TOS PROTOCOL AND PROCEDURE: CANOPY FOLIAGE CHEMISTRY AND LEAF MASS PER AREA MEASUREMENTS

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A	01/13/2014	ECO-01139	Draft release
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LIST OF TABLES AND FIGURES

1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- General safety practices
- Site-specific safety practices
- General equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

1.3 Acknowledgments

This protocol is based closely on standard vegetation sampling methods, as described by the North American Carbon Program (NACP), see description in Smith et al. (2008), as well as 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 information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD [01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD [02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD [03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD [04]	NEON.DOC.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD [06]	NEON.DOC.001271	TOS Protocol and Procedure: Manual Data Transcription

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000906	TOS Science Design Terrestrial Biogeochemistry
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001025	TOS Field Protocol for Plot Establishment
RD [08]	NEON.DOC.001403	NEON Raw Data Ingest Workbook for TOS Terrestrial Biogeochemistry: Chemistry of Soils and Plants
RD [09]	NEON.DOC.001576	Datasheets for Field and Laboratory Protocol: Canopy Foliage Chemistry and Leaf Mass per Area Measurements
RD [10]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration

2.3 Acronyms

Acronym	Definition
^{12}C	Most common isotope of carbon
^{13}C	Less common isotope of carbon
Ca^{2+}	Calcium
DBH	Diameter at Breast Height
K^{+}	Potassium
LMA	Leaf Mass Per Area
LiDAR	Light Detection and Ranging
Mg^{2+}	Magnesium
^{14}N	Most common isotope of nitrogen
^{15}N	Less common isotope of nitrogen
NACP	North American Carbon Program

2.4 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A **procedure** is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 Background

This document describes the required protocols for conducting field sampling of plant canopy tissues for analysis of elemental concentrations (C, N, P, S, K^+, Ca^{2+}, Mg^{2+}), lignin, chlorophyll, isotopic composition ($^{13}C/^{12}C$ and $^{15}N/^{14}N$), and leaf mass per area (LMA). We are interested in quantifying changes in canopy nutrient stocks over time, and using the isotopic composition of leaves (as well as leaf litter, roots, and soils) to follow how the sources of nutrients to and metabolism of the ecosystem change over the lifetime of the Observatory. This dataset is generated in collaboration with the Airborne Observation Platform (AOP), which is largely responsible for mapping plant chemical and physical characteristics using spectral and LiDAR measurements; in part, the ground-based data will be used to ground-truth the airborne measurements as well as to extend the airborne data to create maps of canopy N.

Typically, stocks of C, N, and other elements are expressed as mass per unit area (e.g., g C m^{-2}). This calculation requires knowing the dry mass of leaf tissue per area (i.e., LMA, g m^{-2}) on a per-species basis, the relative abundance of that species per unit area, and the concentration (or amount) of the element per gram of dry leaf tissue (e.g., mg g^{-1}). Isotopic ratios, the measure of a less common isotope (e.g., ^{15}N) relative to the most abundant isotope of an element (e.g., ^{14}N), give insight into the integrated ecosystem processes occurring within soils or other media; the isotopic ratio for a particular ecosystem constituent is determined by the source (of N, in this case) or the degree to which it has been influenced by microbial processes. It is expressed as per mil (‰) using the delta (δ) notation.

Foliar chemistry data provide scientists, managers, and decision-makers with important information on the nutrient stocks and status of an ecosystem. Comparing these data with those from other ecosystem components, including atmospheric deposition, soils, leaf litter, woody tissues, and surface water transformations and transport, allows investigators to evaluate material fluxes across the landscape. As a long-term dataset, they can be used to address how the ecosystem changes 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 nutrient stocks in the plant canopy will likely cascade through the ecosystem, changing the fluxes and transformations of N across the landscape. The overall science design for this component of NEON is described in TOS Science Design Terrestrial Biogeochemistry (RD [03]).

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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 [04]).

4 PROTOCOL

The field protocol used by NEON for collecting leaf canopy samples to analyze chemical constituents and leaf mass per area (LMA) closely follows the approach described by Smith et al. (2002; 2008). Foliar chemistry and LMA vary considerably among species and individuals, therefore, we sample several individuals from up to four dominant species in our study plots to calculate elemental masses per area as accurately as possible. Canopy sampling locations vary for each NEON site; this information will be provided in a separate document to field personnel. Additionally, multiple samples per individual must be collected and pooled, both to obtain enough material for analysis and to collect a representative sample at the scale of the individual.

Forty-meter by forty-meter plots (Distributed and if necessary, Gradient Plots) are distributed across the study area for sampling foliar chemistry and leaf mass per area (LMA). Within the tower airshed, another set of plots (whose sizes differ by location) is established (i.e. Tower Plots) Prior to sample collection, plots where canopy samples will be collected should be identified and marked according to the plot establishment guidelines provided by TOS Field Protocol for Plot Establishment (RD [07]). In addition, individuals identified for sampling within each plot should be flagged according to the guidelines described below, and a schedule determined for the order in which plots should be sampled. This prep work will enable more expedited sampling.

It is critical that the leaf samples be collected from the uppermost part of the canopy (i.e., sun-lit leaves). The AOP remote-sensing instruments scan sun leaves at the top of the canopy; because we are interested in linking the AOP measurements with the terrestrial observations, it is important that the correct leaves be collected. Occasionally, part of the sampling design may include collecting leaf samples at different heights through the canopy in order to understand how nutrient mass (e.g., N) varies along a vertical profile; at many NEON sites, there is likely to be a significant vertical gradient.

In low- (≤ 2 m) and mid-stature (2-6 m) vegetated ecosystems, sun leaves may be obtained using clippers and pole pruners, respectively (see Appendix A). In many forested systems where the canopy is well out of human reach, it will be necessary to shoot down or climb trees to obtain sun leaves. This approach requires participation of a person trained to shoot leaves out of trees with a valid shotgun permit for the sampling location or is a capable, trained tree climber. Use of a slingshot is also common, although, again, this aspect of sampling requires a trained individual. Additional participation of two individuals who can work alongside the shooter to bag, label, and stow samples is required.

