TOS PROTOCOL AND PROCEDURE: 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.

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of sun-lit 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 ground-truth and validate AOP measurements. Such data can help the ecological research community 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, which have value independent of remote sensing observations.

Foliar chemistry data 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 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 NEON Science Design for Terrestrial Biogeochemistry (AD[05]). 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 thus 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 and/or hillslope aspect

affect N availability in the soil, which could 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.

1.3 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]).

1.4 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 Smith et al. (2008), Asner and Martin (2009), and Serbin et al. (2014). 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	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.014037	TOS Protocol and Procedure: Measurement of Herbaceous Biomass
RD[05]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[06]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
RD[07]	NEON.DOC.014038	TOS Protocol and Procedure: Plant Belowground Biomass Sampling
RD[08]	NEON.DOC.001716	TOS Standard Operating Procedure: Toxicodendron Biomass and Handling
RD[09]	NEON.DOC.001717	TOS SOP: TruPulse Rangefinder Use and Calibration
RD[10]	NEON.DOC.000987	TOS Protocol and Procedure: 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: Data Management

2.3 Acronyms

Acronym	Definition
^{12}C	Most common isotope of carbon
^{13}C	Less common isotope of carbon
LMA	Leaf Mass Per Area
LiDAR	Light Detection and Ranging
^{14}N	Most common isotope of nitrogen
^{15}N	Less common isotope of nitrogen
NACP	North American Carbon Program

3 METHOD

The goal of this protocol is to sample the sun-lit vegetation found across a site, essentially capturing the major components of what the AOP sees during overflights. Foliar chemistry and LMA vary considerably both between species and through time; when possible, the same individuals will be sampled over time.

A subset of the 40 x 40 meter "Distributed Base Plots" located across the study area are used for foliar chemistry and LMA sampling. In forest and shrubland sites, these are the same plots used for Vegetation Structure 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, so sticking to sampling within plots allows the sampling strategy to be consistent across the Observatory. Specific Tower and Distributed Plot locations for canopy sampling are provided in a separate document to NEON field ecologists.

In sites dominated by woody cover (e.g., forests and shrubland, Type I sites in Table 1), the general procedure is to sample at least one individual of each species found in the site-level, sun-lit canopy. For the more common species, replicates are taken, spanning whatever gradients are relevant to a site (topography, aspect, soil type, stand age, etc). In high-diversity sites, rare species are only sampled where feasible, meaning individuals with sun-lit leaves can be found in target plots. All samples are collected from Vegetation Structure plots, and 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 were generated by NEON Science and are included in the Sampling Support Library (SSL). More guidance is provided in the Standard Operating Procedures (SOPs) below.

If Type I sites have significant open areas, several representative herbaceous clip strips should also be collected (Table 1). To meet this threshold, a site should have > 25% open (non-woody) cover in at least five Vegetation Structure plots. This assessment should be made by NEON field ecologists qualitatively

using aerial photos/google Earth images as well as site-specific knowledge. If uncertain whether clip strips should be collected, contact NEON Science to discuss.

IMPORTANCE OF SAMPLING SUN-LIT FOLIAGE

It is critical that foliar samples from woody individuals are collected from the outer-most part of the canopy, e.g. **they must be sun-lit leaves**. The AOP remote-sensing instruments scan sun leaves at the top and sun-lit sides of the canopy; because we are interested in linking AOP measurements with terrestrial observations, it is important that only sun-lit leaves be collected. Aside from AOP concerns, sun-lit leaves are the community standard for inter-comparable leaf trait data. Leaves need not be from the very apex of a tree, but they must be collected from sun-lit canopy positions.

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

In many forested systems, the canopy is well out of human reach. In order to obtain sun-lit leaves, it is necessary to use a shotgun, slingshot, line launcher, tree climbers, or employ other methods agreed-upon with NEON Science. Some of these approaches require participation of persons with specialized skills, such as a marksman with a valid shotgun permit for the sampling location, or a capable, trained tree climber. If an individual with specialized training is needed, additional participation of two NEON personnel who can work alongside this individual to subsample, bag, and preserve samples is required. Field Operations should consult with Science to resolve questions about how to obtain sun-lit leaves.

In woody systems with low (≤ 2 m) and mixed-stature (2-6 m) vegetation, sun-lit leaves are obtained using clippers and extendable pole pruners, respectively.

In systems dominated by herbaceous vegetation (i.e., Type II sites, Table 1), bulk herbaceous plant biomass will be harvested from a set of assigned Canopy Foliage plots using clip strips. This clip strip method is very similar to the one described in TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]), but with key high-level differences that are detailed below. Select Type II sites with significant woody cover will also sample woody individuals (Table 1).

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 I: dominated by *woody cover*[a]	Sun-lit leaves from single species	See site-specific lists on the SSL	• focus on sampling taxa from site-specific lists within VST plots • distribute replicates across site gradients • if open areas prevalent (as defined above), take several representative sun-lit clips (max = 8) from VST plots as needed to characterize non-woody diversity	ABBY, BART, 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 II: 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, BLAN*, CPER, DCFS, KONA, KONZ, LAJA, NOGP, OAES, SJER*, STER, TOOL, WOOD

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

Standard Operating Procedures in Section 7 of this document provide detailed step-by-step directions, sampling tips, and best practices for implementing the sampling procedures. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. When unexpected field conditions require deviations from this protocol, consult Section 4.4 of this document and follow instructions therein to ensure quality standards are met.

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

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

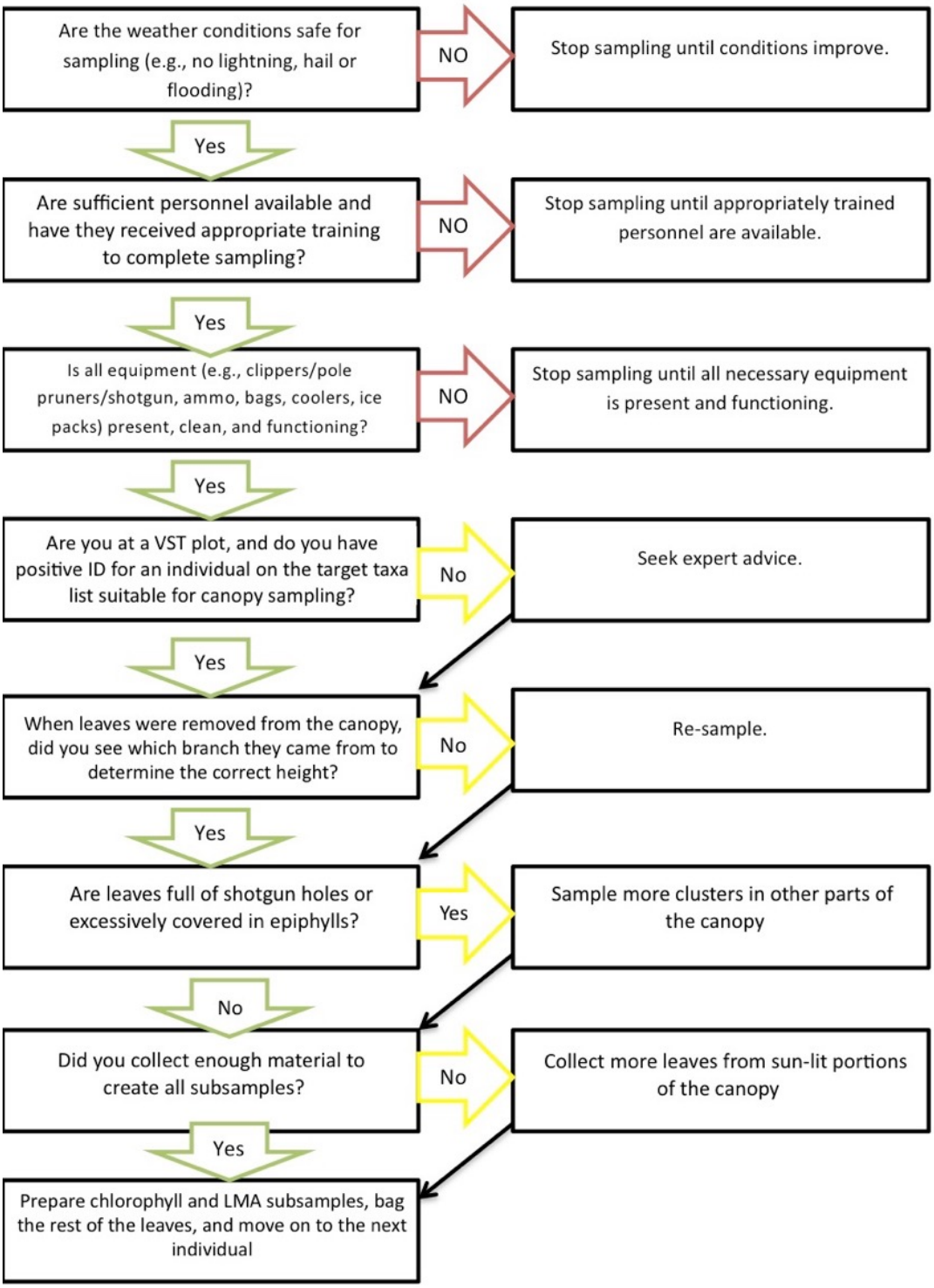


Figure 2. Decision tree to determine if canopy sampling should take place, and when sampling of a woody individual is complete.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

***Timing*:** The timing of airborne data collection by the AOP, which coincides with the approximate timing of peak greenness, largely determines the timing of canopy foliage sampling at a given site. These ground and airborne datasets are typically analyzed together, so effort should be made to coordinate them as closely as possible.

Frequency: Canopy foliage sampling is conducted at each NEON site once every 5 years, with inter-annual schedules chosen by Science and Field Operations leadership to optimize linkages with AOP. The expectation is that a 5-year sampling frequency will sufficiently capture long-term trends in chemical properties of foliar tissues, and also provide sufficient data to conduct calibrations of more frequently collected AOP hyperspectral data.

LINKED BIOGEOCHEMISTRY MEASUREMENTS

The Canopy Foliage Sampling protocol is completed as part of a suite of coordinated TOS measurements aimed at characterizing plant and soil biogeochemical dynamics. The suite of coordinated protocols includes:

- The 'biogeochemistry' component of TOS Protocol: Litterfall and Fine Woody Debris (RD[05]),
- The 'biogeochemistry' component of TOS Protocol: Soil Biogeochemical and Microbial Sampling, including N Transformations (RD[06])
- TOS Protocol: Plant Belowground Biomass Sampling (RD[07]).

Co-execution of these protocols at a given site in the same year is a high priority. When chemical analysis of herbaceous foliage occurs, herbaceous tissue collection for chemistry is a separate bout from the annual clip harvest for biomass.

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling shall be scheduled to begin within **± 2 weeks of the anticipated midpoint of the AOP flight window at the site**. This will increase the chance for overlap between ground and aerial data collection and should coincide with peak greenness at most sites. Field ecologists should verify site-specific peak greenness windows provided in Appendix E and contact Science if the AOP schedule falls outside these windows. 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. At sites where there are multiple peaks in greenness (e.g., some grasslands), Science may provide additional instructions.

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 5-20 days to complete the field sampling component.

4.3 Timing for Sample Processing and Analysis

In order to stabilize and preserve foliage, sun-lit leaves should be placed on cold packs in coolers as soon as they are collected. Additionally, a small subsample for chlorophyll analysis should be 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 collection; oven-drying of chemistry samples 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. 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 1**.

Table 2 Holding times for different foliar samples 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	Begin oven-drying	Within 5 days of collection
Oven-dried Chemistry samples	Subsample and ship to external labs	Within 60 days of collection
Oven-dried Biogeochemistry Archive samples	Ship to bioarchive facility	Within 90 days of collection

4.4 Sampling Timing Contingencies for Foliar Chemistry and LMA

When unexpected field conditions are met, the guidance in **Table 2** should be followed to ensure that basic data quality standards are met:

Table 3. Contingency decisions

Delay/Situation	Action	Outcome for Data Products
LMA and chemistry samples cannot be processed within 24 hours.	Store in the refrigerator (4°C) or, if that is not possible, on ice packs in coolers (change out fresh ice packs every 12 hr). Process upon return to the Domain Support Facility.	None if samples are processed **within 5 days** of collection (see 4.3). If delayed beyond 5 days, may begin to rot and be unsuitable for analysis. Contact NEON Science by issuing a problem ticket if in question.
Samples are not kept cold following collection.	Issue problem ticket to NEON staff; potentially reschedule bout.	Samples likely compromised; potential delay of data products.
It begins to rain during a sampling bout.	Continue to collect foliar samples as long as it is safe and possible to do so. If conditions become unsafe (thunder, lightning, hail, flooding, etc), halt sample collection and attempt to continue the bout when weather conditions improve.	None if bout can be resumed after weather improves. If severe weather persists and bout cannot be completed, issue problem ticket to NEON Science.
Inability to finish a sampling bout within 20 days.	Issue problem ticket to NEON staff; potentially resume existing bout outside target sampling window	Data products likely delayed or reduced in number for that bout.
Delay in starting sample bout, start date is more than two weeks after AOP over flight.	Issue problem ticket to NEON staff to discuss whether or not to sample outside of target sampling window.	Data products likely delayed, with implications for linking ground and airborne datasets for that bout.

4.5 Criteria for Reallocation of Sampling Within a Site

Canopy foliage sampling for chemistry and LMA occurs on the schedule described above at a maximum of 20-40 plots per site, dependent on dominant vegetation type. Ideally, sampling will occur at these plots for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project. However, circumstances may arise requiring that sampling within a site be shifted from one particular plot to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quantity or quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Plots can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot is destroyed by a landslide). 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 non-annual 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, stand-replacing fire, a disease outbreak that is not representative of the site, or any other logistical reasons, should be noted and communicated to Science as soon as possible. For Type I sites, it may be possible to simply choose other plots to sample from, but for Type II sites it is important to communicate issues quickly so that assigned sampling plots can be changed if needed.

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field 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 mixed-stature forest, 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 a shotgun, slingshot, or line launcher, to obtain leaves from the outer, sun-lit portions of the canopy. Field personnel must familiarize themselves with safety procedures for each of these methods.

