TOS PROTOCOL AND PROCEDURE: CANOPY FOLIAGE SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	01/13/2014	ECO-01139	Draft release
B	06/03/2014	ECO-01662	Production release, template change, and other changes as detailed in Appendix A (Rev B only)
C	11/05/2014	ECO-02416	Migration to new template
D	02/17/2017	ECO-04371	• Changed title from " TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass Per Area Measurements" to "TOS Protocol and Procedure: Canopy Foliage Sampling" to be more consistent with other TOS protocols • Incorporated description of canopy foliage sampling at herbaceous sites as well as foliage sampling for genetic archive material into introduction sections • Added safety information relevant to line launcher and slingshot sampling in tall canopies • Revised decision tree for how to sample at tall and mixed-stature plots • Updated equipment lists • Provided more detail on timing and personnel requirements for field and laboratory procedures • Added flow chart overview of sample collection and laboratory processing steps • Added Table 9 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

			• 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 0 as it contains necessary resources for clip strip harvesting for chemistry and LMA

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1 OVERVIEW

1.1 Background

This document describes the required protocols for conducting field sampling of sun-lit plant canopy tissues for analysis of total organic carbon (C) and nitrogen (N), lignin, chlorophyll, major and minor elements, isotopic composition ($^{13}C/^{12}C$ and $^{15}N/^{14}N$), and leaf mass per area (LMA). It also describes a separate procedure for collection of plant tissue to be archived for genetic analyses. 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. Additionally, plant tissue will be collected and archived to facilitate plant genetic analyses by the ecological community.

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.

Moreover, 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-level abundance, will target a representative mix of canopy vegetation samples 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 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.

Beyond information on foliar chemistry, NEON will also collect and curate foliar material for analysis of plant genetic diversity over space and time. Aside from encouragement to collect material from individuals targeted for canopy foliar chemistry (and other protocols), there is nothing that links the genetic archive plant tissue collection to the canopy foliar chemistry collection. Plant tissue collections are integral to next generation phylogenetic and systematics studies including building morphological-genetic relationships, identifying species, and providing a foundation for population genetics and phylogenetic studies over the lifetime of the observatory. NEON will make plant tissue collected from select plant species available for analysis by the ecological community. More information on the plant diversity science design is described in TOS Science Design for Plant Diversity (AD[06]).

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).

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.000912	NEON Science Design for Plant Diversity
AD[07]	NEON.DOC.004104	NEON Science Performance QA/QC 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.014040	TOS Protocol and Procedure: Plant Phenology
RD[06]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[07]	NEON.DOC.014048	TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
RD[08]	NEON.DOC.014038	TOS Protocol and Procedure: Core Sampling for Plant Belowground Biomass
RD[09]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[10]	NEON.DOC.001576	Datasheets for TOS Protocol and Procedure: Canopy Foliage Sampling
RD[11]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[12]	NEON.DOC.001716	TOS Standard Operating Procedure: Toxicodendron Biomass and Handling
RD[13]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[14]	NEON.DOC.001717	TOS SOP: TruPulse Rangefinder Use and Calibration

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

2.4 Definitions

N/A

3 METHOD

The goal of this protocol is to sample the diversity of sun-lit vegetation found across the site, essentially capturing what the AOP sees during overflights. Foliar chemistry and LMA vary considerably both between species and through time; therefore, when possible, the same individuals representing dominant and/or co-dominant species 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 Plots locations for canopy sampling will be provided in a separate document to NEON field personnel.

In forested sites, the top three most abundant *canopy* species on a per-plot basis will be identified, then one individual – preferably tagged, will be sampled from each. This approach should yield measurements from a representative mixture of canopy-dominant species at the 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.

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 (2-10 m) vegetation, sun-lit leaves may be obtained using clippers and 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.

Aside from collection of sun-lit leaf canopy samples for analysis of chemical constituents and LMA, plant tissue from a select set of species will also be collected and archived for genetic analyses. Archival plant tissue need *not* be sun-lit, and will be collected from the three 'Phase I' species selected for phenology observation at each site. This means archive tissue will be collected from the dominant species in the vicinity of the tower (see TOS Protocol and Procedure: Plant Phenology (RD[05]). Archived material, consisting of 30 samples per bout (10 replicates each from the three Phase I Phenology species) will be sampled from both the primary Phenology Plot and a subset of Distributed Base Plots. Samples will be dried with desiccant, stored at room temperature, and sent to a contracted archive facility.

Standard Operating Procedures (SOPs) in Section 7 of this document provide detailed step-by-step directions, 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 0 of this document and follow instructions therein to ensure quality standards are met.

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

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC Plan (AD[07]).

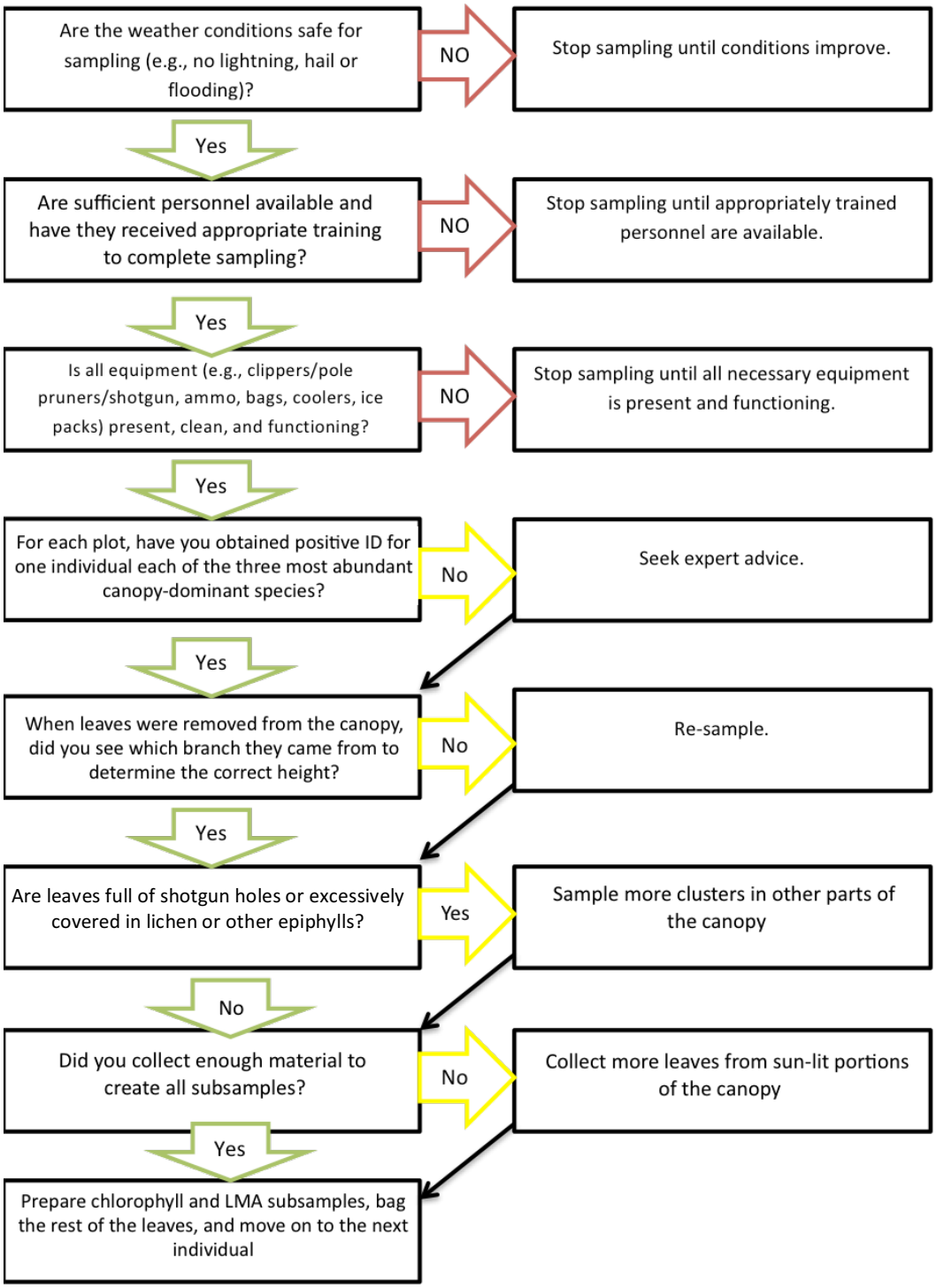


Figure 2. Decision tree to determine if canopy sampling should take place, and when sampling of a woody individual is complete.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

***Timing*:** The timing of airborne data collection by the AOP, which coincides with the approximate timing of peak greenness, largely determines the timing of canopy foliar sampling for chemistry and LMA at a given site. These ground and airborne datasets will be analyzed together, so the efforts must be coordinated.

- Archival sampling for foliar genetic material need *not* concur with AOP and may occur any time during the field season, as long as foliar tissue that is robust and not approaching senescence can be collected.

Frequency: Canopy foliage sampling will be conducted at NEON sites 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 and genetic composition 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 synchronized TOS measurements aimed at characterizing plant and soil biogeochemical dynamics. The suite of synchronized protocols includes:

- The 'biogeochemistry' component of TOS Protocol Litterfall and Fine Woody Debris (RD[06]),
- The 'biogeochemistry' component of TOS Protocol Soil Biogeochemical and Microbial Sampling, including N Transformations (RD[07]), and
- TOS Protocol Core Sampling for Plant Belowground Biomass (RD[08]).

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 must be a separate bout from the annual clip harvest for biomass.

4.2 Criteria for Determining Onset and Cessation of Sampling

Foliar chemistry and LMA: 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 change over the course of the growing season.

Genetic archive: Sampling may begin and end any time during the growing season, as long as foliar tissues that are robust and not approaching senescence (they need **not** be sun-lit) can be collected. This is because genetic composition does not change with leaf canopy position or over the course of the growing season, but healthy, robust leaves are needed to enable high quality genetic analyses. For efficiency, genetic archive sampling may be scheduled to coincide with the foliar chemistry and LMA bout. However, this is not required and may depend on logistical and scheduling constraints.

4.3 Timing for Sample Processing and Analysis

Foliar chemistry and LMA: 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.

Once all laboratory processing is complete, frozen and oven-dried subsamples should be shipped to external laboratory facilities for analysis and archive according to the schedule provided by NEON CLA.

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

4.4 Sampling Timing Contingencies for Foliar Chemistry and LMA

When unexpected field conditions are met, the guidance in **Table 1** should be followed to ensure that basic data quality standards are met:

Table 1. 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 immediately 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 sample 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.

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 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 becomes permanently flooded). 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 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.

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, 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 is also 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[12]) in order to minimize exposure while sampling and handling vegetation and to properly clean equipment.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

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 2. Equipment list: Preparing for chemistry and LMA sampling at one site.

Item No.	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
Durable Items						
MX100703	R	GPS receiver, recreational accuracy	Pre-load sampling plot locations	All	1	N
	R	USB cable	Transfer data to GPS unit	All	1	N
MX100322	R	Laser Rangefinder, ± 30 cm accuracy	Check calibrations and settings	All	1	N
Consumable items						
MX103942	R	All weather copy paper	Print back-up datasheets	All	6	N
	R	Permanent marker	Label bags	All	1	N

Item No.	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
	R	Tin foil	Create pre-folded foil packets to store chlorophyll subsamples	All	1 per sample	N
MX100592	R	Resealable plastic bag, 1 gal	Assemble and pre-label bags to store LMA subsamples	Tall and mixed-statured sites	1 per sample	N
MX 108171	R	Whirl-Pak bags, 2 oz	Assemble and pre-label bags to store chlorophyll subsamples	All	1 per sample	N
MX105089	R	Paper bags, #8	Assemble and pre-label bags to store bulk chemistry samples	All	1 per sample	N

R/S = Required/Suggested.

Table 3. Equipment list: Sampling for chemistry and LMA at one site, **all vegetation types**.