As samples are collected, they should be sealed in freezer resealable plastic bags and placed on cold packs in coolers. Processing must occur within 24-hr following collection, and shipment to contracted analytical facilities must occur shortly thereafter.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD [05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (RD [06]).

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met.

Canopy leaf samples should be collected from positively identified dominant vegetation species. Should it begin raining during sampling, continue to collect leaf samples (as long as conditions do not progress to thunder and lightning, hail, flash flooding, etc, and are safe), but make a note of the weather change in the PDA or datasheets and field notebook. When samples need to be shot down, it is possible that sampling will need to stop earlier than would be necessary for pole pruning or manual clipping; in this case, the appropriate time to stop or pause sampling efforts is at the discretion of the shooter.

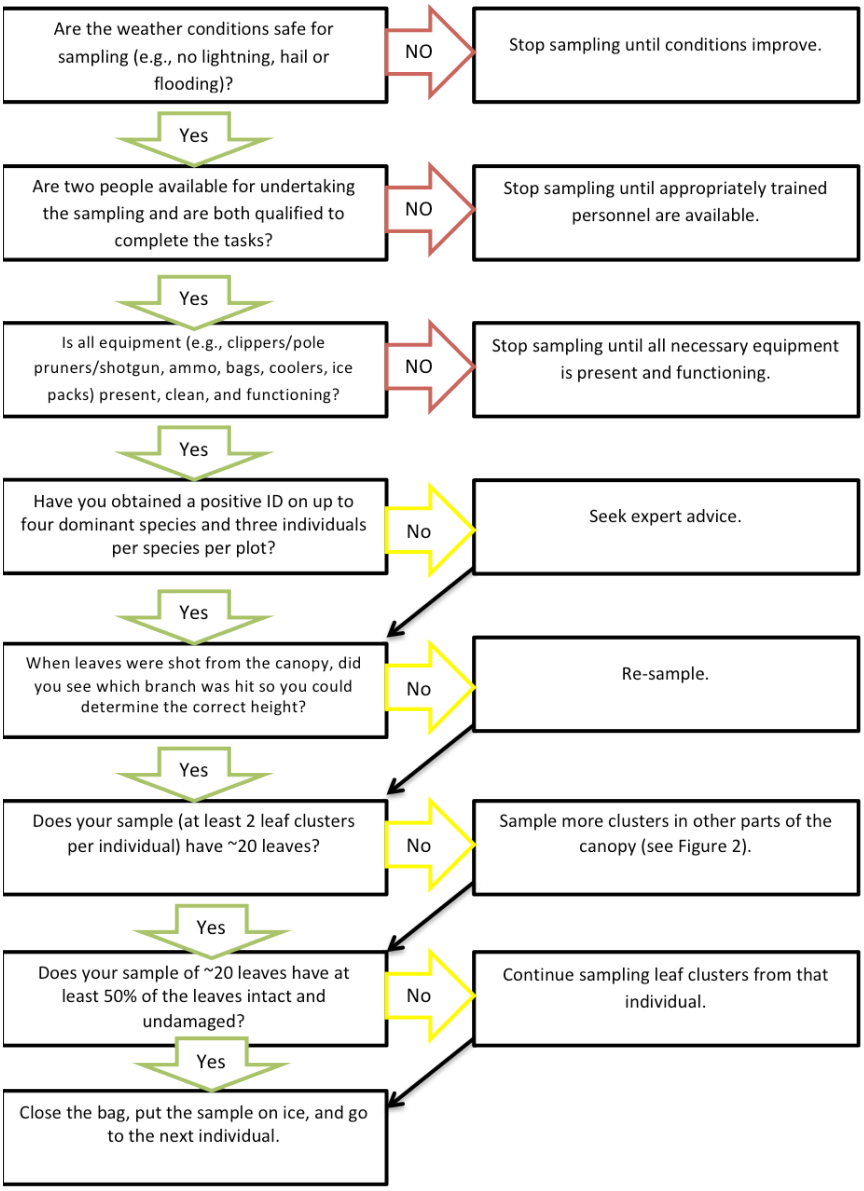

Figure 1. Decision tree

6 SAFETY

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

Personal Protective Equipment (PPE) required for this activity includes the following items:

- Safety glasses

Tree canopy sampling often requires the use of a shotgun to obtain leaves from the top of the canopy. **ONLY THE INDIVIDUAL WHO IS AUTHORIZED TO USE THE SHOTGUN MAY HANDLE IT.** Field personnel must familiarize themselves with safety procedures for shotgun canopy sampling.

CANOPY SAMPLING:

- PPE required during all Canopy Sampling includes the use of a hard hat, safety glasses, work shoes/boots, hearing protection with a minimum of a 28NRR (NEON EHS 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.

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.

7 PERSONNEL REQUIREMENTS

When sampling canopy leaves, field personnel must be familiar with the vegetation species at each site. Field guides, archived samples, and a dedicated plant expert on the domain staff must be available during the field effort. It is always preferable that field personnel stop work and ask questions of relevant experts rather than complete the work incorrectly. Field personnel should be prepared to take extensive notes on any anomalous vegetation species or features that they observe when sampling, or in-field decisions that they make. If a sampled species cannot be identified in the field, use the datasheets to take notes, take a good representative sample, and work with experts in the domain lab to identify upon return from the field.

8 TRAINING REQUIREMENTS

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

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 safety. Refer to (RD [04]). Additionally, field personnel must be trained in proper use of a laser range finder to determine the height from which canopy samples were obtained.

