TOS PROTOCOL AND PROCEDURE: BBC – PLANT BELOWGROUND BIOMASS SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	03/25/2011	ECO-00148	Initial release
B	01/20/2015	ECO-02273	Production release, template change, method improvements
C	02/20/2015	ECO-02702	Migration to new protocol template
D	01/28/2016	ECO-03547	Major changes to protocol include: • All SOPs now implemented together every time protocol is executed, previously SOP D implemented 1X per site. • Timing information updated, and preservation of cores prior to core processing eliminated. • Equipment list updates for lab work • SOP C.1 sieving methods updated based on megapit sampling experience. • Roots from 2 cores within a clipCell are now pooled *after* weighing takes place and prior to grinding for chemical analysis / archive. • "other" non-root biomass no longer quantified. • Method for calculating core `storageHours` now consistent with Herbaceous Biomass protocol. • Updated Sample Shipment procedure (SOP F) to be consistent with Herbaceous Biomass protocol. • To aid co-locating herbaceous clip and fine root coring, added maps of clip cells within plots to appendix G. • References to mini-rhizotrons removed after descope.
E	02/17/2017	ECO-04403	• Added table of common terms and definitions to Section 2.4 • Toxicodendron material condensed and removed when possible, now reference RD[12]. • Added 'Estimated Time' required for protocol sub-tasks to Section 6.4 based on Field Ops experience. • Updated field and lab equipment list based on feedback from Field Ops prototype. • SOP B: Added 'Linked Protocol' call-out box to highlight connection with Herbaceous Biomass. • SOP B: Added `coringPossible` to better document sample collection effort, and added `coreDiameter` to allow future changes in equipment. • SOP C: Cores may be soaked overnight prior to wet-seiving.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• SOP C: Added instructions for using the wire gauge properly to sort roots by diameter. • SOP C: Simplified pooling instructions, and changed minimum mass of pooled sample from 0.250 g to 0.02 g; removed grinding of samples < 1 g (change from 0.75 g). • SOP C/D: Changed all mass measurement requirements to grams, rather than mix of grams and milligrams. • SOP C/D: Changed timing to allow for overnight pause between SOP C and SOP D. • SOP D: Clarified that `sampleVolume` and `subsampleVolume` can be adjusted on a per core basis to optimize root material mass for sorting. • SOP D: Clarified anticipated effort for sorting root/OM aliquots. • Appendix D: Changed dates from DOY to MM/DD format, and updated Ops-IPT approved missing dates.
F	05/17/2018	ECO-05595	• Section 3.1: New section to explicitly call out integration of Belowground Biomass sampling with Herbaceous Clip Harvest. • Section 4.1 and 4.2, Frequency and Timing: Re-organized and simplified to emphasize important scheduling and timing criteria. • Section 6.1, Equipment: Clarified that balances with 0.001 or 0.0001 g accuracy are needed for SOP D; added updated stir-plates or SOP D. • Section 6.4, Estimated Time: Removed labor allocation guidelines, added Table 7 with updated estimated labor per SOP. • SOP B.1: Re-organized workflow to include sample collection method assessment, and added ability to collect a monolith sample type. • SOP B.1: Specified that distance to closest woody stem applies to living stems. • SOP B.2: Split out 'Troubleshooting' into its own section, consistent with Herbaceous Biomass protocol. • SOP B.5: New section detailing modified field sampling layout at agricultural sites. • SOP C: Re-wrote wet-sieving procedure based on domain staff feedback. • SOP C.1: Added guidance for clipping branched root systems according to size category. • SOP C.2: Clarified that Oven Start/End Dates/Times are only needed for initial drying, not additional drying after storage.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• SOP C.4: Updated text and Table 11 with 40-mesh grinding guidance for C:N analysis subsample. • SOP D: New criteria for selecting soil samples for dilution sampling (spatially balanced approach). • SOP F: Updated to reference digital shipment creation and tracking tools. • Multiple sections: Updated text to reflect digital workflow and mobile app structure. • Multiple sections: Added barcoding workflow required for pooled samples shipped for external analysis; optional for other stages of sample collection and processing. • Added Appendix E: Site-specific modifications necessary to aid with consistent sample collection in D18/19.
G	01/22/2019	ECO-05985	• Section 6.1: Added metal weigh pans and glass scint vials to equipment list as an option when static is problematic. • Section 6.4: Changed estimated grinding hours from 8 h to 32 h, updated core sorting hours to 1-10 h per sample. • SOP B/C: New movable label workflow from Field to Lab. • SOP B.2: Added photo of frame method for delineating soil sample collection area. • SOP B.2: Added example label text. • Section 5, SOP B, and SOP C: Added guidance for identifying and processing root samples that may contain *Toxicodendron spp.* • SOP C.1.1: Added ability to pause overnight between sieving and sorting provided conditions are met. • SOP C.1.1: Added photos of sorting container and live vs. dead roots from training materials. • SOP C.1.1: Added illustration of wet-sieving process. • SOP C.1.1: Added example label text. • SOP C.4: Added guidance to prepare root samples prior to grinding to improve milling performance. • SOP C.5: Added Wiley Mill maintenance guidance. • SOP D: Clarified dilution sample number when number of Tower plots is < 20. • SOP F and Appendix G: Added shipping and labeling guidelines for chemical analysis for samples containing *Toxicodendron*. • Appendix B: Changed from 'Reminders' to 'Sampling QC Checklist'. • Appendix E: D19 DEJU modification to use core method despite rock layer at ~25 cm depth.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
H	04/05/2021	ECO-06531	• Updated to new template (NEON.DOC.050006 Rev K) • Section 4: Re-organized content and added sampling onset guidance for agricultural sites with Tower plots planted in multiple crop types. • Section 4.1: Added table showing integration of Plant Belowground Biomass scheduling with other protocols. • Section 4.3: Added table with sample holding times. • Section 4.5: New missed sampling guidance. • Section 7: New high-level workflow diagram indicating key decision points for SOP implementation. • SOP A: Re-organized SOP A.2 and added new table with label requirements by sample type. • SOP B.2: New SOP to describe use of plot prioritization lists during sampling and for Dilution Sample selection. • SOP B.3: Rangefinder is now primary tool to locate sampling cells, consistent with updated Plot Establishment protocol. • SOP B.3: 'distance to woody stems' fields now have a maximum distance of 20 m. • SOP B.3: New `sampleCondition` field to document cold-chain integrity prior to sample processing. • SOP D: Re-organized content to simplify and reduce nested steps; moved sorting activities into a new sub-SOP D.3 to eliminate repeated steps in Wet/Dry sieving sections. • SOP D: Removed live/dead sorting. • SOP D: Removed < 0.5 mm diameter size category. • SOP D: Added `mycorrhizaeVisible` quality flag. • SOP D: 'Toxicodendron Possible' guidance clarified and consistent with data entry application. • SOP D: Added `initialBagMass` and `finalBagMass` workflow for Toxico samples. • SOP E: Dilution Sampling is now SOP E, grinding and pooling moved to SOP F to better reflect lab work flow. • SOP E: Added guidance for `Dilution Sub-sample Fate` field. • SOP F: Added `archiveMass` field to record dry mass of root samples shipped to biorepository. • SOP F and SOP I: Split out Grinding and Pooling and Equipment Maintenance into separate SOPs. • SOP G.1: Added explicit reference to 'QC Checklist' documents linked via the SSL for QC guidance. • Appendix A.5: New Quick Reference section for Grind & Pool tasks. • Appendix G: Cryo-type human readable labels now required for scint vials shipped to external facilities.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
J	02/08/2024	ECO-07041	• Updated NEON logo • Added pointID labels to all figures with plots or subplots, and updated subplotIDs to new naming scheme. • SOP A.2, Table 7: Updated sampleID strings to use only YYYY portion of date. • SOP A.2, A.4, F, H, and Appendix G: Added guidance to use glass scint vials for long-term archive of ground root tissues. • SOP B.2: Added 'medium-risk' sampling strategy for 40m x 40m Tower plots, to be used when there is a risk that 100% of scheduled sampling cannot be completed. • SOP B.3: The 'Sampling Impractical' field is now recorded at the child level and includes a new "obstruction" option; this workflow replaces the deprecated 'rootSamplingPossible' field. • SOP B, B.5, and D: Added option to freeze cores after field collection for later processing, documented with new 'sampleCondition' value "frozen before processing". • SOP D: Added 'sampleCondition' value "gloves not worn" to document if gloves were mistakenly not worn during sieving/sorting. • SOP D.4: New "Troubleshooting" section with guidance for root oddities encountered during sieving/sorting. • SOP D.5: Added 'subsampleCondition' value "spilled during processing" to indicate if sample was dropped/spilled before 'dryMass' was obtained. • SOP D.6: Clarified purpose of QA weighing and lack of mass target for QA weighing. • SOP E: Added option to freeze residual fraction within 72 h of field collection and process dilution sample at a later date. New 'dilutionSampleCondition' field to document freezing. • SOP E: Reduced dilution subsampling effort from n=10 to n=3 based on optimization analyses. • SOP E.2: New serial dilution workflow for large residual fractions. • SOP F: Samples stored > 5 days before grinding and pooling must be re-dried for 24 h at 65 ˚C. • SOP F: New 'poolSampleCondition' field with "gloves not worn" option. • SOP H: Added guidance to contact Science for current packaging/shipping requirements when sending frozen cores to another domain for processing. • Appendix C: Added site-specific modifications for D02 SCBI and D19 HEAL.

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1 OVERVIEW

1.1 Background

Belowground biomass represents a substantial component of the total plant biomass and plant carbon in terrestrial ecosystems, yet belowground biomass stocks and turnover remain very poorly understood both in space and in time. This is in large part due to the inherent difficulties associated with measuring plant parts that are obscured within soil. Developing a better understanding of how much belowground plant biomass there is, as well as how much of that biomass is produced and decomposed within a given year, is therefore important with respect to improving our understanding of how terrestrial ecosystems respond to environmental changes. Here, we define fine roots to be roots with diameter ≤ 10 mm (Burton and Pregitzer 2008). In combination with the belowground biomass soil pit sampling conducted during site construction (RD[09]), the plant belowground biomass sampling described here enables estimation of the amount of belowground plant biomass ≤ 10 mm diameter within the same land surface area from which NEON Tower eddy covariance data are derived; at many sites this will also be the dominant vegetation type(s).

Fine root frequency, biomass, and turnover rates differ substantially across size classes. In general, larger size classes constitute more of the biomass than smaller size classes, but larger roots also turn over much more slowly and therefore contribute less to annual belowground net primary productivity (BNPP) than fine roots do (Steinaker and Wilson 2005, Tierney and Fahey 2007). NEON employs the most common and robust method to measure belowground biomass in both forest and grassland ecosystems: collection of relatively large diameter soil cores (5–10 cm) or similarly sized monoliths (Tierney and Fahey 2007, Burton and Pregitzer 2008). Because large coarse roots occur infrequently in soil, higher volume samples result in more accurate estimates of belowground biomass (Taylor et al. 2013). However, large sample volumes require a significant amount of time to sieve and sort in the laboratory. Given that time is limiting, there is therefore an inherent trade-off between the number and size of samples that must be resolved (Berhongaray et al. 2013). For belowground biomass sampling, NEON typically uses a 76.2 mm (3-inch) outside diameter coring device with 66.5 mm (2.6-inch) inside diameter. Samples are collected to 30 cm maximum depth to be consistent with the sampling depth used for soil biogeochemistry and microbe sampling (RD[07]). Monolith sampling is utilized when soil conditions prevent collecting a core of sufficient depth (e.g., in rocky soils). Within each sampling "cell" selected for belowground biomass sampling, two soil samples are typically collected, for a total minimum sample volume of 2722 cm^3 per sampling cell. If roots up to 10 mm diameter exist at the site, sample volumes of this size should be sufficient to encounter them in the majority of soil samples (Taylor et al. 2013).

To account for differences in BNPP across the spectrum of fine root diameters, researchers typically sort roots within soil samples into various size categories, and then calculate fine root production separately for each size category. Similar to Burton and Pregitzer (2008), NEON sorts root biomass within each soil sample to the following **sizeCategory** bins: < 1 mm, 1–2 mm, and 2–10 mm.

Samples are sieved and washed to remove soil, picked to separate roots from organic material, and roots are then sorted by size category. Picking and sorting roots is time consuming, and similar to other researchers, NEON uses a 1 cm length cutoff to limit the time spent searching for small root fragments – i.e., root fragments < 1 cm length are ignored and discarded for most soil samples. However, root fragments < 1 cm length can contribute > 50% of the total root biomass in some ecosystems (Koteen and Baldocchi 2013). To account for the biomass of root fragments < 1 cm length, NEON employs a dilution technique on a spatially balanced sample of 20 cores/monoliths every time fine root sampling occurs.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

The author is grateful for time and detailed advice provided by Daniel Milchunas and Mark Lindquist at the Shortgrass Steppe LTER program. In addition, SOP D "Dilution Sampling for Fine Root Biomass Fragments" is based on the work of Koteen and Baldocchi (2013). Many thanks to: Tamara Hillman for testing equipment and developing 'movable label' workflow; Kenny McMahon for improving the text describing the sieving and decanting workflow in SOP C.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.002135	Datasheets for TOS Protocol and Procedure: BBC – Plant Belowground Biomass Sampling
RD[06]	NEON.DOC.001925	NEON Raw Data Ingest Workbook for TOS Belowground Biomass Soil Sampling
RD[07]	NEON.DOC.014048	TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Measurements
RD[08]	NEON.DOC.000987	TOS Protocol and Procedure: VST – Measurement of Vegetation Structure
RD[09]	NEON.DOC.001708	TOS Protocol and Procedure: Soil Pit Sampling for Plant Belowground Biomass
RD[10]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration
RD[11]	NEON.DOC.014037	TOS Protocol and Procedure: HBP – Measurement of Herbaceous Biomass
RD[12]	NEON.DOC.001716	TOS Standard Operating Procedure: Toxicodendron Biomass and Handling
RD[13]	NEON.DOC.001710	TOS Protocol and Procedure: LTR – Litterfall and Fine Woody Debris
RD[14]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Sampling
RD[15]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[16]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[17]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment

2.3 Acronyms

Acronym	Definition
BNPP	Belowground net primary productivity
OM	Soil organic matter, often distinguished by presence of plant material.

2.4 Definitions

Clean technique: Procedures to minimize the introduction of chemical or biological contaminants into a sample. Contamination can result from dust particles, non-purified water, sweat, hair, and other environmental sources.

Clip list: A randomized list of clip cells for each 20m x 20m plot or subplot, provided by NEON Science. Working down the list through time ensures that selected sampling locations will generate an unbiased estimate of plant belowground biomass for every bout.

Clip strip: A 2.0m x 0.1m rectangular area, typically centered within each sampling cell that is avoided during plant belowground biomass sampling. Coordinates provided in clip lists correspond to the SW corners of clip strips.

Fulcrum: Software platform used to create NEON digital data entry applications.

Organic matter (OM): For the purposes of this protocol, particulate soil organic matter made up of decayed plant parts of unrecognizable origin – i.e., it is not possible to discern leaf, twig, needle, root origin, etc., but material is clearly plant-derived and not mineral.

Residual fraction: The mixture of organic matter and root fragments < 1 cm length that is left in the bottom of the 250 µm sieve after root fragments ≥ 1 cm length have been picked out of the sample. For a subset of soil samples, root fragments in the residual fraction are quantified via the dilution technique.

Sampling area: Two 0.5m x 0.5m areas that support plant belowground biomass sampling that exist to the north and the south of the clip strip within a given sampling cell.

Sampling cell: A 3.0m x 0.5m rectangular area within a plot that supports plant below-ground biomass core sampling and herbaceous biomass clip harvest sampling. The long-edge of the cell is always oriented north/south.

ServiceNow: Software tool used for problem/incident tracking and resolution.

Sterile technique: Procedures to minimize the introduction of microbial/DNA contaminants into a sample, such as human microbiota or DNA from a different source material or habitat.

3 METHOD

The Standard Operating Procedures (SOPs) presented in this protocol describe tasks that, when taken together, allow estimation of plant belowground fine root biomass across three diameter size classes. These SOPs are:

SOP A: Preparing for Sampling. Instructions to prepare for sampling for subsequent SOPs.

SOP B: Field Sampling for Plant Belowground Biomass. Collecting soil samples from sampling "cells" in the field and recording required data and metadata.

SOP C: Post-Field Sampling Tasks.

SOP D: Laboratory Processing: Sieving, Sorting, and Weighing Roots. Steps to wash, sieve, and separate roots ≥ 1 cm length from mineral soil and organic matter. This SOP also describes steps to dry and weigh roots.

SOP E: Dilution Sampling for Fine Root Fragments. A sub-sampling procedure to quantify the amount of fine root biomass present in small root fragments < 1 cm length. By carrying out this SOP, it is possible to ignore root fragments < 1 cm length in SOP D while still generating accurate fine root biomass estimates, resulting in significant time savings.

SOP F: Grinding and Pooling Biomass for Chemical Analysis and Archive. Pooling, grinding, and splitting samples for shipment to external facilities for chemical analysis and archive.

Plant belowground biomass sampling takes place every 5 years in 400 m^2 sampling units located within Tower plots or subplots (**Figure 1**). Plant belowground biomass core sampling does not occur in Distributed plots. In 20m x 20m Tower plots, two cores are collected from one sampling "cell" per bout. In larger 40m x 40m Tower plots (i.e., four 400 m^2 subplots per plot), cores are collected from each of the two subplots randomly assigned by Science for sampling, and two core samples are collected from one sampling cell per subplot per bout. This strategy means that:

- At sites with thirty 20m x 20m Tower plots, there will be a maximum of n=60 cores collected (2 per plot).
- At sites with twenty 40m x 40m Tower plots, there will be a maximum of n=80 cores collected (4 per plot).
- For both plot types, fewer cores may be collected if root sampling is not possible in some plots/cells (e.g., due to large roots, rocks, etc.).

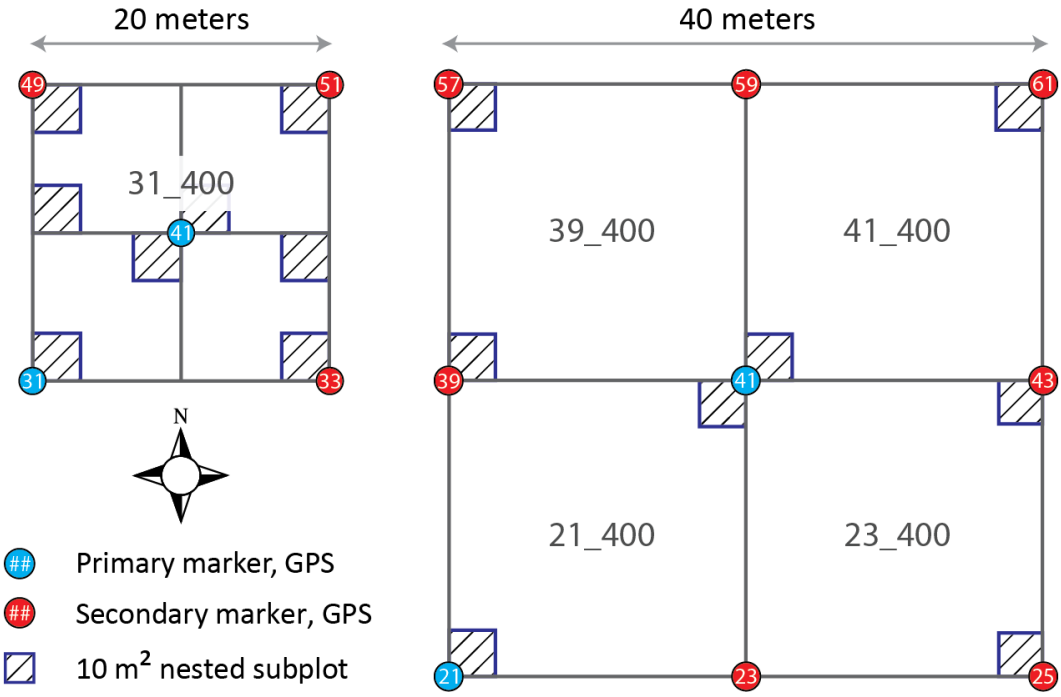

Figure 1. Illustration of two NEON plot sizes used for plant belowground biomass soil sampling. Grey text indicates subplotIDs (XX_YYY format); in each 40m x 40m plot two 400 m^2 subplots are randomly selected by Science for plant belowground biomass sampling. Sampling cells are not provided for and cores are not collected from nested subplots ≤ 10 m^2 (blue hashed squares). Blue and red circles represent plot markers, and the white numbers are pointIDs.

Within each 400 m^2 plot or subplot, sampling cells are 3.0m x 0.5m, and are sequentially numbered (see Appendix F). Coordinates are assigned to the SW corner of a 2.0m x 0.1m clip strip that is centered within each sampling cell. These coordinates are relative to the SW corner of the plot or subplot – i.e., the SW corner of the plot or subplot is defined as having coordinates [0,0] (**Figure 9**, *left*). To determine soil sampling locations, consult a plot-specific "Clip List" to determine which sampling cell was (or will be) used for the peak biomass clip-harvest in the current growing season. Within each sampling cell two soil samples are ideally collected: one from each of the areas to the North AND South of the 2.0m x 0.1m clip strip (**Figure 9**, *right*). To avoid roots and rocks, sampling may occur anywhere within the North and South sampling areas shown in **Figure 9**.

Prior to collecting a soil sample, crowns, corms, rhizomes, and other perennial belowground parts that are not roots are removed from the top 3 cm of soil and discarded (this may also be done in the laboratory prior to sample processing). In some ecosystems, these non-root belowground plant parts may constitute a significant portion of the belowground biomass; however, the NEON protocol is solely focused on measuring fine root biomass.

After sampling from a given cell is completed, site hosts may require that holes be backfilled with an approved material (e.g., purchased sand, soil from another site-host approved location, etc.).

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field staff **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

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

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Plant Belowground Biomass soil samples are collected in a coordinated fashion with other TOS plant and soil biogeochemistry protocols every 5 years to enable spatial and temporal integration with multiple data products generated from a site, and to minimize spikes in required labor within a domain (**Table 1**). Within a year that Plant Belowground Biomass is implemented at a site, samples are collected according to the schedule in **Table 2**.

Table 1. Coordination of Plant Belowground Biomass sampling with other TOS plant and soil protocols through time. Years 1 through 7 are shown to illustrate the temporal grouping of protocols, and the pattern repeats beyond year 7. Dark grey cells indicate synchronized 'chemistry' and 'productivity' protocol groups; medium-grey cells indicate protocols implemented annually in Tower Plots; the lightest grey cells are protocols implemented every 5 y in Tower Plots.

Protocol*	Interval (y)	Plot Type	Number of Plots	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
BGB	5	tower	20 or 30†	X					X	
CFC	5	both	16-20	X					X	
LAI	5	distributed	20	X					X	
LTR-bgc	5	tower	20 or 30†	X					X	
NTR	5	both	10	X					X	
SLS-bgc	5	both	10	X					X	
SLS-bm	5	both	10	X	X	X	X	X	X	X
CDW	5	distributed	20		X					X
HBP	5	distributed	20		X					X
VST	5	distributed	20		X					X
HBP	1	tower	5 to 30†	X	X	X	X	X	X	X
LAI	1	tower	3	X	X	X	X	X	X	X
LTR	1	tower	20 or 30†	X	X	X	X	X	X	X
VST	1	tower	5-10	X	X	X	X	X	X	X
CDW	5	tower	20 or 30†				X			
VST	5	tower	20 or 30†					X		

* Protocol codes and definitions: **BGB** = Belowground Biomass of fine root sampling; **CFC** = Canopy Foliar Chemistry sampling;; **LAI** = Leaf Area Index sampling; **LTR-bgc** = Litterfall biogeochemistry analysis; **NTR** = soil nitrogen mineralization incubation; **SLS-bgc** = Soil biogeochemistry analysis; **SLS-bm** = Soil microbial biomass analysis (PLFA); **CDW** = Coarse Downed Wood tally sampling; **HBP** = Herbaceous Biomass and Productivity sampling; **VST** = Vegetation Structure sampling; **LTR** = Litterfall sampling (no chemistry).

† The total number of Tower Plots sampled varies by site.

Table 2. Sampling frequency for plant belowground biomass sampling procedures on a per SOP per plot type basis.

SOP	Plot Type	Plot Number	Bout Duration	Bouts Per Year	Yearly Interval	Remarks
SOP B	Tower	All	6 weeks (max)	1X per sampling year	5 y	Sampling year is synchronized with protocols listed above.
	Distributed	NA	NA	NA	NA	Distributed plots are not sampled for plant belowground biomass.
SOP D	Tower	All	6 weeks (max)	1X per sampling year	Same as SOP B	SOP quantifies roots ≥ 1 cm length
SOP E	Tower	All	6 weeks (max)	1X per sampling year	Same as SOP B	Dilution sampling quantifies mass of root fragments < 1 cm length.

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling Onset

It is desirable to perform belowground biomass sampling when the root crop is at peak biomass. However, peak belowground biomass does not necessarily correspond with peak aboveground biomass, and in some ecosystems, does not vary in a predictable manner within a growing season from year to year (Milchunas and Lauenroth 2001). Combined with the fact that belowground biomass timecourse data are unavailable for the majority of NEON sites, the timing of plant belowground biomass core sampling is guided by these two factors, listed in order of importance:

1. **Date of peak biomass herbaceous clip harvest**: Schedule plant belowground biomass core sampling such that it is completed within ≤ 7 d of the start of herbaceous clip harvest, or such that plant belowground biomass sampling begins within ≤ 7 d of herbaceous clip harvest completion.
    - If there are two herbaceous biomass peaks, schedule plant belowground biomass sampling relative to the clip harvest with the greatest biomass peak.
    - Site-specific sampling start dates are provided in Appendix B.
2. **Soil moisture**:
    - **Soil hardness**: At some sites, peak herbaceous biomass occurs during hot, dry parts of the year when soils are extremely hard and virtually impenetrable due to high clay content (e.g., D10 CPER). At sites where these conditions occur, the timing of soil sampling may be moved to earlier in the growing season when soil moisture is more conducive to sampling.
        - If soil hardness dictates the timing of sampling, it is not important exactly when in the growing season sampling occurs, but once an acceptable sampling

window is chosen for a given site, all future sampling within that site should be initiated within ± 2 weeks of that sampling window.

- Notify Science staff of the selected sampling start date so that this protocol document may be updated to reflect site-specific sampling dates used for future planning.

- **Standing water**: At sites where plots may be seasonally submerged (e.g., D03 DSNY), soil sampling is ideally scheduled to avoid standing water in potential sampling locations.
  - If a plot is partially submerged but still accessible for terrestrial sampling, "cells" that contain standing water must be rejected for soil sampling, and a new sampling "cell" must be chosen.
  - If plots are fully submerged and the schedule cannot be adjusted to avoid flooding, assess plots for sampling according to SOP B.4.

Sampling Onset – Agricultural Sites

In the event that Tower plots support multiple crop types that reach peak biomass and are harvested at different times, plant belowground biomass sampling should be scheduled to occur when the greatest number of plots are anticipated to be at peak aboveground biomass.

- Additional belowground biomass sampling bouts are NOT scheduled to accommodate multiple crop types harvested at different times.

Sampling Cessation

A given sampling bout should ideally be concluded within ***6 weeks*** of initiation so that the belowground standing crop does not change appreciably during the time that all target plots are sampled.

- This ensures that data collected across all plots within a given sampling bout are as comparable as possible.

4.3 Timing for Laboratory Processing and Analysis

Field Work and Laboratory Processing: After core samples are collected from a given sampling cell, the following points are critical with respect to timing:

- Keep core samples cold until they are processed in the laboratory. This is because root biomass is biologically active after sampling, and fine root structures are delicate and decompose easily. Samples may be kept cold by:
  - Keeping soil samples in a cooler, kept cold with re-usable cold packs. Cold packs should be exchanged for fresh cold packs every 12 hours. Or,
  - Placing soil samples in a 4–8 ˚C refrigerator.
  - Submit an incident if the cold-chain is broken. Storing samples at elevated temperatures will reduce data quality.