Item No.	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
Durable Items						
MX100703	R	GPS receiver, recreational accuracy	Navigate to sampling locations	All	1	N
MX100322	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
MX103218	R	Foliage filter	Allow laser rangefinder use in dense vegetation	All	2	N
MX104359	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 coolers, to be filled with dry ice	Flash-freeze and store frozen chlorophyll subsamples	All	2	N
MX100358	R	Cold packs	Chill foliage samples in the field	All	10	N

Item No.	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
MX106656 MX103211	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
MX100212	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

Item No.	R/S	Description	Purpose	Condition Used	Quantity	Special Handling
	R	Nitrile gloves, powderless	Handle samples	All	1 box	N
	R	Permanent marker	Label bags	All	3	N
MX103232	S	Paper bags, #25	Extra bags to help organize samples	All	10	N
MX105089	R	Paper bags, #8	Extra bags to contain bulk samples	All	10	N
MX100592	R	Resealable plastic bag, 1 gal	Extra bags to contain LMA subsamples	All	10	N
MX100593	R	Resealable plastic bag, 1 qt	Extra bags if pre-labeled LMA subsample bags are too large	All	10	N
MX 108171	R	Whirl-Pak bags, 2 oz	Extra bags to contain chlorophyll subsamples	All	10	N
	R	Pre-made Tin 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
Resources						
RD [10]	R	Field Datasheets, Canopy Foliage Sampling	Back-up to record field data	All	6	N

R/S = Required/Suggested.

Table 4. Additional equipment list: Sampling for chemistry and LMA at one site, **woody vegetation**.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100279	R	Safety glasses	Required PPE, protect eyes	Tall and mixed-stature vegetation	1 per person	N
MX101884	R	Hard hat	Required PPE, predict head	Tall and mixed-stature vegetation	1 per person	N
	R	Hearing protection	Required PPE, protect ears	Shotgun or line launcher sampling	1 per person	N
	R	Work gloves	Required PPE, protect hands	Line launcher or slingshot sampling	1 per handler	N
	R	Shotgun and ammunition (responsibility of Designated Shooter)	Obtain sun-lit foliage samples from tall canopies	Shotgun sampling	1	Y
	R	Line launcher + associated supplies (throw weights, line, cutting tool, pump)	Obtain sun-lit foliage samples from tall canopies	Line launcher sampling	1	Y
	R	Slingshot + associated supplies (throw weights, line, cutting tool)	Obtain sun-lit foliage samples from tall canopies	Slingshot sampling	1	Y
	R	Pole trimmer	Obtain sun-lit foliage samples	Mixed-stature vegetation	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Punch tool (0.5”, 0.75”, or 1.5”)	Obtain standard size broadleaf subsamples for chlorophyll analysis	Broad leaf samples	2	N
MX104442	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
MX103480	R	Hand stamp steel die set	Append canopy-only tags with “Z”	Sampling non-tagged trees	1 set	N
Consumable Items						
MX103940	R	Flagging tape	Flag individuals to be sampled in tall- and mixed-stature vegetation	All	1	N
MX103478	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
MX103224	R	Aluminum nail	Affix tags to stems	Sampling non-tagged trees	10	N
MX107336	R	Aluminum wire	Affix tags to stems	Sampling non-tagged trees	10	N

R/S = Required/Suggested.

Table 5. Additional equipment list: Sampling for chemistry and LMA at one site, **herbaceous vegetation**.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100722	R	Measuring tape, minimum 30 m	Locate clip-harvest strips	Plot slope < 20%, not brushy	1	N
MX100322	R	Compass with mirror and declination adjustment	Locate clip-harvest strips	If using measuring tape	1	N
MX104361	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
MX100543	R	Ruler, 30 cm	Delineate clip harvest strip	All	1	N
MX104442	R	Spring scale, tareable, capacity 30 g	Collect sufficient material for chlorophyll subsample	All	1	N
Consumable Items						
	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

R/S = Required/Suggested.

Table 6. Equipment list: Sampling for **genetic archive material** at one site.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100703	R	GPS receiver, recreational accuracy	Navigate to sampling locations	All	1	N
	R	Pruning shear (sharpened)	Obtain foliar tissue samples	All	1	N
	R	Tweezers	Obtain foliar tissue samples	All	1	
	R	Pole trimmer	Obtain foliar tissue samples	Tall or mixed-stature sites where foliage is out of reach	1	N
	R	Backpack	Transport field equipment	All	1	N
	S	Clipboard	Secure datasheets	All	1	N
MX106656 MX103211	S	Magnifier hand-lens, 10X/20X	Aid in species identification	Uncertain of species ID	1	N
	S	Field guide, regional flora reference guide and/or key	Aid in species identification	Uncertain of species ID	1	N
MX100316	S	Plant press	Press collected individuals for identification	Uncertain of species ID	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Consumable items						
	R	Field notebook	Record field notes	All	1	N
	R	AA battery	Spare battery for GPS receiver	All	2	N
	R	Nitrile gloves, powderless	Handle samples	All	1 box	
	R	Permanent marker	Label bags and envelopes	All	3	
MX100592	R	Resealable plastic bag, 1 gal	Store foliar samples	All	1 box	N
MX103233	R	Coin envelope	Contain foliar tissue samples	All	1 box	N
	R	Color-change desiccant	Dry foliar tissue samples	All	1 bag	N
Resources						
RD [10]	R	Field Datasheets, Canopy Foliage Sampling	Back-up to record metadata	All	6	N

R/S = Required/Suggested.

Table 7. Equipment list: Measuring LMA and drying bulk foliar samples at one site.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100230	R	Drying oven	Dry samples	All	1	N
MX100264	R	Balance, 0.0001 g accuracy	Weigh fresh and dry LMA samples	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 and samples begin to rot	1 each	N
MX103931	S	Plastic tray	Organize samples	All	4	N
Consumable items						
	R	Nitrile gloves, powderless	Handle samples	All	1 box	N
MX103233	R	Coin envelope	Contain samples while oven-drying	Small leaves or needles	1 box	N
MX105089	R	Paper bag, #8	Contain samples while oven-drying	Larger leaves and herbaceous samples	1 box	N
MX103232	S	Paper bags, #25	Organize smaller bags or envelopes in drying oven	All	10	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100689 MX100690	R	Weigh boats, small and large	Contain samples while weighing	All	1 box	N
	R	Permanent marker	Label bags or envelopes	All	2	N
Resources						
	R	Scanner	Scan LMA samples	All	1	N
	R	Image J Software	Calculate scanned area of samples	All	1	N
RD[10]	R	Laboratory Datasheets, Canopy Foliage Sampling	Back-up to record data, plus scanning template with scale bar	All	2	N

R/S = Required/Suggested.

Table 7. Equipment list: Subsampling for chemical analyses and archive.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100264	R	Balance, 0.0001 g accuracy	Weigh and distribute subsamples	All	1	N
MX103235	R	Sample microsplitter, small capacity	Create representative subsamples from ground sample	Herbaceous	1	N
MX103237	R	Hy back pan	Receive sub-samples generated by splitter	Herbaceous	2	N
Consumable items						
	R	Nitrile gloves, powderless	Handle samples	All	1 box	N
MX103233	R	Coin envelope	Contain chemistry samples	Leaves and needles from woody individuals	2 per sample	N
MX101278	R	Scintillation vials with caps, 20 mL	Contain chemistry and archive samples	Herbaceous and archive	1-2 per sample	N
	R	Ethanol, 70%	Clean gloves between samples	All	1 bottle	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Resources						
RD[10]	R	Laboratory Datasheet, Canopy Foliage Sampling	Back-up to record data	All	4	N

R/S = Required/Suggested.

Table 8. Equipment list: Shipping foliar samples from one site.

Item No.	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
MX102297	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
MX100212	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
MX100592	R	Resealable plastic bag, 1 gallon	Double-bag and organize samples prior to shipment; protect manifest	All	6-10	N
Resources						
	R	Shipping Inventory	Inventory of samples being shipped	All	1 per box	N

R/S = Required/suggested

6.2 Training Requirements

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

Field personnel are to be trained in local plant species identification and safe working practices for canopy sampling, including use of clippers, canopy pruning equipment, and shotgun/line launcher safety. 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 at least 98% accuracy for broad leaf samples and 95% accuracy for conifer needles and mixed grass 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 the datasheets 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 LMA AND CHEMISTRY 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 will 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 the dominant species present in each plot.

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 a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

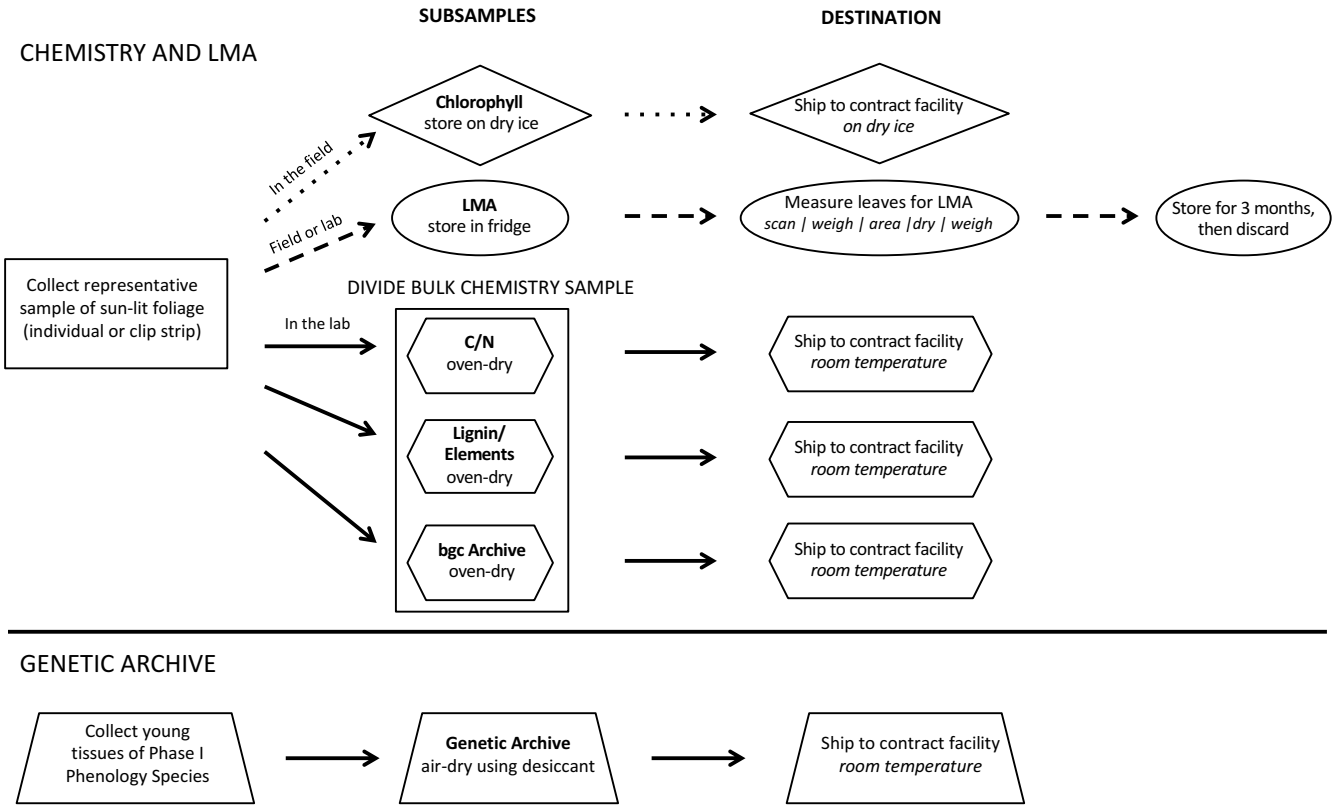
Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Foliar chemistry and LMA: An experienced two-or-three-person team (depending on canopy type) will require approximately 3-5 days to complete field sampling at a predominately woody site. At predominately herbaceous sites, only 1-2 days are likely needed. An additional 2-3 days of active lab work per site are needed for processing samples in the Domain Support lab, 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.

Genetic archive: An experienced two-person team will require approximately 2-3 days to complete field sampling at one site. The estimated time is shorter than for chemistry and LMA sampling because sun-lit leaves are not required and selection of species will be more straight-forward. Field Operations may decide to combine the two foliar sampling efforts, which is acceptable as long as this does not interfere with the timeline for laboratory processing of samples for LMA and chemistry. An additional 1-3 days may be needed to continue drying out tissues in the lab (e.g., changing desiccant).

7 STANDARD OPERATING PROCEDURES

General Workflow for Canopy Foliage Sampling



7.1 Contents and Overview of SOPs

The tasks associated with canopy foliage sampling for LMA and chemistry as well as generating genetic archive samples are divided into a series of eight 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 data 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.

- **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.
- **0:** 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.
- **SOP H:** Details the procedure for sampling, air-drying, and archiving plant tissue for genetic analyses, including what species to collect and where to sample them. SOP H *may or may not* coincide spatially and temporally with SOP B.