9 SAMPLE FREQUENCY AND TIMING

9.1 Sampling Frequency and Timing

The timing, frequency, locations, species selection, and extent of canopy leaf sampling constitute "the science design", and vary by NEON site. The timing of sampling allows researchers to assess terrestrial biogeochemical cycles within a particular window, and, therefore, timing depends on the dominant drivers that affect plant phenology, microbial communities, and other influences on the stocks and fluxes of nutrients in ecosystems. These drivers include, but are not limited to, climate forcing (e.g., solar radiation, air and soil temperature, and precipitation), disturbance (e.g., ice storms, wildfire, land use/land cover change), and plant phenological patterns. The frequency of sampling allows researchers to investigate how nutrient dynamics change within and between seasons or years; for example, at Domain 1, broad-leaf deciduous species will be at peak growth in the summer months (e.g., June, July, August) and senescing in the fall months (e.g., September, October, November). Finally, the extent of canopy sampling allows researchers to evaluate the spatial heterogeneity of nutrient stocks and fluxes; we might expect that differences in soil type, plant communities, or hillslope aspect will affect N availability in the soil, and, thus, primary productivity. At the different NEON sites, sampling will occur more or less frequently, and to a greater or lesser spatial extent, depending on the climatic factors and landscape features, as well as logistical (e.g., site accessibility) and financial constraints.

In addition, timing of airborne data collection by the AOP factors into the timing of canopy foliar sampling for biogeochemistry. Ground and airborne datasets will be analyzed together, so these efforts must be coordinated. The canopy foliar collection schedule will specify targeted timing of the sampling that factors in the phenological state discussed above, and the AOP schedule. Canopy foliar samples will be collected at NEON sites once every 5 years, distributed across the entire site. Our expectation is that a 5-year sampling frequency will sufficiently capture a long-term trend in biogeochemical properties of foliar tissues, and provide sufficient data to conduct calibrations of annually-collected AOP hyperspectral data.

9.2 Criteria for Determining Sampling Dates

Sampling of canopy foliar tissues for biogeochemistry occurs during peak biomass, and coincident with AOP flights. Ground-based sampling should be done ±2 weeks of the AOP flight. Direct communication with AOP staff and NEON HQ staff will be required to properly time the field collection.

9.3 Sampling Frequency

Sampling occurs once every 5 years in tower, distributed, and gradient plots. Specific locations will be provided in the collection schedule.

9.4 Sampling Timing Parameters

Sampling shall occur ± 2 weeks of AOP flights. At sites where there are multiple peaks in biomass (e.g., some grassland sites), NEON staff will provide additional instructions regarding timing of sampling and number of sampling events.

Sampling for foliar chemistry and LMA occurs in conjunction with plant biomass measurements and/or clip harvests (grassland sites).

Sampling occurs until one event (i.e., bout) is complete. At most sites, it will take 1 week with 2-4 technicians dedicated to sampling, plus travel to and from sites, and sample processing (e.g., bagging, labeling, drying, LMA measurements, and shipment).

10 STANDARD OPERATING PROCEDURES

SOP A: Preparing for Sampling

Use Appendix B to determine the appropriate methods for sampling at your site(s).

SOP B: Field Sampling

Field Equipment and Materials

Table 1. Equipment list for sampling one plot

Maximo Item #	Item Description	Purpose	Quantity	Condition-Specific	Special Handling
Suggested	Backpack	Field equipment transport	1	N	N
Suggested	GPS, recreational accuracy	Plot location	1	N	N
Suggested	Rolled flagging to mark dominant species for sampling	Marking individuals to be sampled	1	Tall- and mid-stature vegetation	N
Suggested	Flags to mark clip harvest areas	Sample area marking	20	Short-stature vegetation	N
Suggested	Clippers	Obtaining samples	1	N	N
Suggested	Pole pruner	Obtaining samples from mid-stature forests	1	Mid-stature vegetation	Y
Suggested	Shotgun and ammunition (responsibility of Designated Shooter)	Obtaining samples from tall-stature forests	1	Tall-stature vegetation	Y
Suggested	Laser range finder	Determining sample collection heights from trees	1	Tall- and Mid-stature vegetation	N
Suggested	Glove, Nitrile (Latex-Free), Non-Sterile	Sample handling	1 box	N	N
Suggested	Cooler	Sample preservation and transport	1	N	N
Suggested	Ice packs	Sample preservation	5	N	N
Suggested	Resealable plastic bags (gallon size)	Sample storage	1 box	Broad-leaf vegetation	N
Suggested	Resealable plastic bags (quart size)	Sample storage	1 box	Small leaf and needle leaf vegetation	N
Suggested	Meter tape	Measuring clip harvest areas, heights of measurements	2	N	N
Suggested	Permanent markers	Labeling	3	N	N
Suggested	Pencils	Labeling	3	N	N
Suggested	Field notebook	To record info	1	N	N

Maximo Item #	Item Description	Purpose	Quantity	Condition-Specific	Special Handling
RD [09]	Datasheets	To record info	4	There are specific datasheets for Tall-stature and clip harvest; Mid-stature requires both, see (RD [09])	N
Suggested	PDA	Data entry	1	N	N
Suggested	Extra batteries for GPS (AA)	To power GPS	2	N	N
Suggested	Extra batteries for laser range finder (CR123A)	To power laser range finder	1 set	N	N
Suggested	Extra batteries for PDA (type TBD)	To power PDA	1 set	N	N
Suggested	Dedicated trash bag	Disposing of used gloves and wipes	1	N	N

Select individuals for sampling

1) With GPS unit, find the first plot.
2) Determine the dominant (by percent cover/biomass) species in the plot. The approach will depend on the type and height of vegetation (see Appendix A for pre-characterization/plot establishment determination by NEON site).

