FIELD AND LAB PROTOCOL: CANOPY FOLIAGE CHEMISTRY AND LEAF MASS PER AREA MEASUREMENTS

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REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	01/10/2014	ECO-01139	Draft release. Will be finalized in next rev.

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of the NEON canopy foliage field sampling and laboratory processing protocol. This document provides the content necessary for NEON staff and contractors to carry out these activities at NEON sites and domain facilities. 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 Acknowledgements

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

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

2.3 Acronyms

Acronym	Definition
^{12}C	Most common isotope of carbon
^{13}C	Less common isotope of carbon
DBH	Diameter at Breast Height
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
^{32}S	Most common isotope of sulfur

^{34}S	Less common isotope of sulfur

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 ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$), 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 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 Observatory 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.

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 and 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 RD03, TOS Science Design Terrestrial Biogeochemistry.

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 ([RD04]).

4 PROTOCOL

The field protocol used by NEON for collecting leaf canopy samples to analyze chemical constituents and leaf mass per area (LMA) closely follow 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 a representative sample at the scale of the individual.

Forty-meter by forty-meter 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. Prior to sample collection, plots where canopy samples will be collected should be identified and marked according to the plot establishment guidelines provided by RD07, TOS Field Protocol for Plot Establishment. In addition, identification of the individuals 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 instruments mounted on the AOP carrier scan the 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 gradient.

In low- (≤ 2 m) and mid-stature (2-6 m) vegetated ecosystems, sun leaves may be obtained using clippers and pole pruners, respectively. In many forested systems where the canopy is well out of human reach, it will be necessary to shoot down 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. 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 Ziploc bags and placed on cold packs in coolers. Processing and shipment to contracted analytical facilities must occur within 24-hr following collection.

5 QUALITY ASSURANCE AND CONTROL

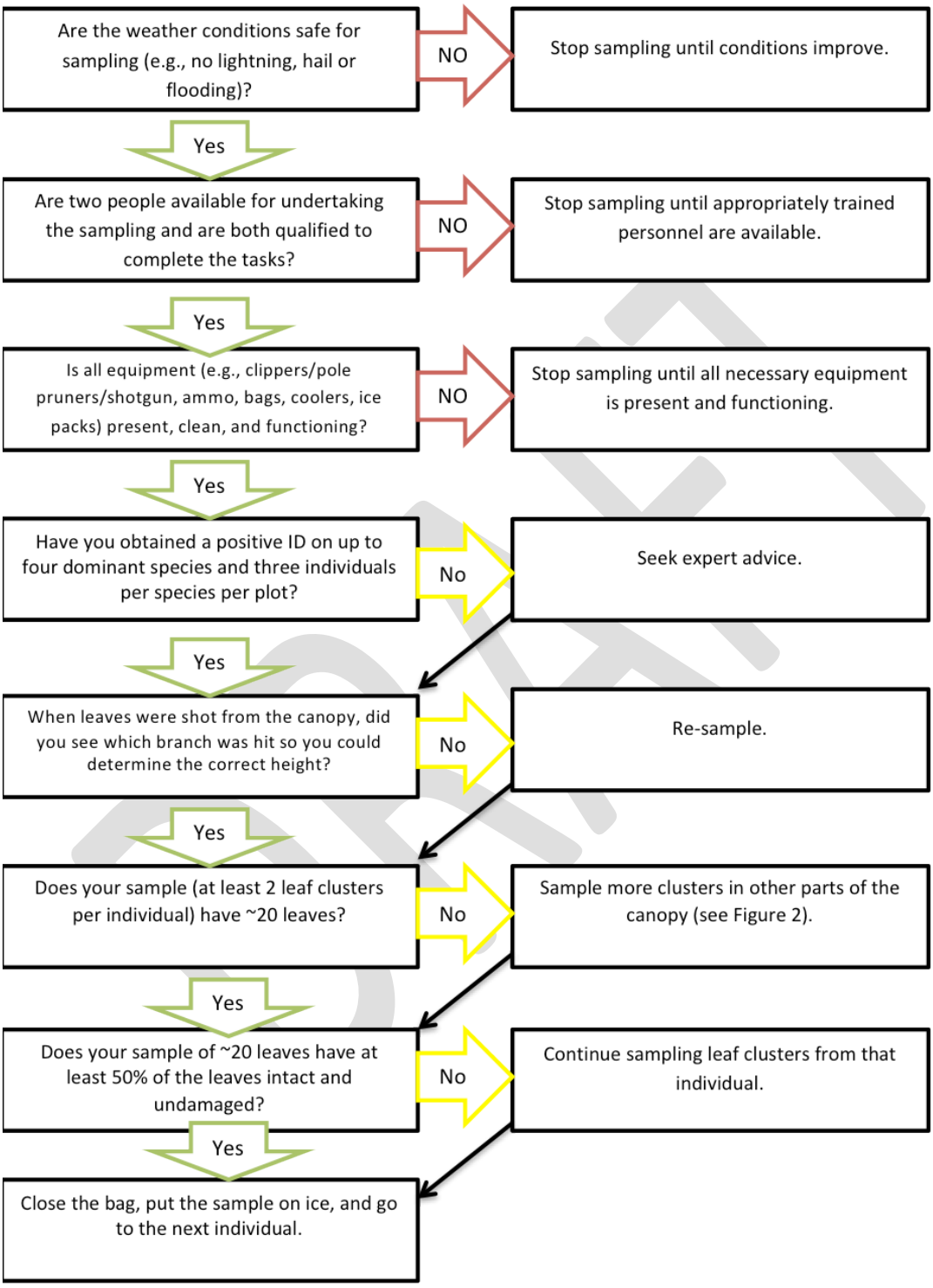
THIS SECTION NEEDS INPUT FROM QA/QC SCIENTIST. *27 March 2013*