7.2 Expected Sample Numbers

Use **Table 9** (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 9. 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 *Short-stature*	4	1	
			Tower *Tall-stature*	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 *Short-stature*	4	2 - 4	
			Tower *Tall-stature*	4	2 - 5	
Genetic Archive	Phase I Phenology Species	Individual	Distributed	7	3	30
			Phenology Loop	3	3	

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

SOP A Preparing for Sampling, Chemistry and LMA

Table 10: Equipment and supply preparation checklist

✓	Item	Action
	GPS unit	Charge and load target plot locations
	TruPulse 360R	Prepare for sampling • Check battery, charge • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination[1]. See RD[14] for details. • Calibrate TruPulse tilt-sensor – only necessary after severe drop-shock; see RD[14] for details.
	Scanner + ImageJ	Check for compatibility of scanner settings and ImageJ • Collect a few local leaves for scanning • Execute sections D.1 **and** D.2 of the LMA procedure • Ensure all steps work smoothly - if not, make adjustments[2]
	Hand clippers & pole pruners	Clean and sharpen blades (if necessary)
	Re-usable cold packs	Place in –20 ˚C freezer
	Dry Ice	Ensure an adequate amount is available to fill two coolers
	Sample bags	Organize and pre-label, see below

[1] Declination changes with time and should be looked up annually per site: http://www.ngdc.noaa.gov/geomag-web/
[2] The most common issue is with file formats – both .jpeg and .tiff in non-compressed form are acceptable, .pdf is not

A.1 Prepare for Data Capture

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

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

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

A.2 Determine Appropriate Methods and Supplies

1. Use 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 increase 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 9 and your knowledge of vegetation types at the site to estimate approximately 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 2oz Whirl-paks.

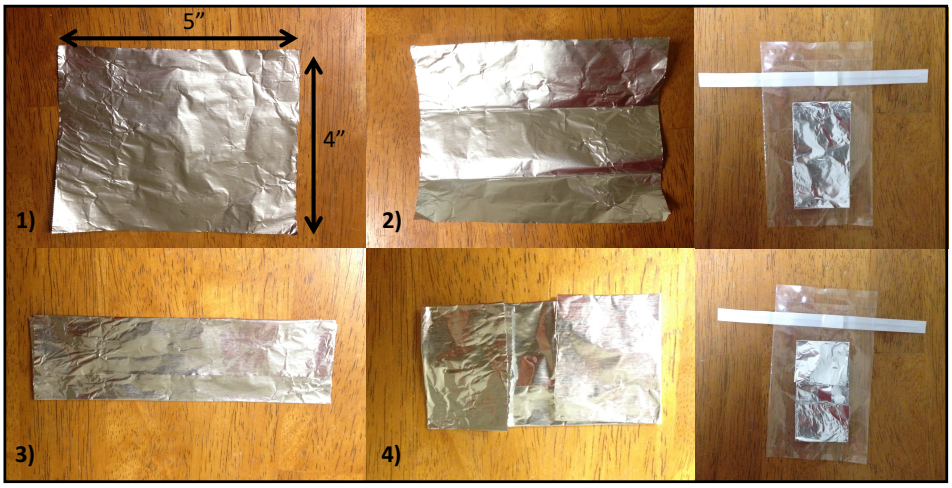


Figure 3 Steps to create foil packets: 1) cut a ~ 5” x 4" rectangle of foil, 2) mark lines to fold into thirds, 3) fold into thirds along the longer edge, 4) fold in the ends to close the packet. Keep a 2oz Whirl-pak bag nearby to make sure packets will fit.

A.4 Prepare to Map and Tag Trees as Needed

1. As outlined in section B.2, in woody plots it is preferable to sample previously tagged individuals for canopy chemistry and LMA, as they are already geo-referenced. However, in some cases it may be necessary to sample non-tagged individuals.
2. In preparation for this occurrence, personnel 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[09]) and be familiar with this procedure before a canopy sampling bout begins.

- Individuals tagged during Canopy Foliage Sampling will NOT be measured during normal implementation of RD[09]. To avoid confusion, these "canopy-only" tagIDs will be appended with a "Z" using the hand stamp and die set (example tagID = 9532**Z**).

A.5 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.6 Organize (and Pre-label) Sample Bag Packets

1. Each sample, be it an 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 label formats, leaving space for **taxonIDs** when planning to sample woody individuals.
2. Each bag packet should contain:
    a. 1 Paper bag (or several for clip strips), to contain the bulk foliage chemistry sample
        - Example label, woody: cfc.GRSM_001.[ ]-1.20160606
        - Example label, herbaceous: cfc.GRSM_001.CLIP-1.20160606
    b. 1 2oz Whirl-pak bag, to the contain the chlorophyll subsample
        - Example label, woody: cfc.GRSM_001.[ ]-1.20160606.chl
        - Example label, herbaceous: cfc.GRSM_001.CLIP-1.20160606.chl

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 label, woody: cfc.GRSM_001.[ ]-1.20160606.lma

SOP B Field Sampling, Chemistry and LMA

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

1. 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.*
2. 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.
3. See Appendix D for vegetation height categories and recommended methods.

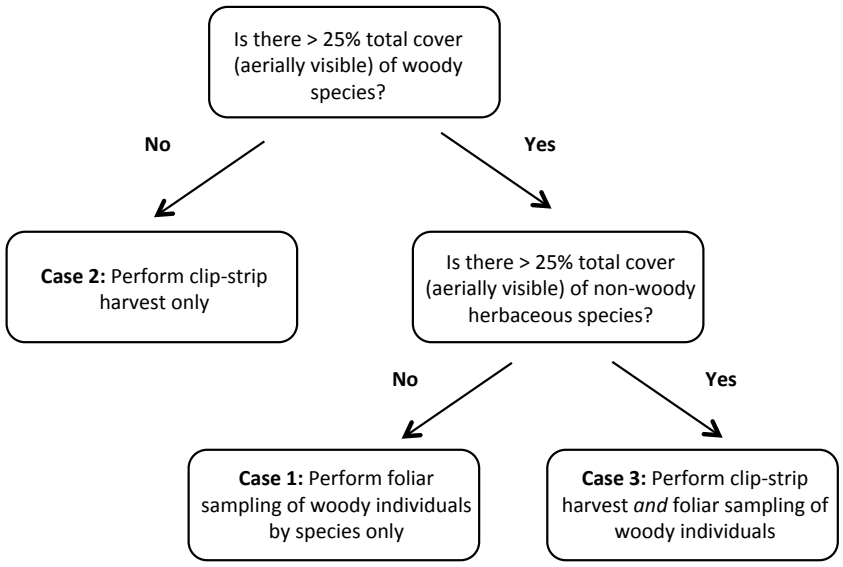


Figure 4 Flow chart to determine appropriate methods for canopy foliage sampling based on plot cover type

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. Identify the **top three canopy-dominant species** in the plot. These should be the species that appear most frequently in the uppermost part of the canopy (e.g. foliage is fully or partially sun-lit) and are visible aerially (e.g. would be "seen" by the AOP). 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. Tagged canopy individuals.

   b. Canopy individuals that are not tagged, but have been sampled for foliar chemistry and LMA in the past.

   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) if possible.

   e. 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 3), which is where tagged individuals are located. However, remember to:
   - Keep sampling 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.
   - Avoid the centroid, attempt to not trample or travel through it.
4. In tall-stature **Tower Plots**, sampling should occur primarily in the two 20m x 20m subplots that are randomly selected for Plant Productivity measurements (Figure 5), as this will be necessary to capture tagged individuals. However, Field personnel must remember to:
   - Keep sampling equipment and coolers in the other two subplots or outside the plot
   - Avoid all nested subplots.
   - Avoid the centroid, attempt not to trample or travel through it.

Box 1. **Guidelines for Choosing Individuals in Predominately Woody Plots**

Observation	Response
There is only one species in the plot (e.g. 100% Red Oak)	Sample a total of 3 individuals from that species, distributed across the plot or subplot
The plot has high overstory diversity and it is unclear which 3 species are the canopy dominants	Drawing on the expertise of the Botanist, do your best to identify the top 3 species, then sample those and move on. *Do not worry if the 4th or 5th most abundant species is sampled*. Remember, the goal is to encompass the range of canopy species variation at the site in order to facilitate robust regressions with AOP data
Some species are being sampled with high replication while others are sampled infrequently	High replication is ok for common species, but at high diversity sites, it is also desirable to even out the level of species replication. Only 1 replicate is not ideal; **in fact, a minimum of n=3 per species is ideal**. As sampling proceeds, take note of what's already been sampled using the WorkTracker in Appendix B. If choosing between two species, one with many replicates and another with only one or two, sample the one with fewer replicates. It is acceptable to sample 1-2 extra individuals per site in order to achieve n = 3 for canopy species that are important at the landscape scale.
It is possible to collect sun-lit foliage from both old and young tagged individuals in a given plot	Consider which age class is most representative of the conditions in that plot and site (e.g., what the AOP will largely "see" from the air) and sample an individual from that age class
An individual from a target species is found in the plot centroid or a Plant Diversity nested subplot	Try to find another individual of that species to sample in a different location 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
It is not possible (or excessively difficult) to sample a tagged individual of the target species	Identify and flag a non-tagged individual and prepare to map and tag it while sampling
There is not enough foliage on a given individual to sample without damaging it	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
Some individuals of a dominant species show signs of disease/sickness/herbivory	Sample diseased/sick individuals if the disease/sickness is a dominant characteristic (> 50%) of the plot. Otherwise, try to avoid diseased individuals where possible

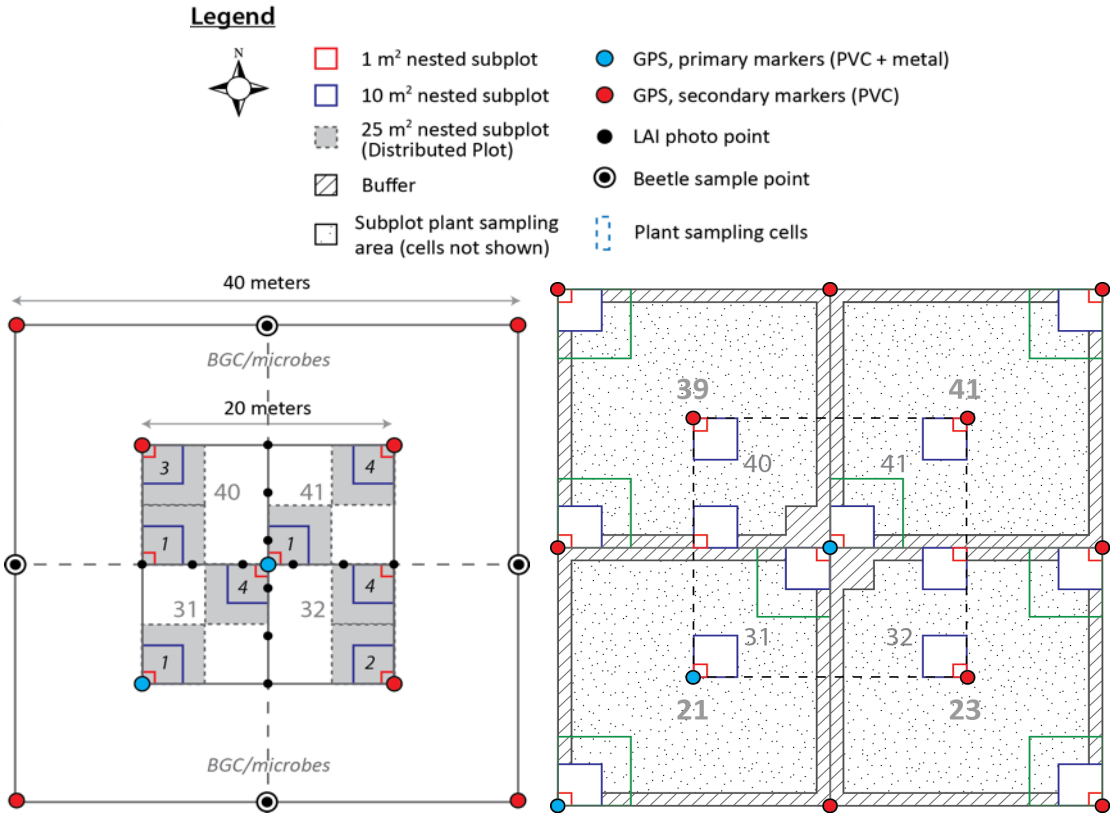


Figure 5 *Left*: Plot layout for Distributed basePlots and short-stature Tower basePlots used for canopy foliage sampling. *Right*: Plot layout of tall-stature Tower basePlots use for canopy foliage sampling

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** (siteID_XXX), **taxonID**, **- sampleNumber** for that plot, and the **collectDate**

    - Example label: **cfc.GRSM_001.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 < 4 m tall, hand clippers should be used to collect leaves.
    - For woody individuals 4-10 m tall, an extendable pole trimmer should be used.
    - For woody individuals > 10 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).
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 (0, Figure 11)
        - 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
        - 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 0, Table 14), then proceed to the next potential strip on the list.
        - **Do NOT record '0' for clip strips rejected because they lie underneath a canopy**. 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. <u>When herbaceous % aerial cover is < 75% AND > 25% of the plot (i.e. mixed cover):</u>
    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.
        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 (0, 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** (SITE_XXX), **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:
   - **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).

