taxa Here?’, or write “No new taxa” in the appropriate 100m$^2$ space on the datasheet.

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 decreases 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.

### B.8 Lab: Data QA/QC

Data should be reviewed regularly throughout the field collection effort and at the end of the sampling bout (AD[06).

## SOP C Morphologically Challenging Species

It is not possible to identify all of the plants encountered. The requisite diagnostic parts of a species may not coincide with the scheduled sampling window, or some species might only be differentiated by an expert in the specific genera. There are numerous tools and recording methods for providing the best taxonomically accurate and consistent data to end-users through time. The following is an overview and specific guidance on handling taxonomic uncertainty (Figure 8).

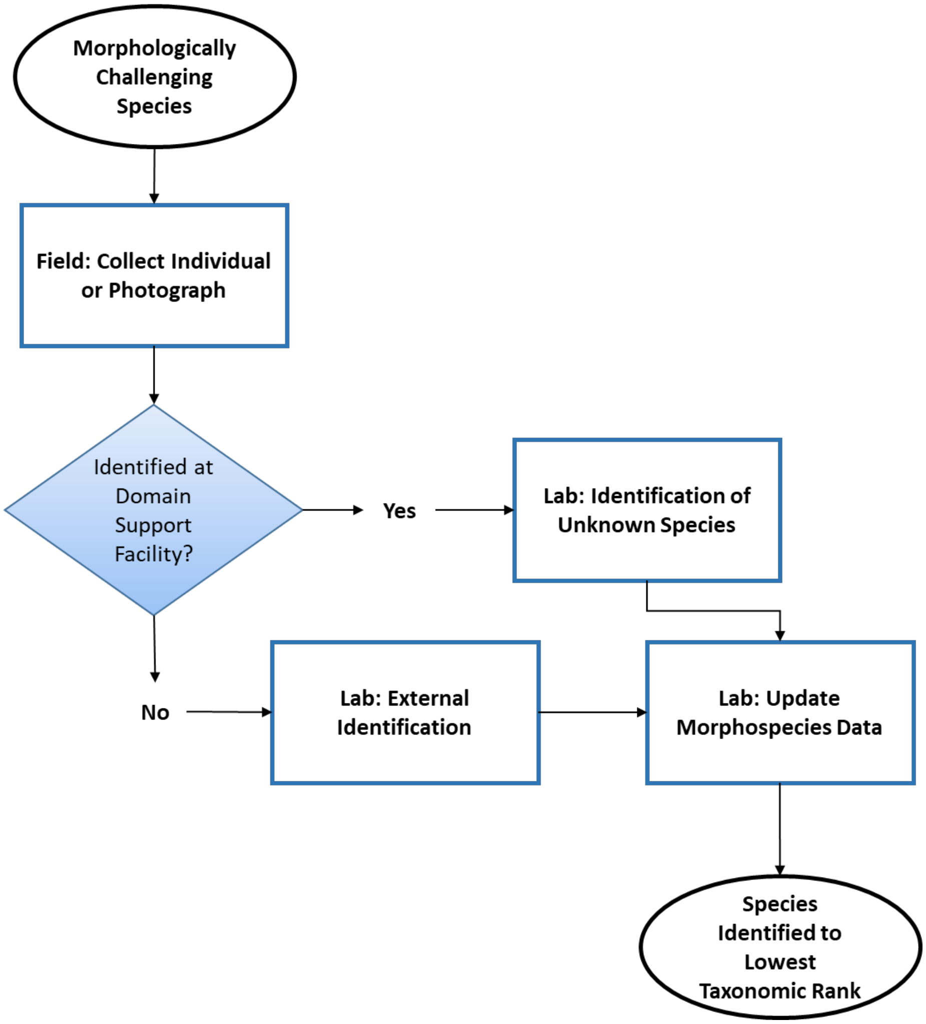

**Figure 9**. Schematic for the unknown plant individuals or morphospecies workflow.

### If you have no idea what the plant is…

- Collect a specimen or take a photograph of diagnostic plant parts (this can reduce searching time and site impacts) and create an unknown morphospeciesID. Even if there are no diagnostic parts, references or experts are likely to at least know the family or genera.

### If diagnostic parts are available…

- Collect a specimen or take a photograph of diagnostic plant parts (this can reduce searching time and site impacts) and create an unknown morphospeciesID. The species can be identified and the morphospeciesID updated.

**If you are approximately 75% certain of the determination…**

- Record the taxonID with uncertainty indicated by the identification qualifier (e.g., Acer rubrum CS). The affinity code can be used to indicate less certainty or similarity.

**If there are two or three consistently indistinguishable species or genera…**

- Enter (or create) the cryptic or slash species pair (e.g., TRSA5/TRAE2). This provides some indication of the observed species present.

**If the individual cannot be identified or differentiated, and diagnostic parts are not available…**

- Record the lowest taxonomic resolution possible of the unknown with an indication of diversity with the scale (i.e., subplot) of observation (e.g., *Arisaema sp.* if there is only one unknown species or *Arisaema spp.* if there are more than one unknown).

### C.1 Field: 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 i.e. only genus is recorded. 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.