- Process collected samples in the laboratory as soon as possible.
    - Ideally, soil samples are processed in the laboratory ***within 24 h*** of collection.
    - It is acceptable to keep cores in cold storage for a ***maximum of 72 h*** before sieving/sorting is initiated. Submit an incident if the 72 h cold-storage maximum is exceeded. Longer storage times will reduce data quality.
    - Alternatively, core samples may be frozen within 72 hours of collection and processed at a later date.
- Once laboratory processing is initiated on a given sample, it is acceptable to pause overnight between sieving and sorting provided that:
    - The sample is refrigerated overnight.
    - No longer than 72 h elapses between field collection and beginning sorting.
    - See **SOP D** for details.
- **Scheduling sieving (SOP D) and Dilution Sampling (SOP E):** It is acceptable to pause overnight between execution of these two SOPs. Dilution sampling does not need to be completed within 72 h of field sampling.

Table 3. Plant belowground biomass holding times by sample type and activity type.

Sample type	Activity	Holding Time
Field-collected soil samples (cold)	Sieve and sort in the laboratory or freeze for later processing	Within 72 h of collection
Oven-dried roots	Weigh and record mass	Within 30 days of core processing (sieving/sorting)
Oven-dried Chemistry samples	Subsample and ship to external labs	Within 90 days of core processing (sieving/sorting)
Oven-dried Archive samples	Subsample and ship to bioarchive facility	Within 90 days of core processing (sieving/sorting)

4.4 Sampling Timing Contingencies

Table 4. Contingency decisions for plant belowground biomass sampling.

Delay/ Situation	Action	Outcome for Data Products
Hours	If delay prevents collecting the second sample from a given cell: 1. Bag and label first sample, 2. Place labeled bags into a cooler. 3. Resume soil sampling in same cell ASAP	None
	If delay occurs between plots or subplots: Resume sampling ASAP.	
1-14 days	If delay prevents collecting the second sample from a given cell: 1. Bag and label first sample, 2. Place labeled bags into a cooler. 3. Process first sample within 72 hours of collection, 4. Resume collection of second sample in same cell ASAP.	Increased uncertainty in belowground biomass estimates.
	If delay occurs between plots or subplots: 1. Process collected samples within 72 hours. 2. Resume soil sampling at additional required plots ASAP.	
14+ days	If delay prevents collecting the second sample from a given cell: 1. Bag and label first sample, 2. Place labeled bags into a cooler. 3. Process first sample within 72 hours of collection, 4. Resume collection of second sample in same cell ASAP.	Potentially substantial increases in uncertainty for belowground biomass estimates. If delay prevents completing sampling from all plots or subplots within a 6 week window, belowground biomass may fluctuate substantially.
	If delay occurs between plots or subplots: 1. Process collected samples within 72 hours. 2. Resume soil sampling at additional required plots ASAP.	

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. Instances that result in canceled sampling must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (Appendix B).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 2**, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 2**).

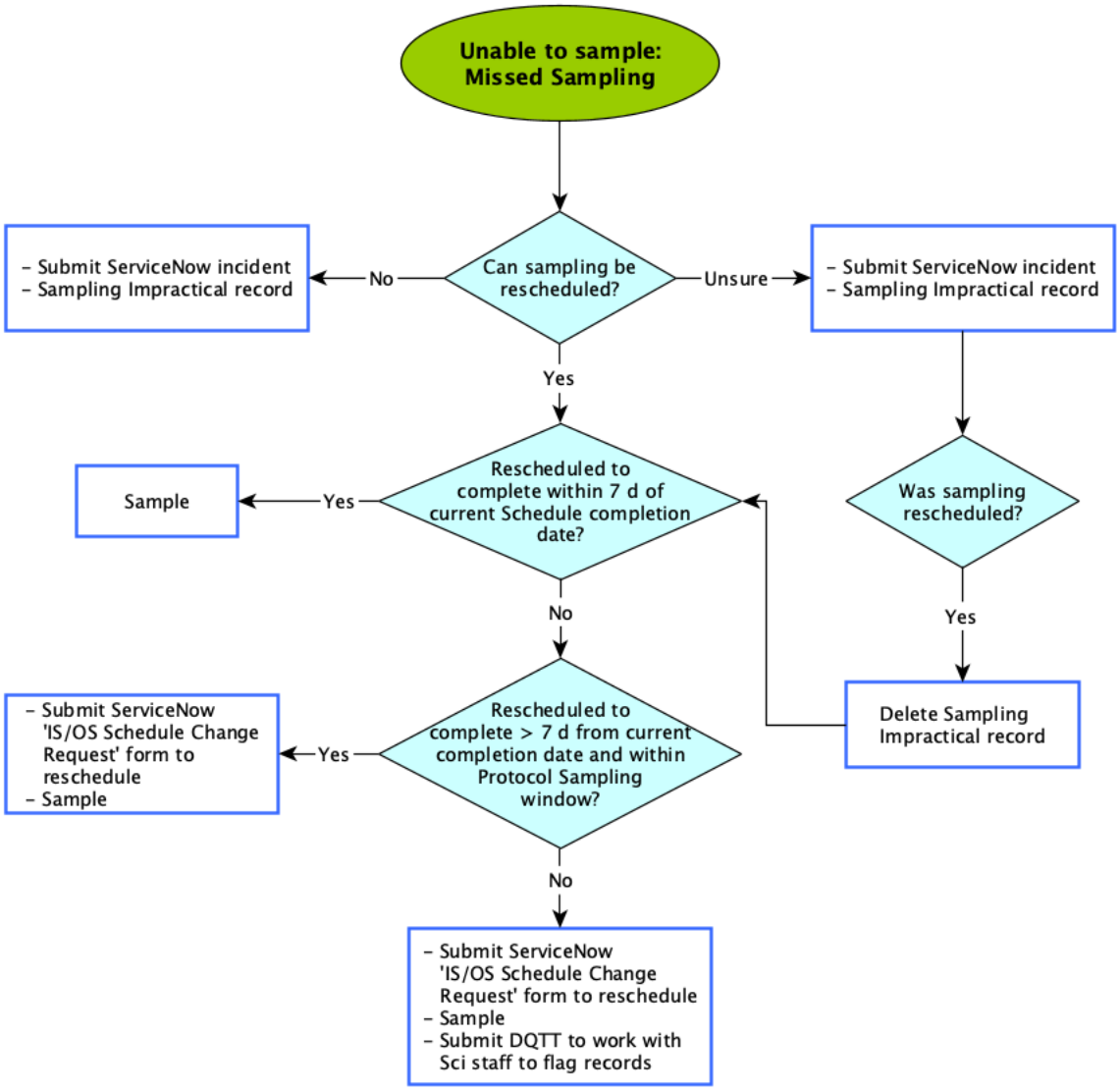

Figure 2. The documentation to account for a Missed Sampling event depends on the situation for each sample not collected per bout.

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to Science by a ServiceNow Incident.
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 2**).
    b. Consult **Figure 2** above to determine required actions if scheduled activities are delayed or canceled. This protocol is the ultimate source of information should any discrepancy exist between this document and other summary materials (e.g., the 'Scheduled Field

Activities – Delays and Cancellations' spreadsheet in the Sharepoint All Collaboration Library).

2. Create a record in the *BBC: Field Sampling [PROD]* app for each **plotID** or **subplotID** that could not be sampled in the field and that cannot be rescheduled.
    a. For sites with n=20, 40m x 40m large-stature Tower Plots: Create two records per scheduled plot, one for each randomly selected subplotID.
    b. For sites with n=30, 20m x 20m small-stature Tower Plots: Create one record per scheduled plotID.
    c. Record the **Collect Date** as the scheduled date.
    d. Select a value for the **Sampling Impractical** field that best fits the reason sampling did not occur (**Table 5**).
    e. Create and save a single 'Soil Sample Field Data' child record and save. The **sampleFate** field should populate to 'not a physical sample' and remain locked.
    f. Missing data in downstream *BBC: Lab Weighing [PROD], BBC: Lab Dilution [PROD]*, and *BBC: Grind and Pool [PROD]* applications are not recorded.

Table 5. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. If more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
Location flooded	Plot is flooded and soil collection conditions are not met
Logistical	Core sample not collected due to logistical reasons (e.g., equipment malfunction, road closure, etc.)
Management	Core sample not collected due to site management activities (e.g., controlled burn)
Extreme weather	Core sample not collected due to hazardous weather conditions (e.g., hurricane, lightning)
Obstruction	Core sample not collected due to an obstruction in the sampling area.
Other	Reason other than one of those listed above prevented collection of samples. Describe briefly in required **remarks**.

3. To document when field sampling was completed and downstream processing was interrupted (e.g., weighing or grinding):
    a. If downstream processing will never occur: In the *BBC: Field Sampling [PROD]* application:
        i. Update **Sampling Impractical** to an appropriate value other than 'OK' in the field child record.

ii. Add **remarks** to the child record: 'Downstream processing canceled'.

b. If downstream processing is delayed but will eventually occur: Create downstream records as needed. No special action required.

c. If multi-step processing cannot be completed (e.g., Dilution Sampling was started but cannot be completed), and a record was created in *BBC: Lab Weighing [PROD], BBC: Lab Dilution [PROD]*, or *BBC: Grind and Pool [PROD]*:

i. In the parent record:

1) Select **sampleFate** = 'not a physical sample'

2) Enter **remarks** = 'Processing canceled'

3) Create a single child-level record.

ii. In the child record created above:

1) **subsampleFate** should default to 'not a physical sample'.

iii. Save the child record then save the parent record.

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 6. Estimated staff and labor hours required for implementation of Plant Belowground Biomass Sampling SOPs.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.2: Preparing for Field Sampling	1 h	1	1 h
SOP A.4: Preparing for Laboratory Sample Processing	0.5 h	1	0.5 h
SOP A.5: Preparing for Dilution Sampling of Fine Root Fragments	4-6 h (first sampling) 0.5 h (subsequently)	1	4-6 h (first sampling) 0.5 h (subsequent)
SOP B: Field Sampling	1 h per plot (20m x 20m) 2 h per plot (40m x 40m)	2	2 h per plot (20m x 20m) 4 h per plot (40m x 40m)
SOP D.1 or D.2: Laboratory Processing: Sieving and Sorting Samples	1 h per core (sieving) 1-10 h per core (sorting)	1 per core	2-11 h per core
SOP D.3 and D.5: Drying, weighing and QA	8 h per bout (initial) 1 h per bout (QA weigh)	1 (initial) 1 (QA weigh)	8 h per bout (initial) 1 h per bout (QA weigh)
SOP E: Dilution Sampling for Fine Root Fragments	1.5 h per core	1 per core	1.5 h per core
SOP F: Grinding and Pooling for External Analysis	16 h per bout	2	32 h per bout
SOP G: Data Entry and Verification	TBD per bout	2	TBD per bout
SOP H: Sample Shipment	1-2 h per bout	1	1-2 h per bout

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and 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.

For the field procedures:

- Soil corer: Safety training is required to properly use the soil corer (e.g., use of heavy gloves and hearing protection). ***!!! There is a serious crushing risk for fingers placed between the slide hammer and the drive head assembly.***
- Soils may contain fungi that may cause illness. Refer to the Operations Field Safety and Security Plan (AD[02]) for details on locations and appropriate precautions.
- A laser rangefinder/hypsometer/compass instrument may be used to navigate to sampling cells within plots. Safety considerations for this instrument include:
    - 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. However, as with any laser device, reasonable precautions should be taken in its operation.
    - It is recommended that you avoid staring into the transmit aperture while firing the laser.
    - Never attempt to view the sun through the scope; looking at the sun through the scope may permanently damage the eyes.

For the laboratory procedures:

- Safety training is required before operating the grinding mill.

For samples that may contain tissue from Toxicodendron spp.:

- Additional safety issues associated with this field procedure include potential exposure to oils from roots of *Toxicodendron spp.* (discussed in Appendix E, AD[02] and RD[12]).

- Throughout this document, the warning pictogram at left is used to identify steps relevant to collecting or processing samples that may contain *Toxicodendron* root tissue.

6 PERSONNEL

6.1 Training Requirements

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

For the field component of this protocol, staff must be trained in navigating to points in the field with a GPS and manual methods. Most critically, staff must be trained to quickly identify commonly encountered types of belowground plant parts at the sites within the region of employment (e.g., crowns, corms, rhizomes, roots, etc.).

Training for both the field and laboratory work must emphasize the importance of consistent, detailed labeling and barcoding of all samples. ***This protocol generates a large number of samples over a short period of time: Accurate sample labeling is imperative.***

6.2 Specialized Skills

For the field work, a minimum of 2 field staff is required for collecting soil core samples due to weight of equipment and samples. When perennial grasses are present, staff must possess a demonstrated ability to identify crown material associated with these plants.

For the laboratory work, staff are required to wash, dry, weigh, grind, and sub-sample belowground biomass samples for shipment to external analytical or archive facilities.

7 STANDARD OPERATING PROCEDURES

SOP Overview

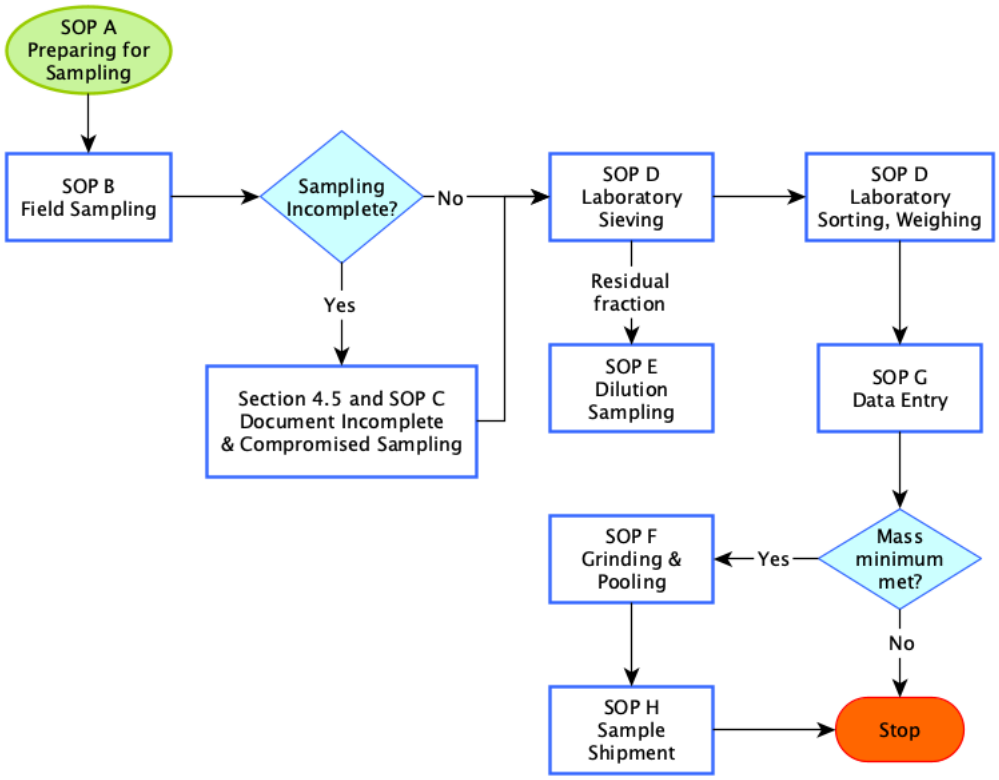

Figure 3. High-level workflow diagram illustrating major components and decision points within the Plant Belowground Biomass protocol.

SOP A: Preparing for Sampling. Tasks completed in the Domain lab, in preparation for the sampling event.

SOP B: Field Sampling for Plant Belowground Biomass. Collect plant belowground biomass soil samples from Tower plots and maintain cold-chain integrity prior laboratory processing.

SOP C: Post-Field Sampling Tasks. Document incomplete sampling efforts and compromised sampling locations.

SOP D: Laboratory Processing: Sieving, Sorting, and Weighing Roots. Separate roots from soil via wet or dry sieving, sort roots to size categories, oven dry, and weigh.

SOP E: Dilution Sampling for Fine Root Fragments. Quantify root fragments < 1 cm length by suspending the residual fraction generated by the sieving procedure in water, subsampling, sorting roots from organic material, drying, and weighing.

SOP F: Grinding and Pooling Biomass for Chemical Analysis and Archive. For samples with sufficient dry root mass, pool, grind, and split to generate subsamples for chemical analysis and archive at external facilities.

SOP G: Data Entry and Verification. Guidelines and requirements for successful data entry and use of QC Checklist. This SOP is NOT a substitute for AOS/TOS Protocol and Procedure: Data Management (RD[04]). Staff must read RD[04]:

- To understand required data quality procedures.
- Prior to transcription from paper data sheets.

SOP H: Sample Shipment. Guidelines and requirements for preparing samples prior to shipment. This SOP is NOT a substitute for NEON Protocol and Procedure: Shipping Ecological Samples and Equipment (RD[17]). Staff must read RD[17] for sample-type-specific packaging and shipment instructions.

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets (RD[05]) should be printed, prepared, and carried along with the mobile devices to sampling locations at all times. If circumstances require use of paper data sheets, refer to the Data Management protocol for data entry procedures (RD[04]).

A.2 Labels and Identifiers

Each soil core or monolith collected in the field is assigned a 'Sample ID', and roots sorted from a sample are assigned 'Subsample IDs'. For grinding, chemical analysis, and archive, subsamples are combined to create a pooled sample that is assigned a 'Pool SampleID', and the pooled sample is then split for chemical analysis (assigned a 'cn Sample ID'), and biogeochemistry archive (assigned a 'bgc Archive ID') (**Figure 4**).

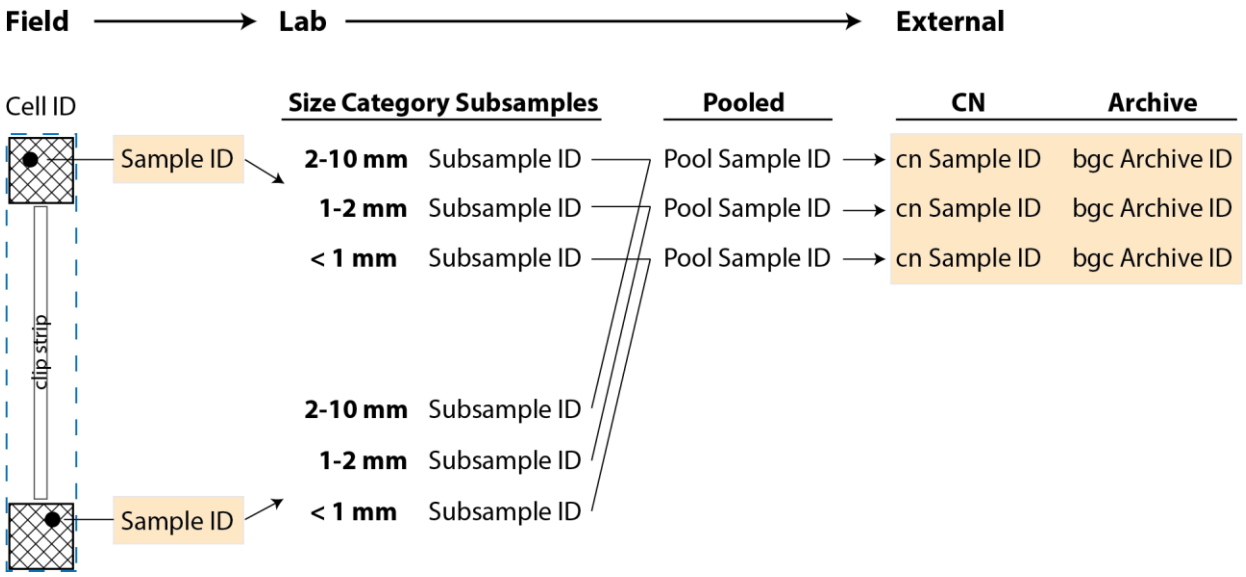

Figure 4. Workflow for generating unique identifiers for samples, subsamples, etc. for a sampling cell from which soils are collected in the field. In the lab, roots are pooled within a size category, ground, and split into CN and Archive samples, then shipped to external facilities. The amber box indicates samples for which barcodes are required.

Proper labeling of samples is critical as sample material passes through the SOPs. Samples are labeled with human-readable information at all steps to improve and aid sample organization, and barcodes are used for most sample types to speed data entry and reduce transcription errors and typos. **Table 7** provides a quick reference to the types of samples this protocol generates and associated labeling and barcode requirements. The rule of thumb is that the primary field sample will ALWAYS need a barcode

due to its importance in generating future samples. Likewise, the final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

See **Appendix G** for label and barcode specifications by sample type and ordering information.

Table 7. Human-readable and barcode labeling requirements for sample types generated by the Plant Belowground Biomass Sampling protocol.

Sample Type	Container Type	Label Type	Required Information	Example
Field-collected cores (refrigerated)	Heavy-duty freezer bag	Human readable: All-weather address label	siteID, plotID/cellID, year of collectDate, soil sample ID (north/south), subplotID	bbc.JORN047251.2023.north.21_400
		Barcode: Type I ***required***	Affix to human-readable label	
Field-collected cores (frozen)	Heavy-duty freezer bag	Human readable: Cryo-safe label	As above.	As above.
		Barcode: Type II ***required***		
Sorted roots	Coin envelope or manila envelope	Human readable: All-weather address label	siteID, plotID/cellID, year of collectDate, soil sample ID (north/south), sizeCategory, subplotID	bbc.JORN047251.2023.north.0-1.21_400
		Barcode: Type I ***strongly recommended***	Affix to envelope/bag	
Dilution	Aluminum tin	Human readable: Permanent tinID	tinID	'47'
		Barcode: Not required	NA	NA
Pooled ground roots	Beaker, microsplitter	Human readable: Labeling tape	siteID, plotID/cellID, year of collectDate, sizeCategory	bbc.JORN047251.2023.0-1
		Barcode: Not required	NA	NA
CN Analysis	Scint vial, 20 mL plastic	Human readable: Cryo-type adhesive label	siteID, plotID/cellID, year of collectDate, sizeCategory, CN	bbc.JORN047251.2023.0-1.cn
		Barcode: Type I ***required***	Affix vertically to vial	
Archive	Scint vial, 20 mL glass	Cryo-type adhesive label	siteID, plotID/cellID, year of collectDate, sizeCategory, AR	bbc.JORN047251.2023.0-1.ar

Sample Type	Container Type	Label Type	Required Information	Example
		Barcode: Type I ***required***	Affix vertically to vial	

Barcode Information

When using barcodes:

- Apply adhesive barcode labels to dry, room temperature bags, envelopes or sample containers at least 30 minutes in advance of their use. Barcodes may also be applied at the start of the season.
- Barcodes are unique, but are not initially associated with a particular sample; if using barcodes, it is encouraged to apply these in advance.
- Barcodes are scanned into the mobile application when indicated in the protocol; only one barcode may be associated with a particular sample, subsample, etc. Do not reuse barcodes.
- If a barcode is associated with multiple subsamples, the data ingest system will throw an error and refuse to pull in entered data.

A.3 Preparing for Field Sampling

1. Make all-weather labels for tracking soil sampling metadata in the field.
    a. Pre-print label template onto all-weather paper (**Figure 5**). Aim for final dimensions of approximately 3” x 5”. Label templates developed by Field Science are available via the SSL.

Bout#_____ Date _____ Bag ____ of ____

clipID: _____ Subplot:_____

CoreID: North South

Figure 5. Example label template that can be printed on all-weather paper prior to field sampling.

    b. Apply barcodes to cut labels. The final 3” x 5” label contains both human-readable information and the barcode, and is placed inside the sample bag with the soil sample. This label may be removed from the bag with the sample and used to track the sample through sieving and sorting.
2. An additional all-weather adhesive label may be affixed to the outside of the plastic bag for easy readability.
    a. Print onto adhesive all-weather labels using a template (Figure 5, or equivalent).
    b. Affix outside labels to clean plastic bags and let cure for approximately 24 h.

3. If it is possible to collect soil cores (as opposed to monoliths): Use local knowledge of the soils present at the site, and determine the type of soil coring bit that is required for the soil conditions at the site (i.e. the degree of relief needed inside the bit) (**Table 8**).

Table 8. Soil core bits and the soil types and conditions for which they should be used.

Bit Type	Intended Soil Conditions or Soil Type
Standard taper	Dry soils
Quick relief	Clay/Loam soils (i.e. "typical soils"); relief inside bit allows for moderate expansion of core inside soil core tube, prevents sample from getting stuck
Heavy duty quick relief	Heavy clay soils; additional relief allows for additional expansion of core inside soil core tube
Extra heavy duty quick relief	Extra heavy expansive clay soils; allows for maximal expansion of core inside soil core tube
Basket retainer bit	Works with basket retainer and basket retainer adapter to retain sandy, non-cohesive soil samples inside the soil core tube

4. Prepare equipment and material according to **Table 9**.

Table 9. Actions required to prepare equipment and materials for belowground biomass soil sampling in the field (SOP B). Equipment listed here are only those items that require preparation actions before sampling; the full equipment list is provided in Appendix H.

Item Description	Action(s)
Mobile data collection device	• Charge and sync
Labels and barcodes	• Prepare according to SOP A.2
GPS unit	• Charge • Load target plot locations
Compass, mirror-sight, adjustable declination	Check/set correct declination*
TruPulse 360R laser rangefinder and clinometer	• Check battery, charge (if possible) • Clean lenses with lens cloth or lens tissue (if necessary) • Check/set correct declination*. See RD[10]. • Calibrate tilt-sensor (only necessary after severe drop-shock; see RD[10]). • Ensure a foliage filter and reflector are available (if necessary).
76.2mm OD (66.5mm ID) soil core tube and bit assembly	Measure 30 cm from the bottom of the bit, and mark on the tube with electrical tape.
Sampling frame (50cm x 50cm)	Assemble a sampling frame from PVC or conduit and elbow-connectors. The sampling frame allows quick delineation of north/south sampling areas in the field.

Item Description	Action(s)
Re-usable cold packs	Place in -20 ˚C freezer
Hand clippers	Clean and sharpen blades (if necessary)
Sand, or other site-specific material	Check with the site host to determine the desired back-fill material. Ensure supply is sufficient for backfilling soil sampling holes.
Belowground biomass "Field Sampling Datasheet"	Print as needed on waterproof copy paper; needed for backup in the event digital data collection workflow fails.
Clip Lists	Print as needed on waterproof copy paper
Tower Plot "Random Subplot List"	Print as needed on waterproof copy paper; only needed for 40m x 40m Tower Plots.

* Declination changes with time and should be looked up annually per site: http://www.ngdc.noaa.gov/geomag-web/

A.3.1 Integrating Belowground Biomass Sampling with Clip Harvest in Agricultural Plots

1. For densely planted, tall-stature crops such as corn, delineate plant belowground biomass sampling areas and the clip strip well before crop maturity. Delineation of sampling areas will be difficult once crops are taller than breast height.

A.4 Preparing for Laboratory Sample Processing (SOP D)

1. Empty and clean root washing station sediment traps.
2. Prepare drying oven for drying root samples:
    a. Set oven temperature to 65°C.
    b. Clear necessary space.
3. Prepare desiccator for temporary storage of dried root samples:
    a. Clear necessary space.
    b. Replace/refresh desiccant as needed.
4. Pre-print adhesive labels for sorting envelopes using a template (e.g., **Figure 6**). Label templates developed by Field Science are available via the SSL.

Date _____ Env. ____ of ____

clipID: _____ Subplot:_____

CoreID: North South

sizeCategory: < 1mm 1-2mm 2-10mm

Figure 6. Label template that can be printed on adhesive labels and applied prior to lab processing.

5. Affix pre-printed adhesive labels to sorting envelopes.

6. ***Prepare barcodes (Required)***: Affix Type I barcodes length-wise to 20 mL scint vials. Do not wrap barcode around the vials; curved surfaces prevent accurate reading of barcodes.
    a. For CN samples: Prepare 20 mL plastic scint vials.
    b. For archive samples: Prepare 20 mL glass scint vials.
7. Print lab weighing datasheets (optional, only if data are not entered directly into digital workflow).
8. Prepare scintillation vials for shipping samples that may contain *Toxicodendron spp.*:

    a. Affix a *Toxicodendron* warning label to the lid of the vial, such as that shown at left.
    b. Allow label adhesive to cure for a minimum of 30 minutes at room temperature.