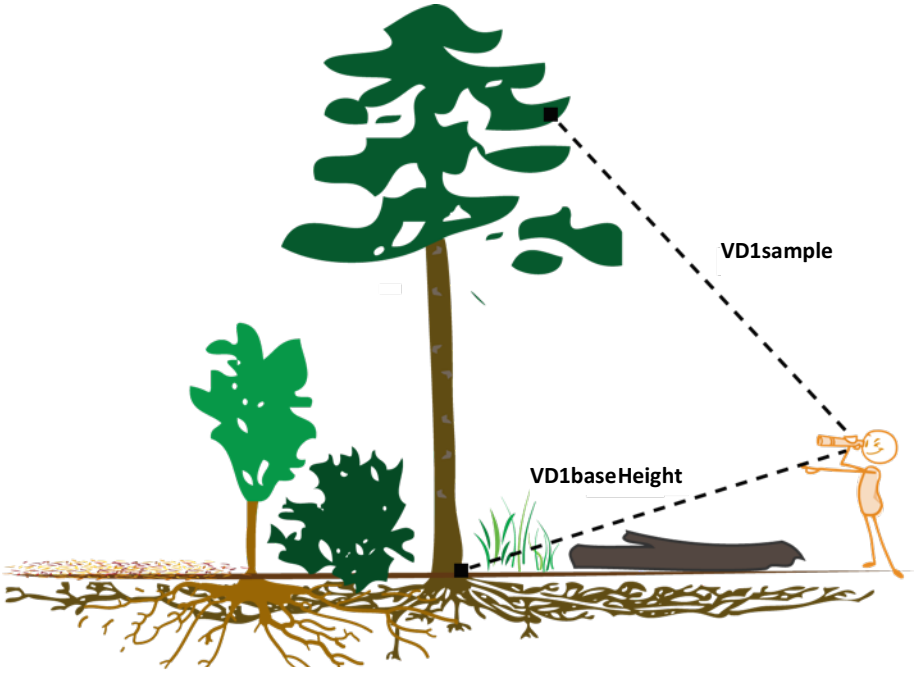


Figure 6 Measuring sample height using a laser rangefinder

   a. Locate a position where the rangefinder operator has simultaneous clean lines of sight to the location from where the sample came, as well as the base of the individual (stem meets ground), preferably on the uphill side if ground is sloped.
   b. Place a reflective surface near the base of the individual to aid accurate readings.
   c. With the laser rangefinder in "VD" mode, aim it at the location where the foliage sample originated and press power (fire) button. Record this value as **VD1sample**.

d. With the laser rangefinder still in VD mode, aim it at the base of the stem (or reflective tape) and press power button. Record this value as **VD1baseHeight**.

e. 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.

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

- How much? Think about what's needed to 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.

7. 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 at least **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).
- Treat leaflets of compound leaves as if they were individual leaves.

8. 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.

9. Package and stow (sub)samples:

a. **Chlorophyll subsample: use ~25% of good-condition, set-aside leaves**

1) For larger leaves (> 0.5" diameter), use a 0.5", 0.75", or 1.5" punch tool to punch 8-10 circles, distributed across all set-aside leaves.

2) For thinner leaves/needles, use clippers or scissors to cut foliage into pieces small enough to fit in a foil packet. *Make sure to 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 not to stack punches. This will help tissue freeze effectively.

5) Place foil packet into a 2oz Whirl-pak bag.

6) Label it with **chlorophyllSampleID** if not already done. The label will consist of the module code (cfc), **plotID** (siteID_XXX), **taxonID**, **- sampleNumber** (for that plot), **collectDate**, and the suffix ".chl"

   - Example label: **cfc.GRSM_001.FAGR-1.20160705.chl**

7) 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 and bout. Ensure to monitor the frozen subsamples.*

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

1) Place material into a resealable plastic bag.

2) Take care not to fold or crush leaves, especially deciduous/broadleaf ones. Folded or crushed leaves will be difficult to use for LMA measurements.

   - It helps to stack individual leaves (Figure 7); woody material may or may not be removed prior to bagging the sample.

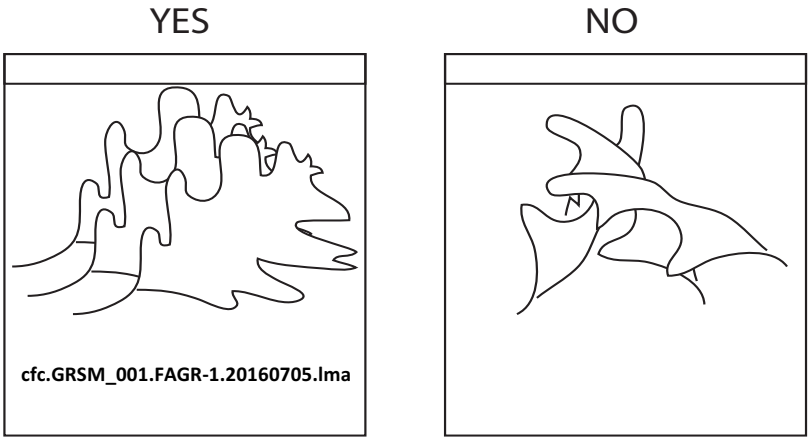


Figure 7. Example of how to package and label a broadleaf LMA subsample.

3) If bag was not pre-labeled, label with **lmaSampleID**. Use the same convention as descried above, but with ".lma" for the suffix.

- Example label: **cfc.GRSM_001.FAGR-1.20160705.lma**

4) Store LMA bag in cooler on ice packs, ensuring not to bend or crush foliage.

c. **Bulk chemistry sample**

1) Place remaining foliage into a kraft paper bag and close.

2) If bag was not pre-labeled, label with **sampleID**. Use the same convention as described above but with no suffix, since this is considered the bulk sample.

- Example label: **cfc.GRSM_001.FAGR-1.20160705**

3) 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.

10. 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 11**, similar to those used in TOS Protocol and Procedure: Measurement of Vegetation Structure (RD[09]), to indicate health of the individual.

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.

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.

Table 11. Tree or shrub status codes and their definitions.

11. 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[09]). If using a mobile device, use the 'Mapping and Tagging' data entry application. If not, use the Canopy Foliage Field Datasheet.

    Briefly:

    a. Attach a pre-numbered aluminum tag, appended with a "Z" using the dicast set (example = 9147**Z**), and record **tagID**. Including the "Z" will mark these as canopy-only trees, which are not sampled in the Vegetation Structure protocol (RD[09]).

    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 most commonly [41], [31], [33], [49], [51]

        2) In tall-stature Tower basePlots, these nine points are most commonly [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[14] for detailed instructions.

        - Remember, these stems are NOT measured in the Vegetation Structure protocol

12. Repeat steps 3 – 11 for all stems that have been marked for sampling in the plot.
13. Remove gloves and stow in a dedicated trash bag.
14. Collect shotgun shells and wadding or any other detritus and remove from plot. Do not contaminate gloved hands while collecting shells and wadding.
15. 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 first 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.
    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 6-15 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 0.1 m x 2 m area for clip-harvesting.

2. Enter required metadata into data entry application or field datasheet: **plotID, subplotID, collectDate, sampleType, clipID**
3. Put on a clean pair of nitrile gloves and use clippers to harvest all herbaceous aboveground biomass *rooted* within the clip strip.

4. 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 *Toxicodendron* is present and will be sampled, follow the guidelines established in TOS Standard Operating Procedure: Toxicodendron Biomass and Handling (RD[12]) to minimize exposure to toxic oils and for guidance on how to clean equipment.
5. Place clipped biomass into kraft paper bags.
6. If not pre-labeled, label bag(s) with **sampleID** as follows: module code (cfc), **plotID** (siteID_XXX), 'CLIP', **- sampleNumber** (for that plot), and **collectDate**
   - Example label: **cfc.WOOD_001.CLIP-1.20160705**
   - Also write the **clipID** and **bagCount** on the paper bag
7. Mix all contents of the sample, then pull out a small, 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, a small handful will suffice.
   - *Remember that for herbaceous samples, you **do not** need to create an LMA subsample in the field, this can be done in the Domain Support facility*.
8. Create the chlorophyll subsample:
   a. Using a clipper or scissors, cut representative foliage into small enough pieces to fit in a foil packet. Keep cutting until you have **0.5-1 g of material**. This will be enough to conduct the chlorophyll analysis but not too much to make flash-freezing difficult.

- *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.*

b. Place small foliage pieces into a pre-made foil packet. Spread out material as much as possible – this will help the tissues freeze effectively. Do not include woody parts.

c. Place foil envelope into a 2oz Whirl-pak bag.

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

   - Example label: **cfc.WOOD_001.CLIP-1.20160705.chl**

e. 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.*

9. Store bulk sample bag 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.

10. 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.

    - Upon returning to the vehicle between plots, transfer frozen chlorophyll subsamples from the first 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.

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^{o} 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).

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, 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. Create a scanning template on your scanner: full color, highest resolution (e.g., 600 dpi), contrast and sharpness maximized. **Acceptable file formats are .tif and .jpeg, NOT .pdf**. Make sure scanned image files are not compressed.
2. Print several copies of the scale bar template from the Canopy Foliage Sampling datasheet package (RD[10]).
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, 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 is included but petiole is removed.
    - Medium-small leaves: 6-12, depending on size and what fits on the scanner. Arrange neatly, remove petioles.
    - 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 <u>in good condition</u> - without holes where possible, mostly green with little dead or damaged parts (especially relevant in grasslands).

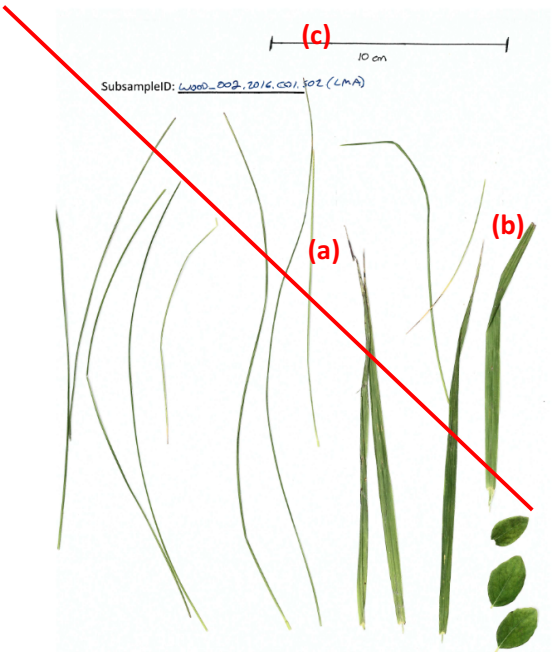


Figure 8 Example of leaf arrangements to avoid when scanning for LMA, including overlapping foliage (a), bent foliage (b), and foliage covering the text (c).

7. Near the top of the scale bar template, record the **lmaSampleID**
    a. Add "scan01," "scan02," etc. if multiple scans will be required for a large leaf.
    b. Can re-use template as long as identifier of the sample being scanned is clear
8. Lay scale bar template face down on top of the leaves/needles, positioned so the written information is NOT covered by foliage. This is necessary for when you crop the image.
9. Scan the sample (Figure 9, left).

