



<i>Title:</i> TOS Protocol and Procedure: Canopy Foliage Sampling		<i>Date:</i> 04/03/2018
<i>NEON Doc. #:</i> NEON.DOC.001024	<i>Author:</i> S. Weintraub	<i>Revision:</i> E

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	<ul style="list-style-type: none"> • 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 Table 11 describing 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

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			<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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

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			<ul style="list-style-type: none"> • 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
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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 ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$), and leaf mass per area (LMA). NEON will quantify 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 will use the isotopic composition of leaves, as well as leaf litter, roots, and soils, to follow spatio-temporal changes in ecosystem C and N cycles.

Plant C and nutrient data will be 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 will be of high utility in helping the ecological research community refine algorithms to map canopy constituents using hyperspectral data. Additionally, foliar data will inform species and site-level estimates of canopy chemical constituents and how those change over time, which will 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 can alter photosynthetic rates, and, thus, the uptake of nutrients like N into leaf biomass. Such changes to canopy nutrient concentrations will likely cascade through the ecosystem, changing fluxes and biogeochemical transformations across the landscape.

The rationale underpinning the timing, frequency, species selection, and spatial extent of canopy foliar sampling is outlined in NEON Science Design for Terrestrial Biogeochemistry (AD[05]). The timing of sampling will allow 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, will allow researchers to track temporal dynamics of foliar chemical and structural change. Species selection, based on plot and site-level abundance, will enable sampling of a representative mix of canopy vegetation species spanning the range of physiological and ecological variability of the site and thus be useful in developing relationships with AOP data. Finally, the extent of canopy sampling will allow researchers to evaluate the spatial heterogeneity of canopy nutrient dynamics. For instance, differences

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in soil type and/or hillslope aspect will 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.

1.2.1 NEON Science Requirements and Data Products

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

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on canopy foliage sampling and trait measurement methods developed by the community, and many scientists working in the field provided valuable input. Relevant papers that describe these methods include 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).

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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: Core Sampling for Plant Belowground Biomass
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

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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 representatively 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; therefore, when possible, the same individuals within each plot will be sampled over time.

A subset of the 40 x 40 meter “Distributed Base Plots” located across the study area will be used for foliar chemistry and LMA sampling. Within the tower airshed, a subset of “Tower Plots” (whose sizes differ by location) will also be utilized. Sampling within plots will facilitate data georeferencing and streamline integration with AOP. It will also simplify longitudinal sampling and allow canopy chemistry data to be linked to other plot-scale soil and vegetation measurements. Specific Tower and Distributed Plot locations for canopy sampling will be provided in a separate document to NEON field ecologists.

In forested sites, the general procedure is to identify the top three most abundant *canopy* species on a per-plot basis, then sample one individual – preferably already tagged for vegetation structure monitoring, from each. However, in certain circumstances, the next most abundant species may instead be sampled, as discussed in detail in 7.2SOP B. This approach should yield measurements from a representative mixture of canopy-dominant species at the plot and landscape scale.

IMPORTANCE OF SAMPLING SUN-LIT FOLIAGE

It is critical that foliar samples from these individuals be 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 fully sun-lit canopy positions.

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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 will be necessary to use a shotgun, slingshot, line launcher, tree climbers, or employ other methods agreed-upon with 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 (2-7 m) vegetation, sun-lit leaves may be obtained using clippers and extendable pole pruners, respectively (see Appendix D). In systems dominated or co-dominated by herbaceous vegetation, bulk herbaceous plant biomass will be harvested 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.

Standard Operating Procedures (SOPs) in Section 7 of this document provide detailed step-by-step directions, sampling tips, and best practices for implementing the sampling procedure. 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



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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 issue tracking system.

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

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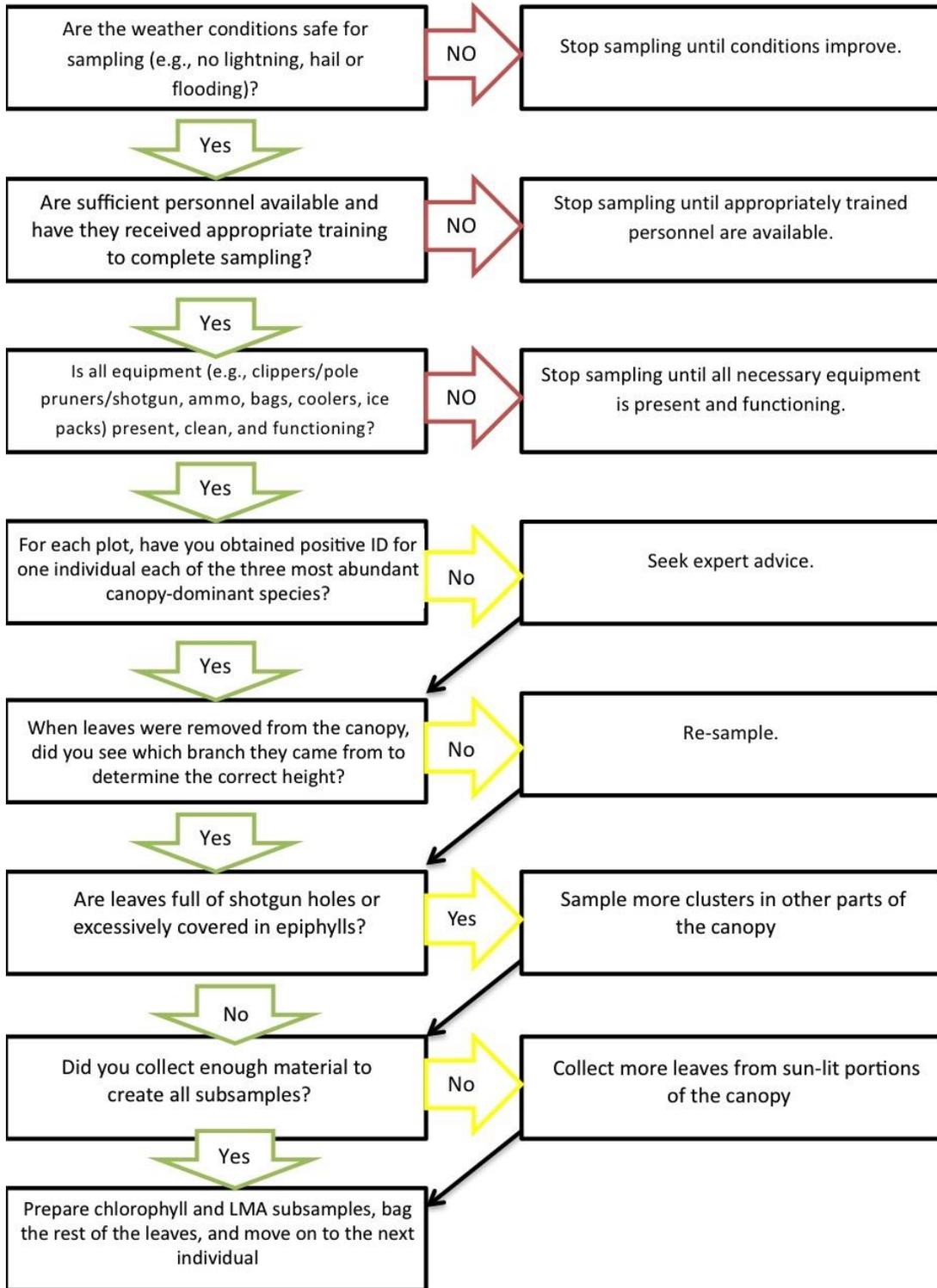


Figure 2. Decision tree to determine if canopy sampling should take place, and when sampling of a woody individual is complete.

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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 foliar sampling for chemistry and LMA 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 will be 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. Sampling will be distributed across the entire permitted area at both core and relocatable sites. The expectation is that a 5-year sampling frequency will sufficiently capture long-term trends in chemical properties of foliar tissues, and will also provide sufficient data to conduct calibrations of annual AOP hyperspectral data.

LINKED BIOGEOCHEMISTRY MEASUREMENTS

The Canopy Foliage Sampling protocol should be 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: Core Sampling for Plant Belowground Biomass (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 range 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 operations should verify site-specific peak greenness windows provided in Appendix F and contact Science if the AOP schedule falls outside these windows. At sites where there are multiple peaks in greenness (e.g., some grasslands), Science may provide additional instructions. Sample bouts should be completed within 10-15 days of 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.

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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 foliar 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.

Holding times for completion of laboratory activities and sample shipment to external laboratory facilities for the different subsample types is described in **Table 1**.

Table 1 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
Biogeochemistry Archive samples	Ship to bioarchive facility	TBD

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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 2. Contingent 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 to do so. Note weather change in datasheets and field notebook. If conditions do 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 10-15 days.	Issue problem ticket to NEON staff; potentially resume existing bout, or reschedule entire bout.	Data products likely delayed or not generated 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 reschedule outside of target sampling window.	Data products likely delayed or not generated for that bout.

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4.5 Criteria for Reallocation of Sampling Within a Site

Canopy foliage sampling for chemistry and LMA will occur on the schedule described above at a maximum of 14-20 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 (relocatable sites). 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 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.

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

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.

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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 gloves. For pressurized line launchers, hearing protection may also be required.
- 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 canopy 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 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.

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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 vegetation type-specific equipment lists.

Table 3. 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							

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Supplier	Supplier Number	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
Ben Meadows Forestry Suppliers	010510-1 14111503	R	All weather copy paper	Print back-up datasheets	All	6	N
		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

R/S = Required/Suggested.

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Table 4. 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

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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

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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.

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Table 5. 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, protect 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 <i>(responsibility of Designated Shooter)</i>	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

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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 trees	1	N
Forestry Suppliers	57522	R	Hand stamp steel die set	Append canopy-only tags with "Z"	Sampling non-tagged trees	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

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Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Ben Meadows	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/S = Required for the specified conditions/Suggested.

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

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Ben Meadows Forestry Suppliers	122731 40108 39943	R	Measuring tape, minimum 30 m	Locate clip-harvest strips	Plot slope < 20%, not brushy	1	N
Ben Meadows Forestry Suppliers	213379 37184 37036	R	Compass with mirror and declination adjustment	Locate clip-harvest strips	If using measuring tape	1	N
Ben Meadows Forestry Suppliers	100952 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							

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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
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.

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Table 7 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

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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
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.

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Table 8. 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	R	Coin envelope	Contain chemistry samples	Small-mass samples	2 per sample	N
Fisher	03-337-23C	R	Plastic scintillation vials with caps, 20 mL	Contain chemistry and archive samples	Large mass and chemistry archive samples	1-3 per sample	N
		R	Permanent marker	Label vials or envelopes	All	2	N

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Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	1.75" x 0.5" return address labels	Label scintillation vials	Large mass and chemistry archive samples	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
Resources							
RD[10]		R	Laboratory Datasheet	Back-up to record data	All	4	N

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

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Table 9. 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							

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

R/S = Required for the specified conditions/suggested

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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. 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 a given individual is capable of analyzing 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 tall or mixed-stature canopies for LMA and chemistry, or when collecting samples for genetic archive, personnel must be familiar with the plant species present at each site. Field guides and a dedicated plant expert on the domain staff must 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 (see below).