Case 1: Predominately tall-stature plots

1) Identify the dominant (≥ 50%) and/or up to 4 co-dominant (≥ 20%) canopy species (i.e., species that make up the majority of the uppermost part of the canopy and/or are visible to the AOP; may or may not be stems ≥ 10 cm DBH) within the plot.
2) Choose up to 5 individuals of the dominant species, and 3 individuals per co-dominant species for sampling foliar chemistry and LMA.
3) In most cases, 5 to 12 trees should be selected per plot

Case 2: Predominately short-stature plots where vegetation is alive

1) If entire plot is relatively homogenous, use the nested plot scheme (Figure 2) to identify the dominant/co-dominant species present in one randomly chosen 10 m^2 nested plot within each subplot (randomly-chosen by flipping a coin or doing rock-paper-scissors).
2) Determine the percent cover of the species within each 10 m^2-nested plot.
3) Using the results from the 4 nested plots, determine the "average case" that is representative of cover within the entire plot.
4) If the plot is not homogenous (i.e., sampling one subplot may not represent the entire plot) subjectively assess the most dominant species by cover (**Must be done by a trained technician.**).

5) As many as 10 dominant (≥ 50%)/co-dominant (≥ 20%) species may exist. Sample up to 5 individuals per dominant species and 3 individuals per co-dominant species across the entire 40m x 40m plot.

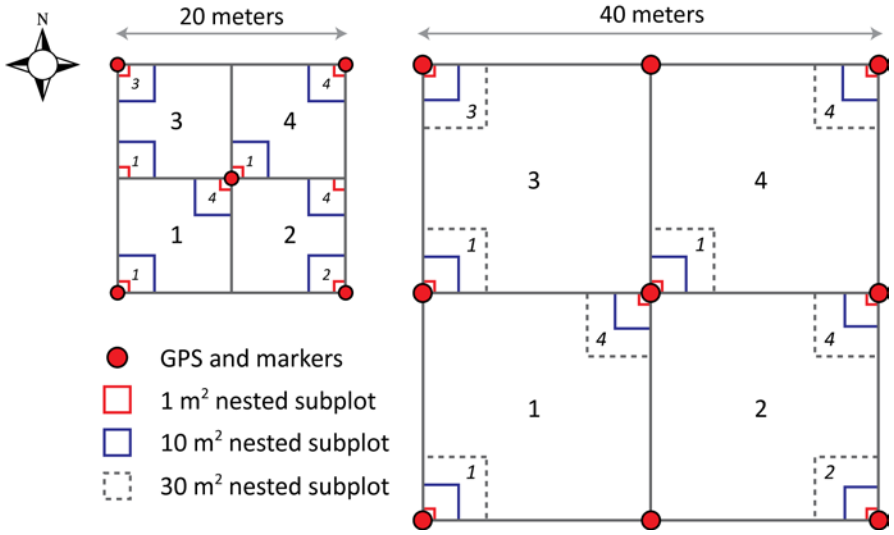

Figure 2. 20m x 20m and 40m x 40m tower plots. Distributed plots have a central subplot where destructive sampling is not permitted

Case 3: Predominately short-stature plots where vegetation is dead

1) For all subplots (i.e., four 20m x 20m subplots/plot) that have short-stature plants present use a randomly-generated angle listed in the table provided by NEON staff as part of the collection schedule.
2) Beginning with module 1, check first entry in the column for "Module1 angles"
3) Standing at the plot center, site the appropriate angle with a compass, and walk in that direction until a suitable patch of herbaceous vegetation is found for clip-harvest. Bear in mind that the minimum distance walked must equal the first "Min Dist from Center" value.
4) If no suitable location is found along the first angle, but suitable vegetation is visible within this module, repeat step ii using the second entry in the "Module1 angles" column, and use the same minimum distance as before.
5) Repeat steps 3 and 4 until a suitable swath (0.2 m x 2 m) of herbaceous veg is found. A suitable swath is defined as presence of grass and/or bare ground without an overhanging tree canopy.
6) Mark this area (2m length of the swath oriented due north-south).
7) Move to Module 2, and use the "Module2 angles" column and the second entry in "Min Dist from Center".
8) In all cases, mark tree and shrub individuals around their stems (or biomass harvest area with flags). Write the sampleID for the sample on the label.

Special considerations pertaining to Cases 1, 2, and/or 3

1) There is only one species in the plot (e.g., 100% Red oak)
    a. Sample a total of 5 individuals within the 40m x 40m plot
2) There is not a clear “dominant” species in the plot (> 50%), only several co-dominant species (e.g., 40% Red oak, 40% Red maple, 20% White pine).
    a. Sample three individuals per co-dominant species.

3) There are less than three individuals present of a species that makes up a large part of the canopy
    a. Sample that individual (or two individuals) and note anomaly in the PDA or field datasheets.
4) There is mixed tall- and short-stature vegetation in the uppermost part of the aerially-visible canopy.
    a. Decide whether or not to use the Peet nested plot scheme to estimate the relative abundance (i.e., 50% White oak, 25% Black oak, 25% Pokeberry) and choose the number of individuals to sample accordingly (e.g., 5 White oak, 3 Black oak, and 3 Pokeberry).
5) There are not enough leaves on one short-stature specimen to provide an adequate sample.
    a. Pool leaves from additional stems or individuals growing in close proximity (< 59 cm). Make a note of this in the PDA.
6) Some individuals of a dominant species show signs of disease/sickness/herbivory
    a. Sample diseased/sick individuals if the disease/sickness is a dominant characteristic (> 50%) of the plot.
    b. Indicate evidence of disease in the "Diseased? (Y/N)" field. If you can identify the disease, write it in the "Notes" field.

Obtain the samples

Case 1: Tree species that require a shotgun (could be Tall- or Mid-stature communities)

1) Make sure that you are wearing appropriate PPE (see Section 6 of this protocol) and adhere to instructions by the marksman regarding where to stand and when shooting is occurring.
2) Decide how the tree canopy will be divided in order to get clusters representative of the entire individual crown, as well as a sample (consisting of multiple leaf clusters) that has ~ 20 leaves (Figure 2).
3) Collect > 20 for large or compound leaves; ~20 for small leaves; >>> 20 needles (i.e., multiple branchlets, comprised of multiple needle fascicles) for coniferous
4) The marksman will shoot down the clusters. Determine the height of each using the Laser Range Finder and the "Height Routine" (see RD [10]) and write the heights down in the datasheets (we will calculate an average height sampled for each individual).
5) If samples came from a range of heights, please note on datasheet.