The procedures associated with this protocol will be audited according to RD[05]. Additional quality assurance will be performed on data collected via these procedures according to 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 (see description in Section 10.4). 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.), but make a note of the weather change in the PDA 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.

5.1 Decision Tree



1. Does your sample (at least 2 leaf clusters per individual) have ~20 leaves?
   a. If YES, go on to #7.
   b. If NO, sample more clusters in other parts of the canopy (see Figure 2).
2. Does your sample of ~20 leaves have at least 50% of the leaves intact and undamaged?
   a. If YES, close the bag, put the sample on ice, and go to the next individual.
   b. If NO, continue sampling leaf clusters at that individual.

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 with sideshields while using chipping hammers, soil corer, or whenever debris may cause an injury to the eye

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 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. 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 local experts will be available resources 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.

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 FIELD STANDARD OPERATING PROCEDURE

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 extent, depending on the climatic factors and landscape features, as well as logistical (e.g., site accessibility) and financial constraints.

Representative spatial sampling from each plant canopy individual is important to capture within-individual variation. In Section 10.4, we detail the approach for sampling within plots and within the canopy of individuals to obtain representative samples.

9.1.1 Criteria for Determining Sampling Dates

9.1.2 Sampling Frequency

Sampling occurs annually within the tower plots and every 5 years within the distributed plots.

9.1.3 Sampling Timing Parameters

Sampling shall occur within three weeks (+/-) of peak biomass at each site and/or coincident with AOP flyovers. At sites where there are multiple peaks in biomass (e.g., some grassland sites), NEON staff will provide additional instructions regarding timing of sampling.

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

Sampling shall occur 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, and shipment).