**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.*

10. Upload scan as .tif or .jpeg file and save to a folder on the local computer.
11. Copy the image file over to the Dropbox folder on the Domain Support Facility N-drive. Image files will sync overnight and be accessible the following day by ImageJ on the Citrix FOPS Desktop. *It they are not, contact the IT Helpdesk for assistance.*

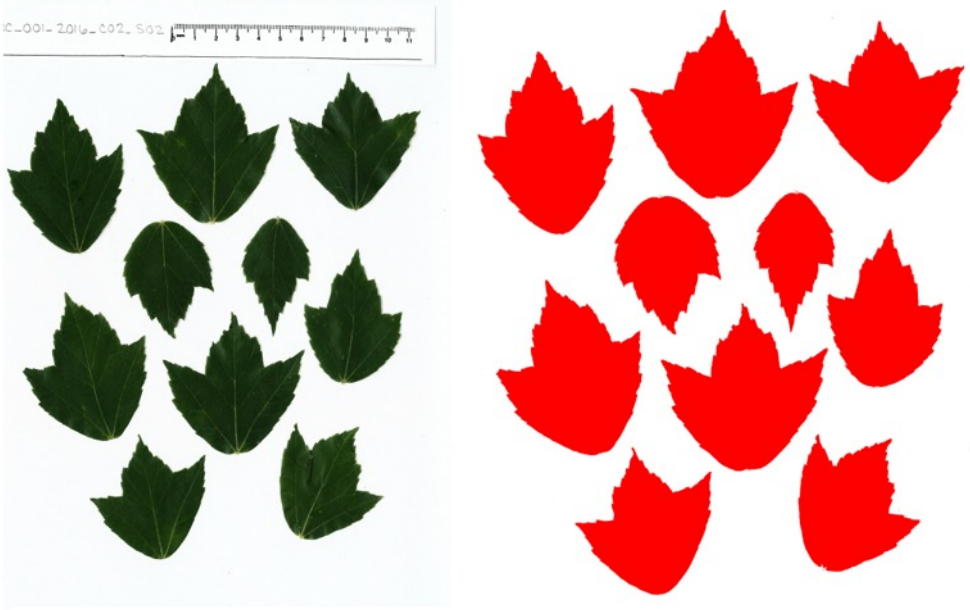


Figure 9 Left: Example of a good quality scanned image of foliage. Right: Image after processing in ImageJ

12. Immediately after scanning, 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.**
    a. Tare a plastic weigh boat.
    b. Transfer sample into weigh boat and note weight to the nearest 0.0001g.
    c. Record **freshMass** (mass in grams of fresh scanned material) and **scanDate** (date of LMA scanning)
13. 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 **lmaSampleID** (e.g., sampleID + ".lma").
    - Example label: **cfc.GRSM_001.FAGR-1.20160705.lma**
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 in the N drive folder.
3. Set the scale that you want to use for area calculations. *Every time you re-open ImageJ you have to reset the scale.*
    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**.
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 will convert the image to black and white (Figure 6, right). This makes it easier to set thresholds for areal calculations.
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, as this will artificially fill them in.

7. If converting to binary and filling holes does not result in a sharp, clear image with no 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 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 when you Convert to Binary. However, do not increase so much that edges become blurry.

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

e. Return to Steps 5 and 6 above to *Convert to Binary* and *Fill Holes*.

8. Next, go to Image → Adjust → Threshold and this will open a new window. The boxes below the scale bars should read 'Default' and 'Red'.

9. Your leaves/needles should now be completely filled out in red on a white background. If needed, adjust the bars so that you have the most red filled in without distorting the shape of the edge of the leaves.

    a. Hit **Set**, then click **OK**. Setting the threshold is telling the software which parts of the image it will be analyzing.

    b. Close the 'Threshold' dialogue box.

10. After the threshold is set, go to Analyze → Set measurements. This will bring up a checklist of available measurements. Make sure that only 'Area,' 'Limit to threshold,' and 'Display Label' are checked. Hit **OK**.

11. 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. Make sure that 'Display Results' and 'Include Holes' are checked and hit **OK** .

12. A table will appear that gives you the individual area of each leaf/needle. Save this table as an excel or .csv file ('File → 'Save As') in same location as the scanned image file.

    a. Use **lmaSampleID** for the file name, add "_scan01", "_scan02" if multiple scans were taken for one subsample.

13. Open this 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:

    - **scannedLeafNumber:** total number of individual leaves/needles/blades scanned
    - **leafArea**: sum total area in mm^2 of all leaves/needles scanned

- **percentGreen**: percent of scanned foliar material that was live and green. A visual approximation, do not spend more than a minute making this estimation

15. Once data has been recorded, you may close the image – do not save changes.
16. Save scanned images and area calculation files for 6 months. 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 0.0001g.
    a. Tare plastic weigh boat.
    b. Transfer sample into weigh boat and note weight to the nearest 0.0001g.
    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.
    - 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 0.0001g.
    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 mm^2
5. Place punches into a coin envelope. Label it with **lmaSampleID** 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 0.0001g.
    a. Tare plastic or aluminum weigh boat.
    b. Transfer punches into weigh boat and note weight to the nearest 0.0001g.
    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 three months. Once it has been verified that data entry has taken place, foliage may be disposed of.

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 for Woody Individual and Herbaceous samples. 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

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**.

E.2 Subsampling Woody Individual Samples 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 ethanol to clean gloved hands in between samples.
2. Place an empty paper bag of the same type as contains the sample on the scale. Tare it.
3. Weigh the sample in its bag to the nearest 0.1 g. *It is not necessary to record dryMass, as this weight is only used in order to ensure enough material is sent to chemistry labs.*
    a. If dry sample mass is $\geq$ **6 grams**, split foliar material equally into three portions.
        - Remove any remaining stems, 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.

b. If the dry sample mass is **2.7 to 6 grams,** apportion the material as follows:

1) Lignin/elements: 1.5-2 grams
2) C/N: 0.2-1 gram
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.

4. Process, stow and label each subsample as follows:

- **Lignin/elements**
  - Container = coin envelope (or small paper bag if needed).
    - Ok to crush/break leaves to fit in container but do not need to grind
  - Identifier = **ligninSampleID**, sampleID + '.lig'
    - Example label: cfc.GRSM_001.FAGR-1.20160705.lig
  - Write neatly on envelope with permanent marker or use a printed label
- **C/N**
  - Container = coin envelope (or small paper bag if needed)
    - Ok to crush/break leaves to fit in container but do not need to grind
  - Identifier = **cnSampleID**, sampleID + '.cn'
    - Example label: cfc.GRSM_001.FAGR-1.20160705.cn
  - Write neatly on envelope with permanent marker or use a printed label
- **Chemistry archive (if applicable)**
  - 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 between samples.
  - Identifier = **bgcArchiveID**, sampleID + '.ar'
    - Example label: cfc.GRSM_001.FAGR-1.20160705.ar
  - Write neatly on laboratory tape wrapped around the entire vial so that it overlaps itself, or use a printed label. **Do not write directly on the vial.**

5. Organize subsamples and group by type in preparation for shipment. Seal coin-envelopes, using tape to close off any leaky corners. Close paper bags with tape or rubber band.

6. Store subsamples in a cool, dry location until they can be shipped to analytical facilities.

E.3 Subsampling Herbaceous Samples 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 ethanol to clean gloved hands in between samples.
2. Place an empty paper bag of the same type as contains the sample on the scale. Tare it.
3. Weigh the sample in its bag(s) to the nearest 0.1 g. *It is not necessary to record dryMass, as this weight is only used to determine whether to grind the entire sample or take a subsample.*
4. Grind the sample in a Wiley mill (0.85mm, 20 mesh size)
   - If dryMass < 20 g, grind the entire sample.
   - If dryMass > 20 g, haphazardly subsample ~ 20 g, then grind it.
   - Ensure no stems, twigs, or other woody material is included in the ground (sub)sample.
   - Clean with compressed air between samples.
5. Use an appropriately sized splitter or microsplitter to generate three subsamples.
   a. Split the sample once and place an entire half into a 20 mL scintillation vial. This will be for the chemistry archive.
   b. Split the remaining material in half again and place each half into it's own 20 mL scintillation vial. These will be for the C/N and lignin/elements analyses.
6. 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.
7. Label each subsample as outlined below, either writing neatly on laboratory tape (wrapped around entire vial so it overlaps itself), or using printed labels. **Do not write directly on vials.**
   - **Lignin/elements**
     - Identifier = **ligninSampleID**, sampleID + '.lig'
     - Example label: cfc.WOOD_001.CLIP-1.20160705.lig
   - **C/N**
     - Identifier = **cnSampleID**, sampleID + '.cn'
     - Example label: cfc.WOOD_001.CLIP-1.20160705.cn

- **Chemistry archive**
    - Identifier = **bgcArchiveID**, sampleID + '.ar'
    - Example label: cfc.WOOD_001.CLIP-1.20160705.ar

8. Organize subsamples and group by type in preparation for shipment.
9. Store subsamples in a cool, dry location until they can be shipped to analytical facilities.

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[13] for complete instructions regarding manual data transcription.

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 Shipping Chlorophyll Subsamples

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

1. Group frozen samples into 1-gallon resealable freezer bags.
2. Use a corrugated cardboard box that meets UN packing group III requirements. Add styrofoam sheets along the walls of the box as insulation. Ensure the styrofoam is not sealed to be airtight. Styrofoam must not be used as outer packing.
3. Put samples to be shipped into insulated box, then weigh it.
4. Add dry ice to completely surround sample bag(s). Ensure there is good contact between dry ice and samples, including packing some dry ice on the top.
5. Re-weigh the box to determine mass of dry ice added.
    a. Some local carriers limit weight of dry ice to 2.5 kg. Check with your local carrier.
    b. If weight limits apply, use cold-soaked packing peanuts to keep samples frozen.
6. Fill empty space with wadded newspaper, styrofoam peanuts, or bubble wrap. Any empty space will allow the dry ice to sublimate faster.
7. Navigate to the "Shipping Information for External Facilities" document on CLA's NEON intranet site. Check whether there are items such as permits or cover letters required to include in the shipment. *Check this document often as instructions are subject to change*.
8. Print out required documents (if needed) to include in shipment box.
9. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site. Include a printed copy of the inventory in the shipment box.
10. Save the file with the following naming convention:

- "DXX_MOD_ShippingInventory_YYYYMMDD_XofX"
- example: D07_CFC_ShippingInventory_20160715_1of2

11. Seal box. Complete packing slip and label for Class 9 dry ice hazard shipment.
12. 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**.
13. Email an electronic copy of the shipping manifest and tracking number to the email addresses specified in the CLA "Shipping Information for External Facilities" document.

G.2 Shipping 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. Navigate to the "Shipping Information for External Facilities" document on CLA's NEON intranet site. Check whether there are items such as permits or cover letters required to include in the shipment. *Check this document often as instructions are subject to change*.
5. Print out required documents (if needed) to include in shipment box.
6. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site. Include a printed copy of the inventory in the shipment box.
7. Save the file with the following naming convention:
    - "DXX_MOD_ShippingInventory_YYYYMMDD_XofX"
    - example: D07_CFC_ShippingInventory_20160715_2of2
8. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.
9. Email an electronic copy of the shipping manifest and tracking number to the email addresses specified in the CLA "Shipping Information for External Facilities" document.

G.3 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.4 Timelines

Frozen (-80℃) chlorophyll subsamples should be shipped out for analysis soon after they are collected, 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 follow the shipping schedule provided by NEON CLA.

G.5 Conditions

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

G.6 Grouping/Splitting Samples

N/A

G.7 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.8 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping inventory enclosed within the shipping container. Shipping inventories should be created using the protocol-specific templates found on CLA's NEON intranet site. If shipping on dry ice, place the hard copy shipping manifest in a resealable plastic bag on top of the packing materials. Send electronic version and shipment tracking information to the email addresses specified in the CLA "Shipping Information for External Facilities" document.

G.9 Laboratory Contact Information and Shipping/Receipt Days

See the "Shipping Information for External Facilities" document on CLA's NEON intranet site.

SOP H Collecting Plant Tissue for Archive Genetic Material

The goal of this SOP is to collect fresh foliar tissue that is robust and not approaching senescence from 10 individuals from each of the three species selected for Phase I of the Phenology observations at a site. Three of those tissue samples should come from individuals tagged for observation in the Phenology Plot loop (see TOS Protocols Plant Phenology (RD[05]) and Vegetation Structure (RD[09])). The remaining seven tissue samples should be collected from individuals found within Distributed Base Plots – preferably those plots with the lowest plotID, as this helps maintain stratified, random sampling. Take samples from tagged individuals when possible, and from the same individuals sampled for canopy chemistry and LMA where appropriate (need not be sun-lit).

