TOS PROTOCOL AND PROCEDURE:
CFC – CANOPY FOLIAGE SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/13/2014	ECO-01139	Draft release
B	06/03/2014	ECO-01662	Production release, template change, and other changes as detailed in Appendix A (Rev B only)
C	11/05/2014	ECO-02416	Migration to new template
D	02/17/2017	ECO-04371	• Changed title from " TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass Per Area Measurements" to "TOS Protocol and Procedure: Canopy Foliage Sampling" to be more consistent with other TOS protocols • Incorporated description of canopy foliage sampling at herbaceous sites as well as foliage sampling for genetic archive material into introduction sections • Added safety information relevant to line launcher and slingshot sampling in tall canopies • Revised decision tree for how to sample at tall and mixed-stature plots • Updated equipment lists • Provided more detail on timing and personnel requirements for field and laboratory procedures • Added flow chart overview of sample collection and laboratory processing steps • Added description of expected sample numbers • Expanded SOP A to include preparing supplies, reviewing linked protocols, and pre-making foil packets for chlorophyll subsamples • Reduced sample targets from 5-12 woody individuals per plot to 3 individuals • Revised criteria for choosing species and individuals for sampling in tall and mixed-stature plots, added Box 1 to help clarify • Inserted instructions for how to sample canopies in herbaceous systems, based on SOP F of TOS Protocol and Procedure: Measurement of Herbaceous Biomass, version F • Removed requirement to collect and pool multiple subsamples from the same woody individual

			• Added instruction for how to create the chlorophyll subsample in the field • Changed instruction for saving the bulk chemistry sample, can now be stored in paper bags • Clarified that Old Standing Dead material should not be removed from herbaceous clip strips • Changed instruction for LMA measurements to reflect that all vegetation types will have leaves/needles scanned, including broadleaf and herbaceous foliage • Added more detailed instruction for how to scan and save images, as well as how to use scans to calculate LMA with ImageJ • Added instructions on how to subsample, store, and ship samples for analysis of chlorophyll, lignin, and major/minor elements to external laboratories • Added SOP H, which has instruction for collection and archive of foliar samples for genetic analysis • Added Appendix E as it contains necessary resources for clip strip harvesting for chemistry and LMA
E	04/03/2018	ECO-05486	• Sampling for Foliar Genetic Archive (SOP H plus all introductory text) has been removed, procedure now a part of the Plant Diversity protocol. • Added instruction on use of scan-able barcode labels throughout protocol • Replaced several figures to address formatting issues; Updated Figure 9 to show petiole inclusion in LMA scanning • Added new Table 1 to specify holding times for different sample types; added new Table 10 to estimate time to complete each SOP • SOP A: Preparing to sample now includes application of scan-able barcodes to sample bags, and use of VST data to assess plot-level canopy dominance and Mapper tool for geolocations of sampled trees; Size of chlorophyll sample bags and packets has been increased to accommodate more material • SOP B, Woody: Provided instruction for sampling in sparsely vegetated sites (total vegetation cover < 25%); expanded Box 1 to include more special cases (leafless plants, canopy vines, low-diversity plots), and removed guidance to sample 3 replicates per species, instead emphasizing goal to sample more species per site; included more detail on size of chlorophyll subsamples

			• SOP B, Herbaceous: Provided additional size options for clip strips; clarified clip strip orientation; added percentCoverClip field; guidance on timing to bag chlorophyll sample and only include live, green foliage • SOP D: Added instruction to include rachis/petiole in leaf scans; expanded tips for getting a good scan; modified instructions for area calculations with ImageJ; changed mass precision to 0.001 g • SOP E: Modified guidelines for chemistry sample grinding, now based on mass instead of sample type; added extra 40 mesh grinding step for CN samples • SOP F: Added language on data management protocol checks and use of scan-able barcodes • SOP G: Specified use of shipping applications
F	01/03/2019	ECO-05989	• Significant change to method for selecting individuals to sample in forested and shrubland sites. Focus on capturing site-level diversity, each site provided with target taxa lists generated by Science and instructed to sample in Vegetation Structure plots. Workflow discussed in depth in Section 3, SOP A, and SOP B. • Modified instructions for collecting herbaceous clip strips to focus on sampling all vegetation in the strip, regardless of rooting location or year of growth. • Included guidance for how to label and handle samples containing *Toxicodendron spp* – relevant to equipment list tables, SOP B, SOP D, and SOP E. • Added additional figures and tables to help clarify procedures throughout. • Edited text for clarity throughout.
G	02/03/2020	ECO-06313	• Updated to new template (NEON.DOC.050006vJ), including addition of documentation for missed sampling using the sampling impractical field • Addition of crown polygon workflow for woody individuals, SOPs A and B • Added more instruction for selecting woody individuals, including min number of plots per site, max number of samples per plot, clearer guidance on what 'sunlit' means, and how to spread out intraspecific replicates. SOPs A and B, Box 1. • Added guidance to sample dead/brown tissue if that is the dominant condition in the plot during sampling and AOP overflight, relevant to clip strips (SOP C).

			• Added instruction to store foliar tissues in sealed plastic bags with moist paper towels, then blot dry before processing in order to remove surface contaminants (Field and Lab SOPs) • LMA section (SOP E), added instruction to place all leaves sunny-side down, to help with area calcs. Added reminder to complete training scans. • Subsampling section (SOP F), added new field to capture the mass of archived foliage. • Two new appendices: Site-specific instructions (Appendix D), covering tree ferns (PUUM) and cacti (SRER), and Best practices for sampling tall canopies (Appendix E). • Reorganization of content to improve readability and clarify instructions throughout, all SOPs • Added several new figures and tables throughout
H	03/23/2020	ECO-06403	• BLAN moved to Type A site • Updated Table 9 to reflect correct sample container • Updated human-readable label type for plastic scintillation vials
J	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites
K	01/18/2023	ECO-06891	• Updated to new template (NEON.DOC.050006vK) • Minor text updates and clarifications throughout • Section 3, introduced term 'mixed sites' for those sampling woody individuals and clip strips • Section 4, revised guidance for timing of bouts to more closely align with airborne data collection, extended holding times for chemistry samples, clarified and updated bout duration guidance • Section 5, added safety details for UAS sampling, removed information on shotgun sampling since NEON does not use this method • SOP A, clarified requirements for scintillation vials, more detail on telescoping pole pruner, additional guidance for bouts using a UAS, more info about crown mapping layers, additional detail on reviewing target taxa lists and sharing sampling plan with Science, how to prepare for bouts with no dry ice • SOP B, added info specific to sampling with a UAS, added new fields 'samplePosition' and 'chlorophyllSamplePrepMethod', updated instructions for creating crown polygons • SOP C, added instruction for cacti in clip strips, added instruction to record plantStatus

			• SOP E, clarified that 5-day holding time especially important for broadleaf samples, updated instruction for setting the threshold, added a few other troubleshooting tips for image analysis • SOP F, clarified that 5-day holding time especially important for broadleaf samples, needle samples not ground to 40 mesh, added conditions for re-drying of samples • SOP G, added instructions for Field Science QC of crown polygons following collection • Appendix C, updated peak greenness dates to reflect newer MODIS data, added footnote for MOAB timing • Appendix E, added best practices for UAS sampling • Appendix G, minor clarifications to equipment lists, including removal of part numbers when exact brand not required

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of sunlit plant canopy tissues for analysis of total organic carbon (C) and nitrogen (N), lignin, chlorophyll, major and minor elements, isotopic composition ($^{13}C/^{12}C$ and $^{15}N/^{14}N$), and leaf mass per area (LMA). NEON quantifies changes in foliar chemical and structural properties over space and time as these are commonly associated with key ecological processes including productivity, decomposition, and herbivory. Similarly, NEON measures the isotopic composition of leaves, as well as leaf litter, roots, and soils, thus enabling end-users to follow spatio-temporal changes in ecosystem C and N cycles.

Plant C and nutrient data are generated in collaboration with the Airborne Observation Platform (AOP), which is largely responsible for mapping plant chemical and physical characteristics across the observatory using hyperspectral and LiDAR measurements. In large part, ground-based foliar data will be used to calibrate and validate plant trait models based on AOP data. Such data can help the ecological research community to develop and refine algorithms to map canopy constituents using hyperspectral data. Additionally, foliar data informs species and site-level estimates of canopy chemical constituents and how those change over time. These estimates have value independent of remote sensing observations.

Foliar chemistry data can provide scientists, managers, and decision-makers with important information on ecosystem nutrient status. Comparing these data with those from other ecosystem components, including atmospheric deposition, soils, leaf litter, and surface water, allows investigators to evaluate material fluxes across the landscape. As a long-term dataset, they can be used to address how ecosystems change with time, as well as in response to drivers such as climate, invasive species, and land use/land cover change. For example, changes in precipitation patterns alter photosynthetic rates, and thus the uptake of nutrients like N and their conversion into leaf biomass. Such changes to canopy nutrient concentrations may cascade through the ecosystem, changing fluxes and biogeochemical transformations across the landscape.

The rationale underpinning the timing, frequency, and spatial extent of canopy foliar sampling is outlined in the NEON Science Design for Terrestrial Biogeochemistry (AD[06]). The timing of sampling allows researchers to assess canopy biogeochemical dynamics within a window of particular importance to ecosystem processes – namely peak greenness, and thus depends on the dominant drivers that affect plant phenology, hydrology, and other stocks and flows of nutrients in ecosystems. The frequency of sampling, with repeated measurements of plots and individuals over time, allows researchers to track temporal dynamics of foliar chemical and structural change. Species selection, based on site-level abundance, enables sampling of a representative mix of canopy vegetation species spanning the range of physiological and ecological variability of the site and is useful in developing relationships with AOP data. Finally, the extent of canopy sampling allows researchers to evaluate the spatial heterogeneity of

canopy nutrient dynamics. For instance, differences in soil type, hillslope aspect, and forest age affect soil N availability, which can translate to the canopy and affect spatial patterns of primary productivity.

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 canopy foliage sampling and trait measurement methods developed by the community, and many scientists working in the field provided valuable input. Relevant papers that describe these methods include Vaieretti et al. (2007), Smith et al. (2008), Asner and Martin (2009), Serbin et al. (2014), and Graves et al. (2018). K. Dana Chadwick and Sarah Graves and were especially helpful in developing the digital crown mapping workflow. Laboratory processes for LMA measurement are modeled on the 'New handbook for standardized measurement of plant functional traits worldwide' (Pérez-Harguindeguy et al., 2013).

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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan
AD[06]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry

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 Data Products Catalog
RD[04]	NEON.DOC.014037	TOS Protocol and Procedure: HBP – Measurement of Herbaceous Biomass
RD[05]	NEON.DOC.001710	TOS Protocol and Procedure: LTR – Litterfall and Fine Woody Debris
RD[06]	NEON.DOC.014048	TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Sampling
RD[07]	NEON.DOC.014038	TOS Protocol and Procedure: BGB – Plant Belowground Biomass Sampling
RD[08]	NEON.DOC.001716	TOS Standard Operating Procedure (SOP): Toxicodendron Biomass and Handling
RD[09]	NEON.DOC.001717	TOS Standard Operating Procedure (SOP): TruPulse Rangefinder Use and Calibration
RD[10]	NEON.DOC.000987	TOS Protocol and Procedure: VST – Measurement of Vegetation Structure
RD[11]	NEON.DOC.001576	Datasheets for TOS Protocol and Procedure: Canopy Foliage Sampling
RD[12]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[13]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment
RD[15]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[16]	NEON.DOC.005346	OS Standard Operating Procedure: FRZ – Preparation and Use of Dry Ice Alternative Freezing Materials

2.3 Acronyms

Acronym	Definition
C	Carbon
N	Nitrogen
LMA	Leaf Mass Per Area
LiDAR	Light Detection and Ranging
UAS	Unoccupied Aerial System
FAA	Federal Aviation Administration

2.4 Definitions

Clean technique: Procedures to minimize the introduction of chemical contaminants into a sample. Contamination can result from: dust or dirt particles, non-purified water, sweat, hair, and other environmental sources.

Hyperspectral imaging: Also known as imaging spectroscopy, involves detection of how objects interact with light from a large number of continuous spectral bands. For vegetation, this allows for characterization of foliar chemical and structural properties.

Sunlit foliage: Foliage that is situated in the outer and upper part of the canopy and/or receives sun light for at least 3 hours of the day.

Shapefile: a vector data format for storing the location, shape, and attributes of geographic features

Fulcrum: Software tool used to create NEON electronic data entry applications.

Field Maps: Software tool used for creating crown polygon shapefiles, also used for site navigation.

Service Now: Software tool used for problem/incident tracking and resolution.

3 METHOD

The goal of this protocol is to sample the sunlit vegetation found across a site, capturing the major components of what the AOP 'sees' during overflights (**Figure 1**). Foliar chemistry and structure vary considerably both between species and through time; when possible, the same individuals will be sampled over time.

Figure 1. Aerial photos of plant canopies at two NEON sites: San Joaquin Experiment Range (SJER, left) and Great Smokey Mountains National Park (GRSM, right). Photos from NEON's Aerial Observation Platform (AOP).

A subset of the Distributed Base Plots located across the study area are used for Canopy Foliage (CFC) sampling. In forest and shrubland sites, these plots are largely the same as the ones used for Vegetation Structure (VST) monitoring. Within the tower airshed, select Tower Plots (whose sizes differ by location) are also utilized. Sampling within plots facilitates data georeferencing and streamlines integration with AOP. It also simplifies longitudinal sampling and allows canopy chemistry data to be linked to other plot-scale soil and vegetation measurements. Lastly, sampling within plots is often mandated by permitting agreements with site hosts. Specific Tower and Distributed Plot locations for CFC sampling are provided in a separate document to NEON field ecologists.

In sites dominated by woody cover (e.g., forests and shrubland, Type A sites in **Table 1**), the general procedure is to sample at least one individual of each species found in the site-level, sunlit canopy. For the more common species replicates are taken, spanning whatever gradients are relevant to a site (topography, aspect, soil type, stand age, etc.). Rare species are sampled, but only where feasible (e.g., target taxa with sunlit foliage present in a CFC plot). Each site has a target sample number proportional to its canopy diversity, as assessed using stem counts. Target taxa lists including per-species sample numbers have been generated by NEON Science and are included in the CFC: Target Taxa Lists Fulcrum application. To help data users unambiguously geolocate foliar samples and link the data with remote

sensing imagery, a digital crown polygon is created for many of the woody individuals sampled. More detail and guidance is provided in the Standard Operating Procedures (SOPs) below.

Table 1. Sampling approaches for each site depending on dominant site vegetation.

Site Vegetation Type	Majority of Samples	Target sample number	Sampling Approach	Sites
Type A: dominated by *woody cover*[a]	Sun-lit leaves from single species	Determined by site-specific lists, posted to Fulcrum	• focus on sampling taxa from site-specific lists within CFC plots • distribute replicates across site gradients • if open areas prevalent (e.g., mixed site), take several representative sun-lit clips (max = 8) from CFC plots as needed to characterize non-woody diversity	ABBY, BART, BLAN, BONA, CLBJ, DEJU, DELA, DSNY, GRSM, GUAN, HARV, HEAL, JERC, JORN, LENO, MLBS, MOAB, NIWO, ONAQ, ORNL, OSBS, PUUM, RMNP, SCBI, SERC, SOAP, SRER, STEI, TALL, TEAK, TREE, UKFS, UNDE, WREF, YELL
Type B: dominated by *herbaceous cover*[b]	Mixed clip strips	20-24	• one clip per 20 x 20 m plot or subplot assigned for CFC sampling	BARR, CPER, DCFS, KONA, KONZ, LAJA, NOGP, OAES, SJER*, STER, TOOL, WOOD

[a]For the purposes of this protocol, includes large cacti and palms –not woody, but functionally analogous
[b]Also includes short-statured woody species that grow like bushes and provide continuous sun-lit ground cover
*This hybrid site will follow both workflows, e.g., clip strip sampling in assigned CFC plots as well as woody individual sampling according to site-specific list. As such, sample numbers will be > 20-24.

If Type A sites have significant open areas, then they are categorized as a mixed site and several representative herbaceous clip strips are also collected (**Table 1**). To meet this threshold, a site should have > 25% open (non-woody) cover in at least five CFC/VST plots. This assessment should be made by NEON field ecologists qualitatively using aerial photos or Google Earth images as well as site-specific knowledge. If uncertain whether a Type A site qualifies as mixed, contact NEON Science to discuss.

IMPORTANCE OF SAMPLING SUNLIT FOLIAGE

It is critical that foliar samples from woody individuals come from the outer-most part of the canopy, e.g. **they must be sunlit leaves** (receiving at least 3 hours of sunlight per day, see 2.4 Definitions). The AOP remote-sensing instruments scan sun leaves at the top and sunlit sides of the canopy. Because we are interested in linking AOP measurements with terrestrial observations, it is important that only sunlit leaves be collected. Aside from AOP concerns, it is important that we sample from a similar light environment to produce inter-comparable leaf trait data across the Observatory. Leaves should be sampled from near the apex of the tree whenever possible. When this is not possible, they must be collected from sunlit, side of canopy positions.

In many forested systems, the canopy is well out of human reach. To obtain sunlit leaves, it is necessary to use tools such as an unoccupied aerial system (UAS) comprised of a drone plus robotic sampler, line launcher, slingshot, tree climbers, or other methods agreed-upon with NEON Science. Some of these approaches require the participation of persons with specialized skills, such as a pilot with a Part #107 Federal Aviation Administration (FAA) license, or a capable, trained tree climber. If an individual with specialized training is needed, additional participation of two or three NEON personnel who can work alongside this individual to subsample, bag, and preserve samples is required. Field personnel should review Appendix E and consult with Science to resolve questions about how to obtain sunlit leaves.

In woody systems with low or medium-stature vegetation (anything less than ~ 6 m tall), sunlit leaves can easily be obtained using clippers and extendable pole pruners.

In systems dominated by herbaceous vegetation (i.e., Type B sites, **Table 1**), bulk herbaceous plant biomass is harvested from a set of assigned CFC plots using clip strips. This clip strip method is similar to the one described in TOS Protocol and Procedure: HBP - Measurement of Herbaceous Biomass (RD[04]), but with key high-level differences that are detailed in SOP C. One of the Type B sites with significant woody cover will also sample woody individuals according to a site-specific target taxa list. This 'hybrid' site is identified in Table 1. Hybrid sites may or may not take clip strips from all assigned CFC plots, depending on whether the canopy is open enough to allow for sunlit herbaceous vegetation.

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

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

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Canopy foliage samples are collected according to the schedule in **Table 2**. Each NEON site conducts CFC sampling once every 5 years. We expect that this will sufficiently capture long-term foliar trends and provide sufficient data to develop trait models from the more frequently collected AOP data. Implementation of this protocol is scheduled on an inter-annual basis at a given site as part of a suite of synchronized TOS measurements aimed at characterizing plant and soil biogeochemical dynamics. Synchronized protocols include:

- TOS Protocol and Procedure: Litterfall and Fine Woody Debris, litter chem component (RD[05])
- TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling, including the biogeochemistry components (RD[06])
- TOS Protocol and Procedure: Plant Belowground Biomass Sampling (RD[07])

Table 2. Sampling frequency for Canopy Foliage procedures.

SOP	Plot Type	Plot Number	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
All	Tower and Distributed	Type A: variable Type B: 20	One to several weeks	1X per sampling year	NA	5 y	Sampling year is synchronized with 'Chemistry Group' protocols. Schedule bout in close coordination with AOP overflight.

Scheduling Considerations

1. **Coordinating with AOP overflights:** The timing of AOP data collection, which coincides with the historical timing of peak greenness, largely determines the timing of canopy foliage sampling at a given site. Ground and airborne datasets are often analyzed together, so effort should be made to coordinate them as closely as possible.
2. **Field Work and Laboratory Processing:** After foliage samples are collected, the following points are critical with respect to timing:
    a. Keep samples cold and moist until they are processed– coolers with ice packs in the field, transfer to 4°C refrigerator upon return to the laboratory
    b. Begin laboratory processing as soon as possible, but not longer than 5 days post-collection. Chlorophyll samples must be shipped within 7 days of collection.

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling shall be scheduled to begin within **the first week of the AOP flight window at the domain**, meaning sample collection should start no earlier than the first day of the AOP window and no later than the 7th day. This will increase the chance for overlap between ground and aerial data collection because the AOP prioritizes foliar sampling sites when first arriving in a domain. This should coincide with peak greenness at many sites and for many plants. However, if field ecologists are concerned that the AOP timing will not align with peak greenness of the majority of site vegetation, they should submit a proposed schedule change as soon as possible so that Science, AOP, and Field Science can discuss the best course of action and whether the field or flight schedules can or should be adjusted.

In agricultural sites, the timing of peak greenness is largely determined by crop type, and this may vary substantially within a site. In general, this is OK and sampling will focus on collecting what is present in assigned plots at the time of the flights. However, if the AOP schedule is misaligned with the *majority* of the site's green up schedule, Field Science should submit a proposed schedule change so that Science and AOP can discuss. Additionally, Yellowstone National Park's Bear Management Plan imparts closures for bears in particular areas of the Park from March 10 to June 30, and the YELL Tower plots are located within a closure area. As such, YELL Tower plot canopy foliage sampling cannot occur in this timeframe.

Sample bouts should be completed as soon as possible after their initiation to ensure that foliar chemical measurements will be relevant toward building relationships with AOP data, since foliar traits may change over the course of the growing season. Bout durations will vary widely depending on sample number and vegetation type, but in general should take between 5-15 sampling days (e.g., 1-3 weeks) to complete the field sampling component. Make all attempts to finish sample collection within 3 weeks of starting the bout. If this is not possible, four weeks is the absolute maximum bout length.

4.3 Timing for Laboratory Processing and Analysis

To stabilize and preserve foliage, samples should be placed on cold packs in coolers as soon as they are collected. Additionally, a small subsample for chlorophyll analysis must be cleaned and then immediately flash-frozen with dry ice and maintained frozen in the dark, since pigments are very sensitive to degradation. In the Domain Support Facility, chlorophyll subsamples should be transferred to an ultra-low temperature freezer and maintained at -80°C, while samples for LMA and chemistry should be placed in a refrigerator and maintained at 4°C until further processing.

Scanning of fresh foliage for LMA measurement must be completed **within 5 days** of canopy foliage sample collection; cleaning and placement of chemistry samples into the drying oven should also begin within this timeframe. Failure to initiate LMA scanning and chemistry sample drying within this window can result in mass loss and rotting, rendering samples unsuitable for analysis. This is especially true for broadleaf sample types. For sites requiring more than 5 days of field work, sampling should either be split up to accommodate this lab work, or multiple teams can be used.

Holding times for completion of laboratory activities and sample shipment to external laboratory facilities for the different subsample types are described in **Table 3**. For detailed shipping information, refer to the protocol for Shipping Ecological Samples, Sensors, and Equipment (RD[14]). Note that chlorophyll samples should be shipped within 7 days of collection. For example, samples collected on July 7th can be shipped up to and including July 14th, any later will be out of protocol compliance.

Table 3. Holding times for different foliage sample types and laboratory activities.

Sample type	Activity	Holding Time
Frozen (-80°C) Chlorophyll samples	Ship to external lab	Within 7 days of collection
Cold (4°C) LMA samples	Scan, weigh, then begin oven-drying	Within 5 days of collection
Cold (4°C) Chemistry samples	Clean, then begin oven-drying	Within 5 days of collection
Oven-dried Chemistry samples	Subsample and ship to external labs	Within 90 days of collection
Oven-dried Biogeochemistry Archive samples	Ship to bioarchive facility	Within 90 days of collection
Leaf mass per area scans	Analyze scanned area using ImageJ	Within 90 days of collection

4.4 Sampling Timing Contingencies

The guidance in **Table 4** should be followed to ensure that data quality standards are met:

Table 4. Contingency decisions for Canopy Foliage sampling.

Delay/Situation	Action	Outcome for Data Products
Samples are not kept cold following collection	Issue Incident ticket to NEON Science; potentially recollect samples or reschedule bout.	Samples likely compromised; potential delay of data products.
Dry ice is not available for field flash-freezing, and sampling cannot be postponed	Plan to freeze chlorophyll samples on ice packs if they can be transferred to an ultra-low freezer at the end of the day and dry ice will be available for shipping.	Flash freeze method deviation will be recorded in the data
LMA and chemistry samples cannot be processed within 5 days	Issue Incident ticket to NEON Science; will work together to determine whether samples should be retained or discarded. Prioritize processing broadleaf samples first, they are more sensitive to mold.	Samples may be compromised; potential delay or reduction in data product instances.

Delay/Situation	Action	Outcome for Data Products
Delay in starting sample bout, start date is more than one week after AOP overflight	Issue Incident ticket to NEON Science; will determine whether to sample outside of target sampling window.	Potential delay of data products, with implications for linking ground and airborne datasets.
Inability to finish field sample collection within 3 weeks of starting	Issue Incident ticket to NEON Science; it is likely that a 4th week of sampling will be recommended, but Science will determine whether to continue or halt sampling.	Potential delay or reduction in data product instances.

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., 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 Site Management App; refer to the Site Management and Disturbance Data Collection protocol for more detail (RD[13]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (Appendix C)
- **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 according to one of the scenarios documented in **Figure 2**, 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**). **For Canopy Foliage sampling, note that creation of Missed Sampling Fulcrum records only applies to Type B sites (Table 1)**. These are the only sites with a specific number of plots assigned for sampling that can be officially missed. Type A sites collect a variable number of individuals from a variable number of plots. As such, documenting Missed Sampling in this way is not realistic or useful.

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Scheduled sampling dates must be communicated to Science by a ServiceNow Incident
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).

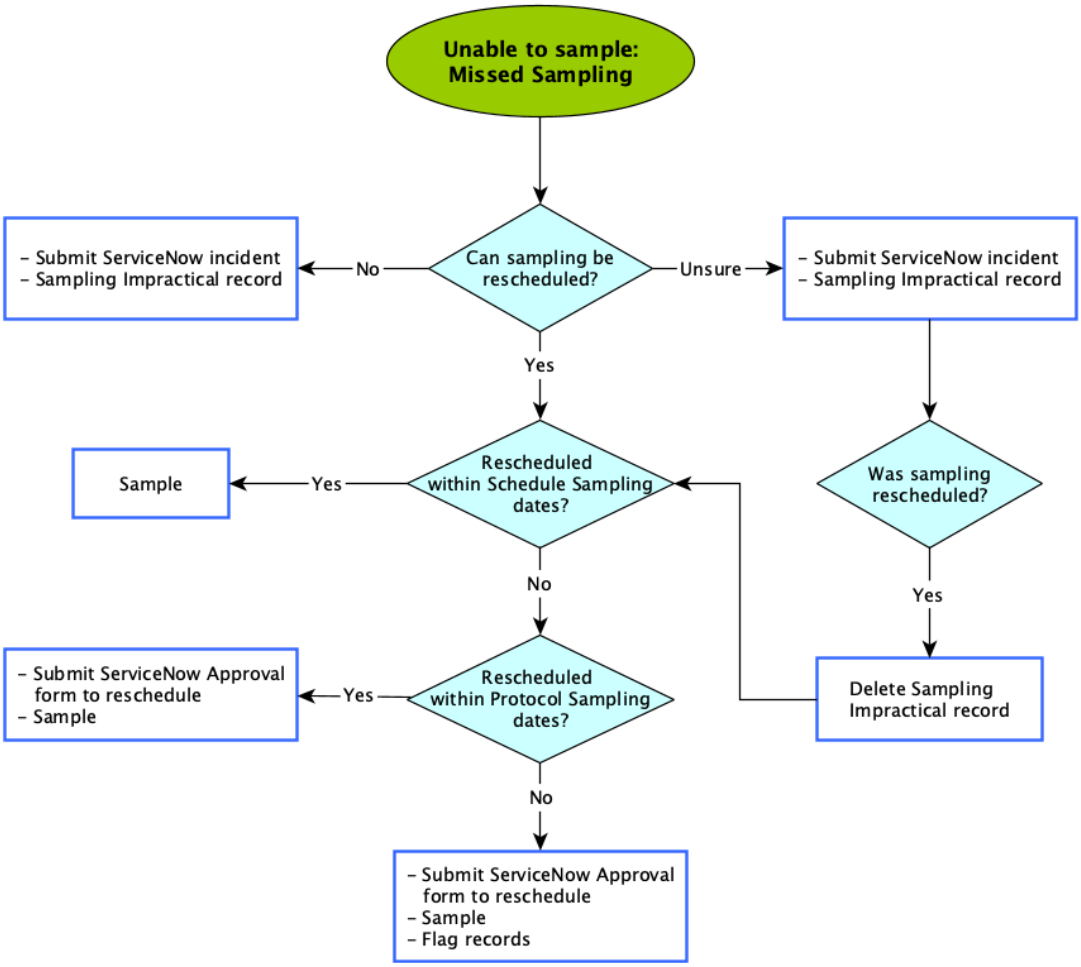

Figure 2. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

b. Consult **Table 5** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science SharePoint library. However, this protocol is the ultimate source of information should any discrepancy exist.