A.5 Preparing for Dilution Sampling of Fine Root Fragments (SOP E)

Item Description	Action(s)
Dilution Sampling Plunger	Assemble plunger from items listed in Appendix G, **Table 19**.
Dilution Sampling Syringe	Beginning with a 40-60 mL syringe (**Table 19**), cut off the tip to create an opening approximately 1 cm in diameter.

1. Assemble a plunger (**Figure 7**), with diameter suitable for the size of beaker selected from **Table 19**; plunger pieces can be assembled from locally available hardware store parts.
    a. Use scissors, a utility knife, or other appropriate tool to cut a circular section out of a piece of acrylic, polycarbonate, or vinyl. The diameter of the circle should be approx. 1 cm less than the diameter of the beaker. If using different beaker sizes, make a plunger for each beaker size.
    b. Create a small hole in the center of the circle just large enough to fit the threaded zinc rod through (hole is approx. ¼”).
    c. Tighten on one nut <1” from the bottom. Then slide the cut disk on, and fasten with another nut.
    d. Drill a ¼” hole completely through the wooden dowel and cut length to a preferred size.
    e. Repeat step 3 to attach the dowel using two nuts.
    f. Coat the nut and tip of the zinc rod at the ‘circle’ end with silicone to avoid breaking the bottom of the beaker when plunging.

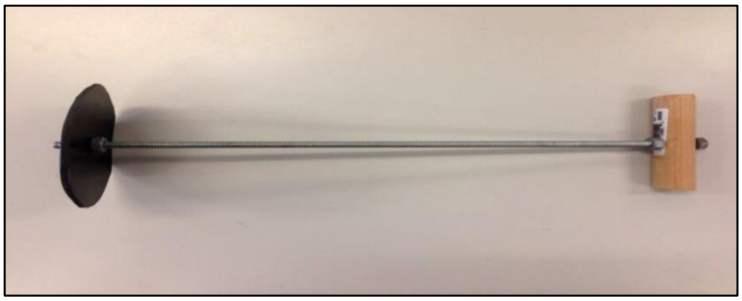

Figure 7. Assembled plunger used to randomize root fragment samples < 1 cm length as part of dilution sampling.

2. Label aluminum weigh tins with unique Tin IDs.
3. Print lab dilution datasheets as necessary (skip if using digital workflow).

SOP B Field Sampling for Plant Belowground Biomass

Overview and Goals

- Collect two plant belowground biomass core samples per sampling cell (see **Figure 9** for diagram of a sampling cell).
- Keep core samples cold until they are processed in the laboratory.
- Core samples may be frozen for processing at a later date provided sufficient labor is available (SOP B.5).
- Collect required field sampling metadata in the *BBC: Field Sampling [PROD]* mobile application.
    - The Belowground Biomass Sampling Fulcrum Manual on the SSL contains detailed data entry instructions.

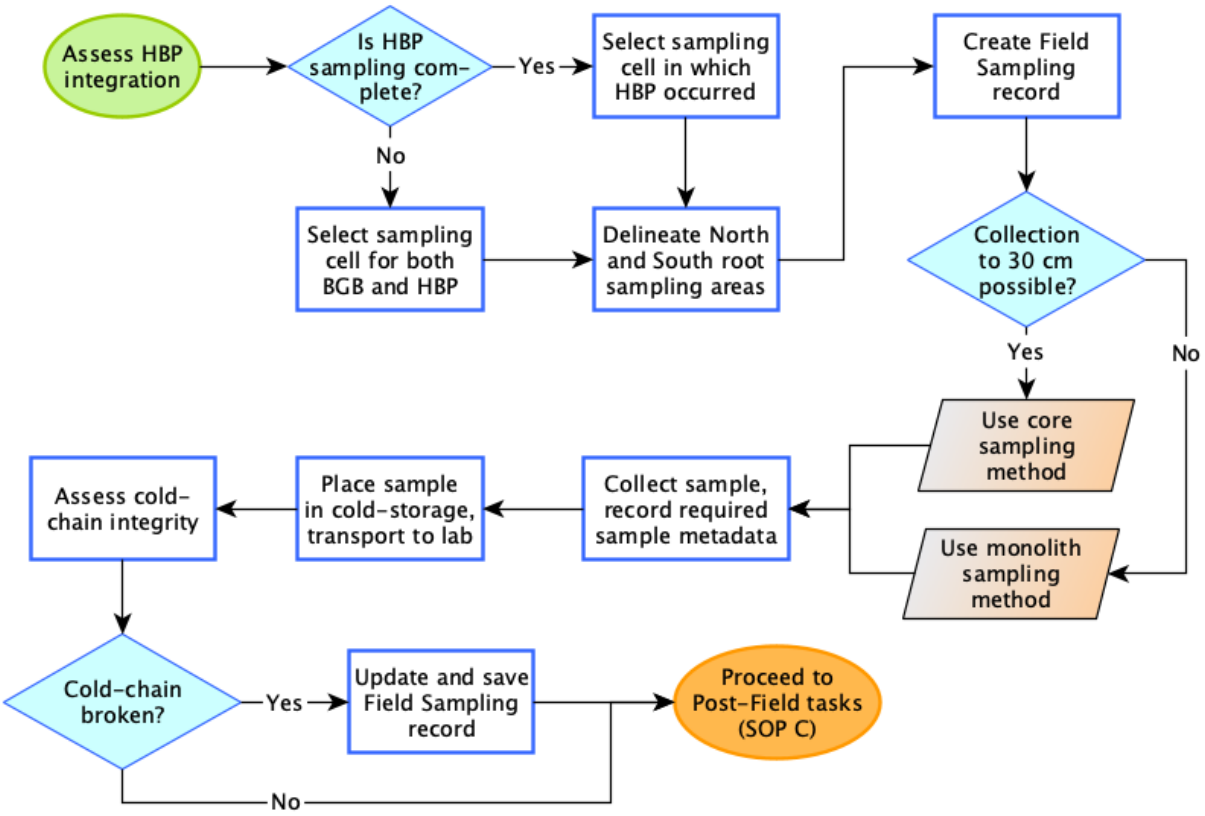

Figure 8. Expanded workflow diagram for Plant Belowground Biomass field sampling. Diagram supports and does not replace protocol text; most common workflow is outlined.

B.1 Spatially and Temporally Linked Protocols

Herbaceous Clip Harvest

- In Tower plots that support herbaceous clip harvest, the Plant Belowground Biomass Sampling protocol and the Herbaceous Biomass protocol (RD[11]) are spatially collocated, and should occur in the same cell in a given sampling year (**Figure 9**, *right*).
    - If plant Belowground Biomass sampling is scheduled prior to Herbaceous Biomass clip harvest sampling, accepting/rejecting sampling cells must be done with both protocols in mind.
    - At grazed sites, do not collect plant belowground biomass cores from underneath exclosures.
    - Ignore collocation guidance at those Tower plots that no longer support herbaceous clip harvest due to optimization.
- If Herbaceous Biomass sampling is scheduled before plant Belowground Biomass sampling:
    - Consult each per plot Clip List to enable co-location of sampling within cells in each plot.
    - Stagger the sampling activities to ensure sufficient oven space for all samples.
    - Always attempt to acquire soil samples from the same cell used for clip harvesting.
- At Agricultural sites:
    - Tall-stature crops may require pre-delineation of sampling areas (SOP A.3).
    - Additional steps are required to ensure that soil sampling areas and agricultural clip strips do not overlap (SOP B.6).

Plant Diversity

Plant Diversity sampling occurs in 3 randomly selected Tower plots each year. In these plots, identify and demarcate a suitable sampling cell for plant belowground biomass/herbaceous biomass sampling prior to performing Plant Diversity sampling.

- This will ensure that the cell is not trampled during Plant Diversity sampling.
- Should plant Belowground Biomass Sampling occur before Plant Diversity sampling, take care to avoid trampling 1 m^2 nested subplots used for Plant Diversity % cover measurements.

B.2 Plot Prioritization

For some combinations of soil and vegetation type, completing scheduled Plant Belowground Biomass sampling may require the full 6 weeks allowed. In the event that the full, scheduled sampling cannot be completed, it is important to generate a spatially-balanced sample set that represents the entirety of the Tower airshed, regardless of completion status.

- *The spatial sampling strategy should be selected before sampling begins*; it is not expected that crews will switch strategies part-way through a bout.
- The general expectation is that all scheduled sampling is completed; however, the 'medium-risk' and 'high-risk' approaches allow us to achieve spatially balanced sampling when circumstances arise that are out of our control and completing sampling is not possible.
- To ensure a spatially-balanced sample, plot prioritization lists are used for both the 'medium-risk' and 'high-risk' sampling strategies.

For low-risk sites at which scheduled sampling is always completed:

1. Because the full, scheduled sampling effort is expected to be completed, visit plots according to optimal logistics. There is no need to use the site-specific Plot Prioritization list.
2. For sites with 20m x 20m small-stature Tower plots:
    a. Collect two soil samples from a single representative sampling cell (i.e., North and South).
    b. For the first 20 plots, flip a coin to randomly select one soil sample per plot for Dilution Sampling (SOP E).
        i. If **Sampling Impractical** = 'Obstruction' for both North and South targets in one of the first 20 plots, select cores from additional plots according to the plot prioritization list to get to n=20 total Dilution Samples.
3. For sites with 40m x 40m large-stature Tower plots:
    a. Collect both North and South soil samples from two representative sampling cells, one sampling cell per assigned subplot (4 soil samples total).
    b. Randomly select one soil sample per plot for Dilution Sampling (SOP E). Successive coin flips or equivalent may be used.

For medium-risk sites with large Tower plots at which at least 50% of full, scheduled sampling will be completed

The medium-risk is not applicable to sites with 20m x 20m small-stature Tower plots. The 'medium-risk' strategy provides efficiency during Round 1 by eliminating some repeat visits to plots. Use of the Plot

Prioritization list during Round 2 ensures spatially balanced root and dilution sampling. To make robust fine root biomass estimates, it is critical that the final dataset be spatially balanced.

1. *Round 1 Collection (certain to be completed):*
    a. Visit all 20 plots and collect 2 cores per plot, one from each subplot.
        i. It is not necessary to follow the Plot Prioritization list for Round 1, as it is assumed you will definitely complete Round 1 if using the medium-risk strategy, and you will therefore eventually collect roots AND process a dilution sample from all plots.
        ii. At approximately 8-10 plots per week, it is assumed it will require approximately 2 weeks to complete Round 1.
    b. To enable easier tracking of which samples have been collected from which plot, systematically collect only North cores (or only South cores).
    c. Process one core per plot for Dilution Sampling (SOP E); randomly choose which subplot the dilution sample comes from.
        i. This strategy means you will process half the dilution samples in the first week of Round 1 collection/processing, and you will process the other half in the second week of Round 1 processing.

2. *Round 2 Collection (may not be completed)*
    a. Aim to visit all 20 plots and collect an additional 2 cores per plot, one from each subplot. For this round it is not assumed you will definitely complete all of the sampling, ***so use the Plot Prioritization list.***
        i. At 8-10 plots per week, Round 2 will also require approximately 2 weeks to complete, but it is not assumed you will necessarily be able to complete all scheduled sampling/processing with existing resources.
    b. Systematically collect only South cores (or North cores if you collected South cores in Round 1).
    c. Dilution samples: Process any additional samples needed to get to a total of n=20 dilution samples.
        i. Use the Plot Prioritization list and select subplots that did not generate a dilution sample in Round 1.

<u>For higher-risk sites at which 50% or more of full, scheduled sampling may not be completed:</u>

Goal: Collect one sample per plot by working down the Plot Prioritization list, then repeat as needed, again collecting one sample per plot in each successive round of sampling until the total number of required samples per plot is met.

For sites with 20m x 20m small-stature Tower plots:

1. *Round 1 Collection*:
    a. Visit plots according to the order specified in the domain-specific Plot Prioritization list in the TOS Sampling Support Library (e.g., files named '*DXX_UniquePlotIDsAndSamplingModules.xlsx*').
    b. Create a record in the *BBC: Field Sampling [PROD]* app. The parent record will have only 1 child record.
    c. Collect either the North or South core sample from a single representative sampling cell (flip a coin to randomly select North/South).
        i. If roots or rocks prevent sample collection, try to collect a sample from the remaining North/South sample area.
        ii. In the *BBC: Field Sampling [PROD]* app, create a child record and select **Sampling Impractical** = 'Obstruction' for the core sample that was unsuccessful.
    d. *Dilution Sampling*: Working in order according to the Plot Prioritization list, process soil samples for Dilution Sampling (SOP E) until n=20 total dilution samples are processed. Round 1 collection will generate all required dilution samples unless **Sampling Impractical** = 'Obstruction' at some sampling locations.
2. *Round 2 Collection*:
    a. Once the Plot Prioritization list has been worked through and a single sample has been collected from each plot, return to the start of the prioritization list and work down the list again to collect the second core sample from the same cells sampled in Round 1.
    b. Create a second *BBC: Field Sampling [PROD]* record for the second sample. The parent record will again have only 1 child record.
    c. *Dilution Sampling*: (If necessary) Process additional Dilution Samples until a total of n=20 are processed.

For sites with 40m x 40m large-stature Tower plots:

1. *Round 1 Collection*:
    a. Visit plots according to the order specified in the domain-specific Plot Prioritization file in the TOS Sampling Support Library (e.g., files named '*DXX_UniquePlotIDsAndSamplingModules.xlsx*').
    b. Randomly select one of the two assigned subplots for sampling.
    c. Within the selected subplot, collect either the North or South soil sample from a single representative sampling cell (flip a coin to randomly select North/South).

i. If roots or rocks prevent sample collection, try to collect a sample from the remaining North/South sample area.

ii. In the *BBC: Field Sampling [PROD]* app, create a child record and select **Sampling Impractical** = 'Obstruction' for the core sample that was unsuccessful.

d. *Dilution Sampling*: Process all collected samples for Dilution Sampling (SOP E).

2. *Round 2 Collection:*

a. Return to the beginning of the plot prioritization list and within each plot, select the subplot that was not sampled in Round 1.

b. Within the subplot, collect either the North or South soil sample from a single representative sampling cell (flip a coin to randomly select North/South).

i. If roots or rocks prevent sample collection, try to collect a sample from the remaining North/South sample area.

ii. In the *BBC: Field Sampling [PROD]* app, create a child record and select **Sampling Impractical** = 'Obstruction' for the core sample that was unsuccessful.

c. (If necessary) *Dilution Sampling:* Process additional soil samples until a total of n=20 dilution samples are processed.

3. Repeat Round 1 and Round 2 until the required 4 samples per plot are collected, making sure to target the same sampling cells in each subplot, and to collect soil from North/South sampling areas that were not previously sampled.

a. Dilution sampling should be complete at this point, no additional samples processed.

B.3 Core Sample Collection

1. Navigate to the plot or subplot to be sampled.

a. See SOP B.2 to determine the order in which plots should be sampled.

b. See SOP B.4 if the entire plot is flooded.

2. Use the plot or subplot-specific Clip List to identify the sampling cell that was (or will be) used for the peak herbaceous biomass clip harvest in the current year.

a. The Clip List provides the randomized list of potential sampling cells per plot or subplot.

b. Coordinates provided for each cell correspond to the SW corner of the clip-strip (red 'x' in **Figure 9**) – i.e. the area from which herbaceous biomass is harvested.

c. If the site host allows, a pin flag may be left behind at the SW corner of the clip strip to aid collocation across protocols.

d. The Clip List indicates which cells have already been harvested or rejected; on the Clip List, mark cells selected for Plant Belowground Biomass Sampling with **status** = 5.

e. If the desired peak biomass sampling cell is submerged by standing water, but the entire plot is not submerged: Reject and work down the Clip List to choose an acceptable cell, and record "peak biomass cell submerged" in the "remarks" field of the Clip List.

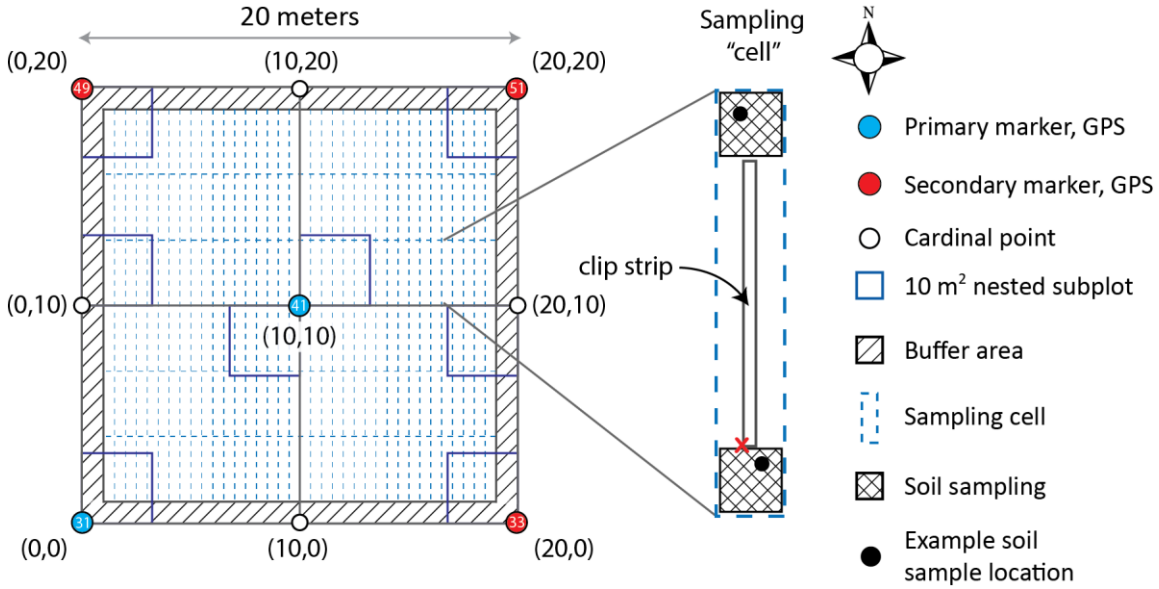

Figure 9. (*Left*) A 20m x 20m Tower plot showing the locations of 3.0m x 0.5m sampling cells used for plant belowground biomass soil sampling; sampling cells that overlap 10 m^2 nested subplots are not sampled and other nested subplots are omitted for clarity. (*Right*) Within a cell selected for root coring, one soil sample is collected from each of the 0.5m x 0.5m areas to the North and South of the clip-strip; the red 'x' indicates the coordinate provided in the Clip List. White numbers within plot markers are pointIDs.

3. Locate the coordinates within the plot that correspond to the SW corner of the clip-strip within the target sampling "cell" (**Figure 9**). The procedure used to locate the **offsetEasting** (X) coordinate depends on the value of the relative **offsetNorthing** (Y) coordinate. If using the rangefinder and a reflective surface to locate the cell, refer to RD[10] for detailed operating instructions.

*If the **offsetNorthing** coordinate is < 10:*

a. Start at the SW corner of the plot or subplot: coordinate (0,0) in **Figure 9**. Use either the rangefinder in **HD** mode, or run a tape East/West toward (20,0) along the south edge of the plot or subplot. If using tape, stretch it taut.

b. Place a pin flag at the desired relative X-coordinate.

c. Standing directly over the pin flag that was just placed, use the rangefinder to locate the Y-coordinate.

i. Make sure the azimuth is 0˚ (True North) when shooting the rangefinder to find the Y-coordinate.

d. Place a pin flag at the clip-strip (X,Y) location – i.e. the SW corner of the clip-strip.

*If the **offsetNorthing** coordinate is > 10:*

a. Start at the plot centroid: coordinate (10,10) in **Figure 9**.

b. *If offsetEasting < 10*: Measure west from (10,10) toward (0,10). If using the rangefinder, make sure the azimuth is 270˚. If using a compass and tape, stretch the tape taut. Place a pin flag at the desired X coordinate.

c. *If offsetEasting > 10*: Measure east from (10,10) toward (20,10). If using the rangefinder, make sure the azimuth is 90˚. Place a pin flag at the desired X coordinate.

offsetEasting coordinate	Rangefinder/Tape Layout[1]
1 < X < 10	From (10,10) → (0,10)
10 < X < 20	From (10,10) → (20,10)

[1] Use the TruPulse in **AZ** mode to guide the tape along the correct azimuth.

d. Standing directly over the pin flag that was just placed, use the rangefinder to locate the Y-coordinate. Make sure the azimuth is 0˚ (True North) when shooting the TruPulse to find the Y-coordinate.

e. Place a pin flag at the Y coordinate, which is the SW corner of the Clip Strip.

4. Assess whether the sampling cell is representative of the plot, and accept or reject the location. ***Remember that you must consider both this protocol, and the Herbaceous Biomass protocol.***

a. Consult the NEON TOS Herbaceous Biomass and Productivity protocol for detailed acceptance/rejection criteria (RD[11]).

b. Obstacles, disturbances, and/or irregularities on the surface may lead to a cell being unrepresentative, and these may include trees, large rocks, ant nests, downed logs, etc.

c. If > 3 consecutive potential cells are rejected as 'unrepresentative,' it is necessary to recalibrate the working definition of 'representative.'

5. Mark the four corners of the South root sampling area within the sampling cell to delineate where the first of the two soil samples should be collected (**Figure 10**). Pin flags, a 50cm x 50cm PVC frame, or equivalent can be employed for this purpose. If using pin flags:

a. Place pin flag "A" 20 cm to the west of the coordinates provided in the Clip List (i.e. the red "x" in **Figure 10**) – use a meter tape or ruler to be accurate.

b. Place pin flag "B" 50 cm to the east of pin flag "A"

c. Place pin flag "C" 50 cm to the south of pin flag "A"

d. Place pin flag "D" 50 cm to the south of pin flag "B"

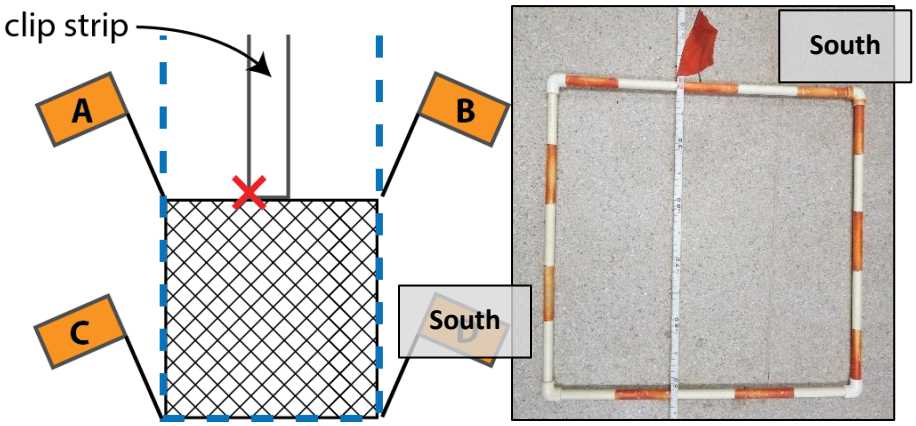

Figure 10. (*Left*) Delineating the South root sampling area (cross hatched) within a sampling cell (dashed blue lines) with pin flags. The clip-strip (black lines) lies immediately to the north of the South root sampling area, and the red "x" marks the coordinates provided in the Clip List. (*Right*) The sampling area may also be delineated using a 50cm x 50cm PVC frame.

6. Mark the four corners of the North root sampling area within the sampling cell to delineate where the second of the two soil samples should be collected (**Figure 11**). Pin flags, PVC frame, or equivalent may be employed. If using pin flags:
    a. Place pin flag "E" 2 m to the north of pin flag "A"
    b. Place pin flag "F" 2.5 m to the north of pin flag "A"
    c. Place pin flag "G" 2 m to the north of pin flag "B"
    d. Place pin flag "H" 2.5 m to the north of pin flag "B"

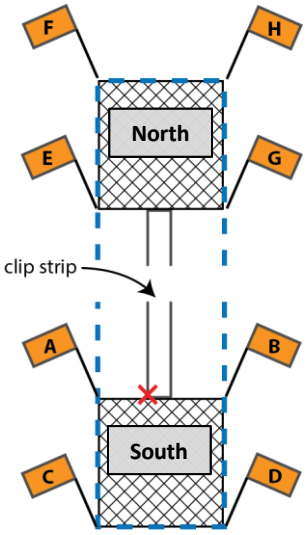

Figure 11. Delineating the North root sampling area with reference to the previously delineated South root sampling area (cross hatched) within a sampling cell using pin flags (dashed blue lines indicate the clip cell boundary). The middle of both the cell and the clip-strip have been omitted for clarity.

7. Within one of the targeted root sampling areas, identify the exact location from which the soil sample will be collected, and determine the **Root Sampling Method** ('core' or 'monolith').
    a. To avoid rocks and roots that may interfere with coring, probe the ground within the target sampling area with a clean chaining pin (or equivalent) to determine a suitable location.
    b. If it appears possible to collect a sample to 30 cm:
        i. Assemble the soil core tube, bit, retainer basket (if necessary), and drive head (see Appendix D), and prepare to collect a ***soil core sample***.
        ii. Note that the corer will handle infrequent smaller diameter rocks (2-5 cm diameter) but cannot handle rocks of this size when they are abundant.
        iii. You will develop site-specific intuition as to when probing indicates coring is possible.
        iv. Consult SOP B.4 "Troubleshooting" if you attempt to collect a core and it is not possible to achieve the desired 30 cm depth.
    c. If collecting a sample to 30 cm depth appears impossible – i.e., probing reveals there is no place within the target coring area where the corer could be inserted without encountering obstacles before reaching 30 cm depth:
        i. Prepare to collect a ***soil monolith sample*** (10cm x 10cm surface area, 30 cm target depth)**.**
        ii. ***!!!*** The soil corer can reliably cut through roots up to 1 cm diameter and larger. If you encounter roots of this size, coring is still the preferred collection method.
8. If it is ***NOT*** possible to collect a soil sample from a soil sampling area, AND the sampling cell is deemed representative of the plot, follow (a) – (c) below; otherwise proceed to the next step.
    a. Create a record in the *BBC: Field Sampling [PROD]* app for the **Plot ID** and **Clip Cell Number**, and create a child-record to record the 'Core Field Data' for the appropriate sampling area ('North' or 'South').
    b. Select **Sampling Impractical** = 'Obstruction', and save the child record.
    c. Return to step (7) above, and attempt to collect a sample from the remaining soil sampling area within the clip cell.
9. Fill in the barcoded, pre-printed waterproof label created in SOP A.3 with the following information:
    a. **Plot ID** and **Sampling Cell Number:** e.g., UKFS047042
    b. **Collect Date**: *YYYYMMDD* format
    c. **Soil Sample ID**: *North* or *South*

d. **Dilution Sample:** *Yes* or *No* (see SOP B.2)

e. **Subplot ID** (see **Figure 1**):

a. For 20m x 20m plots, subplotID = 31_400;

b. For 40m x 40m plots, subplotIDs = 21_400, 23_400, 39_400, or 41_400

10. Create a record in the *BBC: Field Sampling [PROD]* app for the sampled cell, and enter:

a. **Subplot ID**: the identifier for the subplot – e.g., "21_400".

b. **Sampling Protocol Version**: Select the version of the protocol used for sampling, typically the currently released version.

c. **Collect Date/Time**: Use *YYYYMMDD* and *HH:mm* 24-h time format.

d. **Cell Number**: ### format. This number is the last 3 digits of the cell from the Clip List.

11. If root biomass from a *Toxicodendron spp.* may be present in the soil sample:

a. Follow the guidelines established in TOS Standard Operating Procedure: Toxicodendron Biomass and Handling (RD[08]) to minimize exposure to toxic oils and for guidance on how to clean equipment.

b. Label sample bags that may contain *Toxicodendron* so that they will be handled with appropriate caution during downstream processing. A sample warning label such as that shown at left may be employed for this purpose.