H.1 Timing

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

H.2 Field Collection

1. Locate and confirm the identity of individuals belonging to the species selected for Phase I of the Phenology sampling.
    a. Collect material from **3 individuals of each Phase I species from the Phenology Plot loop**. Sample phenology-tagged individuals unless these individuals are small stature annual or perennial species. In this case, collect from individuals of the same species in close proximity to tagged individuals or as available on the Phenology Plot loop.
    b. Collect material from **7 individuals of each Phase I species from Distributed Base Plots**. Prioritize those plots with the lowest plotID; species lists from the plant diversity sampling effort and appropriate habitat types can be used to identify candidate plots. In the case of woody species, material should be collected from individuals tagged for Vegetation Structure.
    c. If seven different Distributed Base Plots do not support each of the species, collections can be made from additional individuals in the Phenology Plot to reach the target sample size.

2. With tweezers and while wearing nitrile gloves, collect approximately 10 cm^2 or 1 g fresh weight (about 0.2 g dried) of leaf material per individual. The leaf material should be collected from young, fresh leaves, but they do not need to be sun-lit (Figure 10).

Figure 10. Collecting young green leaves from a single individual.

3. Before the sample is saved:
    a. Hard, leathery, or succulent leaf material should be cut into small strips.
    b. The surface or epidermis of pruniose or hairy leaves should be removed by scraping with a sharp knife or razor blade.
    c. If leaves are soft and juicy (or even succulent), more tissue, approximately 20 cm^2, should be collected and double the desiccant should be added.
    d. Avoid tissue that is host to parasites (e.g., mildew) or other potential contaminants.
4. Place the tissue in a coin envelope.
5. Label the envelope with a unique **sampleID**. This will include the module code (cfc), **plotID** (siteID_XXX), **taxonID** (USDA plant species code), **sampleNumber** (for that plot), **collectDate**, and the suffix '.gen'
    a. The sample number should correspond to the number of collections within each plot. For example, there will be a minimum of three samples from the Phenology Plot loop.
        - Example label: **cfc.ONAQ_001.BRTE-1.20160605.gen**
6. Record applicable metadata, including **plotID, subplotID, collectDate, taxonID, tagID**, **plantStatus**, **voucher** (yes|no), and **voucherID** (if applicable; see below).

7. Place sample in resealable 1-gallon plastic bag. *Multiple plant tissue samples stored in separate, labeled coin envelopes can be stored in one plastic bag*.
8. Color-change desiccant should be placed in the plastic bag, but outside the coin envelope. The desiccant should be 20-50 times the combined weight of all tissue samples in the bag.
9. Collect an archival-quality voucher specimen from one of the individuals of each species targeted for the genetic collection. The voucher should be from the same individual that the genetic sample was collected from where possible.
    a. Record **voucher** = yes and the **voucherID** for the record associated with that genetic archive sample. **voucherID** is the sampleID plus the word "Voucher"
        - Example label: **cfc.ONAQ_001.BRTE-1.20160605.genVoucher**
    b. The voucher itself must also be labeled such that voucher and tissue can be unambiguously linked. Record the **voucherID** and **sampleID** for the corresponding genetic sample from the same individual.
    c. If no reproductive parts are present, it is acceptable to voucher a target individual at a later date when reproductive parts are available; this may be possible with individuals from the Phenology Plot, given frequent sampling throughout the growing season. However, if collecting a voucher at a later date will not be possible, voucher when the genetic archive tissue is collected.
    d. Do not collect vouchers from *tagged* forb or grass species in the Phenology Plot, but do collect them from tagged trees and shrubs as long as tagged individual are not harmed (see Plant Phenology (RD[05]) and Vegetation Structure (RD[09]) protocols). For herbaceous plants, vouchers should be collected from non-tagged individuals in the Phenology Plot loop or the destructive sampling area of Distributed Base plots.
10. Make sure vouchers of these species are represented in the herbarium collection at the Domain Support Facility.

H.3 Sample Handling

1. Desiccant drying capacity (e.g., color change indicator) must be checked frequently – initially every 6 to 12 hours, less frequently thereafter, to ensure rapid drying. Desiccant may need to be replaced 1-3 times (for succulent or very wet leaves) to fully desiccate the tissue. At particularly humid sites, it may be appropriate to store samples in a desiccant chamber if space is available.
2. Store samples in a cool (ambient), dry location until they can be shipped to the designated archive facility. Bags should be well-sealed to exclude external moisture.

3. Dry and press the voucher specimens for each of the three species sampled. Do not mount the vouchers as they will be shipped to an external facility for archive.

H.4 Shipping Instructions

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

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

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 12. Datasheets associated with this protocol

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

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

APPENDIX B QUICK REFERENCES

OBTAINING CANOPY SAMPLES FOR LMA AND CHEMISTY

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 – 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 – 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.

Work Tracker: Canopy Foliage Sampling for LMA & Chemistry in Primarily Woody Sites

Plot ID	Collect Date	Sampling Crew (measuredBy, recordedBy)	Taxon ID of sampled species (3 per plot)	Remarks

APPENDIX C REMINDERS

SAMPLING FOR LMA AND CHEMISTRY

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?
- ☑ 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 according the schedule provided by NEON CLA.
- ☑ Ship chlorophyll subsamples on dry ice, packaged appropriately.
- ☑ Include shipping inventories.

SAMPLING FOR ARCHIVE GENETIC MATERIAL

While sampling: Remember to…

- ☑ Only collect young, healthy leaves.
- ☑ Foliage need not be sun-lit.
- ☑ Put samples in bags with desiccant as soon as possible.

Sample handling: Be sure to…

- ☑ Continue to change desiccant until the sample is completely dry.
- ☑ Make one voucher specimen for each sampled species.

LABEL REMINDERS

Bulk Chemistry Sample Bag Label
sampleID: cfc.GRSM_001.FAGR-1.20160605

Subsample Labels
chlorophyllSampleID: cfc.GRSM_001.FAGR-1.20160605.chl
lmaSampleID: cfc.GRSM_001.FAGR-1.20160605.lma
cnSampleID: cfc.GRSM_001.FAGR-1.20160605.cn
ligninSampleID: cfc.GRSM_001.FAGR-1.20160605.lig
bgcArchiveID: cfc.GRSM_001.FAGR-1.20160605.ar

Archive Genetic Material Label
sampleID: cfc.ONAQ_001.BRTE-1.20160605.gen
voucherID: cfc.ONAQ_001.BRTE-1.20160605.genVoucher

APPENDIX D VEGETATION HEIGHT CATEGORY TO DETERMINE SAMPLING METHOD BY SITE

Table 13. List of NEON sites, vegetation types found there, and their height categories to determine canopy sampling method.
1 = 0-4 m, manual clip; 2 = 4-10 m, pole pruner; 3 = > 10 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
D05	STEI	woodyWetlands	2,3
D05	TREE	deciduousForest	3
D05	TREE	evergreenForest	3
D05	TREE	mixedForest	3

Domain	Site Name	Vegetation	Height Category
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
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

Domain	Site Name	Vegetation	Height Category
D13	MOAB	shrubScrub	1,2
D13	NIWO	evergreenForest	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
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	srubScrub	2

APPENDIX E RESOURCES FOR CLIP STRIP HARVESTING

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

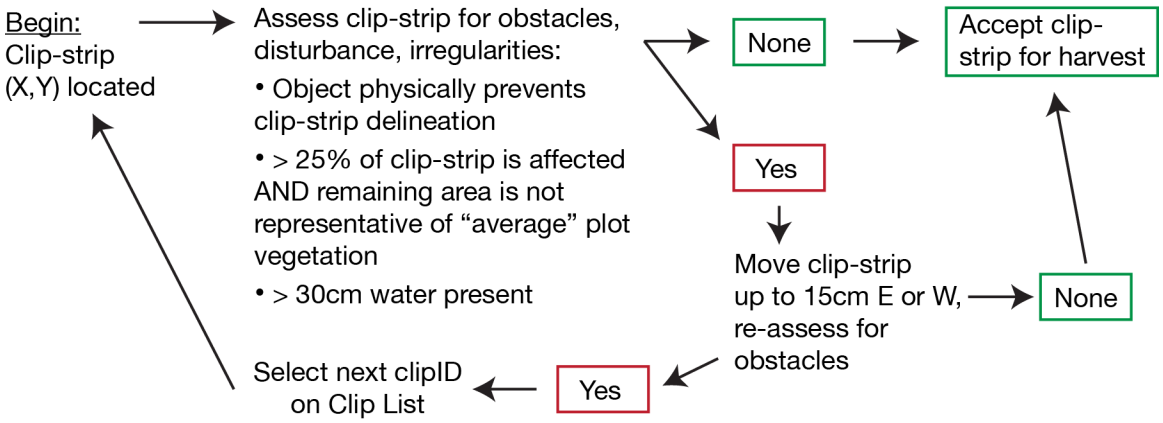

Figure 11. Flow chart to guide assessing potential clip cells for clip-harvest suitability.

Table 14. Codes to document acceptance/rejection of clip-harvest strips on the list of clip strip coordinates.

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

E.2 clipCellNumber Maps by suplotID

In certain situations, Field Operations will be required to locate clip strips within "patches" of herbaceous vegetation when the % cover of herbaceous vegetation over the entire plot is ≥ 25% AND < 75%. To identify the location of clip harvests within herbaceous "patches," first find and utilize the appropriate map below (based on subplotID) to determine which clipCellNumber should be sampled. Then, use **Table 15** 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.

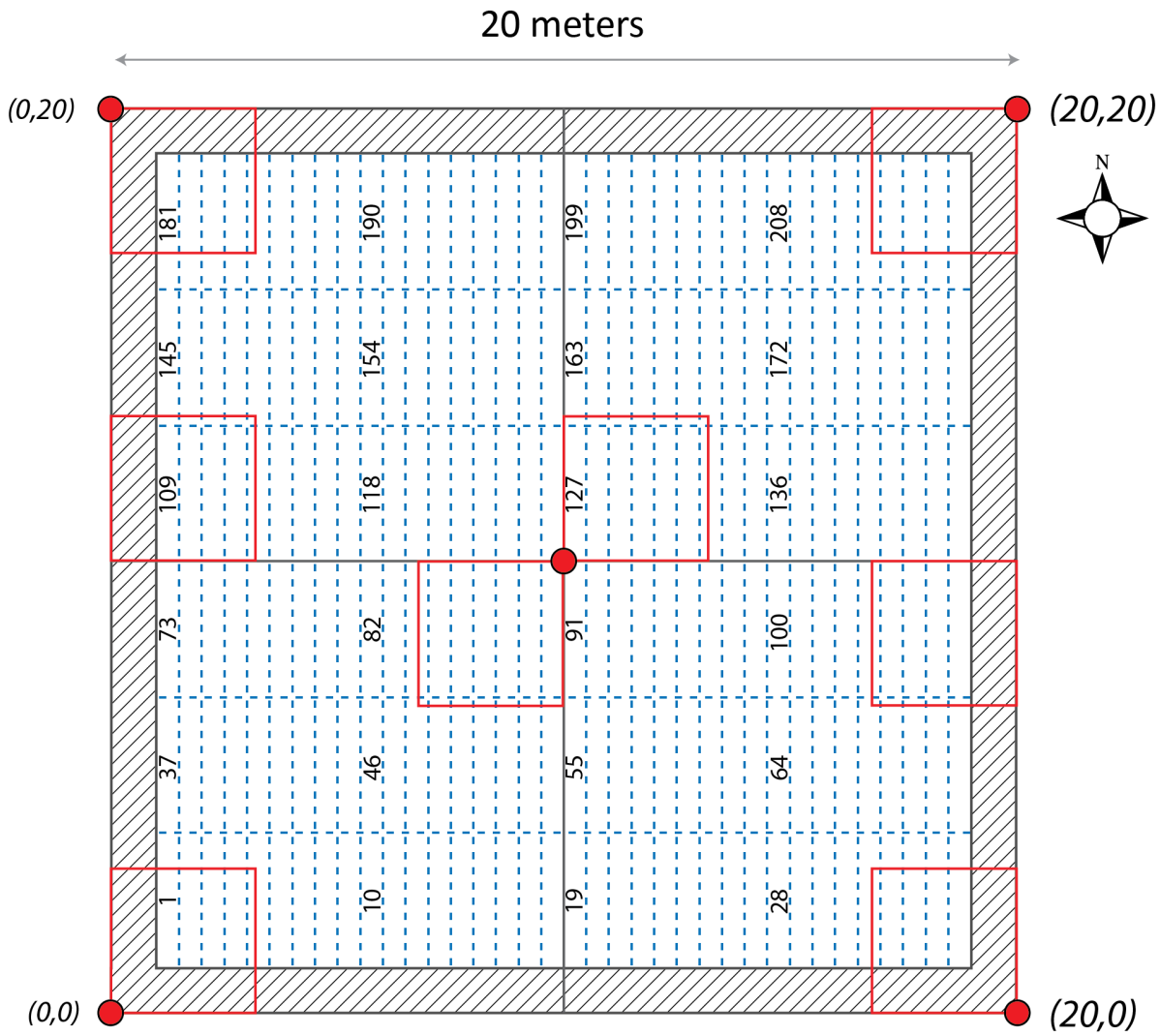

Figure 12. Map of clipCellNumbers in a 20m x 20m base plot (subplotID = 31 in provided Clip Lists). Red squares indicate nested subplots used for diversity sampling; clip cells that significantly overlap red squares are not used for clip sampling. However, clip cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support clip harvest sampling.