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 may be 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 must be a plant expert capable of identifying plot and site-level dominant species.

6.4 Estimated Time

The time required to implement this protocol will vary depending on a number of factors, such as skill level, ecosystem type, 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

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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 3-8 days to complete field sampling at a predominately woody site, with time largely dependent on canopy type. At predominately herbaceous sites, only 2-3 days are likely needed. An additional 2-4 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 two shorter sampling periods, with laboratory processing in between.

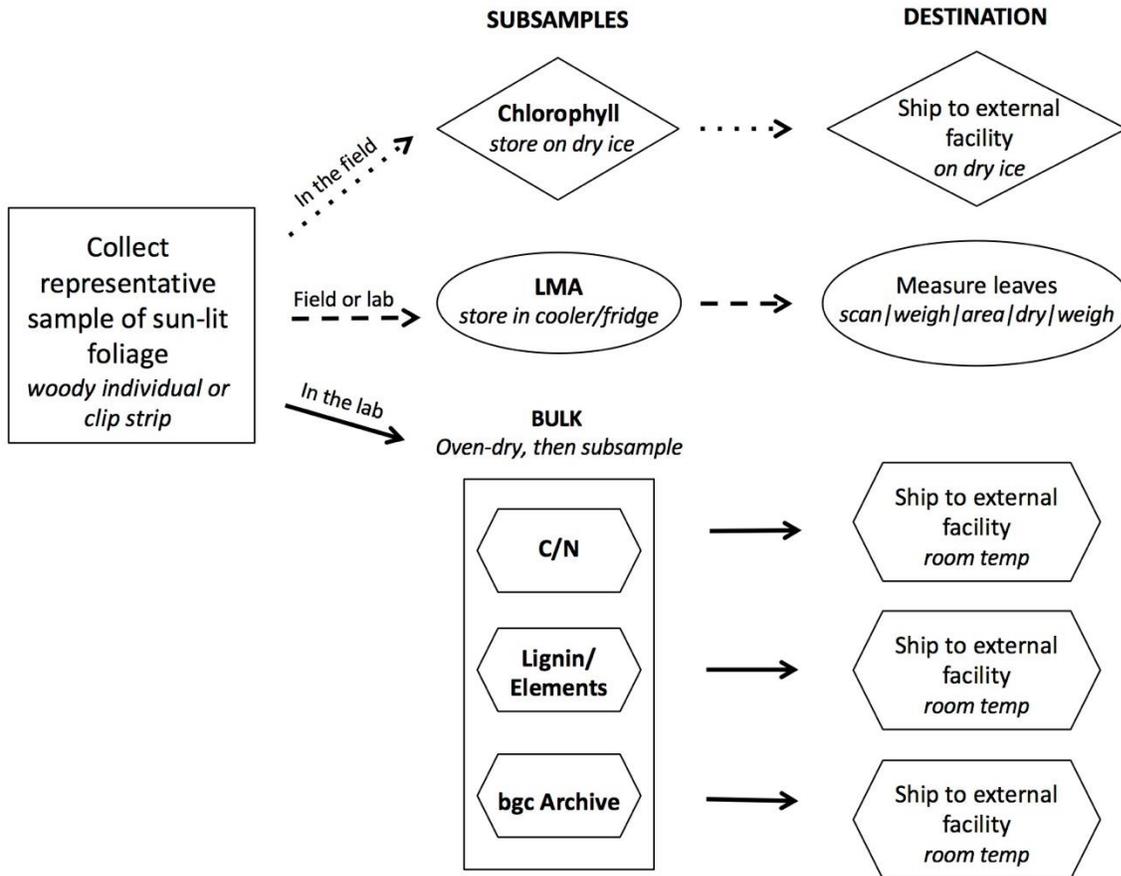
Table 10 Estimated time required to complete field and lab standard operating procedures.

SOP	Estimated total time (hours)	Suggested staff	Total person hours
A Preparing for sampling	8	2	16
B Field Sampling, closed canopy forest	2-4/plot	2 or 3	4-12/plot
B Field Sampling, open canopy and shrublands	1-2/plot	2	2-4/plot
B Field Sampling, herbaceous	1/plot	2	2/plot
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	4	1	4
G Sample Shipment	1/shipment	1	1/shipment

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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 lab in preparation for foliar chemistry and LMA sampling.
- **SOP B:** Instructions for field sample collection. Includes how to determine the type of sampling to employ (B.1), how to select individuals or areas to sample (B.2-B.4), and procedures for obtaining and preserving the samples (B.5-B.7).
- **SOP C:** Explains how to appropriately store samples post-collection and replenish field supplies for subsequent sampling bouts.

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- **SOP D:** Describes steps to 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:** Includes steps for how to oven-dry and subsample foliar samples in preparation for external laboratory chemical analyses.
- **SOP F:** Describes guidelines and requirements for successful data entry.
- **SOP G:** Contains instructions for how to package and ship samples to external laboratories for chemical analysis, including precautions for shipping dry ice.

7.2 Expected Sample Numbers

Use **Table 11** (below) and your knowledge of vegetation and plot types found across the site to estimate how many samples will be collected in total. This will assist with preparation of supplies.

Table 11. Expected sample numbers based on plot and vegetation types.

Sampling Procedure	Site Vegetation Type	Sample Type	Plot Type	# Plots	# Samples per Plot	Total # Samples
LMA & Chemistry	All plots woody (> 75% cover)	Individual	Distributed	10	3	42
			Tower	4	3	
	All plots herbaceous (> 75% cover)	Clip Strip	Distributed	16	1	20 - 24
			Tower <i>Short-stature</i>	4	1	
			Tower <i>Tall-stature</i>	4	2	
	Some/all plots mixed (> 25% of both woody and herbaceous cover)	Both Individual and Clip Strips	Distributed	10	2 - 4	28 – 60*
			Tower <i>Short-stature</i>	4	2 - 4	
			Tower <i>Tall-stature</i>	4	2 - 5	

* Sample numbers will vary based on how many woody individuals are found in each plot and the size of tower plots

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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 <ul style="list-style-type: none"> • Check battery, charge • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination¹. 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 <ul style="list-style-type: none"> • 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 adjustments²
	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.

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A.2 Determine Appropriate Methods and Supplies

1. Use vegetation height category information in Appendix D and your knowledge of the site to determine which method(s) will be appropriate to obtain sun-lit canopy foliage samples.
 - If multiple vegetation types are found at a site, multiple methods will be required.
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:



- **Practice using that device in a nearby area with relevant vegetation.** 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.

A.3 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 11 and your knowledge of site vegetation to estimate roughly how many samples will be collected, then refer to Figure 3 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.

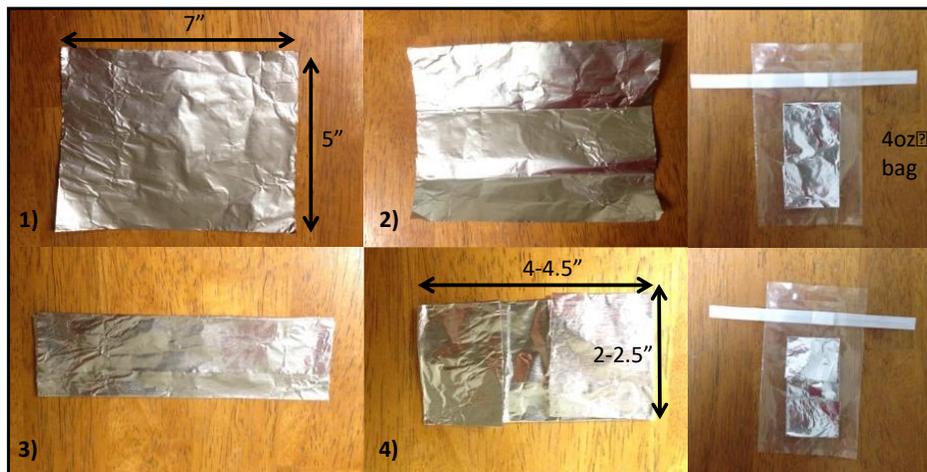


Figure 3 Steps to create foil packets: 1) cut a ~ 7" x 5" rectangle of foil, 2) mark lines to fold into thirds, 3) fold into thirds along the longer edge, 4) fold in the ends to close the packet. Keep a 4oz Whirl-pak bag nearby to make sure packets will fit.

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A.4 Woody - Use VST Data to Assess Canopy Species and Mapper Tool for Geolocations

1. SOP B describes the procedure for selecting woody individuals to sample. In general, this will be the top three canopy-dominant species per plot, with some variance allowed in order to capture all species relevant at the site level. To help prepare for sampling:
 - a. Download or examine the Apparent Individual data collected during Vegetation Structure (VST) sampling for a given site.
 - b. Filter it to a plot designated for canopy foliage sampling.
 - c. Filter again to only include individuals with **canopyPosition** = 1, 2, or 3
 - d. Sort by **taxonID** and see which species show up most frequently in the canopy. Note the top 3-5 canopy taxa for that plot using the WorkTracker in Appendix B; these will be your potential sampling targets.
 - e. Repeat for all canopy foliage sampling plots where data are available.
2. Additionally, it is critical that woody individuals sampled for canopy foliage chemistry have correct mapping data (e.g., pointID, distance, and azimuth), as errors in geolocation data will prevent use of ground-based foliar measurements for scaling and modeling with AOP data.
3. Accordingly, Field Operations should use the VST Mapper tool, accessed through the Sampling Support Library (SSL), to print stem locations and maps for each canopy foliage plot. These maps must be brought to the field and used to ensure each woody individual sampled for foliar chemistry has the correct mapping data (and if not, correct it).

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

1. As outlined in section B.2, in woody plots it is preferable to sample previously tagged individuals as they already have geolocation data. However, in some cases it may be necessary to sample non-tagged individuals.
2. In preparation for this occurrence, Field ecologists sampling in woody plots 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 will NOT be measured during normal implementation of RD[10]. To avoid confusion, “cfcOnly” tagIDs should be appended with a “Z” using the hand stamp and die set (example tagID = 09532Z).
 - b. In the VST data entry application, when **cfcOnlyTag = Y**, a woody individual can be mapped anywhere in a plot, not just the 20 x 20 m core or subplot used for VST.



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A.6 Herbaceous - Prepare to Locate Clip Strips

1. In plots where herbaceous vegetation is at least 25% of the aerial cover, 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 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, and how to locate X,Y-coordinates of clip strip SW corners depending on the ‘offsetNorthing’ coordinate for the clipID.