Table 5 Guidance for responding to delays and cancellations during implementation of the CFC protocol

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
TOS Canopy Foliage	> 1 week (7 days)	IS/OS Schedule Change Request	Submit incident ticket

2. For Type B sites, create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. That is, if data are recorded in the field at the plot or subplot level, a record must be made for each plot or subplot missed.

   a. During Canopy Foliage sampling, record each 20 x 20 m plot or subplot assigned for CFC at a Type B site but not sampled in the CFC: Field Sampling data entry application.

   b. Missing data in downstream applications (e.g., Lab apps) are not recorded. For example, if samples are normally scanned and weighed for LMA, but the samples weren't collected at all, no entries are made in the CFC: LMA application.

3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 6**).

Table 6. Protocol-specific Sampling Impractical reasons entered in the CFC:Field Fulcrum application. Only relevant to Type B sites. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
location flooded	Standing or flowing water too deep to complete sampling
location burned	Location recently burned, not possible 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
wildfire	Sampling location inaccessible due to active wildfire or post fire safety hazards
wildlife hazard	Wildlife hazard, specific hazard described in remarks
safety	Unsafe conditions at sampling location or on route to sampling location
other	Sampling location inaccessible due to other ecological reason described in the remarks

4. For sampling events that occur outside of the defined Protocol Sampling Dates, or more generally when the protocol target conditions are not met, a protocol-specific Flag should also be recorded.

    a. For example, if sampling occurs when the plants are not in peak greenness, this must be indicated with an appropriate choice in the **Plant Status** and/or **Sample Condition** fields. See SOP B and SOP C for details.

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below (**Table 7**) 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, or during the first couple of samples when techniques are being learned). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, submit an Incident ticket.

Table 7. Estimated staff and labor hours required for implementation of Canopy Sampling.

SOP	Estimated time (hours)	Suggested staff	Total person hours
A: Preparing to sample	8-16	1-2	8-32
B: Field Sampling, closed canopy forest, UAS + robotic sampler	0.5/sample	3	1.5/sample
B: Field Sampling, closed canopy forest, line launchers etc	2/sample	2 or 3	4-6/sample
B: Field Sampling, open canopy, shrublands	1/sample	2	2/sample
C: Field Sampling, herbaceous	1/sample	2	2/sample
D: Post-field sampling tasks	3	2	6
E: LMA Initial processing	0.25/sample	2	0.5/sample
E: LMA Image analysis	0.5/sample	1	0.5/sample
F: Drying and Subsampling for chemical analyses	0.5/sample	1	0.5/sample
G: Data entry and verification	2-4	1	2-4
H: Sample Shipment	1/shipment	1	1/shipment

5 SAFETY

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

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

For sampling in tall- and medium-stature forest, meaning woody vegetation is collected using any tool beyond simple hand clippers, Personal Protective Equipment (PPE) required for this activity includes the following items:

- Safety glasses
- Hard hats

Tall canopy sampling may additionally require the use of hearing protection and specialized equipment, such as when using a UAS, line launcher, or slingshot to obtain leaves from the outer, sunlit portions of the canopy. Field personnel must familiarize themselves with safety procedures for each of these methods.

For Unoccupied Aerial System (UAS) Sampling:

- Obey all instructions from the certified drone pilot.
- Be cautious with the grasping arm of the foliage sampler as this is where the cutting blade is.
- Be aware of where the UAS is flying and do not stand directly under it when possible. Hard hats must be worn during UAS operation at all times.
- If the UAS gets stuck in a tree, this may require an emergency release of the sampler from the drone. Good communication will be needed between the pilot and the technician at the base of the tree to coordinate a safe emergency release.

For Slingshot or Line Launcher Canopy Sampling:

- Download instructions provided by the manufacturer and ensure that all members of the sampling team are familiar with them. Do not alter the tools or make custom modifications, use only as specified by the manufacturers.
- PPE required during Line Launcher and Slingshot Canopy Sampling includes the use of a hardhat (or ANSI Certified climbing helmet), safety glasses, and work gloves. For pressurized line launchers, hearing protection may also be required – especially those models that fire blank cartridges. Follow manufacturer recommendations.

- Members of the field team should always stand behind the person handling the line launcher or slingshot, especially once the device is pressurized or otherwise engaged for launching.
- Make sure the area is completely clear of tourists or other scientists before using the tools. Line launchers and slingshots can easily propel a throw weight 300 feet or more.
- For line launchers, use only approved throw weights with the device.

Other Safety Hazards

A laser rangefinder is used to determine height of the sample collected. Avoid staring directly at the laser beam for prolonged periods. The rangefinder is classified as eye-safe to Class 1 limits, which means that virtually no hazard is associated with directly viewing the laser output under normal conditions. As with any laser device, however, reasonable precautions should be taken in its operation. It is recommended that you avoid staring into the transmit aperture while firing the laser.

If *Toxicodendron* spp. are present at a given site, Field Operations should utilize the supplies and procedures outlined in TOS Standard Operating Procedure: Toxicodendron Biomass and Handling (RD[08]) in order to minimize exposure while sampling and handling vegetation and to properly clean equipment. Additional instructions on how to handle canopy foliage samples and subsamples containing *Toxicodendron* spp. are provided in the SOPs below.

A Wiley Mill is used to grind vegetation prior to shipment for analysis and archive. If the mill is not being used in a fume hood, staff should use a dust mask to prevent inhalation of fine particles.

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

Field personnel are to be trained in local plant species identification and safe working practices for canopy sampling, including use of clippers, canopy pruning equipment, and UAS/line launcher safety. In sites with woody vegetation (Type A), field personnel must be trained in proper use of a laser rangefinder in order to map the position of individuals and determine the heights from which canopy samples are obtained.

Additionally, before field personnel can measure leaf mass per area on CFC samples using ImageJ software, they must be able to analyze standard images within the area threshold specified in the training materials (with 98% accuracy for broad leaf samples and 95% accuracy for conifer needles and mixed herbaceous samples). Standard images are located in the NEON Training Center; it is the responsibility of the lead Field Ecologist to ensure this requirement is met.

6.2 Specialized Skills

When sampling in sites with woody vegetation (Type A), personnel must be familiar with the plant species present at each site. Field guides and a person with plant expertise on the domain staff should be available during the field effort. Personnel should be prepared to take extensive notes on any anomalous species or features observed when sampling. If a species cannot be identified in the field, use datasheets or field notebooks to take notes, take a representative sample, and work with experts in the Domain Support Facility to identify it upon return from the field.

When sampling tall canopies where the UAS is not permitted and methods such as tree climber are not permitted or practical, one member of the team must be familiar and practiced with use of a line launcher or slingshot. See Appendix E for best practices related to operation of these tools.

HOW MANY PEOPLE ARE NEEDED FOR FIELD SAMPLING?

Sampling of herbaceous and low-stature canopies can likely be completed without issue by a two-person Field Science team. However, in tall canopies where a UAS, line launcher, or slingshot is needed to obtain sun-lit leaves, *a three-person team is very advantageous*. One person on the team should be a plant expert capable of identifying local species.

7 STANDARD OPERATING PROCEDURES

SOP Overview

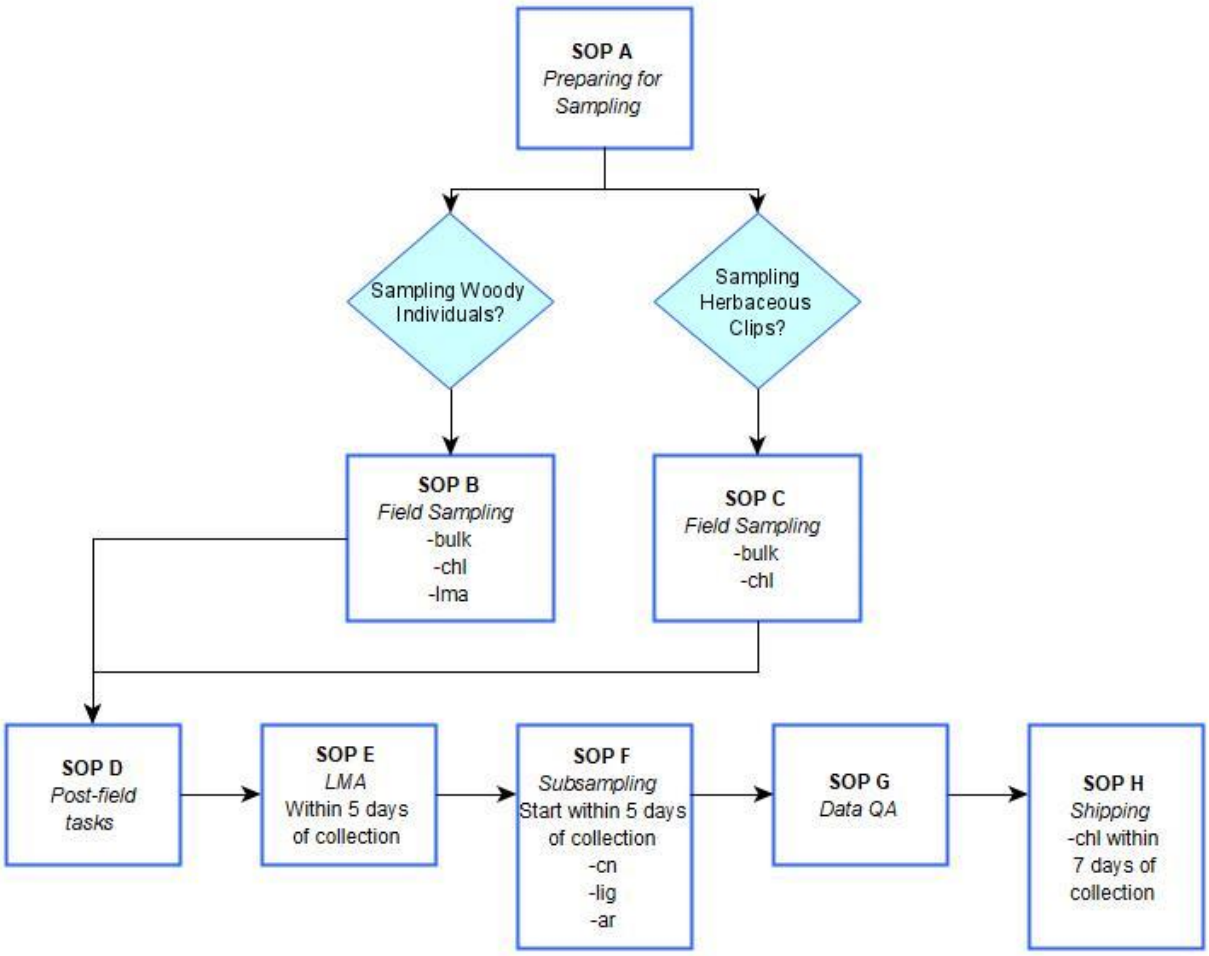

Figure 3. A high level overview diagram of all SOPs. Diamonds represent a decision point that must be assessed.

- SOP A: Tasks to complete in the Domain Support Facility in preparation for CFC sampling
- SOP B: Methods for field sampling of foliar tissues, woody individuals
- SOP C: Methods for field sampling of foliar tissues, herbaceous vegetation
- SOP D: Tasks to complete in the Domain Support Facility following CFC sampling
- SOP E: Measurement of leaf mass per area (LMA) for foliar samples: clean leaves, scan, measure area using ImageJ software, obtain fresh and dry sample weights
- SOP F: Clean, oven-dry, then subsample foliar tissues in preparation for external laboratory chemical analyses
- SOP G: Guidelines and requirements for successful data entry
- SOP H: Package and ship samples to external laboratories and the biorepository

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry and the only mechanism for creating crown polygons when sampling woody individuals. Mobile devices should be fully charged at the beginning of each field day. When creating crown polygons, a GPS-enabled iPad must be available, with mapping layers saved to Field Maps for offline-use prior to sampling.

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 Preparing General Equipment

1. Plan and save sampling routes for field teams using standard site navigation procedures (RD[15]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots.

Table 8. Equipment and supply preparation checklist.

✓	Item	Action
	GPS unit and/or mobile device with Field Maps	Charge and load target plot locations
	TruPulse 360R	Prepare for sampling • Check battery, charge • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination[1]. See RD[09] for details. • Calibrate TruPulse tilt-sensor – only necessary after severe drop-shock; see RD[09] for details.
	Scanner + ImageJ	Check for compatibility of scanner settings and ImageJ • Collect a few local leaves for scanning • Execute sections E.1 **and** E.2 of the LMA procedure • Ensure all steps work smoothly - if not, make adjustments[2]
	Hand clippers & pole pruners	Clean and sharpen blades (if necessary)
	Re-usable cold packs	Place in –20 ˚C freezer
	Dry Ice	Ensure an adequate amount is available to fill two coolers
	Sample bags	Organize and pre-label, see below.

[1] Declination changes with time and should be looked up annually per site: http://www.ngdc.noaa.gov/geomag-web/

[2] The most common issue is with file formats – both .jpeg and .tiff are acceptable, .pdf is not

A.3 Determining Methods and Supplies

1. Use the information in **Table 1** along with knowledge of site vegetation types and heights to determine which method(s) will be needed to obtain sun-lit canopy foliage samples. If multiple vegetation types and/or height mixtures are found at a site, multiple methods may be required. *If this is the first time a Field Ecologist has conducted this work, reach out to other Domains and NEON Science for input*. Recommended methods include:

    - herbaceous vegetation[a] = hand clippers
    - woody individuals[b] = depends on height:
        - 0-2 m = hand clippers
        - 2-6 m = extendable pole pruner
        - 6-10 m = line toss, slingshot, or long-reach pole pruner (30 ft)
        - 10 m = UAS/line launcher/slingshot/tree climbers

[a]*Also includes short-statured woody species that grow like bushes and provide continuous sunlit ground cover*
[b]*For the purposes of this protocol, includes large cacti and palms – they are not woody, but functionally analogous*

2. Review equipment lists to determine whether all required items for the method(s) are available. If relying on a pole pruner, purchase a high-quality telescoping or extendable model to maximize reach into the canopy, some types allow up to a 30-foot reach (**Table 21**).

3. Sites with tall-canopy vegetation where UAS sampling is permitted (**Figure 4,** left) will be contacted by Science and a certified pilot well before the bout to coordinate UAS logistics. Appendix E contains helpful tips on preparing for and executing UAS sampling.

4. If using a line launcher or slingshot to obtain tall canopy foliage:

    - Read through Appendix E to familiarize yourself with the techniques.

    

    Spend a full day practicing use of that device in a nearby area with relevant vegetation (**Figure 4,** right). This will allow for trouble-shooting of issues and increase familiarity with the equipment and technique, greatly increasing efficiency during sampling bouts.

Figure 4. Left: UAS used for canopy foliage sampling. Right: Practicing use of a line launcher.

- Plan to bring two backpacks to the field, one to carry the line launcher/slingshot equipment, and the other to carry supplies for processing foliage.

5. If plots are accessible, sampling can be expedited by pre-selecting and flagging individuals or clip strips, according to the guidelines described below.

A.4 Additional Prep, Woody Individual Sampling

1. Type A sites (**Table 1**) should review the site-specific target taxa lists provided by Science. If any issues are noted, for example species observed in the canopy are not listed, or taxa with high replication in the list are uncommon in the sunlit canopy, notify Science via ServiceNow.
2. Using the lists, the lead Plant Ecologist should create a sampling plan by identifying candidate individuals (required for UAS sampling), or at least a list of plots where likely candidates can be found (all other sampling methods). Guidelines to inform the sampling plan are provided in **Table 9**. Follow these requirements and the steps below to plan for sampling.

Table 9. Guidelines for CFC plot and sample selection.

Variable	Minimum	Maximum	Notes
Plots to sample	8	all	This will ensure samples are spread across the site. Choose from 'CFC' unique plot list, capture site gradients (soil type, elevation, aspect, etc). Aim for coincident soil plots where feasible.
Percent of samples from Tower plots	10%	40%	This will ensure samples are spread across the site, capture site gradients.
Samples per plot	none	4	This will ensure samples are spread across the site and sampled crowns are generally non-overlapping.

a. Download or examine the Apparent Individual data collected during Vegetation Structure (VST) monitoring for a given site.

b. Sort by **Taxon ID**, then hone in on live individuals likely to be in the sunlit canopy. Depending on the site, this could be accomplished using the variables **Canopy Position**, **Growth Form**, **Plant Status**, **Stem Diameter**, **Height**, or other metrics. In general, select the taller trees as these will be the ones in the sunlit portion of the canopy, and only canopy positions 1-3 (1-2 preferred, see **Table 13** in SOP B or the Vegetation Structure protocol for definitions).

c. If VST data are not available for all plots, use familiarity and knowledge from other field work (e.g., Plant Diversity) to determine which CFC plots are likely to have prominent, sunlit canopy individuals of the target taxa. Plan to visit those plots to find candidate individuals to sample and be ready to map and tag before the bout or while sampling (more below).

d. Assemble the list of individuals sampled during the last CFC bout and include those in the sampling plan. They are good sampling targets as long as other criteria are met.

e. For more common species, there will be many individuals to choose from. Use the guidelines below as well as those in **Table 9** to determine which to target:

- Identify candidate individuals from plots that span varied habitats to cover gradients in topography, aspect, soil type, stand age, etc.
- For species replicates, plan to collect samples from different plots, ideally those varying in topography, aspect, soil type, stand age, etc.
- Where possible, select candidate individuals from plots designated for soil sampling. This will maximize cross-protocol data linkages.
- Individuals sampled for CFC should generally have prominent, non-overlapping crowns. That said, when the largest, most canopy-dominant trees are selected for sampling (more likely with UAS sampling), some intermingling of crown edges between neighboring samples is OK.
- Include rare species as candidates, but they will only be sampled where feasible (e.g., possible to sample sunlit leaves in or near a plot in a reasonable amount of time).

3. When planning a bout that involves UAS sampling, the following considerations are critical:

a. Field-based scouting and pre-selection of trees is especially helpful to maximize efficiency and sampling time with the UAS, which tends to be on site for only 1 week.

b. The UAS cannot penetrate the canopy, thus only the tallest trees are acceptable sampling targets. If the tallest trees in plots are not mapped and tagged, this can be done while preparing for the bout (easier given fast pace of UAS collection), or during sampling.

c. Plan for where the UAS will launch relative to each sampling target or plot. A canopy opening at least 4 m wide is needed, most forest roads should be suitable as well as some large gaps with low-density understory. Mark potential launch points on a site map for the UAS team.

d. The UAS can only transmit the camera signal 150-300 m from the launch point, depending on topography and canopy density. For targets located farther than this distance, alternate sampling methods will be required.

4. Record candidate individuals or plots, then send that list to Science via a ServiceNow request as soon as possible (minimum 2 weeks prior to starting the bout). For UAS sampling, locations of target trees will be programmed into the drone. Additionally, regardless of sampling method, target stem locations will be loaded into Field Maps to assist with creation of crown polygons.

5. Target tags should also be recorded in the WorkTracker to keep organized during field collection (see Appendix A). It may also be helpful to print out plot maps from the VST Peregrine application for all plots that will be visited, with target tags marked.
6. Just before the bout begins, download maps of candidate plots to use for crown polygon creation in the field:
    a. If you have never logged in to the NEON ArcGIS Online (AGOL) account, do so to create a profile. Otherwise, skip to STEP (b)
        1) Using a computer and any browser, search for 'AGOL'
        2) Click the first link, then *Sign in* using Enterprise login (**neon**.maps.arcgis.com) and your standard NEON credentials to access the site
        3) Follow prompts to create your profile.
    b. Obtain a GPS-enabled iPad with the ArcGIS Field Maps app installed.
    c. Open Field Maps, then sign into the NEON AGOL account, using Enterprise login (**neon**.maps.arcgis.com) and your standard NEON credentials.
    d. Navigate to the Crown Mapping YEAR group folder, then find the map(s) created for your site. Large sites (> 10 km^2) will have multiple maps, with several plots in each. If you do not have access to the Crown Mapping group, contact Science to change the permissions.
    e. Click on 3 blue dots to *Add Offline Area*
    f. Using two fingers to zoom in or out, change the map area to an appropriate size. It is OK to be very 'zoomed out' such that only markers but not AOP layers are visible. This will allow for download of larger areas that contain multiple plots, with fewer areas to manage (Field Maps has a maximum size for offline scenes).
    g. Using one finger, navigate to a plot or group of plots where CFC samples may be taken, using the plot markers and/or stem location markers as a guide.
    h. When the desired area is selected, *Download Area*. *Rename Area* by clicking on 3 blue dots.
    i. Download as many areas as needed to encompass plots where samples might be taken.
7. Become familiar with the different layers in the map. Click the blue 'stack of books' icon in the top-right corner to view the list of layers as in **Figure 5**. Most sites will have the following layers:
    a. Canopy Height Model (CHM): displays either the absolute and/or relative heights of the trees. Red indicates tall, blue indicates ground or heights < 2 m
    b. High-resolution Camera (Cam): 10 cm resolution, but may appear 'wavy' at this scale due to geolocation corrections

c. False-color spectrometer (FCol): displays reflectance for specific wavelengths from the hyperspectral sensor, as suggested in Graves et al. (2018). Different colors generally correspond to different foliar chemistry and possibly different taxa.

d. Maps also show NEON field markers and stem locations for target tags

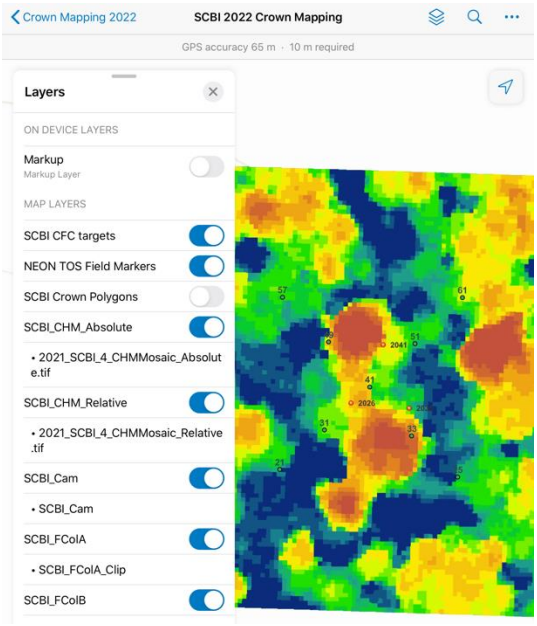

Figure 5. Field Maps interface with the list of layers available to assist with crown polygon creation.

NOTE: It is possible to create crown polygons before the bout begins, for example while scouting or mapping target trees. However, this might result in extra polygons, and those must be deleted upon completion of the bout. Additionally, pay extra attention to polygonID formatting (SITE.TAG.YEAR, see B.4) if creating these ahead of time.

8. Prepare to Map and Tag. While it is desirable to sample individuals tagged for Vegetation Structure monitoring, as this will enhance coincident data, in some cases it will be preferable to sample individuals that have not been tagged. To be ready for this occurrence:

   a. Bring numbered aluminum tags to the field.

   b. Review TOS Protocol and Procedure: Measurement of Vegetation Structure (RD[10]), Mapping and Tagging SOP, and be familiar with this procedure and all associated training materials before a CFC bout begins.

   c. Note the following items as relates to CFC tagging:

      1) Individuals tagged during CFC that are found outside the plot zones reserved for plant productivity measurements, e.g., the plot core or the two randomly selected subplots, will NOT be measured during normal implementation of RD[10]. To communicate this, such definitively "CFC Only" tagIDs should be appended with a "Z" using the hand stamp and die set (example tagID = 09532**Z**).

a) In the Mapping and Tagging data entry application, selecting **CFC Only Tag = Y** for these definitive CFC Only stems allows a woody individual to be mapped anywhere in a plot, not just the 20 x 20 m core or subplots used for VST.

2) Individuals that will (or may) qualify for Vegetation Structure monitoring but simply haven't been measured yet will receive a standard tag without a Z, and should be mapped in the Mapping and Tagging data entry application with CFC Only Tag = N.

A.5 Additional Prep, Herbaceous Vegetation Sampling

1. In Type B sites, or woody sites with significant open areas (**Table 1**), clip strips will be harvested, and mixed community foliar samples will be analyzed for chemistry and LMA.
2. In preparation for this occurrence, Field Operations should review TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD [04]), Field Sampling SOP, and be familiar with the procedures and all associated training materials before a CFC bout begins. This includes:
    - how to use plot or subplot-specific clip lists to identify potential clip strip locations that have not been previously sampled or rejected.
    - how to locate X,Y-coordinates of clip strip SW corners depending on the 'offsetNorthing' and 'offsetEasting' coordinate for the clipID.

A.6 Labels and Identifiers

Regardless of the method of collection, all sample types generated during CFC sampling require a human-readable label, and most require a scannable barcode as well. Barcodes improve sample traceability and data quality. **Table 10** provides a quick reference of sample types, their barcode requirements, as well as the container types and locations of barcodes on containers. All scintillation vials should be plastic, new/unused, and stored in a way to minimize dust or other contamination.

Table 10. Sample types and barcodes used.

Sample Type	Example Identifier	Fulcrum App	Container Type	Barcode Details	Barcode Qty	Location of Barcode
Field sample	cfc.GRSM002.LITU-1.20190606	CFC: Field Sampling	Resealable plastic bag	Type I, required	1 per sample Type A = varies Type B = 20-24	Any location on bag
Chlorophyll subsample (.chl)	cfc.GRSM002.LITU-1.20190606.chl	CFC: Field Sampling	Whirl-pak bag	Type II, required	1 per sample Type A = varies Type B = 20-24	White area of Whirl-pak
LMA subsample (.lma)	cfc.GRSM002.LITU-1.20190606.lma	CFC: LMA	Coin envelope	Type I, optional	1 per sample Type A = varies Type B = 20-24	Lower part of envelope

Sample Type	Example Identifier	Fulcrum App	Container Type	Barcode Details	Barcode Qty	Location of Barcode
Carbon-nitrogen subsample (.cn)	cfc.GRSM002.LITU-1.20190606.cn	CFC: Chemistry Subsampling	20 mL plastic scint vial*	Type I, required	1 per sample Type A = varies Type B = 20-24	Side of vial oriented vertical
Lignin/ elements subsample (.lig)	cfc.GRSM002.LITU-1.20190606.lig	CFC: Chemistry Subsampling	20 mL plastic scint vial*	Type I, required	1 per sample Type A = varies Type B = 20-24	Side of vial oriented vertical
Archive subsample (.ar)	cfc.GRSM002.LITU-1.20190606.ar	CFC: Chemistry Subsampling	20 mL plastic scint vial	Type I, required	1 per sample Type A = varies Type B = 20-24	Side of vial oriented vertical

* it is possible to store -cn or -lig samples in coin envelopes, but only for small amounts of *unground* sample.

About barcode uses and placement

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future samples. Likewise, all samples destined for the Biorepository or an external laboratory must have a barcode affixed to assist in the shipping and receipt of samples. The barcodes that are used for the various CFC sample types are shown in **Figure 6**.

Figure 6. (Left) Example of a Type I barcode. Large-size, field-tolerant, with prefix of 'A' followed by 11 numbers. (Right) Example of a Type II barcode. Large-size, cryo-safe, with prefix of 'B' followed by 11 numbers.

About human-readable labels

The sample identifier convention for CFC bulk field samples (**sampleID**) is detailed in **Figure 7.** To assist with sample preparation, pre-printed labels can be made with certain information omitted.

All downstream subsamples are created from this field sample, and identifiers simply append a character string to the end of the **sampleID**, as defined by the sample type and outlined in **Table 10**. Note that the date in all identifiers is **collectDate**, it does not reflect the downstream processing date.

A.7 Preparing sample containers

1. Refer to **Table 1** and the site-specific target taxa lists on the SSL to determine approximately how many samples will be collected during the bout.