For Shotgun Canopy Sampling:

- **ONLY THE INDIVIDUAL WHO IS AUTHORIZED TO USE THE SHOTGUN MAY HANDLE IT.**
- PPE required during all Shotgun Canopy Sampling includes the use of a hard hat, safety glasses, heavy-duty work shoes/boots, hearing protection with a minimum of a 28NRR (NEON Safety will assist with hearing protection requirements, as needed) and reflective vests.
- NEON employees will coordinate all shooting activities with Authorized Contracted Shooter and will acknowledge more stringent rules and regulations posted by the host or written by the contractor.
- In addition to obeying all directives from the shooter, staff should always stand behind the shooter whenever the shotgun is loaded and the safety is off.

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 Slingshot and Line Launcher Canopy Sampling includes the use of a hardhat (or ANSI Certified climbing helmet), safety glasses and regular work gloves. For pressurized line launchers, hearing protection may also be required; follow manufacturer recommendations.
- Make sure the area is completely clear of tourists or other scientists before using the tools. Slingshots and line launchers can easily propel a throw weight 300 feet or more.
- For line launchers, use only approved throw weights inside the barrel

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.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low temperature freezers, etc. Equipment needs will vary based on the dominant vegetation types present at each site – refer to the tables below for details.

Table 4. Equipment list: Preparing to sample at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
Durable Items							
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy	Pre-load sampling plot locations	All	1	N
		R	USB cable	Transfer data to GPS unit	All	1	N
Forestry Supplier	91567	R	Laser Rangefinder, ± 30 cm accuracy	Check calibrations and settings	All	1	N
Consumable items							
Forestry Suppliers	14111503	R	All weather copy paper	Print back-up datasheets	All	6	

Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
		R	Permanent marker	Label bags	All	1	N
		R	Adhesive barcode labels, weatherproof (Type I)	Label bulk sample with barcode-readable labels	All	1 per sample	N
		R	Adhesive barcode labels, cryogenic (Type II)	Label chlorophyll subsamples with barcode-readable labels	All	1 per sample	N
		R	Aluminum foil	Create pre-folded foil packets to store chlorophyll subsamples	All	1 per sample	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Assemble and pre-label bags to store LMA subsamples	Tall and mixed-statured sites	1 per sample	N
Fisher Scientific	14-955-176	R	Whirl-Pak bags, 4 oz	Assemble and pre-label bags to store chlorophyll subsamples	All	1 per sample	N
ULINE	S-7630	R	Paper bags, #8	Assemble and pre-label bags to store bulk chemistry samples	All	1 per sample	N
Resources							
		R	Vegetation Structure Data	Identify candidate individuals to sample	Type I sites, mostly woody		

R/S = Required/Suggested.

Table 5. Equipment list: Field sampling at one site, **all vegetation types**.

Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
Durable Items							
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy	Navigate to sampling locations	All	1	N
Forestry Supplier	91567	R	Laser Rangefinder, ± 30 cm accuracy	Measuring foliage collection heights; Mapping and tagging; Locating clip strips	Tall trees; non- tagged individuals; herbaceous plots w/ slope > 20% or brushy	1	N
Compass Tools Forestry Supplier	703512 90998	R	Foliage filter	Allow laser rangefinder use in dense vegetation	All	2	N
Grainger	5B317	R	White reflector or reflective tape	Aid in measuring distance to target accurately with laser rangefinder	All	1	N
		R	Large cooler, to be filled with cold packs	Chill bulk chemistry and LMA samples in the field	All	2	N
		R	Smaller cooler, to be filled with dry ice	Flash-freeze and store frozen chlorophyll subsamples	All	2	N
Fisher Grainger	19067113 3UZA9	R	Cold packs	Chill foliage samples in the field	All	10	N

Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
		R	Pruning shear (sharpened)	Obtain foliage samples, remove leaves from woody parts	All	1	N
		S	Scissors	Cut up chlorophyll subsamples, remove leaves from woody parts	All	1	N
		R	Backpack	Transport field equipment	All	1-2	N
		S	Clipboard	Secure datasheets	All	1	N
Forestry Suppliers	61280 61260	S	Magnifier hand-lens, 10X/20X	Aid in species identification	All	1	N
		S	Field guide, regional flora reference guide and/or key	Aid in species identification	All	1	N
Consumable items							
		R	Field notebook	Record field notes	All	1	N
Varies by domain	Varies by domain	R	Dry ice	Freeze chlorophyll subsamples	All	20 lbs	Y
		R	AA battery	Spare battery for GPS receiver	All	2	N
		R	CR123A battery	Spare battery for laser rangefinder	All	2	N
		R	Nitrile gloves, powderless	Handle samples	All	1 box	N
		R	Permanent marker	Label bags	All	3	N

Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
		R	Adhesive barcode labels, weatherproof (Type I) and cryogenic (Type II)	Extra barcode-readable labels	All	1 sheet each	N
ULINE	S-7631	S	Paper bags, #25	Larger bags to organize samples	All	10	N
ULINE	S-7630	R	Paper bags, #8	Extra bags to contain bulk samples	All	10	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Extra bags to contain LMA subsamples	All	10	N
Grainger	5CNK1	R	Resealable plastic bag, 1 qt	Extra bags if LMA subsample bags are too large	All	10	N
Fisher	14-955-176	R	Whirl-Pak bags, 4 oz	Extra bags to contain chlorophyll subsamples	All	10	N
		R	Pre-made Aluminum Foil packets	Extra packets to contain chlorophyll subsamples	All	10	N
		S	Trash bag	Contain paper bags inside cooler to prevent moisture loss; Dispose of trash	All	3	N

R/S = Required/Suggested.

Table 6. Additional equipment list: Field sampling at one site, **woody vegetation**.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Grainger	1VT99 4EY97 5XNWW	R	Safety glasses	Required PPE, protect eyes	Tall and mixed-stature vegetation	1 per person	N
Grainger	5KAX9 4RB37	R	Hard hat	Required PPE, predict head	Tall and mixed-stature vegetation	1 per person	N
		R	Hearing protection	Required PPE, protect ears	Shotgun (and possibly line launcher) sampling	1 per person	N
		R	Work gloves	Required PPE, protect hands	Line launcher or slingshot sampling	1 per handler	N
		R	Shotgun and ammunition *(responsibility of Designated Shooter)*	Obtain sun-lit foliage samples from tall canopies	Shotgun sampling	1	Y
		R	Line launcher + associated supplies (throw weights, line, cutting tool, pump)	Obtain sun-lit foliage samples from tall canopies	Line launcher sampling	1	Y

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	Slingshot + associated supplies (throw weights, line, cutting tool)	Obtain sun-lit foliage samples from tall canopies	Slingshot sampling	1	Y
		R	Extendable pole trimmer (10-15 feet telescoping length)	Obtain sun-lit foliage samples	Mixed-stature vegetation	1	N
		R	Punch tools (0.5”, 0.75”, and 1.5” diameter)	Obtain standard size broadleaf subsamples for chlorophyll analysis	Broad leaf samples	2	N
Forestry Suppliers	93010	R	Spring scale, tareable, capacity 30 g	Determine mass of bulk chemistry sample, collect sufficient material	All	1	N
		R	Hammer	Nail tags to trees	Sampling non-tagged individuals	1	N
Forestry Suppliers	57522	R	Hand stamp steel die set	Append canopy-only tags with “Z”	Sampling non-tagged individuals	1 set	N
Consumable Items							
Grainger Forestry Supplier	9WKP4 57880	R	Flagging tape	Flag individuals to be sampled in tall- and mixed-stature vegetation	All	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Forestry Supplier	152580	R	Round numbered aluminum tag, silver, 0001-6000 and 8001-9999	Add tags to sampled individuals if they do not already have them	Sampling non-tagged trees	10	N
Forestry Supplier	2BJAAF	R	Aluminum nail	Affix tags to stems	Sampling non-tagged trees	10	N
Grainger	16Y067	R	Aluminum wire	Affix tags to stems	Sampling non-tagged trees	10	N
Resources							
RD [10]		R	Field Datasheet: Woody Individual	Back-up to record field data	All	6	N
		R	Stem maps from VST Mapper tool	Verify geolocation data for sampled individuals	All	1 per VST plot	N

R/S = Required for the specified conditions/Suggested.

Table 7. Additional equipment list: Field sampling at one site, **herbaceous vegetation**.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Forestry Suppliers	122731 40108 39943	R	Measuring tape, minimum 30 m	Locate clip-harvest strips	Plot slope < 20%, not brushy	1	N
Forestry Suppliers	213379 37184 37036	R	Compass with mirror and declination adjustment	Locate clip-harvest strips	If using measuring tape	1	N
Forestry Suppliers	39167	R	Chaining pins or other suitable anchor	Anchor measuring tape	If using measuring tape	2	N
		R	Pre-marked string and stake set	Delineate clip harvest strip	All	2	N
Amazon Grainger	41N620 41N620	R	Ruler, 30 cm	Delineate clip harvest strip	All	1	N
Forestry Suppliers	93010	R	Spring scale, tareable, capacity 30 g	Collect sufficient material for chlorophyll subsample	All	1	N
Consumable Items							

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	Survey marking flag, PVC or fiberglass stake	Delineate clip-harvest strip areas	All	4	N
ULINE	S-21339	R	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	Samples contain *Toxicodendron spp*	1 per container	N
Resources							
		R	Per plot/subplot clip-strip coordinate lists	Identify clip-strip locations	All	2	N
RD [10]		R	Field Datasheet: Herbaceous	Back-up to record field data	All	6	N

R/S = Required for the specified conditions/Suggested.

Table 8 Equipment list: Measuring LMA and drying bulk foliar samples at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Amazon		R	Scanner, CanoScan LiDE 120	Scan foliage	All	1	N
		R	Punch tools (0.5”, 0.75”, and 1.5” diameter)	Contingency plan for measuring LMA of broadleaf vegetation	If scanning is prohibitively slow & samples begin to rot	1 each	N
Fisher	1523911	S	Plastic tray	Organize samples	All	4	N
Consumable items							
		R	Nitrile gloves, powderless	Handle samples	All	1 box	N
ULINE	S-14720	R	Coin envelope	Contain samples while oven-drying	Small leaves or needles	1 box	N
ULINE	S-7630	R	Paper bag, #8	Contain samples while oven-drying	Larger leaves and herbaceous samples	1 box	N
ULINE	S-7631	S	Paper bags, #25	Organize smaller bags or envelopes in drying oven	All	10	N
Fisher	08-732-115 08-732-112	R	Weigh boats, small and large	Contain samples while weighing	All	1 box	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	Permanent marker	Label bags or envelopes	All	2	N
		R	Adhesive Type I barcode labels	Label bags or envelopes with barcode-readable labels	All	1 per sample	N
Avery	5662	S	Clear address labels	Facilitate re-use of scanning template	All	10	N
		S	Dry erase marker, fine tip	Facilitate re-use of scanning template	All	2	N
		R	Transparency sheets	Protect scanner from contamination with toxic oils	Samples contain *Toxicodendron* spp	2	N
ULINE	S-21339	R	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	Samples contain *Toxicodendron* spp	1 per container	N
Resources							
		R	Image J Software	Calculate scanned area of samples	All	1	N
RD[10]		R	Laboratory Datasheets	Back-up to record data, plus scanning template with scale bar	All	2	N

R/S = Required for the specified conditions/Suggested.

Table 9. Equipment list: Subsampling for chemical analyses and biogeochemistry archive.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
		R	Grinding Mill, Wiley, plus 20 mesh and 40 mesh inserts	Grind subsamples	All	1	N
Fisher	NC9052925	R	Sample microsplitter, small capacity	Create representative subsamples from ground sample	Large-mass samples	1	N
Fisher	NC0516918	R	Hy back pan	Receive sub-samples generated by splitter	Large-mass samples	2	N
Consumable items							
		R	Nitrile gloves, powderless	Handle samples	All	1 box	N
ULINE	S-14720	S	Coin envelope	Contain chemistry samples	Unground samples	2 per sample	N
Fisher	03-337-23C	R	Plastic scintillation vials with caps, 20 mL	Contain chemistry and archive samples	All	1-3 per sample	N
		S	Permanent marker	Label bags and/or envelopes	All	2	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	Standard address labels	Label scintillation vials	All	2-3 sheets	N
		R	Adhesive Type I barcode labels	Label vials or envelopes with barcode-readable labels	All	1 per sample	N
		R	Ethanol, 70%	Clean gloves between samples	All	1 bottle	N
ULINE	S-21339	R	Sample warning pictogram label	Identify acute toxins that may cause serious eye or skin irritation	Samples contain *Toxicodendron* spp	1 per container	N
Resources							
RD[10]		R	Laboratory Datasheet	Back-up to record data	All	4	N

R/S = Required for the specified conditions/Suggested.

Table 10. Equipment list: Shipping foliar samples from one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Use	Quantity	Special Handling
Consumable items							
		R	Cardboard box	Package samples for shipment	Dry sample types: C-N, lignin-elements, and archive	3	N
ULINE	S-16478	R	Insulated shipper, UN packing group III	Package frozen samples for shipment (e.g. chlorophyll)	Frozen samples	1	N
		R	Dry ice shipping label	Label shipments containing dry ice	Frozen samples	1	N
Varies by Domain	Varies by Doman	R	Dry ice, pelletized	Keep samples frozen during transport	Frozen samples	10 lbs	Y
		R	Packing tape	Package samples for shipment	All	1 roll	N
		R	Cushioning material (e.g. wadded newspaper)	Package samples for shipment	All	As needed	N
Grainger	8YAT5	R	Resealable plastic bag, 1 gal	Double-bag and organize samples prior to shipment; protect manifest	All	6-10	N
Resources							

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Use	Quantity	Special Handling
		R	Shipping Manifest	Inventory of samples being shipped	All	1 per box	N

R/S = Required for the specified conditions/suggested

6.2 Training Requirements

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

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 shotgun/line launcher safety. In sites with tall canopies, field personnel must be trained in proper use of a laser range finder to determine the height from which canopy samples were obtained. Additionally, before field personnel can conduct leaf mass per area measurements using ImageJ software, it must be verified that they can analyze standard images within the area threshold specified in the training materials (e.g., with 98% accuracy for broad leaf samples and 95% accuracy for conifer needles and mixed herbaceous samples).