A.7 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 possibly pre-label) these bag packets. If pre-labeling, refer to the relevant sections in SOP B for sample identifier formats, leaving space for **taxonIDs** when planning to sample woody individuals.
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., the scanner won’t work on a curved surface), and ensure no wrinkles or folds are present.
3. Each bag packet should contain:
 - a. 1 Paper bag (or several for clip strips), to contain the bulk foliage chemistry sample
 - Example identifier, woody: cfc.GRSM001.[]-1.20160606
 - Example identifier, herbaceous: cfc.GRSM001.CLIP-1.20160606
 - Type I scan-able barcode label (1 per sample)
 - b. 1 4oz Whirl-pak bag, to contain the chlorophyll subsample
 - Example identifier, woody: cfc.GRSM001.[]-1.20160606.chl
 - Example identifier, herbaceous: cfc.GRSM001.CLIP-1.20160606.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 shut
 - c. 1 foil packet, to protect the chlorophyll subsample inside the Whirl-pak
 - d. 1 gallon plastic bag, to contain the LMA subsample (**woody plots only**)
 - Example identifier, woody: cfc.GRSM001.[]-1.20160606.lma
 - No scan-able barcode required, this is applied during execution of SOP D

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SOP B Field Sampling

This SOP for field sampling has three main components, including how to determine the type of sampling to employ (B.1), how to select individuals or areas to sample (B.2-B.4), and procedures for obtaining and preserving the samples (B.5-B.7). For selecting individuals or areas to sample, the methods employed differ depending upon the vegetation type (woody, herbaceous, mixed). For obtaining samples, the methods differ by structure (tall, mixed, and low stature). Field personnel should follow the section that describes the vegetation at the NEON site where they are sampling.



Note that for non-woody herbaceous sites, the procedure to collect samples is very similar to the one detailed in TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]) but with key high-level differences detailed below. Methods for delineating clip strips within plots are largely the same as detailed in RD[04] and Field personnel should refer to that protocol with any questions.

B.1 Determine Canopy Sampling Method for a Plot

- Using a GPS unit (if needed), navigate to a plot designated for canopy sampling. *If field personnel are quite familiar with the plot, step 2 (below) may be completed in the lab.*
- Determine appropriate canopy foliage sample method(s) based on plot vegetation cover.
 - Refer to the flow chart below (Figure 4) and the cases outlined in this SOP to determine how to execute foliage sample collection.
 - For plots with < 25% plant cover, determine the most aerially dominant cover class (woody or herbaceous), then plan to use that sampling method for the plot.
- See Appendix D for vegetation height categories and recommended methods.

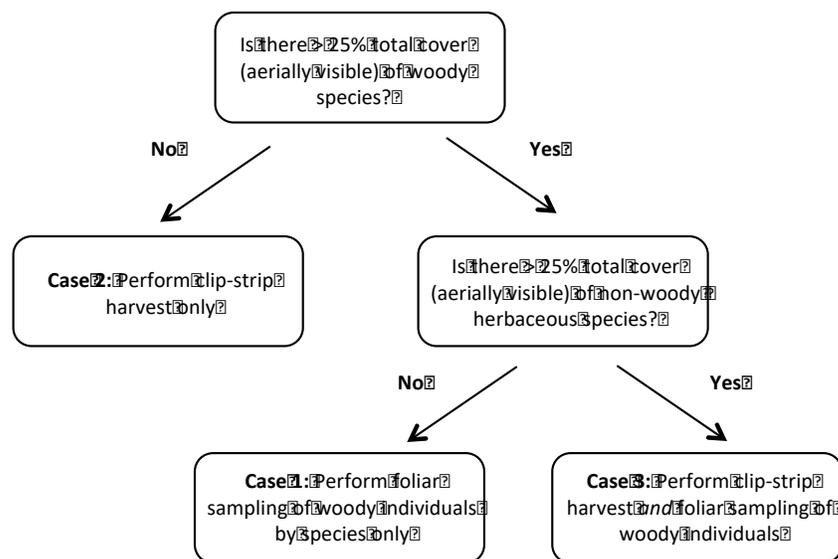


Figure 4 Flow chart to determine appropriate methods for canopy foliage sampling based on plot cover type

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B.2 Case 1: Preparing to Sample in Predominately Woody Plots

This procedure pertains to plots where woody species are dominant by percent cover, meaning they comprise the majority of aerially visible cover and herbaceous vegetation is minor (<25%) or absent.

1. Using the VST data as a guide plus an on-site ocular estimate, determine the **top three canopy-dominant species** in the plot. These are the species that appear most frequently in the uppermost part of the canopy (e.g. foliage is fully or partially sun-lit) and are thus visible aerially. Stems may or may not be ≥ 10 cm DBH.
2. Select **one** individual to sample from each of the top three canopy-dominant species.

PRIORITIES FOR CHOOSING INDIVIDUALS (IN THIS ORDER):

- a. Canopy individuals tagged for vegetation structure measurement and previously sampled for foliar chemistry and LMA.
- b. Canopy individuals tagged for vegetation structure measurement but *not* previously sampled for foliar chemistry and LMA.
- c. Canopy individuals that are not tagged and have not been sampled before.

Additionally:

- d. Choose individuals spread across the plot/subplot (i.e. not clumped). *Crowns of sampled trees should not overlap.*
 - e. 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.
 - f. **See Box 1 for additional guidelines for choosing individuals to sample.**
3. In Distributed Base Plots, sampling should occur primarily in the 20 x 20 m plot core (Figure 5), as this is where tagged individuals are located. 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).
 4. In tall-stature Tower Plots, sampling should occur primarily in the two 20m x 20m subplots randomly selected for Plant Productivity measurements (Figure 5), as this will be necessary to capture tagged individuals. 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).

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Box 1. Guidelines for Choosing Individuals in Predominately Woody Plots

Observation	Response
<i>There are less than 3 unique species in the plot</i>	If the canopy is monodominant, sample a total of 3 individuals from that species, distributed across the plot or subplot. If the canopy is composed of two species, sample each once, and then take a third sample from whichever species is more common, distributed across the plot or subplot
<i>The plot has high overstory diversity and it is unclear which 3 species are the canopy dominants</i>	Drawing on the expertise of the lead Plant Ecologist, do your best to identify the top 3 species, then sample those and move on. <i>Do not worry if the 4th or 5th most abundant species is sampled.</i> Remember, a high-level goal is to encompass the range of canopy species variation across the site in order to facilitate robust regressions with AOP data
<i>Some canopy species are being sampled with high replication while others are sampled infrequently or not at all</i>	A degree of replication is acceptable for common species, but at high diversity sites, it is also desirable to sample a larger total number of species, collecting at least one individual from all significant members of the site-level canopy. As sampling proceeds, take note of what's already been sampled using the WorkTracker in Appendix B. If choosing between two species in a high-diversity plot, one with many replicates and another with none or one, sample the latter.
<i>It is possible to collect sun-lit foliage from both old and young tagged individuals in a given plot</i>	Consider which age class is most representative of the conditions in that plot and site and sample an individual from that age class
<i>An individual from a target species is found in the plot centroid or a Plant Diversity nested subplot</i>	Try to find another individual of that species to sample in a different location in the plot. If this is not possible, sample that individual only if no damage to the centroid or subplot is anticipated. Otherwise, choose the next most abundant species in the plot to sample
<i>It is not possible (or excessively difficult) to sample a tagged individual of the target species</i>	Identify and flag a non-tagged individual and prepare to map and tag it while sampling
<i>There is not enough foliage on a given individual to sample without damaging it</i>	Try to find another individual of that species to sample. If this is not possible, choose the next most abundant species in the plot to sample
<i>Some individuals of a dominant species show signs of disease/sickness/herbivory</i>	Sample diseased/sick individuals if the disease/sickness is a dominant characteristic (> 50%) of the plot. Otherwise, try to avoid diseased individuals where possible

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Box 2. Guidelines for Choosing Individuals in Predominately Woody Plots (<i>continued</i>)	
Observation	Response
<i>The plot is sparsely vegetated (e.g., aridlands) and does not contain three woody individuals</i>	Sample as many woody individuals as are found in the plot, one or two is acceptable.
<i>Woody individuals do not have true leaves, or leaves are extremely minute (ex: Ephedra spp)</i>	Sample the plant part that is functionally analogous to a leaf (e.g., photosynthetic stem) and treat as leaf throughout.
<i>Vines or lianas (ex: Vitus spp) are a dominant component of aerially visible canopy cover in the plot</i>	It is acceptable to select and 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 <i>and</i> mapped to provide geolocation data for the sample. Note the situation in the remarks field.

5. Place flagging tape around the stems of individuals chosen for sampling. Flagging should be removed once the bout is complete.
6. With a permanent marker, write the **sampleID** on the flagging tape. This will consist of the module code (cfc), **plotID** (siteIDXXX), **taxonID**, - **sampleNumber** for that plot, and the **collectDate**
 - Example identifier: **cfc.GRSM001.FAGR-1.20160705**



7. The actual method of obtaining sun-lit foliage will vary based on the height of the woody vegetation being sampled – see Appendix D for vegetation height categories in NEON sites.
 - For woody individuals < 2 m tall, hand clippers should be used to collect leaves.
 - For woody individuals 2-7 m tall, an extendable pole trimmer should be used.
 - For woody individuals > 7 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.

B.3 Preparing to Sample in Predominately Herbaceous Plots (Case 2)

This procedure pertains to plots where herbaceous plants are dominant by percent cover/biomass and woody plant cover is minor (<25%) or absent. In this case, the process for delineation of clip strips is the same as that detailed in TOS Protocol and Procedure: Measurement of Herbaceous Biomass (RD[04]).

1. One clip strip will be harvested per Distributed basePlot or ‘Short-stature’ Tower basePlot, and it will come from the non-destructive plot core. If sampling a ‘Tall Stature’ Tower Plot, two clip strips will be collected, one from each of the two randomly selected subplots used for Plant Productivity measurements (Figure 5).