2. Each sample, be it a woody individual or herbaceous clip strip, will require a field 'bag packet' to collect foliar material. Assemble these bag packets ahead of time. Each bag packet should have the following containers, labeled and barcoded as described in **Table 10**:

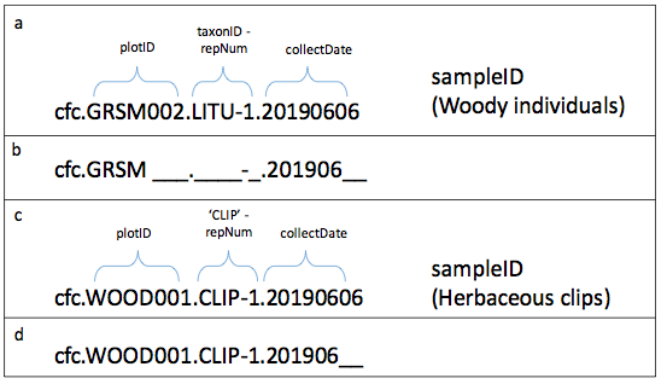

Figure 7. Annotated sampleIDs for woody individuals (a) and herbaceous clips (c). SampleIDs for pre-printing labels for woody individuals (b) and herbaceous clips (d), including areas to leave blank and fill in upon field sampling.

a. 1 gallon-size resealable plastic bag (or several for clip strips), to contain the field sample.

b. 1 4oz Whirl-pak bag, to the contain the chlorophyll subsample.

c. 1 foil packet, to protect the chlorophyll subsample inside the Whirl-pak. Pigments are sensitive to light so each chlorophyll subsample must be wrapped in foil. See **Figure 8** for instructions on how to create packets, work on a clean surface and wear clean Nitrile gloves.

d. 1 resealable plastic bag, to contain the LMA subsample (woody samples only)

- No scannable barcode required, this is applied during execution of SOP E

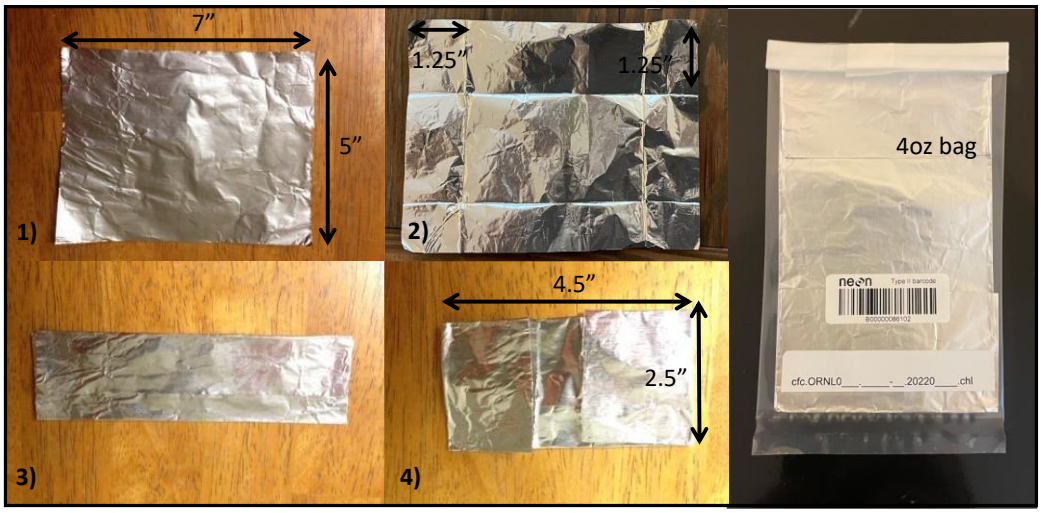

Figure 8. Steps to create foil packets: 1) cut a 7” x 5" foil rectangle, 2) mark lines to fold into thirds, 3) fold into thirds along the longer edge, 4) fold in the ends to close the packet. Will take up most space in a 4oz Whirl-pak bag.

3. Scannable barcodes should be applied to dry, room temperature containers in advance of use in the field (at least 30 minutes prior, but may be applied at the start of the season).
    a. Barcodes 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.
4. Containers for downstream samples (new, clean plastic scintillation vials and coin envelopes) can either be barcoded and pre-labeled ahead of time, or this can wait until SOP F is conducted.
    a. Barcode labels must 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 or vertically along a vial, not horizontally wrapping around a vial.
    b. Plastic scintillation vials must have their human-readable label applied using the cryogenic labels listed in **Table 24**. Only this type should be used as standard address labels do not adhere well to the plastic. The label should be oriented vertically so it is easier to read.
5. Each plastic bag that will contain a field sample or LMA subsample (if applicable) will contain a moist paper towel, added in the field. The purpose is to maintain turgor pressure and moisten leaf surfaces to facilitate cleaning. Prepare deionized water and sufficient paper towels to bring to the field.
    a. It is ok to pre-moisten paper towels the day before use; if so, store them in a sealed plastic bag and handle wearing Nitrile gloves.

A.8 Preparing for collections without dry ice

1. If dry ice is not available to take to the field for chlorophyll subsample flash-freezing, plan to freeze those subsamples on ultra-cold ice packs instead. This will be recorded in the data.
    a. Refer to RD[16] for detailed instructions on how to prepare and use these alternate materials
    b. In this case, ‘dry ice’ references in SOP B and SOP C actually refer to ultra-cold ice packs
2. Make sure that you will be able to be transfer samples to an ultralow freezer or a dry ice cooler at the end of the sampling day, and that dry ice will be available for shipping within the holding times specified in **Table 3**. If either of these conditions cannot be met, issue an Incident for Science, who will likely advise that we forgo chlorophyll subsampling.

SOP B Field Sampling, Woody Individuals

Fulcrum App = **CFC: Field Sampling**. Fulrcum Manual is available in the NEON SSL.

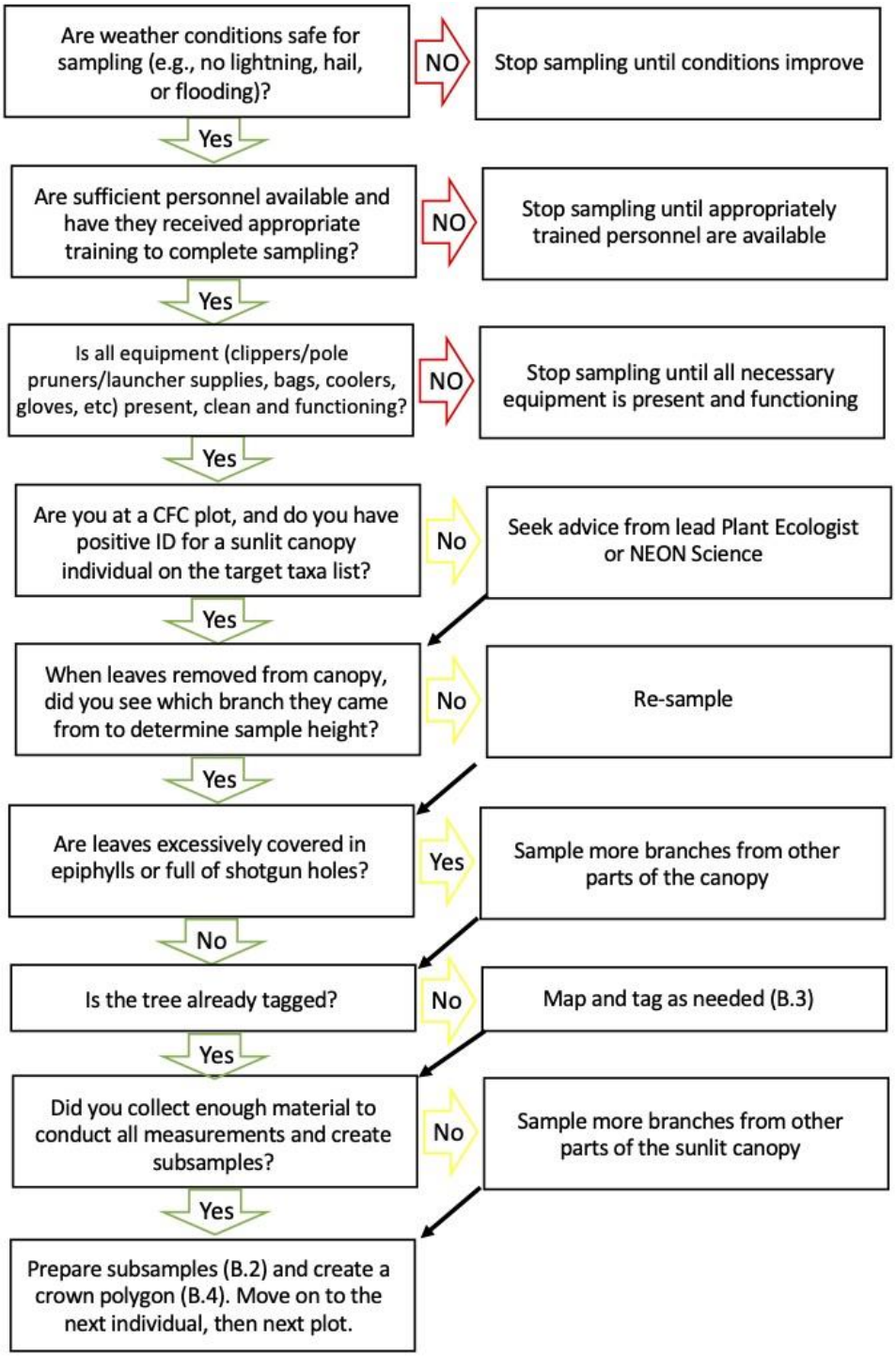

Figure 9. Overview diagram of SOP B, Field Sampling for Woody Individuals.

B.1 Collecting Foliar Material

1. Using the Work Tracker and sampling plan developed in SOP A, navigate to a plot where there is at least one candidate for sampling.
2. Ensure there is one dry ice *and* one ice pack cooler available at the plot to preserve fresh samples. A second of each cooler type can be left in the vehicle for sample storage.
3. Survey all candidate individuals in the plot and determine which (if any) are suitable for sampling. Recall that crowns must be sunlit (**Figure 10**). To facilitate robust linkages with AOP remote sensing data, two sampled crowns should generally not overlap. However, if one or both are very large, canopy-dominant trees (more common when sampling with the UAS), modest crown overlap between neighboring samples is acceptable.

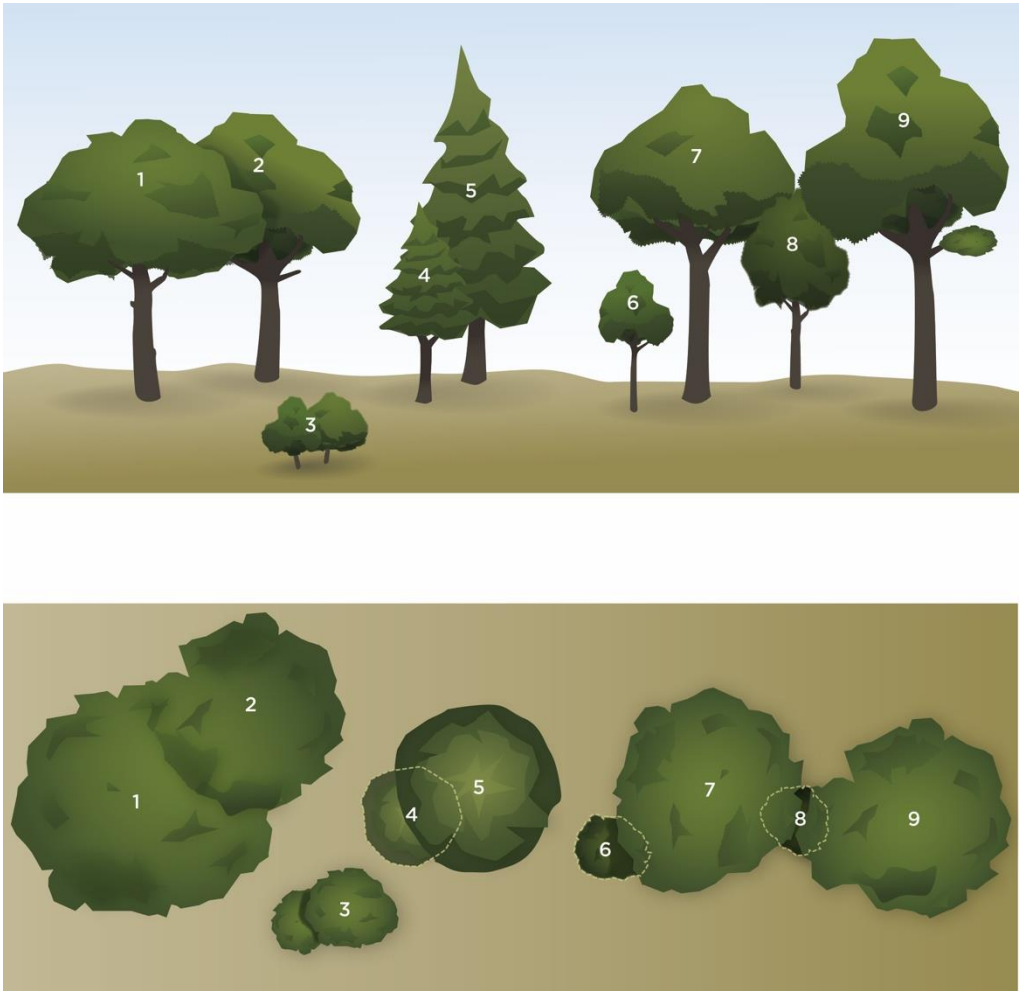

Figure 10. Cross section (top) and birds-eye view (bottom) of a forested site. The best CFC trees are 1, 2, 5, 7, and 9 as they are the most prominent, sunlit crowns. However, both 1 and 2 should not be sampled since their crowns overlap, unless they are very large trees that dominate the plot canopy. 6 and 4 are not ideal choices since much of their crowns are shaded from above by larger trees. 8 is not acceptable, this individual cannot be seen from above. 3 could be acceptable if it's the only option for sampling a rare species, or one that rarely reaches the canopy.

4. While choosing individuals, take note:
    a. It is ok to sample individuals not identified as candidates in your sampling plan.
    b. Because of links with AOP, aim for prominent crowns that can be 'seen' from above. A tree with one sunlit branch but most of the crown overtopped neighbors should not be sampled.
    c. For common taxa, do not take species replicates from the same plot, unless the individuals differ strongly in age, health, or some other important condition.
    d. All else equal, give priority to sampling individuals tagged (or soon to be tagged) for VST measurements as well as those sampled previously for canopy foliage. However, only give this priority when all other requirements are met. Individuals sampled in previous bouts may no longer be suitable candidates. In such cases, choose different individuals.
    e. If a prominent crown is in the plot but the stem is just outside, it may be ok to sample, but this depends on the site host agreement. Discuss with your Domain Manager first.
    f. **See Box 1 for additional guidelines for choosing individuals to sample.**
5. In Distributed Base Plots, sampling will occur primarily in the 20 x 20 m plot core, as this is where tagged individuals are located (**Figure 11**, left). However, remember to:
    - Keep equipment and coolers out of this component of the plot. Stage supplies in the external buffer zone reserved for soil sampling or outside the plot.
    - Avoid the 1m x 1m nested subplots used for Plant Diversity sampling.
6. In tall-stature Tower Plots, sampling will occur primarily in the two 20m x 20m subplots randomly selected for Plant Productivity measurements, as this will be necessary to capture tagged individuals (**Figure 11**, right). However, remember to:
    - Keep sampling equipment and coolers in the other two subplots or outside the plot.
    - Avoid trampling in the 10 m^2 and smaller nested subplots.

Remember: It is acceptable to sample outside of these areas if needed to procure sunlit foliage from a prominent canopy individual of the target taxa. This will require Mapping and Tagging (more below).

7. Place flagging tape around the stems of individuals chosen for sampling. Flagging should be removed once sampling is complete.
8. The actual method of obtaining sun-lit foliage will vary based on the height of the woody vegetation being sampled. For vegetation > 6 m tall, refer to **Appendix E** for sampling tips. Field Science personnel must use appropriate PPE for the chosen method (see Safety, section 5).
    - 0-2 m = hand clippers
    - 2-6 m = extendable pole pruner
    - 6-10 m = line toss, slingshot, or long-reach pole pruner (30 ft)
    - 10 m = UAS/line launcher/slingshot/tree climbers

Box 1. Guidelines for choosing woody individuals

Observation	Response
A species that is not on the target taxa list is encountered	As long as the individual has a sunlit crown, sample it. The goal is to capture as much diversity at the site level as possible
It is possible to collect sunlit foliage from both old and young tagged individuals of a target species	In most cases, the older trees will be the prominent, sunlit individuals, so they should be sampled. If young individuals dominate the site and are commonly found in sunlit positions, sample them as well
A candidate individual from a target species is found in a Plant Diversity nested subplot	Try to find another individual of that species to sample in a different location or plot. If this is not possible, sample that individual only if no damage to the subplot is anticipated
There is not enough foliage on a given individual to sample without damage	Sample a different individual of that species from the list of candidates
Some individuals of a target species show significant signs of disease/sickness/herbivory	Sample diseased/sick individuals if the disease/sickness is a dominant characteristic (> 50% of individuals show some level of disease at the site). Otherwise, try to avoid diseased individuals
The site experienced a recent disturbance (e.g., fire, windthrow)	Sample from plots affected by this disturbance if it was widespread. If only a small area experienced it, do not sample there. Use the appropriate values of **plantStatus** (more below) to describe anomalous conditions as a result of this disturbance
The site is sparsely vegetated (e.g., aridlands), woody individuals are rare	Attempt to sample to the site-specific target sample number, but if not enough woody individuals are present, take fewer samples
Woody individuals do not have true leaves, or leaves are tiny (Ephedra spp)	Sample the plant part that is functionally analogous to a leaf (e.g., photosynthetic stem) and treat as leaf throughout
Vines or lianas (ex: Vitus spp) are a dominant component of site canopy	It is acceptable to sample a vine overtopping a supporting stem, especially if the tree or shrub underneath is dead or mostly dead. If the vine has not been previously tagged, it must be tagged during the course of CFC sampling, and the supporting stem **must** be identified *and* mapped. Note this situation in the remarks field
Individuals are functionally similar but not actually woody (Palmettos, cactus)	Sample as if they were woody individuals, including mapping and tagging as needed. See **Appendix D** for instructions on cacti.
Individual has the wrong taxonID in VST Mapping and Tagging	Create a new record with the correct taxonID in the VST: Mapping and Tagging application, then associate this new record with the CFC record.

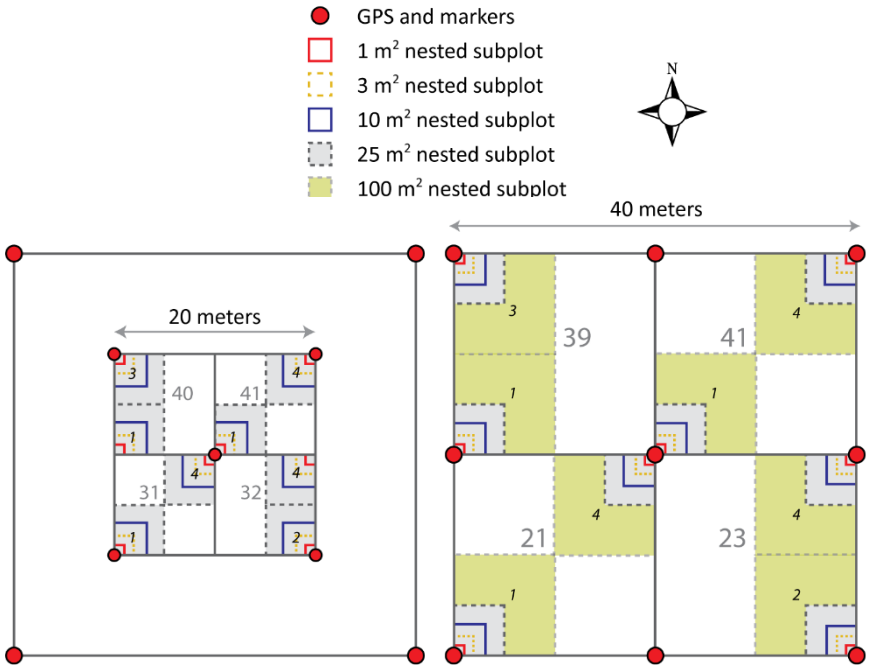

Figure 11. (Left) Plot layout for Distributed basePlots and short-stature Tower basePlots. (Right) Plot layout of tall-stature Tower basePlots.

HOW LONG TO SPEND ON ONE TREE?

Ideally, Field personnel should not spend more than 1.5 hours attempting to sample a single tree. If this time is exceeded due to difficulty accessing the sunlit canopy, consider abandoning that tree. This is especially true if no sample material has been collected. If partial material has been collected, it may be worth pushing on if it seems sampling can be completed without much additional time.

9. If sampling with the UAS, arrange the sampling team as follows:
    a. Set up a processing station near the UAS launch point.
    b. At minimum one, and if possible two, NEON technicians remain at the launch point/processing station with the UAS pilot. They will assist with UAS sampler control, retrieval of foliage following collection, and subsampling as outlined below.
    c. One other NEON technician, ideally the person with the most botanical expertise, will be at the plot and use a walkie-talkie to direct the pilot to the target tree(s). They should prepare to use cardinal directions to help the UAS pilot navigate. Once a branch is collected, this person records field metadata and creates a crown polygon (B.4).
10. Retrieve foliage as it is brought down from the canopy. Any person handling foliage must wear a clean pair of Nitrile gloves, so sweat and dirt from their hands do not contaminate the sample. It is ok to handle the woody parts (branches) without gloves, but not leaves or needles.

11. If not done already, create a record for the individual in the CFC: Field application and start to populate sample metadata.

12. For tall-statured vegetation, determine the height where foliage came from using the laser range finder (**Figure 12**). Remember that no metal should be worn on the upper body while using the rangefinder, including glasses, watches, rings, or earrings. *You will not use the 3-shot height routine,* instead follow the instructions provided below.

    Each sample requires a pair of distinct 'vertical distance' measurements to calculate height:

    - **VD(#) sample** = vertical distance between observer and canopy foliage sample
    - **VD(#) baseHeight** = vertical distance between observer and base of the stem (usually a negative number

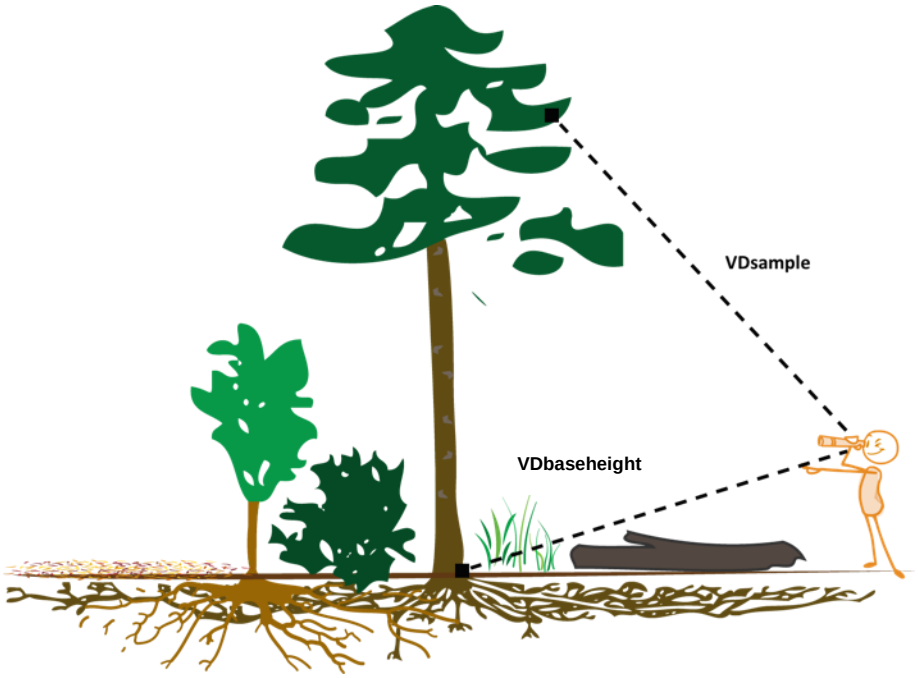

Figure 12. Measuring sample height using a laser rangefinder.

a. Locate a position where the rangefinder operator has simultaneous clean lines of sight to the location from where the sample came, as well as the base of the individual (stem meets ground), preferably on the uphill side if ground is sloped (**Figure 12**).

    1) For UAS sampling, it may be difficult to record height while the tool is in use because it is difficult to see and/or it creates wind. In this case, measure the crown apex after sampling is complete as this is a good approximation of sample height.

b. Place a reflective surface near the base of the individual to aid accurate readings.

c. With the laser rangefinder in "VD" mode, aim it at the location where the foliage sample originated and press power (fire) button. Record this value as **VD1sample**.

d. With the laser rangefinder still in VD mode, aim it at the base of the stem (or reflective tape) and press power button. Record value as **VD1baseHeight**. This value is generally negative except when measuring a tree from a downslope position.

e. If leaves came from a range of heights, take multiple measurements (up to three). Enter each value in the CFC: Field application (VD1, VD2, VD3 sample and base height), or in a separate row if using paper datasheets.

13. For short-statured vegetation, do the following instead:

a. **VD(#)sample**:
   - Using a meter tape/stick, measure total height above ground of sampling location.

b. **VD(#)baseHeight**:
   - Enter '0'

14. Select a subset of sampled leaves/needles in good condition (**whole, healthy, green**) and set aside to generate subsamples for chlorophyll and LMA measurements.

a. Use a sorting tray if available, this helps with organization (especially for small leaves/needles). Wipe tray clean with ethanol between samples.

- How much foliage? Think about what is needed to make a good-quality, 8 x 11" leaf scan with no overlap between leaves (**Figure 13**) - roughly 1-5 large leaves, 7-15 medium leaves, and 20-100 small leaves or needles, plus extra for the chlorophyll subsample

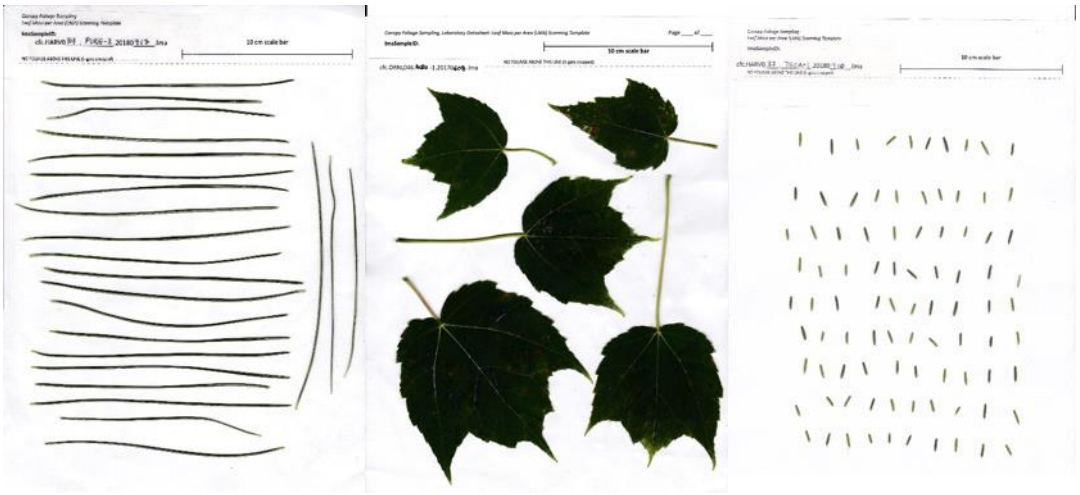

Figure 13. Example LMA foliage scans from Domain 1, to help give a sense of foliage quantities needed.

15. Use a spring scale or portable digital scale to weigh the remaining foliage, ensuring enough is left for all chemical analyses. Foliage need not be whole/pristine, but ensure it is not excessively covered in epiphylls (fungi or lichen growing on the surface).

a. To get an accurate weight, either tare the scale with an empty bag first, or weigh the bag alone and add this to the target weight.

b. Ranges are provided – err on the high end if procuring material is easy (as this will simplify lab processing workflows), but the low end is OK for difficult to procure samples.

- Target **30-40 g fresh foliage** for broad-leaf species
- Target **15-30 g fresh foliage** for needle-leaf species
- These masses do not include woody parts

c. In cases where a scale is not available, target the following leaf quantities. Note that this is not the preferred method since mass per leaf varies substantially with species:

- Large leaves: 15-30
- Medium leaves: 30-50
- Small leaves and needles: >> 100 (e.g., several branchlets comprised of multiple needle fascicles for coniferous plants).

16. If enough material is available, bag and stow all (sub)samples according to the steps below. If more material is required, procure additional sun-lit foliage, then combine with previously collected material and record additional collection height(s) as needed.

17. While 1 member of the crew prepares subsamples (B.2), other members can proceed to map and tag as needed (B.3) and create a polygon of the sampled crown (B.4).

B.2 Subsampling and Metadata

1. Reminder to use fresh Nitrile gloves for each sample. In addition:

   a. Be aware of what you are touching to avoid introducing contaminants to the sample. Do not touch face, hair, bare ground, etc. while processing. If in doubt, change gloves.

   b. If using a tool (clipper/blade) for subsampling, clean it with ethanol between samples.

2. Package and stow chlorophyll subsample:

   a. Use ~25% of good-condition (green), set-aside foliage

   b. Clean foliage with DI water. Surface contaminants likely do not contain chlorophyll, but they may contribute to the mass, decreasing the accuracy of our measurements.

      1) Moisten a paper towel, ring it out so not dripping, and gently wipe foliage to remove surface contaminants. For needles, it may be easier to do this while still on the branch.

      2) If leaves have small hairs or other features that make wiping difficult, can rinse samples with DI water, then blot dry by placing foliage inside a folded paper towel and pressing.

   c. For larger leaves (>= 0.5” wide), use a punch tool to extract circles distributed across all set-aside, cleaned leaves (**Figure 14**, left).