6.3 Specialized Skills

When sampling in sites dominated by woody individuals, 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 shotguns are not allowed and tree climbers are not used, one member of the team must be familiar and practiced with use of a line launcher or slingshot. The Canopy Foliage Sampling discussion board in the NEON SSL contains useful 'tips and tricks' related to supplies and best practices for operation of some 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 team. However, in tall canopies where a slingshot, line launcher, or marksman is needed to obtain sun-lit leaves, *a three-person team is very advantageous*. One person, either a NEON technician or outside contractor, uses the selected tool to obtain sun-lit leaves, while two other NEON personnel collect, subsample, and preserve foliage samples as they come down from the canopy. One person on the team should be a plant expert capable of identifying local species.

6.4 Estimated Time

The time required to implement this protocol varies greatly depending on a number of factors, such as skill level, ecosystem type and biodiversity, environmental conditions, and distance between sampling plots. The timeframes provided below are estimates based on completion of the tasks by skilled teams,

i.e., not the time it takes at the beginning of the field season. Use these estimates as a framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

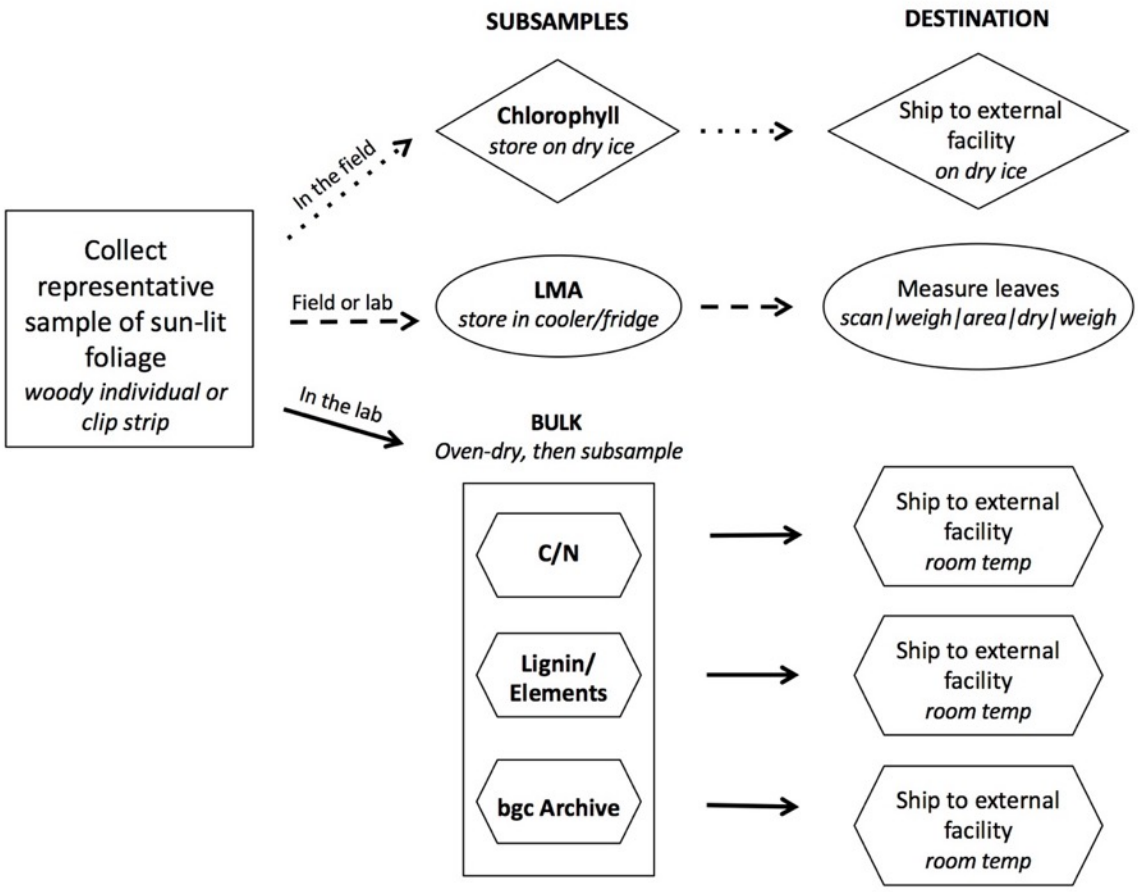
An experienced two-or-three-person team will require approximately 5-20 days to complete field sampling at a predominately woody site, with time largely dependent on canopy height and diversity. At predominately herbaceous sites, sampling can likely be completed within 5 days. An additional 2-5 days of active lab work per site are needed for processing samples in the Domain Support Facility, not including the waiting period when samples are oven-drying. In order to complete all field and lab tasks while not letting foliage sit longer than 5 days between collection and processing, it may be necessary to split field collection bouts into several sampling periods, with laboratory processing in between, or use multiple teams.

Table 11 Estimated time required to complete field and lab standard operating procedures.

SOP	Estimated total time (hours)	Suggested staff	Total person hours
A Preparing for sampling	8-16	1-2	8-32
B Field Sampling, closed canopy forest	2/sample	2 or 3	4-6/sample
B Field Sampling, open canopy, shrublands	1/sample	2	2/sample
B Field Sampling, herbaceous	1/sample	2	2/sample
C Post-field sampling tasks	3	2	6
D Laboratory Processing, LMA	0.5/sample	2	1/sample
E Laboratory Processing, chemistry subsampling	0.5/sample	1	0.5/sample
F Data entry and verification	2-4	1	2-4
G Sample Shipment	1/shipment	1	1/shipment

7 STANDARD OPERATING PROCEDURES

General Workflow for Canopy Foliage Sampling



7.1 Contents and Overview of SOPs

The tasks associated with canopy foliage sampling are divided into a series of seven separate SOPs.

- **SOP A:** Tasks to complete in the Domain Support Facility in preparation for canopy foliage sampling.
- **SOP B:** Methods for field sampling of foliar tissues – separate instructions for woody individuals and herbaceous vegetation.
- **SOP C:** Store samples post-collection and replenish field supplies for subsequent sampling bouts.
- **SOP D:** Measure leaf mass per area (LMA) for foliar samples: scan leaves, measure area using ImageJ software, obtain fresh and dry sample weights, and execute the contingency plan (if needed) for broad-leaf samples.

- **SOP E:** Oven-dry and subsample foliar samples in preparation for external laboratory chemical analyses.
- **SOP F:** Guidelines and requirements for successful data entry.
- **SOP G:** Package and ship samples to external laboratories for chemical analysis, including precautions for shipping dry ice.

SOP A Preparing for Sampling

Table 12: Equipment and supply preparation checklist

✓	Item	Action
	GPS unit	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 D.1 **and** D.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.1 Prepare for Data Capture

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

If the site is dominated by woody individuals and has been sampled for canopy foliage before, print a list of the tagIDs that were previously sampled to bring to the field. This will facilitate the re-sampling of these same individuals (where possible) in the current bout.

A.2 Determine Appropriate Methods and Supplies

1. Use the information in **Table 1** along with knowledge of site vegetation types and height information 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.
    - herbaceous vegetation[a] = hand clippers
    - woody individuals[b] = depends on height:
        - 0-2 m = hand clippers
        - 2-6 m = extendable pole pruner
        - > 6 m = line launcher/slingshot/tree climbers /certified marksmen/other

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

2. Review equipment lists and determine whether all required items are available
3. If using a line launcher or slingshot to obtain tall canopy foliage:
    - Check the Canopy Foliage Sampling discussion board in the NEON SSL for useful 'tips and tricks' related to use of these tools.

    - Spend an entire day practicing use of that device in a nearby area with relevant vegetation (Figure 3). This will allow for trouble-shooting of issues and increased familiarity with the equipment, greatly increasing efficiency during sampling bouts.
    - Plan to bring two back packs to the field, one to carry the line launcher/slingshot equipment, and the other to carry supplies for processing foliage.
4. If plots are accessible, sampling can be expedited by pre-selecting and flagging individuals or clip strips, according to the guidelines described in SOP B.

Figure 3 Practicing use of a line launcher.

A.3 Woody Individual Sampling - Use VST Data to Identify Sampling Candidates

1. Type I sites (Table 1) should review the site-specific target taxa lists provided by Science in the NEON Sampling Support Library (SSL).

2. The lead Plant Ecologist will help make a sampling plan by identifying candidate individuals, roughly twice as many as the level of replication given in the site-specific lists since not all candidates will be sample-able.
    a. If available, download or examine the Apparent Individual data collected during Vegetation Structure (VST) monitoring for a given site.
    b. Sort by **taxonID**, then hone in on individuals likely to be in the sun-lit canopy. Depending on the site, this could be accomplished using the variables **canopyPosition**, **growthForm**, **stemDiameter**, **height**, or other metrics.
    c. If VST data are not available for the all or part of the site, use familiarity and knowledge from other field work to determine which eventual VST plots are likely to have prominent, sun-lit canopy individuals of the target taxa. Plan to visit those plots to find candidate individuals to sample.
3. For more common species, there will be many individuals to choose from. Use these guidelines to determine which to target:
    a. Individuals sampled for foliar chemistry should have prominent, non-overlapping crowns. There is no cap on sample number per plot, but overly dense sampling within plots should be avoided.
    b. Identify candidate individuals from plots that span varied habitats so that replicates will cover gradients in topography, aspect, soil type, stand age, etc.
    c. Where possible, select candidate individuals from plots designated for soil sampling. This will maximize cross-protocol data linkages.
    d. If the site has been sampled for canopy foliage before, include tagIDs that were previously sampled as candidates since resampling of individuals is desirable.
    e. In high-diversity sites, do include rare species as candidates, but they will only be sampled where feasible (e.g., possible to sample sun-lit leaves in reasonable time).
4. Record candidate individuals in the WorkTracker, Appendix B.

A.4 Woody Individual Sampling - Use VST Mapper to Verify Geolocations

1. It is critical that individuals sampled for canopy foliage have correct mapping data (e.g., pointID, distance, and azimuth), as errors in this geolocation data will prevent use of ground-based foliar measurements for scaling and modeling with AOP data.
    a. Accordingly, use the VST Mapper tool to print stem locations and maps for each vegetation structure plot where canopy foliage sampling may occur.

b. Bring these maps to the field and use to ensure each woody individual sampled for canopy foliage has the correct mapping data (and if not, correct it).

A.5 Woody Individual Sampling - Prepare to Map and Tag Individuals as Needed

1. It is desirable to sample individuals previously tagged for Vegetation Structure monitoring as they already have geolocation and other useful data. However, in some cases it may be necessary to sample non-tagged individuals.
2. In preparation for this occurrence, Field Ecologists sampling in primarily woody sites must bring numbered aluminum tags to the field and be ready to map and tag any non-tagged individuals that are sampled. Moreover, they should review TOS Protocol and Procedure: Measurement of Vegetation Structure, SOP B: Classification, Mapping and Tagging (RD[10]) and be familiar with this procedure before a canopy sampling bout begins.

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

   1) In the Mapping and Tagging data entry application, selecting **cfcOnlyTag = Y** for these definitive cfcOnly 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.

b. 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 **cfcOnlyTag = N.**

A.6 Herbaceous Vegetation Sampling - Prepare to Locate Clip Strips

1. In sites with primarily herbaceous vegetation, or woody sites with significant open areas (> 25% cover in at least 5 VST plots), 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 steps (2) – (5) of TOS Protocol and Procedure: Measurement of Herbaceous Plant Biomass, SOP B.1: Sample Collection in the Field (RD [04]) before a canopy foliage sampling 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.7 All Sample Types: Make Foil Packets for Chlorophyll Samples

1. Pigments are very sensitive to light, thus, it is necessary to wrap each chlorophyll subsample in a foil packet. Make foil packets ahead of time to increase field sampling efficiency.
2. Use **Table 1**, site-specific target taxa lists, and your knowledge of site vegetation to estimate approximately how many samples will be collected for a bout. Then, refer to **Error! Reference source not found.** and the instructions below to create foil packets.
    - Make one per estimated sample, plus 5-10 extras in case some are lost.
    - Remember to check that foil packets are the right size to fit inside 4oz Whirl-paks

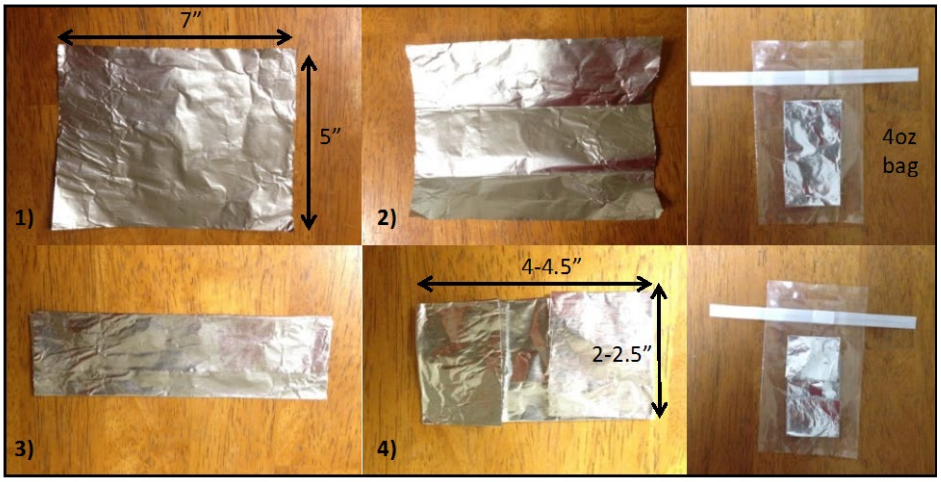


Figure 4 Steps to create foil packets: 1) cut a ~ 7” x 5" rectangle of foil, 2) mark lines to fold into thirds, 3) fold into thirds along the longer edge, 4) fold in the ends to close the packet. Keep a 4oz Whirl-pak bag nearby to make sure packets will fit.