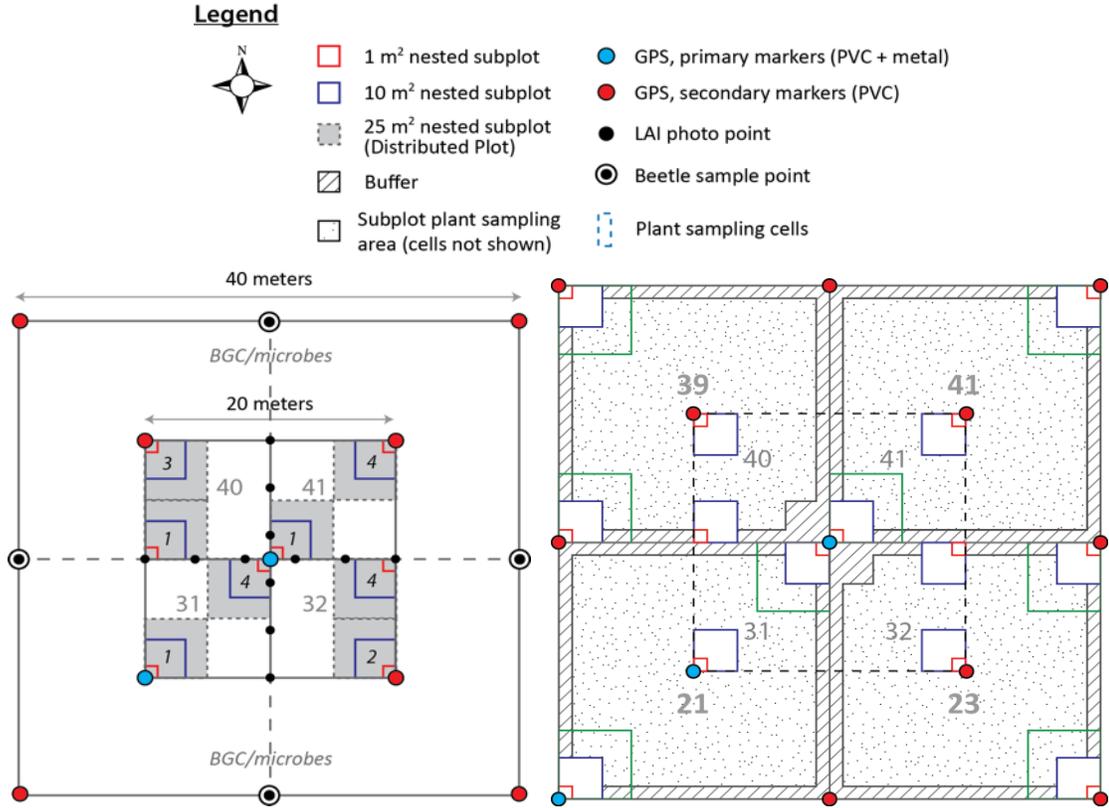


Figure 5 Left: Plot layout for Distributed basePlots and short-stature Tower basePlots used for canopy foliage sampling. Right: Plot layout of tall-stature Tower basePlots use for canopy foliage sampling

2. Select the first potential clip harvest location using the plot-specific clip list.
3. 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 (Appendix E, Figure 10)

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- 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 Appendix E, Table 17), 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.
4. 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.

B.4 Preparing to Sample in Mixed Woody and Herbaceous Plots (Case 3)

This procedure pertains to plots where woody and herbaceous species are co-dominant (by percent cover/biomass), e.g., each type comprises > 25% of the total aerially visible cover. For these plots, the procedures outlined above in sections B.2 and B.3 will both be followed, but with a targeted, non-random procedure for selecting herbaceous clip strip locations (detailed below).

1. Follow the steps outlined in Section B.2 to select and mark one individual each from the **top three canopy-dominant species** in the plot/subplot. Choose individuals according to the criteria outlined above. If there are less than three individuals in the plot/subplot, it is acceptable to only sample one or two.
2. When herbaceous % aerial cover is < 75% AND > 25% of the plot (i.e. mixed cover):
 - a. One clip strip will be harvested per Distributed basePlot or ‘Short-stature’ Tower basePlot, and it will come from the non-destructive plot core. If sampling a ‘Tall Stature’ Tower Plot, two clip strips will be collected, one from each of the two randomly selected subplots used for Plant Productivity measurements (Figure 5).
 - b. Select clip harvest location(s) using the following targeted, non-random procedure:
 - 1) Assign a number to each continuous “patch” of herbaceous vegetation in the plot/subplot.

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- 2) Randomly select a patch to sample using a coin flip or random number list.
- 3) Find the approximate center of the patch, then use a map of the clip cells (Appendix E, E.2) to select a clip strip that is close to the patch center.
- 4) Assess suitability using criteria described above in B.3. Continue assessing possible clip strips near the patch center until an acceptable one is found.
- 5) Record which strip is chosen for canopy foliage sampling on the clip list.

B.5 Obtaining the Samples in Tall-stature Woody Vegetation

This procedure should be followed when canopy samples are collected from locations well out of human reach. Skip to B.6 for short-stature woody vegetation and B.7 for herbaceous vegetation.



1. 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. 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.
3. While the marksman, arborist, or others are preparing to obtain foliage from the outer, sun-lit portion of the canopy, record sample metadata, including:
 - **plotID** (SITEXXX), **subplotID**, **sampleNumber** (unique to each plot), **collectDate**, **tagID** (if tagged), and **taxonID** (USDA plant species code)
4. 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. This pair of gloves can be worn for the entire plot.

HOW LONG TO SPEND ON ONE TREE?

Ideally, Field personnel should not spend more than 45 minutes attempting to sample a single tree. If this time is exceeded due to difficulty accessing the sun-lit canopy, it is acceptable to reject that tree and choose another, according to the criteria outlined in section B.2

5. 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:

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- **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)

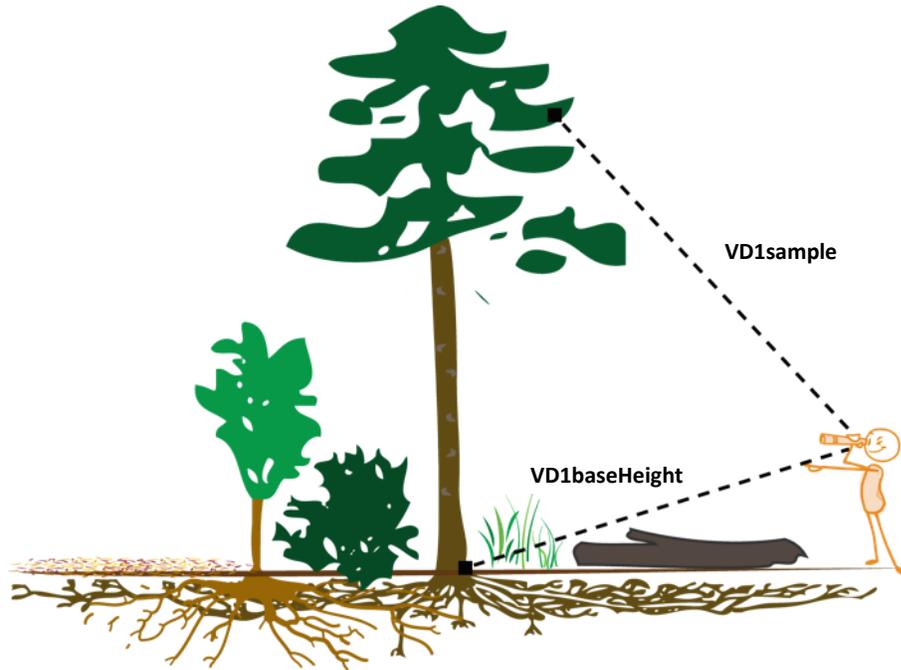


Figure 6 Measuring sample height using a laser rangefinder

6. 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.
7. Select a subset of leaves/needles in good condition (**whole, healthy, green**) and set them aside to generate subsamples for chlorophyll and LMA measurements.

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- How much? Think about what’s needed to fill an 8 x 11” scanner with no overlap between leaves - roughly 2-4 large leaves, 10-15 medium leaves, and 20-30 small leaves or needles, plus some extra for the chlorophyll subsample.
8. 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).
 9. 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.
 10. Package and stow (sub)samples:
 - a. **Chlorophyll subsample: use ~25% of good-condition (green), set-aside leaves**
 - 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 punches. This will help tissue freeze effectively.
 - 5) Place foil packet into a 4oz Whirl-pak bag.

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- 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), **collectDate**, and the suffix “.chl”
 - Example identifier: **cfc.GRSM001.FAGR-1.20160705.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. LMA subsample: use remainder of good-condition, set-aside leaves.

- 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 7); woody material may or may not be removed prior to bagging the sample.

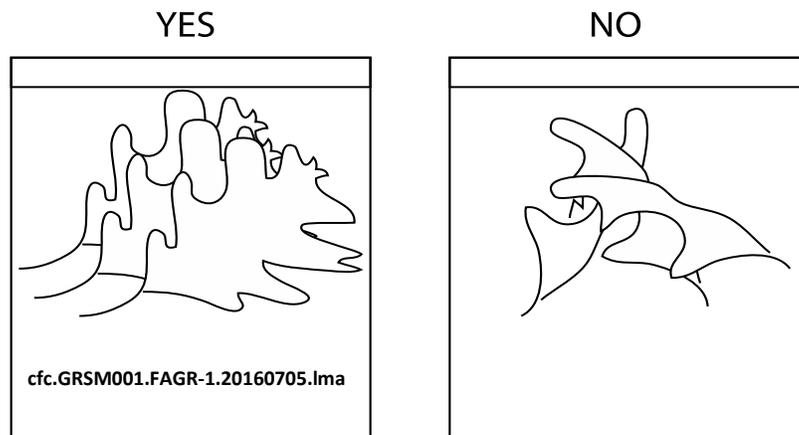


Figure 7. Example of how to package and label a broadleaf LMA subsample.

- 3) If bag was not pre-labeled, label with **lmaSampleID**. Use the same convention as described above, but with “.lma” for the suffix.
 - Example identifier: **cfc.GRSM001.FAGR-1.20160705.lma**
- 4) Store LMA bag in cooler on ice packs, ensuring not to bend or crush foliage.

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c. **Bulk chemistry sample**

- 1) Place remaining foliage into a kraft paper bag and close.
- 2) If bag was not pre-labeled (or the label was incomplete), label with **sampleID**. Use the same convention as described above but with no suffix, since this is considered the bulk sample.
 - Example identifier: **cfc.GRSM001.FAGR-1.20160705**
- 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.

11. Enter other required metadata into the data entry application or Field Datasheet.

- **plantStatus** is a code for assessing the health of a woody individual. Use codes in **Table 13**, similar to those used in TOS Protocol and Procedure: Measurement of Vegetation Structure (RD[10]), to indicate health of the individual.

Table 13. Tree or shrub status codes and their definitions.

Code	Description
1	Live – any live Individual that is of typical healthy status for the ecosystem in question; that is, if trace amounts of insect damage to foliage are typical on the majority of individuals, use this code rather than codes below.
4	Live, insect damaged – note ‘crown’ or ‘bole’ damage in remarks , and indicate type of insect causing damage if possible (e.g., Mountain Pine Beetle, Gypsy Moth, etc.)
5	Live, disease damaged – note ‘crown’ or ‘bole’ damage in remarks , and indicate type of disease causing damage if possible (e.g., Blister Rust, rot, canker, other (specify), unknown).
6	Live, 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)
7	Live, other damage – note ‘crown’ or ‘bole’ damage in remarks , and note cause if possible.

12. If the sample was collected from a non-tagged individual, record **vstTag** = ‘no’ in the data entry application or Field Datasheet and prepare to map and tag it, as specified in SOP B of the Vegetation Structure protocol (RD[10]). If using a mobile device, record values with the ‘Mapping and Tagging’ data entry application. If not, use the Canopy Foliage Field Datasheet.



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Briefly:

- a. Attach a pre-numbered aluminum tag, appended with a “Z” using the dicast set (example = 09147Z), and record **tagID**. Including the “Z” will mark these as canopy-only trees, which are not sampled in the Vegetation Structure protocol (RD[10]).
 - b. Record the **pointID** where the laser rangefinder is positioned. **Only pointIDs that are GPS measured and monumented are acceptable for mapping and tagging.**
 - 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.
 - o Remember, these stems are NOT measured in the Vegetation Structure protocol
13. Repeat steps 3 – 12 for all stems that have been marked for sampling in the plot.
 14. Remove gloves and stow in a dedicated trash bag.
 15. Collect shotgun shells and wadding or any other detritus and remove from plot. Do not contaminate gloved hands while collecting shells and wadding.
 16. Repeat until all designated Distributed Base and Tower plots have been sampled.
 - Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the plot dry ice cooler, the active ‘flash-freezer,’ to a second dry ice cooler for storage. Having one cooler maintained empty of samples will yield better flash-freezing results as new subsamples will have good contact with dry ice.