      - 0.5” diameter punch tool = 20-25 circles
      - 0.75” diameter punch tool = 15-18 circles

- 1.5” diameter punch tool = 5-6 circles

Figure 14. Examples of chlorophyll subsample packets, broad leaves (Left) and needle leaves (Right).

d. For skinny/small leaves or needles, use clippers or scissors to cut foliage into pieces small enough to fit in and fill a foil packet. *Remove all non-foliar material, including stems, petioles, needle sheaths, and anything woody* (**Figure 14**, right).

e. Place punches or small pieces into a pre-made foil packet. Do not fold leaves, instead cut them into small enough pieces to fit as one layer in the packet. Spread out tissues and minimize stacking to help foliar material freeze effectively.

f. Place foil packet into a 4oz Whirl-pak bag.

g. Label it with **chlorophyllSampleID**. The identifier will consist of the module code (cfc), plotID (siteIDXXX), taxonID, - sampleNumber (for that plot and date), collectDate, and suffix “.chl”

   1) Example identifier: **cfc.GRSM001.QUAL-1.20190705.chl**

h. Scan the chlorophyll sample barcode – it should appear in the field **chlorophyllSampleCode**.

   1) For UAS sampling, the mobile device used for data collection may be at the plot while subsampling occurs at the processing station. In this case, scan the chlorophyll barcode once the person working at the plot has returned to the processing station.

i. Place freshly collected subsample into a dry ice cooler and **flash-freeze it by completely covering with dry ice**. If using dry ice blocks, sandwich the subsample. If using pelletized dry ice, bury it. Make sure there is good contact between the sample and dry ice.

   1) If ultra-cold ice packs are used instead of dry ice, make sure this is indicated in the **chlorophyllSamplePrepMethod** field

**Chlorophyll subsamples must stay frozen at all times. Keep any eye on the dry ice - if it is running low, attempt to replenish it over the course of the day or bout.*

3. Package and stow LMA subsample, using remainder of good-condition, set-aside leaves.

   a. If not done already, place a paper towel moistened with DI water into the sample bag. This will help foliage maintain turgor pressure. The paper towel should be wet but not dripping, ring out as needed.

b. Place material into a resealable plastic bag. Take care not to fold or crush leaves, especially broadleaf ones. Folded or crushed leaves will be difficult to use for LMA measurements.

- It helps to stack individual leaves (**Figure 15**)
- Woody material may or may not be removed prior to bagging the sample. In fact, since foliage will be blotted dry prior to LMA scanning, for needles and small leaves this may be easier while still on the stem.

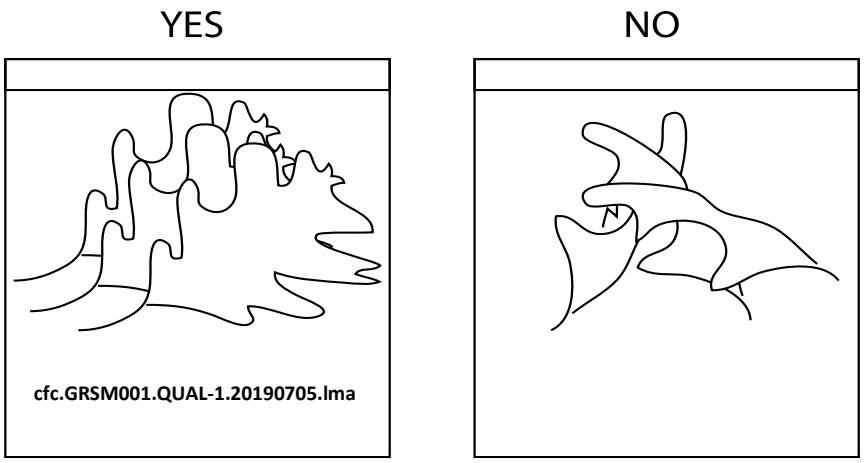

Figure 15. Example of how to package and label a broadleaf LMA subsample.

c. Label the bag with the **lmaSampleID**. Use the same convention as descried above, but with ".lma" for the suffix.

1) Example identifier: **cfc.GRSM001.QUAL-1.20190705.lma**

d. Store LMA bag in cooler on ice packs, ensuring not to bend or crush foliage. It helps to put a layer of flat cardboard over the ice packs to ensure a flat surface for LMA samples to rest on, and to make sure they don't get damaged by accidentally getting too cold.

4. Package and stow the bulk chemistry sample

a. If not done already, place a paper towel moistened with DI water into the sample bag. This will help foliage maintain turgor pressure and aid in foliage cleaning back in the lab. Paper towel should be wet but not dripping, ring out as needed.

b. Place remaining foliage into a plastic bag and seal.

1) For species where stripping foliage from the stem takes a long time (ex: hemlock needles), or where cleaning will be easier with foliage on the stem, it is ok to take the bulk sample back to the lab in this condition.

c. Complete the label by writing the **sampleID**. Use the same convention as described above but with no suffix, since this is considered the bulk sample.

1) Example identifier: **cfc.GRSM001.QUAL-1.20190705**

d. Scan the bulk sample barcode – it should appear in the field **sampleCode**.

1) For UAS sampling, the mobile device used for data collection may be at the plot while sample processing occurs at the processing station. In this case, scan the sample barcode once the person working at the plot has returned to the processing station.

e. Store bulk sample bag in a cooler with ice packs – it matters less if this foliage gets crushed.

5. Record metadata about the woody individual by capturing the fields described below.

- **plantStatus**: This field is for assessing the status of a woody individual at the time of CFC sampling. Use choices in **Table 11**, similar to those used in VST but with additional canopy sampling options. If multiple apply, select the one that is most likely to impact the data and take note of other conditions in remarks.
- **canopyStatus**: This field is for assessing whether an individual is part of the true/continuous canopy of that plot or is instead found in an opportunistic sunlit gap. It will be helpful for remote sensing applications. Use choices in **Table 12**.
- **canopyPosition**: This field is for assessing the canopy position of a woody individual at the time of CFC sampling. Select from the choices in **Table 13**, similar to VST. Ideal CFC samples fall in categories 1 and 2, but 3 will be a common choice in some forests with even stand age and canopy heights. 4 is acceptable just for rare species that can only be sampled in a gap.
- **samplePosition:** This field is for assessing where on the woody individual leaves or needles were collected. Choices are: top of crown, side of crown, mixture.

Table 11. Plant status options and their definitions for woody individuals.

Choice	Description
Ok	Any live Individual that is of typical mature, healthy, peak-green status for the ecosystem in question; that is, if trace amounts of insect/disease/physical damage are typical on the majority of individuals, use this code rather than codes below.
Insect damaged	Insect damage more than is typical for the site. Note 'crown' or 'bole' damage in **remarks**, and indicate type of insect causing damage if known (e.g., Mountain Pine Beetle, Gypsy Moth, etc.)
Disease damaged	Disease damage more than is typical for the site. Note 'crown' or 'bole' damage in **remarks**, and indicate type of disease causing damage if known (e.g., Blister Rust, rot, canker, etc.)
Physically damaged	Note 'crown' or 'bole' damage in **remarks**, and indicate type of physical damage if possible (e.g., broken stem, bole scar, girdling, snow/ice damage, crushed, lightning, crown scorch, bole scorch)
Other damage	Note details in **remarks**. If a crown is heavy with vines/lianas, use this option and describe in **remarks**.
Leaves not fully expanded	If plot was recently burned or suffered another severe disturbance, or the individual is sampled early in the season and has leaves that are not yet mature
Leaves beginning to senesce	If sampling occurs when leaves are past the peak green condition and starting to senesce

Table 12. Canopy status choices and their definitions.

Choice	Description
True canopy	Individual is part of the local canopy; crown height is similar to neighboring sunlit plants
Sunlit gap	Individual is not part of the canopy; sample is only sunlit because it is found in a gap and neighboring sunlit plants are significantly taller

Table 13. Canopy position choices and their definitions. Modified from the USFS Forest Inventory Analysis program Crown Class definitions (U.S. Forest Service 2012).

Choice	Description
1 - Open grown	Full sun, not touching other plants, with crowns that have received full light from above and from all sides throughout most of its life
2 – Full sun	Crowns receiving full light from above and partly from the sides. Their crown form or shape appears to be free of influence from neighboring plants
3 - Partially shaded	Crowns receive full light from above, but little direct sunlight penetrates their sides
4 – Mostly shaded	Individuals that receive little direct light from above and none from the sides

B.3 Mapping and Tagging

If the sample was collected from a non-tagged individual, it will need to be mapped and tagged, similar to the Vegetation Structure protocol (RD[10]). If the sample was collected from a tagged-only individual, it will also need to be mapped. If using a mobile device, record values in the Mapping and Tagging application. If not, use the Canopy Foliage Field Datasheet. For samples previously tagged but not mapped, make a new record that includes both the mapping and tagging data.

1. Attach a pre-numbered aluminum tag to the individual.

   a. If 100% certain that it will be a canopy-only individual (e.g., found outside the zones used for plant productivity measurements, the plot core or two randomly selected subplots), append tag with a "Z" using the dicast set (example = 09147**Z**).

   b. If the individual will (or may) qualify for VST measurements but sampling has simply not yet occurred, use a standard tag

2. Record **tagID** in the application or datasheet.
3. Record whether it is (or will soon be) a VST tag - if uncertain, select Unknown.
4. Map the individual.
   a. Record the **pointID** where the laser rangefinder is positioned. Only pointIDs that are GPS measured and/or monumented are acceptable for mapping and tagging. Make sure the rangefinder is positioned directly over the selected point.
      1) In Distributed basePlots and short-stature Tower basePlots, the five acceptable points are [41], [31], [33], [49], [51]
      2) In tall-stature Tower basePlots, the nine acceptable points are [41], [21], [23], [25], [39], [43], [57], [59], [61]
      3) Avoid using pointID [41] unless absolutely necessary, as mapping from this pointID leads to increased plot trampling due to its location in the plot center.
5. Use the laser rangefinder to determine **stemDistance** and **stemAzimuth** while at the given pointID; see RD[09] and RD[10] for detailed instructions.
   - Remember, stems sampled only for canopy foliage and appended with a Z are NOT measured in the Vegetation Structure protocol

B.4 Creating Crown Polygons

This workflow is similar to the procedure outlined in Graves et al (2018) and relies on a combination of mapping techniques, image interpretation, and location information from a GPS receiver (if available). The goal is to delineate a shapefile polygon that contains pixels from only the crown of the sampled individual, as 'seen' from above.

Polygons should encircle > 1 pixel or square in the CHM and FCol layers (1 pixel = 1 m^2 in those layers). If the vegetation is smaller than this (for instance, shrubs in dry/cold sites), do not create a crown polygon, but double-check the mapping data using the procedure outlined above (B.3). Highly accurate mapping data are required since there will be no polygon. If errors in previously mapped individuals are found, create an updated Mapping and Tagging record.

In forested sites, the majority of samples should have crown polygons associated with them. The workflow for creating crown polygons is as follows:

1. Open the Field Maps application, then the offline map of interest in the On Device folder.
2. Your approximate location should appear as a blue dot in the imagery. Depending on the site, the signal will be stronger or weaker.
    a. In dense, closed-canopy forest, position will be approximate, and the blue dot may jump around a lot. If this happens, plan to ignore it and draw polygons with survey techniques.
    b. In more open areas with better GPS signal, the blue dot is likely to be within a few meters of your actual location and can be used as a geolocation reference.
3. Review the different layers present in the map by clicking the blue 'stack of books' icon in the top-right corner (**Figure 5**). Recall that most sites will have the following layers:
    a. Canopy Height Model (CHM): displays absolute and/or relative heights of the trees. Red and orange are tall, yellow and green are medium, dark blue indicates ground or heights < 2 m
    b. High-resolution Camera (Cam): each pixel (square) is 0.1 m, but image sharpness varies
    c. False-color spectrometer (FCol): shows reflectance for specific wavelengths from the hyperspectral sensor, different colors generally correspond to different chemistry and possibly different taxa.
    d. Maps also show NEON field markers and stem locations for target tags
4. Turn the different layers on and off by tapping each slider button to see how that changes interpretation of the map. You will need to zoom in to be able to see the AOP raster layers. At large scale, only the plot and stem markers will show.
    a. Note that conditions might have changed slightly since the AOP imagery was collected – for example, there may be newly dead trees, new gaps, growth, etc.
5. Walk around and assess the crown of the individual for which you are creating a polygon. If neighboring crowns overlap, focus on the parts of the crown that are sunlit and contain 'pure pixels' of only the target individual.
    a. Note size and position of the crown relative to neighbors, plot markers, and other reference points (such as large gaps). Where is the bulk of the crown mass relative to the stem?

b. If the GPS signal is strong, watch the path the blue dot travels in the image as you walk around the crown. In some systems, this will clearly show crown location in the imagery. In most it will not, as the GPS point will be jumping around due to poor signal.

6. For a stem location displayed on the map, verify that the marker is where it should be given observations on the ground. If it is, that should be helpful in deciding which pixels belong to the crown of that stem.

7. For stem locations not displayed on the map, use the VST Mapping and Tagging data to mark where the stem (or crown) should be in the imagery.

   a. Identify the **pointID** that was used for mapping.

   b. Use the *Measure* tool (ruler, in 3 blue dots menu in the top-right) to draw the distance in meters from that point to the stem (**Figure 16**, left). One end of the line should be on the TOS marker used for mapping, the other at the location of the stem or crown. Adjust the end points as needed.

   c. Field Maps does not measure azimuth. As a first cut, approximate this when drawing. Then place a clear protractor or compass on top of the iPad and refine the angle as needed.

   d. Drop a pin at the location where the stem/crown should be by holding down a finger on the screen, save to *My places* with tagID, taxonID as the label (**Figure 16**, right). How does it line up with the imagery and your visual plot assessment?

      1) If the pin falls in 'dark pixels' or does not point to a clear crown, map again from a different pointID.

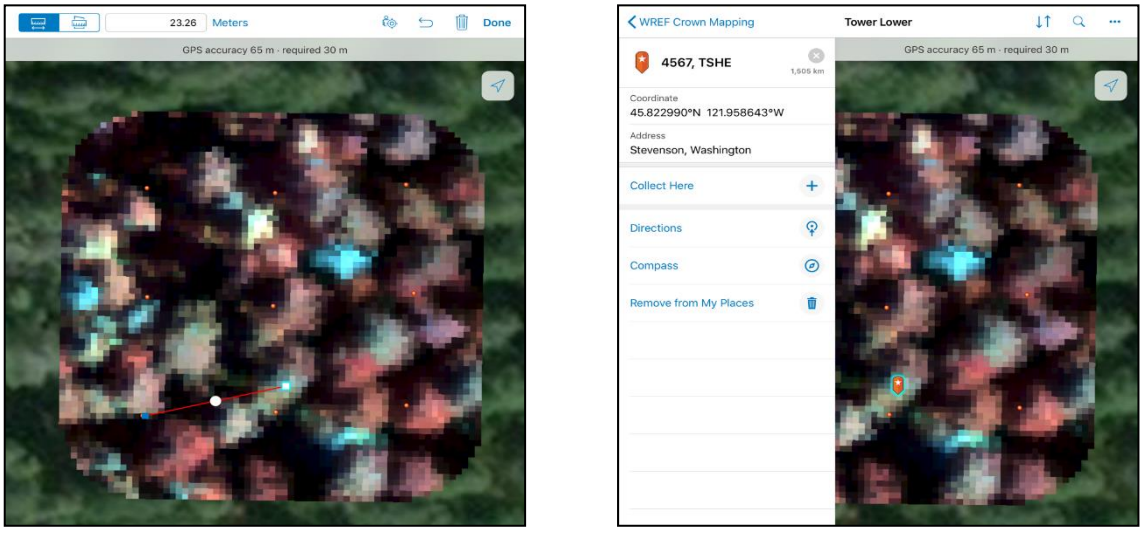

Figure 16. (Left) Using the Measure tool to draw mapping data; (Right) dropped pin in approximate crown location.

8. If more help is needed to identify a crown in the image, measure its size and shape. If irregular, measure the length of the longest diameter and note angle. Use these measurements to help with image interpretation; look for groups of pixels with the size and orientation measured.
9. Taking all this information into account, determine which pixels to include in the crown polygon. In general, look for groupings of pixels that are similar (but not identical) in color/hue, as this indicates similar height, chemistry, etc. Also look for pixels with a sensible position relative to plot markers and significant features such as large gaps. Things to consider:
    a. Repeatedly toggle on and off all the layers in the map. Certain ones may be helpful in specific situations but not others. Find which ones have the most information and contrast for the area you are working. For example:
        1) The CHM layer will be very helpful if heights of neighboring trees are variable
        2) The FCol spectrometer layers can help pick out contrasts between different species neighbors with similar heights
    b. Zoom out and in several times. It's helpful to get both the zoomed-out perspective while also looking closely at the pixels.
    c. In the FCol layers, dark/black pixels are shadows, have no information, and should generally not be circled. Keep in mind that shorter, shaded trees or samples taken from gaps may appear as dark pixels in the imagery. This shadowing effect depends on viewing geometry during the AOP overflight and height of neighbors. For individuals with **canopyPosition** = 4 or **canopyStatus**= sunlit gap, creation of a useful shapefile polygon may not be possible.
10. Decide which pixels will be captured in the crown polygon. If uncertain, make a relatively small, conservative polygon that may not encircle the entire crown*. It is better to be confident that you have a cluster of sunlit pixels from a given tree (e.g., 'pure pixels') vs making a polygon that encompasses the entire crown*.
11. Whenever possible, create polygons on top of the CHM layer, as it is most geospatially accurate. However, if this layer is not useful, use Cam or FCol instead.
12. To create a crown polygon, use the + sign (bottom right) to create a new feature.
    a. *Add Points* to draw the polygon, one point at a time.
        1) If you dropped a pin, can select it, then *Collect Here* to start adding points at that location.
        2) If you need to delete a point, click it (it will turn white), then select the 3 blue dots, then *Delete Selected Point*
    b. Add a minimum of 6 points; there is no maximum.
    c. Polygon should have a sensible, generally round (ish) shape as in **Figure 17**.

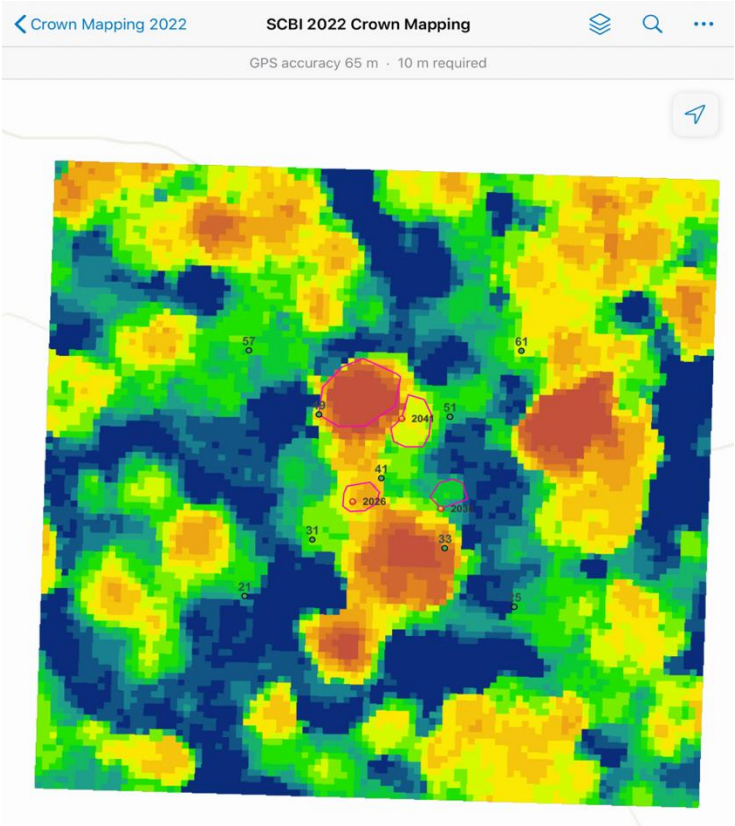

Figure 17. Example distributed base plot with 4 completed crown polygons

d. Once polygon is complete, enter metadata in Field Maps

   1) **crownPolygonID – SITE.TAG.YEAR** – required, either copy-pasted directly from Fulcrum if using a single mobile device or transcribed if not.

   2) **taxonID –** required

   3) **mappingLayer** – CHM, FCol, Cam – required. This lets end users know which layer was the reference for polygons, given potential small geospatial offsets between them

   4) **mapName** – required for large sites with multiple maps (ex: Elkhorn, Murray Hill, Distributed). Not a published field but helps Science complete polygon QC

   5) **notes** – optional field, add anything relevant

e. *Submit* the polygon shapefile.

f. In the CFC:Field application, record metadata about the polygon. This includes how well you think the crown is captured by filling out the **crownPolygonConfidence** field (**Table 14**).

13. If a polygon needs to be discarded, click on it, then *Delete*

14. If a polygon needs to be edited, click *Edit*, then move/delete points as needed.

15. Repeat for as many crowns as need mapping.

a. If a crown polygon is not created at the time of sampling, it is possible to go back and create one a later time. This should ideally occur before the CFC Fulcrum records lock so that crown polygon metadata can be added – within 46 days of Fulcrum record creation.

b. Recall that back in the DSF, you need to 'sync offline layers' for each offline map in order for polygons to transfer from the device to the AGOL server.

Table 14. Polygon confidence choices and their definitions and use cases.

Choice	Description	When to use
High	Crown center and most crown pixels are captured by the polygon.	Mapping clearly points to a given tree of reasonable height and crown diameter in the imagery, whose boundaries can be easily delineated due to high contrast with neighbors. A strong GPS signal helps with high confidence but is not required
Medium	Crown center is not in the center of the polygon, but polygon includes some pixels that belong to the crown	Mapping seems to align with a given tree in the imagery, but trees are dense and with similar properties such that one cannot be certain. Or, a single tree is identified, but its boundaries are hard to delineate due to low contrast with neighbors that have similar canopy height or foliar properties. Poor GPS signal can contribute
Low	Not sure if crown center is captured in the polygon, and polygon may include pixels that do not belong in the crown.	Mapping does not align with a specific tree in the imagery (e.g., falls in a gap), aligns with a tree of the wrong shape/size, or whose borders are very difficult to discern from neighbors. Low-confidence polygons may arise from issues with monumented GPS points used to mark NEON plot boundaries - contact Science if you suspect this.

B.5 Transitioning Between Plots and Tracking Progress

1. Repeat all steps in the above sections for all individuals to be collected in a given plot. All samples should be nested under the same parent Fulcrum record.
2. Collect trash and detritus and remove from plot.
3. Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the plot dry ice cooler, the active 'flash-freezer,' to a second dry ice cooler for storage. Having one cooler maintained empty of samples will yield better flash-freezing results as new subsamples will have good contact with dry ice.
4. Update the Work Tracker and take stock of which species have been sampled and at what level of replication. Based on progress toward acquiring target samples spanning relevant site gradients, decide which plot to visit next.
5. Make a reasonable effort to sample rarer canopy species at the site level, but do not spend an excessive amount of time in this pursuit. If rare species from the target taxa list are not sampled, collect extra replicates of common species to achieve the site-specific total sample count.
6. Repeat until the site-specific total sample count is achieved.

SOP C Field Sampling, Herbaceous Cover

Fulcrum App = **CFC: Field Sampling**. Fulrcum Manual is available in the **NEON SSL**

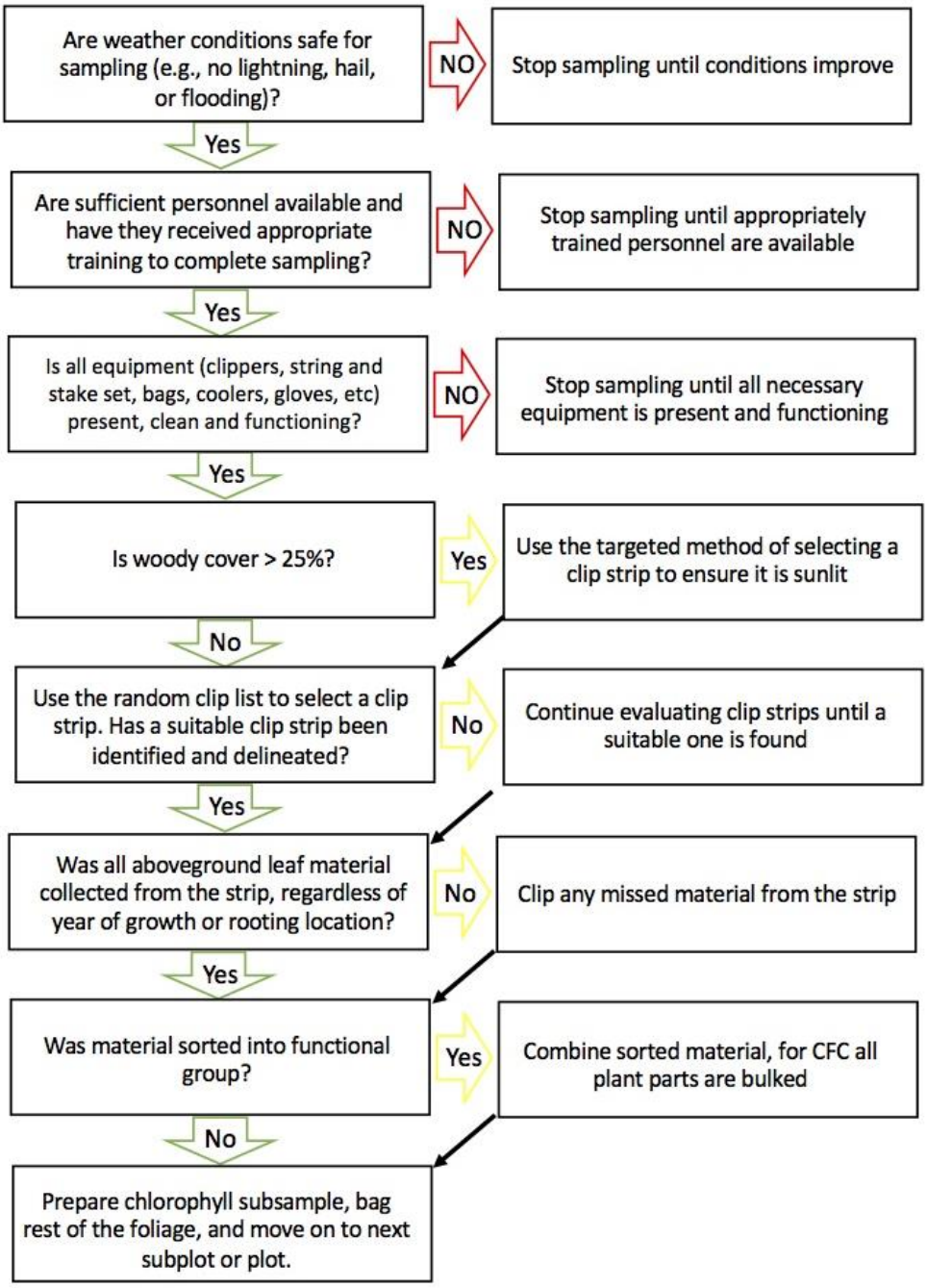

Figure 18. Overview diagram of SOP C, Field Sampling for Herbaceous Cover.

C.1 Collecting Foliar Material

1. The number of clip strips harvested and their locations will depend on site type (**Table 1**).
    a. Type B and Hybrid site, one sunlit clip strip per assigned CFC Distributed basePlot or 'Short-stature' Tower basePlot, from the non-destructive plot core. For 'Tall Stature' Tower Plots, two clip strips, one from each of the two randomly selected subplots used for Plant Productivity measurements (**Figure 11**).
        1) In the Hybrid site, do not take a clip from the plot/subplot if the canopy is so dense that none of the herbaceous vegetation is in the sun.
    b. Type A sites that qualify for herbaceous sampling due to prevalence of open cover, 1-8 clip strips from any of the possible CFC plots, as many as needed to represent herbaceous assemblages and bioclimatic gradients found across the site.
2. Navigate to a plot where clip strip sampling will occur.
3. Ensure there is one dry ice *and* one ice pack cooler available at the plot to preserve fresh samples. A second of each cooler type can be left in the vehicle for sample storage.
4. If plot woody cover is absent or < 25%, continue to STEP #5 below to use a randomized method to select a clip strip for harvest. If plot woody cover is > 25%, skip to STEP #10 and follow the targeted method to guarantee selection of a sunlit clip strip.
5. Select the first potential clip harvest location using the plot-specific clip list.
6. Use the clip list to locate the desired target coordinates for the selected clip strip.
7. Locate and mark the relative X,Y-coordinates of the clip strip SW corner within the plot or subplot. This procedure is outlined in detail in RD [04], Herbaceous Biomass (HBP).
8. Assess whether clip strip location is suitable for sampling (**Figure 19**).
    - Is vegetation in the clip strip location broadly representative of herbaceous biomass in the plot? If not, reject it.
    - Is the vegetation under an overstory canopy? If so, reject it
    - Is there enough vegetation biomass in the clip harvest cell to generate all samples and subsamples? If not, reject it.
    - A clip strip may also be rejected if obstacles, disturbances, and/or irregularities are encountered, particularly those that prevent delineation of the clip strip. These may include trees, large rocks, ant nests, etc.
9. If the clip strip is rejected, record why in the 'status' column of the clip list (use codes in **Table 15**), then proceed to the next potential strip on the list and repeat steps above.

- **Do NOT record '0' for clip strips rejected because they lie underneath a canopy or have insufficient biomass for this protocol.** These may still be used for regular herbaceous biomass sampling and should therefore not be permanently rejected.