A.8 All Sample Types: Organize and Pre-label Sample Bag Packets

1. Each sample, be it a woody individual or clip strip, will require a ‘bag packet’ to collect foliar material. To save time and keep things organized in the field, pre-arrange and pre-label these bag packets.
    - For pre-labeling, refer to the examples below and SOP B for more details on sample identifier formats. Leave space for any pieces of information that may not be known until sample is collected (ex: **plotID**, **taxonID, sampleNumber** per plot/date; Figure 5).
2. While creating the bag packets, also affix scan-able barcode labels to sample bags as specified below. Since barcodes are not initially associated with a particular sample, it is fine to make these up in advance. Recall that barcode labels should be oriented such that it is possible to scan them (e.g., not on a curved surface), and ensure no wrinkles or folds.

Figure 5 Example bag to contain the bulk foliage sample including barcode and human-readable label

3. Each bag packet should contain:
    a. 1 Paper bag (or several for clip strips), to contain the bulk foliage sample (Figure 5)
        - Example complete identifier, *woody*: cfc.GRSM002.LITU-1.20190606
        - Example complete identifier, *herbaceous*: cfc.WOOD001.CLIP-1.20190606
        - **Type I** scan-able barcode label (1 per sample)
    b. 1 4oz Whirl-pak bag, to the contain the chlorophyll subsample
        - Example complete identifier, woody: cfc.GRSM002.LITU-1.20190606.chl
        - Example complete identifier, *herbaceous*: cfc.WOOD001.CLIP-1.20190606.chl
        - **Type II** (cryogenic) scan-able barcode label (1 per sample) – place in appropriate location such that barcode will be scan-able when bag is folded
    c. 1 foil packet, to protect the chlorophyll subsample inside the Whirl-pak
    d. 1 gallon plastic bag, to contain the LMA subsample (**woody samples only**)
        - Example complete identifier, *woody*: cfc.GRSM002.LITU-1.20190606.lma
        - No scan-able barcode required, this is applied during execution of SOP D

4. If samples are likely to contain *Toxicodendron spp*, remember to bring sample warning labels to apply to sample bags in the field.

SOP B Field Sampling

This SOP is divided into two sections: how to sample woody individuals (B.1) and how to sample herbaceous vegetation using clip strips (0). Field personnel should follow the section that applies to the vegetation at the NEON site where they are sampling. Some sites will use both (see Table 1).

B.1 Sampling Woody Individuals

1. Using the Work Tracker and sampling plan developed in SOP A, navigate to a VST plot where there is at least one candidate for sampling. 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.
2. Survey all candidate individuals in the plot and determine which (if any) are suitable for sampling. Recall that crowns must be sun-lit and no two sampled crowns should overlap. This is needed in order to facilitate robust linkages with AOP remote sensing data.
3. While choosing individuals, note:
    a. It is ok to sample individuals not identified as candidates in your sampling plan.
    b. 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.
    c. Use stem locations and maps from the VST Mapper tool to ensure selected individuals have the correct mapping data (pointID, distance, azimuth) – if not, correct values in the field datasheet or Mapping and Tagging data entry application.
    d. **See Box 1 for additional guidelines for choosing individuals to sample.**
4. In Distributed Base Plots, sampling should occur primarily in the 20 x 20 m plot core, as this is where tagged individuals are located (Figure 9, 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 and microbial sampling or outside the plot.
    - Avoid the 1m x 1m nested subplots used for Plant Diversity sampling, as well as the centroid (attempt not to trample or travel through it).
5. In tall-stature Tower Plots, sampling should 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 9, right). However, remember to:
    - Keep sampling equipment and coolers in the other two subplots or outside the plot.
    - Avoid nested subplots, as well as the centroid (attempt not to trample or disturb).

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

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 sun-lit crown, go ahead and sample it. The goal is to capture as much diversity at the site level as possible
It is possible to collect sun-lit foliage from both old and young tagged individuals of a target species	Consider which age class is most representative of conditions at the site and sample an individual from that age class. If the species is common and several replicates will be taken, sample both old and young individuals
A candidate individual from a target species is found in the plot centroid or 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 centroid or subplot is anticipated
It is not possible (or excessively difficult) to sample a tagged individual of the target species	Identify and flag a non-tagged individual and prepare to map and tag it while sampling
There is not enough foliage on a given individual to sample without damaging it	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 where possible
The site experienced a recent disturbance (e.g., fire, windthrow)	Sample from plots affected by this disturbance if it was widespread. If only one or two plots experienced it, do not sample there
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 extremely minute (ex: 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 cover	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 canopy foliage sampling, and the supporting stem **must** be identified *and* mapped to provide geolocation data for the sample. Note this situation in the remarks field
Individuals are similar functionally to woody individuals but not actually woody (ex: Palmettos, cactus)	Sample as if they were woody individuals, including mapping and tagging as needed

6. Place flagging tape around the stems of individuals chosen for sampling. Flagging should be removed once sampling is complete.
7. The actual method of obtaining sun-lit foliage will vary based on the height of the woody vegetation being sampled:
    - For woody individuals < 2 m tall, hand clippers should be used to collect leaves.
    - For woody individuals 2-6 m tall, an extendable pole trimmer should be used.
    - For woody individuals > 6 m tall, methods will vary by site and may include shotgun, slingshot, line launcher, trained tree climbers, or other methods deemed appropriate for the site and agreed upon with Science
8. Ensure Field Operations personnel are wearing appropriate PPE, depending on the chosen method of sampling. If a marksman will shoot leaves out of the canopy, adhere to their instructions regarding where to stand when shooting is occurring.
9. While one person is preparing to obtain foliage from the outer, sun-lit portion of the canopy, record sample metadata, including:
    - **plotID** (SITEXXX), **sampleNumber** (unique to each plot and sampling date), **collectDate**, and **tagID** (if already tagged).
10. Retrieve foliage as it is brought down from the canopy. Ensure the person handling it wears a clean pair of Nitrile (Latex-free) gloves, so sweat and dirt from their hands do not contaminate the sample.

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 sun-lit canopy, consider abandoning that tree and choosing another from the list of candidate individuals for that species. 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 that the remaining sample material can be collected in a reasonable amount of time.

11. For tall-statured vegetation, determine the height where foliage came from using the Laser Range Finder (Figure 6). *You will not use the 3-shot height routine,* instead following 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

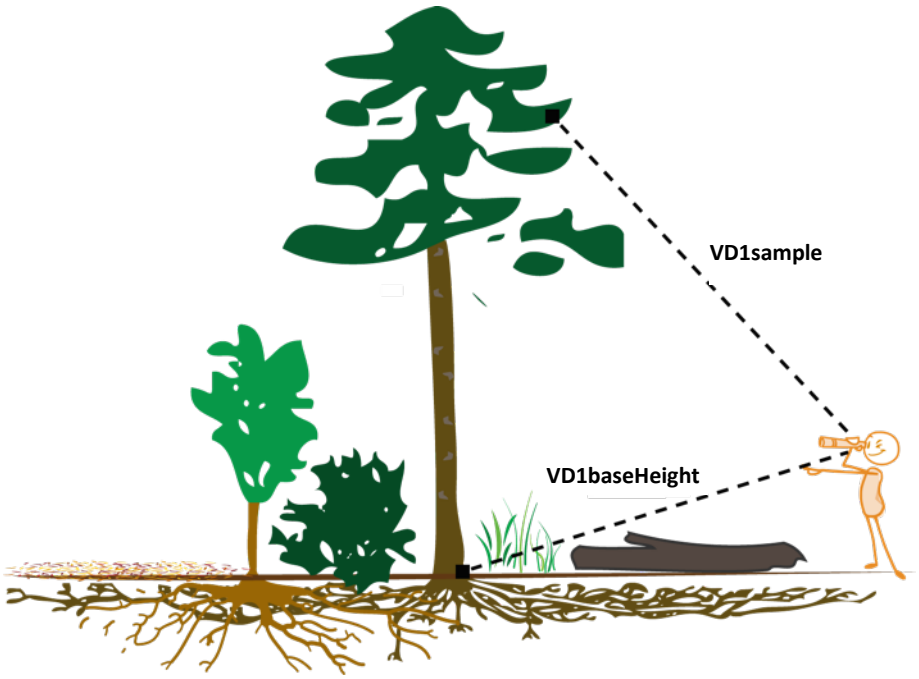


Figure 6 Measuring sample height using a laser rangefinder

12. 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.
    a. Place a reflective surface near the base of the individual to aid accurate readings.
    b. 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**.
    c. With the laser rangefinder still in VD mode, aim it at the base of the stem (or reflective tape) and press power button. Record this value as **VD1baseHeight**.
    d. If leaves came from a range of heights, take multiple measurements (up to three). Enter each value in the data entry 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 the ground of the sampling location.
    b. **VD(#)baseHeight**:
        - Enter '0'

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

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

Figure 7 Example LMA foliage scans from Domain 1, to help give a sense of foliage quantities needed

15. Use a spring scale to weigh the remaining foliage, ensuring enough material is left for all chemical analyses. Foliage need not be whole/pristine, but ensure that it is not excessively covered in epiphylls (fungi or lichen growing on the surface) and contains no shotgun holes.

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

        - Target roughly **30 g fresh foliage** for broad-leaf species
        - Target **15-20 g fresh foliage** for needle-leaf species
        - These masses do not include woody parts

    b. If a spring scale is not available, target the following leaf quantities:

        - 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. Package and stow (sub)samples:

    a. **<u>Chlorophyll subsample: use ~25% of good-condition (green), set-aside leaves</u>**

        1) For larger leaves (>= 0.5" diameter), use a punch tool to extract circles distributed across all set-aside leaves.
            - 0.5" diameter punch tool = 20-25 circles
            - 0.75" diameter punch tool = 15-18 circles
            - 1.5" diameter punch tool = 5-6 circles
        2) For thinner 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.*
        3) Place punches or small pieces into a pre-made foil packet.
        4) Spread out foliar material as much as possible in the foil packet, trying to minimize the stacking of foliage. This will help tissue freeze effectively.
        5) Place foil packet into a 4oz Whirl-pak bag.
        6) Label it with **chlorophyllSampleID** if not already done. The identifier will consist of the module code (cfc), **plotID** (siteIDXXX), **taxonID**, **-sampleNumber** (for that plot and date), **collectDate**, and the suffix ".chl"
            - Example identifier: **cfc.GRSM001.QUAL-1.20190705.chl**
        7) If using a data entry application, scan the chlorophyll sample barcode – it should appear in the field **chlorophyllSampleCode**.
        8) 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.

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

    b. **<u>LMA subsample: use remainder of good-condition, set-aside leaves.</u>**

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

- It helps to stack individual leaves (Figure 8); woody material may or may not be removed prior to bagging the sample.

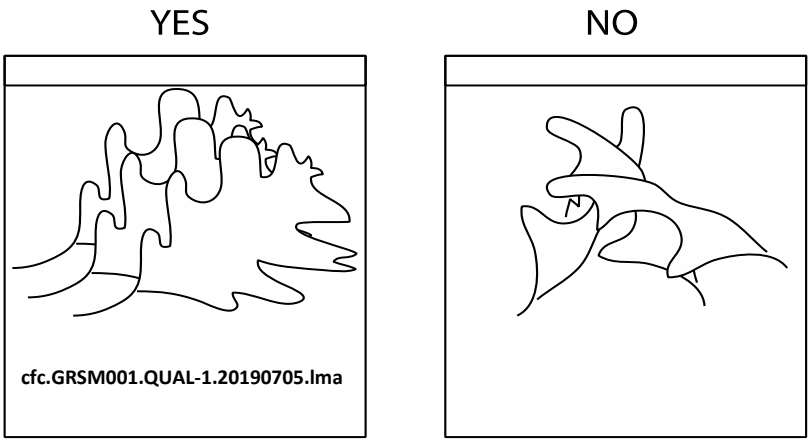


Figure 8. Example of how to package and label a broadleaf LMA subsample.

3) If bag was not pre-labeled, label with **lmaSampleID**. Use the same convention as descried above, but with ".lma" for the suffix.
   - Example identifier: **cfc.GRSM001.QUAL-1.20190705.lma**
4) Store LMA bag in cooler on ice packs, ensuring not to bend or crush foliage.

c. **Bulk chemistry sample**

1) Place remaining foliage into a paper bag and close.
2) 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.
   - Example identifier: **cfc.GRSM001.QUAL-1.20190705**
3) If using a data entry application, scan the bulk sample barcode – it should appear in the field **sampleCode**.
4) Store bulk sample bag in a cooler with ice packs – it does not matter if foliage gets crushed. If possible, line the cooler with a trash bag in order to preserve moisture of the samples.

18. Record **plantStatus** in the data entry application or Field Datasheet.

- This field is for assessing the status of a woody individual at the time of canopy foliage sampling. Use choices in Table 13, similar to those used in TOS Protocol and Procedure: Measurement of Vegetation Structure (RD[10]), but with additional canopy sampling options. If multiple options apply, select the one that is most likely to impact the data.

Table 13. Woody individual status options and their definitions.

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 'crown' or 'bole' damage in **remarks** and cause if possible.
Leaves not fully expanded	If plot was recently burned or suffered another severe disturbance and the individual sampled 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

19. If the sample was collected from a non-tagged individual, it will need to be tagged – either with a Z if 100% certain that it will be a canopy-only individual (e.g., found outside the zones reserved for plant productivity measurements, the plot core or the two randomly selected subplots), or with a standard tag if it will (or may) qualify for VST measurements but sampling has not yet occurred.

20. Record whether it's a canopy-only tag in the data entry application or Field Datasheet - if uncertain, defer to **cfcOnlyTag** = N. Then prepare to map and tag, as specified in SOP B of the Vegetation Structure protocol (RD[10]). If using a mobile device, record values in the Mapping and Tagging application. If not, use the Canopy Foliage Field Datasheet.

    Briefly:

    a. Attach a pre-numbered aluminum tag, appended with a "Z" using the dicast set (example = 09147**Z**) or not as appropriate. Record **tagID**.

    b. Record the **pointID** where the laser rangefinder is positioned. **Only pointIDs that are GPS measured and/or monumented are acceptable for mapping and tagging** (Figure 9).