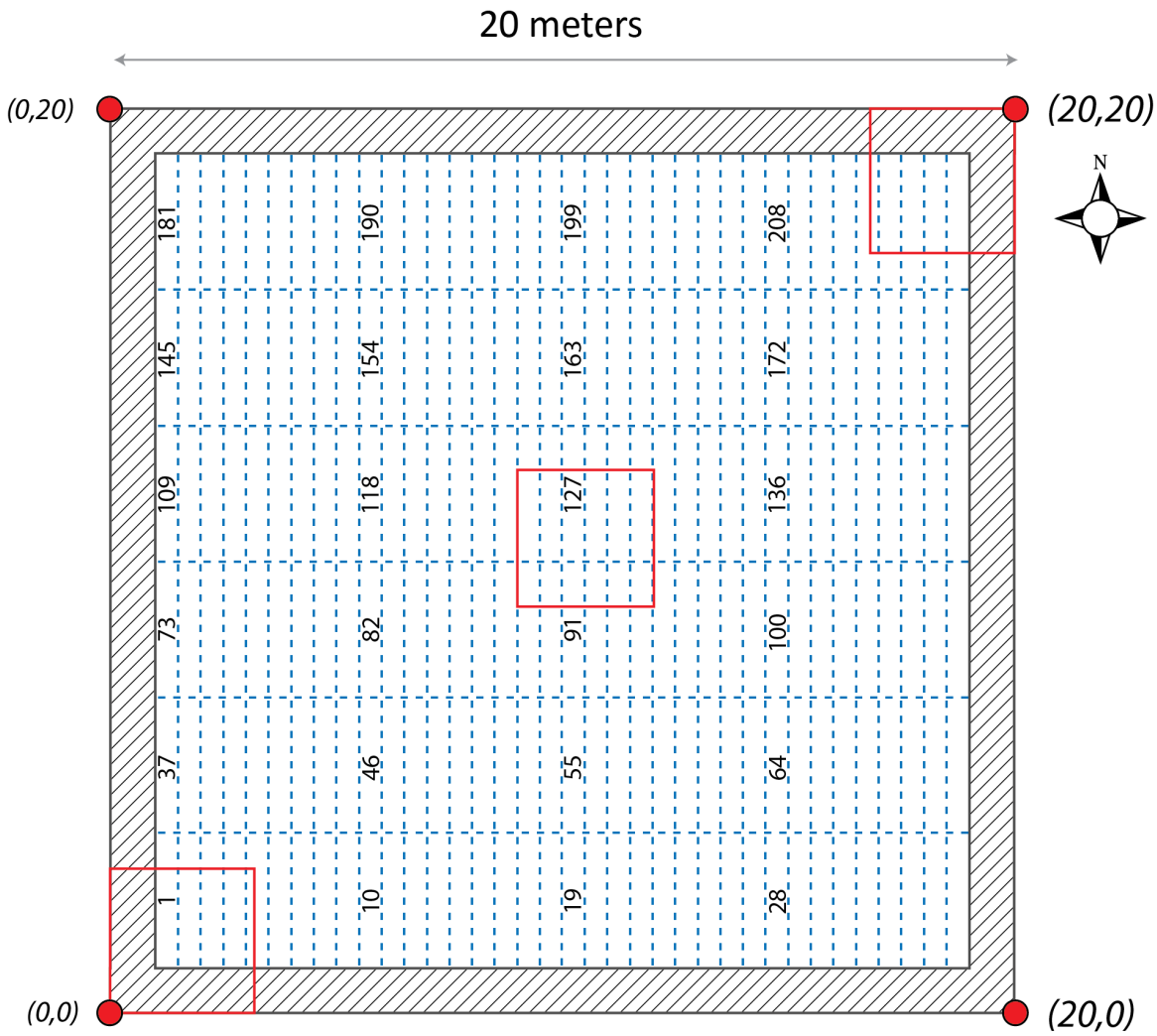

Figure 13. Map of clipCellNumbers for **subplotID = 21** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

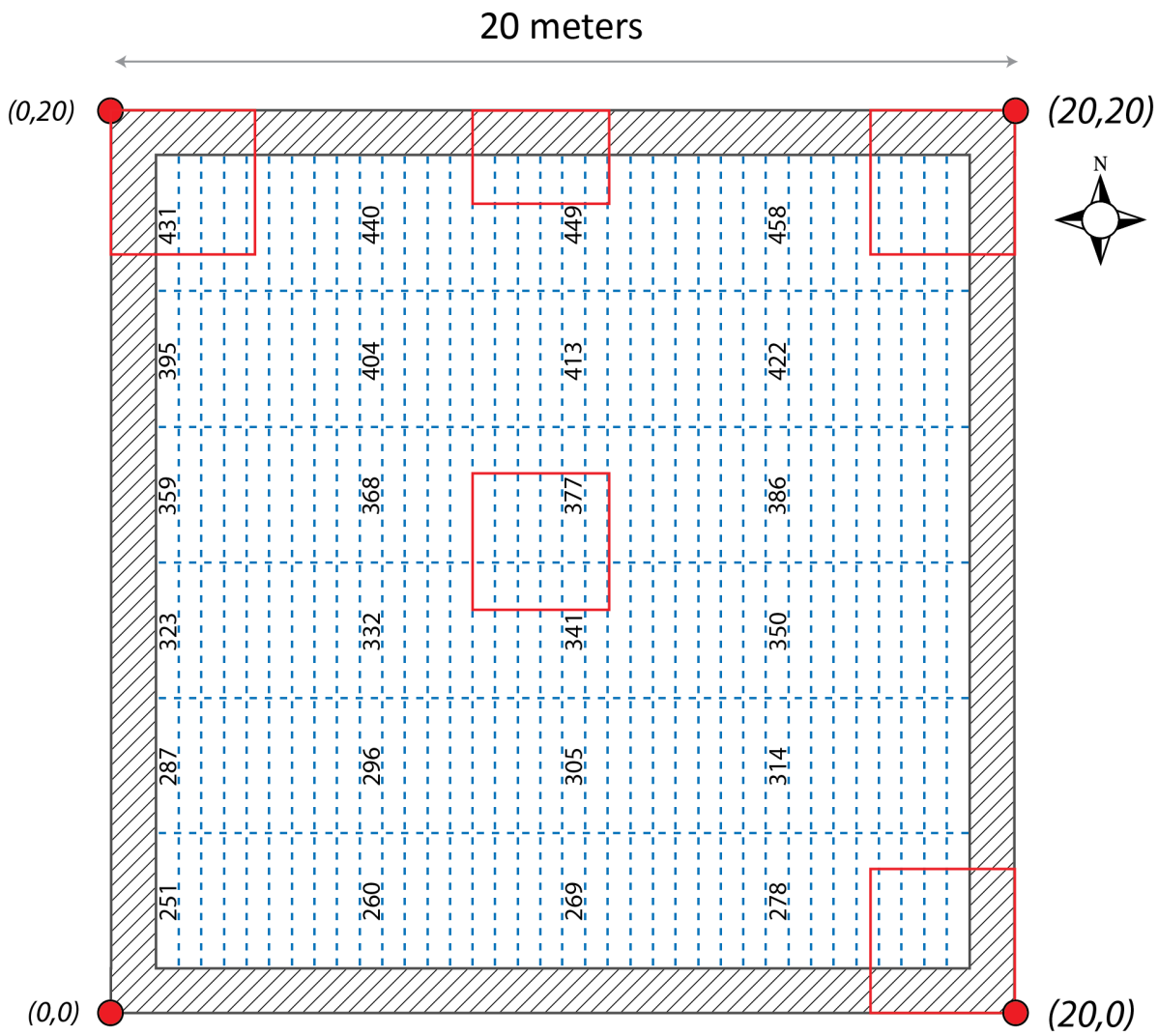

Figure 14. Map of clipCellNumbers for **subplotID = 23** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

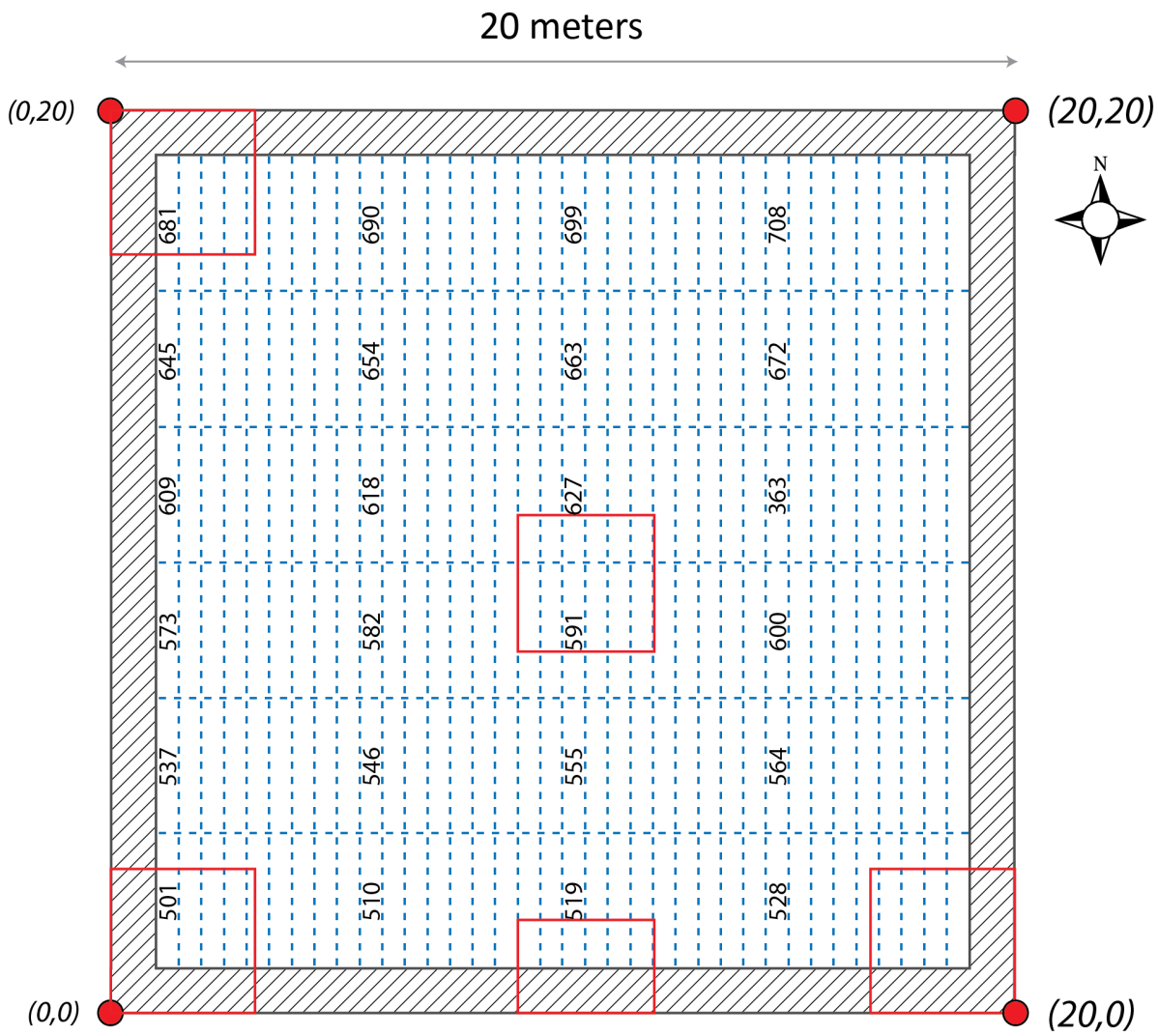

Figure 15. Map of clipCellNumbers for **subplotID = 39** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