**Table 7**. 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 |
|---|---|---|
| CF | cf. family | Roughly equals but "not sure" about the family |
| AF | aff. family | "Similar to, but is not" the family |
| CG | cf. genus | Roughly equals but "not sure" about the genus |
| AG | aff. genus | "Similar to, but is not" the genus |
| CS | cf. species | Roughly equals but "not sure" about the species |
| AS | aff. species | "Similar to, but is not" the species |
| CB | cf. subspecies | Roughly equals but "not sure" about the subspecies |
| AB | aff. subspecies | "Similar to, but is not" the subspecies |
| CV | cf. variety | Roughly equals but "not sure" about the variety |
| AV | aff. variety | "Similar to, but is not" the variety |

## C.2 Field: 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.

## C.3 Field: 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. Do not make a collection if there is a possibility that the species might be threatened, endangered, or similar.

### Individuals that cannot be identified to species

Species that cannot be identified and do not possess sufficient parts to allow identification in the lab or with external help are periodically 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 a 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.

**Lumping**

A list of those genera within which species cannot be consistently differentiated is made available to botanists in the field (on the Sampling Support Library) and data users (on the portal). The ability to 'lump' – combine these species at the genus level (or family, but this is discouraged) – allows consistent comparability of cover and richness data within plots and a site through time while field expertise and timing of diagnostic parts (e.g., flowering) will change through time. Some sites always lump a particular genus. However, to take advantage of taxonomic expertise and to provide the most detailed data possible for data users, it is acceptable to identify those typically lumped at genus to species when an accurate determination is possible. Providing the list of frequently lumped species to the end user allows them to lump data to account for otherwise inflated representations of species turnover through time. Record lumped information as follows:

- Enter the genus followed by the sp. and the appropriate taxonID when there is only one individual present.
- Enter the genus followed by the spp. and the appropriate taxonID when there is more than one individual present that by lumping logic represents more than one species.
  These entries are coded much the same as any determination that can't be made to species (see above). Aside from the instance of more than one individual, there is little difference.

The lumping, for example, recognizes that botanists at Domain 10 at the Central Plains Experimental Range site cannot differentiate species of the genus *Astragalus* (enter ASTRA, *Astragalus spp.*). However, should an astragalus expert work the site for some years, the lumping system captures that expertise and allows the end user to handle the data appropriate to their analyses.

### 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. It can be useful to collect duplicates if the specimen is likely to be destroyed during subsequent identification and/or if the specimen is to be included in the herbarium. 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.

1. Create a morphospeciesID in the morphospecies application on the mobile device
    a. **Date:** The date the morphospecies was created.
    b. **Year:** The year the morphospecies was created.
    c. **Domain ID:** Select the domainID.
    d. **Site ID:** Select the siteID.
    e. **Technician ID:** The name of the person who observed the plot data and named the morphospecies.
    f. **Morphopecies ID**: Enter a descriptive name that is memorable should the morphospecies be found in other plots. These morphospeciesID's 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>
    g. **Morphospecies Description:** Enter a description of the individual that might be useful when keying the plant in the lab (e.g. pubescent ligules, acidic moist habitat).
    h. **Photos**: The mobile application allows pictures to be linked directly to the record. Take pictures to support identification of the collected specimen or in the case that a specimen can't be located outside the plot.
2. Enter the morphospeciesID in the data
    a. Record the lowest taxon rank known (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 Remarks field.
3. Collecting a specimen

a. Given NEON's long-term monitoring efforts, unknowns of herbaceous species should be collected from outside the 20 m x 20 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 reproductive parts 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. 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 is not possible, take photograph(s) of the individual (including flowers and other parts crucial to identification). If working with datasheets, record photographic information in the morphospeciesIDRemarks field. Once downloaded, 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 - five 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[12]).

### C.4 Lab: Identification of Unknown Species

Identification of plant specimens requires knowledge of morphological characteristics of different plant families, plant keys, a clean bench space in the lab, and a dissecting microscope and kit. Collected unknown or morphospecies are to be identified such that the true determination can be incorporated into the data. There are multiple options depending on available time:

- Morphospecies can be identified the same day as the field collection.
- Morphospecies can be pressed and dried for determination at a later date (RD[12]). The Type I field barcode should be pressed with the specimen, and the human readable sampleID should be written directly on the newsprint in which the specimen is pressed.
- Morphospecies can be refrigerated for no more than two – five days until they can be pressed and/or identified as above.

Following identification, specimens can be discarded or included in the reference herbarium.

Determinations must be entered in the morphospecies application. If the morphospecies name was used few times, determinations may also be updated in the plant diversity 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.

### C.5 Lab: External Identification

Contract(s) have been established to facilitate the identification of morphospecies in cases where determinations can't be made by NEON staff or confirmation of determinations is required.

**Selection of specimens for shipping and identification by external botanist**

NEON botanists should endeavor to identify all unknowns with dissecting scopes and dichotomous keys in the lab. When uncertainty persists, specimens can be shipped for identification. Only unknown plants for which distinguishing parts are available should be sent for identification. For example, at some sites a basal rosette is sufficiently generic that the individual could belong to numerous genera or even families. These unknown specimens should not be sent, but identified to the lowest possible taxonomic rank in the morphospecies data. Alternatively, the unique morphology of a basal rosette at a site might make determination possible. These unknown specimens should be sent to the contractor.

**Specimen preparation and labeling**

All specimens to be shipped must be dried and pressed, labeled with a sampleID that will be used as a primary key, to enable shipping through the NEON shipping applications, and for appropriate packaging.