B.6 Obtaining the Samples in Short-stature Woody Vegetation (Shrubs and Small Trees)

1. 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. Put on a pair of Nitrile (Latex-free) gloves. You can wear 1 pair of gloves for an entire plot.
3. Use a pole trimmer, clippers, and/or gloved hands to obtain sun-lit leaf clusters.
4. Use a meter tape/stick OR the laser rangefinder (if samples are out of reach) to measure the height(s) at which foliage was taken. Record in data entry application or field datasheet.

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- a. **VD(#)sample:**
 - Meter tape/stick: This total height above the ground of the sampling location.
 - Laser Rangefinder: The vertical distance between the sampling location and the rangefinder (see B.5, step 4).
- b. **VD(#)baseHeight:**
 - Meter tape/stick: Enter '0'
 - Laser Rangerfinder: The vertical distance between the rangefinder and the base of the individual (typically a negative number, see B.5, step 4).
- 5. Follow steps 7-16 in section B.5 above to complete sampling in the plot, then move to the next plot until all designated Distributed Base and Tower plots have been sampled

B.7 Obtaining the Samples in Herbaceous Vegetation

1. 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. Navigate to the clip strip that was identified for canopy sampling and 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.
 - d. The two sets of marks on the two string-and-stake sets now clearly delineate a designated area for clip-harvesting.
 - e. 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.*

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3. Enter required metadata into data entry application or field datasheet: **plotID, subplotID, collectDate, sampleType, clipID, clipDimensions**
4. Visually estimate and record percent foliage cover of the clip strip as **percentCoverClip**. Don't spend more than 1 minute making this visual estimate.
5. Put on a clean pair of nitrile gloves and use clippers to harvest all herbaceous aboveground biomass *rooted* within the clip strip.



6. Key items to remember:
 - **DO NOT** sort biomass into functional groups.
 - **DO NOT** remove old standing dead (OSD) material from the sample.
 - **DO NOT** clip herbaceous vegetation that passes through/leans over into the clip strip but is not rooted in the strip (this includes non-woody vines).
 - **DO** clip all herbaceous plants rooted within the strip > 1-2 cm in height. That is, include leaves in the harvest that exit the strip but originate from stems rooted in the strip.
 - **DO** clip leaves of woody stemmed plants with ddh < 1cm that are produced in the current year AND originate from nodes that fall within the clip strip. It is not necessary that the individuals are rooted in the clip strip as long as the most recent node from which the current year growth originates falls within the strip.
 - **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 9 below, using representative foliage), then continue clipping the rest of the strip. This will help limit chlorophyll degradation.
 - If *Toxicodendron* is present and will be sampled, 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.
7. Place clipped biomass into kraft paper bags.
8. If not pre-labeled, label bag(s) with **sampleID** as follows: module code (cfc), **plotID** (siteIDXXX), 'CLIP', - **sampleNumber** (for that plot), and **collectDate**
 - Example identifier: **cfc.WOOD001.CLIP-1.20160705**
 - Also write the **clipID** and **bagCount** on the paper bag
9. 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. Not much foliar material is needed, one handful will suffice.

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- 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.

10. Create the chlorophyll subsample:

- 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.



- Use a spring scale to achieve the target mass for the first couple of subsamples, but after this initial ‘calibration,’ it is ok to estimate sample quantity by eye.
- 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.
 - Place foil envelope into a 4oz Whirl-pak bag.
 - 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-1.20160705.chl**
 - If using a data entry application, scan the chlorophyll sample barcode – it should appear in the field **chlorophyllSampleCode**.
 - 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.*

- 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.
- 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.
- 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.

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- Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the plot dry ice cooler, 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.

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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.
 - a. If you are working remotely, keep samples on fresh cold packs (change every 12 hr)
3. Store Whirl-pak bags containing frozen chlorophyll subsamples in a -80 °C ultra-low temperature freezer until they are shipped to the designated analytical facility.
 - 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
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.
4. Recharge batteries for the GPS unit (if necessary).
5. Recharge or replace batteries for the Laser Rangefinder (if applicable).

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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 IT Intranet Page > HelpDesk > Domain Facility Users > Hot Topics and download the ‘CFC Scanning Instructions’ document. Use this to set up the scanner and create a scanning template. 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 scanning template is ready to go. 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 sites) 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.

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- Medium-small leaves: 6-12, depending on size and what fits on the scanner. Arrange neatly, include petioles/rachis.
- Needles: 20-50, depending on size and what fits on the scanner. Arrange neatly.
- Herbaceous samples: as many live, green leaves/blades as can comfortably fit on the scanner. Try to ensure a representative sample. If needed, can use clear tape to secure foliage to screen. It is ok to cut long blades of grass if needed.



5. 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 8).
 - c. Choose representative leaves in good condition - without holes where possible, mostly green with little dead or damaged parts (especially relevant in grasslands).

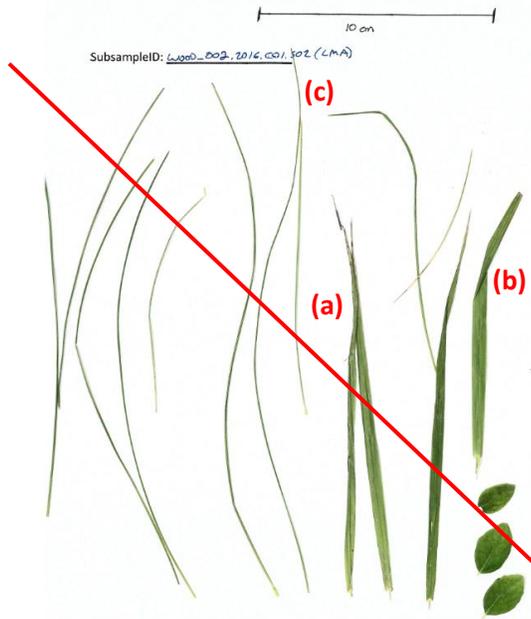


Figure 8 Example of leaf arrangements to avoid when scanning for LMA, including overlapping foliage (a), bent foliage (b), and foliage covering the text (c).

6. Near the top of the scale bar template, record the **ImaSampleID**
 - 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 ImaSampleID field with clear tape, then use a dry erase marker to record each identifier and erase in between samples.
7. 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.



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8. Scan the sample (**Error! Reference source not found.**, left) and 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 9 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.*



9. Upload scan as .tif or .jpeg file and save to a folder on the local computer.
10. Copy the image file over to the Dropbox folder on the Domain Support Facility N-drive to have as a back-up. Image files will sync overnight and then be accessible the following day on the Citrix FOPS Desktop. *It they are not, contact the IT Helpdesk for assistance.*
11. Immediately after scanning, clean the scanner glass with a tissue to remove dust/resin, then measure the weight of the fresh material scanned and enter it into the date entry application or lab datasheet. **Be careful not lose any material; it needs to be dried and weighed again.**

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- a. Tare a plastic weigh boat.
 - b. Transfer sample into weigh boat and note weight to the nearest 0.001g.
 - c. Record **freshMass** (mass in grams of fresh scanned material) and **scanDate** (date of LMA scanning)
12. Place all scanned leaf/needle material into a coin envelope (or paper bag if it is a large sample), *being careful not to lose any*. Label it with **ImaSampleID** (e.g., sampleID + “.Ima”).
- Example identifier: **cfc.GRSM001.FAGR-1.20160705.Ima**
13. 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 **ImaSampleCode**
14. Place coin envelopes or paper bags 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

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) and draw a line that measures 10 cm by tracing the scanned scale bar.
 - b. 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) If you check the global scale box this will keep your scale settings for all of the images you process later on while the software is open.
 - 6) Click **OK**.

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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 artificially fill them in
7. If this produces 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 9, 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

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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 set the size (mm²) to '10-infinity' and this will eliminate any smaller particles (noise) from the area calculation.
 - a. If you have real holes that are contained by black areas in your image, make sure that "include holes" is NOT checked.
 - b. If there are simply lighter-colored areas that look like holes, do check that option.
 - c. Make sure that 'Display Results' is checked and that the 'Show dialog' box displays Masks. Hit **OK**.
 11. A table will appear that gives you the individual area of each leaf/needle. Additionally, a new image 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, save the area table as an excel or .csv file ('File → 'Save As') in same location as the scanned image file. Use **ImaSampleID** for the file name, add "_scan01", "_scan02" if multiple scans were taken for one subsample.
 - b. If the mask is not accurate, close the image without saving and conduct area calculations again by adjusting minimum, contrast, and threshold as 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.
 14. In the data entry application or lab datasheet, record:

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- **scannedLeafNumber**: total number of individual leaves/needles/blades scanned
- **leafArea**: sum total area in mm² of all leaves/needles scanned
- **percentGreen**: percent of scanned foliar material that was live and green. A visual approximation, do not spend more than one 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. Once it has been verified that data entry has taken place, 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 and note weight to the nearest 0.001g.
 - c. Make sure all material is removed from the coin envelope or bag, use tweezers if needed.
 - d. Record **dryMass** (the dry weight of a sample in grams)
3. 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.
4. Store LMA coin envelopes or paper bags in a cool, dry cabinet for 6 months. Once it has been verified that data entry has taken place, foliage may be disposed of.

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.

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- Use the same punch size for all leaves in the same sample.
2. Record the weight of the fresh material punched. Be careful not to lose any punches; they need to be dried and weighed again.
 - a. Tare plastic or aluminum weigh boat.
 - b. Transfer punches into weigh boat and note weight to the nearest 0.001g.
 - c. Record **freshMass** (mass in grams of fresh punched material)
 3. In “remarks” field, note ‘LMA punch method’
 - a. If using paper datasheets, also record punch diameter (0.5, 0.75, 1.5”) and punch number in the ‘remarks’ field, so these can be entered into the data entry application at a later date
 4. Enter punch diameter and number into the data entry application and it will calculate **leafArea**, e.g., the total area of all punches in mm²
 5. Place punches into a coin envelope. Label it with **lmaSampleID** and affix a Type I adhesive barcode label as described above.
 6. 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).
 7. Save remaining fresh sample in refrigerator for up to one week in case there is a problem.
 8. Remove subsamples from the oven once they have been dried at 65°C for 48h. Record **ovenEndDate**.
 9. Immediately upon removing from oven, weigh punches to nearest 0.001g.
 - a. Tare plastic or aluminum weigh boat.
 - b. Transfer punches into weigh boat and note weight to the nearest 0.001g.
 - c. Make sure all material is removed from the coin envelope or bag, use tweezers if needed.
 - a. Record **dryMass** (dry weight of the punches in grams)
 10. 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.
 11. Store LMA coin envelopes in a cool, dry cabinet for 6 months. Once it has been verified that data entry has taken place, foliage may be disposed of.