12. Remove plants and litter from the sampling area, then remove non-root belowground plant parts from the top 3 cm of soil:

a. Use hand clippers to remove aboveground plant leaves and stems from the exact area from which a sample will be collected and remove litter down to the soil surface.

b. Score the ground with the soil core bit or soil knife so it is clear exactly where the soil sample will be collected.

c. Loosen the soil with a soil knife and remove the soil from around any perennial non-root plant parts growing within the scored area (e.g., corms, rhizomes, crowns, biological soil crust, etc.).

i. If perennial graminoid crowns are present, remove soil until the transition from crown to root is visible.

ii. If biological soil crust is present, score the soil just below the moss/lichen layer and carefully remove the crust. The crust can be placed back over the coring hole on top of the backfill material.

d. Clip all *non-root* material from within the scored area, and discard; (c) and (d) may be done in the laboratory if field conditions are not conducive.

13. Collect a soil sample to 30 cm maximum depth:

*If using the **core** sampling method:*

a. Position the soil core bit back over the scored area, and make sure the soil core assembly is vertical. If the plot is sloped, the soil core assembly should still be vertical.

b. Use the slide hammer to pound the soil core tube to 30 cm maximum depth (*which should be marked on the soil core tube with electrical tape or similar*).

***!!!* Once the soil corer is in the ground, do not turn the unit counterclockwise, as this will unscrew the bit from the core tube underground, resulting in loss of the bit.**

c. Remove the slide hammer attachment and push the core tube back and forth sharply several times to loosen it within the soil profile.

d. Remove the core tube from the ground, carefully extract the core into a plastic bag, and place the label inside the bag.

*If using the **monolith** sampling method:*

a. Use the soil knife and a ruler to measure and cut a sample with 10cm x 10cm surface area. Use a rubber mallet to drive the soil knife vertically (if necessary).

b. Cut and remove soil sample as you work, and place into a plastic bag with a label inside.

i. When rocks are encountered, remove when possible.

ii. Removal of larger rocks may enlarge the hole. This is acceptable, but soil sample should only be collected from the target 10cm x 10cm area as it extends downward from the surface. The intent is to enable calculation of root density (g cm^{-3}) and root mass per area (g m^{-2}).

c. Collect soil and roots from the 10cm x 10cm sampling area to a maximum depth of 30 cm.

i. See SOP B.4 “Troubleshooting” **Table 10** if a sampling depth of 30 cm cannot be attained.

14. Create a child record for the **Soil Sample ID** (*North* or *South*), and measure and enter the required sampling data.

a. **Sampling Impractical**: enter ‘OK’.

b. **Toxicodendron Possible**: enter ‘Yes’ if the sample may contain roots from *Toxicodendron* based on your assessment of the surrounding vegetation.

c. Obtain the dimensions of the hole from which the sample was collected:

*If using the **core** sampling method:*

i. **Core Diameter**: measure the inside diameter of the coring device, nearest 0.05 cm. For the standard corer listed in Appendix G, the value is 6.65 cm.

ii. **Root Sample Depth**; measure the average depth below the surface to which the soil sample was collected, nearest 1 cm. Push past any loose soil that fell back into the hole, and measure a representative depth.

*If using the **monolith** sampling method:*

i. **Monolith Length** and **Monolith Width**: the actual length and width of the 10cm x 10cm surface area from which the sample was collected, nearest 1 cm. Dimensions may be recorded to the nearest 0.1 cm, if possible.

ii. **Root Sample Depth**: measure the average depth below the surface to which the soil sample was collected, nearest 1 cm, as above for ***core*** sampling.

d. **Litter Depth**: average litter depth for the entire 'North' or 'South' soil sampling area, nearest 1 cm.

i. If litter is < 1 cm average depth, record 0.5 cm if litter is patchy but present.

ii. Record 0 cm if litter is absent from the 50cm x 50 cm root sampling area.

e. **Woody Stem Distance, DBH ≥ 10 cm**: distance to closest *living* woody stem with DBH ≥ 10 cm, nearest 0.1 m. Leave blank if no qualifying stems are within 20 m of the sampling location.

f. **Woody Stem Distance, DBH ≥ 1 cm**: distance to closest *living* woody stem with 1 cm ≤ DBH < 10 cm, nearest 0.1 m. Leave blank if no qualifying stems are within 20 m of the sampling location. Ignore individuals with DBH < 1 cm.

g. **Bare Ground**: % of entire 'North' or 'South' soil sampling area that is made up of soil (particles < 5 mm diameter) and/or rock (mineral particles > 5 mm diameter), nearest 10%.

h. **Sample Barcode** (required): scan in the sample barcode affixed to the waterproof label.

i. **Sample Condition**: indicator for sample cold-chain integrity. Defaults to 'cold-chain unbroken'; update as necessary if the cold-chain is broken before the sample is sieved.

j. Save the child record, then save the parent record.

15. Place the sample label in the bag, seal, and place the bagged soil sample into cold storage. Maintain cold until samples can be processed in the laboratory (see SOP B.5).

16. Backfill the sample hole with site-host approved material (if required by site host).

17. Collect additional samples per Plot Prioritization guidance (see SOP B.2).

B.4 Troubleshooting

Table 10. Potential issues encountered during plant Belowground Biomass sampling and issue resolution.

Issue	Resolution
30 cm depth not reached due to obstacles	• Attempt sample collection at up to 3 total locations within the target coring area. • Collect a sample to the greatest depth possible. • Record the final sampling depth.
A sample cannot be collected from a representative sampling area	• Record **Sampling Impractical** = 'Obstruction' • Move on to the next sampling area within the Sampling Cell, the next Sampling Cell, or the next plotID, whichever is applicable.
Flooded plot	Resolution strategies in order of preference: 1. Schedule plant belowground biomass sampling at a time of year when probability of flooding is minimized and potentially decouple from Herbaceous Biomass clip-harvest sampling (Section 4.2). 2. Attempt to collect soil samples from plots with water < 30 cm depth. **a.** Use the basket adapter with sandy soils if this would be helpful to prevent soil falling out of the collection tube. b. Keep sample if soil is cohesive enough such that either of the following are true: i. The bore hole can be accurately measured for sampling depth (equivalent to sample length). ii. The sample itself can be accurately measured for length. c. Discard sample if the depth of the bore hole or the length of the sample cannot be reasonably measured. That is, discard the sample if either the bore hole has collapsed and/or filled with sediment, or the sample lacks structural integrity and cannot be measured.
Cold-chain is broken before sample is sieved	If the cold-chain is broken for < 12 h: 1. Update **Sample Condition** = 'cold-chain broken – less than 12 h' in the *BBC: Field Sampling [PROD]* app. 2. Save the record and process the sample as normal. If the cold-chain is broken for > 12 h: 1. Update **Sample Condition** = 'cold-chain broken – more than 12 h' in the *BBC: Field Sampling [PROD]* app. 2. Submit an incident in ServiceNow to document length of time cold-chain was broken and determine next steps.

B.5 Sample Preservation

1. Keep samples in a cooler with cold packs to minimize cellular activity, reduce decomposition, and preserve sample mass.
2. Change cold packs for fresh ones every 12 h or transfer to a 4-8 ˚C refrigerator prior to laboratory processing.
    a. **Sample Condition**: Update as appropriate in the *BBC: Field Sampling [PROD]* app if the cold-chain is broken. See SOP B.4 Troubleshooting for required actions.
3. Sieving and sorting must be initiated in the laboratory according to SOP D ***within 72 h*** of collection in the field.
    a. Alternatively, core samples may be frozen within 72 h of collection and stored for processing at a later date. Ensure that labor will be available for processing frozen samples before choosing this workflow.
        i. If samples are to be frozen prior to processing, they should be frozen as soon as possible – i.e., 72 h is the maximum amount of time allowed between collection and freezing.
        ii. To maintain sample integrity, samples should only be frozen/thawed **once** before laboratory processing.
    b. Samples must be processed within 3 months of freezing.
4. To freeze core samples:
    a. Freeze core samples at either –20 ˚C or –80 ˚C. If freezing at –80 ˚C, freeze at –20 ˚C first to avoid overloading the ultracold freezer.
    b. Use Type II barcodes and cryo-safe human readable labels. Other labels will readily fall off sample bags when handled at freezer temperatures (**Table 7**).
    c. ***Example use case 1***: Employ short-term freezing of root core samples to improve efficiency of field and lab work (i.e., samples are frozen for a week to several weeks). When samples are frozen after collection in the field, it is not necessary to coordinate field sampling and lab processing around the 72 h cold-storage limit. Instead, staff can focus on collecting core samples in the field followed by processing those samples when the scheduled field effort is complete. It is critical to avoid collecting more samples than there is available labor to process.
    d. ***Example use case 2***: Freeze samples as soon as possible after collection in the field (and always within 72 h) to ship to another domain for laboratory processing. Consult the Shipping Protocol for packaging guidelines to ensure the samples remain frozen during transit.

e. ***Example use case 3***: Employ longer-term sample freezing to complete field sampling for multiple high-priority protocols/activities. Laboratory processing is then completed later when there are fewer competing priorities. Here, it is again critical to avoid collecting more samples than there is available labor to process.

!!! **IMPORTANT**: Record the **Collect Date** and **time** in the Field app AND **Oven Start Date** and **time** in the Lab Weighing app so that the number of hours the samples were stored cold can be calculated.

B.6 Plant Belowground Biomass at Agricultural Sites

Delineation and flagging of sampling areas for both Plant Belowground Biomass Sampling and the Agricultural Biomass SOP should be carried out at the same time regardless of which protocol is executed first.

1. Bring a 3 m long folding ruler, or equivalent rigid measuring device, and 0.5m x 0.5m frames used to lay out the belowground biomass sampling areas.
2. Locate the SW corner of the clip strip as in SOP B.3. At agricultural sites, *this Clip List coordinate will serve as the SW corner of the clip cell rather than the clip strip*.
3. Rotate clockwise until you are facing perpendicular to crop rows (**Figure 12**).
4. Use the rigid measuring stick to lay out the 3 m long left side of a 3.0m x 0.5m clip cell.
5. Use the 0.5m x 0.5m frames to layout the plant belowground biomass sampling areas at either end of the clip cell. Flag the lower-left corner of the cell and the upper-right corner of the cell.
    a. Flagging should remain if soil sampling occurs prior to agricultural clip harvest.
6. Delineate a clip strip of the appropriate dimensions; the long edge of the clip strip should remain perpendicular to crop rows (**Figure 12**).

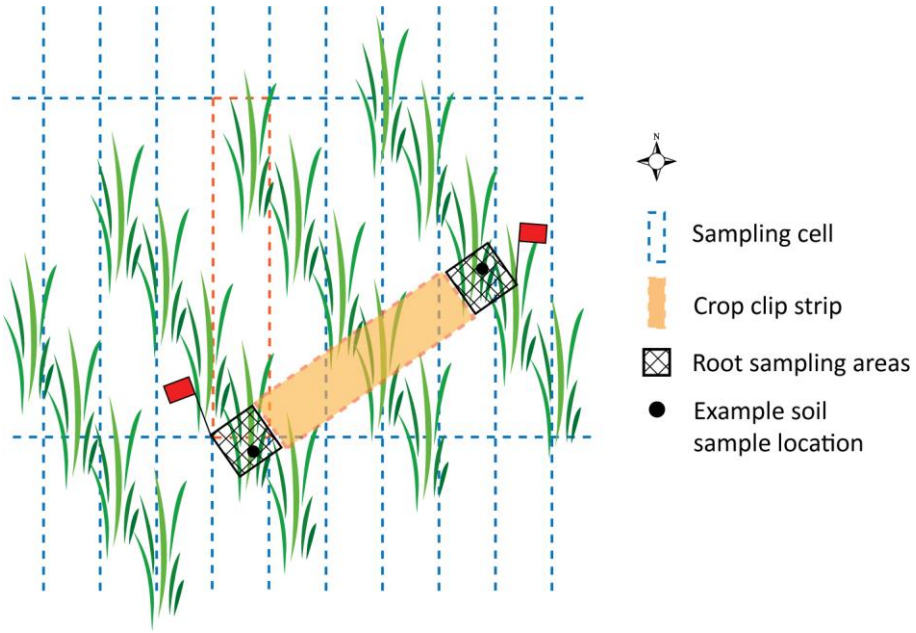

Figure 12. Modified sampling cell layout when integrating plant belowground biomass sampling and herbaceous biomass clip harvest at agricultural sites. Orange dashes indicate the rotated clip cell. The red flag on the left is placed at the coordinate provided in the Clip List.

SOP C Post-Field Sampling Tasks

C.1 Document Incomplete and Compromised Sampling

Plant Belowground Biomass sampling occurs on the schedule described in Section 4.1 at up to 30 Tower Plots per site. Ideally, sampling occurs at these sampling locations for the lifetime of the Observatory. However, circumstances may arise that require that sampling within a site be shifted from one particular location to another. In general, sampling is compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, an incident should be submitted.

There are two main pathways by which sampling can be compromised. Sampling locations can become unsuitable to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

For Plant Belowground Biomass sampling, criteria for considering a plot compromised include:

- If sampling cannot be completed in a plot for 2 consecutive bouts. Because bouts are scheduled every 5 y, it is necessary to examine the **Sampling Impractical** field from previous bouts to determine whether a sampling location has become compromised.

If sampling at a given plot is not possible during a given bout an incident should be submitted.

To document locations not sampled during the current bout:

1. Review the completed sampling effort and create **Sampling Impractical** records as described in Section 4.5 for plots at which sampling was scheduled, and where sampling was not completed or was not attempted.
2. To determine whether a sampling location is compromised according to the criteria above:
    a. Review the **Sampling Impractical** field in the *BBC: Field Sampling [PROD]* app and the 'bbc_percore' table in Portal data to identify plots where root samples could not be collected after sampling was attempted.
    b. Create an incident with the following naming convention to document the missed sampling: 'TOS Sampling Incomplete: BBC – [Description of root cause]'
        i. Example: 'TOS Sampling Location Compromised: BBC – Could not access plot for two consecutive bouts due to localized flooding'
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.

SOP D Laboratory Processing: Sieving, Sorting, and Weighing Roots

Goals

- (Optional) Thaw previously frozen core sample.
- Isolate fine roots > 1 cm length from soil, sort to **Size Category**, then dry and weigh.
- For cores identified for dilution sampling, retain the residual fraction (root fragments < 1 cm length + organic material) for processing via SOP E.
- Collect required laboratory data:
    - Enter required data in the *BBC: Lab Weighing [PROD]* application.
    - The Belowground Biomass Sampling Fulcrum Manual on the SSL provides detailed data entry instructions.

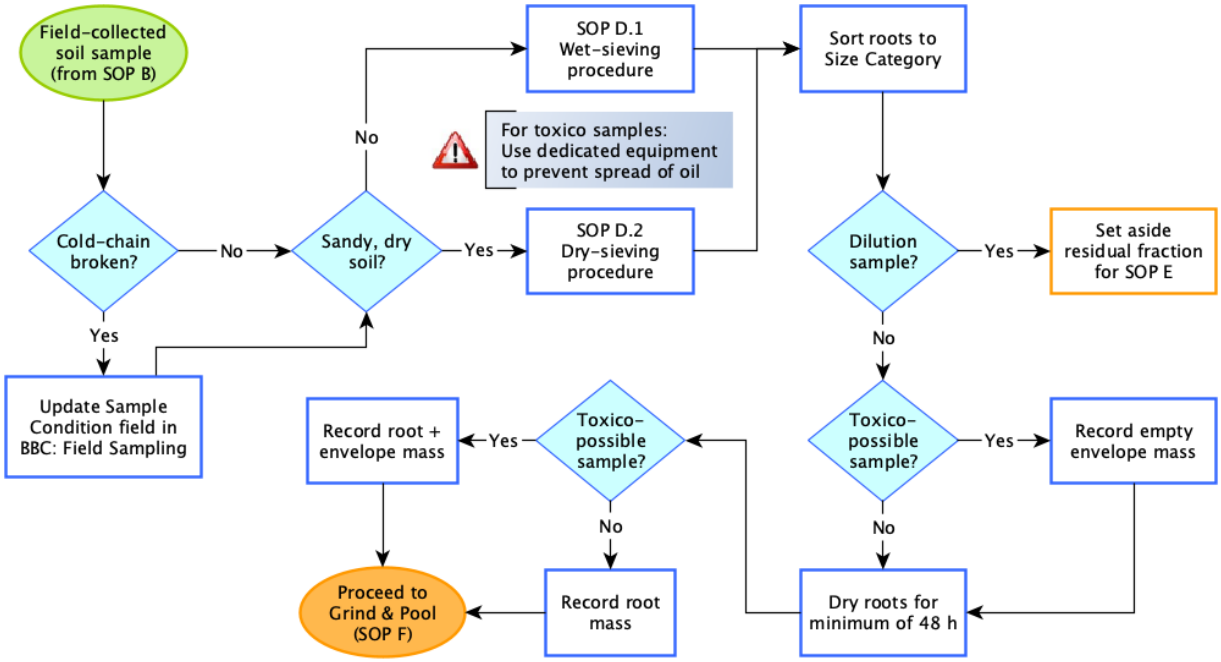

Figure 13. Expanded workflow diagram for Plant Belowground Biomass sieving, sorting, and weighing in the laboratory. Diagram supports and does not replace protocol text; most common workflow is outlined.

Overview

Use time estimates for lab processing steps provided in Section 4.6 to plan field work so that a backlog of soil samples does not develop and the ***72 h maximum cold storage*** requirement between core collection in the field and beginning sieving/sorting can be met.

Time sensitive processing steps are illustrated in **Figure 13** and include:

1. Determine whether soil samples will be wet-sieved or dry-sieved.
    - **Wet-sieving**: If the soil samples have a large amount of root mass, soils are finely textured, or the soil is difficult to break apart by hand without fragmenting roots, wet-sieving may be the most efficient procedure for separating roots from soil.
        - Soak hard and/or clay-rich soil samples for 1-12 h before wet-sieving.
    - **Dry-sieving**: If the soil samples have little root mass and are sandy or coarsely textured, and have low moisture content, dry-sieving soils may be the most efficient procedure.
2. Track and appropriately label samples with **Toxicodendron Possible** = 'Yes'. If samples with *Toxicodendron* are possible at a site, it is strongly advised to use a set of dedicated equipment for affected samples to minimize exposure to toxic oils (buckets, sieves, sorting trays, etc.).
3. Wash and sieve soil cores to separate mineral soil from root biomass and organic matter, and separate roots ≥ 1 cm length from the residual fraction (roots < 1 cm length).
4. Sort sieved roots to size class.
5. Set aside the residual fraction from a spatially-balanced subset of 20 samples for dilution processing (SOP E). The residual fraction is discarded for samples not selected for dilution sampling.
    - See SOP B.2 for guidance on selecting samples for dilution sampling.
    - It is acceptable to pause overnight between execution of SOP D and SOP E. Store labeled residual fractions overnight at 4 ˚C in a sealed container (e.g., labeled 50 mL tube).
6. Dry fine root biomass ≥ 1 cm length to constant weight.

Once roots are dry the following steps may be completed as time allows:

7. Weigh and record dry weight biomass.
8. Set aside dried, qualifying root materials for grinding.

Gloves

Fine root samples generated from this procedure are analyzed for isotopes (^{13}C and ^{15}N); as such, ***disposable latex or nitrile gloves are required during sieving, sorting, and grinding*** to prevent contamination of the sample with your hands. ***!!!*** Gloves also prevent exposure to *Toxicodendron* roots (use latex).

D.1 Wet Sieving Soil Samples for Fine Root Biomass

1. (Optional) Thaw previously frozen sample(s) overnight at room temperature for 8-12 h. Only thaw the number of samples that can be processed with available time/resources.
    a. Alternatively, samples may be thawed for 24-72 h in a refrigerator.
    b. Note: It is not a problem if samples are not completely thawed before placing into the 5-gallon bucket in water (step 3).
2.  Identify soil samples that may contain *Toxicodendron* and use a dedicated set of equipment for these samples throughout this procedure to prevent spread of toxic oils.
3. For wet sieving:
    a. To break up core samples prior to sieving, soak samples in water in a 5 gallon plastic bucket (or equivalent), for ≤ 15 min to up to 12 h. Water depth should be sufficient to cover the sample. Adjust soak time based on soil texture and hardness; soil aggregates should be easily broken up in water with gentle manual pressure.
        i. Hard, dry, and/or clay-rich samples may be soaked overnight in the bucket to facilitate workflow scheduling.
        ii. Transfer the label from the field sample bag to the bucket. A duct tape 'tab' on the bucket handle can be used as a place to stick the label.
        iii. ***!!!*** To avoid data loss, labels must follow samples through the sieving process.
    b. ***Soil Sample Barcodes*** (from the field): Retain the barcode and group with downstream root subsamples when they are placed in the oven for drying.
4. Before beginning the wet-sieving routine:
    a. ***Determine whether the soil sample has been selected for dilution sampling*** (SOP B.2). If selected, the residual fraction must be saved to generate a dilution sample.
    b. Check sieve integrity. Damaged sieves will reduce data quality and should not be used.
    c. Prepare the root-washing station (SOP A.4) and assemble the sieve stack.
        i. The 2 mm sieve should be on top of the 250 µm sieve, and the stack should be placed over one of the washing station grates.
    d. Label a sorting tray with the **sampleID**. Include 'D' on the label if the sample will be processed for Dilution Sampling.
        i. The adhesive label from the field can be transferred again from the bucket to the sorting tray (minimizes transcription errors).
        ii. Two sorting trays may be useful: One for roots from the 2 mm sieve, and the other for roots from the 250 µm sieve.

5. Gently break up the field-collected sample in the bucket.
    a. Massage the sample in the bucket with gentle manual pressure to break up large aggregates and organic matter (OM) pieces.
    b. Thoroughly mix the slurry in the bucket by hand to separate small roots from mineral soil particles. At this point, roots and small pieces of OM should be floating on the surface.
6. Remove large roots from the surface of the slurry in the bucket (**Figure 14**).
    a. Using hands or forceps, pick large visible roots from the bucket and place in the 2 mm sieve (i.e., the top of the sieve stack).
    b. Wash mineral particles from the roots and be sure to rinse hands over the stack so as not to lose root particles.
    c. Transfer clean roots to a sorting tray with water and lid – i.e., a clear plastic bin, white enamel pan or equivalent (**Figure 15**). Sorting is carried out in a subsequent step.

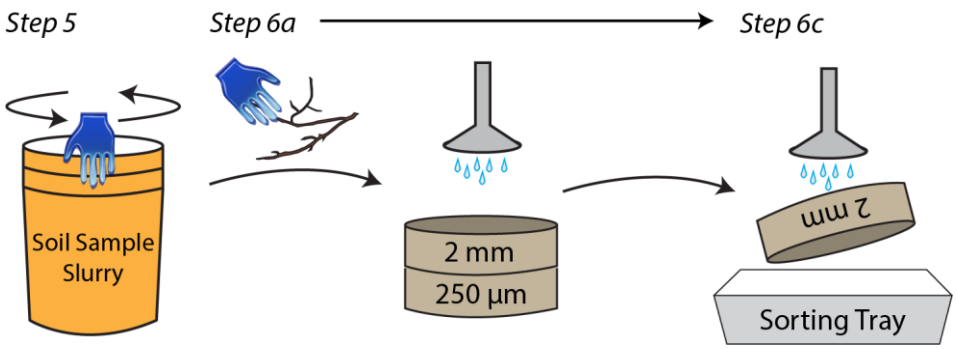

Figure 14. Manual removal of large roots from the surface of the soil sample slurry, followed by transfer from the 2 mm sieve to the sorting tray. Numbers correspond to protocol steps above.

Figure 15. Example of a plastic bin sorting container with a small amount of water to aid root separation.

7. To begin separating remaining roots and OM from the soil slurry in the bucket, pour PART of the slurry through the top of the sieve stack (**Figure 16**).
    a. BE CAREFUL NOT TO OVERFLOW THE 250 µm SIEVE!
    b. Quickly remove and rinse large rocks from the surface of the 2 mm sieve as you go.
    c. When the 250 µm sieve is full, transfer the entire contents into a large plastic decanting tray, or equivalent, for decanting and separation of mineral particles. Set aside the contents in the decanting tray until the entire soil sample has been passed through the sieve stack.
    d. When checking the 250 µm sieve, rinse roots trapped in the 2 mm sieve and transfer clean roots from the 2 mm sieve into the sorting tray from step (6.c) above; transfer by turning the 2 mm sieve upside-down over the sorting container and using the washing station nozzle.
    e. Continue pouring aliquots of the sample slurry from the bucket through the sieve stack, repeating (a)-(d) immediately above, until the entire sample has passed through the stack.

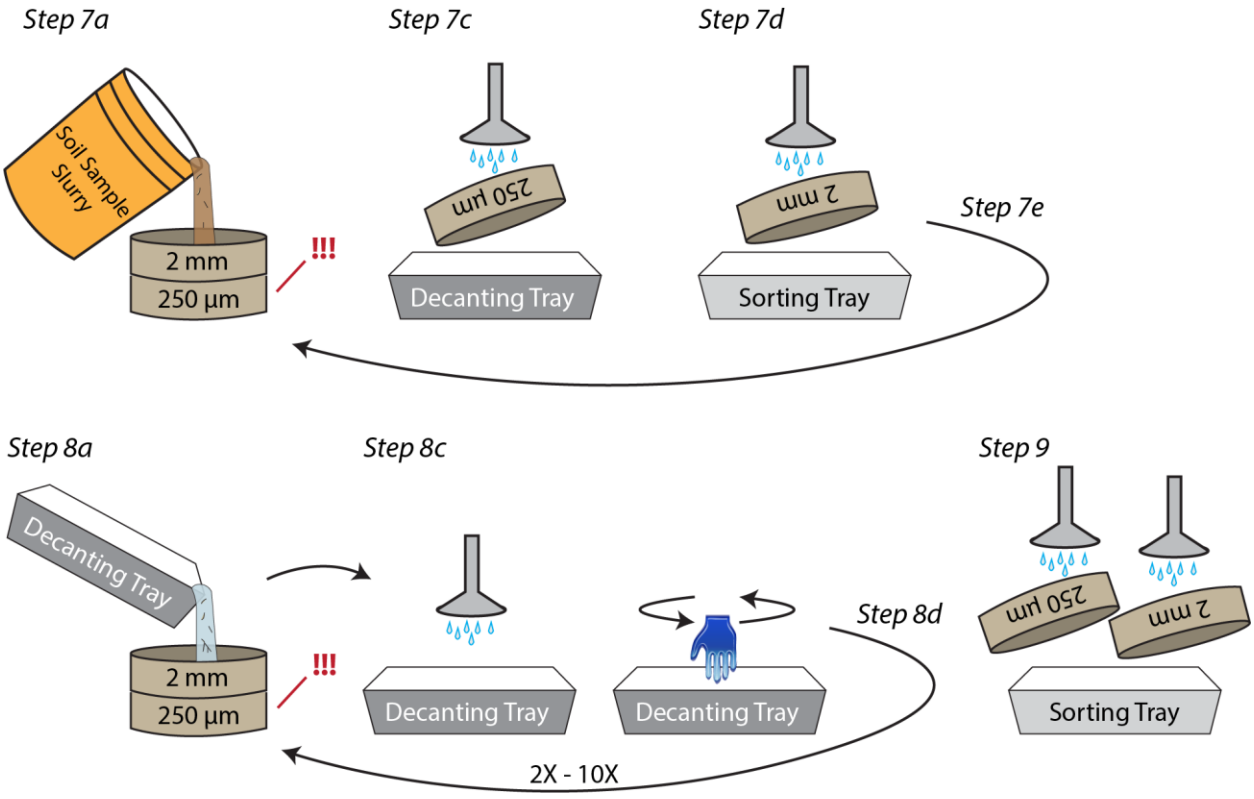

Figure 16. Wet-sieving the soil sample slurry (*step 7*) followed by decanting the 250 µm sieve contents to separate organic material and roots ≥ 1 cm length from mineral soil (*step 8*). Mineral soil remains in the decanting tray, and roots and organic material are transferred to the sorting tray (*step 9*).