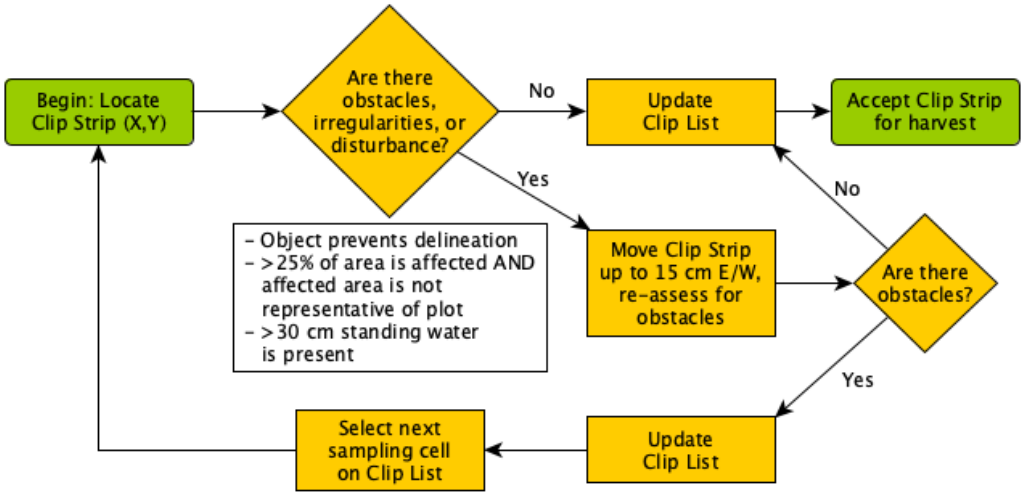

Figure 19. Flow chart to guide assessing potential clip cells for clip-harvest suitability.

Table 15. Codes to document acceptance/rejection of clip-harvest strips on the list of clip strip coordinates.

Code	Definition
0	Rejected; disturbance, obstacle, and/or irregularity encountered within the clipID cell
1	Accepted, no exclosure
2	Accepted, exclosure
3	Rejected temporarily, inundated
4	Rejected temporarily, uncommon plant
5	Co-located belowground biomass core sampling

10. If needed, select clip harvest location(s) using the following targeted, non-random procedure. Otherwise, skip to STEP #13.
    a. Assign a number to each continuous "patch" of herbaceous vegetation (**Figure 20**).
    b. Randomly select a patch to sample using a coin flip or random number list.
    c. Find the approximate center of the patch. Then use the appropriate map of plot clip cells (**Appendix F**) to select a clip strip that is close to the patch center.

Avoid selecting clip strips in or adjacent to the 1m x 1m nested subplots used for Plant Diversity sampling. Also avoid the plot centroid.

11. Assess suitability using criteria described above. Continue assessing possible clip strips near the patch center until an acceptable one is found.
12. Locate and mark the relative X,Y-coordinates of the selected clip strip SW corner within the plot or subplot. This procedure is outlined in detail in RD [04], Herbaceous Biomass (HBP)

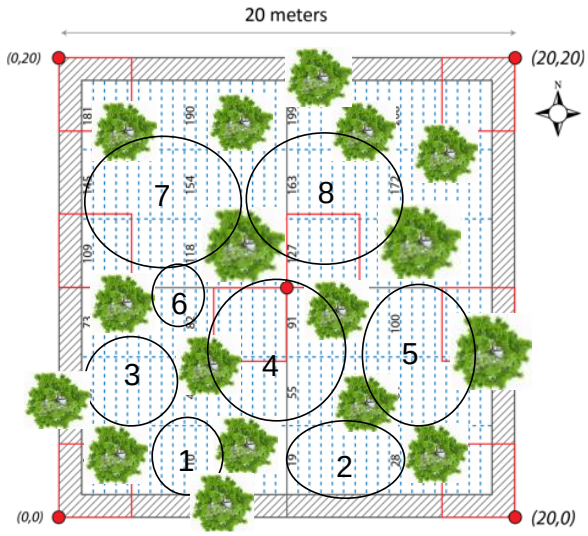

Figure 20. Divide plot into 'patches' of sun-lit herbaceous vegetation; assign numbers to facilitate random sampling.

13. Once an acceptable clip strip has been found, record that it was selected for CFC on the clip list.
14. Delineate the strip:
    a. Using one of the pre-marked string and stake sets, line up one of the marks with the flag and push one stake into the ground.
    b. Stretch the string and second stake from South to North end of the clip strip, using a compass or the Laser Rangefinder to orient the string in a North/South direction.
    c. Keep the compass or Rangefinder at least 50 cm from metal plot markers, eyeglasses, wristwatches, tent stakes, etc.
    d. Use a ruler to place the second string-and-stake set 10 cm to the right (east) of the first set. Check that distance between the two strings is exactly 10 cm at both ends.
    e. The two sets of marks on the two string-and-stake sets now clearly delineate a 0.1 x 2 m designated area for clip-harvesting (**Figure 21**).

Figure 21. Delineated clip strip.

15. If this size clip strip does not capture sufficient vegetation (possible in aridlands, tundra, or croplands), delineate a larger strip for sampling encompassing more of the clip cell. Use any of the three size choices available in the HBP Protocol (RD[04]), namely:

    - 2 m x 0.1 m, 2 m x 0.5 m, or 1.5 m x 0.65 m

*Since geolocation data is very important for canopy foliage sampling, do not deviate from the standard procedure for delineating clip strips, even in agricultural sites. Thus, the long end of the strip does **not** need to be perpendicular to row crops when sampling for canopy foliage.*

16. Enter required metadata into CFC: Field application or datasheet: **plotID, subplotID, collectDate, sampleType, clipID, clipDimensions**

    - subplotIDs are in reference to clip lists and HBP sampling (as in RD[04]), not in reference to Plant Diversity sampling.

17. Visually estimate and record percent foliage cover of the clip strip (as opposed to bare ground) in the field **percentCoverClip**. Don't spend more than ~ 1 minute making this visual estimate.

18. Put on a clean pair of Nitrile gloves and use clippers to harvest all herbaceous aboveground biomass present within the clip strip.

19. Key items to remember:

    - **DO** clip all vegetation that is inside the strip, regardless of where it is rooted in the strip or whether it was produced in the current year's growth. The only exception is cacti – if any are present in the clip strip, assess cover including this biomass but do not sample it and note 'cacti present but not sampled' in **remarks**
    - ***DO NOT*** sort biomass into functional groups.
    - ***DO NOT*** remove old standing dead (OSD) material from the sample.
    - ***DO NOT*** include twigs or other woody parts from these plants.

- If, after 15 minutes, the clip strip is still being harvested, stop and create the chlorophyll subsample (C.2, using representative foliage), then continue clipping the rest of the strip. This will help limit chlorophyll degradation.

20. If a *Toxicodendron* spp. Is present and will be sampled:

    a. Follow the guidelines established in TOS Standard Operating Procedure: Toxicodendron Biomass and Handling (RD[08]) to minimize exposure to toxic oils and for guidance on how to clean equipment

    b. Label sample bags that may contain *Toxicodendron* spp. So that they will be handled with appropriate caution during downstream processing. A sample warning label may be employed for this purpose.

21. Place a paper towel moistened with DI water into the bag(s) that will contain the sample. This will help foliage maintain turgor pressure and aid in foliage cleaning back in the lab. Paper towel should be wet but not dripping, ring out as needed.
22. Place clipped biomass into plastic bag(s) and seal.
    a. For very large, bulky samples (ex: corn crops), if you are running out of cooler space it is acceptable to subsample the entire bulk sample in the field, but this is not preferred. Make sure the subsample is representative, this will be easiest with crops.
23. Label bag(s) with **sampleID** if not already done. This will consist of the module code (cfc), plotID (siteIDXXX), 'CLIP-', sampleNumber (for that plot), and collectDate.
    - Example identifier: **cfc.WOOD001.CLIP-1.20190705**
    - Also write the **clipID** and **bagCount** on the samples bag(s)

C.2 Subsampling and Metadata

1. Remember to wear clean Nitrile gloves while handling foliage, change between samples
2. Mix all contents of the sample, then pull out a representative subsample of bulk herbaceous material from which to generate a chlorophyll subsample.
    a. An approximate representation of community composition is acceptable, do not spend more than five minutes on this task.
    b. A small amount of foliar material is needed, one handful will suffice.
    c. A sorting tray may help with organization of leaves if available, to ensure that tissues selected are broadly representative of the species composition in the clip strip.

*For herbaceous samples, you **do not** need to create an LMA subsample in the field, this can be done in the Domain Support facility (SOP E).*

3. Create the chlorophyll subsample (**Figure 22**):
    a. Clean foliage with DI water. Surface contaminants likely do not contain chlorophyll, but they may contribute to the mass, decreasing the accuracy of our measurements.
        1) Moisten a paper towel, ring it out so it is not dripping, and gently wipe the foliage to remove surface contaminants.
        2) If foliage is so fine that wiping is difficult, can rinse samples with DI water, then blot dry by placing foliage inside a folded paper towel and pressing.
    b. Using a clipper or scissors, cut representative foliage into small enough pieces to fit in a foil packet. Keep cutting until you have 0.5-1 g of material. This will be enough to conduct the chlorophyll analysis but not too much to make flash-freezing difficult.

1) For the first few samples, weigh them using a spring scale or portable digital scale to ensure masses are in this range.

c. In general, make sure that only **live, green vegetation** is included – do not include woody parts, dead foliage, or flowers.

1) However, if the plot has only dead/brown foliage cover, due to agricultural management or AOP flying outside of the peak greenness window, it is ok to sample dead/brown tissues if that is the dominant condition.

d. Place small foliage pieces into a pre-made foil packet. Spread out material as much as possible – this will help the tissues freeze effectively.

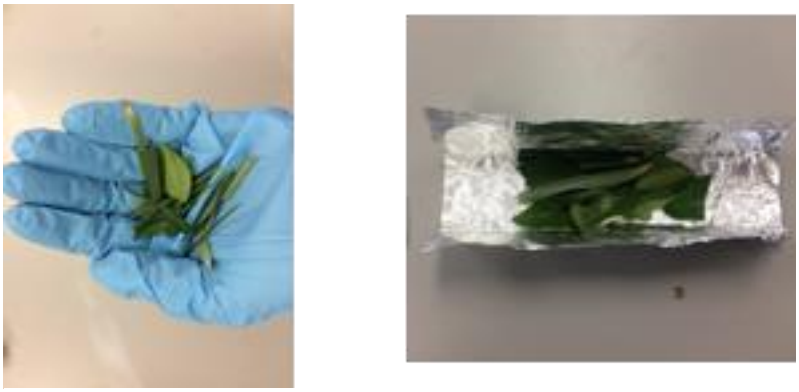

Figure 22. Example of chlorophyll subsample packet, herb clip samples.

e. Place foil envelope into a 4oz Whirl-pak bag.

f. Label whirlpak with **chlorophyllSampleID**. The will consist of the module code (cfc), plotID (siteIDXXX), 'CLIP-', sampleNumber (for that plot), collectDate, and the suffix ".chl"

1) Example identifier: **cfc.WOOD001.CLIP-1.20190705.chl**

g. Scan the chlorophyll sample barcode– it should appear in the field **chlorophyllSampleCode**.

h. If sample contains *Toxicodendron* spp., add a sample warning label sticker (an example is shown at the left).

4. Place freshly collected subsample into a dry ice cooler and **flash-freeze it by completely covering with dry ice**. If using dry ice blocks, sandwich the subsample. If using pelletized dry ice, bury it. Ensure good contact between sample and dry ice.

a. If ultra-cold ice packs are used instead of dry ice, make sure this is indicated in the **chlorophyllSamplePrepMethod** field

**Chlorophyll subsamples must stay frozen at all times. If dry ice is running low, attempt to replenish it over the course of the day and bout. Be sure to monitor the frozen subsamples.*

5. Scan the barcode for the bulk sample in the CFC: Field data entry application – it should appear in the field **sampleCode**. Recall that if a sample overflows into multiple bags, only one may have

a barcode label; the others will have only sample identifiers + bagCount. Alternatively, a barcode may be placed on a larger vessel that contains multiple bags.

6. Document atypical sample conditions:
    a. If sample contains *Toxicodendron* spp., select 'YES' in the **toxicodendronPossible** field.
    b. If the sample is made up of mostly dead/brown tissues, due to agricultural management or AOP flying well outside of the peak greenness window, note 'most leaves senesced' in the **plantStatus** field and 'dried and brown tissue, representative of the plot' in the **sampleCondition** field.
    c. If the sample has leaves that are just starting to senesce or are not fully expanded, make the appropriate selection in the **plantStatus** field. Otherwise, plantStatus should be 'OK.'
7. Store bulk sample bag(s) in a cooler with ice packs. Try to minimize crushing of the foliage since the LMA subsample will come from this bag.

C.3 Transitioning Between Plots and Tracking Progress

1. Repeat if an additional clip strip will be harvested in the plot (large-stature Tower plots), then move to the next plot until all CFC-designated baseplots have been sampled.
2. Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the plot dry ice cooler, which is the active 'flash-freezer,' to the second dry ice cooler for storage. Having one cooler maintained empty of samples will yield better flash-freezing results as new subsamples will have good contact with dry ice.
3. If certain plots are unable to be sampled during the course of the bout and sampling cannot be rescheduled, note those plots and be prepared to document missed sampling as discussed in the next SOP.

SOP D Post-Field Sampling Tasks

D.1 Sync Devices

1. Make sure that mobile devices used for data collection have Wi-Fi and sync to the cloud.
    a. Fulcrum: Press button with up and down blue arrows (top right), then Sync.
    b. Field Maps: 3 blue dots in the offline map icon, then Sync. You will immediately have access to polygon shapefiles in AGOL, so review and editing can be done on the computer.

D.2 Sample Preservation

1. Upon returning to the Domain Support Facility, make sure LMA subsample leaves are stored flat in their resealable plastic bags. It is okay to open bags in order to re-arrange crumpled foliage but squeeze as much air as possible out of the bags before resealing.
2. Store LMA and bulk foliage sample bags in refrigerator (4°C) until foliage can be processed.
    a. If you are working remotely, keep samples on fresh cold packs (change every 12 hr).
3. Store Whirl-pak bags containing frozen chlorophyll subsamples in a -80°C ultra-low temperature freezer until they are shipped to the designated analytical facility (within 7 days of collection).
    a. If you are working remotely, keep samples on dry ice, replenish before it sublimates.
4. LMA and bulk chemistry samples cannot be kept in the refrigerator for longer than 5 days. If so, they may begin to lose mass, potentially mold, and be unusable.
    a. If mold or other deterioration is evident after storing them in the refrigerator post-collection, issue an incident ticket; samples may be unusable.
    b. Review SOP E for instructions on LMA scanning and SOP F for instructions on sample drying and subsampling so you are prepared for the next time-sensitive steps.

D.3 Refreshing the Field Sampling Kit

1. Make sure the following consumables are available in sufficient quantity for the next bout:
    - Plastic bags, appropriate sizes as needed; nitrile gloves; permanent markers; flagging tape; aluminum foil; scannable barcode labels
2. Return cold packs to the -20° freezer to refreeze.

D.4 Equipment Maintenance and Cleaning

1. Clean blades of hand clippers and pole trimmer with water, then ethanol. Dry completely.
2. Clean, re-organize, and stow any line launcher or slingshot supplies (if applicable).

3. Clean any items that may have been in contact with *Toxicodendron* spp. as detailed in RD[08].

4. Recharge mobile devices and replace batteries for the laser rangefinder (as applicable).

D.5 Document Incomplete Sampling Within a Site

Canopy Foliage sampling is 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 (gradient 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. Plots can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot is compromised after road-building activities). Alternatively, plots may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

Since Canopy Foliage Sampling is a 5-year protocol and must provide data encompassing the range of variability present at a site, any plot in which sampling becomes compromised – due to landslide, flooding, or other logistical reasons, should be communicated to Science as soon as possible. For Type A sites, other plots can be chosen to sample, but for Type B sites it is important to communicate issues quickly so that plots can be re-assigned if needed.

<u>Missed Sampling in Type A sites:</u>

1. Review Fulcrum records to determine if site-specific target sample numbers were met ± 10%
2. If not, create a SN incident with the following naming convention to document missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]'
    a. Example: 'TOS Sampling Incomplete: CFC – Insufficient staff to meet target sample number'

<u>Missed Sampling in Type B and Hybrid site</u>:

1. Review Fulcrum records and unique plot lists to determine which plots/subplots were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]'
    a. Example: 'TOS Sampling Incomplete: CFC – Could not access plot due to closed road'
3. Staff scientists review periodically to determine whether a sampling location is compromised.
4. Create records for missed sampling locations using the **samplingImpractical** field as described in Section 4.5

SOP E Leaf Mass Per Area Measurements

Fulcrum App = **CFC: LMA**. Fulcrum Manual is available in the NEON SSL

Leaf mass per area (LMA) is generally measured by scanning fresh leaves/needles with a flatbed scanner, then using image analysis software to quantify the scanned area and determining the dry weight of the scanned material.

- Foliage should be scanned within 5 days of collection. This is especially important for broadleaf samples as needle samples tend to be less susceptible to mold. Foliage not scanned within 5 days of collection should be marked as such using **lmaSampleCondition**.
- Scanned area measurements should be completed within 90 days of collecting the field sample. While image scans do not expire, it is important to process them in a timely manner so that measurements can appear on the NEON data portal following expected timelines.

BACK-UP METHOD FOR LMA MEASUREMENT

For small leaves (< 0.5" wide), conifer needles, and grass blades, scanning plus image analysis is our only viable option and must be performed. For broad-leaf samples that are ≥ 0.5" wide, it is also possible to punch circles of known area from leaves, then oven-dry and weigh the punches. This will streamline the measurement process but is not preferable as within-leaf heterogeneity from veins and other features is not well-captured. Thus, leaf punching (section E.4) will be reserved as an option only if scanning of large leaves takes an unacceptable amount of time, such that a large number of samples remain in the fridge after 5 days of storage and foliar samples may soon mold and become unusable.

IMPORTANT! *Before starting on 'real' samples, navigate to the Sampling Support Library and find the Scanner Instructions document. Use this to set up the scanner. Then, ensure a trial of sections E.1* ***and*** *E.2 has been conducted with non-sample leaf material. This will save time and effort by allowing detection of issues with scanner and software settings prior to analysis of actual samples.*

E.1 Scanning Leaves and Needles

1. Ensure the scanner settings match what is listed in the Scanner Instructions document (output = 600 dpi, brightness = 25, image quality = high, save images to local N-drive folder). Remember that acceptable file formats are .tif and .jpeg, NOT .pdf.
2. Print several copies of the scale bar template from the Canopy Foliage Sampling datasheet package (RD[11]). Use a ruler to verify that the template prints true to size, e.g. exactly 10 cm. Sometimes, default printer settings can result in a compressed page view and thus the scale bar will be shorter than intended. If this happens, adjust printer settings and re-print.
3. Remove an LMA subsample (or entire herbaceous bulk sample) from the refrigerator.

a. If working with an herbaceous bulk sample, wear Nitrile gloves while handling foliage so you do not contaminate the rest of the material that will be used for chemistry measurements. Remove a handful of representative foliar material for the LMA subsample.

4. Working on a clean surface, remove leaves/needles from bag and blot dry. They should be moist from storage conditions; use a dry paper towel to simply remove surface moisture. Use care for fine leaves/needles and only clean and dry as much material as is needed for scanning.

5. Arrange an appropriate amount of material on the flatbed scanner. **Place all foliage sunny side down (facing the scanner glass)**. Use these leaf quantity guidelines for different size categories:

- Large leaves: as many as can fit on the scanner without overlapping, may only be one.
    - If needed, cut leaf into smaller pieces, and conduct multiple scans to get the entire leaf area. Include mid-vein and petiole (the stalk that joins the leaf to the stem).
- Medium leaves: 7-15, depending on size and what fits on the scanner. Arrange neatly, include petioles and rachis (the main axis of a compound leaf structure) if present.
- Needles: 20-100, wide variation depending on needle size. Arrange neatly, flat needles same side up. *Do not need to cover the entire 8 x 11" area* (see **Figure 13**).
- Herbaceous samples: as many live, green leaves/blades as can comfortably fit on the scanner. Try to ensure a representative sample, meaning the quantity of leaves used for LMA should be roughly proportional to species abundances in the clip strip. If needed, use clear tape to secure foliage to screen. It is OK to cut long blades of grass into smaller strips.

6. If sample contains *Toxicodendron* spp., wear single-use cotton gloves and place a transparency sheet down on the scanner glass to protect it from contamination with toxic oils.

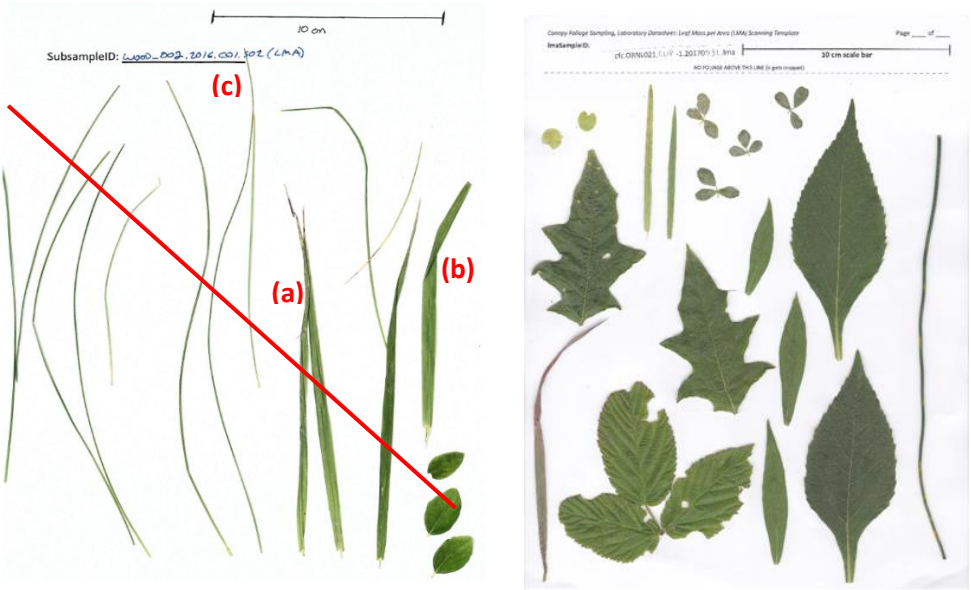

Figure 23. (Left) Example of leaf arrangements to avoid when scanning for LMA, including overlapping foliage (a), bent foliage (b), and foliage covering the text (c). (Right) High-quality scan for a clip strip

7. Additionally, it is important to make sure:

   a. There is white space around each individual leaf/needle.

   b. Foliage is not bent or overlapping (**Figure 23**).

   c. Choose representative leaves in good condition - without holes where possible, mostly green with little dead or damaged parts (especially relevant in grasslands). *The exception is when the entire sample was dead/brown because that is what was representative.*

8. Near the top of the scale bar template, record the **lmaSampleID**

   a. Add “scan01,” “scan02,” etc. if multiple scans will be required for a large leaf.

   b. Can re-use template as long as identifier of the sample being scanned is clear. One strategy is to cover the **lmaSampleID** field with clear tape or a transparent label, then use a dry erase marker to record each identifier and erase in between samples.

9. Lay scale bar template face down on top of the leaves/needles, positioned so the written information is NOT covered by foliage. This is necessary for when you crop the image.

10. Scan the sample (**Figure 24**, left).

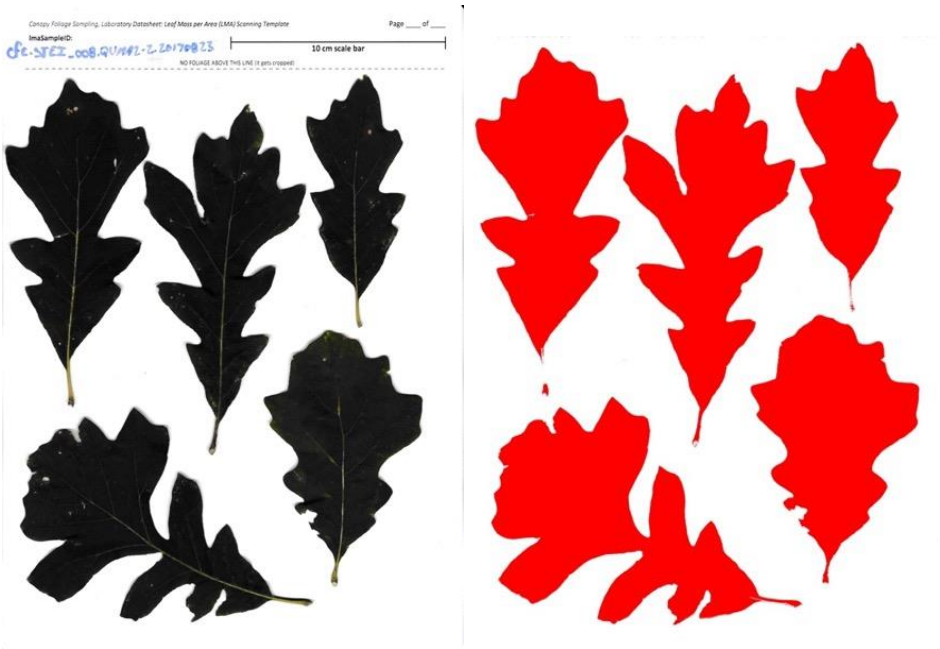

Figure 24. (Left) Example of a good quality scanned image of foliage. (Right) Image after processing in ImageJ.

11. Examine the scan.

   a. If the image contains significant shadows, attempt to reduce by 1) increasing contrast on the scanner settings, 2) cutting very curly leaves into smaller pieces to help flatten, or 3) placing

a heavy object on top of the scanner to flatten the leaves. *Do not spend > 15 minutes making these adjustments*.

b. If the foliage shows water drops, remove material, dry thoroughly, then scan again.

NOTE: All material set aside for LMA may not actually be used for scanning. If there is extra tissue, combine it with the bulk chemistry sample. Or, if sure there is sufficient mass for all subsamples, discard extra foliage.

12. Upload scan as .tif or .jpeg file and save to a folder in the Domain Support Facility N-drive.
13. Immediately after scanning, weigh samples to nearest **0.001g**
    a. Tare a plastic weigh boat.
    b. Transfer sample into weigh boat. *Use care not to spill/lose any scanned material*.
    c. Weigh to the nearest **0.001g**.
    d. Record **freshMass** (mass in grams of fresh scanned material) and **scanDate** (date of LMA scanning) in the data entry application or lab datasheet.
14. Transfer all scanned foliar material into a coin envelope (or paper bag if it is a large sample), *being careful not to spill/lose any*. Label it with **lmaSampleID** (e.g., sampleID + ".lma").
    - Example identifier: **cfc.GRSM001.QUAL-1.20190705.lma**
15. Barcodes can help with sample tracking but are not required for LMA. If using, affix a Type I adhesive barcode label to the bag or coin envelope, then scan this barcode into the field **lmaSampleCode.**

16. If sample contains a *Toxicodendron* spp., add sample warning label to bag or coin envelope.
17. Place coin envelope or paper bag into drying oven and record **ovenStartDate** (date and time a sample was placed into drying oven). Samples must oven-dried at 65°C for at least 48 hours.
    - If there is no space in the drying oven, place them in a cool, dry area until space is available, then transfer to oven as soon as possible (ideally within one week).
    - Can write **ovenStartDate** on envelopes/bags if it helps organize oven-drying workflow
18. For herbaceous clip strip samples where LMA material is taken from the bulk sample bag, make sure to begin processing the bulk sample once scanning is complete and within 5 days of collection, as detailed in SOP F.
19. Clean the scanner glass with a tissue to remove dust/resin. If dry tissue is insufficient to remove resin, use a small amount of glass cleaner. Move on to next sample until all have been scanned.

E.2 Measuring Leaf Area

IMPORTANT! *Before personnel can measure LMA on real samples, they must analyze standard images within the area threshold specified in the training materials, as described in Section 6.1. Standard images are located in the NEON Training Center.*

1. Open ImageJ, which can be accessed through the Citrix FOPS Desktop.
2. Open the leaf/needle scan you want to process in ImageJ by clicking on the File → Open and browse for the image.
3. If the leaves are clearly not in 'peak green' condition, for example their coloration indicates leaves not fully expanded or starting to senesce, make sure this is captured in the **plantStatus** entry in the field data.
4. Set the scale that you want to use for area calculations. Reset the scale for each image.
    a. Click on the line segment tool (box with line).
    b. Draw a line that measures 10 cm by tracing the scanned scale bar.
    c. With line still selected, click Analyze → Set Scale. This will bring up a new window.
        1) Leave 'Distance in pixels' as they are
        2) In the 'Known Distance' box, type in the distance (in mm) of the line (100).
        3) Leave the 'Pixel aspect ratio' at 1.0.
        4) In 'Units of length' box, type in 'mm'.
        5) Click OK.
5. Now that the scale is set, use one of the selection tools – either rectangular, oval, or polygon (left most buttons of the tool bar), to select the part of the image that contains only the needles or leaves that need to be measured. Make sure to exclude all text and lines.
    a. Go to Image → Crop. This will crop the image so you will only be analyzing foliage.
6. Next go to Process → Binary → Make Binary, which converts the image to black and white.
7. Go to Process → Binary → Fill Holes. This will fill in any areas within the leaves or needles that may have a different (often lighter) color value due to irregularities in the original scan.
    a. Do not Fill Holes if your leaves have actual, large holes. This will wrongly fill them in.
8. If these procedures yield a sharp, clear, black image with no artificial holes or white space, proceed to STEP 10 below. If not, conduct the procedure outlined in STEP 9 first.