9.2 Equipment and Materials

Table 1. Leaf Canopy Sampling Equipment and Supplies

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Backpack (suggested)		Include a "Y" or "Yes" if specific	Include a "Y" or "Yes" if hazardous
	GPS, recreational accuracy			
	Flagging to mark dominant species for sampling			
	Clippers			
	Pole pruner (if applicable; mid-stature forests)			
	Shotgun and ammunition (if applicable; tall stature forests)			
	Laser range finder			
	1-2 boxes powderless gloves			
	Coolers with ice packs			
	Ziploc freezer bags (gallon and quart sizes)			
	Meter tap			
	Permanent markers			
	Pencils			
	Field notebook			
	PDA			
	Extra batteries for GPS, Laser Range Finder, and PDA – CR123A for Laser Range Finder			
	Trash bag			

Table 2. Sample Shipping

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Shipping coolers		Include a "Y" or "Yes" if specific	Include a "Y" or "Yes" if hazardous
	Ice packs			
	Packing tape			

Table 3. LMA Measurements

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Punch tools (0.5", 0.75", and 1.5" diameter)		Include a "Y" or "Yes" if specific	Include a "Y" or "Yes" if hazardous
	4" x 5" coin envelopes			
	White paper (8 x 11")			
	Scanner			
	Drying oven			

9.3 Preparation

1) Check the canopy sampling kit to make sure that all supplies are packed and ensure batteries for GPS and PDA units are charged.

9.4 Sample Collection in the Field

1) Select individuals for sampling
   a) With GPS unit, find the first plot (tower or distributed).
   b) Determine the dominant (by percent cover/biomass) species in the plot. The approach will depend on the type and height of vegetation (see Table 2 for pre-characterization/plot establishment determination by NEON site).
   c) Predominately tall-stature plots:
      i) 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.
      ii) Choose up to 5 individuals of the dominant species, and 3 individuals per co-dominant species for sampling foliar chemistry and LMA.
      iii) In most cases, 5 to 12 trees should be selected per plot
   d) Predominately short-stature plots where vegetation is alive:
      i) If entire plot is relatively homogenous, use the Peet nested plot scheme (Figure 1) to identify the dominant/co-dominant species present in one randomly chosen 10 m^2 nested plot within each module (randomly-chosen by flipping a coin or doing rock-paper-scissors).
      ii) Determine the percent cover of the species within each 10 m^2-nested plot.
      iii) Using the results from the 4 nested plots, determine the "average case" that is representative of cover within the entire plot.
      iv) If the plot is not homogenous (i.e., sampling one module may not represent the entire plot) subjectively assess the most dominant species by cover (**Must be done by a trained technician.**).
      v) 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.