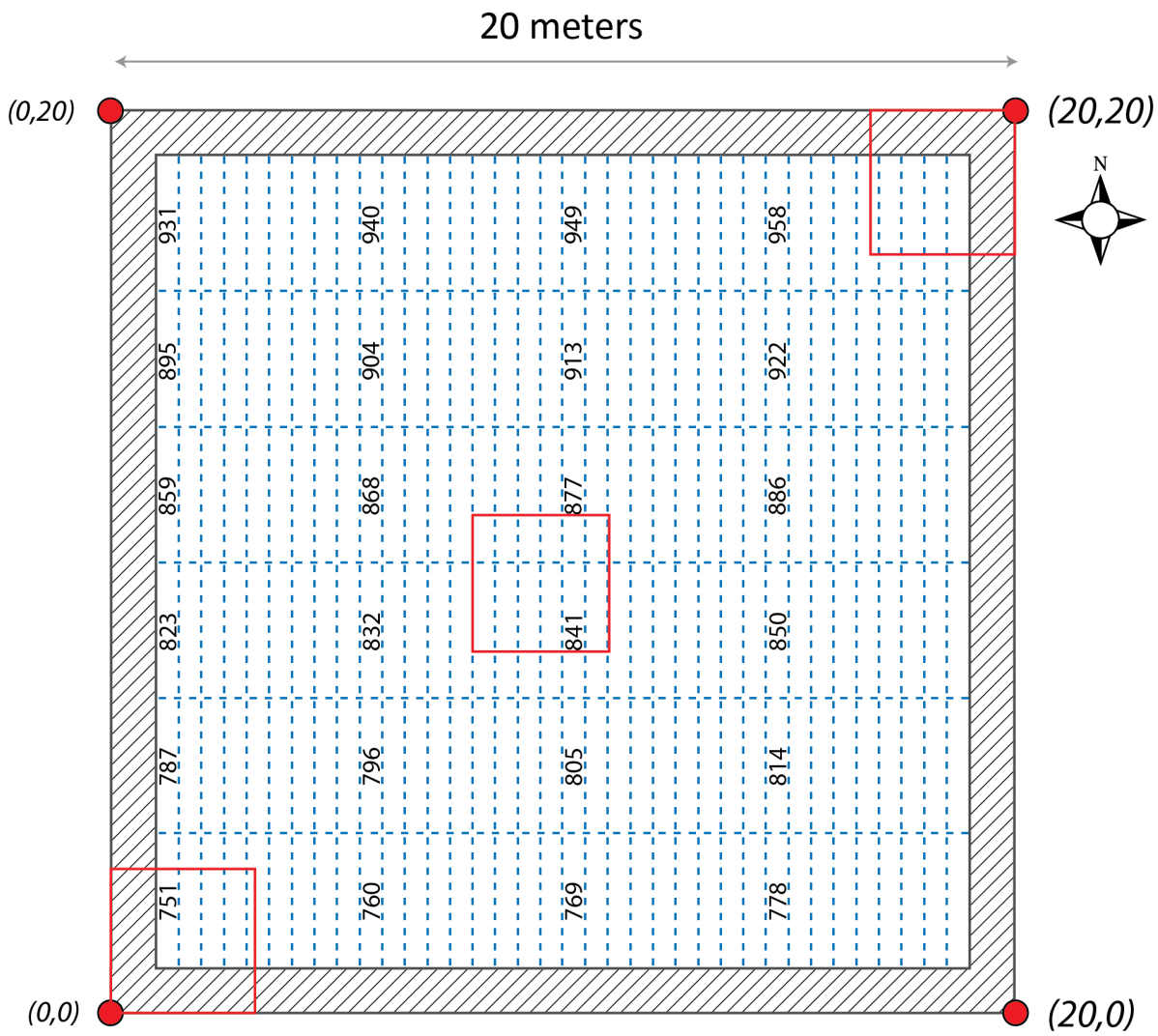

Figure 16. Map of clipCellNumbers for **subplotID = 41** in a 40m x 40m Tower base plot. Clip cells that overlap nested subplots indicated by red squares are not used for clip sampling.

E.3 Coordinates for clipCellNumbers by subplotID

Table 15. List of clipCellNumbers by subplotID and associated easting and northing coordinates. Coordinates correspond to the SW corner of a 0.1m x 2m Clip Strip, and indicate the distance in meters relative to the SW corner of the plot (subplotID = 31) or subplot (subplotID = 21, 23, 39, 41).

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

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
38	38	288	538	788	1.7	4.5
39	39	289	539	789	2.2	4.5
40	40	290	540	790	2.7	4.5
41	41	291	541	791	3.2	4.5
42	42	292	542	792	3.7	4.5
43	43	293	543	793	4.2	4.5
44	44	294	544	794	4.7	4.5
45	45	295	545	795	5.2	4.5
46	46	296	546	796	5.7	4.5
47	47	297	547	797	6.2	4.5
48	48	298	548	798	6.7	4.5
49	49	299	549	799	7.2	4.5
50	50	300	550	800	7.7	4.5
51	51	301	551	801	8.2	4.5
52	52	302	552	802	8.7	4.5
53	53	303	553	803	9.2	4.5
54	54	304	554	804	9.7	4.5
55	55	305	555	805	10.2	4.5
56	56	306	556	806	10.7	4.5
57	57	307	557	807	11.2	4.5
58	58	308	558	808	11.7	4.5
59	59	309	559	809	12.2	4.5
60	60	310	560	810	12.7	4.5
61	61	311	561	811	13.2	4.5
62	62	312	562	812	13.7	4.5
63	63	313	563	813	14.2	4.5
64	64	314	564	814	14.7	4.5
65	65	315	565	815	15.2	4.5
66	66	316	566	816	15.7	4.5
67	67	317	567	817	16.2	4.5
68	68	318	568	818	16.7	4.5
69	69	319	569	819	17.2	4.5
70	70	320	570	820	17.7	4.5
71	71	321	571	821	18.2	4.5
72	72	322	572	822	18.7	4.5
73	73	323	573	823	1.2	7.5
74	74	324	574	824	1.7	7.5
75	75	325	575	825	2.2	7.5
76	76	326	576	826	2.7	7.5
77	77	327	577	827	3.2	7.5
78	78	328	578	828	3.7	7.5
79	79	329	579	829	4.2	7.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
80	80	330	580	830	4.7	7.5
81	81	331	581	831	5.2	7.5
82	82	332	582	832	5.7	7.5
83	83	333	583	833	6.2	7.5
84	84	334	584	834	6.7	7.5
85	85	335	585	835	7.2	7.5
86	86	336	586	836	7.7	7.5
87	87	337	587	837	8.2	7.5
88	88	338	588	838	8.7	7.5
89	89	339	589	839	9.2	7.5
90	90	340	590	840	9.7	7.5
91	91	341	591	841	10.2	7.5
92	92	342	592	842	10.7	7.5
93	93	343	593	843	11.2	7.5
94	94	344	594	844	11.7	7.5
95	95	345	595	845	12.2	7.5
96	96	346	596	846	12.7	7.5
97	97	347	597	847	13.2	7.5
98	98	348	598	848	13.7	7.5
99	99	349	599	849	14.2	7.5
100	100	350	600	850	14.7	7.5
101	101	351	601	851	15.2	7.5
102	102	352	602	852	15.7	7.5
103	103	353	603	853	16.2	7.5
104	104	354	604	854	16.7	7.5
105	105	355	605	855	17.2	7.5
106	106	356	606	856	17.7	7.5
107	107	357	607	857	18.2	7.5
108	108	358	608	858	18.7	7.5
109	109	359	609	859	1.2	10.5
110	110	360	610	860	1.7	10.5
111	111	361	611	861	2.2	10.5
112	112	362	612	862	2.7	10.5
113	113	363	613	863	3.2	10.5
114	114	364	614	864	3.7	10.5
115	115	365	615	865	4.2	10.5
116	116	366	616	866	4.7	10.5
117	117	367	617	867	5.2	10.5
118	118	368	618	868	5.7	10.5
119	119	369	619	869	6.2	10.5
120	120	370	620	870	6.7	10.5
121	121	371	621	871	7.2	10.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
122	122	372	622	872	7.7	10.5
123	123	373	623	873	8.2	10.5
124	124	374	624	874	8.7	10.5
125	125	375	625	875	9.2	10.5
126	126	376	626	876	9.7	10.5
127	127	377	627	877	10.2	10.5
128	128	378	628	878	10.7	10.5
129	129	379	629	879	11.2	10.5
130	130	380	630	880	11.7	10.5
131	131	381	631	881	12.2	10.5
132	132	382	632	882	12.7	10.5
133	133	383	633	883	13.2	10.5
134	134	384	634	884	13.7	10.5
135	135	385	635	885	14.2	10.5
136	136	386	636	886	14.7	10.5
137	137	387	637	887	15.2	10.5
138	138	388	638	888	15.7	10.5
139	139	389	639	889	16.2	10.5
140	140	390	640	890	16.7	10.5
141	141	391	641	891	17.2	10.5
142	142	392	642	892	17.7	10.5
143	143	393	643	893	18.2	10.5
144	144	394	644	894	18.7	10.5
145	145	395	645	895	1.2	13.5
146	146	396	646	896	1.7	13.5
147	147	397	647	897	2.2	13.5
148	148	398	648	898	2.7	13.5
149	149	399	649	899	3.2	13.5
150	150	400	650	900	3.7	13.5
151	151	401	651	901	4.2	13.5
152	152	402	652	902	4.7	13.5
153	153	403	653	903	5.2	13.5
154	154	404	654	904	5.7	13.5
155	155	405	655	905	6.2	13.5
156	156	406	656	906	6.7	13.5
157	157	407	657	907	7.2	13.5
158	158	408	658	908	7.7	13.5
159	159	409	659	909	8.2	13.5
160	160	410	660	910	8.7	13.5
161	161	411	661	911	9.2	13.5
162	162	412	662	912	9.7	13.5
163	163	413	663	913	10.2	13.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
164	164	414	664	914	10.7	13.5
165	165	415	665	915	11.2	13.5
166	166	416	666	916	11.7	13.5
167	167	417	667	917	12.2	13.5
168	168	418	668	918	12.7	13.5
169	169	419	669	919	13.2	13.5
170	170	420	670	920	13.7	13.5
171	171	421	671	921	14.2	13.5
172	172	422	672	922	14.7	13.5
173	173	423	673	923	15.2	13.5
174	174	424	674	924	15.7	13.5
175	175	425	675	925	16.2	13.5
176	176	426	676	926	16.7	13.5
177	177	427	677	927	17.2	13.5
178	178	428	678	928	17.7	13.5
179	179	429	679	929	18.2	13.5
180	180	430	680	930	18.7	13.5
181	181	431	681	931	1.2	16.5
182	182	432	682	932	1.7	16.5
183	183	433	683	933	2.2	16.5
184	184	434	684	934	2.7	16.5
185	185	435	685	935	3.2	16.5
186	186	436	686	936	3.7	16.5
187	187	437	687	937	4.2	16.5
188	188	438	688	938	4.7	16.5
189	189	439	689	939	5.2	16.5
190	190	440	690	940	5.7	16.5
191	191	441	691	941	6.2	16.5
192	192	442	692	942	6.7	16.5
193	193	443	693	943	7.2	16.5
194	194	444	694	944	7.7	16.5
195	195	445	695	945	8.2	16.5
196	196	446	696	946	8.7	16.5
197	197	447	697	947	9.2	16.5
198	198	448	698	948	9.7	16.5
199	199	449	699	949	10.2	16.5
200	200	450	700	950	10.7	16.5
201	201	451	701	951	11.2	16.5
202	202	452	702	952	11.7	16.5
203	203	453	703	953	12.2	16.5
204	204	454	704	954	12.7	16.5
205	205	455	705	955	13.2	16.5

clipCellNumber subplotID = 31	clipCellNumber subplotID = 21	clipCellNumber subplotID = 23	clipCellNumber subplotID = 39	clipCellNumber subplotID = 41	easting offset	northing offset
206	206	456	706	956	13.7	16.5
207	207	457	707	957	14.2	16.5
208	208	458	708	958	14.7	16.5
209	209	459	709	959	15.2	16.5
210	210	460	710	960	15.7	16.5
211	211	461	711	961	16.2	16.5
212	212	462	712	962	16.7	16.5
213	213	463	713	963	17.2	16.5
214	214	464	714	964	17.7	16.5
215	215	465	715	965	18.2	16.5
216	216	466	716	966	18.7	16.5

APPENDIX F PEAK GREENNESS WINDOWS BY SITE

Table 16. 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

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	8/8
18	BASC	7/2	8/20
19	DEJU	6/2	8/18
19	BONA	6/1	8/15
19	HEAL	6/8	8/17
20	OLAA	12/23	2/4