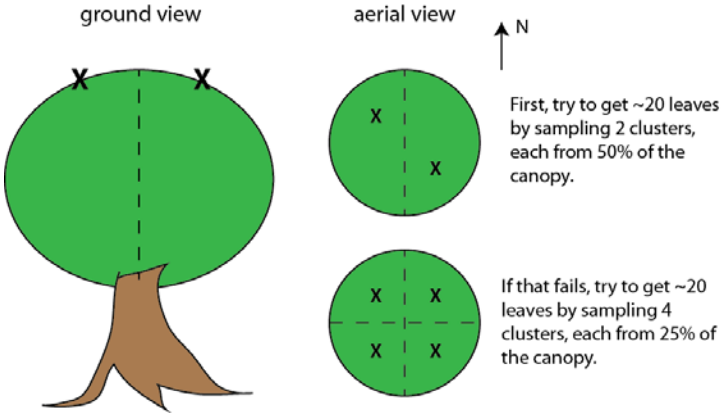

Figure 3. Division of an individual plant canopy crown for sampling. The same principles apply for shrubs. If further division is required to obtain the requisite samples, continue to do so symmetrically.

6) Wear a clean pair of Nitrile (Latex-free) gloves for each individual tree
7) Retrieve the sample
8) Bag the sample in an appropriately sized resealable plastic bag, noting that at least 50% of the leaves on the clusters are intact and do not contain shotgun holes. (If necessary, shoot down more leaf clusters).
9) Label the bag with the plotID, sampleID, eventDate, scientificName, measuredBy (technician name), and recordedBy (technician name)
10) Enter required information into the field datasheet
11) Remove gloves and stow in a dedicated trash bag
12) Collect shotgun shells and wadding and remove from plot
13) Do not contaminate gloved hands by collecting shells and wadding while samples are still being collected from the same tree

Case 2: Vegetation species clipped by hand (shrubs)

1) Put on a pair of Nitrile (Latex-free) gloves. You only need 1 pair of gloves per shrub/grass individual, but you MUST put on a new pair for each individual; do not reuse gloves.
2) Decide how the shrub or grass canopy will be divided in order to get clusters representative of the entire individual crown, as well as a sample (consisting of multiple leaves or blades) that has $\geq$ 20 leaves or blades (Figure 3).
3) Use the clippers to obtain the leaf clusters or grass blades. Note the height of each sample using the meter tape.
4) Write height values in the datasheet.
5) Bag the sample in an appropriately sized resealable plastic bag
6) Label the bag with the plotID, sampleID, eventDate, scientificName, measuredBy (technician name), and recordedBy (technician name)
7) Enter the required information into the datasheet
8) Remove gloves and stow in a dedicated trash bag

Case 3: Manual clip-harvest (grass species, alive or dead)

1) Put on a clean pair of Nitrile (Latex-free) gloves. You only need 1 pair of gloves per clip strip, but you MUST put on a new pair for each strip; do not reuse gloves.
2) Use the clippers to obtain the leaf clusters or grass blades.
3) Take 4 height measurements using the meter tape. Write these height values in the datasheet.
4) Clip entire area (0.2 m x 2 m) of grass, to 1-2 cm above the ground surface. **You do not need to separate by species**.
5) Place bulk sample into resealable plastic bags.
6) Make sure that each bag containing a subsample of the 0.2 m x 2 m harvest area has the same label (including plotID, sampleID, eventDate, and technicans' names (measuredBy)), so that subsamples can be recombined into one sample in the lab. Label the series of subsamples "1/n, 2/n…n/n" where 'n' is the total number of subsample bags for the sample. This will help the person sorting through them in the lab to figure out how many subsamples to expect per sampleID.
7) Fill out the datasheets.
8) Remove gloves and stow in a dedicated trash bag.
9) Special considerations
    a. There is bare soil.

i. Harvest the aboveground biomass that is present, and indicate presence of bare soil in datasheet or PDA.

SOP C: Sample Preservation

1) Upon return to the lab, make sure that leaves (especially broad-leaf deciduous species) are stored flat in the resealable plastic bags.
2) It helps to stack them individually before transferring to a bag.
3) If the samples cannot be processed and shipped within 24 hr, store in sealed resealable plastic bags in a refrigerator (4°C).). If you are working remotely, samples must be kept on fresh ice packs (change every 12 hr).

SOP D: Laboratory Processing of Foliar Samples: Subsampling and Drying Field Samples

Following field collection, canopy foliar samples must be subsampled for Leaf Mass per Area (LMA) measurement, nutrient and isotopic analysis, and archiving. The exceptions are clip harvest collections, which must be dried and weighed prior to subsampling.