8. Once the entire sample from the bucket has been passed through the sieve stack, repeatedly decant the sample in the decanting tray through the sieve stack to separate roots + OM (retained on both sieves) from soil minerals (retained in the decanting tray)(**Figure 16**). To decant:
    a. Let the mineral soil settle to the bottom then carefully but quickly pour off the water, roots and OM from the top and into the sieve stack.
    b. BE CAREFUL NOT TO OVERFLOW THE 250 µm SIEVE!
    c. Add more water to the decanting tray, and stir into a slurry to release more roots and OM from the mineral soil.
    d. Continue to rinse and pour through the sieve stack until only mineral soil is in the bin/tray. This may require between 2-10 rinses depending on soil type.
9. Transfer washed roots from both sieves to the sorting tray (**Figure 16**).
10. Thoroughly clean the sieves and sorting tray with water between core/monolith samples.
11. Check sediment traps in the root washing station; if traps are full, dispose of sediment in an approved receptacle.
    a. Pouring off water in the morning after sediment has settled overnight is an effective method for retaining as much sediment as possible in the buckets prior to disposal.
12. (*OPTIONAL*) It is acceptable to pause overnight between sieving and beginning the sorting process provided that:
    a. The sample is kept refrigerated and sealed in a container with a label (to avoid spills and loss of roots). For example, the entire sorting container may be covered with a lid, plastic bag, or plastic wrap with the waterproof label inside and then placed in the refrigerator, AND
    b. No longer than 72 h elapses between sample collection in the field and completing the sieving.

D.2 Dry Sieving Soil Samples for Fine Root Biomass

1. (Optional) Thaw previously frozen sample(s) overnight at room temperature for 8-12 h. Only thaw the number of samples that can be processed with available time/resources.
    a. Alternatively, samples may be thawed for 24-72 h in a refrigerator.
    b. Note: Samples will need to be completely thawed before dry-sieving is possible.
2. Before beginning the dry-sieving routine:
    a. Determine whether the soil sample has been selected for dilution sampling (SOP B.2). The residual fraction must be saved to generate a dilution sample.

b. Check sieve integrity. Damaged sieves will reduce data quality and should not be used.

c. Assemble the sieve stack: The 2 mm sieve on top, the 250 µm sieve in the middle, and a clean sieve pan on the bottom.

d. Label a light-colored sorting tray with the **sampleID**. Include 'D' on the label if the sample will be processed for dilution sampling.

i. The adhesive label from the field can be transferred to the sorting tray to minimize transcription errors.

3. Pass 10% – 20% of the sample through the sieve stack with shaking to separate roots from mineral soil and soil organic matter.

a. The 2 mm sieve is useful for catching and removing large rocks from the sample, as well as larger roots

b. The 250 µm sieve is useful for capturing any roots that have passed through the 2 mm sieve. Roots ≥ 1 cm in length are not likely to pass through this finer mesh.

4. From each sieve:

a. Break up aggregates and organic matter pieces using gentle manual pressure.

b. Manually remove larger rocks from the top of the 2 mm sieve – but don't spend more than several minutes.

c. Add the contents of the 2 mm and 250 µm sieves to a clean, empty 250 µm sieve and set aside.

5. Repeat steps (2) and (3) until the entire sample has been processed.

6. All roots from the sample should now be in a single 250 µm sieve. Gently wash sediment from the pooled root sample; sediment clinging to roots can significantly inflate weighed root biomass.

7. Transfer roots to a light-colored tray for sorting and picking (SOP D.3).

a. Roots may be placed in a 65 ˚C oven for 1-2 h to ease transfer to the sorting tray.

8. Thoroughly clean the sieves and sorting tray with water and dry between soil samples.

D.3 Sort Roots to Size Category

1. Use forceps to pick all roots ≥ 1 cm length from the sorting tray, and sort to **Size Category** as you go. Use a wire gauge to determine the **Size Category;** the largest diameter of a root fragment should be used to classify the size (**Figure 17**).

a. **Size Categories** are: < 1 mm, 1–2 mm, 2–10mm

b. ***VERY IMPORTANT:*** To determine root diameter, you must pass the root through the gap ***in the side*** of the wire gauge; DO **NOT** insert the root through the larger hole. The

wire gauge may be mounted on the side of the sieve using one of the larger gaps, enabling quick access for size classification. Label gauges for easy reference.

c. Calipers must be used to determine whether large roots are ≤ 10 mm diameter.

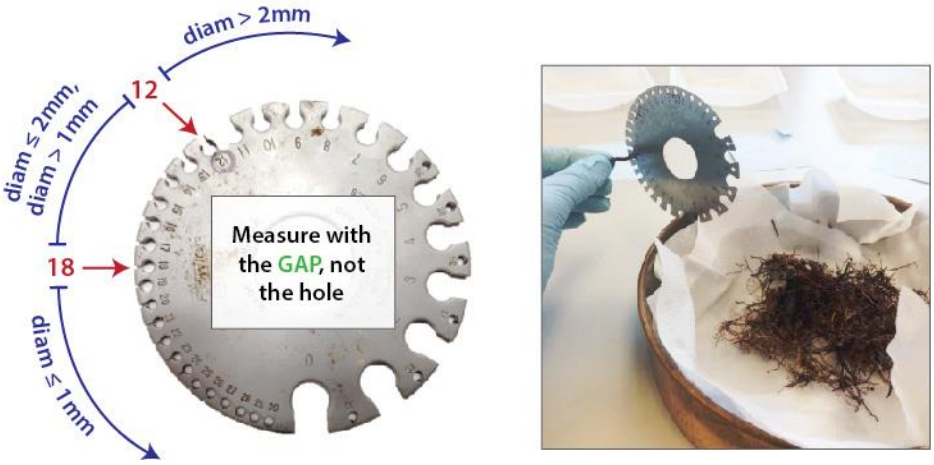

Figure 17. (*Left*) A circular wire gauge showing the 12 gauge and 18 gauge gaps used to sort roots to **Size Category**. (*Right*) A circular wire gauge mounted on the side of the sieve for convenient diameter checking.

2. Clip apart branched root systems into respective **Size Category** classes (see **Figure 18**):
    a. Clip only at branch points.
    b. **Size Category** is assessed at the largest end of the clipped segment.
    c. Do not clip at a given branch point if there are no 'downstream' changes in **Size Category**.
    d. Ignore branches that result in root fragments < 1 cm length.
3. Visually inspect sorted roots to determine whether mycorrhizal fungi are visible to the naked eye (either arbuscular or ectomycorrhizal types).
    a. Record **mycorrhizaeVisible** = 'Yes' or 'No' on the labeled envelope in the next step; this is a quality flag field to indicate whether mycorrhizae may contribute to the final recorded **dryMass** value.
    b. Do not attempt to separate mycorrhizae from roots.

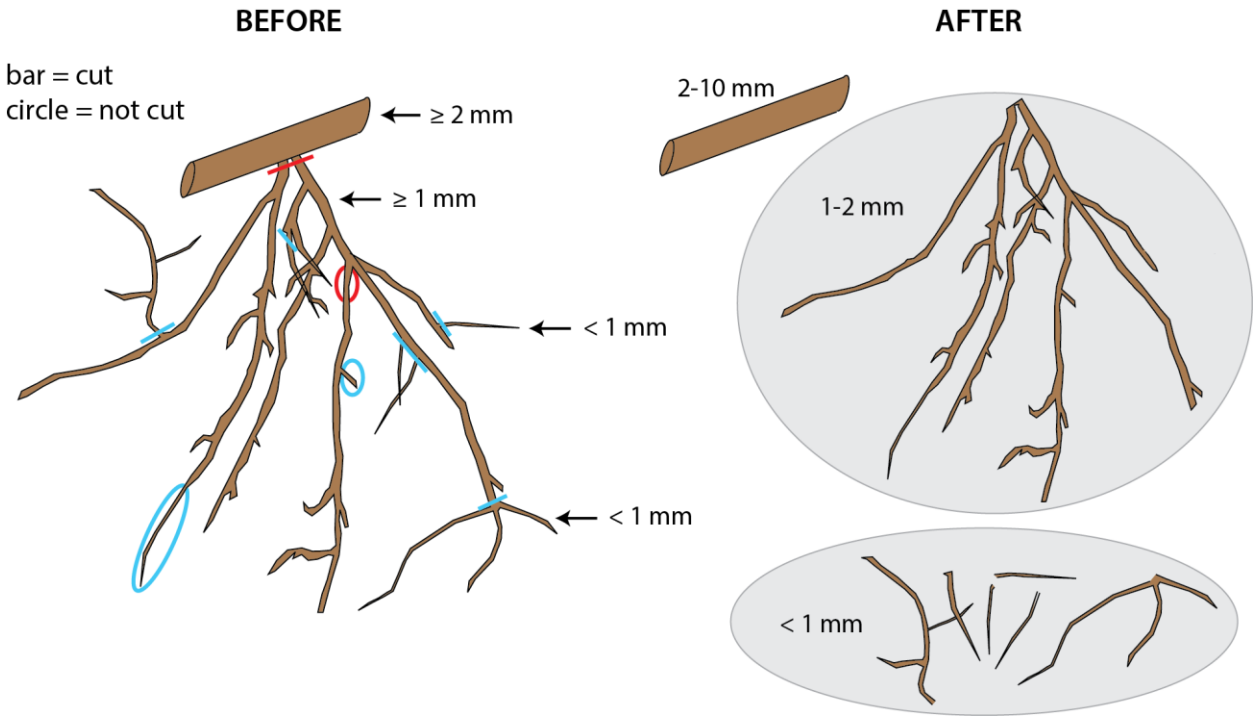

Figure 18. Clipping a branched root system to **Size Category**. The red bar indicates the 2 mm diameter break-point, and the blue bars indicate the 1 mm diameter break-point. The red circle is not clipped because there are no downstream changes in Size Category. The left blue circle is not clipped because Size Category is assessed at the largest end and clipping only occurs at branch points; the right blue circle is not clipped because the fragment is < 1 cm length.

4. For each core sample, label up to 3 coin envelopes with the information below. The total number of envelopes needed depends on the number of **Size Categories** the sample generated. For large amount of root biomass within a given size category, use a clasp envelope instead.

    a. For samples that may contain *Toxicodendron spp.* roots:

        i. Add *Toxicodendron* warning label to envelope (example sticker at left).

        ii. Dry labeled envelopes in a 65 ˚C oven for 1 h, then cool to room temperature in a desiccator.

        iii. Weigh each empty envelope using the same high-precision microbalance that is used for roots (0.001 or 0.0001 g accuracy), and record the **initialBagMass** mass on the envelope. This step enables determining the **dryMass** at a later step without further direct handling.

        iv. Clean durable equipment that may have contacted *Toxicodendron* tissue (e.g., sieves, forceps) as described in RD[12].

b. ***Root Subsample Barcodes (strongly recommended)***: Add a Type I barcode to a minimum of one root envelope per core sample. This will enable rapid scanning of samples into mobile applications for downstream steps.

c. Label envelopes with human-readable information:

i. **Plot ID and Clip Cell Number**: e.g., SRER047042

ii. **Collect Date** year: year roots were sampled in the field; *YYYY* format

iii. **Soil Sample ID**: either '*North*' or '*South*'

iv. **Size Category**: 0-1, 1-2, 2-10

v. **Subplot ID**:

1) For 20m x 20m plots, subplotID = 31_400

2) For 40m x 40m plots, subplotID = 21_400, 23_400, 39_400, or 41_400

vi. ***Example label text***: SRER047042.2023.North.0-1, subplot=21_400

5. Place sorted roots into the labeled envelopes.

6. **If the sample has been selected for dilution sampling:** Set aside the residual fraction for processing via SOP E (the residual fraction = root fragments < 1 cm mixed with organic material that is left over in the sorting tray after all roots ≥ 1 cm length have been picked out).

a. See SOP B.2 for guidance on selecting a spatially-balanced set of dilution samples. The residual fraction may be discarded for cores not selected for dilution sampling.

b. The residual fraction may be stored in a sealed 50 mL tube at 4 – 8 ˚C and processed the next day. The residual fraction may also be frozen and processed at a later date.

c. Create a record in the *BBC: Lab Dilution [PROD]* app for the Dilution Sample that will be generated from the soil Sample ID.

d. Scan the Sample ID barcode from the field to populate the record with required plot and sample data.

i. *Optional manual workflow*: Manually select required plot-level and soil sample information to identify the Dilution Sample.

e. Save the incomplete Lab Dilution record.

7. Gather roots from the same soil sample together to keep them organized.

a. Place envelopes containing root samples into a paper bag to keep samples organized (lunch sack size works well); OR

b. If there are very few roots, coin envelopes may be paper clipped together.

c. ***Barcode Workflow***:

i. Keep the physical barcode originally associated with the field-collected soil sample – i.e., the Sample ID barcode on weatherproof paper – with the root subsamples as they are dried and weighed.

ii. The Sample ID barcode will aid in bringing up the correct record during Dry Mass data entry.

D.4 Troubleshooting Root Sorting

Table 11. Common issues encountered during root sorting and potential solutions.

Issue	Resolution
Root sleeve – i.e., only exterior cortex present and interior tissue has rotted away	• To the best of your ability, assign **Size Category** as if the interior of the root were present.
Root fragment > 1 cm length with diameter bisected by coring device	• Measure the diameter as it exists, do not estimate what the diameter would have been had the fragment not been damaged.
Corm, rhizome, or other non-root structure in core sample	• Discard. Although these structures can represent significant biomass, they are not the focus of this protocol because their turnover/decomposition rates are very different from roots.

D.5 Drying and Weighing Root Samples

Washed roots should be placed in the drying oven as soon as possible following sieving.

1. For any samples that may contain *Toxicodendron spp*., ensure that the mass of the empty envelope or bag has been recorded as the **initialBagMass** in SOP D.3.
2. Label groups of envelopes containing washed roots from the same soil sample with the date and time they are placed in the drying oven.
    a. These data are the **Oven Start Date** and **Time** required during data entry.
    b. ***Critical step:*** Labeling bags allows assessment of how long different batches of bags have been in the oven, especially when roots sampled on different days occupy the same oven.
3. Place labeled bags into a drying oven for a minimum of 48 h (longer is okay, but not required).
    a. Dry all root diameters at 65 ˚C.
4. Remove bags of dried biomass from the drying oven, and label bags with **ovenOutDate**/Time. Dried roots may be weighed immediately, or stored and then weighed:

a. After removing from the drying oven, dried roots may be weighed as soon as they have returned to room temperature. Roots will absorb moisture from the air if left in ambient room conditions (particularly in humid environments).

    i. If using this method, it is helpful to remove bags from the oven and weigh one at a time.

    ii. Dried roots may also be placed in a desiccator to cool and may be weighed one at a time from the desiccator. A makeshift desiccator can be constructed from a large tupperware with a layer of drying crystals in the bottom.

b. Alternatively, dried roots may be stored for up to 30 days in ambient room conditions prior to weighing. Samples treated in this manner must be returned to the drying oven for 24 h prior to weighing, and must be weighed as above after removal from the oven.

    i. If samples were initially dried and kept in storage, it is not necessary to record any additional drying times.

5. Organize all samples from the same **Plot ID**.

    a. Weighing samples from the same **Plot ID** at the same time, and keeping samples grouped, will greatly facilitate subsequent grinding and pooling steps (SOP F).

6. Weigh each fine root sample using a mass balance (minimum 0.001 accuracy) and a weigh boat. Balances with glass doors are required because samples may be very light and air currents may affect perceived sample mass.

    a. For large volumes of biomass that do not readily fit into a large weigh boat, use the following strategies:

        i. Use a large plastic tray (or equivalent) instead of a weigh boat (see equipment list).

        ii. Crush or chop the biomass to reduce volume so it will fit into a weigh boat.

        iii. Avoid splitting the biomass into subgroups for weighing, as uncertainty values must be added each time a subgroup is created.

    b. Aluminum weigh pans may be employed when weighing small masses of roots that may be affected by static when weighed with a plastic weigh boat.

    c. For samples that may contain *Toxicodendron* roots, sample envelopes should have a warning label such as that shown at left. To record mass for these samples:

        i. Do NOT remove the root biomass from the envelope.

        ii. Weigh the **finalBagMass** (envelope + roots) and record to 0.001 or 0.0001 grams.

iii. Combined with the **initialBagMass** (from SOP D.3), the **finalBagMass** is used to automatically calculate the **dryMass** when entered into the *BBC: Lab Weighing [PROD]* app.

7. In the *BBC: Lab Weighing [PROD]* app, create a record for each sampling cell from which biomass was clipped in the field (i.e., each **Plot ID, Subplot ID** and **Date** combination), and enter required parent-level data:

    a. ***Barcode Workflow***: Scan the Sample ID barcode from the field to rapidly select the desired **Plot ID, Subplot ID** and **Date** for the record.

    b. **Site ID**: the site from which root samples were collected (auto-populated if using barcodes).

    c. **Plot ID**: from the list, select the plot, subplot and date associated with the root samples (auto-populated if using barcodes).

    d. **Sample Condition**: Choose from 'OK', 'frozen before processing', 'error: gloves not worn', and 'frozen and glove error'. Defaults to 'OK'.

        i. Gloves should be worn during the sieving/sorting process. Document deviations here.

    e. **Root Mass Presence**: For each Soil Sampling Area (***North*** and ***South***), indicate which Size Categories are present in the sample.

        i. If no roots were found, select '***No (zero)***' mass for that category.

    f. **Mycorrhizae Presence**: Select the Size Categories that contain mycorrhizae visible to the naked eye.

8. Create a child-level record for each dried root sample from a given Clip Cell, and enter:

    a. **Oven Start Date/Time**: date (*YYYYMMDD* format) and time (24-h format) the sample was initially placed in the drying oven.

        i. Enter only for initial drying event. Do not enter additional dates/times for samples stored at room temperature, and then re-dried prior to weighing.

    b. **Oven End Date/Time**: date and time the sample was initially removed from the drying oven.

    c. **Weigh Date**: date Dry Mass was weighed for the sample, *YYYYMMDD* format.

    d. If **Toxicodendron Possible** = 'Yes':

        i. **Initial Bag Mass**: Record the mass of the empty envelope that was written on the envelope in SOP D.3 (nearest 0.001 or 0.0001 g).

        ii. **Final Bag Mass**: Record the mass of the roots + bag/envelope (nearest 0.001 or 0.0001 g).

e. **Dry Mass**: Enter the dried root mass, greatest precision possible (nearest 0.001 or 0.0001 g), calculated automatically if **Toxicodendron Possible** = 'Yes'.

f. **Subsample Fate**: Defaults to 'active'. Select other value as appropriate.

g. **Subsample Condition**: Choose from 'OK' and 'spilled during processing'.

h. ***Barcode Workflow***: Link barcode(s) from a minimum of one root subsample for which Sample Mass Presence = 'Yes'.

i. Save child-level record.

j. Repeat step (8) for all root samples from the same soil sample.

k. Save the parent-level record.

9. Once all masses have been recorded for a given sampling bout:

   a. Keep field sample barcodes with groups of dried root subsamples to facilitate QA.

   b. Perform QA on a subset of samples (SOP D.6), or

   c. Return dried fine roots to temporary storage in a desiccator at ambient conditions. Samples in temporary storage can then be weighed for QA as time permits.

D.6 Data Quality Assurance

To quantify uncertainty associated with weighing dried biomass, a random selection of dried samples is re-weighed by a different technician than the person who originally weighed the biomass.

1. For each sampling event at a given site, randomly select 10% of dried, previously weighed samples for re-weighing.

   a. If QA weighing does not occur within 1 hour of the initial weighing, return the selected samples to the drying oven for 24 h prior to QA weighing. In humid environments, samples will pick up moisture from the atmosphere.

   b. The purpose of QA weighing is to quantify uncertainty associated with the weighing process. As such, there is no criterion for how close the QA mass must be to the original mass.

2. For root samples selected for QA, select the appropriate parent record in the *BBC: Lab Weighing [PROD]* app, and edit to create a new child-level '***QA***' record.

   Barcode workflow: Scan the Sample ID barcode from the field for the root subsample for which QA is desired. This will bring up the appropriate parent-level Lab Weighing record.

3. Enter required data into the new QA child-level record:

   a. **QA Dry Mass**: select the 'Y' option from the drop-down.

b. **QA Sample List**: select the root subsample for which QA Dry Mass will be recorded from the list of previously weighed and entered root masses.

c. **Weigh Date**: date QA Dry Mass was weighed, *YYYYMMDD* format.

d. **Dry Mass**: dried QA root sample mass, greatest precision possible (0.001 or 0.0001 g).

e. Save the child-level QA record.

f. Save the parent record.

4. Return to step (2) above for additional QA samples.

5. After QA weighing, return plant material to temporary storage.

SOP E Dilution Sampling for Fine Root Fragments

Goals

- Quantify the ratio of root fragments < 1 cm length to organic material in the residual fraction for selected soil samples (**Figure 19**).
- Collect required dilution sampling data:
    - The preferred method for data collection is the *BBC: Lab Dilution [PROD]* application.
    - The Belowground Biomass Sampling Fulcrum Manual on the SSL contains detailed data entry instructions.

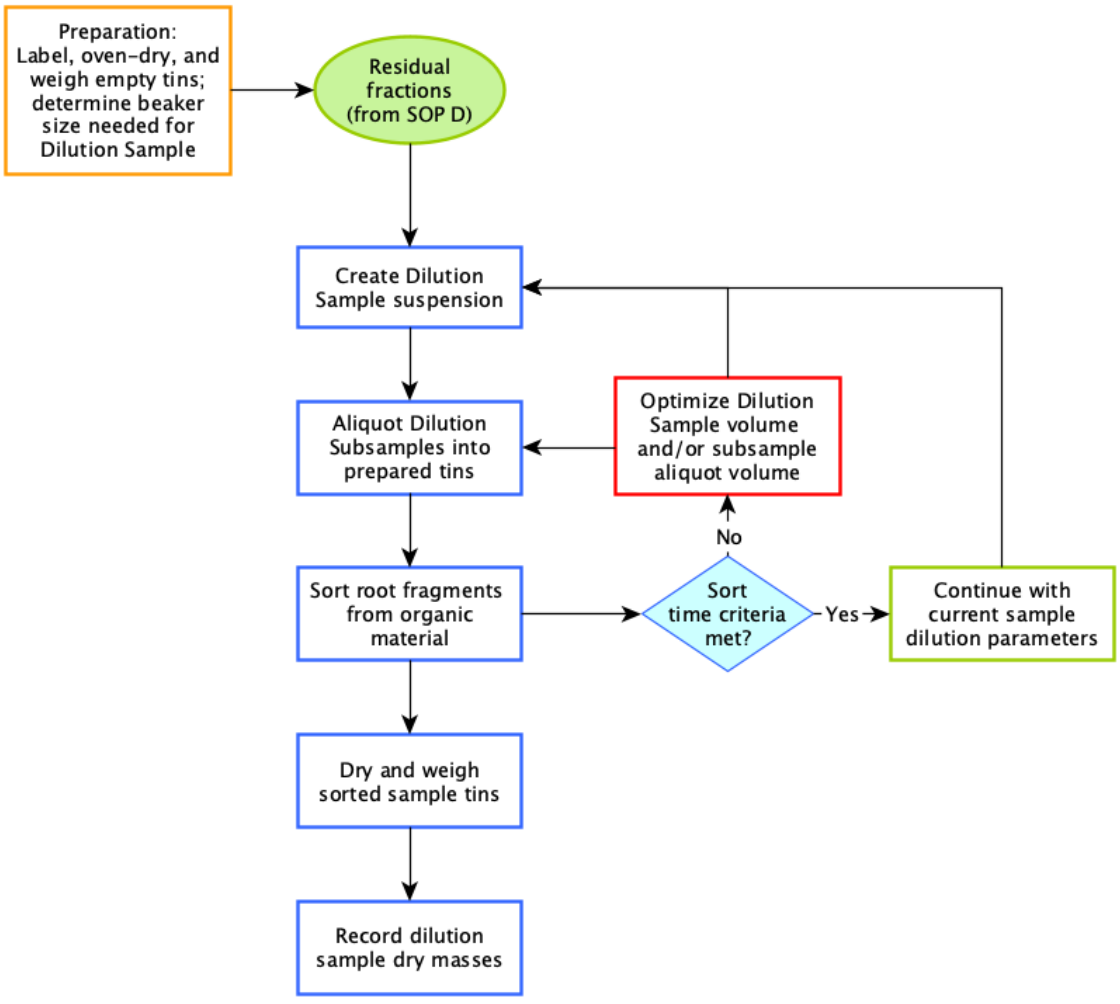

Figure 19. Expanded workflow diagram for Plant Belowground Biomass dilution sampling and quantification of root fragments < 1 cm length. Diagram supports and does not replace protocol text; most common workflow is outlined.

Overview

1. **When samples are processed**: Dilution sampling for quantifying fine root fragments < 1 cm length begins after a soil sample has been sieved and all roots ≥ 1 cm length have been picked from the residual fraction (see SOP D).
    a. Sieving and sorting must be initiated within 72 h of sample collection in the field. Dilution sampling is expected to be initiated with minimal delay following sieving and sorting, but it is not expected that dilution sampling be completed within 72 h of sample collection.
    b. It is acceptable to pause overnight between execution of SOP D and SOP E. Store labeled residual fractions overnight at 4 ˚C in a sealed container (e.g., labeled 50 mL tube).
    c. Alternatively, residual fractions may be labeled and frozen within 72 h of sample collection in the field then processed at a later date.
2. **How samples are processed**:
    a. The entire residual fraction *containing all root fragments < 1 cm length from the soil sample* is suspended in water and vortexed to homogenize, creating a **Dilution Sample**.
    b. **Dilution Subsamples** (n=3) are extracted from the vortexing **Dilution Sample**.
    c. **Dilution Subsamples** are sorted to root and soil organic matter components, and then dried and weighed to enable calculation of total root fragment mass in the residual fraction.
        i. ***!!! Masses must be recorded to minimum 0.001 g accuracy; 0.0001 g accuracy is preferred.***
    d. **Sample Volume** and **Aliquot Volume** are optimized on a per soil type basis to generate root fragment and soil organic material masses that are sufficiently large such that reliable masses can be weighed, but that are not so large that sorting to completion requires more than an average of 15 min per Dilution Subsample.
3. **Digital workflow**:
    a. Records in the *BBC: Lab Dilution [PROD]* app are created for dilution samples in SOP D. When data entry is required, data may be entered directly into the digital workflow by editing an existing record, or may be recorded on paper for multiple samples, then transcribed.
    b. ***Before oven-drying***: Previously created records are edited to create child-level records containing Dilution Subsample IDs. The Tin ID is added for each child record and the record is saved.
    c. ***After oven-drying***: Each child record is edited to add **Dry Mass** and the record is saved.

E.1 Dilution Sampling Steps

For root samples identified for dilution sampling via SOP B.2, the steps below describe how to separate root fragments from soil organic matter and quantify root fragment biomass with a relatively time-efficient technique.

1. (Optional) Thaw previously frozen residual fraction samples overnight at room temperature. Only thaw the number of samples that can be processed with available time/resources.
2. Transfer the residual fraction from SOP D to a clean 250 µm sieve, and carefully wash with the root washer nozzle. The residual fraction should be free from mineral soil particles at this point.
3. Transfer the consolidated residual fraction – i.e. all roots < 1 cm length + associated organic matter – to a beaker and suspend the sample in ***distilled water***.

   !!! Use distilled water from this point forward, including water used to rinse the sieve that is then collected. Mineral build-up on weighing tins has been shown to significantly alter perceived root and organic matter masses.

   a. Based on the size of the residual fraction, choose either a 1 L, 2 L, or 4 L beaker. Note that the size of the beaker can be varied from core to core, depending on the size of the residual fraction.

   TIP: The goal is to sufficiently dilute the residual fraction so that not too many roots need to be picked and sorted, but not dilute so much that there are too few roots to weigh accurately once they are dry. If in doubt, use the 1 L beaker, and dilute further if necessary.

   TIP: For residual fractions that require a beaker larger that 4 L, or if a 4 L beaker is unavailable, carry out a serial dilution as described in SOP E.2.

   b. Manually transfer as much of the residual fraction as possible to the beaker. Use a scoopula, spatula or equivalent.

   c. Transfer any remaining residual fraction from the 250 µm sieve to the beaker; use a squirt bottle and ≤ 500 mL of distilled water to rinse the sieve.

   d. Carefully fill the beaker with distilled water to approximately ¾ full (e.g., 750 mL, 1.5 L, or 3 L). Add water to the sample to bring up to the target volume. It is helpful to fill to one of the pre-marked graduations on the beaker, as an accurate volume at this step will be used to estimate the total mass of root fragments < 1 cm length.