9. If converting to binary and filling holes still yields artificial holes or white space (likely for conifer needles with light-colored undersides), or if some sections of the leaves/blades disappear upon conversion (likely for grass or cactus blades with light colored segments), **you must close the file without saving and do the following**.
    a. Re-open the original scanned color image and complete STEPS 2-5.
    b. Go to Image → Color → Split channels. You can then select the channel that produces that best, sharpest and most clear image. It is most often the blue channel. If the images all look similar, work with the blue channel.

c. Go to Image → Adjust → Brightness/Contrast. Increase both Minimum and Contrast; this should help to fill in artificial holes and light-colored sections. The goal is to have the foliage be as dark as possible without exaggerating any shadows created during scanning or overly blurring the edges.

d. When the image is as crisp and filled-in as possible, click Set. A dialogue box will open, click **OK**. Close the 'Brightness/Contrast' dialogue box.

e. Next, go to Image → Adjust → Threshold and this will open a new window. The boxes below the scale bars should read 'Default' and 'Red,' and your leaves/needles will be red on a white background (**Figure 24**, right).

1) Use the slider bars to adjust which pixels to include in the area calculation. Generally, the top slider will be left alone, as this should be set as far left as possible (to include darkest pixels). Move the bottom slider right to include lighter pixels, or left to include darker pixels. Adjust to include the most leaf area without distorting the leaf perimeter.

2) Due to reflections off the scanner bed or light-colored areas in the leaf, it may be impossible to include certain pixels using just the Threshold slider bars without also including unwanted pixels around the margins. The paintbrush tool can be used to include some of these small areas:

a) Select the color picker tool from the toolbar  and click on an area that is already highlighted red

b) Select the paintbrush tool  and carefully color over pixels that represent actual leaf area. Adjust the brush width by double clicking on the paintbrush tool icon

f. When satisfied, hit **Apply**. Setting the threshold is telling the software which parts of the image it will be analyzing. Close the 'Threshold' dialogue box.

10. Go to Analyze → Set measurements. This will bring up a checklist of available measurements. Make sure that only 'Area' is checked. Hit **OK**.

11. Go to Analyze → Analyze Particles. In the window:

a. Set the size (mm2) to '10-infinity' in order to eliminate smaller particles (noise) from the area calculation.

b. If dealing with tiny leaf/needle segments (e.g., hemlock needles), 10-infinity may be too high. Consider using a lower minimum such as '4-infinity.'

c. If you have real holes that are contained by black areas in your image, make sure that "include holes" is NOT checked.

d. If there are simply lighter-colored areas that look like holes, do check that option.

e. Make sure that 'Display Results' is checked and that the 'Show dialog' box displays Masks. Hit **OK**.

12. A table will appear that gives the individual area of each leaf/needle. Additionally, a new image (mask) will pop up that displays the parts of the image included in area calculations.

13. Compare this mask and table with the original image to confirm accuracy.

    a. If the mask looks correct, excluding all but the most delicate features (e.g., bristle tips), save the area table as an excel or .csv file ('File → 'Save As') in the same location as the scanned image file. Use **lmaSampleID** for the file name, add "_scan01", "_scan02" if multiple scans were taken for one subsample.

    b. If the area table has many more rows than expected, all with tiny area values, it means the software is picking up small non-leaf particles and counting them as leaves. Go back to STEP 11 but increase the minimum from 10 to 100, 500, etc - keep going up until the number of rows matches the number of leaves/needles scanned.

    c. If the mask looks inaccurate, close the image without saving and conduct area calculations again by adjusting minimum, contrast, and threshold as described in STEP 9 above.

        1) If after 3 attempts, the mask is still imperfect but only very minor flaws remain, save calculations and proceed.

        2) If after 3 attempts, the mask differs substantially from the true image with significant artificial holes, do not save and contact Science for assistance.

14. Open the saved excel or .csv file and sum areas of all leaves/needles scanned. If there were multiple scans for one subsample, make sure to sum them all. Count the number of leaves/needles scanned using the number of rows in the .csv file.

15. In the data entry application or lab datasheet, record:

    - **scannedLeafNumber:** total number of individual leaves/needles/blades scanned
    - **leafArea**: sum total area in mm^2 of all leaves/needles scanned
    - **percentGreen**: percent of scanned foliar material that was live and 'green' (not senesced or damaged). A visual approximation, do not spend more than a minute making this estimation

16. Once data has been recorded, you may close the image – do not save changes.

17. Save scanned images and area calculation files for 6 months. After this time, if data entry and ingest has occurred successfully, these files may be removed.

E.3 Drying and Weighing Samples

1. Remove subsamples from the oven once they have been dried at 65°C for a minimum of 48h and record **ovenEndDate** (date and time a sample was removed from the drying oven).

- For herbaceous samples, more than 48 hours may be required to fully dry the material. Use the procedure and datasheet outlined in TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]), SOP E, to monitor the drying progress. Remove samples from the oven only after they have achieved constant mass.

2. Allow samples to cool, then weigh samples to nearest **0.001g**.
    a. Tare a metal or plastic weigh boat.
    b. Transfer sample into weigh boat. Make sure all material is removed from the coin envelope or bag, use tweezers if needed.
    c. Weigh to the nearest **0.001g**.
    d. Record **dryMass** (mass in grams of dried scanned material) in the CFC: LMA data entry application or lab datasheet

3. If sample contains *Toxicodendron* spp., use extra care when handling foliage and wear single-use cotton gloves. Clean durable equipment that comes in contact with Toxicodendron spp (e.g., tweezers) as described in RD[08].
4. Return sample to coin envelope or paper bag. The weigh boat may be reused if it remains free of dry material, but re-tare it between samples.
5. Store LMA coin envelopes or paper bags in a cool, dry cabinet for 6 months. After this time, if data entry and ingest has occurred successfully, these samples may be discarded.

E.4 Leaf Punching Option for Broad-leaf Deciduous Species

Only use this option if scanning large leaves takes so long that samples may soon mold and become unusable. Note that leaves must be large enough to use a 0.5”, 0.75”, or 1.5” diameter punch tool.

1. Working on a clean surface, remove leaves from bag and blot dry. They should be moist from storage conditions; use a dry paper towel to simply remove surface moisture. Only clean as much material as is needed for punching.
2. Use the largest diameter punch tool that will allow a minimum of three punches per leaf (or leaflet if dealing with large, compound leaves). Punch 3-5 circles per leaf/leaflet, depending on size of leaf, with one punch in the center including the mid-vein. Aim for 15-30 punches total per lmaSampleID.
3. Use the same punch size for all leaves in the same sample.
4. Record the weight of the fresh material punched.
    a. Tare a metal or plastic weigh boat.
    b. Transfer punches into weigh boat. *Use care not to lose any punches.*
    c. Weight to the nearest **0.001g**.
    d. Record **freshMass** (mass in grams of fresh punched material) in the CFC: LMA data entry application or lab datasheet

5. In the data entry application, indicate 'leaf punch method' and enter punch diameter and number. It will then calculate **leafArea**, e.g., the total area of all punches in mm2
    a. If using paper datasheets, note 'LMA punch method' and record punch diameter (0.5, 0.75, 1.5") and punch number in the **remarks** field, so that these can be entered later
6. Place punches into a coin envelope. Label it with **lmaSampleID** and affix a Type I adhesive barcode label (if desired) as described above.
7. Place coin envelopes into drying oven and record **ovenStartDate**. Samples must oven-dried at 65°C for at least 48 hours.
    a. If there is no room in the drying oven, place them in a cool, dry area until space is available, then transfer to oven as soon as possible (ideally within one week).
8. Save remaining fresh sample in refrigerator for up to one week in case there is a problem.
9. Remove subsamples from the oven once they have been dried at 65°C for 48h. Record **ovenEndDate**.
10. Allow samples to cool, then weigh punches to nearest **0.001g**.
    a. Tare a metal or plastic weigh boat.
    b. Transfer punches into weigh boat. Make sure all material is removed from the coin envelope or bag, use tweezers if needed.
    c. Weigh to the nearest **0.001g**.
    d. Record **dryMass** (dry weight of the punches in grams) in the CFC: LMA data entry application or lab datasheet
11. Return sample to coin envelope or bag. The weigh boat may be reused if it remains free of dry material, but re-tare it between samples.

Store LMA coin envelopes in a cool, dry cabinet for 6 months. After this time, if data entry and ingest has occurred successfully, these samples may be discarded.

SOP F Drying and Subsampling for Chemical Analyses

Fulcrum App = **CFC: Chemistry Subsampling**. Fulcrum Manual is available in the NEON SSL

Bulk canopy foliage samples will be cleaned, oven-dried and split into three subsamples destined for different analytical and archive facilities. Separate instructions for preparing subsamples are provided depending on the mass of dry foliar material available. *Recall that an additional subsample for chlorophyll was already generated in the field and is held at -80 °C until shipment to the designated analytical facility.*

- Foliage must be cleaned and placed in the oven within 5 days of collection. This is especially important for broadleaf samples as needle samples tend to be less susceptible to mold. Foliage not processed within 5 days of collection should be marked as such using **sampleCondition**.

In low humidity conditions, static may make it difficult to transfer ground plant material to plastic scintillation vials. Use of an anti-static gun may help manage cling.

F.1 Cleaning, Drying and Weighing

1. Remove bulk foliage sample from refrigerator.
2. Verify that an LMA subsample has been created.
    a. If not, set aside enough fresh foliage to fill a scanner bed and place into a labeled, resealable plastic bag containing a DI-moistened paper towel. Store in the refrigerator. Wear a clean pair of Nitrile gloves so as not to contaminate the bulk sample.
3. Wearing Nitrile gloves, remove bulk sample material from resealable plastic bag. Clean foliage by blotting with a paper towel to remove surface contaminants (dust, pollen, etc) that can influence chemistry values.
    a. Aim to spend 2-3 minutes per sample, 15 minutes max if cleaning is difficult
    b. Blot where possible, especially if foliage has hairs or waxes. This will remove contaminants without destroying the leaf. Either place foliage in between 2 paper towels and press, or put foliage on a clean work surface, press down on top of leaf, then flip and do the other side.
    c. For small leaves or needles attached to stems, clean them first, then remove from stem prior to drying. Small pieces of bark remaining on needle tips should be removed when possible, although do not spend excessive time.
    d. In general, more water should not be needed as the leaf surfaces will be moist from condensation in the sample bag. If not, can moisten a paper towel with house DI water, then blot or gently wipe with that
    e. For large samples (herbaceous), only clean as much as is needed for chemistry measurements (max 100 g); subsample representatively. As herbaceous samples have lots

of fine tissues, it may be more efficient to use a spray bottle filled with house DI for cleaning. Spray down tissues and let dripping water clean them off, then shake - no blotting.

f. Change paper towels between samples. Ok to re-use gloves but use ethanol to clean gloved hands in between samples.

g. For samples that contain *Toxicodendron* spp., no cleaning will occur, proceed to next step.

4. Transfer clean foliage to a paper bag, then place into drying oven set to 65°C and dry for a minimum of 48 hours. Record **ovenStartDate** (data and time the sample went in to the oven).
   a. Write **ovenStartDate** on bag if it helps organize the oven-drying workflow
   b. For herbaceous samples, more than 48 hours may be required to fully dry the material. Use the procedure and datasheet outlined in TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]), SOP E, to monitor the drying progress. Remove samples only after they have achieved constant mass.
   c. If a fresh herbaceous sample weighs more than 60 g, it has more than enough material and extra foliage may be removed and discarded, ensuring the sample is still representative.
5. After 48 hours in the oven (or when samples have achieved constant mass), remove paper bags from the oven and record **ovenEndDate**.
6. Place an empty paper bag of the same type as contains the samples on the scale. Tare it.
7. Weigh each sample in its bag to the nearest 0.1 g, then record the mass on the paper bag. This mass will not be captured on a datasheet, it is only used to determine which subsampling procedure will be used to prepare the chemistry subsamples.
8. Group samples together according to whether they have more or less than 10 g dry mass. This will allow similar size samples to be processed together for efficiency.
   - For dry mass < 10 g, follow section F.2 to prepare the chemistry subsamples
   - For dry mass > 10 g, follow section F.3 instead

9. For samples that contain *Toxicodendron* spp., follow the steps described in F.4, regardless of sample mass.
10. Samples that have been stored for > 5 days prior to processing must be re-dried at 65 °C for a minimum of 24 hours before grinding and subsampling to ensure consistent sample condition for long term archive and to prevent any continued decomposition or microbial activity.

F.2 Subsampling with Small Mass (< 10 g)

1. Wear a pair of Nitrile gloves when handling and subsampling foliage. It is acceptable to re-use gloves but use ethanol to clean gloved hands in between samples.
2. Split the foliar material into two or three subsamples by hand, following the mass guidelines for each subsample in **Table 16**. Try to ensure that the splits are representative.

Table 16. Guidelines for chemistry subsampling with small mass (< 10 g dry).

dryMass (g)	Samples to create (g)		
	C/N	lignin/ elements	Archive (ground)
< 2.7	0.2 – 0.7	1 – 2	-
2.7 – 6	0.2 – 1	1.5 – 2	1 – 3
6-10	equal	equal	equal

3. Make sure to:
    - Remove twigs and other non-foliage woody parts. Remove flowers, if possible, but don't spend too much time if many small flowers as they won't affect chemistry values much.
    - Process, stow and label each subsample as instructed below, including the directive to grind the chemistry archive sample to 20 mesh.
    - Refer to **Table 10** for more on sample vials and barcodes.
4. C/N and lignin/elements
    a. Container = plastic scint vial, coin envelope, or small paper bag (whatever is easiest and results in less material loss). *Non-scint vial containers are only acceptable for unground material.* It is OK to crush foliage so that it will fit in the chosen container.
    b. Identifier = **cnSampleID** (sampleID + .cn) or **ligninSampleID** (sampleID + .lig)
        1) Example CN: cfc.GRSM001.QUAL-1.20190705.cn
        2) Example lignin: cfc.GRSM001.QUAL-1.20190705.lig
    c. Use the cryogenic labels listed in **Table 24** for scint vials, applied vertically so the identifier is easier to read (do not write directly on vials as this can rub off). For envelopes or paper bags, OK to write on identifiers using permanent marker. *To create printed labels ahead of time, export sampleIDs from the CFC: Field application, then add .cn or .lig suffixes.* If labels are applied after plant tissues, clean vial exterior to remove dust so labels will adhere.
    d. Affix a Type I adhesive barcode label to each container without covering the sample identifier. If using scint vials, make sure barcode is placed lengthwise so it can be scanned. Scan each barcode and ensure it appears in **cnSampleCode** or **ligninSampleBarcode.**
5. Chemistry archive (when sufficient material)
    a. Container = 20 mL plastic scintillation vial
    b. **Necessary to grind and homogenize foliar material using a Wiley mill** (0.85mm, 20 mesh size). Use a paint brush to transfer any particles left in the grinding compartment into the sample vial, then clean the mill in between samples. Use canned air or a vacuum to remove

leaf particles, and ethanol if needed for resinous foliage. Be sure to clean mill accessories (mesh, splitter, etc) as well in between samples.

c. Record the mass of the archive material.

1) Tare the scint vial, then add foliage.

2) Record mass in the field **bgcArchiveMass**, to the nearest 0.01 g.

d. Identifier = **archiveSampleID (**sampleID + .ar)

1) Example: cfc.GRSM001.QUAL-1.20190705.ar

e. Use a cryogenic label for sample identifier, see above tip for printing but use .ar suffix instead. Do not write directly on the vial as it can rub off. If labels are applied after plant tissues, clean vial exterior to remove dust so labels will adhere.

f. Affix a Type I adhesive barcode label lengthwise along the vial, without covering the sample identifier. Scan this barcode – it should appear in the field **archiveSampleCode.**

6. Organize subsamples and group by type in preparation for shipment. Ensure scint vial caps are on and closed, seal coin-envelopes and use tape to close off any leaky corners, or close paper bags with tape or rubber band.

7. Store subsamples in a cool, dry location until they can be shipped to analytical facilities or the biorepository (see **Table 3** for holding times).

F.3 Subsampling with Large Mass (> 10 g dry)

1. Wear a pair of nitrile gloves when handling and subsampling foliage. It is acceptable to re-use gloves but use ethanol to clean gloved hands in between samples.

2. Grind the sample in a Wiley mill (0.85mm, 20 mesh size)

- DryMass **< 20 g**, grind the entire sample.
- DryMass **> 20 g**, haphazardly subsample ~ 20 g, then grind it. Attempt to select as representative a subsample as possible with respect to leaf types and particle sizes.
- Remove twigs and other non-foliage woody parts. Remove flowers if possible, but don't spend too much time if many small flowers as they won't affect chemistry values much.

3. Use an appropriately sized splitter or microsplitter to generate three subsamples.

a. Place the 20 mL plastic scintillation vial that will be used for archive onto a balance (0.01 g accuracy) and tare it.

b. Split the sample once and place an entire half into that vial.

c. Place on the balance and record the mass in **bgcArchiveMass** field, to the nearest 0.01 g.

d. Split the remaining material in half again and place each half into its own 20 mL plastic scintillation vial. These will be for the C/N and lignin/elements analyses. Masses are not required for these subsamples.

e. If all three vials are full after splitting, leftover material may be discarded.

4. The C/N lab requires minimal material, but lignin/element analyses require more, such that the lignin/elements vial should be at least 1/3 full. If this is not the case, grind additional material (if available), or pour the archive sample back into the splitter and keep splitting until there is enough material in the lignin/elements vial. Adjust **bgcArchiveMass** as needed.

a. **DO NOT** create sub-samples with a scoopula/spatula. These tools should only be used to transfer entire subsamples into vials, otherwise the subsamples will not be representative with respect to particle sizes.

5. Ensure that each subsample is labeled as outlined below (also see **Table 10**). Do not write directly on vials as the label can rub off, instead use the cryogenic labels listed in **Table 24**, applied lengthwise or vertically so the identifier is easier to read**.** *For label printing ahead of time, export sample identifiers from the CFC: Field application, then add appropriate suffixes.* If labels are applied after plant tissues, clean vial exterior to remove dust so labels will adhere.

6. Also affix a Type I adhesive barcode label lengthwise along each vial without overlapping the sample identifier. Scan each barcode, which should appear in the fields noted below.

- **C/N**
    - Identifier = **cnSampleID** (sampleID + .cn)
    - Example: cfc.WOOD001.CLIP-1.20190705.cn
    - Barcode should appear in **cnSampleCode** field
- **Lignin/elements**
    - Identifier = **ligninSampleID** (sampleID + .lig)
    - Example: cfc.WOOD001.CLIP-1.20190705.lig
    - Barcode should appear in **ligninSampleBarcode** field
- **Chemistry archive**
    - Identifier = **archiveSampleID** (sampleID + .ar)
    - Example: cfc.WOOD001.CLIP-1.20190705.ar
    - Barcode should appear in **archiveSampleCode** field

7. **For leaf samples, take the material <u>from the C/N sample only</u> and re-grind it in the Wiley Mill with the 40-mesh attachment (0.42 mm mesh).** Do not re-grind lignin/elements or chemistry archive subsamples, only the C/N laboratory requires very finely ground material for analysis.

a. Keep grinding leaf C/N samples to 40 mesh until no more material is observed passing through the mill, grind another 30 seconds, then stop and consider the C/N subsample complete. Do not collect leftover material that is adhered to the mill.

b. Needle C/N samples cannot be ground to 40 mesh as they are too resinous and get stuck in the mill. Instead, mark these C/N sample caps with a red sharpie dot to alert the lab.

8. Clean the mill and all accessories (mesh, splitter, etc) in between samples. Use canned air or a vacuum to remove leaf particles, and ethanol if needed for resinous foliage.

9. Once all subsamples have been created, organize and group by type in preparation for shipment. Ensure scint vial caps are on and closed.

10. Store subsamples in a cool, dry location until they can be shipped to analytical facilities or the biorepository (see **Table 3** for holding times).

F.4 Subsampling with *Toxicodendron* spp.

1. If sample contains or may contain *Toxicodendron* spp, no grinding will take place. However, subsampling for the different chemical analyses and archive will still occur.

2. Conduct all subsampling activities in a clean fume hood. Use caution when handling the sample so as not to expose yourself or others to leaves containing toxic oils. Wear single-use cotton gloves as described in RD[08] and follow the guidelines in RD[08] to clean any equipment, clothing, or skin that comes in contact with foliage.

3. Homogenize the sample prior to manual subsampling by crushing/shaking the contents of the brown paper sample bag(s). It may be helpful to transfer sample to a larger-size paper bag first if it is held in a small paper bag.

    a. If the sample is very large (**> 20 g)**, haphazardly subsample ~ 20 g first, then use this for further subsampling. The rest can be discarded.

4. Split the homogenized foliar material into three subsamples. Try to ensure that the splits are representative but with minimal handling of the foliage.

    a. Sample mass **< 10 g**: follow guidelines in section F.2, Table 14, to apportion material for the different subsample types. Use forceps to avoid having to touch the material where possible. *Do not grind the archive subsample*.

    b. Sample mass **> 10 g**: split the sample in half and use one of those halves to create the chemistry archive sample. Split the remaining material in half again – use one portion to create the C/N sample, another to create the lignin/elements sample.

5. Place unground foliage into containers. For the chemistry samples, it is acceptable to use scintillation vials, paper bags, or coin envelopes, whichever is easiest to work with. For the archive sample, you must use a 20 mL plastic scintillation vial. Apply human-readable labels and barcodes to each container as described in sections F.2 and F.3 (also see **Table 10**).

6. Place a sample warning label on each subsample container, either on the lid for scintillation vials or directly applied to bags or coin envelopes.
7. Clean all durable supplies and surfaces that may have come in contact with sample material as described in RD[08]. Discard all consumable items.
8. Organize and group subsamples by type in preparation for shipment.
9. Store subsamples in a cool, dry location until they can be shipped to analytical facilities or the biorepository (see **Table 3** for holding times).

SOP G Data Entry and Verification

Mobile applications are the preferred mechanism for data entry, and the only mechanism to create digital crown polygons. Data should be entered into the protocol-specific applications 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[12] for complete instructions regarding manual data transcription.

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data are accurate and complete. Certain QA checks can be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before the sampling window closes. Invalid metadata (e.g. collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location.

Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess inadvertently duplicated data and transcription errors to maintain data ***validity*** and ***integrity***. See the Data Management Protocol (RD[12]) for more discussion of QA measures.

Before samples ship to external facilities and/or their digital records load to the NEON database, the data must undergo thorough quality checks. The steps needed to accomplish this are outlined in the CFC QC Checklist, which is available on the NEON SSL.

In addition, crown polygons created in the field require a basic level of office QA checks:

1. Log in to the AGOL software, as described in section A.4
2. Go to *Groups*, then navigate to the Crown Mapping one relevant to the sampling year
3. Go to *Content*, then find the map for your site. Select it, then *Open in Map Viewer Classic*
4. In the navigation pane on the left, find the layer called SITE Crown Polygons. Click the icon that looks like a spreadsheet. This will bring up the attribute table, each polygon is a row.

5. At a minimum, verify that identifier formatting and count match polygonIDs listed in Fulcrum. Formatting issues can be fixed by double-clicking relevant cells in the table. Mismatched counts may mean devices need to be synced, or that polygons for tags not sampled need to be deleted
6. Other quality checks as time permits: review each polygon for odd shapes, polygons very offset from stem locations (when those are included on the map), circling of dark/low pixels. *Science will do these checks, so if not done by Field staff and oddities exist, Science will be reaching out.*
    a. To view a specific polygon, select it in the attribute table, then click the 3 horizontal lines button on the top-right of the attribute table, then *Center on Selection*.
    b. You may need to zoom out to be able to view the AOP raster layers. Turn on and off the different layers by selecting/deselecting in the navigation pane.
    c. If a polygon needs to be edited or deleted, this can either be done on the tablet (see B.4), or in the AGOL user interface. To edit on the computer, select a polygon, then *Edit* – another click on the polygon outline allows for editing/deleting the individual points.
    d. If things look off but Field staff are not sure how to correct, briefly record the issue in the notes field in the attribute table and contact Science via ServiceNow for input.

Sample Identifiers & Barcodes

By default, each (sub)sample produced by this protocol receives a sample identifier, which contains information about the location, date, and sample type. Each (sub)sample will also be associated with a scannable barcode, which will not contain information about sample provenance, but will improve sample tracking and reduce transcription errors introduced by writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, thus it is encouraged to apply these in advance. Use the appropriate barcode label type with each container (i.e., cryogenic Type II barcode labels only used for samples that are stored at -80°C, etc). Note that a barcode label is applied *in addition to* a sample identifier (hand-written or printed).

Barcodes are scanned into the data entry application when a sample is placed into a container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data.

SOP H Sample Shipment

Follow the instructions in NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment (RD[14]) in order to ship samples to external laboratories for chemical analysis and to the biorepository for archive. Follow the sample shipping timelines outlined in **Table 3**.

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APPENDIX A QUICK REFERENCES

OBTAINING CANOPY FOLIAGE SAMPLES

STEP 1 – Determine method(s) that will be used to sample the canopy, then assemble supplies and bag packets. Additionally:

- For **woody individual sampling -** practice with the chosen tool on relevant vegetation, create candidate individual lists using VST data, download maps in Collector for shapefile polygon creation, be prepared to map and tag.
- For **herbaceous clip strip** sampling - ensure familiarity with clip list workflow

STEP 2 – Woody individual sampling: obtain sunlit leaves from a canopy individual. Measure and record the height(s) where samples came from. Map and tag if needed. Create a crown shapefile polygon.

STEP 3 – Herbaceous clip strip sampling: identify a clip strip using either the standard, random method or the targeted method if woody cover > 25%. Once a strip is chosen, delineate and cut all foliar material in the clip strip.

* Some sites will be doing a mix of STEPS 2 and 3*

STEP 4 – Set aside a small, representative subsample of healthy green leaves for chlorophyll analysis: 5-25 punches for woody samples (depending on punch size), 0.5-1 g for herbaceous clip samples. Clean tissues then flash-freeze immediately using dry ice. These will be shipped to an external lab within 7 days of collection.

STEP 5 - In woody sites, set aside an additional subsample of in-tact, healthy, whole green leaves for LMA analysis. Store in a chilled cooler, with a moist paper towel in the sample bag.

STEP 6 – Place bulk sample in a chilled cooler, with a moist paper towel in the sample bag.

STEP 7 - Ensure all data have been recorded and scan barcode labels in to the correct sample ID's. Keep samples frozen/cold (as appropriate) until back at the Domain Support Facility.

LABORATORY PROCESSING AND SHIPMENT OF CANOPY SAMPLES

STEP 8 – Clean and dry leaves, then make LMA measurements, recording data for all variables:

scan fresh leaves | record fresh mass | calculate scanned area in imageJ |dry leaves | record dry mass

STEP 9 – Clean, then oven-dry remaining bulk sample. Split into three subsamples: one for C/N analysis, one for lignin and major/minor element analysis, and one for chemistry archive

STEP 10 – Ensure all samples are labeled correctly and receive a barcode scanned to the correct sample ID's. Ship subsamples to appropriate external laboratory facilities for analysis

WorkTracker: Canopy Foliage Sampling in Type B Sites (print as many as needed)

Target TaxonID *Common taxa get multiple rows*	Sampling candidates plotID, tagID *Circle the individuals that get sampled*					Sampling Crew	Collect Date	Notes
	1	2	3	4	5			

APPENDIX B REMINDERS

Pre-sampling: Check…

- ☑ If using an outside contractor or a NEON UAS pilot, has their availability been confirmed?
- ☑ Does sampling schedule overlap with the AOP overflight?
- ☑ Is all required equipment available?
- ☑ For woody individual sampling, did you create a list of candidate individuals and/or plots using the VST data and share that with Science? Practice with the chosen sampling tool? Create offline maps in Field Maps for crown polygons? Ensure that field samplers know how to map and tag if needed?
- ☑ For herbaceous sampling, are clip lists available for assigned CFC plots? Do field samplers know how to use them?
- ☑ Are several coolers available for dry ice and ice packs? Pre-labeled bags and pre-assembled foil packets?
- ☑ Are there any special permit requirements or quarantine restrictions for the target site?

At the plot: Be sure to…

- ☑ Determine and flag target individuals to sample (woody).
- ☑ Ensure the location of the clip strip is suitable (herbaceous).