1. Specimens must be dried and pressed (RD[12]).
2. Generate the sampleID by entering the specimen into the voucher collection application.
    a. Enter the lowest taxonomic rank possible.
    b. Enter the morphospecies.
    c. Enter other available information (i.e., habitat, life stage) into the application.
3. Label the specimen with a human readable version of the sampleID.

**Specimen shipping**

After the specimen is dried, enter information in the NEON shipping application and prepare unknown specimens:

1. Place dried, fumigated/frozen, and pressed specimens and sampleID label in newsprint (12"x18") or similar paper that was used for drying and pressing; one specimen per newsprint fold. Make sure fruits are secure and put loose pieces in temporary packets. Be sure all parts fit within the newsprint.

2. Place specimens in newsprint in stacks of 10-20.
3. Place each stack between two genus covers and tape them together.
4. No more than two or three of these stacks should be placed between two 12”x18” cardboard flats.
5. Tie each end using an herbarium slip knot.
6. Wrap the bundle with wrapping paper to ensure loose pieces are not lost.
7. Write “NEON specimens”, domain, site and contact information on each bundle.
8. Place bundle(s) in sturdy box marked “Herbarium Specimens” and “Fragile”.

**Lab: Updating data**

When specimens and determinations are returned, enter the relevant information into the morphospecies application. Specimens can then be discarded or included in the reference herbaria or the external archive as appropriate.

## SOP D Voucher Specimens

Plant species are collected at NEON: 1) collection of species that can't be identified in the field allows the identification of species (see section B.4 above), 2) reference herbarium vouchers are stored in Domain Support Facility herbaria for training and quality assurance purposes (RD[12]), 3) archive vouchers are archived at the external bioarchive to contribute to a long-term record of plant diversity observations as part of the NEON archive program; find the list of species to be collected on the Sampling Support Library. These lists identify species designated noxious in Arizona that can be shipped to the archive but most not include reproductive parts. Collect these noxious weed specimens without seeds or flowers or remove these parts prior to shipping. If this is not possible, collect alternative species from the voucher archive list.

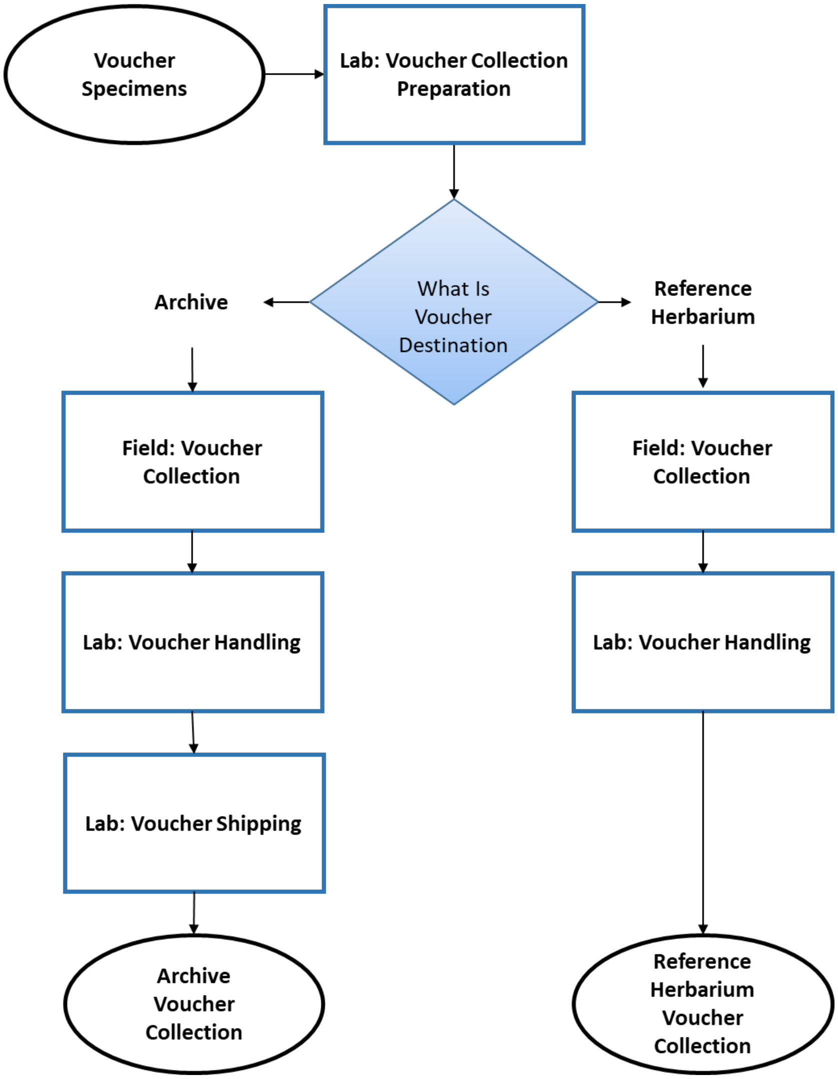

**Figure 10**. Schematic of plant voucher collection, handling, and shipping.

## D.1 Field: Voucher Collection

The following guidelines should be considered when collecting voucher specimens of all types:

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 or plant press if pressing in the field.
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 the herbarium.
5. Record pertinent label information in the voucher application for specimens destined for the Domain Support Facility herbarium, external archive, or external identification. Record:
    - **Domain ID.** The domain in which the specimen is collected.
    - **Site ID.** The site at which the specimen is collected.
    - **Plot ID** (if applicable). The plot number from which the specimen is collected.
    - **Location – if not at plot**. If the voucher is not collected in (or near) a plot, record coordinates, uncertainty (if available) and elevation.
    - **Date.** YYYY-MM-DD
    - **Identified By.** The ‘Collector Name’, name of the person responsible for recording original occurrence.
    - **Recorded By.** The name of individual recording information.
    - **Collected By.** The name of the individual who collected the specimen.
    - **Sample Tag.** Record a NEON sample tag number if present.
    - **Taxon ID.** The NEON taxonID to lowest possible taxonomic rank.
    - **Taxon ID Remarks.** Notes about the specimen.
    - **Identification Qualifier** (if appropriate). The standardized term to qualify the identification of the organism when doubts about taxonomic identity exist.
    - **Identification References** (if appropriate). The name of the reference used to identify the specimen.
    - **MorphospeciesID** (if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.
    - **Plant description**. A description of notable specimen characteristics e.g., Very small yellow flowers turning white with age, small lanceolate leaves. Flattened round fruit.
    - **Life stage**. The age class of the individual (e.g., ‘fruiting’, ‘seedling’).
    - **Locality**. Natural language description of the place where the organism was collected, e.g., Blue Mountains, 50m west of summit of Grandfather Mountain.

    - **Habitat description**. A category or description of the habitat in which the specimen occurred.
    - **Associated taxa**. NEON taxonID of plant species associated or found in proximity to the collected specimen.
    - **Voucher (Collection) number**. An identifier given to the specimen at the time it was recorded; typically a collector-specific running number (sometimes called record number).
    - **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 after time and middle initial is entered.

6. Generate a human readable label and (optionally) a field barcode label (Type I or Type II) and place it in the bag for reference herbarium vouchers, archive vouchers, or morphospecies vouchers.

### D.2 Lab: Voucher Handling

Plant voucher specimens are collected for reference and training herbaria at the Domain Support Facility and to provide a physical record of specimens at the external archive. To preserve fragile plant parts necessary for quality vouchers, specimens should be pressed the same day as collected or (less ideal) after no more than two – five days of refrigerator storage.

- Reference herbarium vouchers should be pressed, dried, mounted and labeled (RD[12]). In addition to the herbarium label, a new Type II ‘archive barcode’ should be generated in the specimen-specific record in the voucher application and attached to the herbarium sheet next to the label.
- Archive vouchers must be pressed, dried (but not mounted), and a final herbarium label should be generated (RD[12]). In addition to the herbarium label, a new Type II barcode should be generated; leave the backing on the label such that it can be adhered to the herbarium sheet when the specimen is mounted.
- Pass all samples destined for the internal reference herbarium, the external archive, or external identification through the -80°C freezer to kill any pests (RD[12]).

### D.3 Lab: Voucher Shipping

The dried and pressed plant voucher specimens will be shipped in newsprint between cardboard with both the printed label and the Type II barcode that provide voucher information. See the shipping guidance for more information (RD[15]).

After the specimen is dried, enter information in the NEON shipping application and prepare specimens:

1. Place dried and pressed specimens, the voucher label, and the Type II 'archive barcode' in newsprint (12"x18") or similar paper that was used for drying and pressing; one specimen per newsprint fold. Make sure fruits are secure and put loose pieces in temporary packets. Be sure all parts fit within the newsprint.
2. Write a human readable version of the sampleID on the newsprint.
3. Place specimens in newsprint in stacks of 10-20.
4. Place each stack between two genus covers and tape them together.
5. No more than two or three of these stacks should be placed between two 12"x18" cardboard flats.
6. Tie each end using an herbarium slip knot.
7. Wrap the bundle with wrapping paper to ensure loose pieces are not lost.
8. Write "NEON specimens", domain, site and contact information on each bundle.
9. Place bundle(s) in sturdy box marked "Herbarium Specimens" and "Fragile".
10. Be sure to include the CLA memo describing the duration of freezing with the shipment (RD[15]).

## SOP E Genetic foliar Tissue

The goal of this SOP is to collect fresh – not approaching senescence – and robust foliar tissue for the genetic archive. Genetic foliar tissue  is collected 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 on the Phenology Plot loop (see TOS Protocols Plant Phenology (RD[08]). 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 phenology, vegetation structure, canopy chemistry, and LMA. One of the ten individuals from which tissue was collected must be vouchered.