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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 these 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 kraft 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 < 10 g dry mass or > 10 g dry mass. This will allow similar size samples to be processed together for efficiency. When sample dry mass is < 10 g, follow steps outlined in E.2 below to prepare the chemistry subsamples. If the dry mass is > 10 g, follow steps outlined in E.3 instead.

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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.
 - a. If dry sample mass is **6 to 10 grams**, split foliar material equally into three portions.
 - b. If dry sample mass is **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)
 - c. If dry sample mass is **< 2.7 grams**, prioritize getting adequate lignin/elements and C/N subsamples and do not create a chemistry archive subsample (Table 14).
2. Regardless of mass, make sure to:
 - Remove any remaining twigs and other non-foliage woody parts.
 - Process, stow and label each subsample as instructed below, including the directive to grind the chemistry archive sample.

Table 14 Guidelines for chemistry subsampling with low mass (< 10 g dry)

dryMass (g)	Samples to create (g)		
	C:N	Lignin	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. 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 need to grind
 - Identifier = **cnSampleID** (sampleID + .cn) or **ligninSampleID** (sampleID + .lig)
 - Example CN: cfc.GRSM001.FAGR-1.20160705.cn
 - Example lignin: cfc.GRSM001.FAGR-1.20160705.lig

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- Write identifiers neatly on envelopes or paper bags using permanent marker, or use return 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.*
- 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)**
 - 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.
 - Identifier = **archiveSampleID** (sampleID + .ar)
 - Example: cfc.GRSM001.FAGR-1.20160705.ar
 - Use a return 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.
 - 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**.
- 4. 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.
- 5. 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)
 - If dryMass is < **20 g**, grind the entire sample.
 - If dryMass is > **20 g**, haphazardly subsample ~ 20 g, then grind it. Attempt to select as representative a subsample as possible with respect to leaf particle sizes.

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- Ensure no twigs or other woody material is included in the ground (sub)sample.
3. Use an appropriately sized splitter or microsplits 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.
5. Label each subsample as outlined below using return 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.

- **Lignin/elements**

- Identifier = **ligninSampleID** (sampleID + .lig)
- Example: cfc.WOOD001.CLIP-1.20160705.lig
- Barcode should appear in **ligninSampleBarcode** field

- **C/N**

- Identifier = **cnSampleID** (sampleID + .cn)
- Example: cfc.WOOD001.CLIP-1.20160705.cn
- Barcode should appear in **cnSampleCode** field

- **Chemistry archive**

- Identifier = **archiveSampleID** (sampleID + .ar)
- Example: cfc.WOOD001.CLIP-1.20160705.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.

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- 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.

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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).

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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.

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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 airtight). 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.

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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.

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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°C) 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 analytes are far more stable. Field Personnel should plan to ship samples within the holding times specified in Table 1.

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](#)

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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.

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APPENDIX B QUICK REFERENCES

OBTAINING CANOPY SAMPLES FOR LMA AND CHEMISTRY

STEP 1 – Determine method(s) that will be used to sample the canopy.

STEP 2 – In woody plots, identify and mark the trees/shrubs that will be sampled – one from each of the three most abundant canopy dominant species at the plot scale

STEP 3 – In herbaceous plots, identify a clip strip using the clip list.

* If sampling mixed plots, do STEPS 2 and 3*

STEP 4 – In woody plots, obtain sun-lit canopy leaves from each individual. Measure and record the height(s) where samples came from.

STEP 5 – In herbaceous plots, cut all vegetation in the clip strip.

STEP 6 – Set aside a small, representative subsample for chlorophyll analysis and flash-freeze it immediately using dry ice. In woody sites, set aside an additional subsample of in-tact, whole leaves for LMA analysis. Store all non-chlorophyll foliar material in a chilled cooler.

STEP 7 –Ensure all data have been recorded on paper datasheets and/or in data entry applications. If using scan-able barcode labels, ensure all have been scanned 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 9 – Make LMA measurements, recording all variables:

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

STEP 10 – 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 11 –If using scan-able barcode labels, ensure all have been scanned to the correct sample ID’s. Ship all subsamples to appropriate facilities.

OBTAINING FOLIAR TISSUE FOR ARCHIVE GENETIC MATERIAL

STEP 12 – If tissue for genetic archive was not collected along with canopy foliage sampling for chemistry and LMA, collect the requisite number of samples from Phase I Phenology species.

STEP 13 – Dry samples with desiccant and ship to the archive facility along with vouchers.

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APPENDIX C REMINDERS

Pre-sampling: Check...

- If using an outside contractor, has their availability been confirmed?
- Does sampling schedule overlap with the first AOP overflight?
- Is all required equipment available?
- 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 appropriate individuals to sample based on plot-level abundance (woody).
- Ensure the location of the clip strip is suitable (herbaceous).

While sampling: Remember to...

- Only collect sun-lit leaves.
- Ensure majority of leaves are healthy 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)

Sample handling in the field: Be sure to...

- Set aside a representative subsample for chlorophyll analysis; flash-freeze it immediately.
- In woody sites, also set aside an LMA subsample.
- Keep the non-chlorophyll foliar material cool but not frozen.
- Change gloves between plots.

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? Did all include a scale bar?
- For larger leaves, were midveins included but petioles removed?
- Was scannedLeafNumber and leafArea recorded?

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- 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?
- Were they of sufficient quantity?

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.FAGR-1.20160605

Subsample Identifiers

chlorophyllSampleID: cfc.GRSM001.FAGR-1.20160605.chl

lmaSampleID: cfc.GRSM001.FAGR-1.20160605.lma

cnSampleID: cfc.GRSM001.FAGR-1.20160605.cn

ligninSampleID: cfc.GRSM001.FAGR-1.20160605.lig

bgcArchiveID: cfc.GRSM001.FAGR-1.20160605.ar

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APPENDIX D VEGETATION HEIGHT CATEGORY TO DETERMINE SAMPLING METHOD BY SITE

Table 16. List of NEON sites, vegetation types found there, and their height categories to determine canopy sampling method. 1 = 0-2 m, manual clip; 2 = 2-7 m, pole pruner; 3 = > 7 m, shotgun/tree climbers/slingshot/other

Domain	Site Name	Vegetation	Height Category
D01	BART	deciduousForest	3
D01	BART	evergreenForest	3
D01	BART	mixedForest	3
D01	HARV	deciduousForest	3
D01	HARV	evergreenForest	3
D01	HARV	mixedForest	3
D01	HARV	woodyWetlands	2,3
D02	BLAN	deciduousForest	3
D02	BLAN	pastureHay	1
D02	SCBI	deciduousForest	3
D02	SCBI	pastureHay	1
D02	SERC	cultivatedCrops	1,2
D02	SERC	deciduousForest	3
D03	DSNY	pastureHay	1
D03	DSNY	woodyWetlands	2,3
D03	JERC	cultivatedCrops	1,2
D03	JERC	deciduousForest	3
D03	JERC	evergreenForest	3
D03	JERC	mixedForest	3
D03	OSBS	emergentHerbaceousWetlands	1
D03	OSBS	evergreenForest	3
D03	OSBS	woodyWetlands	2,3
D04	GUAN	evergreenForest	3
D04	LAJA	cultivatedCrops	1,2
D04	LAJA	evergreenForest	3
D04	LAJA	grasslandHerbaceous	1
D04	LAJA	pastureHay	1
D05	STEI	deciduousForest	3
D05	STEI	mixedForest	3

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Domain	Site Name	Vegetation	Height Category
D05	STEI	woodyWetlands	2,3
D05	TREE	deciduousForest	3
D05	TREE	evergreenForest	3
D05	TREE	mixedForest	3
D05	TREE	woodyWetlands	2,3
D05	UNDE	deciduousForest	3
D05	UNDE	mixedForest	3
D05	UNDE	woodyWetlands	2,3
D06	KONA	cultivatedCrops	1,2
D06	KONZ	deciduousForest	3
D06	KONZ	grasslandHerbaceous	1
D06	UKFS	deciduousForest	3
D06	UKFS	grasslandHerbaceous	1
D07	GRSM	deciduousForest	3
D07	GRSM	evergreenForest	3
D07	MLBS	deciduousForest	3
D07	ORNL	deciduousForest	3
D07	ORNL	evergreenForest	3
D07	ORNL	pastureHay	1
D08	DELA	evergreenForest	3
D08	DELA	woodyWetlands	2,3
D08	LENO	deciduousForest	3
D08	LENO	woodyWetlands	2,3
D08	TALL	deciduousForest	3
D08	TALL	evergreenForest	3
D08	TALL	mixedForest	3
D09	DCFS	grasslandHerbaceous	1
D09	NOGP	emergentHerbaceousWetlands	1
D09	NOGP	grasslandHerbaceous	1
D09	WOOD	emergentHerbaceousWetlands	1
D09	WOOD	grasslandHerbaceous	1
D10	CPER	grasslandHerbaceous	1

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Domain	Site Name	Vegetation	Height Category
D10	RMNP	evergreenForest	2,3
D10	STER	cultivatedCrops	1,2
D11	CLBJ	deciduousForest	2,3
D11	CLBJ	grasslandHerbaceous	1
D11	OAES	grasslandHerbaceous	1
D11	OAES	shrubScrub	2
D12	YELL	evergreenForest	3
D12	YELL	grasslandHerbaceous	1
D12	YELL	shrubScrub	2
D13	MOAB	evergreenForest	2,3
D13	MOAB	shrubScrub	1,2
D13	NIWO	evergreenForest	2,3
D13	NIWO	grasslandHerbaceous	1
D14	JORN	shrubScrub	2
D14	SRER	shrubScrub	2
D15	ONAQ	evergreenForest	2,3
D15	ONAQ	shrubScrub	2
D16	ABBY	evergreenForest	3
D16	ABBY	grasslandHerbaceous	1
D16	ABBY	mixedForest	2,3
D16	ABBY	shrubScrub	2
D16	WREF	evergreenForest	3
D17	SJER	evergreenForest	2,3
D17	SJER	grasslandHerbaceous	1
D17	SJER	shrubScrub	2
D17	SOAP	evergreenForest	3
D17	SOAP	shrubScrub	2
D17	TEAK	evergreenForest	3
D17	TEAK	shrubScrub	2
D18	BARR	emergentHerbaceousWetlands	1
D18	BARR	sedgeHerbaceous	1
D18	TOOL	dwarfScrub	1

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Domain	Site Name	Vegetation	Height Category
D18	TOOL	sedgeHerbaceous	1
D18	TOOL	shrubScrub	2
D19	BONA	deciduousForest	2,3
D19	BONA	evergreenForest	2,3
D19	BONA	mixedForest	2,3
D19	BONA	shrubScrub	2
D19	BONA	woodyWetlands	2,3
D19	DEJU	evergreenForest	3
D19	DEJU	shrubScrub	2
D19	DEJU	woodyWetlands	2,3
D19	HEAL	dwarfScrub	1
D19	HEAL	evergreenForest	3
D19	HEAL	shrubScrub	2
D20	PUUM	evergreenForest	2,3
D20	PUUM	shrubScrub	2