        1) In Distributed basePlots and short-stature Tower basePlots, these five points are [41], [31], [33], [49], [51]

        2) In tall-stature Tower basePlots, these nine points are [41], [21], [23], [25], [39], [43], [57], [59], [61]

c. 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

21. Repeat steps 7-19 for all individuals to be collected in a given plot. Change gloves before handling foliage from the next individual.
22. Collect all trash and detritus and remove from plot.
23. Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the plot dry ice cooler, which is 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.
24. Before moving on to the next plot, 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.

    a. Make a reasonable effort to sample some of the more rare canopy species at the site level, but do not spend an excessive amount of time in this pursuit. If several rare species from the target taxa list cannot be sampled, collect extra replicates of more common species in order to achieve the site-specific total sample count.

25. Repeat the steps above until the site-specific total sample count is achieved.

B.2 Sampling Herbaceous Cover

1. Navigate to a plot where clip strip sampling will occur.
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. For Type II sites (Table 1), one clip strip will be harvested per assigned Distributed basePlot or 'Short-stature' Tower basePlot, and it will come from the non-destructive plot core, while two clip strips will be collected per 'Tall Stature' Tower Plot, one from each of the two randomly selected subplots used for Plant Productivity measurements (Figure 9).

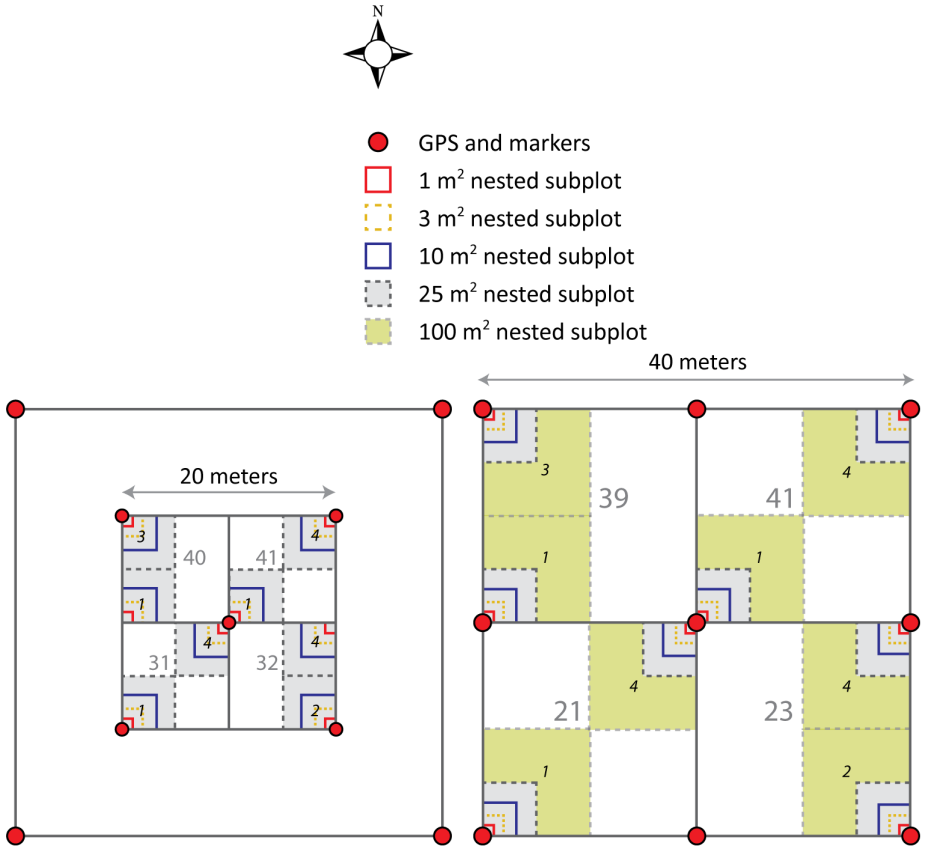


Figure 9 *Left*: Plot layout for Distributed basePlots and short-stature Tower basePlots used for canopy foliage sampling. *Right*: Plot layout of tall-stature Tower basePlots use for canopy foliage sampling

4. If woody cover is absent or < 25% of plot cover, follow STEP 5 below to use a randomized method to select a clip strip for harvest. If woody cover is > 25% of plot cover, skip to the targeted method outlined in STEP 6 to guarantee selection of a sun-lit clip strip.
5. Select the first potential clip harvest location using the plot-specific clip list.

a. Use the clip list to locate the desired target coordinates for the selected clip strip.

a. Locate 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], SOP B.1, steps (3) – (5).

b. Assess whether clip strip location is suitable for sampling (8Appendix D, Figure 14)

- 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.

c. If the clip strip is rejected, record why in the 'status' column of the clip list (use codes in 8Appendix D, Table 16), then proceed to the next potential strip on the list.

- **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.

d. When a clip strip has been found that is deemed acceptable, record that it has been selected for canopy foliage sampling on the clip list and mark its location.

6. Select clip harvest location(s) using the following targeted, non-random procedure:
    a. Assign a number to each continuous "patch" of herbaceous vegetation in the plot/subplot.

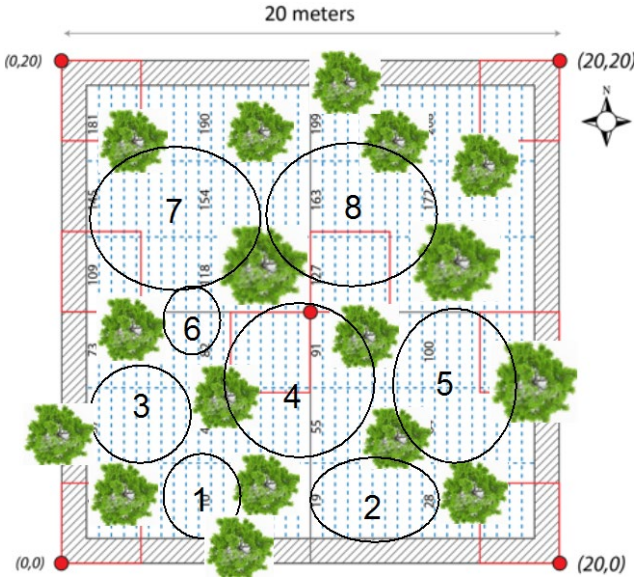


Figure 10 Divide plot into 'patches' of sun-lit herbaceous vegetation; assign numbers to facilitate random sampling

    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 a map of the clip cells (8Appendix D, D.2) 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.*
    d. Assess suitability using criteria described above. Continue assessing possible clip strips near the patch center until an acceptable one is found.
    e. Record which strip is chosen for canopy foliage sampling on the clip list and mark its location
7. Once a clip strip has been selected for canopy sampling, delineate it.

a. Using one of the pre-marked string and stake sets, line up one of the marks with the pin 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.

   - Keep the compass or Rangefinder at least 50 cm from non-aluminum metal plot markers, eyeglasses, wristwatches, tent stakes, etc.

c. 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.

Figure 11 Delineated clip strip

d. The two sets of marks on the two string-and-stake sets now clearly delineate a designated area for clip-harvesting (Figure 11).

8. If this size clip strip does not capture sufficient vegetation (possible in aridlands, tundra, or croplands), delineate a larger strip for sampling. Use any of the three size choices available in the Herbaceous Biomass 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.*

9. Enter required metadata into data entry application or field datasheet: **plotID, subplotID, collectDate, sampleType, clipID, clipDimensions**

   a. **subplotIDs** are in reference to clip lists and Herbaceous Biomass sampling (as in RD[04]), not in reference to Plant Diversity sampling.

10. Visually estimate and record percent foliage cover of the clip strip as **percentCoverClip.** Don't spend more than 1 minute making this visual estimate.

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

12. 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.

- ***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 (STEP 17 below, using representative foliage), then continue clipping the rest of the strip. This will help limit chlorophyll degradation.

13. 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* so that they will be handled with appropriate caution during downstream processing. A sample warning label may be employed for this purpose.
14. Place clipped biomass into paper bags.
15. If not pre-labeled, label bag(s) with **sampleID** as follows: module code (cfc), **plotID** (siteIDXXX), 'CLIP', **- sampleNumber** (for that plot and date), and **collectDate**
    - Example identifier: **cfc.WOOD001.CLIP-1.20190705**
    - Also write the **clipID** and **bagCount** on the paper bag
16. Mix all contents of the sample, then pull out a representative subsample of bulk herbaceous material from which to generate a chlorophyll subsample. An approximate representation of community composition is acceptable, do not spend more than five minutes on this task. A small amount of foliar material is needed, one handful will suffice.
    - *Remember that for herbaceous samples, you **do not** need to create an LMA subsample in the field, this can be done in the Domain Support facility*.
17. Create the chlorophyll subsample:
    a. 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.
    b. Place small foliage pieces into a pre-made foil packet. Spread out material as much as possible – this will help the tissues freeze effectively. Make sure that only **live, green vegetation** is included – do not include woody parts, dead foliage, or flowers.
    c. Place foil envelope into a 4oz Whirl-pak bag.

d. If not already done, label bag with **chlorophyllSampleID**. The will consist of the module code (cfc), **plotID** (siteIDXXX), 'CLIP', **sampleNumber** (for that plot), **collectDate**, and the suffix ".chl"
    - Example identifier: **cfc.WOOD001.CLIP-1.20190705.chl**

e. If using a data entry application, scan the chlorophyll sample barcode – it should appear in the field **chlorophyllSampleCode**.

f. If sample contains *Toxicodendron spp*, add sample warning label sticker.

g. 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.

**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.*

18. If using a data entry application, scan the barcode for the bulk sample – 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 paper bags.
19. If sample contains *Toxicodendron spp*, select 'YES' in the **toxicodendronPossible** field in the data entry application. If using paper datasheets, note this in **remarks**.
20. 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. If possible, line the cooler with a trash bag in order to preserve sample moisture.
21. Repeat if an additional clip strip will be harvested in the plot, then move to the next plot until all designated Distributed Base and Tower plots have been sampled.
22. 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.

SOP C Post-Field Sampling Tasks

C.1 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 ok 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. 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.
    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 will 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 a problem ticket; samples may be unusable.
    b. Review SOP D for instructions on LMA scanning and SOP E for instructions on sample drying and subsampling for chemistry.

C.2 Refreshing the Field Sampling Kit

1. Make sure the following consumables are available in sufficient quantity for the next round of canopy foliage chemistry sampling:
    - Paper and plastic bags, appropriate sizes as needed; Rite-in-the-Rain paper for printing field datasheets; Nitrile gloves; permanent markers; Flagging tape; Scannable barcode labels
2. Return cold packs to the -20° freezer to refreeze.

C.3 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 contaminated by contact with *Toxicodendron spp* as detailed in RD[08].
4. Recharge or replace batteries for the GPS unit and Laser Rangefinder (as applicable).

SOP D Laboratory Processing: Leaf Mass per Area Measurements

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. For small leaves (< 0.5" wide), conifer needles, and grass blades, scanning plus image analysis is our only viable option and must be performed.

BACK-UP METHOD FOR LMA MEASUREMENT

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 D.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 will soon mold and become unusable.

NOTE: *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 D.1* ***and*** *D.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.*

D.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 bulk chemistry sample for herbaceous samples) from the refrigerator.
4. Arrange an appropriate amount of material on the flatbed scanner. 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. Make sure mid-vein and petiole are included.

- Medium leaves: 7-15, depending on size and what fits on the scanner. Arrange neatly, include petioles/rachis.
- Needles: 20-100, wide variation depends on needle size. Arrange neatly, flat needles same side up. *Do not need to cover the entire 8 x 11" area* (see Figure 7).
- Herbaceous samples: as many live, green leaves/blades as can comfortably fit on the scanner. Try to ensure a representative sample. If needed, use clear tape to secure foliage to screen. It is ok to cut long blades of grass if needed.

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

6. 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 12).
   c. Choose representative leaves <u>in good condition</u> - without holes where possible, mostly green with little dead or damaged parts (especially relevant in grasslands).

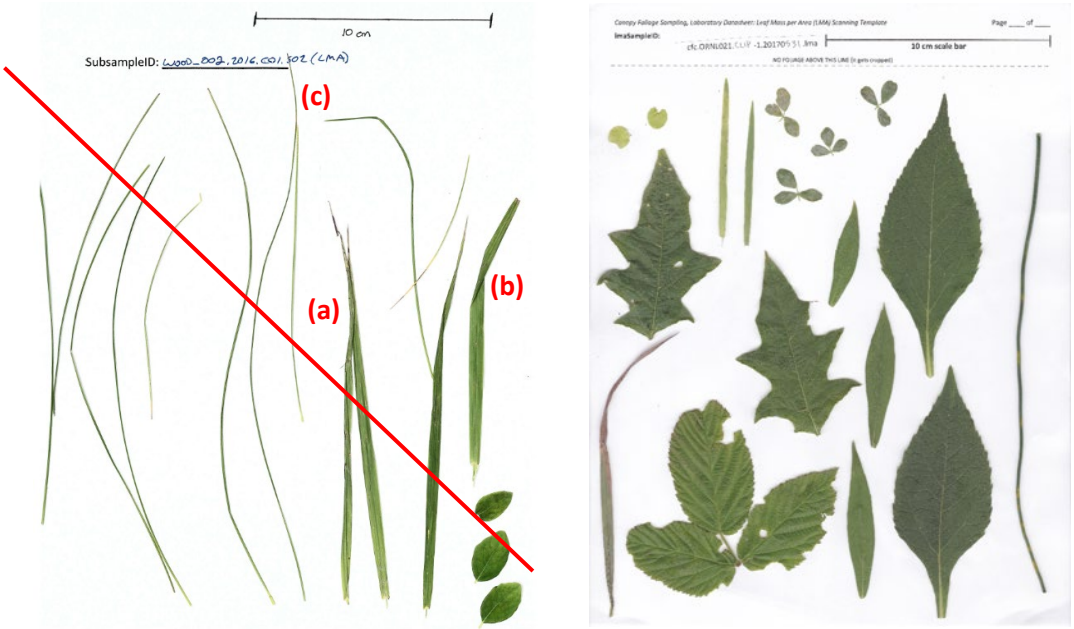


Figure 12 Left: Example of leaf arrangements to avoid when scanning for LMA, including overlapping foliage (a), bent foliage (b), and foliage covering the text (c). Right: High-quality scan for a clip strip sample (mixed foliage).