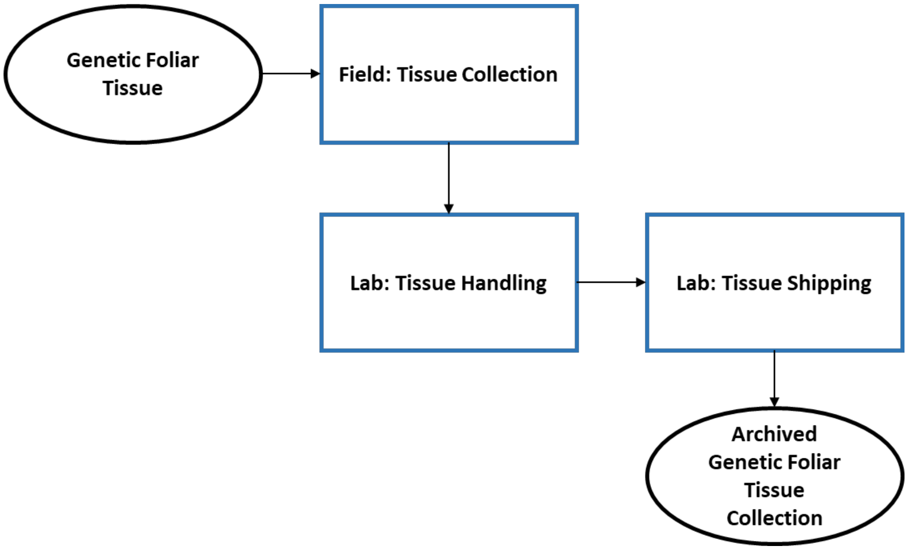

**Figure 11**. Schematic of genetic foliar tissue collection, handling, and shipping.

### E.1 Field: Genetic Foliar Tissue 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 and Foliar Chemistry protocols.
    c. If phenology sampling has been discontinued at a site, collect tissue from those species initially identified for phenology observations from the tower airshed (3 individuals per species) and across the site (7 individuals per species).

**Figure 12**. Collecting young green leaves from a single individual

2. With forceps 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 (Figure 11).
3. Before the sample is stored:
   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. Record in the Fulcrum application:
   - **Domain ID.** The domain in which the specimen is collected.
   - **Site ID.** The site at which the specimen is collected.
   - **Plot ID** (if applicable). The plot number from which the specimen is collected.
   - **Location – if not at plot**. If the voucher is not collected in (or near) a plot, record coordinates, uncertainty (if available) and elevation.
   - **Date.** YYYY-MM-DD
   - **Identified By.** The 'Collector Name', name of the person responsible for recording original occurrence.
   - **Recorded By.** The name of individual recording information.
   - **Collected By.** The name of the individual who collected the specimen.
   - **Tag ID** (if applicable). The NEON tag on the individual if one exists.

- **Taxon Code.** The NEON taxonID to lowest possible taxonomic rank.
- **Identification Qualifier** (if appropriate). The standardized term to qualify the identification of the organism when doubts about taxonomic identity exist.
- **Identification References** (if appropriate). The name of the reference used to identify the specimen.
- **MorphospeciesID** (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.
- Generate **Genetic Sample ID.** The application creates this from location, date, and time.

6. Label the envelope with a unique **geneticSampleID** generated by the Fulcrum application. This includes 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
7. Generate an adhesive field barcode label (Type I or Type II) and affix to the envelope, without covering the human-readable label.

TIPS & TRICKS

**Write the NRCS code on the coin envelope**
- Labeling the envelope code with the species Identification helps track the collection effort

8. Place sample in resealable 1-gallon plastic bag. *Multiple genetic foliar 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 <u>one</u> 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.
    a. In many cases it will not be possible to obtain a quality voucher when young foliar tissue is available. In such cases, collect nine tissue samples early in the season and the 10th genetic foliar tissue sample when a quality voucher can be derived from the same individual.
11. 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[08]) 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.
12. See **SOP D Voucher Collection** for directions on voucher collection.

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

## E.2 Lab: Genetic Foliar Tissue Handling

### Drying Samples

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. While drying, 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.

### Sample Preparation

Samples must be transferred from the coin envelopes to the 10 mL cryo vials for shipping and eventual storage at the external archive (Figure 11).

1. Wearing nitrile gloves and using tweezers, transfer the dried genetic foliar tissue to the 10 mL cryo vials.
2. Generate a new – 'archive barcode' - Type II barcode in the sample record in the Fulcrum application.
3. Affix the barcode lengthwise to the vial

**Figure 13**. Simport 10 mL cryo storage vial with Type II barcode label.

## E.3 Lab: Genetic Foliar Tissue Shipping

The foliar genetic tissue must be shipped according to specific instructions and frozen to prevent spread of pests and any noxious species material according to the following steps:

1. Pass all samples through the -80°C freezer to kill any pests. This can be done prior to the steps below or before samples are boxed for shipping.
2. Place samples in fiberboard cryo boxes with fiberboard dividers (Appendix D).
3. Prepare samples for shipping according to shipping guidance (RD[15]) and with NEON's Fulcrum shipping applications.
4. Note that the shipping application should be used to document the samples in each cryo box, but also to document sample location according to the well-coordinates.
5. Be sure to include the CLA memo describing the duration of freezing with the shipment (RD[15]).

## SOP F Post-Field Sampling Tasks

### F.1 Refresh the sampling kit

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

### F.2 Document Incomplete Sampling within a Site

The plant diversity protocol and associated SOPs are scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and Appendix C. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a terrestrial sampling plot is compromised after road-building activities. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

Criteria for moving a plot include:

- If 50% or more of the 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.
- Cumulative impacts to vegetation within the plots such that the data are substantially impacted. Endeavor to minimize disturbance and report within plot sampling impacts (RD[13]).

If sampling at a given plot is not possible during a given bout a problem ticket should be submitted by Field Science staff.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'TOS Sampling Incomplete: DIV – [Root Cause Description]'
    a. Example: 'TOS Sampling Incomplete: DIV – Could not access plot due to permanently closed road'
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.

## SOP G 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. 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 for entering data can be found on the Sampling Support Library. 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 are 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.

## SOP H Sample Shipment

Please reference the shipping information in specific SOPs and the guidance for shipping for more information (RD[15]).