APPENDIX E RESOURCES FOR CLIP STRIP HARVESTING

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

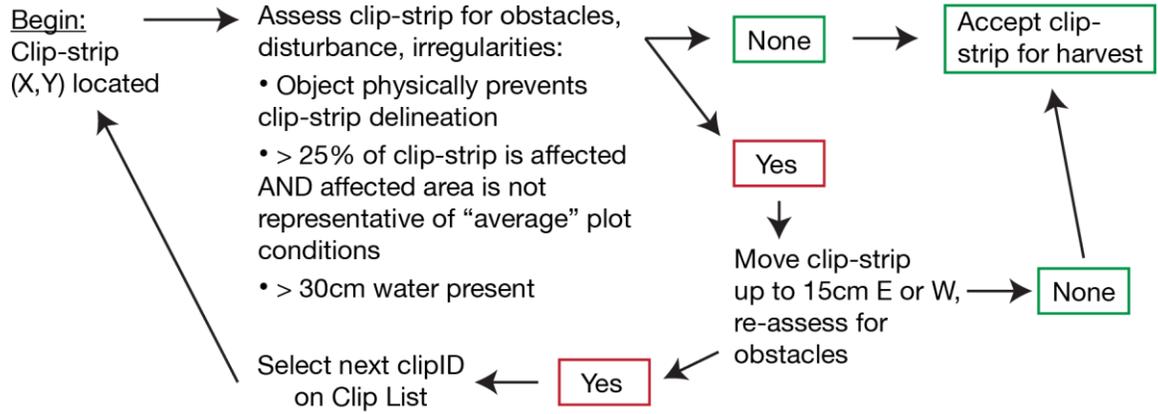


Figure 10. Flow chart to guide assessing potential clip cells for clip-harvest suitability.

Table 17. 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 enclosure
2	Accepted, enclosure
3	Rejected temporarily, inundated
4	Rejected temporarily, uncommon plant
5	Co-located belowground biomass core sampling

E.2 clipCellNumber Maps by subplotID

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 $\geq 25\%$ 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 18** to find the easting and northing values associated with that clip strip so it can be delineated at a known location relative to the SW corner of the 20m x 20m plot or subplot.

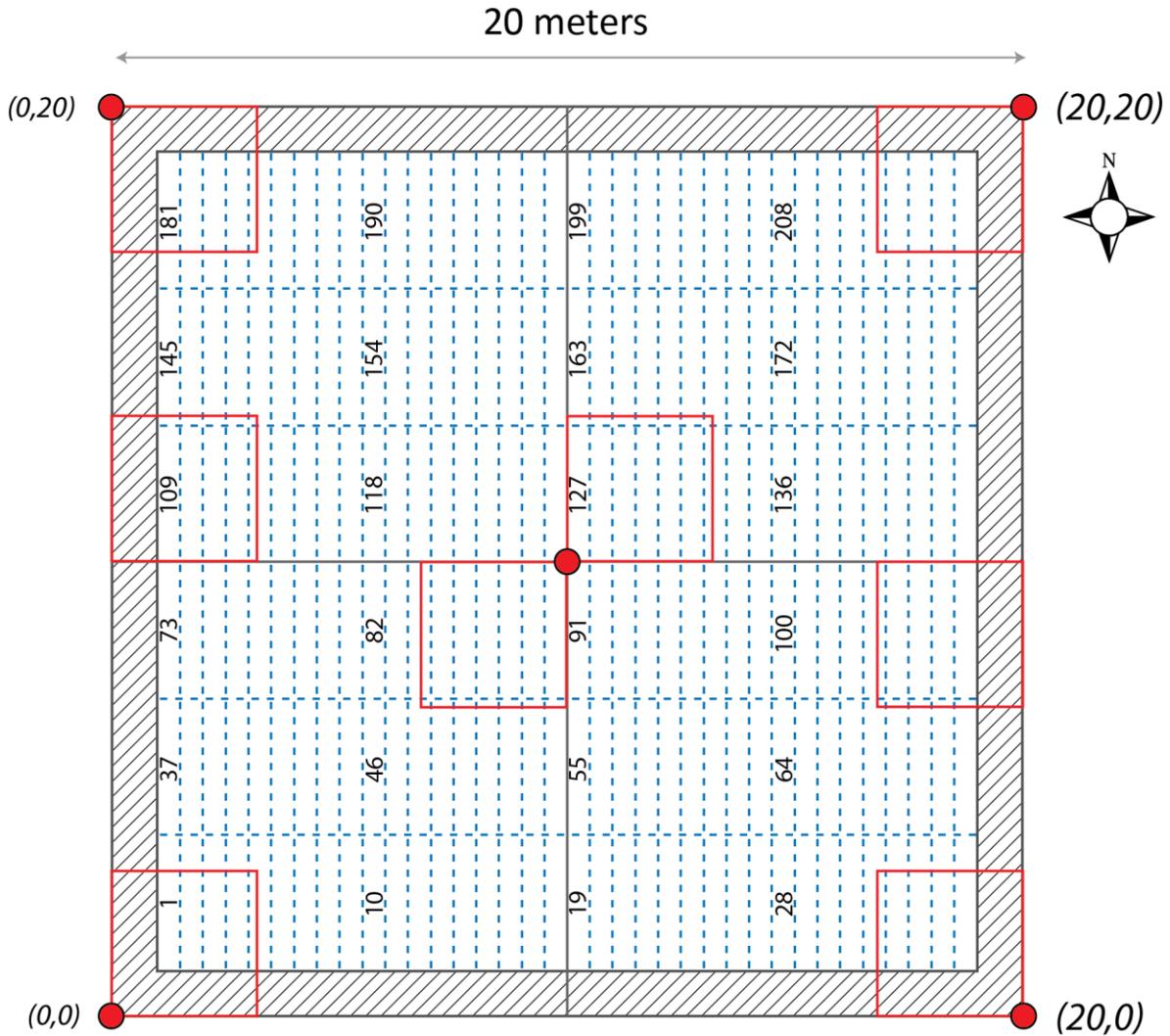


Figure 11. Map of clipCellNumbers in a 20m x 20m base plot (subplotID = 31 in provided Clip Lists). Red squares indicate nested subplots used for diversity sampling; clip cells that significantly overlap red squares are not used for clip sampling. However, clip cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support clip harvest sampling.

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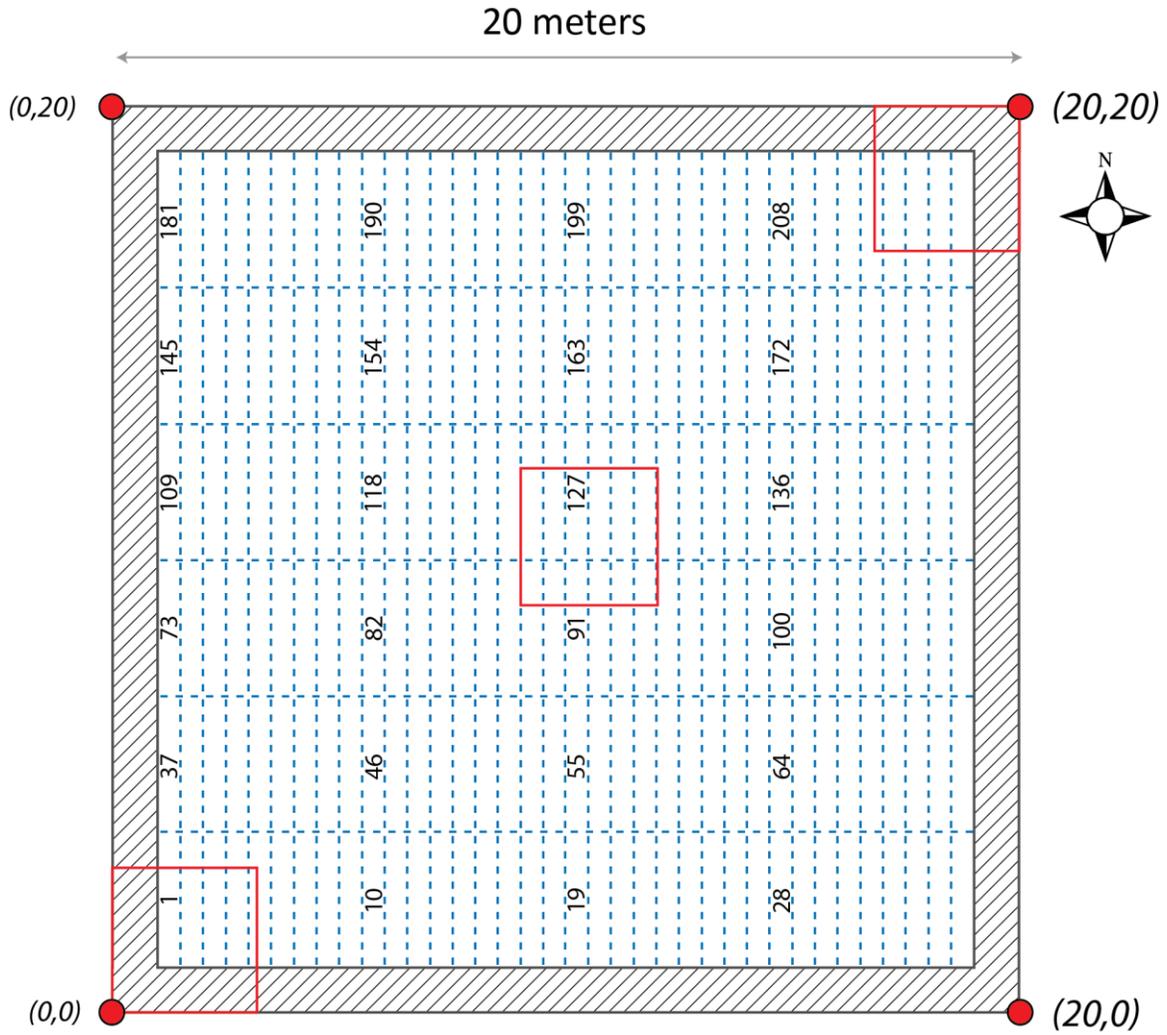


Figure 12. Map of clipCellNumbers for subplotID = 21 in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