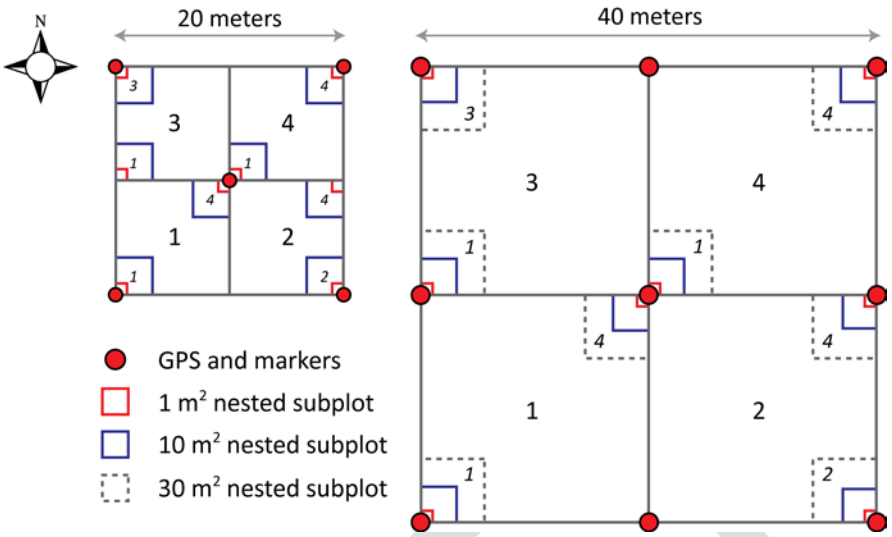


Figure 1. Peet plots for 200 m^2 distributed plots

e) Predominately short-stature plots where vegetation is dead:
   i) For all modules (i.e., 4 20m x 20m modules/plot) that have short-stature plants present use a randomly-generated angle listed in the table provided by NEON staff as part of the work order.
   ii) Beginning with module 1, check first entry in the column for "Module1 angles"
   iii) 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 = the first "Min Dist from Center" value.
   iv) If no suitable 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.
   v) Repeat steps iii and iv 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.
   vi) Mark this area (2m length of the swath oriented due north-south).
   vii) Move to Module 2, and use the "Module2 angles" column and the second entry in "Min Dist from Center".
f) In all cases, mark tree and shrub individuals around their stems (or biomass harvest area, 2c, with flags). Write the unique ID for the sample on the label (see Figure 3).
g) Special considerations:
   i) There is only one species in the plot (e.g., 100% Red oak)
   ii) Sample a total of 5 individuals within the 40m x 40m plot
   iii) 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).
   iv) There are less than three individuals present of a species that makes up a large part of the canopy
   v) Sample that individual (or two individuals) and note anomaly in the PDA.
   vi) There is mixed tall- and short-stature vegetation in the uppermost part of the aerially-visible canopy.

(1) 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).

vii) There are not enough leaves on one short-stature specimen to provide an adequate sample.
(1) Pool leaves from additional stems or individuals growing in close proximity (< 59 cm). Make a note of this in the PDA.

viii) Some individuals of a dominant species show signs of disease/sickness/herbivory
(1) Sample diseased/sick individuals if the disease/sickness is a dominant characteristic (> 50%) of the plot.
(2) Indicate evidence of disease in the "Diseased? (Y/N)" field. If you can identify the disease, write it in the "Notes" field.

2) Obtain the samples
a) For tree species that require a shotgun:
i) 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).
(1) > 20 for large or compound leaves
(2) < 20 for small leaves
(3) If coniferous, multiple branchlets (comprised of multiple needle fascicles) should be collected (>>> 20 needles)

ii) The marksman will shoot down the clusters. Determine the height of each using the Laser Range Finder and the "Three Shot Routine" or VD mode (see protocol provided by Courtney Meier) and write the heights down in the data sheets (we will calculate an average height sampled for each individual).
(1) If samples came from a range of heights > 50 cm please note.

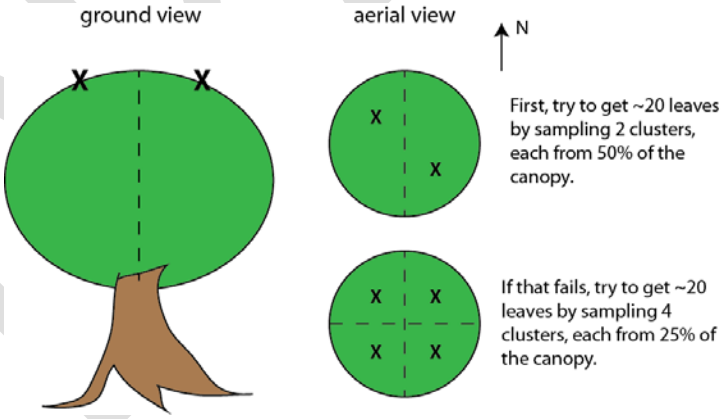


Figure 2. Division of an individual plant canopy crown for sampling.
The same principles apply for shrubs. If further division is required to obtain the requisite samples, continue to do so symmetrically.

iii) Retrieve the sample, and should each wear a clean pair of powderless gloves. You only need 1 pair of gloves per individual tree (a sample consisting of multiple clusters), but you MUST put on a new pair for each individual; do not reuse gloves.

iv) Bag the sample in an appropriately sized Ziploc, noting that at least 50% of the leaves on the clusters are intact and do not contain any shotgun holes. (If necessary, shoot down more leaf clusters).
   (1) Label the bag with the Plot ID, unique ID, date, and technician initials (Figure 3)
   (2) Enter required information into the PDA (see Appendix A).
   (3) Remove gloves and stow in a dedicated trash bag.

v) Collect shotgun shells and wadding and remove from plot

vi) Do not contaminate gloved hands by collecting shells and wadding while samples are still being collected from the same tree.