## 8 REFERENCES

Didan, K. 2015. MOD13Q1 MODIS/Terra Vegetation Indices 16-Day L3 Global 250m SIN Grid V006. NASA EOSDIS Land Processes DAAC. https://doi.org/10.5067/MODIS/MOD13Q1.006.

Peet, R. K., T. R. Wentworth, and P. S. White. 1998. A flexible, multipurpose method for recording vegetation composition and structure. Castanea 63(3):262-274.

Stohlgren, T. 2007. Measuring plant diversity, lessons from the field. Oxford University Press, New York.

# APPENDIX A QUICK REFERENCES

## Quick Reference: Plant Diversity

1. Determine if sampling full plot with all of the multi-scale subplots or only the 1m$^2$ subplots
2. Delineate plot and place 1m$^2$ subplot frame in corner
3. 1m$^2$ subplot: Record 'Other Variables'
4. 1m$^2$ subplot: Record cover and height <300cm of plant species
5. 1m$^2$ subplot: Check cover values with application tools; values likely to exceed 100%
6. 10m$^2$ subplot: Search for species not found in nested 1m$^2$ subplot
7. 100m$^2$ subplot: Search for species not found in nested 1 or 10m$^2$ subplots
8. Morphologically challenging species:

**If you have no idea what the plant is…**

- Collect a specimen or take a photograph of diagnostic plant parts (this can reduce searching time and site impacts) and create an unknown morphospeciesID. Even if there are no diagnostic parts, references or experts are likely to at least know the family or genera.

**If diagnostic parts are available…**

- Collect a specimen or take a photograph of diagnostic plant parts (this can reduce searching time and site impacts) and create an unknown morphospeciesID. The species can be identified and the morphospeciesID updated.

**If you are approximately 75% certain of the determination…**

- Record the taxonID with uncertainty indicated by the identification qualifier (e.g., Acer rubrum CS). The affinity code can be used to indicate less certainty or similarity.

**If there are two or three consistently indistinguishable species or genera…**

- Enter (or create) the cryptic or slash species pair (e.g., TRSA5/TRAE2). This provides some indication of the observed species present.

**If the individual cannot be identified or differentiated, and diagnostic parts are not available…**

- Record the lowest taxonomic resolution possible of the unknown with an indication of diversity with the scale (i.e., subplot) of observation (e.g., *Arisaema sp.* if there is only one unknown species or *Arisaema spp.* if there are more than one unknown).

### Quick Reference: Archive Vouchers

1. Retrieve the list of species to be collected for the external archive from the SSL

Field:

2. Collect a disease-free specimen that includes the roots, stems, flowers, fruits, and seeds.
3. Place specimen in collection bag or plant press
4. Collect duplicate samples if identification will require destructive sampling
5. Record relevant data in the application
6. The sampleID should be of the format: pla.OAES.20151014.10:30.dtb.V123
7. Label specimen with a ‘field barcode’ - a Type I barcode stuck to the specimen or included in the bag or press

Lab:

8. Vouchers should be dried and pressed with the Type I ‘field barcode’ and the sampleID written on the newsprint
9. Vouchers for the external archive should not be mounted, but an herbarium label that includes an ‘archive barcode’ a new Type I barcode generated for the label.

**Quick Reference: Genetic Foliar Tissue**

1. Acquire list of target plant species – Phase I Phenology species
2. Collect material from 3 individuals of each Phase I species from the Phenology Plot loop.
3. Collect material from 7 individuals of each Phase I species from across the site.

Field:

4. Wear nitrile gloves and use forceps to handle genetic foliar tissue
5. Collect approximately 10 cm$^2$ or 1 g fresh weight of leaf material per individual.
6. The leaf material should be collected from young, fresh leaves
11. Place the tissue in a coin envelope
12. Record in the application
13. The sampleID should follow example: gen.OAES.20171014.10:35
14. Generate an adhesive barcode label (Type I) and affix to the envelope
15. Place samples in resealable 1-gallon plastic bag
16. Color-change desiccant should be placed in the plastic bag, but outside the coin envelope.
17. Change desiccant until tissue is dry
18. Collect a 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.
19. In many cases it will not be possible to obtain a quality voucher when young foliar tissue is available. In such cases, collect nine tissue samples early in the season and the 10th genetic foliar tissue sample when a quality voucher can be derived from the same individual.

Lab:

20. When dry, transfer tissue to cry vial
21. Label cryo vial with a new ‘archive barcode’ – a Type II barcode

## APPENDIX B REMINDERS

### Preparation

- ☑ Identify start date based on schedule and fine-tune based on phenology sampling observations.
- ☑ Review equipment lists and update handheld computers.
- ☑ Review species lists by site and plot, and review with the reference herbarium and floras.
- ☑ Review options for recording unknown and uncertain species determinations.
- ☑ Identify Phase I phenology species from which foliar tissues should be collected.

### Plot sampling

- ☑ Use appropriate tools to establish the plot.
- ☑ Double check plot number entry in handheld computer.
- ☑ Avoid walking on/trampling all parts of the plot, including the 40x40m area that surrounds the diversity sampling footprint.
- ☑ Make observations from outside the 20x20m plot when possible, and don’t trample the 1m$^2$ subplots.
- ☑ Estimate cover of both ‘variables’ and vascular plant species in 1m$^2$ subplots, and indicate which, if any, species > 300cm.
- ☑ Review list of species observed and total estimates of cover of plants and ‘other’ variables.
- ☑ Search 10 and 100m$^2$ subplots for species not found in nested subplots. While searching make every attempt to minimize impacts.
- ☑ Generate unique and memorable morphospeciesID.
- ☑ When morphospecies are identified, update the morphospeciesID in the morphospeciesID table.