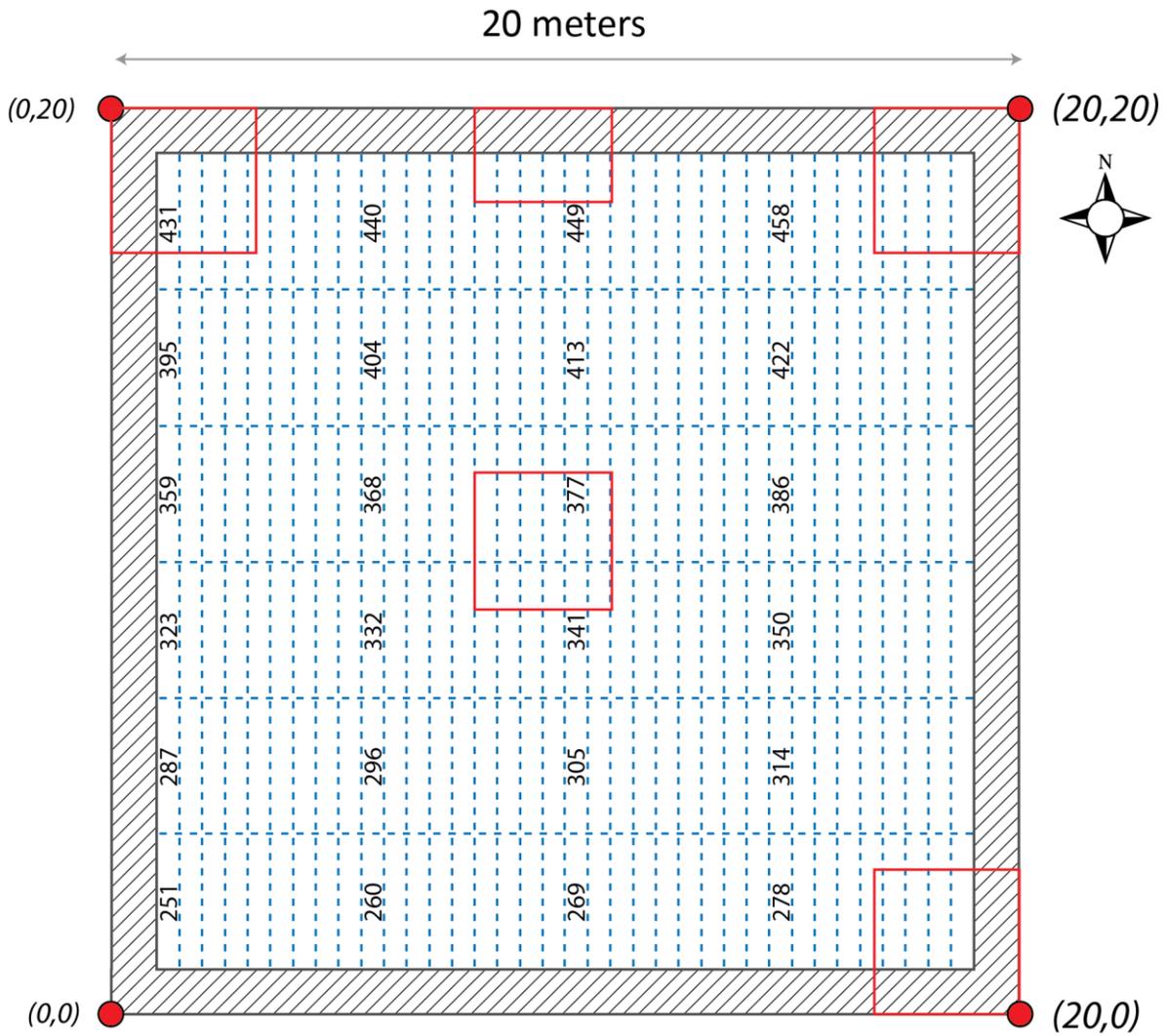


Figure 13. Map of clipCellNumbers for **subplotID = 23** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

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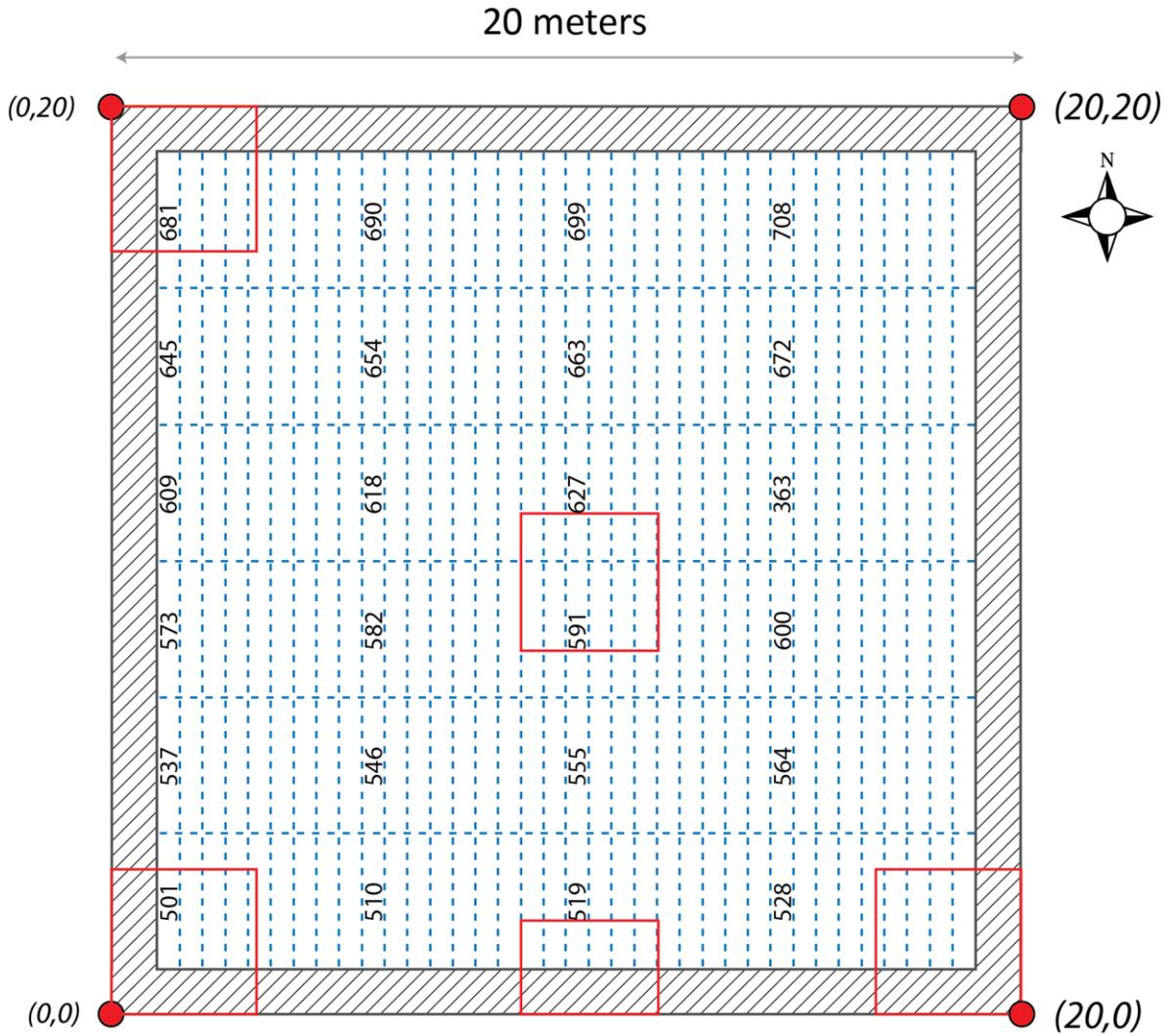


Figure 14. Map of clipCellNumbers for **subplotID = 39** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

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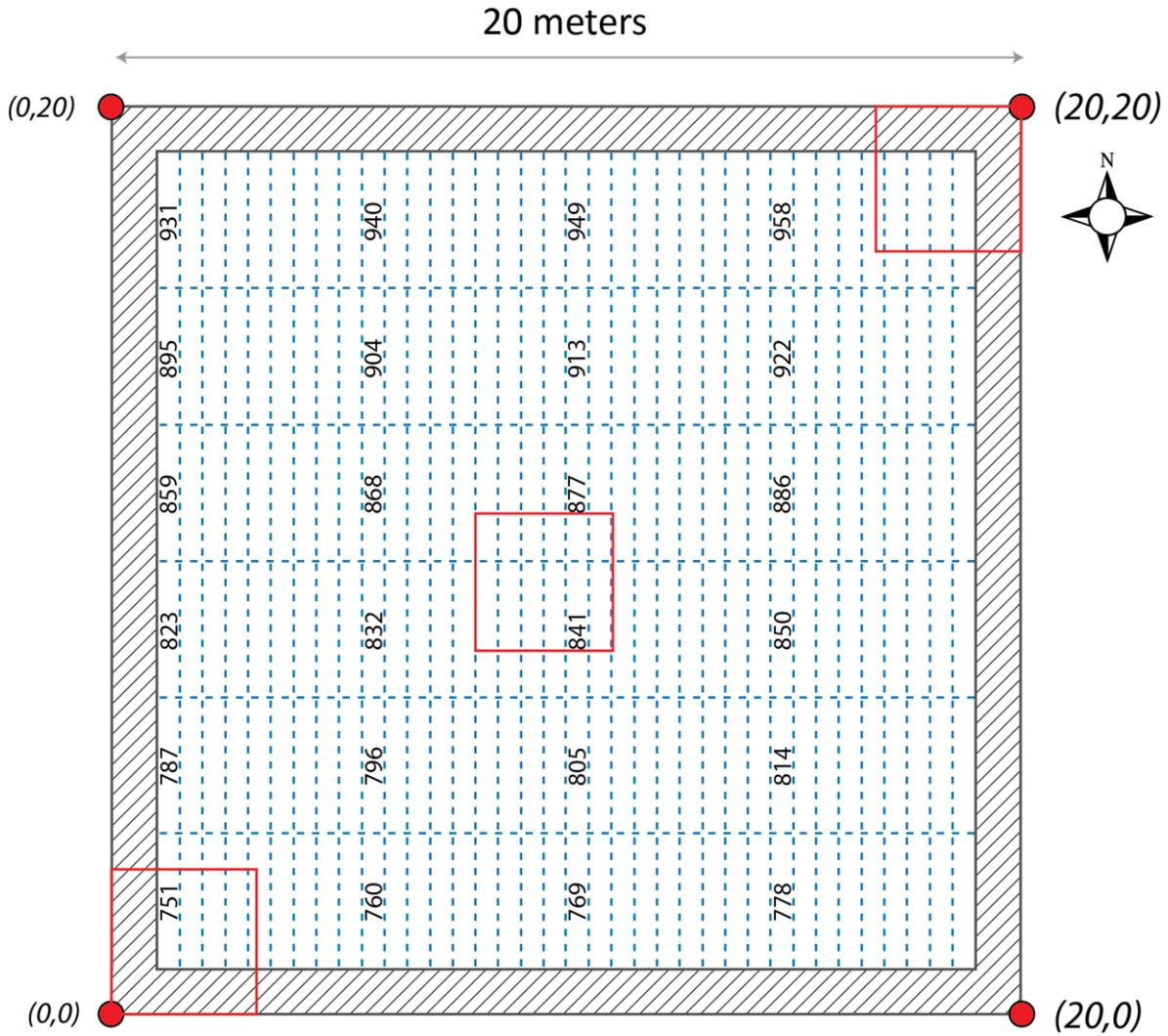


Figure 15. Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

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E.3 Coordinates for clipCellNumbers by subplotID

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

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

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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

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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

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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

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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

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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

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APPENDIX F PEAK GREENNESS WINDOWS BY SITE

Table 19. 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.

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

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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