While sampling: Remember to…

- ☑ Only collect outer canopy, sunlit leaves.
- ☑ Ensure majority of leaves are healthy, green, and not excessively covered in epiphylls.
- ☑ Ensure enough leaf material is collected to generate all required subsamples.
- ☑ Record heights where samples came from.
- ☑ If sampling a non-tagged woody individual, it must be mapped and tagged ("Z"-appended for canopy-only individuals)
- ☑ Create a digital crown polygon for all woody individuals

Sample handling in the field: Be sure to…

- ☑ Set aside a representative subsample for chlorophyll analysis; clean tissues, then flash-freeze immediately. 5-25 punches for woody samples (depending on punch size), 0.5-1 g for herbaceous clip samples
- ☑ In woody sites, also set aside an LMA subsample.
- ☑ Store the bulk and LMA sample in a chilled cooler.
- ☑ Change gloves between samples.

Sample handling in the lab: Remember…

- ☑ Transfer the chlorophyll subsample to a -80°C ultra-low temperature freezer
- ☑ Store LMA and bulk chemistry samples in the refrigerator at 4°C for no longer than 5 days or samples may become unusable.

Processing for LMA: Check…

- ☑ Foliage dried before scanning?
- ☑ Scans are clear and of good quality (no overlapping leaves)? Did all include a scale bar?
- ☑ For larger leaves, were midveins and petioles included?
- ☑ Has the person conducting calculations in ImageJ analyzed standard images to the acceptable thresholds outlined in the CFC training materials?
- ☑ Non-peak green foliage conditions are noted in **plantStatus** in the field data?
- ☑ Was scannedLeafNumber and leafArea recorded?
- ☑ Were areas from multiple scans summed as needed?
- ☑ Were samples weighed fresh, just after scanning, and again after at least 48 hr of oven drying?

Subsampling for chemistry: Verify…

- ☑ Was foliage cleaned before drying?
- ☑ Were three oven-dry subsamples created from the bulk sample: C/N, lignin/elements, and archive?
- ☑ Were they of sufficient quantity?
- ☑ Were large-mass and archive subsamples ground in the mill, 40 mesh for leaf C/N and 20 mesh for the other types?
- ☑ Did all subsamples that may contain *Toxicodendron* spp. receive the warning label?

SAMPLE IDENTIFIER FORMAT REMINDERS

Bulk Chemistry Sample Identifier

sampleID: cfc.GRSM001.QUAL-1.20190605

Subsample Identifiers

chlorophyllSampleID: cfc.GRSM001.QUAL-1.20190605.chl
lmaSampleID: cfc.GRSM001.QUAL-1.20190605.lma
cnSampleID: cfc.GRSM001.QUAL-1.20190605.cn
ligninSampleID: cfc.GRSM001.QUAL-1.20190605.lig
bgcArchiveID: cfc.GRSM001.QUAL-1.20190605.ar

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The table below lists historical peak greenness windows for each NEON site, derived by NEON AOP using reflectance data from 2003-2021 collected by the Moderate Resolution Imaging Spectroradiometer (MODIS) instrument. Note that for YELL, Tower plot sampling may not occur before June 30th due to a Bear Management closure. Should AOP fly YELL when Tower Plots are off limits, samples may still be collected, but exclusively from Distributed Plots.

Table 17. Historical peak greenness windows for NEON sites.

Domain	Site Name	Start Peak Greenness	End Peak Greenness
1	HARV	5/27	8/18
1	BART	5/29	8/23
2	SCBI	5/14	8/11
2	SERC	5/10	8/20
2	BLAN	5/12	8/5
3	OSBS	6/5	8/22
3	DSNY	4/24	8/4
3	JERC	5/25	8/17
4	GUAN	9/8	12/13
4	LAJA	8/30	11/11
5	UNDE	5/31	8/27
5	STEI	6/1	8/22
5	TREE	6/4	8/18
6	KONZ	5/25	7/14
6	KONA	5/25	7/14
6	UKFS	5/14	8/12
7	ORNL	5/1	8/2
7	MLBS	5/31	8/20
7	GRSM	5/18	8/12
8	TALL	5/3	7/20
8	DELA	4/21	8/17
8	LENO	4/20	8/12
9	WOOD	6/8	7/12
9	DCFS	6/8	7/12
9	NOGP	6/1	7/15
10	CPER	5/25	6/23
10	STER	7/23	8/15
10	RMNP	6/26	7/30
11	CLBJ	4/27	7/8
11	OAES	4/25	6/30
12	YELL	6/2	7/26
13	NIWO	7/5	8/3
13	MOAB	5/7	9/13

Domain	Site Name	Start Peak Greenness	End Peak Greenness
14	SRER	8/4	9/1
14	JORN	8/12	9/10
15	ONAQ	4/20	5/22
16	WREF	6/3	8/6
16	ABBY	6/5	7/24
17	SJER	2/23	4/4
17	SOAP	5/31	7/21
17	TEAK	6/26	8/1
18	TOOL	6/27	7/22
18	BARR	7/5	8/21
19	DEJU	6/9	8/11
19	BONA	6/8	8/12
19	HEAL	6/25	7/25
20	PUUM	6/29	8/14

* For MOAB, although the MODIS data indicate a long peak green window, the preference is to sample earlier in the season, ideally before 5/31, to sample more types of plants in peak green condition

APPENDIX D SITE-SPECIFIC INFORMATION

D.1 D20 – PUUM – Pu'u Maka'ala Natural Area Reserve

When tree ferns are sampled for CFC, strip the green leaflets (pinnae) off the rachis and subsample those for LMA, chlorophyll and the bulk sample. Avoid the midvien when it is thicker than 2 mm.

D.2 D12 – YELL – Yellowstone National Park

This site is not permitted to sample foliage using any methods other than clippers or a pole pruner. Since forested plots contain fairly dense, tall stands of trees, it is necessary to use an extendable, telescoping pole pruner with as long a reach as possible (ideally 30 ft or longer) in order to collect sunlit samples.

D.3 D14 – SRER – Santa Rita Experimental Range

When cacti are sampled for CFC, follow the general guidelines below. The goal is to enable sampling of these important desert plants without causing long-term damage to them or injury to the samplers.

Segmented type cacti such as Prickly Pear (*Opuntia spp*):

1. Remove a single sunlit pad with a sharp knife
2. Remove spines with tweezers
3. Cut pad in half lengthwise
4. Use one half for chemistry measurements:
    a. Create the chlorophyll subsample - clean pad with deionized water, then use a sharp knife to remove small strips and place into foil packet
    b. Place the rest of that half into the bag for the bulk sample
5. Use other half to create the LMA sample
6. Process in the lab same as other sample types.

Barrel type cacti:

1. Select a small section of tissue that is at the apex of the stem
2. Remove spines with tweezers from this area, then carefully remove a few small strips of photosynthetic tissue using a scalpel
3. Remove just enough to create the chlorophyll subsample and a bulk chemistry sample. We will not measure LMA on these types of cacti

Note: Avoid protected species, cholla cacti (*Cylindropuntia spp*) whose spines pose too much of a hazard, and small cactus individuals that would be severely damaged by sampling.

In the lab, it can take 2-3 months to dry cacti tissue completely at 65 ˚C. Last time SRER was sampled for canopy foliage, prickly pears took 4-5 weeks to dry in the oven. This is OK, be patient and allow tissues to dry completely (e.g., stop losing mass) before removing from the oven. Do not increase oven temps.

APPENDIX E BEST PRACTICES FOR TALL CANOPY SAMPLING

Sampling sunlit foliage from tall trees in a closed-canopy forest is a difficult task. If available, three-person teams are best for this work. Whenever possible, we utilize a UAS to collect foliage from the top of sunlit crowns. Below are some helpful tips on preparing for and executing this kind of sampling in partnership with a certified NEON UAS pilot.

When the UAS cannot be used due to permitting or site host restrictions, line launchers and slingshots are often employed. Mastering these tools requires significant practice. The first few days of sampling will be slow-going, but it should improve thereafter. Along with proficiency using the tools comes better ability to recognize which trees will be most amenable to sampling based on their position in the canopy. Here we include some best practices gathered from NEON Field Ecologists and external researchers. Keep in mind that all sites are different, and the suggestions below may need to be modified for different sites and species.

E.1 Tips for UAS Sampling and Working with a Pilot

1. Samples arrive quickly and having two people at the processing station helps the team keep up with subsampling. Ensure there is a second hole punch and a good system for keeping track of samples as they move through, especially if the lead botanist remains at the plot.
2. At the launch point/processing station, work in the shade whenever possible to prevent tissues from wilting and to slow dry ice sublimation.
3. Prioritize conifers and pines for UAS sampling, since they are more difficult to sample with the line launcher.
4. For full days of sampling, the UAS batteries will need to be recharged at least once. Start at plots that are farther away in the morning when all batteries are full, then plan to sample at plots closer to the tower or other charging infrastructure in the afternoon.
5. Plot maps from Peregrine are helpful to identify and visualize sampling targets. They can also be used to record sample heights if the UAS is going faster than possible to create Fulcrum records.
6. For each target tree, find a gap where the canopy is visible from the ground. Have at least one viewing location in mind per tree before you give the OK to launch the drone. It may be helpful to place a reflector at the base of the tree.
7. Estimating meters for the drone to move in the air tends to result in over estimations. Use shorter distances than you might think and half-meter increments when communicating with the pilot. It is helpful if they rotate the claw to get a visual on the target tree before adjusting.
8. If the pilot has a centroid point location programmed, direct them to trees by giving our mapping information, remapping the tree from the center as needed. If a tree is leaning, provide an azimuth for the direction in which it leans.

E.2 Line Launcher or Slingshot Sampling - Getting a Line Over the Desired Branch

1. Manual Toss of Throw Weight
   - Canopy height: 6-10m
   - Advantages: Simple, few pieces of equipment needed
   - Disadvantages: Limited range

   1) Attach a throw weight to the end of a line
   2) Toss the throw weight over the desired branch

2. Sherrilltree Big Shot Slingshot
   - Canopy height: up to 40 m
   - Advantages: Very customizable range, easy to take multiple shots
   - Disadvantages: Less precise for aiming; smaller people will require the trigger

   1) Attach slingshot head into pole
   2) Lay weight into pouch, grasp trigger loop with index and middle finger
   3) With your head on one side of the pole and firing pouch on the opposite, pull the firing pouch down the shaft of the pole while changing from a standing position to down on one knee
   4) Aim and release pouch
   5) See next section for important tips on rope management

3. TreeStuff Air Powered Trees Access (APTA) Line Launcher
   - Canopy height: up to 40 m
   - Advantages: Able to shoot fairly high with moderate precision
   - Disadvantages: Cumbersome to take multiple shots, keeping pressure can be an issue

   1) Close the APTA pressure valve (perpendicular to barrel)
   2) Pressurize the chamber to 60 - 140 psi depending on the height of tree you are sampling. Mechanical or hand (bicycle) bumps are both viable options for pressurizing the device.
      a) Some rough estimates: 20 m trees = 70-80 psi, 30 m trees = 110-120 psi
      b) A small amount of air (~ 5 psi) is lost each time a pressurizing device is removed from the APTA, so it's helpful to slightly exceed the pressure that you think will be necessary.
   3) Load the throw weight into the APTA “ring-first”

4) If needed, use a stick (ramrod) to push the throw weight firmly to the base of the APTA barrel, or simply shake the barrel up and down and few times.

5) Place some cushion between the end of the barrel and your shoulder to avoid injury from kickback – material could include a pool noodle, foam packing material, or a towel.

6) Aim the APTA over the branch that you would like to sample.

   a) Use a fairly steep angle, this prevents extraneous horizontal movement of the throw line and helps keep it mainly over the tree you're targeting.

7) Pull back the pressure valve quickly and smoothly to fire

8) See next section for important tips on rope management

4. Sherrilltree Big Launcher Line Launcher

- Canopy height: up to 60 m
- Advantages: Able to shoot very high with precision aim; easy to take multiple shots
- Disadvantages: Best for very tall trees, range not customizable; requires hearing protection

1) Previous shooting experience is helpful for aiming

2) Load a blank cartridge

3) Aim over the branch that you'd like to sample - a steep angle is advised

4) Engage the trigger to launch the line.

   a) The launch is very powerful, which is why it can reach such tall heights. Ensure sampling team is well behind the person using the launcher

   b) There are two power charge options for differing distances.

5) Do not bother using the reel to organize the rope, simply flake it (see next section)

E.3 Line Launcher or Slingshot Sampling - Tips for Procuring Foliage Samples

1. Suggested roles for a three-person team:

   a. One person shoots/manipulates one end of the throw line.

   b. A second person manipulates the other end of the throw line and any cutting attachments.

   c. The third person watches the branch and line and directs the others as needed. This individual can also watch for falling branches and collect them or prepare to subsample while the other two cut the branch.

2. Good rope management is a key factor for successful use of launchers and slingshots. Keep these points in mind and realize that there will be a learning curve:

a. For the rope that is attached to the throw weight, use a length of maximum canopy height x 2.5. If your trees require sawing to procure foliage (instead of simply tugging), you will need another piece of rope for the other end of the cutting rig. This can be a shorter piece.

b. Before shooting the rope, you must 'flake' it. This means pulling small sections of the line taut as you drop it onto a tarp or into a bucket or bag. This is crucial as it prevents tangles. Do not coil the rope accidentally while flaking, make sure it moves in different directions.

c. If you are using a bucket to keep the rope in, you may tie one end of the throw line to it.

d. Keep the flaked rope to the side and slightly in front of the shooter. This prevents it from snagging on the shooter/ vegetation following launch.

3. Before shooting, the throw weight will need to be attached to the long piece of rope. Some may prefer Quick Links instead of directly tying knots to allow for an efficient switch, otherwise knots such as a bowline loop and half hitch can work.

4. To save time and prevent rope tangles, it is useful to "catch" the throw line (while wearing work gloves) once it has reached the desired branch. This helps control the descent of the throw weight and minimize the number of branches that the line drapes over. *Ideally, the line only drapes over one branch.*

5. If the line drapes over multiple branches, it is possible to adjust to isolate just one branch. To do this, carefully pull the throw bag back through the canopy, then let it fall down after it goes back over a few branches.

   a. Throw bags may get stuck while doing this maneuver. Some have found that replacing the bag with a fist-sized rock is just as effective and leaves less opportunity for equipment loss.

6. With practice or using the adjustment described above, the throw weight will go up and over the desired branch and return to the ground. At this point there are two options:

   a. Some trees (see below) may have brittle branches, or leaves that easily fall off the branch upon shaking. In this, simply tugging firmly or shaking the rope (wearing work gloves) will bring down some foliage. If so, great.

   b. Other trees will require an additional piece of equipment, such as a saw or piece of chain, to cut down a branch or strip foliage, respectively. If this is the case, detach the throw weight, then attach the saw or chain to the piece of rope draped over the branch (using Quick Links). To the other end of the saw or chain, attach the second, shorter piece of rope.

      1) There are various brands and styles of flexible saws that can be used for cutting, some of which tend to work better for different kinds of trees. See **Table 21** for suggestions, it is helpful to order several different options to try out on the local vegetation.

      2) Additionally, the saws tend to break so make sure to have several on hand.

3) Use colorful duct tape to cover the attachment points. The tape both strengthens the attachment point and provides a visual clue for when the saw or chain is directly over the target branch. Binoculars are also helpful.

7. Raise the rope so that the equipment is at the target branch and manipulate the line to get the saw or chain into the desired location.

8. A team of two can work together to manipulate the two sections of rope to cut down the target branch or use the chain to strip off leaves. If available, another member of the team can collect leaves/branches immediately as they fall from the canopy.

   a. Use care if cutting a larger branch, make sure no one is standing where it will fall.

   b. Be prepared to lose some equipment to the trees (throw weights, saws, etc). It is inevitable, have several spares on hand.

E.4 Line Launcher or Slingshot Sampling - Species Specific Experiences

This list will be expanded when additional feedback is received from NEON Field Ecologists.

Acer rubrum – Holds on to leaves well, need to focus on looping over the ends of the branches and stripping them. This is basically the only way to get leaves. Not very shakable. Chain is useful.

Amalanchier laevis – Similar to *Acer sp*, very bendable branches. Use a chain to help strip branches.

Betula lenta – Lots of individual leaves fall when shaken and/or stripped.

Halasia tetraptera – Pretty easy, tends to fall in small clumps.

Juniperus virginiana – Once a line is in the tree, little clumps start falling. The longer you shake, the more little clumps fall. Very, very shakable and very easy.

Liriodendron tulipifera – If a branch is looped well, it is easy to break it off the tree; therefore, try to only shoot a small branch. Leaves do fall off in small clumps, but branches are difficult to strip. Shakable.

Liquidambar styraciflua – Will drop clumps if branch is well looped. Branches easily break, so try to choose a small one. Shakable.

Nyssa sylvatica – Pretty easy, tends to fall in small clumps. If you get on a branch that is too large, you will not be able to shake any leaves free. Aim for end of branch and shake. Stripping will yield way more leaves than you need, but works well.

Pinus echinata – Very difficult! Aim for end of branches, preferably a very ‘twiggy’ one so you can break or saw one of those. Branches are very bendy, so shaking doesn’t work well.

Quercus alba – Tend to be brittle where clumps of leaves fall if lines can be crisscrossed and stripped. Not very shakable if you’re on a larger branch, aim for branch tip.

Quercus montana – Tend to be brittle where clumps of leaves fall if lines can be crossed and pulled. Also quite shakable.

Quercus rubra – Tend to be brittle where clumps of leaves fall if lines can be crisscrossed and stripped. Only slightly shakable. Aim for a twiggy area and try to break branches.

Tsuga canadensis – Once the line is in the tree, shaking produces lots of little clumps.

APPENDIX F RESOURCES FOR CLIP STRIP SAMPLING, TARGETED METHOD

When woody cover is >25% of the plot, clip strips will be selected using a targeted method that identifies sunlit "patches" of herbaceous vegetation. To identify the location of clip cells within these sunlit herbaceous patches, Field Science will need to find and utilize the appropriate map below (based on subplotID) to determine which clipCellNumber should be sampled. Then, they will use **Table 18** to find the easting and northing values associated with that clip strip so it can be delineated at a known location relative to the SW corner of the 20m x 20m plot or subplot.

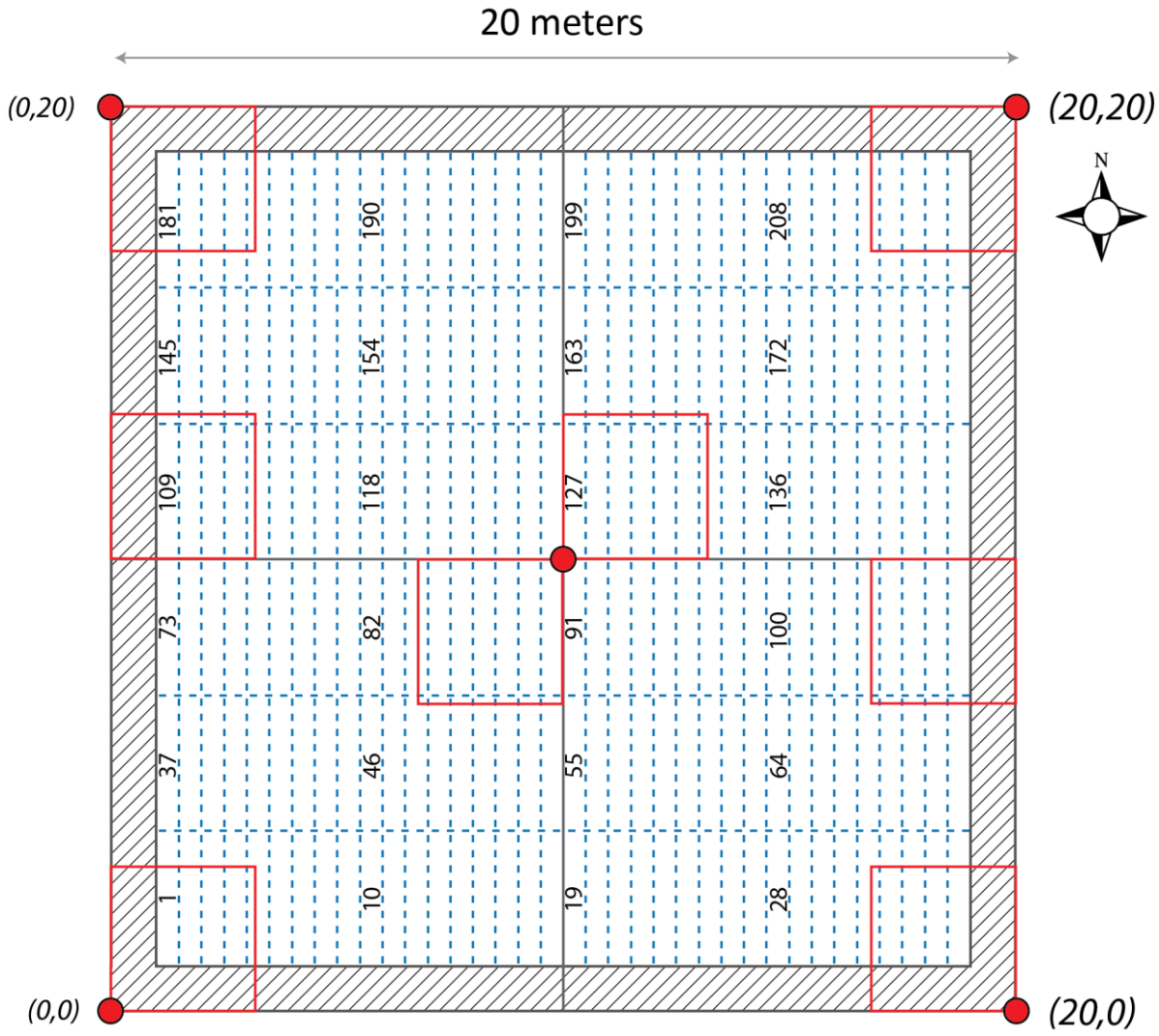

Figure 25. Map of clipCellNumbers in a 20m x 20m base plot (**subplotID = 31** in provided Clip Lists). Red squares indicate nested subplots; clip cells that significantly overlap red squares are not used for clip sampling. However, clip cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support clip harvest sampling.

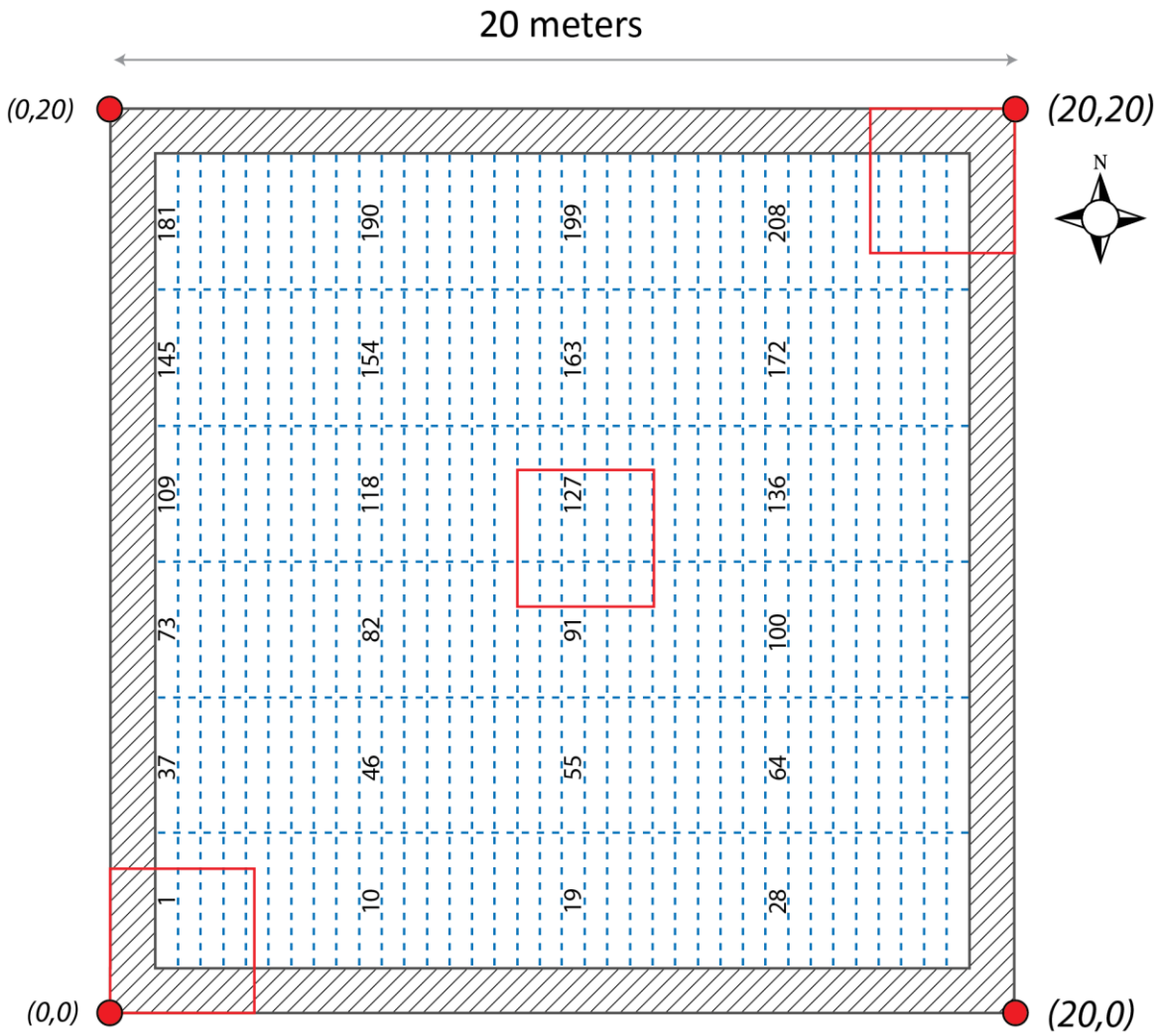

Figure 26. Map of clipCellNumbers for **subplotID = 21** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

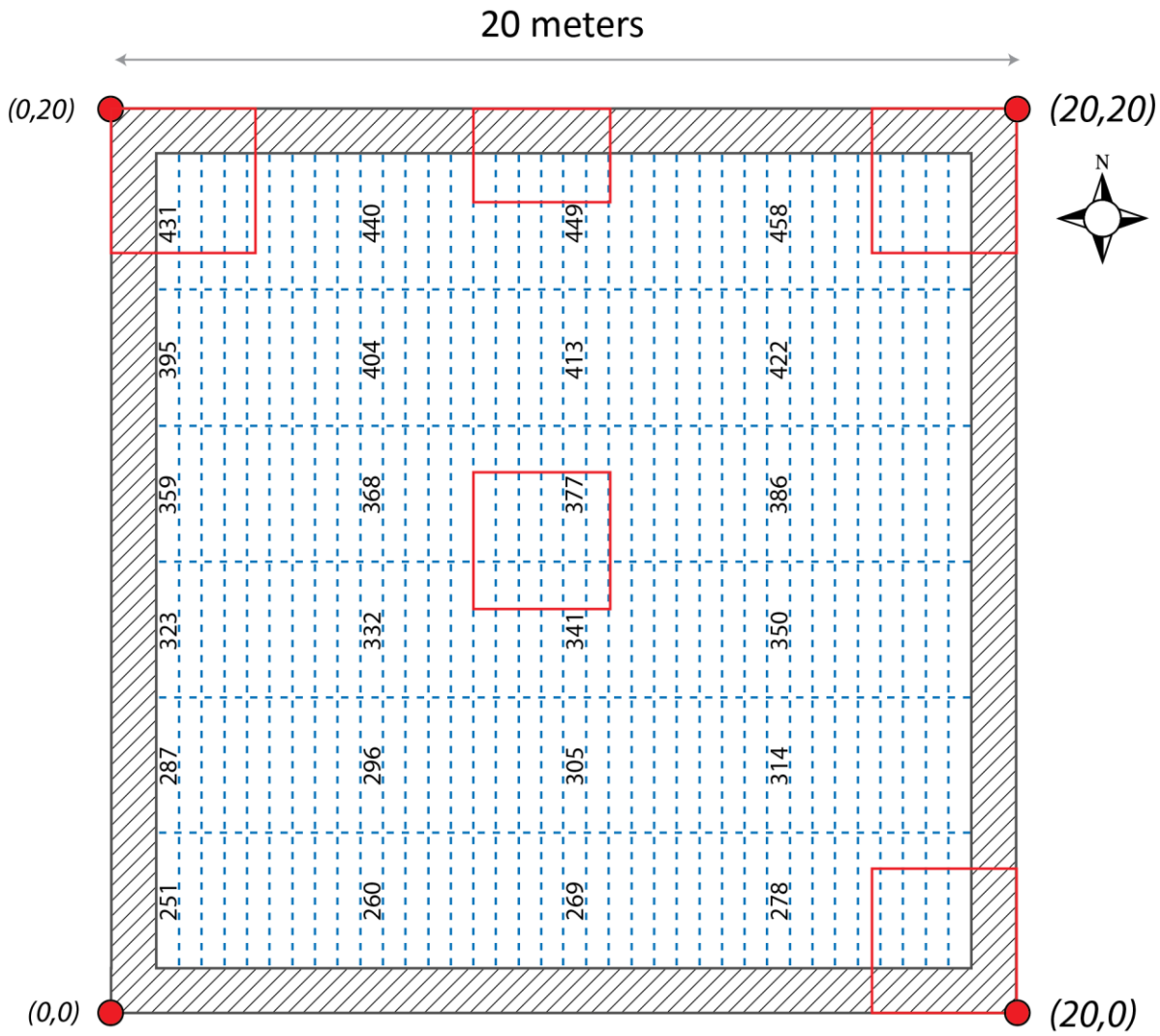

Figure 27. Map of clipCellNumbers for **subplotID = 23** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