4. Record required **Dilution Sample** metadata.

   a. **Sample Volume**: total volume of water + residual fraction in the beaker; best precision possible, e.g., nearest 10 mL

   b. **Processed Date**: date dilution sampling is carried out, *YYYYMMDD* format.

c. **Dilution Sample Fate**: set to 'lost' if equipment breakage occurs during subsequent steps and sample is compromised.

d. **Dilution Sample Condition**: Select 'OK' or 'frozen before processing'.

5. Label 3 pairs (n=6 total) of aluminum weighing tins to hold the 3 Dilution Subsamples.

   a. Tins should be pre-numbered with a unique **Tin ID** (e.g. A, B, C, etc.)(see **Figure 21**). The **Tin ID** is tracked with the sample, rather than labeling each tin with sample information.

   b. For each pair of tins, one is for root fragments, and the other is for organic material.

6. Pre-weigh each empty, dry tin with a microbalance; nearest 0.001 g (minimum), or nearest 0.0001 g (preferred).

   a. Tins should be oven-dried at a minimum of 65 ˚C for 15-30 min prior to weighing to remove adsorbed moisture (use whichever temperature is most convenient based on existing oven temperatures). The microbalance will detect moisture adsorbed from the air in humid environments.

   b. Tins must return to room temperature before weighing. Store in a desiccator between drying and weighing to ensure moisture does not re-adsorb to the surface while cooling.

   c. Associate tin data with previously created Lab Dilution records:

      i. **Tin ID**: the unique number assigned to the tin.

      ii. **Empty Tin Mass**: the mass of the clean, dry, empty tin.

      iii. (Paper workflow) **Dilution Subsample Number**: 1-3, technician assigned, needed to track pairs of tins from the same Dilution Subsample (**Figure 21**).

7. Work in pairs to generate 3 **Dilution Subsamples** from the aqueous suspended **Dilution Sample** in the beaker (**Figure 20**). Consult the training video for a visual demonstration of the following steps:

   a. **[Person1]** Turn the plate mixer on high, and vortex the aqueous suspended Dilution Sample thoroughly (approx. 10 s from the start of vortexing).

   b. **[Person1]** Turn off the mixer, and quickly plunge the suspension to stop the vortex and randomize the sample in the water.

   c. **[Person2]** Take a 20 mL Dilution Subsample from the middle of the water volume in the beaker using the customized syringe, and transfer to one of the 'OM' tins.

      i. Take care to keep the syringe vertical during transfer. Tilting the syringe may allow air to enter the aperture and sample will spill out.

   NOTE: In addition to adjusting the beaker **Sample Volume** in step (3) above, the Dilution Subsample **Aliquot Volume** obtained with the syringe can also be adjusted from 20 mL

to optimize the amount of material needed for sorting and weighing. For example, collect 10 mL if the suspension is particularly dense.

d. **[Person2]** Back off the plunger in the syringe to the 5 mL mark. Rinse the interior of the syringe with the squirt bottle and transfer the rinse to the same tin.

Figure 20. (*Left*) A dilution subsample, comprised of unsorted root fragments and organic matter, in an aluminum sorting tin. (*Right*) Sorted root fragments in a second, paired 'root' tin. The amount of material in the left tin is a good target when creating dilution subsamples.

8. Record required Dilution Subsampling metadata:
    a. **Aliquot Volume**: the volume of the subsample taken with the syringe; nearest 1 mL. The volume of water from the squirt bottle should **NOT** be added to this number.
    b. (Paper workflow) **Subsample Type**: the type of material the tin will hold after picking and sorting is complete; the tin initially receiving the mixed sub-sample should be **Subsample Type** = '*OM'*, and the tin into which roots are sorted should be **Subsample Type** = '*ROOT*.' Repeat steps (5) and (6) until 3 sub-samples have been transferred to 3 '*OM*' tins (**Figure 21**).
9. For each of the three (3) Dilution Subsamples, carefully pick and sort root fragments from organic material and transfer the roots to the '*ROOT*' tin of the pair (**Figure 21**; see Section 2.4 for 'organic material' definition).
    a. A small amount of water in the *'ROOT'* tin aids in transferring root material.
    b. ***Aim for no more than 15 min sorting time per tin pair, 10-15 min is ideal***; adjust the **Sample Volume** in the beaker and the **Aliquot Volume** in the syringe as necessary.

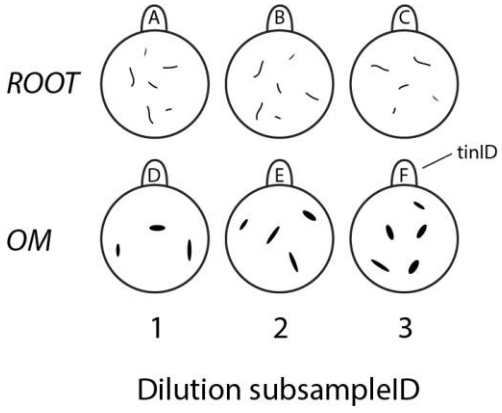

Figure 21. Three pairs of labeled aluminum weighing tins for separating roots from OM. Dilution subsamples are initially transferred to the *OM* tins via syringe, and roots are then sorted into the *ROOT* tins. Each tin has a unique Tin ID.

10. Carefully transfer tins to a 65 ˚C drying oven for a minimum of 48 h.
    a. Record **Oven Start Date/Time**: the date and time the samples were placed in the drying oven.

Tips:

- Use a tray to move batches of tins in the laboratory.
- Heavy duty metal trays may be placed directly in the drying oven with all of the samples.
- Do not leave samples on light-weight trays in the drying oven. Light-weight metal trays occasionally twist when heated which will cause samples to spill.
- ***!!!*** Place a large piece of cardboard over tins to prevent oven fans from blowing very light dried samples out of the tins.

11. The residual fraction suspended in the beaker can be discarded once tins containing dilution subsamples are safely in the oven.
    a. Up to this point, the suspended residual fraction can be used to replace any tins that are spilled during processing.
12. Repeat steps (2) – (9) for additional soil samples.
13. Once tins are dry, weigh the total mass of each 'tin+ROOT' or 'tin+OM' with a microbalance (0.0001 g precision preferred, 0.001 g precision acceptable).
    a. Tins must return to room temperature prior to weighing.
        i. Place tins in a desiccator to cool to room temperature. Warm tins create air currents within the microbalance enclosure that affect perceived mass.

ii. If it takes longer than 2-3 minutes for the tin + sample to return to room temperature before weighing, place tins in a desiccator to cool, then weigh.

b. Weigh one at a time from desiccator, and record required 'Lab Dilution' data:

i. **Oven End Date/Time**: the date and time samples were removed from the drying oven.

ii. **Dilution Sub-sample Fate**: Record 'lost' if a dilution sub-sample was spilled or otherwise compromised during processing and drying.

iii. **Dry Mass**: the mass of the dry 'tin+ROOT' or 'tin+OM' material; nearest 0.001 g (minimum), nearest 0.0001 g (preferred).

E.2 Serial Dilution Method for Dilution Sampling

The steps below are an expansion of Step (3) in SOP E.1, to be employed when the residual fraction is relatively massive and large volumes of distilled water (> 2 L) are required to create a dilution sample such that each dilution subsample can be sorted within the timing guidelines (15 min per tin pair, n=3 tin pairs total). Note that serial dilution is not needed if large 4 L or 5 L beakers are available in the laboratory.

1. Begin with a 2-factor serial dilution if it is not known whether 2-factor or 3-factor is appropriate for the residual fraction(s) at hand (**Figure 22**).

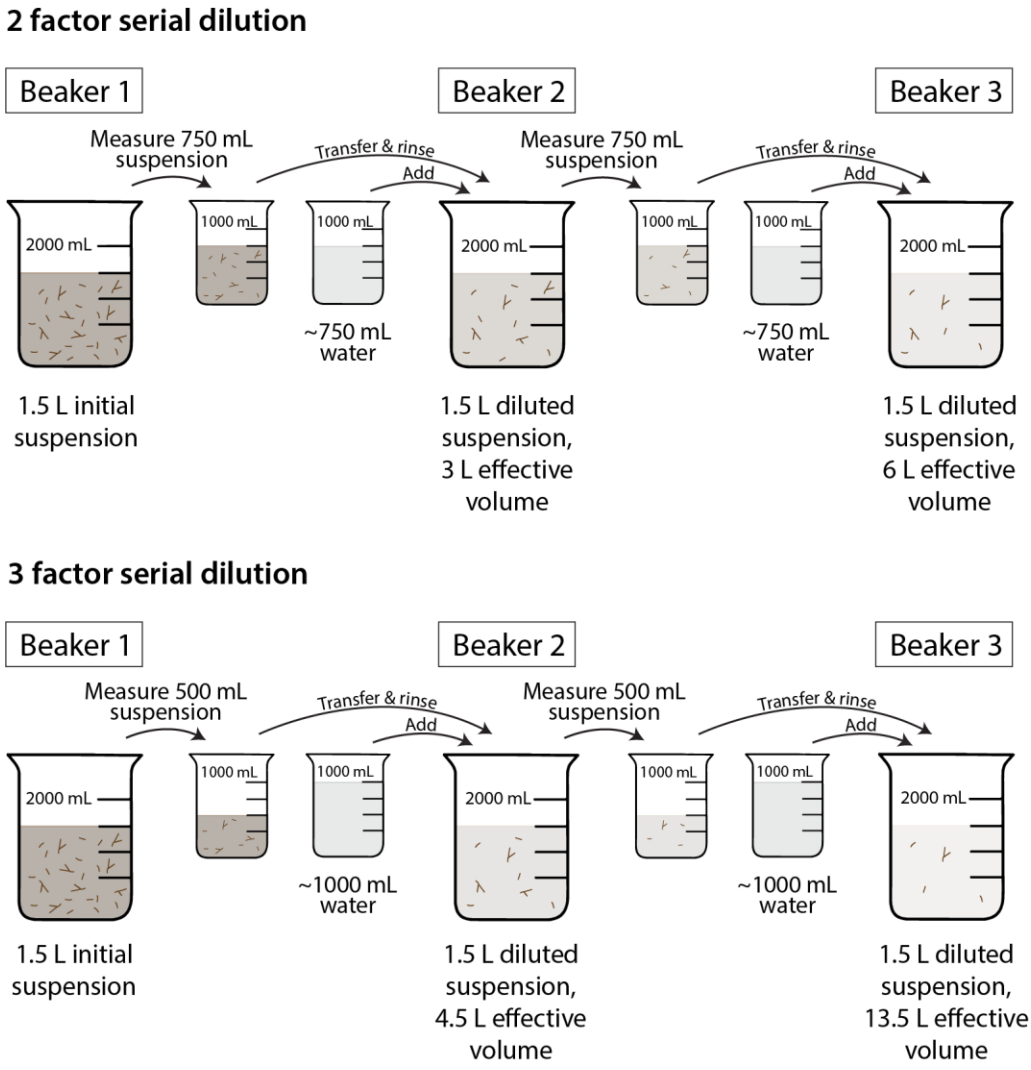

Figure 22. Serial dilution workflow for creating large effective sample volumes using 1 L and 2 L beakers.

2. Referring to **Figure 22**, suspend the residual fraction in 1.5 L distilled water in Beaker 1 (2 L) and create a homogeneous suspension:

    a. *Option 1 (preferred):* Use a magnetic stir plate and stir bar to create a vortex and maintain the vortex for a minimum of 10 s. Turn off the mixer, and quickly plunge the suspension to stop the vortex and randomize the sample in the water.

    b. *Option 2*: If the initial suspension is too thick to allow the stir bar to create a vortex, use a glass stirring rod to create a vortex, then quickly plunge the suspension to stop the vortex and randomize the sample in the water.

3. Place a 1 L transfer beaker in a tray and quickly and carefully pour a portion of the suspension into the transfer beaker. In the event of a spill, the tray will allow the sample to be

quantitatively recovered. A transfer beaker is used because volumes are more accurate in a smaller beaker.

a. For a 2-factor dilution, pour 750 mL.

b. For a 3-factor dilution, pour 500 mL.

c. If the amount poured into the transfer beaker is > 25 mL different from the target volume:

i. Return the volume in the transfer beaker to Beaker 1 and use distilled water and a squirt bottle to rinse the transfer beaker into Beaker 1. The volume in Beaker 1 will now exceed 1.5 L.

ii. Pass the suspension in Beaker 1 back through a 250 µm sieve and resuspend the sample to 1.5 L total volume in Beaker 1.

iii. Make another attempt at quantitatively transferring the desired suspension volume to the transfer beaker.

4. Pour the contents of the 1 L transfer beaker into Beaker 2 (2 L), and thoroughly rinse the transfer beaker with distilled water using a squirt bottle.

5. Bring the final volume of Beaker 2 up to 1.5 L with distilled water. The amount will vary slightly based on the volume of rinse water used.

a. For a 2-factor dilution: The total volume of distilled water to add should be slightly less than 750 mL.

b. For a 3-factor dilution: The total volume of distilled water to add should be slightly less than 1000 mL.

6. If the contents of Beaker 2 appear sufficiently dilute, the diluted suspension in Beaker 2 may be used to generate the required n=3 dilution subsamples. The effective dilution sample volume is calculated as:

a. *Effective dilution sample volume (mL) = (dilution factor) x (final suspension volume in mL)*

b. For a 2-factor dilution: *Effective dilution sample volume = 2 x 1500 = 3000 mL*

c. For a 3-factor dilution: *Effective dilution sample volume = 3 x 1500 = 4500 mL*

7. (Optional) If the diluted suspension in Beaker 2 is still too dense, create a second dilution from Beaker 2 to Beaker 3 (**Figure 22**, steps are similar to 2-5 above). The effective dilution sample volume is calculated as:

a. For a 2-factor dilution: *Effective dilution sample volume = 4 x 1500 = 6000 mL*

b. For a 3-factor dilution: *Effective dilution sample volume = 9 x 1500 = 13,500 mL*

8. (Optional) Generate the required n=3 dilution subsamples from Beaker 3.
9. In the Fulcrum *BBC: Lab Dilution [PROD]* app, record the effective dilution sample volume as calculated above in the **Sample Volume (mL)** field.
    a. *Note:* Submit an Incident if the effective dilution sample volume is too large for existing Fulcrum rules.

SOP F Grinding and Pooling Biomass for Chemical Analysis and Archive

Goals

- Grind dried biomass and ship to external facilities for chemical analysis and archive.
- Collect required laboratory data:
    - Enter required data in the *BBC: Grind and Pool [PROD]* application.
    - The Belowground Biomass Sampling Fulcrum Manual on the SSL provides detailed data entry instructions.

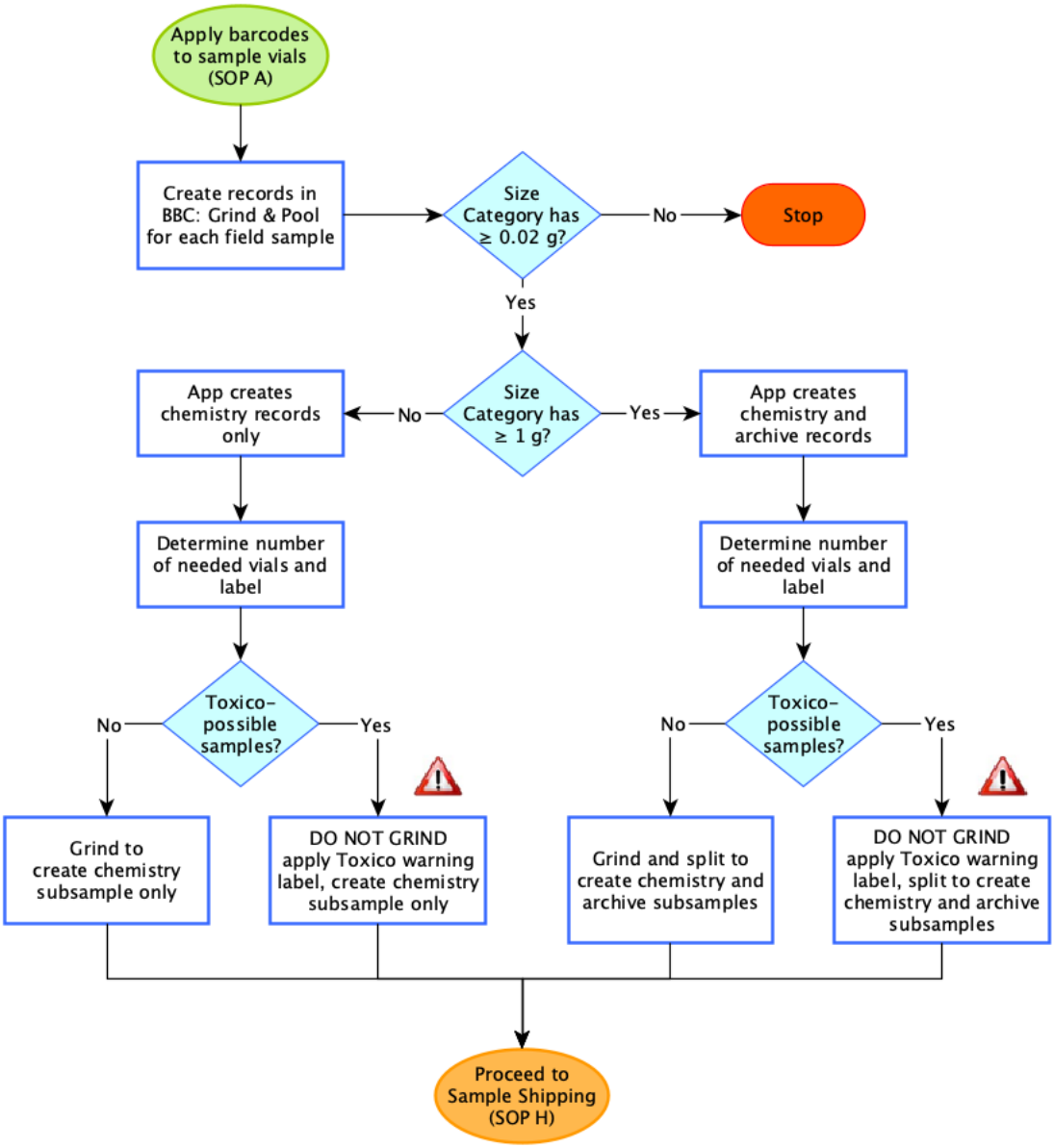

Figure 23. Expanded workflow diagram for Plant Belowground Biomass pooling, grinding, and splitting dried root samples in the laboratory. Diagram supports and does not replace protocol text; most common workflow is outlined.

Overview

1. **Which samples are processed**: All dried root samples with ≥ 0.02 g mass are processed via this SOP once QA masses have been recorded.
    a. Samples with > 1 g mass are additionally processed for the bioarchive.
2. **How samples are processed**: Pooled root samples are created and then the pooled sample is split for shipment to chemical analysis and archive facilities (see **Figure 4**).
    a. ***Wear gloves*** throughout the grinding and pooling procedure. Samples analyzed for isotopes are easily contaminated.
    b. To create a pooled root sample, roots within the same **Size Category** are pooled across the '*North*' and '*South*' samples that originate from the same **Sampling Cell Number**.
    c. The *BBC: Grind and Pool [PROD]* app employs the logic in **Table 12** to determine which pooled samples should be created.
    d. The pooled sample is created, ground, and split into representative subsamples.
    e. A maximum of 3 pooled root samples are created and ground per unique **Sampling Cell Number** (one for each **Size Category**).
3. ***Mandatory barcode workflow***: Sample containers shipped for external analysis or archive must have barcodes in addition to human-readable information on each container.
    a. Barcodes are required by the Stork Shipping Tool, and enable automatic creation of shipping manifests, as well as receipt and tracking forms for all relevant parties.
        - *Note*: Stork is only accessible from computers on the internal NEON network.
    b. Apply barcodes to vials a minimum of 30 minutes before vials are used (see SOP A.2).
        - Use 20 mL plastic scint vials for CN samples.
        - Use 20 mL glass scint vials for archive samples.
    c. Barcodes on sample containers are linked to upstream root and soil collection information via the *BBC: Grind and Pool [PROD]* app.

Procedural steps:

1. Samples that have been stored for > 5 days prior to processing for chemistry and archive must be re-dried at 65 ˚C for a minimum of 24 h before grinding and subsampling.
    a. This step ensures consistent sample condition for long-term archive and prevents any continued decomposition or microbial activity.

2. Use the *BBC: Grind and Pool [PROD]* app to determine, based on the total mass of each ***pooled*** root sample, which processing steps are required (see **Table 12**).
    a. Create a parent-level record corresponding to each sampling cell from which cores were collected in the field.
    b. ***Barcode Workflow***: Scan the barcode affixed to a dried root subsample envelope to bring up the Domain, Site, and list of samples available for pooling. If > 1 envelopes are barcoded, any envelope from the same soil sample may be scanned.
    c. Create and save a child-level record for each **Size Category**.
    d. The app displays the **CN Sample ID** and the **BGC Archive ID** fields, when sufficient sample is available according to the logic in **Table 12**.
    e. Save the parent record.

Table 12. Splitting and processing guidelines for fine root samples, based on pooled sample mass.

dryMass	Samples to create		Processing guidelines
	C:N sample	Archive sample	
< 0.02 g	-	-	Discard: Do not process sample for C:N analysis or archive. The **poolSampleCondition** field will default to "insufficient mass".
0.02 – 0.4 g	X		Do not grind; place entire pooled sample in 20 mL plastic scint vial. Use gloved hand to crush if necessary.
0.4 – 1 g	X		Grind entire sample to 40 mesh for C:N analysis and place ground sample in 20 mL plastic scint vial.
> 1 g	X	X	Grind entire sample to 20 mesh; use splitter to generate a 0.4 g subsample that is ground to 40 mesh for C:N analysis and placed in a 20 mL plastic scint vial; archive remainder of 20 mesh grind in a 20 mL glass scint vial.

3. Once records have been created for all samples:
    a. Filter and download the data for the records that match the samples, and
    b. Use a return-address template to print **CN Sample ID** and **BGC Archive ID** labels for those pooled root samples that have sufficient mass to warrant processing.
    c. Label vials for shipment to external analysis, and if applicable, archive. Orient labels vertically so the label does not overlap the mandatory barcode.

        i. For vials that may contain tissue from *Toxicodendron spp.*: Use vials with a warning sticker prepared in SOP A.2.

    d. ***Barcode Workflow***: Retain a barcode affixed to an envelope for each group of root samples from the same soil sample, and keep with the associated vials. The barcode is

subsequently used to more rapidly link vial barcodes with the correct Grinding and Pooling records.

4. For root samples that may contain tissue from *Toxicodendron spp.*: Do NOT grind and split as per standard samples. Subsample for chemical analysis and archive according to steps (a) – (g) below, and skip steps (5) and (6).

   a. Use caution when handling the sample to avoid exposure to tissue containing toxic oils. Wear single-use cotton gloves as described in RD[12] and follow the guidelines in RD[12] to clean any equipment, clothing, or skin that comes in contact with such tissue.
   b. Conduct all subsampling activities in a clean fume hood to contain dust particles.
   c. Homogenize the sample by cutting roots into approximately 1 cm length fragments using scissors.
      i. The sample may be transferred from the envelope to an appropriately sized metal or plastic weigh pan to facilitate homogenizing.
      ii. Clean the weigh pan with a tissue between samples and re-use.
   d. Manually split the homogenized root material into two subsamples according to the logic in **Table 12**.
      i. Try to ensure the splits are representative, and
      ii. Handle with forceps to prevent unnecessary contact.
   e. If a **BGC Archive Sample** was created: Record the **archiveMass**, nearest 0.01 grams.
   f. Place unground chemistry and archive subsamples into labeled, barcoded 20 mL scintillation vials and seal.
      i. Use plastic scint vials for CN samples and glass scint vials for archive samples.
   g. Clean all durable supplies and surfaces that may have come into contact with sample material as described in RD[12].
   h. Continue to step (9).

5. Prepare roots for grinding. It is important that large diameter roots and long lengths of root are not introduced into the grinding chamber.
   a. For roots with diameter ≤ 1 mm: No preparation is necessary.
   b. For root samples with 1 mm < diameter ≤ 2 mm: Cut into approximately 1 cm fragments.
   c. For root samples > 2 mm diameter:
      i. Cut into 1 cm fragments

ii. Manually break-up with a clean mortar and pestle to prevent introducing large-diameter woody root pieces into the grinding chamber.

iii. Clean the mortar and pestle with 95% ethanol and a kim-wipe between samples.

6. Grind and split oven-dried pooled root samples according to the logic in **Table 12**. See Training Materials or user manual for detailed operating instructions for the Wiley Mill.

    a. Do NOT load sample into the mill while it is powered off.

    b. Funnel root fragments into the grinding chamber at a measured rate. Material should not be funneled into the grinding chamber faster than ground material leaves the chamber.

    c. Use an appropriately sized splitter or microsplitter to generate representative sample splits. ***DO NOT create splits with a scoopula or a spatula****; these tools should only be used to transfer an ENTIRE split into a vial*.

    d. If an archive sample will be created:

        i. Grind the entire sample to 20 mesh, then use a splitter to re-grind a 0.4 g subsample to 40 mesh for CN analysis.

        ii. If possible, grind enough root material for a full vial for archive. If a split is too large to fit into a vial in its entirety, continue splitting until the desired volume is obtained.

    e. Record the **CN Sample Condition** to indicate the mesh used to grind the chemistry sample.

        i. The value should be ’40 mesh’; select a different value in the event of an error.

7. If a **BGC Archive Sample** was created:

    a. Tare the empty glass vial, then fill it with the ground archive sample.

    b. Record the **archiveMass**; nearest 0.01 grams.

    c. Record the **BGC Archive Condition** to indicate the mesh used to grind the archive sample.

        i. The value should be ’20 mesh’; select a different value in the event of an error.

8. Seal ground samples into vials. Excess ground biomass may be discarded at this point.

9. Clean grinding tools and splitter thoroughly between samples:

    a. For a grinding mill: Power off, unplug, remove protective glass, and clean grinding chamber with compressed air. Clean glass with a kimwipe and ethanol. *Never remove protective glass with mill plugged in – doing so could result in serious injury.*

b. Clean mortar and pestle with a kimwipe and ethanol.

10. **Mandatory Barcode Workflow**: Link vial barcodes with *BBC: Grinding and Pooling* records previously created in step (1).

    a. ***Barcode Workflow***: Scan the root envelope barcode associated with a group of vials to bring up the desired parent record.

    b. Open and edit each child-level record, and scan in the required **CN Sample Barcode**, and if sufficient sample was present, the required **BGC Archive Barcode**.

    c. **Pool Sample Condition**: Select "error: gloves not worn" if samples were inadvertently pooled and ground without gloves.

    d. Save each child record.

    e. Save the parent record.

11. See SOP H for shipping instructions.

    a. Store samples in a cool, dry location until they can be shipped to analytical facilities or the biorepository.

SOP G Data Entry and Verification

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

Given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available as a backup to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

G.1 Digital Data Workflow

Data Quality Control (QC) is a very important task for all data entered into Fulcrum applications. ***Use the Plant Belowground Biomass 'QC Checklist' documents linked via the SSL*** to guide and focus QC activities. Below is a high-level summary of important QC activities by Fulcrum application:

Data collected in the field:

1. The **Clip ID**, **Collect Date** and sampling area (North/South) are used to construct the soil **Sample ID** in the *BBC: Field Sampling [PROD]* app. Make sure these data are entered correctly before finalizing Field Sampling records.
2. Finalizing *BBC: Field Sampling [PROD]* records and syncing will make **Sample ID**s created in this application available for further data entry in the following downstream applications: *BBC: Lab Weighing [PROD]*, *BBC: Lab Dilution [PROD]*, and *BBC: Grind and Pool [PROD]*.
    a. If corrections to either the Sampling Cell number, Collect Date, or sampling area are required after a Sample ID has been selected in a downstream application:
        i. Make correction(s) in the *BBC: Field Sampling [PROD]* app and save.
        ii. Open, edit, and save each downstream parent and child record to propagate the update.
    b. Consult the plant Belowground Biomass Sampling Fulcrum User Manual on the SSL for more detail.