Case 1: Canopy foliar samples (broad-leaf, small leaf, and needle leaf species)

Subsampling

Applies to all types of samples except those collected by clip harvest (alive or dead grass collections)

1) Wear a new pair of Nitrile (Latex-free) gloves for each sample
2) For archive samples: place ~1/3 of leaves or needles collected into an unsealed coin envelope
3) Do not include woody material or stems (i.e., pick off needles or compound leaves from woody parts, being careful to leave the needles or leaves intact)
4) Transfer information from field resealable plastic bag to coin envelope
5) Coin envelopes from the same plot can be placed together in paper lunch bags (label the lunch bags with site, plotID, eventDate, and "Archive")
6) For LMA samples: place ~1/3 of leaves (but at least 6 leaves) in a new resealable plastic bag, seal bag
7) Store LMA samples in refrigerator until time to make measurements (must be within 4 days of collection or they will rot)
8) For nutrient/isotopic analysis samples: place 1/3 of leaves collected into an unsealed coin envelope
9) Transfer information from field resealable plastic bag to coin envelope
10) Coin envelopes from the same plot can be placed together in paper lunch bags (label lunch bag with site, plotID, eventDate, and "Nutrients/Isotopes")

Drying

Applies to samples for archiving and nutrient/isotopic analysis; not LMA subsample

1) Get appropriate datasheet for sample drying from Datasheets for Field and Laboratory Protocol: Canopy Foliage and Leaf Mass per Area Measurements (RD [09])
2) For archive and nutrient/isotopic subsamples: place grouped (in lunch bag) unsealed coin envelopes into drying oven
3) For ease, paper bags can be put on cafeteria trays for loading into oven (not required)
4) Dry 5 empty, unused paper bags
5) Dry at 60°C for 48h, recording appropriate information in datasheet fields
6) When drying is done, seal coin envelopes and store in cool, dry cabinet prior to shipment

Case 2: Grasses Sampled by Clip Harvest (Alive or Dead Collections)

Drying and Weighing

1) Get appropriate datasheet for sample drying from Datasheets for Field and Laboratory Protocol: Canopy Foliage and Leaf Mass per Area Measurements (RD [09])
2) Transfer samples from resealable plastic bags to paper bags
3) Take care not to lose any vegetal material in the transfer; you will have to get quantitative weights of these samples.
4) Label paper bags with all information on field resealable plastic bags
5) Group paper bags by clip strip (i.e., "1/n, 2/n…n/n")
6) Place grouped paper bags into drying oven
7) For ease, paper bags can be put on cafeteria trays for loading into oven (not required)
8) Dry 5 empty, unused paper bags
9) Dry at 60°C for 48h, recording appropriate information in datasheet fields
10) When drying is done, weigh samples (+paper bag, which you will subtract in the datasheet) and record weights to nearest 0.01 g in datasheet
11) Weigh the dried 5 empty paper bags as a group and record weight to nearest 0.01 g in datasheet
12) Store samples in a cool, dry cabinet prior to shipment

Table 2. Equipment List for processing plant foliage from one site

Maximo Item #	Item Description	Purpose	Quantity	Condition -Specific	Special Handling
Required	4" x 5" Coin envelopes	Drying samples	1 box	N	N
Required	Paper bags	Drying samples	1 box	N	N
Suggested	Cafeteria trays	Organizing samples	4	N	N
Suggested	Pencils	Labeling	2	N	N
Suggested	Permanent markers	Labeling	2	N	N
Required	Scale	Weighing samples	1	N	N
Required	Drying oven	Drying samples	1	N	N
RD [09]	Datasheets	Recording sample info	---	N	N

SOP E: Laboratory Processing of Foliar Samples: Leaf Mass per Area (LMA) Measurements

Case 1: Leaf punching broad-leaf deciduous

Leaves must be big enough for either 1/2", ¾", or 1.5" diameter punch

1) Use the largest punch tool possible for each leaf. Punch 3-5 circles per leaf depending on size of leaf. One punch should be centered on the leaf, with the mid-vein in the middle. Aim for 15-30 punches per sampleID (i.e., from your ~6 leaves per subsample for LMA).
2) Place punches in coin envelopes
3) Save remaining fresh sample in refrigerator
4) Enter required metadata and sample information into datasheet
5) Place coin envelopes in a cool dry area until samples can be dried at 60°C for 48 hours.

Case 2, Part 1: Scanning needle-leaf species (and small leaves that are <1/2" area)

1) Scan 20 needles (or 10 leaves, depending on what fits within scanning area) per sample, laid in two columns on scanner screen
2) Create scanning template: full color, high resolution (600 dpi), with contrast and sharpness to the highest level, how you do this will depend on the scanner you are using
3) Write sampleID on white sheet of paper and lay it on the screen with needles underneath as well as a scale bar showing 20 cm for calibration purposes later on (you want this to show in your scan)
4) Upload scans as .tif files and save all of them in appropriate NEON network drive (TBD)
5) After scanning, place scanned needles or leaves in coin envelopes labeled with sampleID, eventDate, and your name (measuredBy)
6) Enter required metadata and sample information into datasheet
7) Place the coin envelopes in a cool dry area until samples can be dried at 60°C for 48 hours

Case 2, Part 2: Measuring the area of needle-leaf species (and small leaves that are < ½" area)

1) First, to download the 'image J' software
2) Go to http://imagej.nih.gov and you can download it for Windows, Mac OS X, and Linux, you can also download the source code for the program or view it online. Make sure you have the up to date version of Java installed on your computer before downloading
3) Open the needle scan you want to process in image J by clicking on the File→Open and browse for the image
4) Set the scale that you want to use for you area calculations (mm)
5) Click on the line segment tool (box with line) and draw a line on that measures at least 100mm. With the line still selected click Analyze→Set Scale, this will bring up a new window.

6) Leave the pixels as they are and in the 'Known Distance' box type in the distance in mm of the line (e.g. 100mm), and then in the units box, type in 'mm'.
7) Leave the pixel aspect ratio at 1.0. 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. Click OK. Every time you re-open Image J you have to reset the scale.
8) Now that you have set your scale, use the rectangular selection tool (far left of tool bar) and select the part of the image that contains only the needles or leaves that need to be measured. Go to Image→Crop and this will crop the image so that you will only be analyzing what you want.
9) Next go to Process→Binary→ make binary, which will convert your image to black and white (this makes it easier to set your area thresholds).
10) Go to Process→Binary→Fill Holes and this will fill in any areas within the pine needles that may have a different color value due to irregularities in the original scan.
11) If converting the image to binary does not result in a good image then go to Image → Color → split channels. You can then select the channel that produces that best image (it is most often the blue channel).
12) Next, go to Image→Adjust→Threshold and this will open a window. The boxes below the scale bars should read 'Default' and 'Red'.
13) Adjust the scale bars until your needles are completely filled out in red on a white background. If analyzing a leaf, make sure to adjust the bars so that you have the most red filled in without distorting the shape of the edge of the leaves. Hit Set. Setting the threshold is telling the software which parts of the image it will be analyzing.
14) After the threshold is set, go to Analyze→Set measurements. This will bring up a checklist of available measurements. Make sure that 'area' is checked. Also be sure to check 'Limit to threshold' and 'Display Label'. Hit 'OK'.
15) Go to Analyze→Analyze Particles: In the window set the size (mm^2) 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'.
16) A chart will appear that gives you the individual area of each pine needle. Save this as a text file.
17) Close the image without saving any of the changes.