7. 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.
8. 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.
9. Scan the sample (Figure 13, left).
10. 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*.

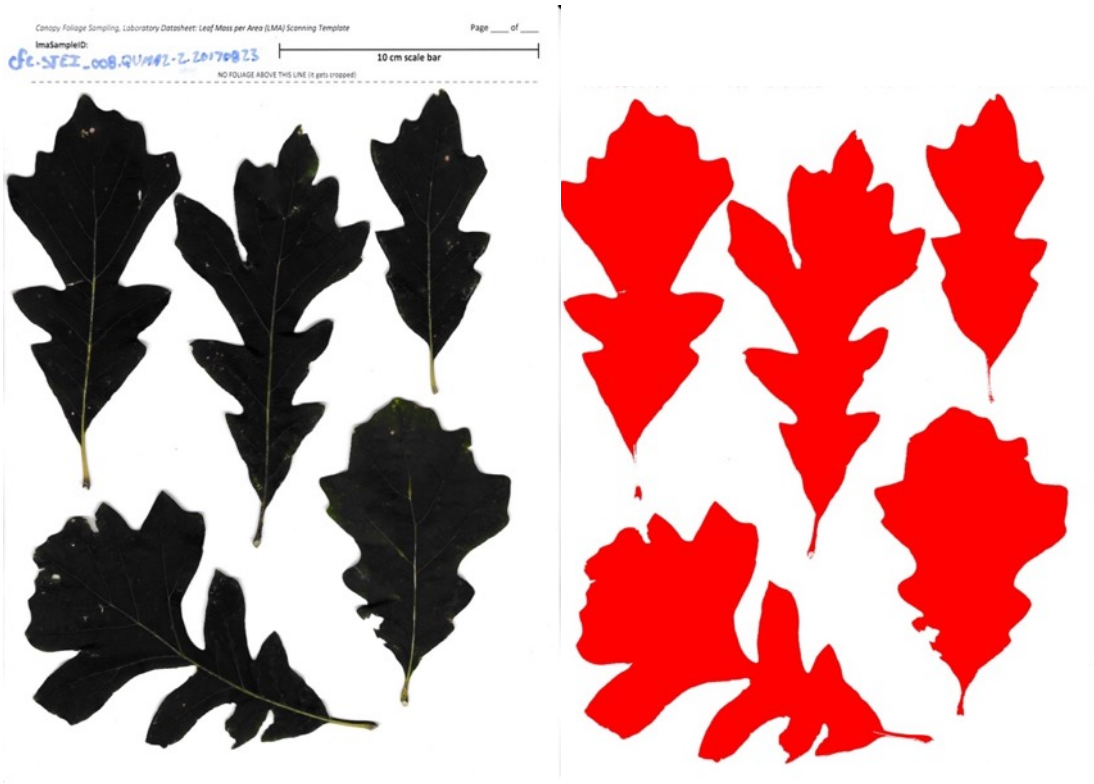


Figure 13 Left: Example of a good quality scanned image of foliage. Right: Image after processing in ImageJ

**The entire LMA subsample may not be used for scanning. If there is extra material, combine it with the bulk chemistry sample. Or, if sure there is sufficient mass for all subsamples, extra foliage may be discarded.*

11. Upload scan as .tif or .jpeg file and save to a folder in the Domain Support Facility N-drive.
12. 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.
13. 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**
14. Also affix a Type I adhesive barcode label to the bag or coin envelope. If using a data entry application, scan this barcode – it should appear in the field **lmaSampleCode**

15. If sample contains a *Toxicodendron spp*, add sample warning label to bag or coin envelope.
16. 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 a minimum of 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
17. Clean the scanner glass with a tissue to remove dust/resin. If dry tissue is insufficient to remove resin, a small amount of glass cleaner can be applied. Move on to next sample until all samples have been scanned.

D.2 Measuring Leaf Area

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. 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**.

4. 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.
5. Next go to Process → Binary → Make Binary, which converts the image to black and white.
6. 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.
7. If these procedures yield a sharp, clear, black image with no artificial holes or white space, proceed to STEP 9 below. If not, conduct the procedure outlined in STEP 8 first.

8. 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 2-4.
    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 13, 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 **Set**, then click **OK**. Setting the threshold is telling the software which parts of the image it will be analyzing. Close the 'Threshold' dialogue box.

9. Go to Analyze → Set measurements. This will bring up a checklist of available measurements. Make sure that only 'Area' is checked. Hit **OK**.
10. 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.
        1) 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.'
    b. If you have real holes that are contained by black areas in your image, make sure that "include holes" is NOT checked.
    c. If there are simply lighter-colored areas that look like holes, do check that option.
    d. Make sure that 'Display Results' is checked and that the 'Show dialog' box displays Masks. Hit **OK**.
11. 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.
12. Compare this mask 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 mask is inaccurate, close the image without saving and conduct area calculations again by adjusting minimum, contrast, and threshold as described in STEP 8 above.

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

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

13. 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.

14. 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. A visual approximation, do not spend more than a minute making this estimation

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

16. 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.

D.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. Immediately upon removing from the oven, weigh samples to nearest **0.001g**.

a. Tare 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 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.

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

Only use this option if scanning large leaves takes so long that samples will 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. 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.
    - Use the same punch size for all leaves in the same sample.
2. Record the weight of the fresh material punched.
    a. Tare 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 data entry application or lab datasheet
3. 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 into the data entry application later
4. Place punches into a coin envelope. Label it with **lmaSampleID** and affix a Type I adhesive barcode label as described above.

5. Place coin envelopes into drying oven and record **ovenStartDate**. Samples must oven-dried at 65°C for 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).
6. Save remaining fresh sample in refrigerator for up to one week in case there is a problem.
7. Remove subsamples from the oven once they have been dried at 65°C for 48h. Record **ovenEndDate**.
8. Immediately upon removing from oven, weigh punches to nearest **0.001g**.
    a. Tare 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.**
    a. Record **dryMass** (dry weight of the punches in grams) in the data entry application or lab datasheet
9. 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.
10. 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 E Laboratory Processing: Drying and Subsampling for Chemical Analyses

Bulk canopy foliage samples will be 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.

E.1 Drying and Weighing

1. Remove bulk foliage sample from refrigerator.
2. Verify that an LMA subsample has been created. If not, set aside enough fresh foliage to fill a scanner bed and place into a labeled, resealable plastic bag, stored in the refrigerator.
3. Place paper bag 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.
4. After 48 hours (or when samples have achieved constant mass), remove paper bags from the oven and record **ovenEndDate**.
5. Place an empty paper bag of the same type as contains the samples on the scale. Tare it.
6. 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.*
7. 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 E.2 to prepare the chemistry subsamples
    - For dry mass > 10 g, follow section E.3 instead

8. For samples that contain *Toxicodendron spp*, follow the steps described in E.4, regardless of sample mass.

E.2 Subsampling with Small Mass (< 10 g dry) for Chemical Analyses

1. Wear a pair of Nitrile (Latex-free) gloves when handling and subsampling foliage. It is acceptable to re-use gloves but use 70% ethanol to clean gloved hands in between samples.
2. Split the foliar material into two or three subsamples by hand, following the guidelines in Table 14 and listed below. Try to ensure that the splits are representative.
    a. Dry sample mass **< 2.7 grams**, prioritize getting adequate C/N and lignin/elements subsamples and do not create a chemistry archive subsample.
    b. Dry sample mass **2.7 to 6 grams,** apportion the material as follows:
        1) C/N: 0.2-1 gram
        2) Lignin/elements: 1.5-2 grams
        3) Chemistry Archive: 1-3 grams (ground to 20 mesh)
    c. Dry sample mass **6 to 10 grams**, split foliar material equally into three portions.

Table 14 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. Regardless of mass, make sure to:
    - Remove any remaining twigs and other non-foliage woody parts.
    - Process, stow and label each subsample as instructed in STEP 4, including the directive to grind the chemistry archive sample to 20 mesh.
4. Subsample processing instructions:
    - **C/N and lignin/elements**
        - Container = coin envelope, small paper bag, or plastic scint vial (whatever is easiest to fit the material into)
            - Ok to crush/break leaves to fit in container but do not grind in mill
        - Identifier = **cnSampleID** (sampleID + .cn) or **ligninSampleID** (sampleID + .lig)

- Example CN: cfc.GRSM001.QUAL-1.20190705.cn
- Example lignin: cfc.GRSM001.QUAL-1.20190705.lig

o Write identifiers neatly on envelopes or paper bags using permanent marker, or use address labels for scint vials (do not write directly on vials as this can rub off). *To create printed labels, export sample identifiers from the Field data entry application, then add .cn or .lig suffixes.*

o 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. When using a data entry application, scan each barcode and ensure it appears in the correct field, either **cnSampleCode** or **ligninSampleBarcode.**

- **Chemistry archive (when sufficient material)**

o Container = 20 mL scintillation vial

- **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 after grinding, then clean with compressed air (and ethanol if needed for resinous foliage) between samples.

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

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

o Use an address label for identifier, see above tip for printing but use .ar suffix instead. Do not write directly on the vial as it can rub off.

o Affix a Type I adhesive barcode label lengthwise along the vial, without covering the sample identifier. When using a data entry application, scan this barcode – it should appear in the field **archiveSampleCode.**

5. Organize subsamples and group by type in preparation for shipment. Seal coin-envelopes, using tape to close off any leaky corners. Close paper bags with tape or rubber band.
6. Store subsamples in a cool, dry location until they can be shipped to analytical facilities.

E.3 Subsampling with Large Mass (> 10 g dry) for Chemical Analyses

1. Wear a pair of Nitrile (Latex-free) gloves when handling and subsampling foliage. It is acceptable to re-use gloves but use 70% 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.
- Ensure all twigs and other woody materials have been removed prior to grinding.

3. Use an appropriately sized splitter or microsplitter to generate three subsamples.
    a. Split the sample once and place an entire half into a 20 mL scintillation vial. This will be for the chemistry archive.
    b. Split the remaining material in half again and place each half into it's own 20 mL scintillation vial. These will be for the C/N and lignin/elements analyses.
    c. 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 chemistry archive sample back into the splitter and keep splitting until there is enough material in the lignin/elements vial.
    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. Label each subsample as outlined below using address labels. Do not write directly on vials as the label can rub off**.** *For label printing, export sample identifiers from Field data entry application, then add appropriate suffixes.* Also, affix a Type I adhesive barcode label lengthwise along each vial without overlapping the sample identifier. If using a data entry application, 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

6. Take the material **from the C/N sample only** 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 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.

7. Clean mill with compressed air (and ethanol if needed for resinous foliage) between samples.
8. Once all subsamples have been created, organize and group by type in preparation for shipment.
9. Store subsamples in a cool, dry location until they can be shipped to analytical facilities.

E.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 fairly representative but with minimal handling of the foliage.

    a. Sample mass **< 10 g**: follow guidelines in section E.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 scintillation vial. Label and apply a barcode to each container as described in section E.3.5.
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 biorepository.

SOP F Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[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***.

All QA measures needed for this protocol are described in the Data Management Protocol (RD[12]).

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 scan-able 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.

If available, adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season).

Barcodes are unique, but are not initially associated with a particular sample, 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.

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

SOP G Sample Shipment

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

G.1 Prepare Shipping Inventory

Creating a shipping inventory: Whenever samples are shipped, they must be accompanied by a hard-copy inventory enclosed within each shipping container. In addition, a corresponding electronic version of the file will be emailed to the laboratory and NEON CLA contact once the shipment is finalized, using the Stork Shipment Verification Tool.

1. Navigate to the "Shipping Information for External Facilities" document on the CLA intranet site. There, you will find instructions on whether any special items (permits, cover letters, etc) are required to include in the shipment. Check this document each time a new shipment is prepared as it is subject to change.
2. Print out required documents as needed to include in shipment box.
3. Prepare a shipping inventory detailing the contents of the shipment, using the appropriate shipping applications (this requires the use of the Shipping: Shipment Creation, Shipping: Shipment Review, and the Stork Shipment Verification Tool). Print a copy of the inventory (which can be downloaded from the Stork Shipment Verification Tool) to include in each shipment container.
4. While organizing samples for shipment, verify that each sample listed in the inventory is present and will be included in the shipment.

G.2 Ship Chlorophyll Subsamples

Subsamples for chlorophyll analysis must be kept frozen and shipped on dry ice within 7 days of collection. Dry ice is a Class 9 regulated material and must be shipped according to CFR 49 Subchapter C, Hazardous Material Regulations. Dry ice releases carbon dioxide gas, which can build up pressure and rupture packaging. To prevent this, ensure packing allows release of this pressure (e.g., it is not air-tight). Dry ice must be packaged using **UN packing group III** compliant materials. The maximum amount of dry ice per package is 200 kg. Refer to the Chemical Hygiene Plan and Biosafety Manual (AD[03]) for additional requirements on commercial shipment of hazardous or dangerous material.

1. Group frozen samples into 1-gallon resealable freezer bags.
2. Use a corrugated cardboard box that meets UN packing group III requirements. Add styrofoam sheets along the walls of the box as insulation. Ensure the styrofoam is not sealed to be airtight. Styrofoam must not be used as outer packing.

3. Put samples to be shipped into insulated box, then weigh it.
4. Add dry ice to completely surround sample bag(s). Ensure there is good contact between dry ice and samples, including packing some dry ice on the top.
5. Re-weigh the box to determine mass of dry ice added.
    a. Some local carriers limit weight of dry ice to 2.5 kg. Check with your local carrier.
    b. If weight limits apply, use cold-soaked packing peanuts to keep samples frozen.
6. Fill empty space with wadded newspaper, styrofoam peanuts, or bubble wrap. Any empty space will allow the dry ice to sublimate faster.
7. Insert the hard copy shipping inventory, along with any other required documents, into a resealable plastic bag and add to shipping container prior to sealing.
8. Seal box. Complete packing slip and label for Class 9 dry ice hazard shipment.
9. Address shipment and send samples standard overnight to the destination specified in the CLA "Shipping Information for External Facilities" document. **Do NOT ship on Friday**.
10. Submit the shipment on the Stork Verification Tool (http://den-raven-1.ci.neoninternal.org/shipping/) to email the shipping inventory as well as receipt and tracking forms to all relevant parties.