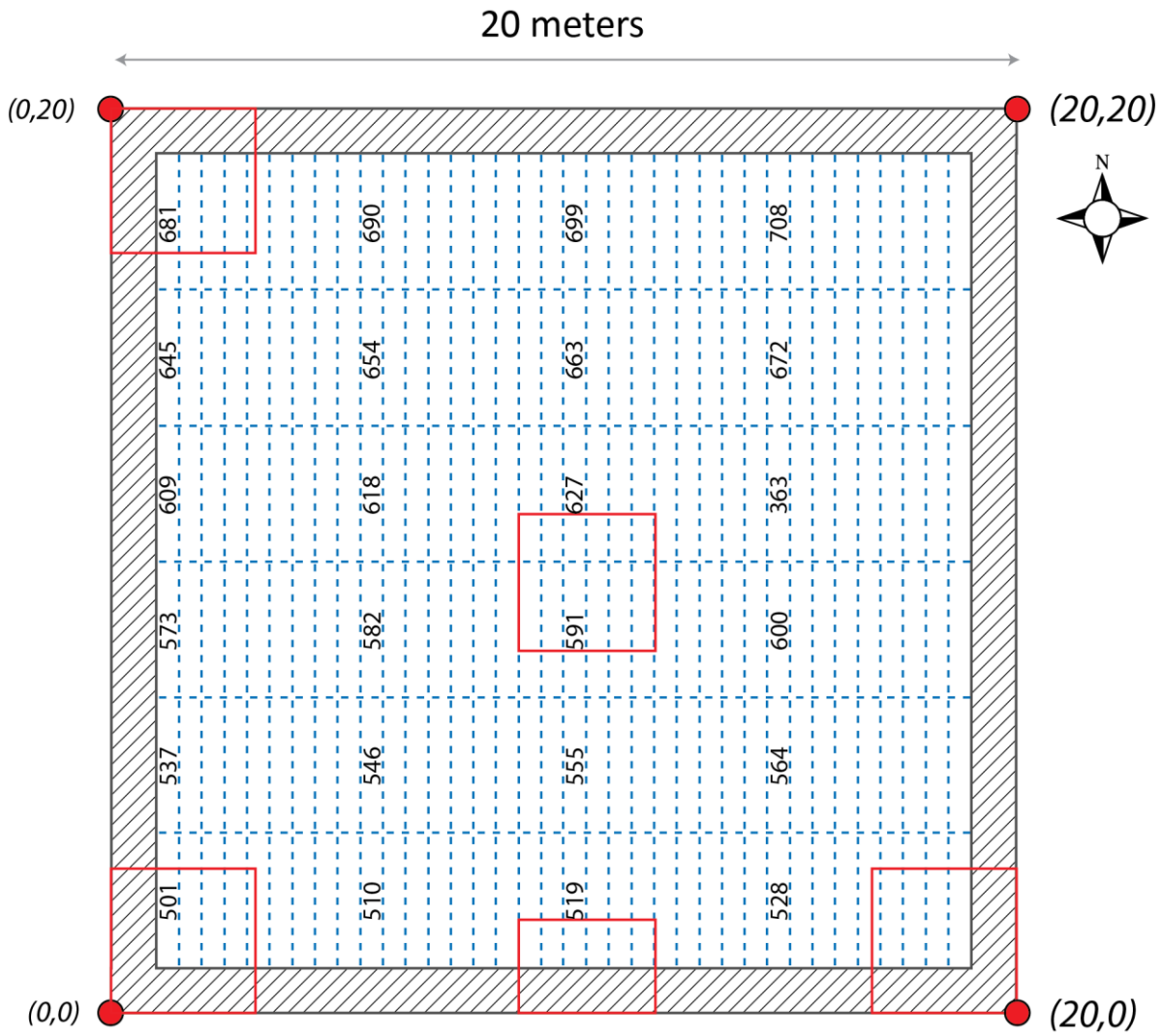

Figure 28. Map of clipCellNumbers for **subplotID = 39** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

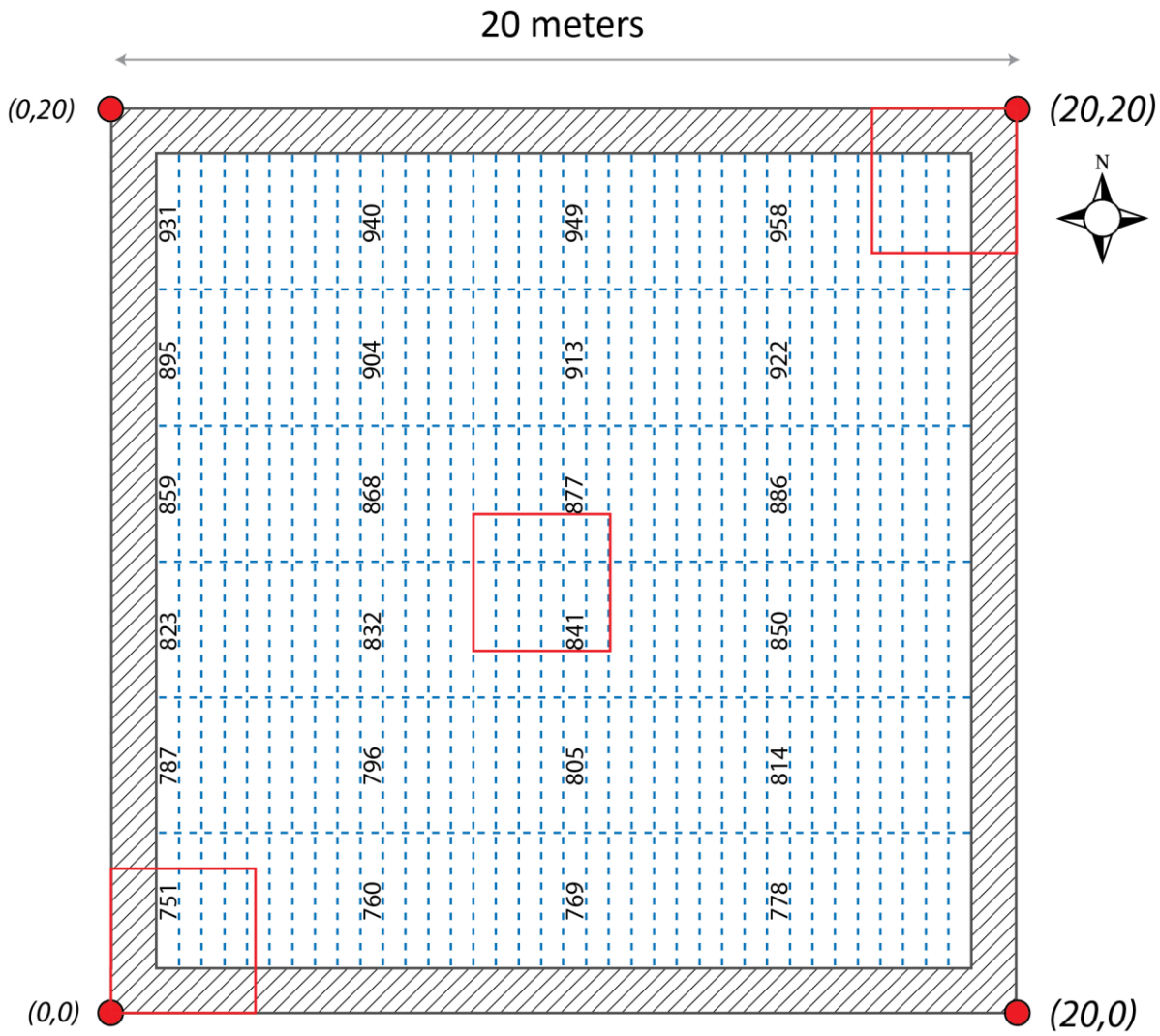

Figure 29. Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

Table 18. List of clipCellNumbers by subplotID and associated easting and northing coordinates. Coordinates correspond to the SW corner of a 0.1m x 2m Clip Strip, and indicate the distance in meters relative to the SW corner of the plot (subplotID = 31) or subplot (subplotID = 21, 23, 39, 41).

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
1	1	251	501	751	1.2	1.5
2	2	252	502	752	1.7	1.5
3	3	253	503	753	2.2	1.5
4	4	254	504	754	2.7	1.5
5	5	255	505	755	3.2	1.5
6	6	256	506	756	3.7	1.5
7	7	257	507	757	4.2	1.5
8	8	258	508	758	4.7	1.5
9	9	259	509	759	5.2	1.5
10	10	260	510	760	5.7	1.5
11	11	261	511	761	6.2	1.5
12	12	262	512	762	6.7	1.5
13	13	263	513	763	7.2	1.5
14	14	264	514	764	7.7	1.5
15	15	265	515	765	8.2	1.5
16	16	266	516	766	8.7	1.5
17	17	267	517	767	9.2	1.5
18	18	268	518	768	9.7	1.5
19	19	269	519	769	10.2	1.5
20	20	270	520	770	10.7	1.5
21	21	271	521	771	11.2	1.5
22	22	272	522	772	11.7	1.5
23	23	273	523	773	12.2	1.5
24	24	274	524	774	12.7	1.5
25	25	275	525	775	13.2	1.5
26	26	276	526	776	13.7	1.5
27	27	277	527	777	14.2	1.5
28	28	278	528	778	14.7	1.5
29	29	279	529	779	15.2	1.5
30	30	280	530	780	15.7	1.5
31	31	281	531	781	16.2	1.5
32	32	282	532	782	16.7	1.5
33	33	283	533	783	17.2	1.5
34	34	284	534	784	17.7	1.5
35	35	285	535	785	18.2	1.5
36	36	286	536	786	18.7	1.5
37	37	287	537	787	1.2	4.5
38	38	288	538	788	1.7	4.5
39	39	289	539	789	2.2	4.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
40	40	290	540	790	2.7	4.5
41	41	291	541	791	3.2	4.5
42	42	292	542	792	3.7	4.5
43	43	293	543	793	4.2	4.5
44	44	294	544	794	4.7	4.5
45	45	295	545	795	5.2	4.5
46	46	296	546	796	5.7	4.5
47	47	297	547	797	6.2	4.5
48	48	298	548	798	6.7	4.5
49	49	299	549	799	7.2	4.5
50	50	300	550	800	7.7	4.5
51	51	301	551	801	8.2	4.5
52	52	302	552	802	8.7	4.5
53	53	303	553	803	9.2	4.5
54	54	304	554	804	9.7	4.5
55	55	305	555	805	10.2	4.5
56	56	306	556	806	10.7	4.5
57	57	307	557	807	11.2	4.5
58	58	308	558	808	11.7	4.5
59	59	309	559	809	12.2	4.5
60	60	310	560	810	12.7	4.5
61	61	311	561	811	13.2	4.5
62	62	312	562	812	13.7	4.5
63	63	313	563	813	14.2	4.5
64	64	314	564	814	14.7	4.5
65	65	315	565	815	15.2	4.5
66	66	316	566	816	15.7	4.5
67	67	317	567	817	16.2	4.5
68	68	318	568	818	16.7	4.5
69	69	319	569	819	17.2	4.5
70	70	320	570	820	17.7	4.5
71	71	321	571	821	18.2	4.5
72	72	322	572	822	18.7	4.5
73	73	323	573	823	1.2	7.5
74	74	324	574	824	1.7	7.5
75	75	325	575	825	2.2	7.5
76	76	326	576	826	2.7	7.5
77	77	327	577	827	3.2	7.5
78	78	328	578	828	3.7	7.5
79	79	329	579	829	4.2	7.5
80	80	330	580	830	4.7	7.5
81	81	331	581	831	5.2	7.5
82	82	332	582	832	5.7	7.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
83	83	333	583	833	6.2	7.5
84	84	334	584	834	6.7	7.5
85	85	335	585	835	7.2	7.5
86	86	336	586	836	7.7	7.5
87	87	337	587	837	8.2	7.5
88	88	338	588	838	8.7	7.5
89	89	339	589	839	9.2	7.5
90	90	340	590	840	9.7	7.5
91	91	341	591	841	10.2	7.5
92	92	342	592	842	10.7	7.5
93	93	343	593	843	11.2	7.5
94	94	344	594	844	11.7	7.5
95	95	345	595	845	12.2	7.5
96	96	346	596	846	12.7	7.5
97	97	347	597	847	13.2	7.5
98	98	348	598	848	13.7	7.5
99	99	349	599	849	14.2	7.5
100	100	350	600	850	14.7	7.5
101	101	351	601	851	15.2	7.5
102	102	352	602	852	15.7	7.5
103	103	353	603	853	16.2	7.5
104	104	354	604	854	16.7	7.5
105	105	355	605	855	17.2	7.5
106	106	356	606	856	17.7	7.5
107	107	357	607	857	18.2	7.5
108	108	358	608	858	18.7	7.5
109	109	359	609	859	1.2	10.5
110	110	360	610	860	1.7	10.5
111	111	361	611	861	2.2	10.5
112	112	362	612	862	2.7	10.5
113	113	363	613	863	3.2	10.5
114	114	364	614	864	3.7	10.5
115	115	365	615	865	4.2	10.5
116	116	366	616	866	4.7	10.5
117	117	367	617	867	5.2	10.5
118	118	368	618	868	5.7	10.5
119	119	369	619	869	6.2	10.5
120	120	370	620	870	6.7	10.5
121	121	371	621	871	7.2	10.5
122	122	372	622	872	7.7	10.5
123	123	373	623	873	8.2	10.5
124	124	374	624	874	8.7	10.5
125	125	375	625	875	9.2	10.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
126	126	376	626	876	9.7	10.5
127	127	377	627	877	10.2	10.5
128	128	378	628	878	10.7	10.5
129	129	379	629	879	11.2	10.5
130	130	380	630	880	11.7	10.5
131	131	381	631	881	12.2	10.5
132	132	382	632	882	12.7	10.5
133	133	383	633	883	13.2	10.5
134	134	384	634	884	13.7	10.5
135	135	385	635	885	14.2	10.5
136	136	386	636	886	14.7	10.5
137	137	387	637	887	15.2	10.5
138	138	388	638	888	15.7	10.5
139	139	389	639	889	16.2	10.5
140	140	390	640	890	16.7	10.5
141	141	391	641	891	17.2	10.5
142	142	392	642	892	17.7	10.5
143	143	393	643	893	18.2	10.5
144	144	394	644	894	18.7	10.5
145	145	395	645	895	1.2	13.5
146	146	396	646	896	1.7	13.5
147	147	397	647	897	2.2	13.5
148	148	398	648	898	2.7	13.5
149	149	399	649	899	3.2	13.5
150	150	400	650	900	3.7	13.5
151	151	401	651	901	4.2	13.5
152	152	402	652	902	4.7	13.5
153	153	403	653	903	5.2	13.5
154	154	404	654	904	5.7	13.5
155	155	405	655	905	6.2	13.5
156	156	406	656	906	6.7	13.5
157	157	407	657	907	7.2	13.5
158	158	408	658	908	7.7	13.5
159	159	409	659	909	8.2	13.5
160	160	410	660	910	8.7	13.5
161	161	411	661	911	9.2	13.5
162	162	412	662	912	9.7	13.5
163	163	413	663	913	10.2	13.5
164	164	414	664	914	10.7	13.5
165	165	415	665	915	11.2	13.5
166	166	416	666	916	11.7	13.5
167	167	417	667	917	12.2	13.5
168	168	418	668	918	12.7	13.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
169	169	419	669	919	13.2	13.5
170	170	420	670	920	13.7	13.5
171	171	421	671	921	14.2	13.5
172	172	422	672	922	14.7	13.5
173	173	423	673	923	15.2	13.5
174	174	424	674	924	15.7	13.5
175	175	425	675	925	16.2	13.5
176	176	426	676	926	16.7	13.5
177	177	427	677	927	17.2	13.5
178	178	428	678	928	17.7	13.5
179	179	429	679	929	18.2	13.5
180	180	430	680	930	18.7	13.5
181	181	431	681	931	1.2	16.5
182	182	432	682	932	1.7	16.5
183	183	433	683	933	2.2	16.5
184	184	434	684	934	2.7	16.5
185	185	435	685	935	3.2	16.5
186	186	436	686	936	3.7	16.5
187	187	437	687	937	4.2	16.5
188	188	438	688	938	4.7	16.5
189	189	439	689	939	5.2	16.5
190	190	440	690	940	5.7	16.5
191	191	441	691	941	6.2	16.5
192	192	442	692	942	6.7	16.5
193	193	443	693	943	7.2	16.5
194	194	444	694	944	7.7	16.5
195	195	445	695	945	8.2	16.5
196	196	446	696	946	8.7	16.5
197	197	447	697	947	9.2	16.5
198	198	448	698	948	9.7	16.5
199	199	449	699	949	10.2	16.5
200	200	450	700	950	10.7	16.5
201	201	451	701	951	11.2	16.5
202	202	452	702	952	11.7	16.5
203	203	453	703	953	12.2	16.5
204	204	454	704	954	12.7	16.5
205	205	455	705	955	13.2	16.5
206	206	456	706	956	13.7	16.5
207	207	457	707	957	14.2	16.5
208	208	458	708	958	14.7	16.5
209	209	459	709	959	15.2	16.5
210	210	460	710	960	15.7	16.5
211	211	461	711	961	16.2	16.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
212	212	462	712	962	16.7	16.5
213	213	463	713	963	17.2	16.5
214	214	464	714	964	17.7	16.5
215	215	465	715	965	18.2	16.5
216	216	466	716	966	18.7	16.5

APPENDIX G 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 19. Equipment list – Preparing to sample.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		GPS receiver, recreational accuracy	Pre-load sampling plot locations if planning to use GPS for navigation	1
		USB cable	Transfer data to GPS unit	1
Forestry Supplier 91567	Y	TruPulse 360R Laser Rangefinder, ± 30 cm accuracy	Check declination and calibrations	1
Request from NEON HQ	Y	GPS-enabled iPad with ArcGIS Field Maps application installed	Download offline maps to create crown polygons, woody individual sampling (Type B sites)	1
See Table **21**	N	Tall-canopy sampling supplies when UAS not used	Practice with your device, tall stature woody individual sampling	1 set
		All weather copy paper	Print back-up datasheets	6
		All-weather address labels	Pre-print labels for field sample bags	2-3 per sample
		Permanent marker	Label bags	1
Request from NEON HQ	Y	Adhesive barcode labels, weatherproof (Type I)	Label bulk sample with barcode-readable labels	1 per sample
Request from NEON HQ	Y	Adhesive barcode labels, cryogenic (Type II)	Label chlorophyll subsamples with barcode-readable labels	1 per sample
		Aluminum foil	Create pre-folded foil packets to store chlorophyll subsamples	1 per sample

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Resealable plastic bag, 1 gal	Assemble and pre-label bags to store bulk and LMA subsamples	1-2 per sample
		Whirl-pak type bags, 4 oz size	Assemble and pre-label bags to store chlorophyll subsamples	1 per sample
		Paper towels	Moisten and place in sample bags; to maintain hydration of foliage in transit	1-2 per sample
		Deionized water, from DSF filtration unit	Moisten paper towels	1 liter
		Vegetation Structure Data	Identify candidate individuals to sample	

Table 20. Equipment list – Field Sampling, all vegetation types.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		GPS receiver, recreational accuracy	Navigate to sampling locations. Optional – also possible to use Field Maps.	1
Forestry Supplier 91567	Y	TruPulse 360R Laser Rangefinder, ± 30 cm accuracy	Measure foliage collection heights; Map and tag; Locate clip strips in steep or brushy plots	1
Forestry Supplier 90998	Y	Foliage filter	Allow laser rangefinder use in dense vegetation	2
		White reflector or reflective tape	Aid in accurate measurement of distance to target with laser rangefinder	1
		Sighting compass with mirror and declination adjustment	Navigating to and within plots; locating clip harvest strips (herbaceous)	
		Large cooler, to be filled with cold packs	Chill bulk chemistry and LMA samples in the field	2
		Smaller cooler, to be filled with dry ice	Flash-freeze and store frozen chlorophyll subsamples	2

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Cold packs	Chill foliage samples in the field	10
		Cryogenic safe gloves	Handle chlorophyll samples as they are put on dry ice	1 pair
		Pruning shear (sharpened)	Obtain foliage samples, remove leaves from woody parts	1
		Scissors	Cut up chlorophyll subsamples, remove leaves from woody parts	1
		Ethanol	Clean shears or scissors and sorting tray between samples	
		Sorting tray	Help organize foliage while subsampling	1
		Backpack	Transport field equipment	1-2
		Clipboard	Secure datasheets	1
		Magnifier hand-lens, 10X/20X	Aid in species identification	1
		Field guide, regional flora reference guide and/or key	Aid in species identification	1
		Field notebook	Record field notes	1
		Dry ice	Freeze chlorophyll subsamples	20 lbs
		AA battery	Spare battery for GPS receiver	2
		CR123A battery	Spare battery for laser rangefinder	2
		Nitrile gloves, powderless	Handle samples	1 box
		Permanent marker	Label bags	3
Request from NEON HQ	Y	Adhesive barcode labels, weatherproof (Type I) and cryogenic (Type II)	Extra barcode-readable labels	1 sheet each
		Resealable plastic bag, 1 gal	Extra bags to contain bulk and LMA samples	10
		Resealable plastic bag, 1 qt	Extra bags if LMA subsample bags are too large	10

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Whirl-pak type bags, 4 oz size	Extra bags to contain chlorophyll subsamples	10
		Pre-made Aluminum Foil packets	Extra packets to contain chlorophyll subsamples	10
		Paper towels	Moisten and place in sample bags; clean chlorophyll tissue	1-2 per sample
		Deionized water, from DSF filtration unit	Moisten paper towels	1 liter

Table 21. Equipment list – Field Sampling, extra equipment for woody individuals.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Safety glasses	Required PPE, protect eyes	1 per person
		Hard hat	Required PPE, predict head	1 per person
		Hearing protection, type depends on sampling device	Required PPE to protect ears for some types of sampling, see Section 5	1 per person
		Work gloves	Required PPE to protect hands for some types of sampling, see Section 5	1 per person
DJI M600 + DeLeaves	Y	Unoccupied aerial system for foliage collection *(responsibility of dedicated UAS team)*	Obtain sunlit foliage samples from tall trees at the top of the canopy	1
		Line launcher – for example, Treestuff APTA or Sherrilltree Big Launcher	Obtain sunlit foliage samples from tall trees	1
		Floor bike pump	Used for pressurizing the APTA line launcher	1

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Portable mechanical pump. Suggest Ryobi P737 model, has worked well in the past.	Used for pressurizing the APTA line launcher	1
		Cushioning for between shoulder and launcher – pool noodle, foam packing material, towels, etc	Prevent injury to shoulder from APTA kickback	1
		Slingshot – for example SherrilTree Big Shot	Obtain sunlit foliage samples from tall trees	1
		Throw weight. Suggest Weaver Arborist, brand has worked well in the past	Used as a projectile for certain types of line launchers and slingshots, as well as hand tossing	5
		Throwline, 1.75 or 2.2 mm diameter	Throwline for launching method of choice or hand tossing	See Appendix E
		Heavy-duty twisted-link chain	For stripping leaves instead of cutting branches	3 feet
		Saw, Commando or Wire type	Saw option to cut branches	5
		Saw, Hand or Pocket Chain type	Saw option to cut branches	5
		Quick Links	Easy attachment of launcher/slingshot cutting tools	10
		Extendable pole trimmer, up to 30 feet telescoping length preferred – such as DocaPole GoSaw	Obtain sunlit foliage samples, shorter canopies	1
		Punch tools - 0.5”, 0.75”, and 1.5” diameter. Can buy online or from local craft store	Obtain standard size broadleaf subsamples for chlorophyll analysis	At least 2
		Portable balance – spring scale or digital. Ability to measure to nearest 1 g, minimum 60 g capacity.	Determine mass of bulk chemistry sample, collect sufficient material	1
		Hammer	Nail tags to trees	1

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Hand stamp steel die set	Append canopy-only tags with "Z"	1 set
Request from NEON HQ	Y	GPS-enabled iPad with ArcGIS Field Maps installed and offline maps downloaded for site	Create digital crown polygons	1
		Clear protractor	Create digital crown polygons, assist with mapping on top of imagery	1
		Flagging tape	Flag individuals to be sampled in tall- and mixed-stature vegetation	1 roll
		Round numbered aluminum tag, silver, 0001-6000 and 8001-9999	Add tags to sampled individuals if they do not already have them	10-20
		Aluminum nail	Affix tags to stems	10-20
		Aluminum wire	Affix tags to stems	1 roll
Sherrilltree 36479	Y	Blank cartridges, heavy load red	Used for shooting the Big Launcher	1-2 boxes
Sherrilltree 36487	Y	Blank cartridges, light load green	Used for shooting the Big Launcher	1-2 boxes
RD [10]	Y	Field Datasheet: Woody Individual	Back-up to record field data	6
		Stem maps from VST Mapper tool	Help locate target trees	1 per target plot

Table 22. Equipment list – Field Sampling, extra equipment for herbaceous clips.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Measuring tape, minimum 30 m	Locate clip-harvest strips	1
		Chaining pins or other suitable anchor	Anchor measuring tape	2

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Pre-marked string and stake set	Delineate clip harvest strip	2
		Ruler, 30 cm	Delineate clip harvest strip	1
		Portable balance – spring scale or digital. Ability to measure to nearest 0.1 g	Collect sufficient material for chlorophyll subsample	1
		Survey marking flag, PVC or fiberglass stake	Delineate clip-harvest strip areas	4
ULINE S-21339	Y	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	1 per container
		Per plot/subplot clip-strip coordinate lists	Identify clip-strip locations	2
RD [10]	Y	Field Datasheet: Herbaceous	Back-up to record field data	6

Table 23. Equipment list – Leaf Mass Per Area Measurements.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
CanoScan LiDE 120	Y	Digital scanner	Scan foliage	1
		Punch tools (0.5”, 0.75”, and 1.5” diameter)	Contingency plan for measuring LMA of broadleaf vegetation	1 each
		Plastic tray	Organize samples	4
		Nitrile gloves, powderless	Handle samples	1 box
		Coin envelope	Contain samples while oven-drying	1 box
		Paper bag, #8	Contain samples while oven-drying	1 box
		Paper bags, #25	Organize smaller bags or envelopes in drying oven	10

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Weigh boats, small and large. Plastic or metal acceptable.	Contain samples while weighing	1 box
		Permanent marker	Label bags or envelopes	2
Order from NEON HQ	Y	Adhesive Type I barcode labels	Label bags or envelopes with barcode-readable labels	1 per sample
		Address labels	Label bags or envelopes with sample identifiers	1 per sample
		Clear address labels	Facilitate re-use of scanning template	5
		Dry erase marker, fine tip	Facilitate re-use of scanning template	2
		Transparency sheets	Protect scanner from contamination with toxic oils	2
ULINE S-21339	Y	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	1 per container with *tox spp*
		Paper towels	Dry foliage before scanning	1-2 per sample
Provided by NEON HQ	Y	Image J Software	Calculate scanned area of samples	1
RD[10]	Y	Laboratory Datasheets	Back-up to record data, plus scanning template with scale bar	2

Table 24. Equipment list – Subsampling for Chemical Analyses.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
	Y	Grinding Mill, Wiley, plus 20 mesh and 40 mesh inserts	Grind subsamples	1

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
		Sample microsplitter, small capacity	Create representative subsamples from ground sample	1
		Hy back pan	Receive sub-samples generated by splitter	2
		Anti-static gun	Remove static charge from scint vials to prevent cling	1
		Nitrile gloves, powderless	Handle samples	1 box
		Paper towels	Clean foliage by blotting prior to oven drying	1-2 per sample
		Deionized water, from DSF filtration unit	As needed to moisten paper towels or spray down foliage for cleaning	Varies
		Spray bottle, rinsed 5x with deionized water	Spray down foliage for cleaning, herbaceous samples where blotting impractical	1
		Coin envelope	Contain chemistry samples	2 per sample
Fisher 0333723C; Thomas 9718J20	Y	Plastic scintillation vials with caps, 20 mL.	Contain chemistry and archive samples. Must be new/unused and stored in a way to minimize dust or other contamination, if not do not use.	1-3 per sample
		Permanent marker	Label bags and/or envelopes	2
Fisher 15-930-C	Y	Cryogenic adhesive labels, 0.5” x 1.25”	Label scintillation vials, this type needed in order to stick to the plastic	2-3 sheets
Order from NEON HQ	Y	Adhesive Type I barcode labels	Label vials or envelopes with barcode-readable labels	1 per sample
		Ethanol	Clean gloves between samples, clean mill if samples are resinous	1 bottle

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
ULINE S-21339	Y	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	1 per container with *tox spp*
		Canned air or shop vacuum	Clean mill between samples, remove leaf particles	1
RD[10]	Y	Laboratory Datasheet	Back-up to record data	4