Cases 1 and 2: Drying and Weighing Samples

Applies to leaf punches and needles/small leaves.

1) Get LMA drying datasheet for broad-leaf species or needle/small leaf (scanned) species (RD [09]).
2) Place all samples in coin envelopes in drying oven at 60°C for 48h
3) Record necessary fields in the appropriate lab datasheet
4) Immediately after drying, weigh samples to nearest 0.0001g

5) Tare plastic weigh boat, dump sample into weigh boat and weigh recording to the nearest 0.0001g.
6) Return sample to coin envelope and store in cool, dry location (you can reuse the weigh boat, if it remains free of dry material, but re-tare for each sample)
7) Leaf punch samples: calculate weight of a single punch area: divide dryWeightGrams by numberLeavesScanned and record singlePunchWeightGrams in datasheet.
8) Needle (or small leaf species): calculate weight of a single needle for each sample and record→ singleNeedleWeightGrams (or singleLeafWeightGrams) = dryWeightGrams / numberNeedlesScanned.
9) Once weighed, return leaf punches and/or needles/small leaves to their labeled coin envelopes
10) Store in a cool, dry cabinet

Laboratory Equipment and Materials

Table 3. Equipment list for measuring LMA on samples from one site

Maximo Item #	Item Description	Purpose	Quantity	Condition -Specific	Special Handling
Required	Punch tools (0.5”, 0.75”, and 1.5” diameter)	Obtaining a standard size tissue sample	1 ea	Y	N
Required	4” x 5” Coin envelopes	Sample drying	1 box	N	N
Required	White paper (8” x 11”)	Mounting samples	1 ream	N	N
Required	Scanner	Scanning samples	1	Y	N
Required	image J Software (see text for download instructions)	Scanning samples	1	Y	N
Required	Scale (to 0.0001 g precision)	Weighing dried samples	1	N	N
Required	Small plastic weigh boats	Drying and weighing samples	1 box	N	N
RD [09]	Datasheets	Recording info	---	N	N

SOP F: Sample Shipment

1) Samples will be shipped oven-dried, in coin envelopes, to contracted laboratory and archiving facilities
2) When ready to ship, take samples out of cabinets and seal envelope.
3) Ship coin envelopes in small, narrow boxes (the boxes that the coin envelopes come in work well, see Table 4), with coin envelopes (seal up) placed upright in boxes
4) Place these boxes within thick-walled shipment boxes and ship ground to the destinations specified in the collection schedule
5) Use bubble wrap or other shipping material to secure boxes within shipment boxes

Laboratory Equipment and Materials

Table 4. Equipment list for samples from one site

Maximo Item #	Item Description	Purpose	Quantity	Condition-Specific	Special Handling
Suggested	Shipping boxes	Sample shipment	2(+)	N	N
Suggested	Packing tape	Sample shipment	1 roll	N	N
Suggested	Small narrow boxes (e.g., 5” w x 5” h x 12” l) for arranging samples	Sample organization and shipment	4	N	N
Suggested	Shipping material (e.g., bubble wrap)	Sample shipment	TBD	N	N

SOP G: Data Entry and Verification

Data entry, from hardcopy data sheets into electronic data ingest files, must occur within 14 days of data generation. Data ingest file pertaining to this protocol is the NEON Raw Data Ingest Workbook for TOS Terrestrial Biogeochemistry: Chemistry of Soils and Plants (RD [08]). Follow QA/QC and file scanning and storage instructions described in the TOS Protocol for Manual Data Entry (AD[06])).

APPENDIX A PROTOCOL CHANGE SUMMARY

The following changes have been made between V2 and V3 protocols:

- SOP B: Biomass clip harvests for alive and dead grasses are described together and collected using the same approach. Live grasses are no longer grouped in with shrub collection.
- SOP D: Added procedure for drying and processing biomass clip harvests for alive and dead grasses.

APPENDIX B VEGETATION HEIGHT CATEGORY TO DETERMINE CANOPY SAMPLING METHOD BY SITE

(1 = 0-2 mm, manual clip; 2 = 2-6 m, pole pruner; 3 = > 6 mm shotgun)

Domain	Site Name	Vegetation	Height Category
1	Bartlett	Deciduous.Forest	3
1	Bartlett	Evergreen.Forest	3
1	Bartlett	Mixed.Forest	3
1	Harvard	Deciduous.Forest	3
1	Harvard	Evergreen.Forest	3
1	Harvard	Mixed.Forest	3
1	Harvard	Woody.Wetlands	2,3
1	Plum.Island	Deciduous.Forest	3
2	Blandy	Deciduous.Forest	3
2	Blandy	Pasture.Hay	1
2	SCBI	Deciduous.Forest	3
2	SCBI	Evergreen.Forest	3
2	SCBI	Pasture.Hay	1
2	SERC	Deciduous.Forest	3
2	SERC	Emergent.Herbaceous.Wetlands	1
2	SERC	Woody.Wetlands	2,3
3	Disney	Pasture.Hay	1
3	Disney	Woody.Wetlands	2,3
3	Jones	Cultivated.Crops	1,2
3	Jones	Deciduous.Forest	3
3	Jones	Evergreen.Forest	3
3	Jones	Grassland.Herbaceous	1
3	Jones	Mixed.Forest	3
3	Ordway	Emergent.Herbaceous.Wetlands	1
3	Ordway	Evergreen.Forest	3
3	Ordway	Grassland.Herbaceous	1
3	Ordway	Woody.Wetlands	2,3
4	Guanica	Evergreen.Forest	3
4	Lajas	Cultivated.Crops	1,2
4	Lajas	Evergreen.Forest	3
4	Lajas	Grassland.Herbaceous	1