G.3 Ship C/N, Lignin/Element, and Chemistry Archive Subsamples

1. Take canopy foliar samples (coin envelopes and/or scintillation vials) out of cabinets or oven. Make sure each container is clearly labeled and sealed.
2. Group samples into 1-gallon resealable bags. Place bags into thick-walled shipment boxes.
3. Use bubble wrap, wadded newspaper, or other packing material to pad and secure samples within shipment boxes. Add enough packing so they will not move around as box is handled.
4. Add the hard copy shipping inventory, along with any other required documents, to the shipping container prior to sealing.
5. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
6. Submit the shipment on the Stork Verification Tool (http://den-raven-1.ci.neoninternal.org/shipping/) to email the shipping inventory as well as receipt and tracking forms to all relevant parties.

G.4 Supplies and Containers

Samples should be shipped in boxes approximately 12” L x 12” W x 12” D, though larger or smaller boxes may be used if the sample number requires it. For samples requiring dry ice, ensure these meet UN packing group III requirements. Unfilled space around samples should be minimized.

G.5 Timelines

Frozen (-80℃) chlorophyll subsamples should be shipped out for analysis within 7 days of collection, as pigments are sensitive to degradation. Oven-dried subsamples for C/N and lignin/elements analyses can be stored in a closed, dry cabinet for weeks to months prior to shipment, as these analyses are far more stable. Field Personnel should plan to ship samples within the holding times specified in Table 2.

G.6 Conditions

Samples are either shipped frozen on dry ice or at room temperature, as specified above.

G.7 Grouping/Splitting Samples

N/A

G.8 Return of Materials or Containers

If using insulated shipper kits or other reusable containers, include return ground shipping forms for the laboratory to return shipping materials.

G.9 Laboratory Contact Information and Shipping/Receipt Days

See the “Shipping Information for External Facilities” document on CLA’s NEON intranet site

8 REFERENCES

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Smith, M.-L., D. Y. Hollinger, and S. Ollinger (2008). Estimation of forest canopy nitrogen, p 197-203, In Hoover, C.M., editor. *Field Measurements or Forest Carbon Monitoring,* Springer, The Netherlands.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 15. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001576	Datasheets for TOS Protocol and Procedure: Canopy Foliage Sampling

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

APPENDIX B 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 and create candidate individual lists using vegetation structure data.
- For herbaceous clip strip sampling, ensure familiarity with clip list workflow

STEP 2 – Woody individual sampling: obtain sun-lit canopy leaves from a woody individual selected for sampling. Measure and record the height(s) where samples came from. Map and tag if needed.

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. 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.

STEP 6 – Place bulk sample in a chilled cooler.

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 SHIPPING OF CANOPY SAMPLES

STEP 8 – Make LMA measurements, recording all variables:

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

STEP 9 – Oven-dry remaining bulk sample, then 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 all subsamples to appropriate external laboratory facilities for analysis.

WorkTracker: Canopy Foliage Sampling in Primarily Woody 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 C REMINDERS

Pre-sampling: Check…

- ☑ If using an outside contractor, has their availability been confirmed?
- ☑ Does sampling schedule overlap with the AOP overflight?
- ☑ Is all required equipment available?
- ☑ For woody individual sampling, was a list of candidate individuals assembled using the vegetation structure data? Did you practice with the chosen sampling tool?
- ☑ Are several coolers available for dry ice and ice packs?
- ☑ 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, sun-lit 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)

Sample handling in the field: Be sure to…

- ☑ Set aside a representative subsample for chlorophyll analysis; flash-freeze it 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 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 will become unusable.

Processing for LMA: Check…

☑ Were scans clear and of good quality (no overlapping leaves)? Did all include a scale bar?

☑ For larger leaves, were midveins and petioles included?

☑ 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…

☑ 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 C/N and 20 mesh for the other types?

☑ Did all subsamples that may contain *Toxicodendron spp* receive the warning label?

Shipping: Remember to…

☑ Ship materials within the holding times provided in Table 1

☑ Ship chlorophyll subsamples on dry ice, packaged appropriately.

☑ Include shipping inventories.

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 D RESOURCES FOR CLIP STRIP HARVESTING

D.1 Assessing clip-strip suitability and recording decision on the clip list.

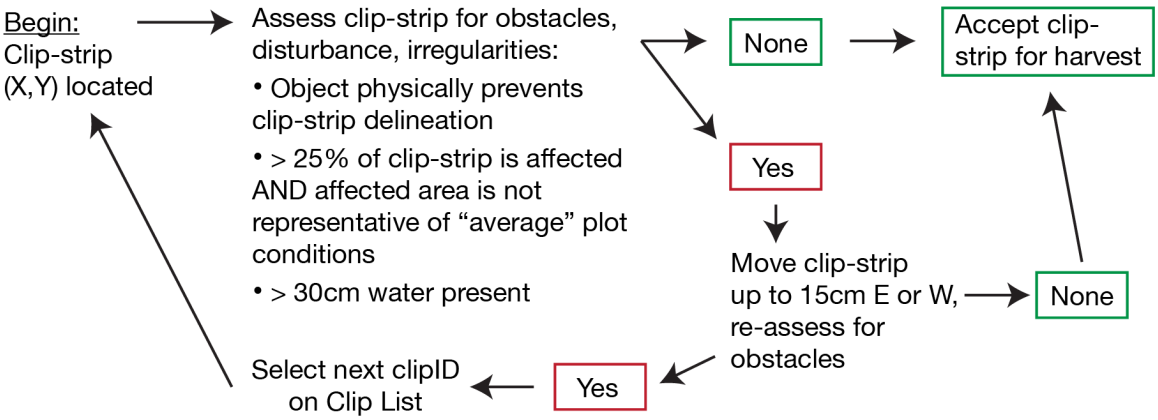

Figure 14. Flow chart to guide assessing potential clip cells for clip-harvest suitability.

Table 16. 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

D.2 clipCellNumber Maps by suplotID

In certain situations, Field Operations will be required to locate clip strips within "patches" of herbaceous vegetation when the % cover of herbaceous vegetation over the entire plot is ≥ 25% AND < 75%. To identify the location of clip harvests within herbaceous "patches," first find and utilize the appropriate map below (based on subplotID) to determine which clipCellNumber should be sampled. Then, use **Table 17** 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.

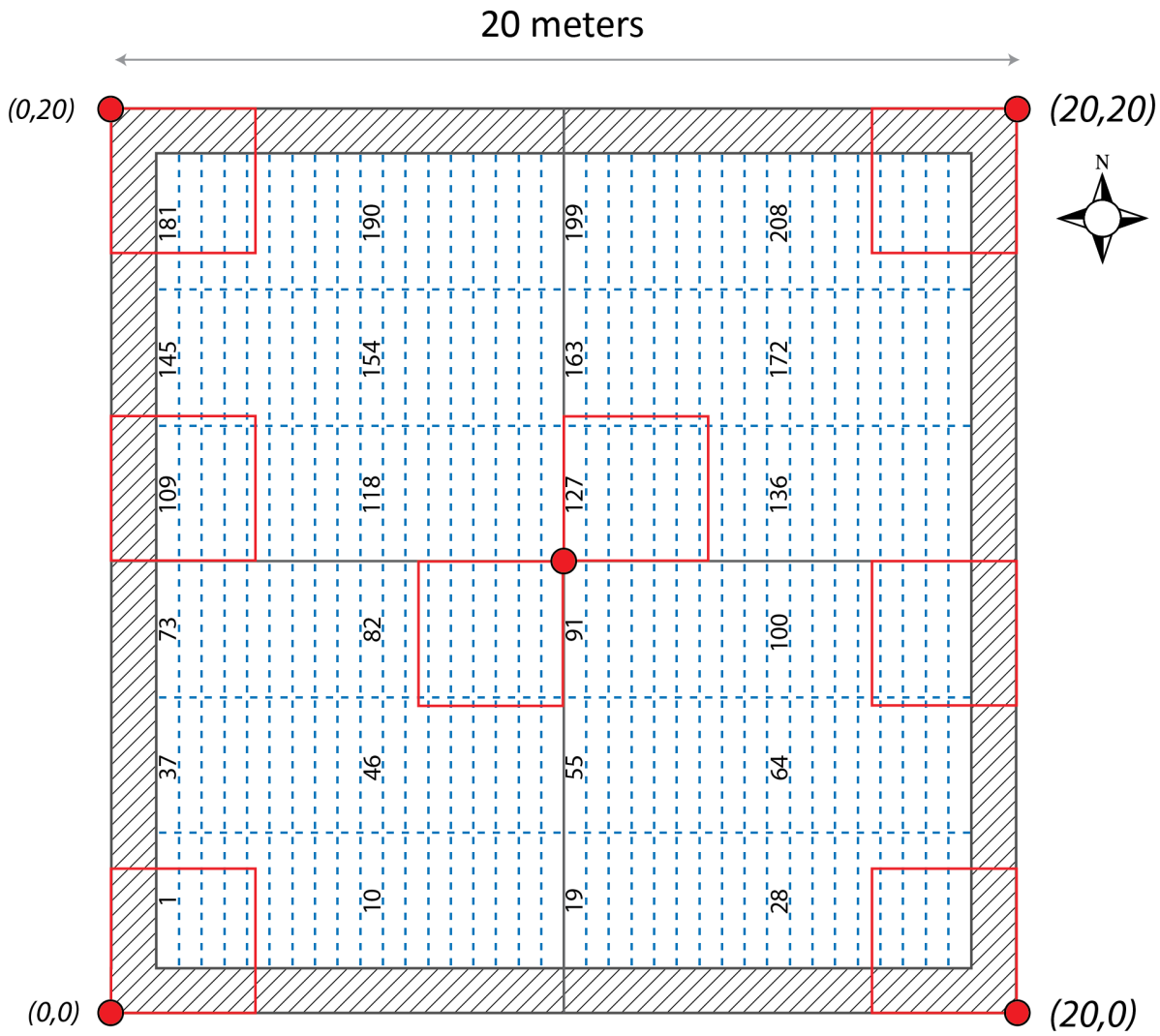

Figure 15. Map of clipCellNumbers in a 20m x 20m base plot (subplotID = 31 in provided Clip Lists). Red squares indicate nested subplots used for diversity sampling; clip cells that significantly overlap red squares are not used for clip sampling. However, clip cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support clip harvest sampling.

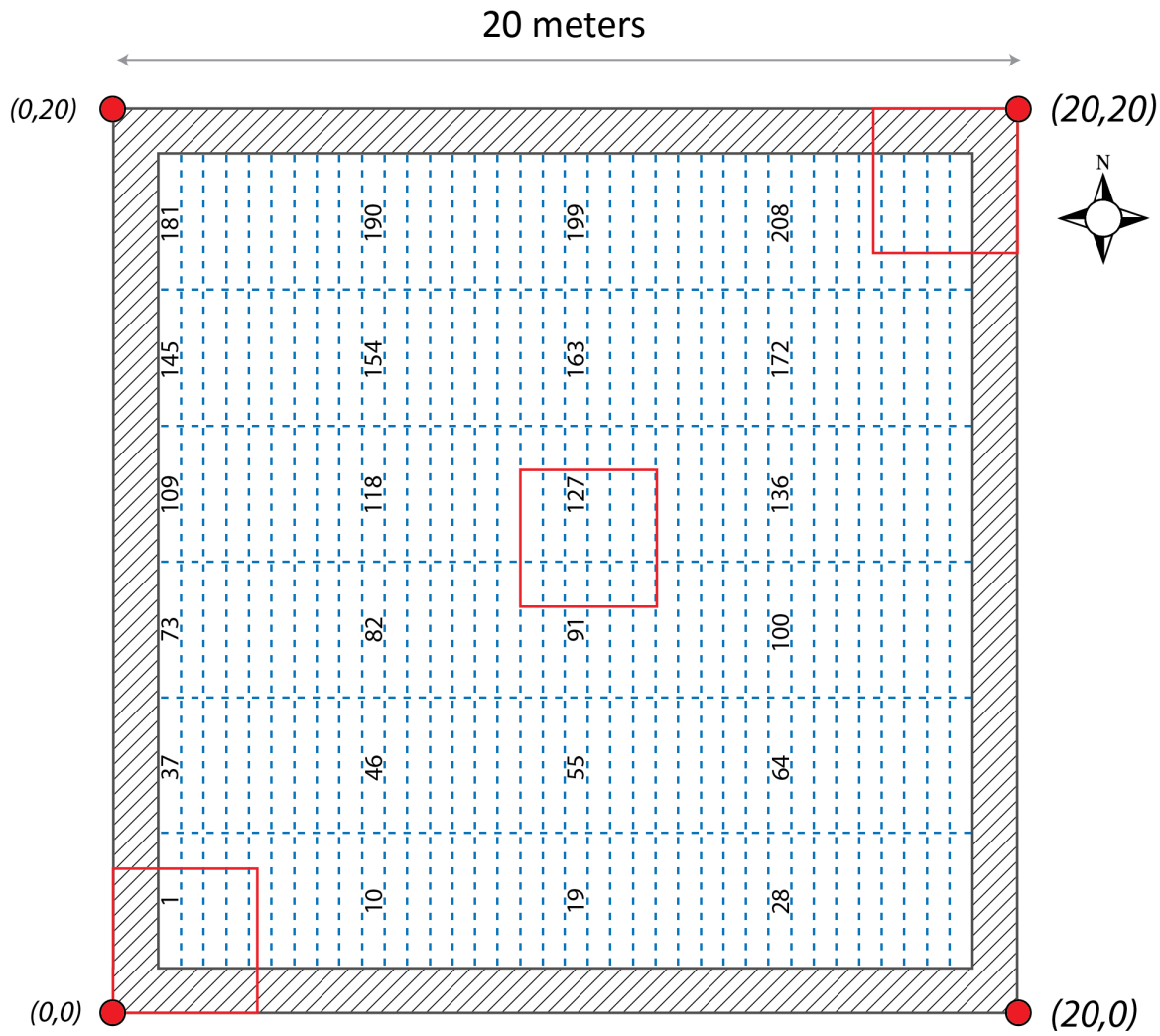

Figure 16. Map of clipCellNumbers for **subplotID = 21** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