### Plant voucher collections

- ☑ Collect vouchers with reproductive parts.
- ☑ Record voucher information in Fulcrum.
- ☑ Apply human readable sampleID and Type II barcode to the voucher.
- ☑ Don’t let the voucher rot; store in cooler and refrigerator, then press and dry the specimen as soon as possible.
- ☑ Mount and label specimens for the reference herbarium at the Domain Support Facility.
- ☑ Do not mount or generate a NEON herbarium label for vouchers collected for the external archive. External archive shipping information TBD.

## Genetic foliar tissue collections

☑ For each of the three Phase I species: collect 3 tissue samples from 3 individuals near the phenology loop, and 7 tissue samples from 7 individuals across the site.

☑ Voucher one individual from which tissue is collected for each of the 3 species.

☑ Wear nitrile gloves.

☑ Cut hard, leathery, or succulent tissue into strips and remove epidermis of pruniose species.

☑ Record data in handheld computer.

☑ Place tissue in coin envelope with human readable sampleID and Type II barcode.

☑ Place coin envelope in plastic bag with silica.

☑ When back at lab, replace silica as needed to ensure tissue is dry.

☑ Shipping instructions TBD.

## APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are estimated from satellite MODIS-EVI phenology data averaged from 2005-2014 (Didan 2015). Dates presented here are only a guide, and are derived according to the logic presented in Section 4.2. Because individual years may vary widely from the average dates provided below, 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.

**Table 8**. Domain- and site-specific, bout number, and per bout sampling start and end dates.

| 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 | August | 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 | 1 | May | June | | |
| | 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 | May | August | | |

## APPENDIX D 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 9**. Equipment list – Materials and supplies required for one crew for the plot-based plant diversity sampling procedure.

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| **Durable Items** | | | | | | |
| Ben Meadows Forestry Suppliers | 100952<br>39167 | N | Chaining pins or other suitable anchor | Anchor measuring tapes | 4-6 | N |
| | | N | Cooler | Chill perishable plant vouchers in field and/or vehicle | 1-5 | N |

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| B&H | OLTG4B | N | Digital camera and SD card, 12 megapixel | Capture images of plants for species identification | 1 | N |
| Amazon<br>Cabela's<br>REI | IK270217<br>895022 | N | GPS receiver, recreational accuracy | Navigate to sampling location | 1 | N |
| Fisher Scientific<br>Grainer | 19067113<br>3UZA9 | N | Ice pack | Chill perishable plant vouchers in field | Many | N |
| Forestry Suppliers | 61260 | N | Magnifier hand-lens, 20X | Aid in species identification | Many | N |
| Ben Meadows<br>Forestry Suppliers | 122732<br>39945 | N | Measuring tape, minimum 50 m | Delineate plot boundary | 3 | N |
| | | N | Pruning shear | Collect voucher specimens | 1 ea. | N |
| | | N | Sampling frame, 1m$^2$ | Delineate 1m$^2$ subplot | 1 | N |
| | | N | Small carabiner and ring binder | Organize and carry unknown plant vouchers | 1 | N |
| | | N | Weeder | Collect voucher specimens | 1 | N |
| | | N | Meter stick | Evaluate plant height against 300cm | 1 | N |
| | | N | Handheld computer | Data collection | 1 | N |
| **Consumable items** | | | | | | |

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | N | AA battery | Spare battery for GPS receiver | 2 | N |
| | | N | Adhesive label | Label unknown and voucher specimens | 1 sheet | N |
| | | N | All weather copy paper | Print datasheets | Many | N |
| | | N | Digital camera battery | Spare battery | 1 | N |
| | | N | Field notebook | Record field notes | 1 | N |
| Grainger Forestry Suppliers | 9WKP4<br>57880 | N | Flagging tape | Delineate sampling area | 1 | N |
| Grainger | 5CNK5<br>8YAT5 | N | Resealable plastic bag, 1 gal | Organize and carry unknown plant vouchers and genetic foliar tissue | > 40 | N |
| | | N | Survey marking flag, PVC or fiberglass stake | Delineate sampling area | Many | N |
| | | N | Barcode labels, type II | Genetic foliar tissue and voucher tracking | Many | N |
| **Resources** | | | | | | |
| RD[05] | | | Field datasheet | Record data | 1 | N |

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | N | Field guide, regional flora reference guide and/or key | Identify unknown species | 1 | N |
| | | | Field guide, species list | Identify unknown species | 1 | N |

**Table 10.** Equipment list – Laboratory processing

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| **Durable Items** | | | | | | |
| Forestry Suppliers | 53872 | N | Botany dissection kit | Identify unknown species | 1 | N |
| Forestry Suppliers<br>Bioquip | 53741<br>3127 | N | Cardboard ventilator | Pressing plants | Many | N |
| | | N | Microscope | Aid in species identification | 1 | N |
| Fisher Scientific | 11350121 | N | Paper blotters | Press collected individuals for identification | Many | N |
| Forestry Suppliers<br>Bioquip | 53674<br>3115 | N | Plant press | Press collected individuals for identification | 2 | N |

| Supplier | Supplier Number | Exact Brand | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | N | Scissors or pruning shear | Prepare voucher specimen for mounting | 1 ea. | N |
| **Consumable items** | | | | | | |
| | | | Tabloid newspaper pages | Press collected individuals for identification | Many | N |
| Simport | T310-10A | Y | 10 mL Cryo vial | Cryo storage of genetic foliar tissue | Many | N |
| VWR | 89214-738 | Y | Fiberboard storage box | Hold cryo vials | 1 per sampling year | N |
| VWR | 82007-150 | Y | Fiberboard box dividers | Hold cryo vials | 1 per sampling year | N |
| **Resources** | | | | | | |
| | | R | Field guide, regional flora reference guide and/or key | Identify unknown species | | N |