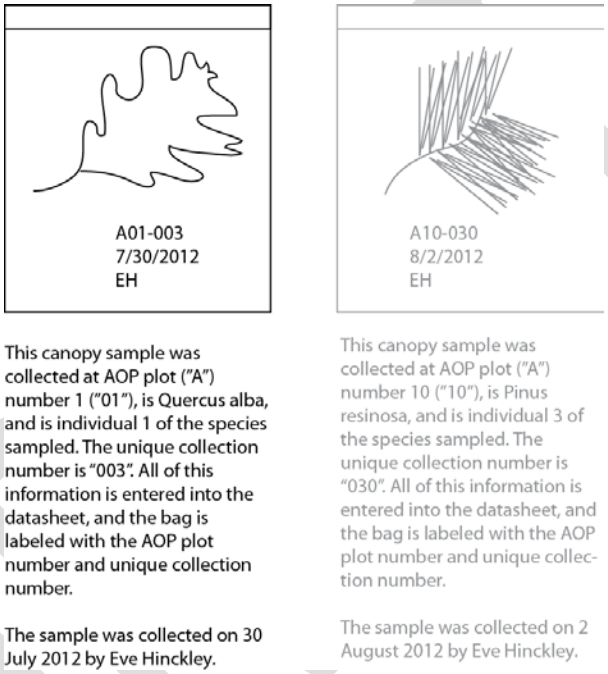


Figure 3. Examples of how to label the sample bags with the unique ID for each sample

b) For vegetation species clipped by hand (shrubs and live grasses):
   i) 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 ≥ 20 leaves or blades (Figure 2).
   ii) Use the clippers to obtain the leaf clusters or grass blades. Wear a clean pair of powderless gloves and note the height of each sample using the meter tape. Write these height values in the data sheets.
      (1) 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.
   iii) Measure reflectance on the leaf sample; wear a clean pair of powderless gloves.
   iv) Bag the sample in an appropriately sized Ziploc.
      (1) Label the bag with the unique ID (Figure 3)
      (2) Enter the required information into the PDA (see Appendix A).
      (3) Remove gloves and stow in a dedicated trash bag.

v) Special considerations
(1) In a short-stature plot, one of the dominant or co-dominant species has < 20 leaves.
(a) Sample from a cluster of this species (e.g., several Echinacea plants within a 10 m2 area) rather than one individual. Identify 4-5 clusters, rather than individuals, to sample within the 40m x 40m plot.

c) For dead vegetation (grasses) biomass harvest strips clipped by hand (requires 2 trained field personnel):
i) Use the clippers to obtain the leaf clusters or grass blades. Wear a clean pair of powderless gloves and take 4 height measurements using the meter tape. Write these height values in the data sheets.
(1) You only need 1 pair of clean gloves per swath.
ii) Clip entire area (0.2 m x 2 m) of grass, down to the ground surface. Grass will be dry. **You do not need to separate by species**, but place bulk sample into Ziploc bags.
iii) Make sure that each bag containing a subsample of the 0.2 m x 2 m harvest area has the same label, 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.
iv) Measure reflectance on the leaf sample; wear a clean pair of powderless gloves.
v) Bag the sample in an appropriately sized Ziploc.
(1) Label the bag with the unique ID (Figure 2)
(2) Enter the metadata into the PDA (see Appendix B)
(3) Remove gloves and stow in a dedicated trash bag.
vi) Special considerations
(1) There is bare soil.
(a) Harvest the aboveground biomass that is present, and indicate presence of bare soil in appropriate field in PDA.