Lab Weighing and Grinding and Pooling:

1. The **Sample ID**, and **Size Category** data are used to construct the **Subsample ID** that is associated with a given Dry Mass value.
2. The downstream *BBC: Grind and Pool [PROD]* application uses masses from available Subsample IDs to determine which subsamples are pooled, processed and shipped to external facilities.
    a. If corrections to data used to construct Subsample IDs are required:
        i. Make correction(s) in the *BBC: Lab Weighing [PROD]* app and save.
        ii. Open, edit, and save each downstream parent and child record to propagate the update.

Lab Dilution data:

1. The **Sample ID** from the *BBC: Field Sampling [PROD]* application is used to construct the **Dilution Sample ID**.
2. If corrections to either the Sampling Cell number, Collect Date, or sampling area are required in the *BBC: Field Sampling [PROD]* app after a Sample ID has been selected in the Lab Dilution app:
    a. Make correction(s) in the *BBC: Field Sampling [PROD]* app and save.
    b. Open, edit, and save each Lab Dilution parent and child record in order to propagate the update.

See the Data Management Protocol (RD[04]) for detailed, protocol-specific Data Management SOPs. See training materials on the SSL for detailed data ingest guidance via the NEON digital workflow.

G.2 Field Datasheets

1. Transcribe data from the plant Belowground Biomass Field Datasheets (RD[05]) to the *BBC: Field Sampling [PROD]* application.
    - Consult the Belowground Biomass Fulcrum Manual on the SSL to determine appropriate values and formats for each field in the ingest table.
2. If a representative Sampling Cell did not support belowground biomass soil sampling, noted as **Sampling Impractical** = 'Obstruction' in the **remarks** field of the Field Datasheet, enter in the *BBC: Field Sampling [PROD]* app:
    - **Sampling Impractical** = 'Obstruction'
3. Update permanent digital versions of the Clip Lists with **date** and **status** = '5' data recorded in the field.

G.3 Lab Datasheets

- Transcribe data from the 'Lab Weighing' datasheet into the *BBC: Lab Weighing [PROD]* application.
    - If a soil sample contained no fine root biomass within a given **Size Category**, select 'No' in the appropriate sample Presence/Absence field.

Transcribe data from the 'Lab Dilution' datasheet into the *BBC: Lab Dilution [PROD]* application.

SOP H Sample Shipment

Information included in this SOP conveys instructions for preparing and labeling samples up to the point at which they are ready to be placed in a box for shipment.

- **Timelines**: See Section 4.3, **Table 3**. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
- **Grouping/Splitting Samples**: Samples originating from the same sampling cell should be grouped together for shipment, if possible.
- **Samples Containing *Toxicodendron***: Samples that contain or may contain tissue from *Toxicodendron spp.* require labeling to ensure the receiving lab is aware of the contents.

  - Label 20 mL scint vials that may contain *Toxicodendron* tissue with a warning sticker affixed to the lid (example sticker at left).
  - Use the 'Shipment Remarks' field in the Shipping App to indicate "***X samples in the shipment contain Toxico, handle with care.***"

- **Shipment Preparation**:
  - Frozen cores shipped to another domain for processing must be packaged and shipped such that they arrive frozen at the destination domain support facility. Packing and shipping guidance is in development; submit a ServiceNow request to Science for the most recent guidance.
  - Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment to ship dried, ground samples to external laboratories or the biorespository (RD[17]).
    - CN samples: Ship in 20 mL plastic scint vials.
    - Archive samples: Ship in 20 mL glass scint vials.

SOP I Equipment Maintenance

1. Balances should be calibrated with a standard calibration weight set:
    a. After initial installation.
    b. Any time the balance is moved to a new surface.
    c. Every 6 months.
    d. If you suspect readings are inaccurate for any reason.
2. The Wiley Mini Mill
    a. Check stationary blades: Stationary blades may move during the course of processing large-diameter roots and require adjustment.
        i. Check for tip-to-tip clearance between all rotor blades and stationary blades by placing a piece of paper with average thickness against each stationary knife in turn.
        ii. Turn the rotor shaft by hand counter-clockwise so that all four rotor blades pass the stationary blade.
        iii. Blades should touch the paper but not cut it.
    b. Adjust stationary blades if warranted based on check above (**Figure 24**).
        i. Loosen stationary blade clamping screw.
        ii. Tighten stationary blade adjustment screw so that stationary blade is moved closer to rotor blades.
        iii. Tighten stationary blade clamping screw.
        iv. Repeat for other stationary blade.
        v. Perform stationary blade check above in 2.a . Stationary blades MUST NOT TOUCH ROTOR BLADES DURING OPERATION. SEVERE DAMAGE WILL RESULT.
    c. Blade sharpening or replacement
        i. Blade sharpening or replacement may be required if adjusting the position of stationary blades does not improve mill performance.
        ii. Contact Troemner for an RMA at 1-800-352-7705 and select option for Technical Service.
        iii. Order the online Sharpening Service from Thomas Scientific.
        iv. Allow 4 weeks for service to be completed.

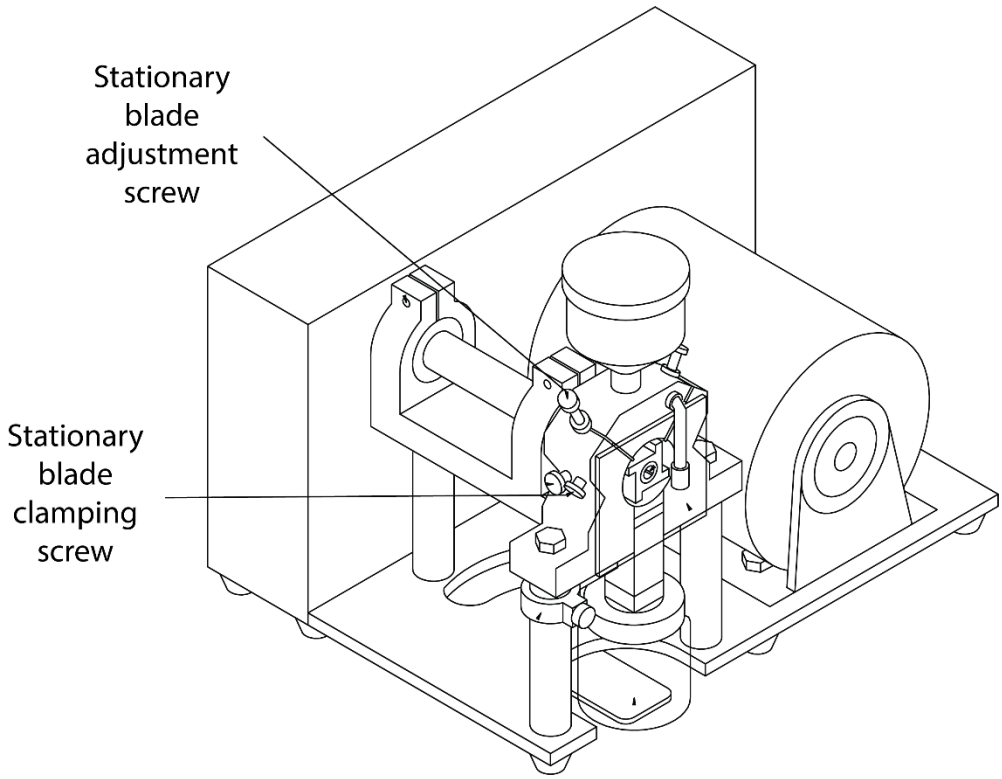

Figure 24. Wiley Mini Mill diagram showing position of stationary blade adjustment and clamping screws.

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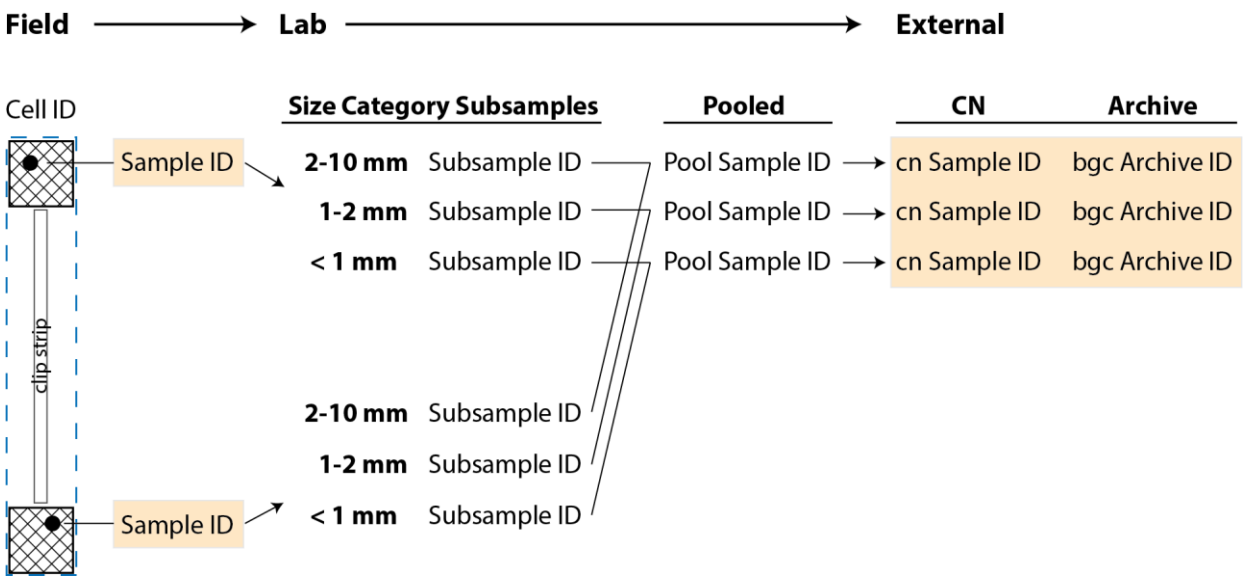
APPENDIX A QUICK REFERENCES

A.1 Sample Relationships



A.2 Field Sampling

1. Use Plot Prioritization lists on the SSL to determine the order of sampled plots and whether a Dilution Sample will be generated from the sample(s).
2. Select the first available sampling cell from the Clip List, and assess for suitability. Be sure to check if Herbaceous Biomass sampling has already occurred in the current season, and if a cell has already been clipped, choose the clipped cell to co-locate sampling. Ignore if working in a Tower plot that no longer supports HBP sampling.
3. Collect one soil core or monolith sample from the North sampling area, and another core or monolith sample from the South sampling area.
    a. *Note*: Only one core may be collected per sampling area if using the 'medium-risk' or 'high-risk' sampling strategy.
4. Measure litter depth and distance to nearest qualifying woody stems.
5. Measure and record the depth of the sample hole.
6. Create a label + barcode for each soil sample on waterproof paper, and be sure to record all required sampling metadata.
7. Record the date and time the soil sample was placed in the cooler in the field.
8. *(Optional)* Freeze samples upon return to the laboratory for processing at a later date.

QUALITY DEPENDS ON PROPER:

- Labeling and barcoding of soil samples.
- Measurement of sample hole depth.
- Maintaining samples in cold storage.

Use barcodes: Labeling problems can cause downstream errors and waste significant time.

A.3 Laboratory Processing

1. Monitor and track samples that will be processed for Dilution Sampling; these samples should have been previously identified in the field according to SOP B.2.
2. *(Optional)* Thaw previously frozen sample(s).
3. Soak samples prior to sieving in a plastic bin or bucket.
4. Process one small aliquot of the sample slurry through the sieve stack at a time – ***avoid overflowing the fine bottom sieve!*** Wear gloves.
5. Use a wire gauge to determine root **Size Category** – ***always measure root diameter through the gap in side of the wire gauge***. Do NOT pass the root through the hole of the gauge.
6. Sort roots by **Size Category**.
7. Dry sorted roots for a minimum of 48 h at 65 ˚C.

QUALITY DEPENDS ON PROPER:

- Passing the samples through the sieves – DO NOT OVERFLOW!
- Removal of mineral soil and organic material from roots.
- Use of the side-gap in the wire gauge for **Size Category** sorting.

A.4 Dilution Sampling

1. Retain the residual fraction from soil samples selected for Dilution Sampling.
2. Label all sample tins to ensure that samples can be tracked.
3. Work in pairs to quickly obtain representative subsamples of the suspended residual fraction.
4. Adjust the size of the beaker **Sample Volume** and the size of the **Aliquot Volume** sampled from the beaker with the syringe to keep sorting time manageable.
    a. Use distilled or filtered water.
    b. Aim for approximately 10-15 minutes per tin pair.
5. Dry sorted root fragments and OM for a minimum of 48 h at 65 ˚C.
6. Use a desiccator to cool samples before weighing them.

QUALITY DEPENDS ON:

- Choosing an appropriately sized beaker for suspending the residual fraction. Too concentrated will take too long to sort, and too dilute will result in masses too light to accurately weigh.
- Dispersing the residual fraction evenly throughout the sample volume in the beaker to generate representative aliquots.
- Accurately distinguishing roots from organic material.

A.5 Grinding and Pooling

1. Pre-label all vials with label + barcode. ***Barcodes are required.***
    a. CN samples: Use 20 mL plastic scint vials.
    b. Archive samples: Use 20 mL glass scint vials.
2. Pool roots by Size Category from the North and South samples that come from the same cell. Wear gloves.
3. Process all samples with mass ≥ 0.02 grams. Discard samples with mass < 0.02 g once mass has been recorded and QC is complete.
4. DO NOT GRIND samples that may contain *Toxicodendron spp.*
5. For pooled root samples with 0.02 g ≤ mass < 1 g: Create one chemistry analysis subsample only.
6. For pooled root samples with mass ≥ 1 g: Create chemistry analysis and archive subsamples. For pooled samples that do not contain *Toxico*:
    a. Grind the entire sample to 20 mesh.
    b. Use a splitter to re-grind a 0.4 g subsample to 40 mesh for chemistry analysis.

QUALITY DEPENDS ON:

- Using a microsplitter or splitter to generate sub-samples, NOT a spatula or scoopula.
- Wear gloves to prevent contamination of samples intended for ^{13}C and ^{15}N isotope analysis.
- Use barcodes to track samples shipped to external facilities.

APPENDIX B SITE-SPECIFIC DATES FOR SAMPLING ONSET

The dates in the table below are estimated from satellite MODIS-EVI phenology data averaged from 2012-2021 (Didan 2023), with the exception that dates for D04 and D20, which are relatively invariant with respect to greenness, are derived from precipitation data. Dates presented here are only a guide, and are derived according to the logic presented in Section 4.2. Because individual years may vary widely from the average dates provided below, it is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling, as described in Section 4 of this protocol.

Core sampling for plant Belowground Biomass is ideally timed to broadly coincide with the peak aboveground biomass clip harvest due to the scientific utility of relatively coincident estimates of both aboveground and belowground biomass. As such, dates listed in **Table 13** below are the estimated dates after which greenness begins to decrease at each site, and in theory, after which the majority of above and belowground biomass has been produced. However, soil moisture also influences the timing of sampling, and as such, dates below may need to be adjusted at a given site based on soil moisture conditions within a given year.

Table 13. Estimated average dates after which greenness begins to decrease for each NEON site based on MODIS-EVI phenology data. Ideally, soil sampling and aboveground biomass clip harvests should occur on or near these dates.

Domain	Site	Start Date (MM/DD)	Additional Information
01	BART	07/17	Date is earlier than indicated by MODIS due to understory consistently senescing before overstory.
	HARV	07/17	Date is earlier than indicated by MODIS due to understory consistently senescing before overstory.
02	BLAN	08/05	Modified start date based on Field Science feedback.
	SCBI	08/04	
	SERC	08/11	
03	DSNY	07/28	Flooded plots are likely at this time; Field Science to choose consistent alternative sampling start date.
	JERC	08/18	
	OSBS	07/25	
04	GUAN	10/15	Start date targets middle of wet season and is based on precipitation data from the Ensenada station (2005-2021). Months

Domain	Site	Start Date (MM/DD)	Additional Information
			receiving > 60 mm precipitation are considered wet-season.
	LAJA	10/15	Same logic for start date as GUAN site.
05	STEI	08/12	
	TREE	08/12	
	UNDE	08/13	
06	KONA	08/11	For plots planted with agricultural crops, sample 2-4 weeks before crop harvest. If plot is fallow with no cover crop, use provided date for scheduling.
	KONZ	08/11	
	UKFS	08/11	
07	GRSM	08/09	
	MLBS	08/11	
	ORNL	07/23	
08	DELA	07/22	
	LENO	07/25	
	TALL	07/20	
09	DCFS	08/05	Modified start date based on Field Science feedback.
	NOGP	07/31	Modified start date based on Field Science feedback.
	WOOD	08/10	Modified start date based on Field Science feedback.
10	CPER	06/20	Earlier start date than MODIS date based on soil moisture.
	RMNP	08/06	
	STER	2-4 wks before crop harvest	If plot is fallow with no cover crop, sample at peak green of surrounding vegetation.
11	CLBJ	10/01	MODIS-EVI data difficult to interpret; start date based on Field Science feedback.

Domain	Site	Start Date (MM/DD)	Additional Information
	OAES	11/11	MODIS-EVI data difficult to interpret; start date based on Field Science feedback.
12	YELL	07/23	
13	MOAB	08/13	MODIS-EVI data difficult to interpret; start date based on Field Science feedback.
	NIWO	08/01	Earlier start date than MODIS date based on Field Science feedback.
14	JORN	09/07	Visual estimate from MODIS timecourse data.
	SRER	08/13	MODIS-EVI data variable; start date based on Field Science feedback.
15	ONAQ	06/10	Visual estimate from MODIS timecourse data.
16	ABBY	06/27	Date adjusted earlier than MODIS-EVI based on Field Science assessment of understory peak biomass for GASH, MANE2.
	WREF	06/27	Date adjusted earlier than MODIS-EVI based on Field Science assessment of understory peak biomass for GASH, MANE2.
17	SJER	04/10	
	SOAP	06/26	Date earlier than MODIS-EVI date due to shift in vegetation following fire that is not captured by MODIS time series.
	TEAK	07/27	Date earlier than MODIS-EVI due to effect of more frequent dry years.
18	BARR	07/27	Earlier start date than MODIS date based on Field Science feedback.
	TOOL	07/26	Earlier start date than MODIS date based on Field Science feedback.
19	BONA	07/26	Earlier start date than MODIS date based on Field Science feedback.
	DEJU	07/27	Earlier start date than MODIS date based on Field Science feedback.

Domain	Site	Start Date (MM/DD)	Additional Information
	HEAL	07/28	Earlier start date than MODIS date based on Field Science feedback.
20	PUUM	05/21	Start date based on precipitation data and targets end of wet season for logistical reasons.

APPENDIX C SITE-SPECIFIC SAMPLING INFORMATION

Table 14. Summary of site-specific belowground biomass sampling modifications and supporting rationale.

Domain	Site(s)	Modification Type	Modification	Standard Rule	Rationale for Change
D02	SCBI	Collection method	Use core method despite large rocks. Use **Sampling Impractical** = 'Obstruction' as appropriate.	Monolith method	Monolith method would require large holes due to size of rocks.
D18/19	BARR TOOL BONA DEJU HEAL	Clarification: Definition of soil surface	Site-specific criteria to determine where the soil surface begins.	Soil surface and litter layer are differentiated by former lacking discernable plant parts.	Fibric plant structures persist into soil organic layer; roots grow into living moss layers.
D19	DEJU	Collection method	Use core method to collect samples despite rock layer that prevents collecting to 30 cm depth.	Monolith method is used when rock prevents collecting to 30 cm depth.	Monolith method intended for large, discontinuous rocks. Continuous rock layer at ~25cm depth at DEJU cannot be avoided via monolith method.
D19	HEAL	Collection method	Monoliths may be cut to 10 x 4 x 30 cm to better approximate the volume of a Giddings core.	Standard monoliths are 10 x 10 x 30 cm	Standard monolith size assumes rocks are present; no rocks at HEAL but cannot use Giddings corer because of bryophyte compression.
D19	HEAL	Sieving / sorting	For root mats collected on 2 mm sieve, pick roots > 1 mm diameter and clean to remove OM. Assign cleaned mats that also contain < 1 cm length fragments to < 1 mm size category.	Fragments < 1 cm length are separated from roots with diameter < 1 mm and > 1 cm length.	Vast majority of root fragments < 1 cm length are also < 1 mm diameter due to abundant bryophytes. Very time consuming to separate.

Domain	Site(s)	Modification Type	Modification	Standard Rule	Rationale for Change
D19	HEAL	Sieving /sorting	Sort OM sludge that mostly contains root fragments < 1 mm diameter and < 1 cm length for 15 min max to remove fragments > 2 cm length. Process remaining sludge as dilution sample if applicable or discard.	Sort all root fragments > 1 cm length from OM.	OM is very difficult to differentiate from bryophyte root fragments with a 1 cm cutoff, and both OM and bryophyte root fragments are abundant. Dilution sampling is more appropriate for the task.

C.1 D18/19 Site-specific Modifications

1. To determine where the soil surface begins, use the presence of roots, color, and texture:
    a. The presence of roots determines the position of the soil surface. This means we will begin collecting a soil sample at depths where the substrate may still be comprised of identifiable plant parts (live or dead).
    b. When vascular plant roots are absent, identify the boundary between mostly live or mostly dead plant material, and call this boundary the top of the organic soil horizon.
        i. Dead plant material may still appear fibric and very much like a discernable plant part at this boundary.
        ii. Finding the boundary can be difficult because live plant material, roots, and dead plant material will often transition along a continuum from the surface downward.
        iii. To sample consistently, use color (green to brown), texture (soft and friable material should be mostly dead), and presence of roots.
2. To collect a soil sample:
    a. Use clippers or equivalent to remove surface vegetation and reveal the soil surface.
        i. Remove vegetation from a surface area of approximately 10 cm x 10 cm until roots are apparent or until the surface of the organic soil layer is apparent (using criteria above).
    b. Collect a soil sample to 30 cm maximum depth or refusal, whichever comes first.
    c. Remove the soil sample and process according to SOP B.
3. To process soil samples with a fibric surface soil:

a. Cut and separate fibric organic material from more mineral-rich and decomposed organic soil before sieving – fibric material will not sieve easily and should not be passed through the sieve stack. Typical organic soil will pass through the sieve stack.

b. From the fibric fraction, manually pluck larger roots and then float and wet-pick the remaining material to remove smaller diameter roots and fragments ≥ 1 cm length.

APPENDIX D SOIL CORER ASSEMBLY

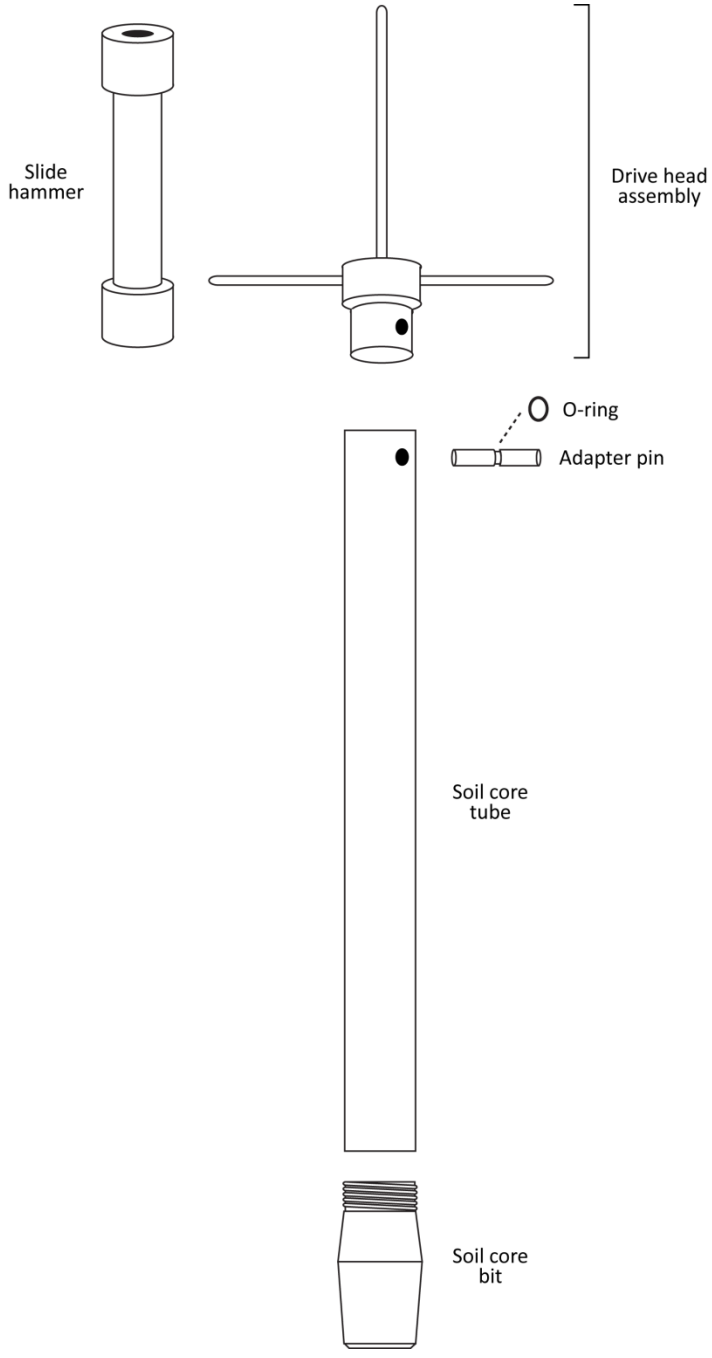


Figure 25. Component parts of the Giddings soil core assembly.

APPENDIX E MANAGING EXPOSURE TO *TOXICODENDRON* SPECIES

General guidelines for preventing and mitigating exposure to toxic oils from *Toxicodendron* species can be found in RD[12].

The following are protocol-specific best-practice techniques for minimizing exposure to toxic oil during plant Belowground Biomass Sampling.

Table 15. Equipment list – Minimizing exposure to toxic oils from roots of *Toxicodendron spp*. that may be encountered during plant Belowground Biomass Sampling.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
	N	Labeled clippers, dedicated to clipping *Toxicodendron spp.* (see **Table 18**)	Prevent spread of toxic oils to multiple clippers	1
	N	Labeled sieve set(s), dedicated to sieving samples containing *Toxicodendron.* (Set contains 2mm sieve and 250 µm sieve. See **Table 18**.)	Prevent spread of toxic oils to multiple sieves.	As needed
	N	Labeled forceps, blunt tip, stainless steel; dedicated to *Toxicodendron* samples	Prevent spread of toxic oils to multiple forceps.	As needed
Consumable Items				
		See RD[12]		

1. **Prior to field work:**
    a. Count out coin envelopes or clasp envelopes for storing and drying root samples that will likely contain *Toxicodendron* biomass. Don't mix samples containing *Toxicodendron* biomass with any other samples.
    b. Pre-weigh (to nearest 0.01 g) and label each envelope that will be used for storing and drying soil samples containing *Toxicodendron* biomass. Once the weight of each empty envelope is written on the envelope, the biomass inside the bag will never have to be touched after it is initially placed in the bag.
2. **To collect soil samples containing *Toxicodendron* biomass in the field:**
    a. Before collecting the soil sample, use a pair of clippers dedicated solely to clipping *Toxicodendron spp.* to clip and remove any aboveground *Toxicodendron* biomass that would be contacted while sampling.

b. Write 'Toxico' or equivalent on the label of any soil sample that may contain *Toxicodendron* roots.

c. Bring a clean, new plastic bag to the field for storing and transporting contaminated gloves, soil sampling equipment, and clippers after use.

3. **To process and weigh samples that may contain *Toxicodendron* biomass in the laboratory:**

a. Use sieves and forceps dedicated to processing root samples containing *Toxicodendron* biomass. Wash sieves and forceps with Tecnu (or equivalent) following each use.

b. Minimize potential spread of toxic oil by putting envelopes containing *Toxicodendron* roots into the same drying oven every time.

c. When drying is complete, clean drying oven shelves used for drying *Toxicodendron* biomass with hot water and Tecnu. Wear appropriate PPE when cleaning.

d. Record weight of bag + dried biomass to nearest 0.001 g or 0.0001 g, and also record weight of individual empty bag (to minimum of 0.001 g) on data sheets. Dried *Toxicodendron* biomass should never leave the bag.