# APPENDIX E SITE-SPECIFIC INFORMATION

## E.1 D20 – PUUM - Puu Makaala Natural Area Reserve

The dense tropical flora at the Pu'u Maka'ala Natural Area Reserve requires multiple protocol modifications:

- When the density of uluhe “false staghorn fern” (*Dicranopteris linearis*) is such that differentiating between stems rooted within and out of a 1m$^2$ subplot for the purposes of percent cover is difficult or near impossible, record the coverage of uluhe within the frame regardless of rooting point. If the rooting point can be determined without significant disturbance to the fern or other vegetation, then measure cover per normal protocol guidelines.
- In cases where tree fern (*Cibotium* spp. and *Sadleria* spp.) fronds are dead and have fallen, yet remain propped and attached to the standing stem, consider prostrate fronds as litter. The nature of tree fern frond tissue does not readily allow for dead fronds to separate from the main trunk. Often, these fronds begin to decompose while minimally attached to standing material, and functionally behave as litter.
- In cases where the 1m$^2$ subplot is populated by root material <5mm diameter, determine the species and combine the root cover with other parts that contribute to cover. If the species cannot be determined, enter the percent cover of the roots in Other and include “fine root mat” in the Remarks.

## APPENDIX F PLOT DELINEATION

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 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 1m$^2$ and 10m$^2$ subplots should be 1 m and 3.16m 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.

### F.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 is 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.

Alternatively, steps 7-11 above can be done by the following:

1. Anchor the third tape at point 40 (10m north of point 31/10m south of point 49) and extend it east to point 42 (10m north of point 33/10m south of point 51.
2. Anchor a fourth tape at point 32 (10m east of point 31/10m west of point 33) and extend it north to point 50 (10m east of point 49/10m west of point 51).

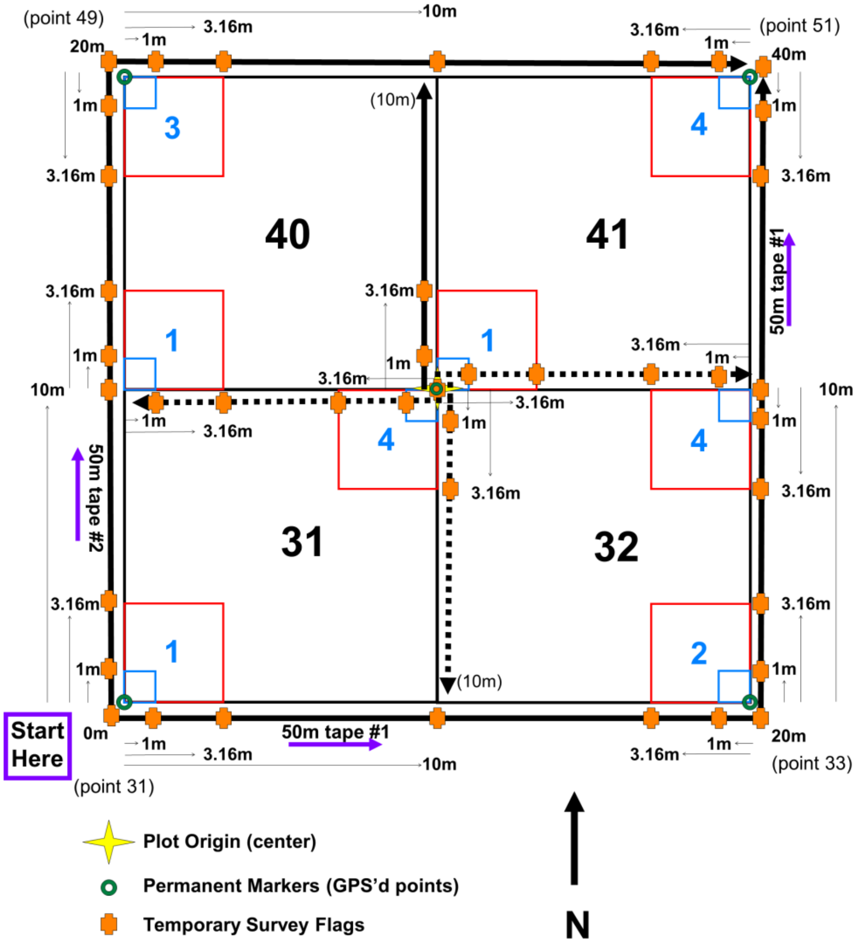

**Figure 14**. The plot has permanent markers and also requires temporary flags that are placed each time the plot is measured. The figure includes 1 and 10m2 subplots at the center of the plot that are relevant to other protocols but not the current version of the plant diversity protocol.

## F.2 Plot delineation with some lack of precision in plot and some obstacles (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 F.1 (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 are 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 is 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 and/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 and/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.

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.