9.5 Sample Preservation

1) Make sure that leaves (especially broad-leaf deciduous species) are stored flat in the Ziploc bags (see Figure 4).
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 Ziploc bags in a 4°C refrigerator. If you are working remotely, samples must be kept on fresh ice packs.

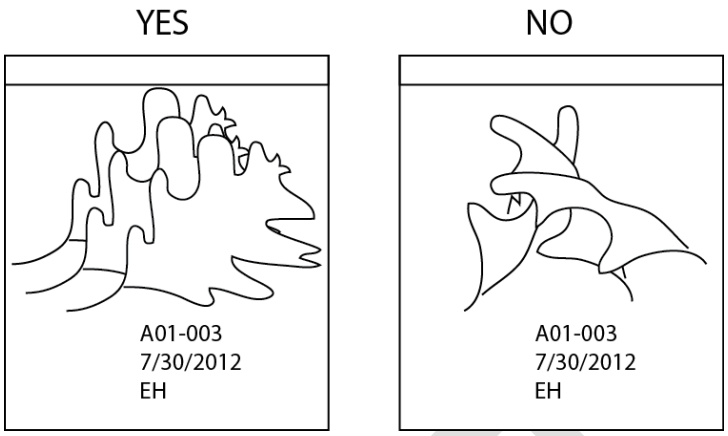


Figure 4. Desired arrangement of leaves bagged for shipment.
For needle-leaved and other non-broadleaf deciduous species, please bag as flat and neatly as possible to minimize damage to the tissues.

9.6 Sample Shipping

1) Do not send any woody material (i.e., small branches) with the samples. If these are present, they can be removed and the Ziploc bag resealed, removing air, prior to shipment.
2) Samples will be shipped to contracted laboratory facilities. A sub-sample may be processed locally at the domain laboratory for Leaf Mass Per Area (LMA), following Appendix C.

9.7 Data Handling

1) Upload PDA data (TBD not used in 2013).

9.8 Refreshing the Sampling Kit

1) Restock the sampling kit so that its contents match the list above. Reorder disposables (and any other equipment/supplies) as needed.

9.9 Equipment Maintenance, Cleaning and Storage

1) Clean clippers and pole pruning shears (if applicable) with ethanol.
2) If shotgun was used, shooters should clean and store as appropriate.
3) Make sure that GPS, Laser Range Finder, and PDA have charged batteries.

Appendix A DATA SHEET: NEON CANOPY SAMPLING FOR CHEMISTRY AND LMA

DATA SHEETS: NEON PLANT CANOPY SAMPLING

AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
____
Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
____

AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
____
Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
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AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
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Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
____

AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
____
Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
____

AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
____
Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
____

AOP Plot #____________________
Genus species____________________
Individual #____________________
Canopy hts sampled (m) __________
____
Unique ID____________________
Date (YYYY/MM/DD)____________________
Tech name____________________
Notes____________________
____

Appendix B DATA SHEET: NEON ABOVEGROUND BIOMASS HARVEST FOR CHEMISTRY

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

AOP Plot #______________________
Module #______________________
X, Y coords of swath center________
Canopy hts sampled (m) __________
____
Unique ID______________________
eventDate (YYYY/MM/DD)______________
# of subsamples__________________
RecordedBy_____________________
Remarks_______________________
____

Appendix C VEGETATION HEIGHT CATEGORY TO DETERMINE CANOPY SAMPLING METHOD BY SITE

Table 4. Vegetation height category to determine canopy sampling method by site. (1 = 0-2 m, manual clip; 2 = 2-6 m, pole pruner; 3 = > 6 m 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

Domain	Site Name	Vegetation	Height Category
4	Guanica	Evergreen.Forest	3
4	Lajas	Cultivated.Crops	1,2
4	Lajas	Evergreen.Forest	3
4	Lajas	Grassland.Herbaceous	1
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

Domain	Site Name	Vegetation	Height Category
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
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

Domain	Site Name	Vegetation	Height Category
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
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

Domain	Site Name	Vegetation	Height Category
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

Appendix D PROTOCOL FOR PROCESSING FOLIAR SAMPLES FOR LEAF MASS PER AREA (LMA)

Note: As of March 2013, we do not know if this processing will be done by domain labs or contracted facilities. We are including the protocol here, in the event that this work will be done by Field Operations personnel.