Domain	Site Name	Vegetation	Height Category
4	Mameyes.Ponce	Evergreen.Forest	3
4	Mameyes.Ponce	Grassland.Herbaceous	1
5	Steigerwaldt	Deciduous.Forest	3
5	Steigerwaldt	Emergent.Herbaceous.Wetlands	1
5	Steigerwaldt	Grassland.Herbaceous	1
5	Steigerwaldt	Mixed.Forest	3
5	Steigerwaldt	Shrub.Scrub	2
5	Treehaven	Deciduous.Forest	3
5	Treehaven	Emergent.Herbaceous.Wetlands	1
5	Treehaven	Evergreen.Forest	3
5	Treehaven	Mixed.Forest	3
5	Treehaven	Woody.Wetlands	2,3
5	UNDERC	Deciduous.Forest	3
5	UNDERC	Mixed.Forest	3
5	UNDERC	Woody.Wetlands	2,3
6	Konza	Deciduous.Forest	3
6	Konza	Grassland.Herbaceous	1
6	KUFS	Deciduous.Forest	3
6	KUFS	Grassland.Herbaceous	1
7	GSMNP	Deciduous.Forest	3
7	GSMNP	Evergreen.Forest	3
7	Mountain.Lake	Deciduous.Forest	3
7	ORNL	Deciduous.Forest	3
7	ORNL	Evergreen.Forest	3
7	ORNL	Pasture.Hay	1
8	Choctaw	Deciduous.Forest	3
8	Choctaw	Woody.Wetlands	2,3
8	Dead.Lake	Evergreen.Forest	3
8	Dead.Lake	Woody.Wetlands	2,3
8	Talladega	Deciduous.Forest	3
8	Talladega	Evergreen.Forest	3
8	Talladega	Mixed.Forest	3
9	Dakota.Coteau	Grassland.Herbaceous	1
9	NGPRL	Emergent.Herbaceous.Wetlands	1
9	NGPRL	Grassland.Herbaceous	1

Domain	Site Name	Vegetation	Height Category
9	Woodworth	Emergent.Herbaceous.Wetlands	1
9	Woodworth	Grassland.Herbaceous	1
10	CASTNET	Evergreen.Forest	3
10	CASTNET	Grassland.Herbaceous	1
10	CPER	Grassland.Herbaceous	1
10	Sterling	Cultivated.Crops	1,2
10	Sterling	Grassland.Herbaceous	1
11	Klemme	Grassland.Herbaceous	1
11	Klemme	Shrub.Scrub	2
11	LBJ	Deciduous.Forest	2,3
11	LBJ	Grassland.Herbaceous	1
11	UOKBS	Deciduous.Forest	2,3
11	UOKBS	Grassland.Herbaceous	1
12	Bozeman	TBD	TBD
12	Loch.Leven	TBD	TBD
12	Yellowstone	Evergreen.Forest	3
12	Yellowstone	Grassland.Herbaceous	1
12	Yellowstone	Shrub.Scrub	2
13	Winter.Park	TBD	TBD
13	Moab	Barren.land	1
13	Moab	Evergreen.Forest	2,3
13	Moab	Shrub.Scrub	2
13	Niwot	Evergreen.Forest	3
13	Niwot	Grassland.Herbaceous	1
14	CAPLTER	Shrub.Scrub	2
14	Jornada	Shrub.Scrub	2
14	Santa.Rita	Shrub.Scrub	2,3
15	Onaqui	Evergreen.Forest	2,3
15	Onaqui	Shrub.Scrub	2
15	Murray	TBD	TBD
15	Red.Butte	Deciduous.Forest	3
15	Red.Butte	Evergreen.Forest	3
15	Red.Butte	Shrub.Scrub	2,3
16	Wind.River	Evergreen.Forest	3

Domain	Site Name	Vegetation	Height Category
16	Thayer	TBD	TBD
16	Abby Road	TBD	TBD
17	SJER	Evergreen.Forest	2,3
17	SJER	Grassland.Herbaceous	1
17	SJER	Shrub.Scrub	2
17	Soaproot.Saddle	Evergreen.Forest	3
17	Soaproot.Saddle	Shrub.Scrub	2
17	Teakettle	Evergreen.Forest	3
17	Teakettle	Shrub.Scrub	2
18	Barrow	Emergent.Herbaceous.Wetlands	1
18	Barrow	Sedge.Herbaceous	1
18	Toolik	Dwarf.Scrub	1
18	Toolik	Sedge.Herbaceous	1
18	Toolik	Shrub.Scrub	2
19	Caribou Creek	Evergreen.Forest	2,3
19	Caribou Creek	Deciduous.Forest	2,3
19	Caribou Creek	Mixed.Forest	2,3
19	Caribou Creek	Woody.Wetlands	2,3
19	Delta.Junction	Evergreen.Forest	3
19	Delta.Junction	Shrub.Scrub	2
19	Delta.Junction	Woody.Wetlands	2,3
19	EightMile	Shrub.Scrub	2
19	EightMile	Evergreen.Forest	3
19	EightMile	Dwarf.Scrub	1
19	Poke Flat	Evergreen.Forest	2,3
19	Poke Flat	Deciduous.Forest	2,3
20	Olaa	TBD	TBD
20	PuuWaaWaa	TBD	TBD
20	PuuWaaWaa	TBD	TBD