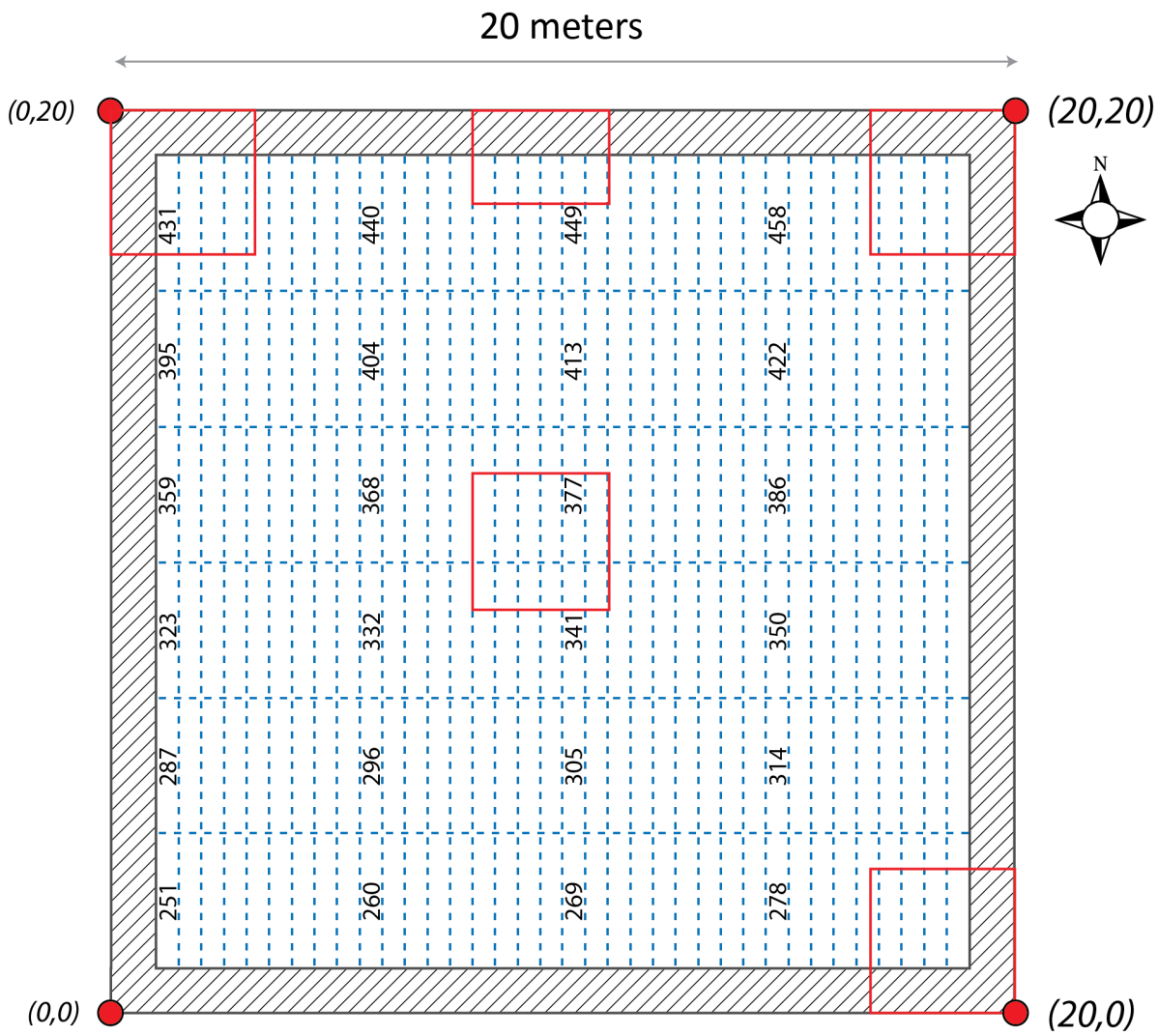

Figure 17. Map of clipCellNumbers for **subplotID = 23** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

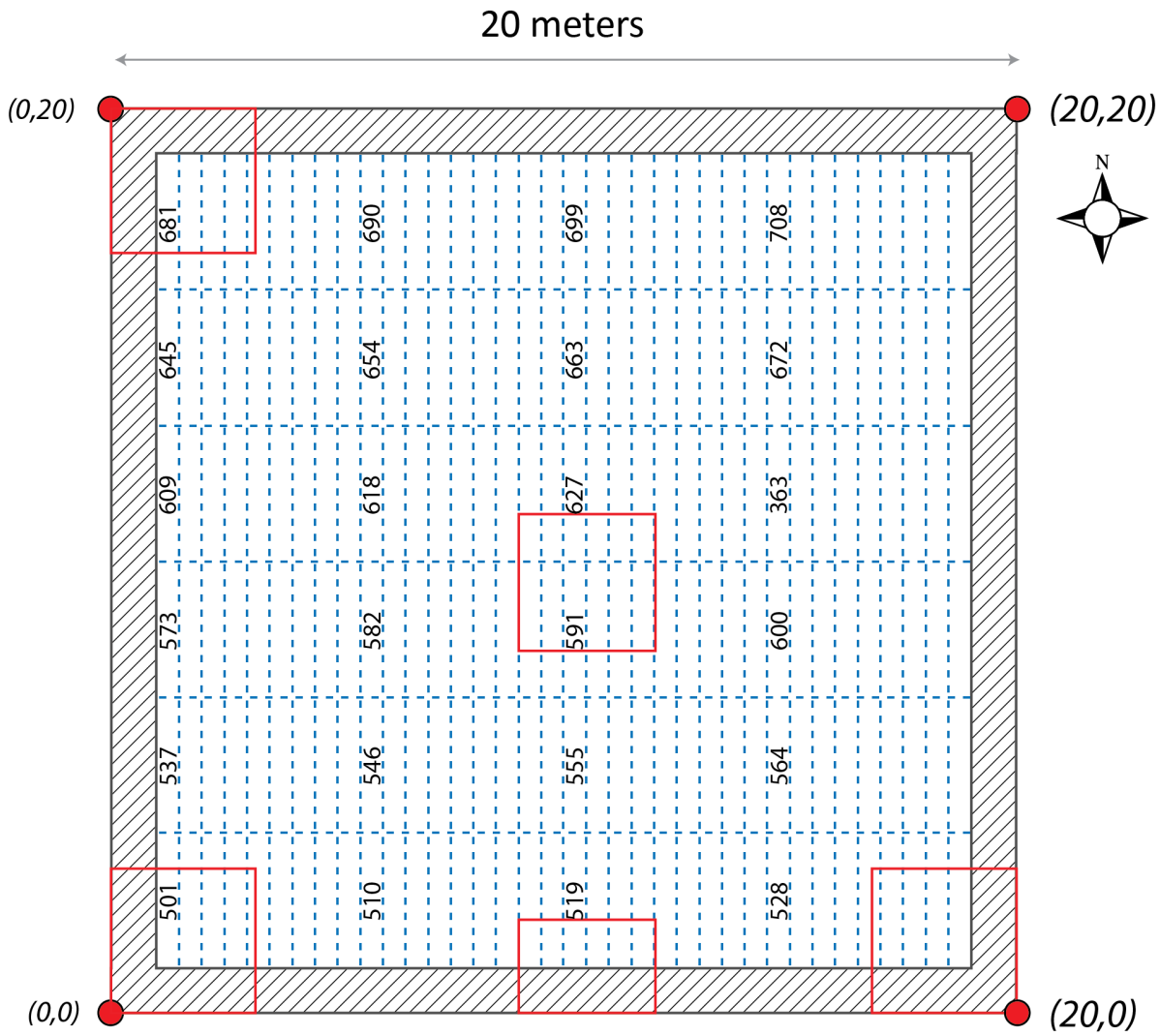

Figure 18. Map of clipCellNumbers for **subplotID = 39** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

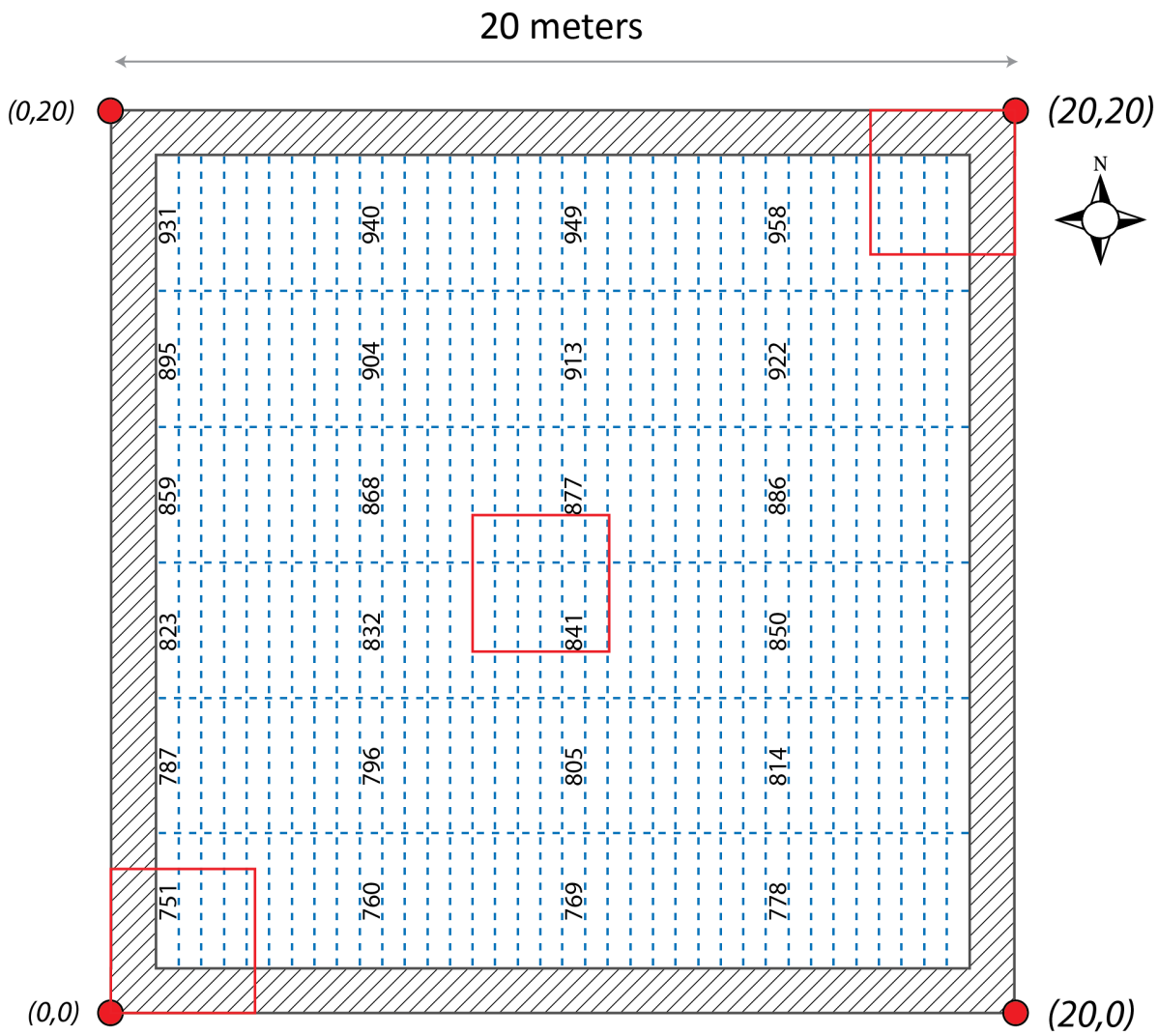

Figure 19. Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

D.3 Coordinates for clipCellNumbers by subplotID

Table 17. 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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
38	38	288	538	788	1.7	4.5
39	39	289	539	789	2.2	4.5
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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
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
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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
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
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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
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
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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
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
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 E PEAK GREENNESS WINDOWS BY SITE

Table 18. List of historical peak greenness windows for each NEON site, derived by NEON AOP using reflectance data from 2001-2015 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.

Domain	Site Name	Start Peak Greenness	End Peak Greenness
1	HARV	5/25	9/15
1	BART	5/26	9/14
2	SCBI	5/7	9/23
2	SERC	5/15	9/25
2	BLAN	5/2	9/8
3	OSBS	6/25	9/28
3	DSNY	8/21	10/6
3	JERC	7/17	9/2
4	GUAN	10/21	12/4
4	LAJA	9/5	11/29
5	UNDE	5/29	9/11
5	STEI	5/26	9/10
5	TREE	5/28	9/15
6	KONZ	5/21	9/3
6	KONA	5/21	9/3
6	UKFS	5/6	9/10
7	ORNL	4/29	9/5
2	MLBS	5/24	9/18
7	GRSM	5/18	9/22
8	TALL	4/28	9/20
8	DELA	4/11	9/15
8	LENO	4/23	9/10
9	WOOD	6/8	8/20
9	DCFS	6/8	8/20
9	NOGP	5/25	8/9
10	CPER	5/22	7/4
10	STER	5/2	8/20
10	RMNP	6/14	9/26
11	CLBJ	4/20	7/12
11	OAES	4/14	5/1
12	YELL	6/8	7/25
13	NIWO	6/30	8/31
13	MOAB	5/2	9/5
14	SRER	8/1	9/8

14	JORN	8/10	9/28
15	ONAQ	4/19	6/20
16	WREF	6/6	7/16
16	ABBY	6/6	7/20
17	SJER	2/12	4/3
17	SOAP	5/23	7/9
17	TEAK	6/21	11/4
18	TOOL	6/13	8/16
18	BARR	6/3	8/23
19	DEJU	6/2	8/18
19	BONA	6/1	8/15
19	HEAL	6/8	8/17
20	PUUM	12/23	2/4