LMA Procedure

Sample Processing:

Subsampling:

- Archive – ~1/3 of leaves
- Leaf Mass per Area (LMA) – ~1/3 of leaves (but at least 6)
- Nutrient/Isotopic analysis – ~1/3 of leaves

Archive and Nutrient/Isotopic Analysis: leaves from each sample placed in an unsealed coin envelope; coin envelops from same plot placed into paper lunch bags; samples put in oven at 60°C for 48h.

LMA: kept in Ziploc in refrigerator

Sample Analysis:

Archive: maintained at NEON

Nutrient/Isotopic Analysis: coin envelopes taped shut and bagged (ziplocs) according to plot

- Cross check envelopes with master list of samples (from field)
- Shipped to contracted laboratory(ies) for analysis
- Will receive back mg N/leaf or mg C/g leaf values

LMA: samples kept in refrigerator until further processing. (**Must** be processed within 4 days of collection for most species; they will rot.)

LMA measurement protocol:

- Leaf punch procedure for broad-leaf deciduous: leaves need to 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 sample. Place punches in coin envelopes
  2) Save remaining fresh sample in refrigerator
  3) Enter required information into PDA for each sample:
     - Sample ID
     - Date leaf punches done
     - Size of punch used
     - Total # of punches per sample
     - # of leaves those punches came from

4) Place coin envelopes in a cool dry area until samples can be dried at 60 degrees C for 48 hours.

- Scan procedure for needle-leaf species (and others that are <1/2" area): Scan 20 needles (or 10 leaves, depending on what fits within scanning area) per sample, laid in two columns on scanner screen
  1) 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.
  2) Write Sample ID 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)
  3) Upload scans as .tif files and save all of them in appropriate NEON network drive (TBD).
  4) After scanning, place scanned needles or leaves in coin envelopes labeled with sample ID
  5) Metadata to record for each sample:
     - Sample ID
     - Date needles scanned
     - Total # of needles/leaves scanned per sample
  6) Place the coin envelopes in a cool dry area until samples can be dried at 60 degrees C for 48 hours.
- Procedure for measuring the Area of needles/leaves < ½" area:
  1) First, to download the necessary software, 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.
  2) Open the needle scan you want to process in image J by clicking on the File→Open and browse for the image.
  3) Set the scale that you want to use for you area calculations (mm) : To do this 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. Leave the pixels as they are in the Known Distance box type in the distance in mm of the line e.g. 100, and then in the units box type in mm. 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 re-set the scale.**
  4) 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.
  5) Next go to Process→Binary→ make binary, which will convert your image to black and white (this makes it easier in the long run to set your area thresholds). After that to again 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. 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).

6) Next got to Image→Adjust→Threshold and this will open a window, the boxes below the scale bars should read Default and Red. 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.
7) 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.
8) Got 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.
9) This will bring up a chart that gives you the individual area of each pine needle. Save this as a text file.
10) Close the image without saving any of the changes.

- <u>Procedure for Drying and Weighing Samples</u>
  1) Place all samples in coin envelopes in drying oven at 60 degrees C for 48h
  2) Immediately after drying, weigh samples to nearest 0.0001g
     - Tare plastic weigh boat, dump sample into weigh boat and weigh recording to the nearest 0.0001g.
     - Return sample to coin envelope and store in cool, dry location.
  3) Calculate weight of a single needle for each sample and record→ single needle weight (g) = weight of sample (g) / # needles weighed.