APPENDIX F SAMPLING CELL NUMBER COORDINATES AND MAPS

Plant Belowground Biomass Sampling and peak biomass clip harvest sampling ideally take place in the same sampling cell in a given Tower plot. NEON Field Science staff must track the sampling cell associated with root sampling and peak biomass clipping on the Clip Lists provided by Science. When the Herbaceous Biomass clip harvest (RD[11]) precedes soil sampling in the field, it is necessary to physically locate the sampling cell in which the peak biomass clip occurred.

F.1 Maps of Sampling Cell Number by subplotID

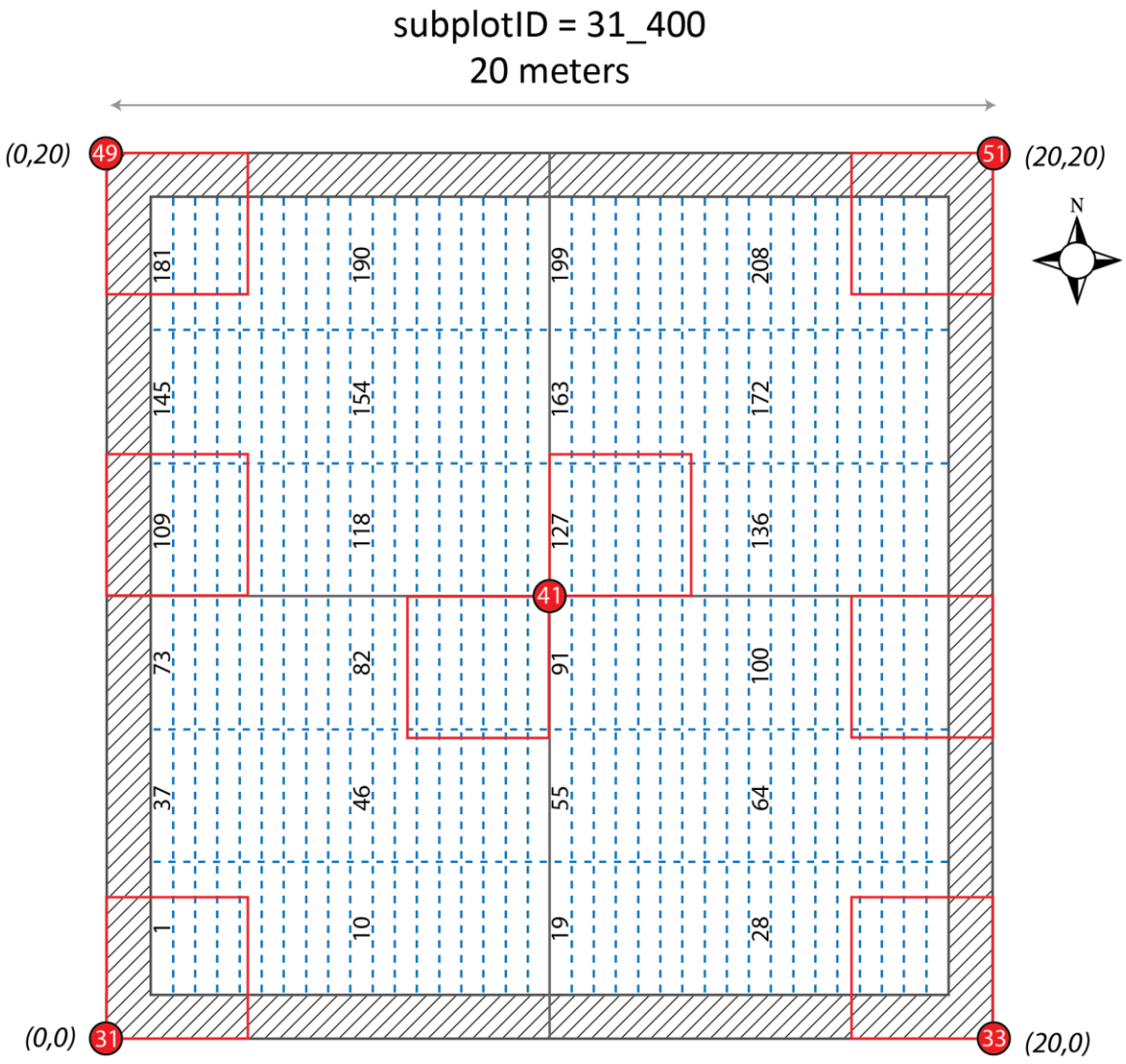


Figure 26. Map of Sampling Cells and numerical identifiers in a 20m x 20m base plot (subplotID = 31_400). Red squares indicate nested subplots used for diversity sampling; sampling cells that significantly overlap red squares are not used for fine root soil sampling or clip sampling. However, cells with minimal overlap (e.g., 48-54, 68-72, 145-149) do support these sampling activities. Red circles with white numbers represent plot markers with associated pointIDs.

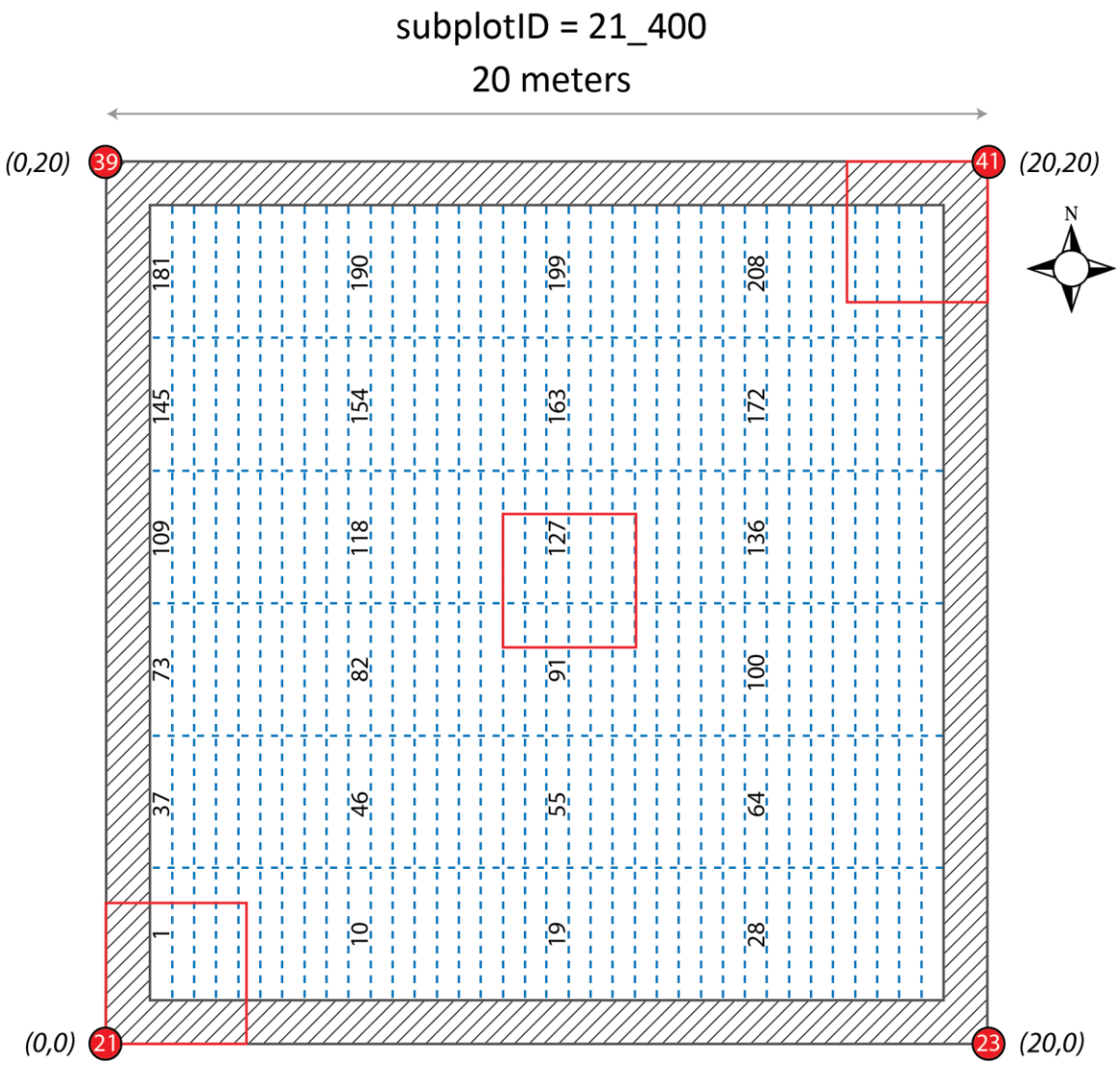


Figure 27. Map of Sampling Cells and numerical identifiers for **subplotID = 21_400** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil sampling or clip sampling. Red circles with white numbers represent plot markers with associated pointIDs.

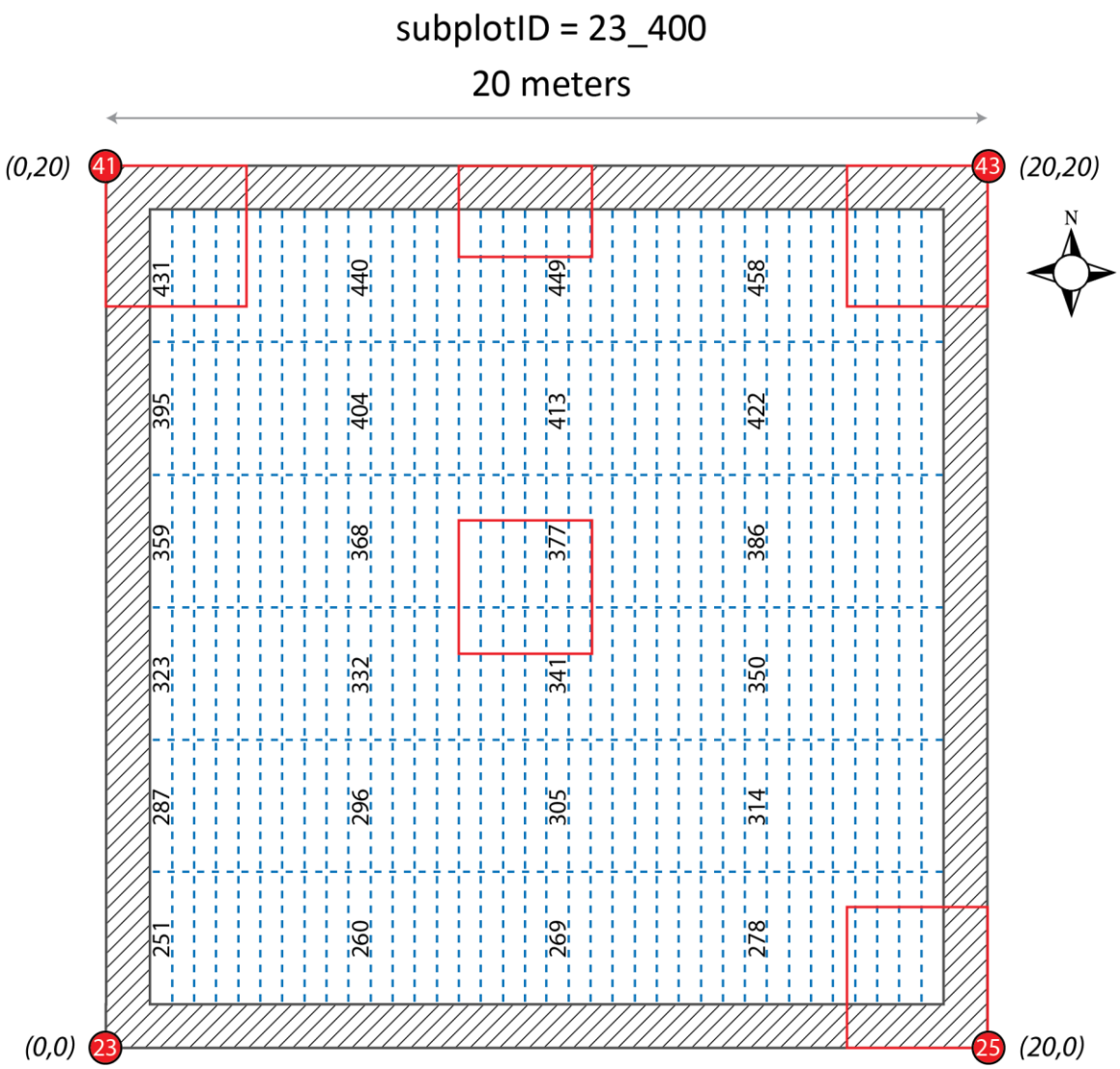


Figure 28. Map of Sampling Cells and numerical identifiers for **subplotID = 23_400** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil sampling or clip sampling. Red circles with white numbers represent plot markers with associated pointIDs.

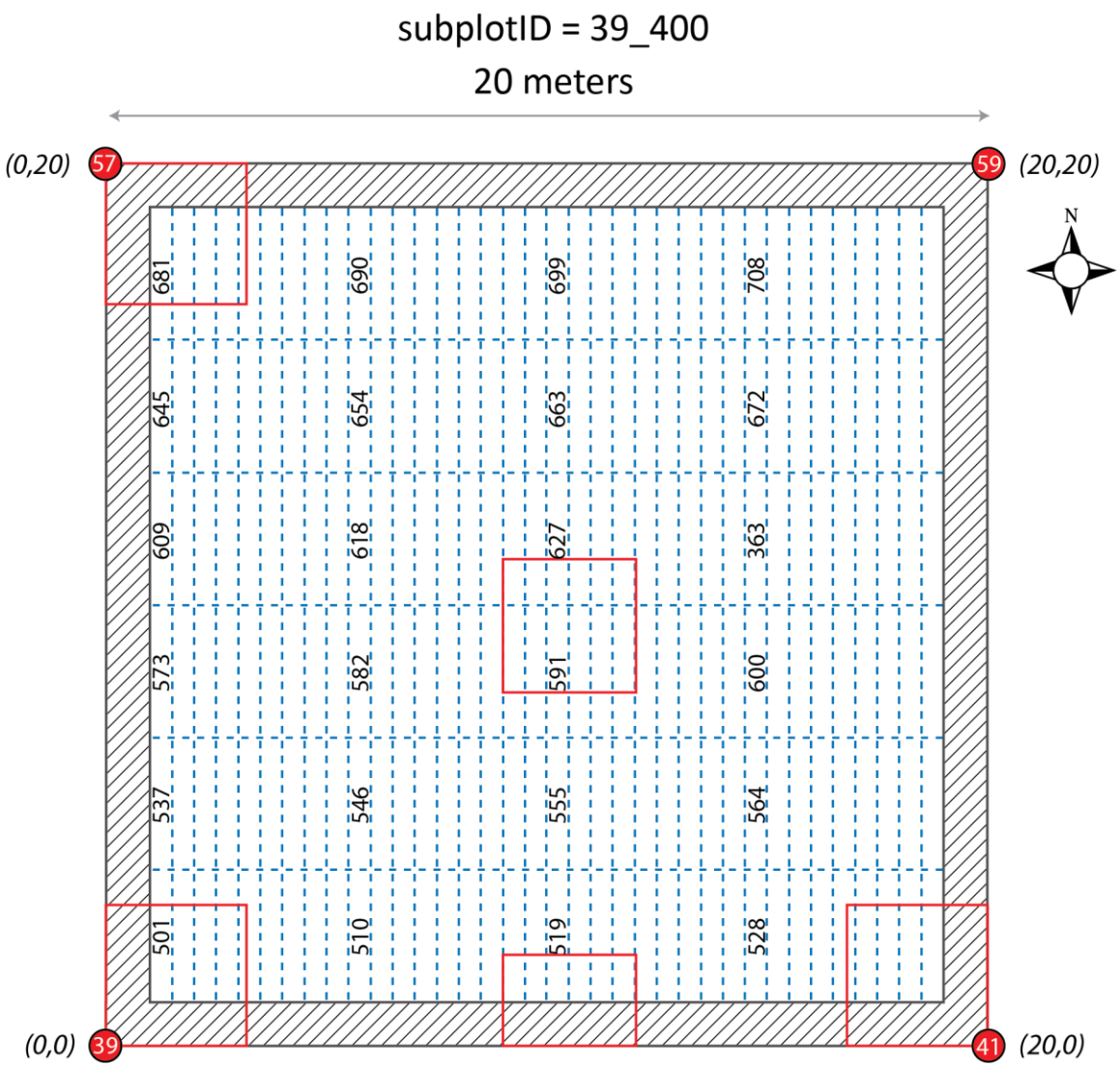


Figure 29. Map of Sampling Cells and numerical identifiers for **subplotID = 39_400** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil sampling or clip sampling. Red circles with white numbers represent plot markers with associated pointIDs.

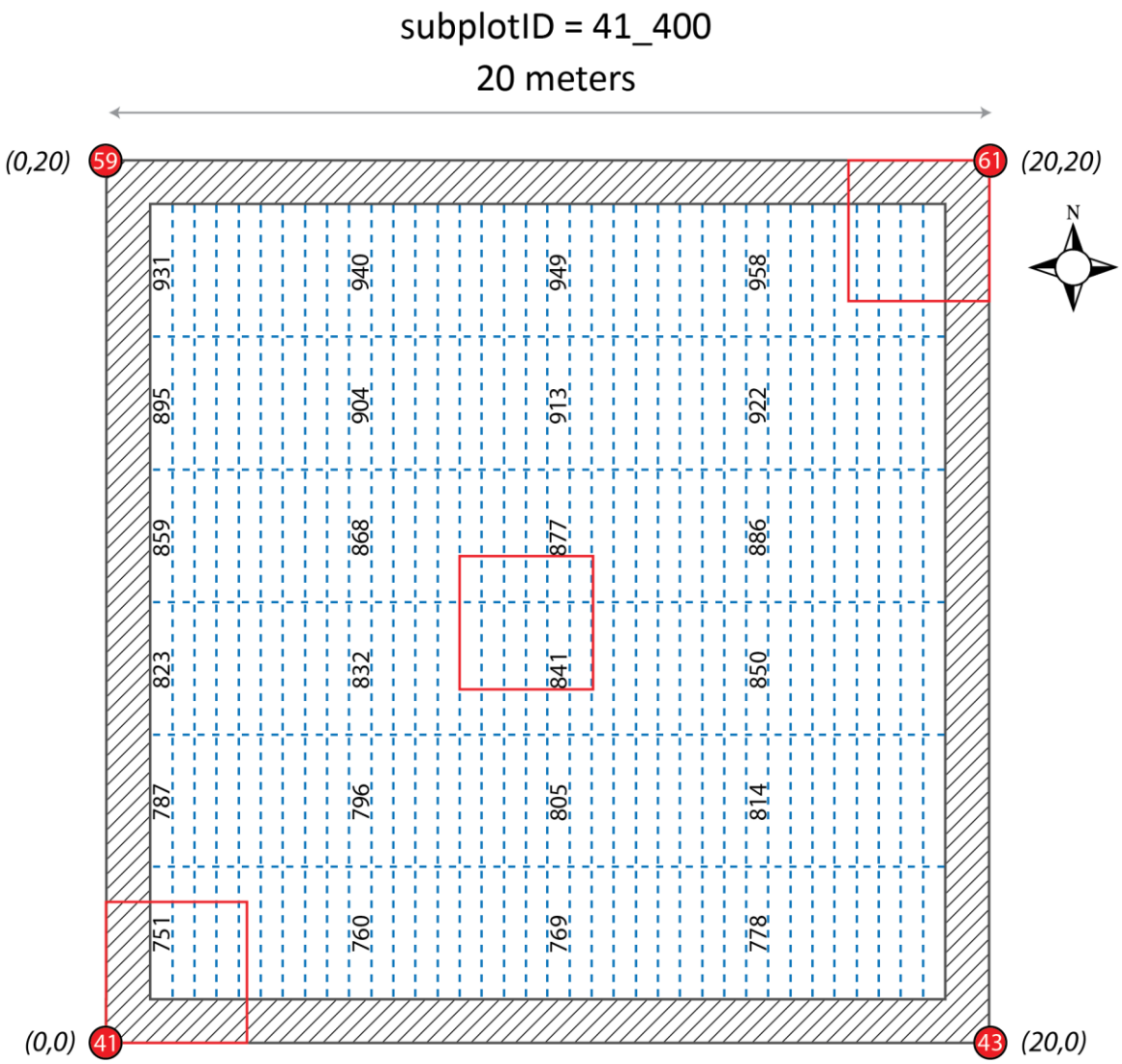


Figure 30. Map of Sampling Cells and numerical identifiers for **subplotID = 41_400** in a 40m x 40m Tower base plot. Cells that overlap nested subplots indicated by red squares are not used for fine root soil sampling or clip sampling. Red circles with white numbers represent plot markers with associated pointIDs.

F.2 Coordinates for Sampling Cells by subplotID

Table 16. List of Sampling Cells and numerical identifiers by subplotID and associated easting and northing coordinates. Coordinates correspond to the SW corner of a 0.1m x 2m Clip Strip, and indicate the distance in meters relative to the SW corner of the plot (subplotID = 31_400) or subplot (subplotID = 21_400, 23_400, 39_400, and 41_400).

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
1	1	251	501	751	1.2	1.5
2	2	252	502	752	1.7	1.5
3	3	253	503	753	2.2	1.5
4	4	254	504	754	2.7	1.5
5	5	255	505	755	3.2	1.5
6	6	256	506	756	3.7	1.5
7	7	257	507	757	4.2	1.5
8	8	258	508	758	4.7	1.5
9	9	259	509	759	5.2	1.5
10	10	260	510	760	5.7	1.5
11	11	261	511	761	6.2	1.5
12	12	262	512	762	6.7	1.5
13	13	263	513	763	7.2	1.5
14	14	264	514	764	7.7	1.5
15	15	265	515	765	8.2	1.5
16	16	266	516	766	8.7	1.5
17	17	267	517	767	9.2	1.5
18	18	268	518	768	9.7	1.5
19	19	269	519	769	10.2	1.5
20	20	270	520	770	10.7	1.5
21	21	271	521	771	11.2	1.5
22	22	272	522	772	11.7	1.5
23	23	273	523	773	12.2	1.5
24	24	274	524	774	12.7	1.5
25	25	275	525	775	13.2	1.5
26	26	276	526	776	13.7	1.5
27	27	277	527	777	14.2	1.5
28	28	278	528	778	14.7	1.5
29	29	279	529	779	15.2	1.5
30	30	280	530	780	15.7	1.5
31	31	281	531	781	16.2	1.5
32	32	282	532	782	16.7	1.5
33	33	283	533	783	17.2	1.5
34	34	284	534	784	17.7	1.5
35	35	285	535	785	18.2	1.5
36	36	286	536	786	18.7	1.5

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
37	37	287	537	787	1.2	4.5
38	38	288	538	788	1.7	4.5
39	39	289	539	789	2.2	4.5
40	40	290	540	790	2.7	4.5
41	41	291	541	791	3.2	4.5
42	42	292	542	792	3.7	4.5
43	43	293	543	793	4.2	4.5
44	44	294	544	794	4.7	4.5
45	45	295	545	795	5.2	4.5
46	46	296	546	796	5.7	4.5
47	47	297	547	797	6.2	4.5
48	48	298	548	798	6.7	4.5
49	49	299	549	799	7.2	4.5
50	50	300	550	800	7.7	4.5
51	51	301	551	801	8.2	4.5
52	52	302	552	802	8.7	4.5
53	53	303	553	803	9.2	4.5
54	54	304	554	804	9.7	4.5
55	55	305	555	805	10.2	4.5
56	56	306	556	806	10.7	4.5
57	57	307	557	807	11.2	4.5
58	58	308	558	808	11.7	4.5
59	59	309	559	809	12.2	4.5
60	60	310	560	810	12.7	4.5
61	61	311	561	811	13.2	4.5
62	62	312	562	812	13.7	4.5
63	63	313	563	813	14.2	4.5
64	64	314	564	814	14.7	4.5
65	65	315	565	815	15.2	4.5
66	66	316	566	816	15.7	4.5
67	67	317	567	817	16.2	4.5
68	68	318	568	818	16.7	4.5
69	69	319	569	819	17.2	4.5
70	70	320	570	820	17.7	4.5
71	71	321	571	821	18.2	4.5
72	72	322	572	822	18.7	4.5
73	73	323	573	823	1.2	7.5
74	74	324	574	824	1.7	7.5
75	75	325	575	825	2.2	7.5
76	76	326	576	826	2.7	7.5
77	77	327	577	827	3.2	7.5
78	78	328	578	828	3.7	7.5

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
79	79	329	579	829	4.2	7.5
80	80	330	580	830	4.7	7.5
81	81	331	581	831	5.2	7.5
82	82	332	582	832	5.7	7.5
83	83	333	583	833	6.2	7.5
84	84	334	584	834	6.7	7.5
85	85	335	585	835	7.2	7.5
86	86	336	586	836	7.7	7.5
87	87	337	587	837	8.2	7.5
88	88	338	588	838	8.7	7.5
89	89	339	589	839	9.2	7.5
90	90	340	590	840	9.7	7.5
91	91	341	591	841	10.2	7.5
92	92	342	592	842	10.7	7.5
93	93	343	593	843	11.2	7.5
94	94	344	594	844	11.7	7.5
95	95	345	595	845	12.2	7.5
96	96	346	596	846	12.7	7.5
97	97	347	597	847	13.2	7.5
98	98	348	598	848	13.7	7.5
99	99	349	599	849	14.2	7.5
100	100	350	600	850	14.7	7.5
101	101	351	601	851	15.2	7.5
102	102	352	602	852	15.7	7.5
103	103	353	603	853	16.2	7.5
104	104	354	604	854	16.7	7.5
105	105	355	605	855	17.2	7.5
106	106	356	606	856	17.7	7.5
107	107	357	607	857	18.2	7.5
108	108	358	608	858	18.7	7.5
109	109	359	609	859	1.2	10.5
110	110	360	610	860	1.7	10.5
111	111	361	611	861	2.2	10.5
112	112	362	612	862	2.7	10.5
113	113	363	613	863	3.2	10.5
114	114	364	614	864	3.7	10.5
115	115	365	615	865	4.2	10.5
116	116	366	616	866	4.7	10.5
117	117	367	617	867	5.2	10.5
118	118	368	618	868	5.7	10.5
119	119	369	619	869	6.2	10.5
120	120	370	620	870	6.7	10.5

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
121	121	371	621	871	7.2	10.5
122	122	372	622	872	7.7	10.5
123	123	373	623	873	8.2	10.5
124	124	374	624	874	8.7	10.5
125	125	375	625	875	9.2	10.5
126	126	376	626	876	9.7	10.5
127	127	377	627	877	10.2	10.5
128	128	378	628	878	10.7	10.5
129	129	379	629	879	11.2	10.5
130	130	380	630	880	11.7	10.5
131	131	381	631	881	12.2	10.5
132	132	382	632	882	12.7	10.5
133	133	383	633	883	13.2	10.5
134	134	384	634	884	13.7	10.5
135	135	385	635	885	14.2	10.5
136	136	386	636	886	14.7	10.5
137	137	387	637	887	15.2	10.5
138	138	388	638	888	15.7	10.5
139	139	389	639	889	16.2	10.5
140	140	390	640	890	16.7	10.5
141	141	391	641	891	17.2	10.5
142	142	392	642	892	17.7	10.5
143	143	393	643	893	18.2	10.5
144	144	394	644	894	18.7	10.5
145	145	395	645	895	1.2	13.5
146	146	396	646	896	1.7	13.5
147	147	397	647	897	2.2	13.5
148	148	398	648	898	2.7	13.5
149	149	399	649	899	3.2	13.5
150	150	400	650	900	3.7	13.5
151	151	401	651	901	4.2	13.5
152	152	402	652	902	4.7	13.5
153	153	403	653	903	5.2	13.5
154	154	404	654	904	5.7	13.5
155	155	405	655	905	6.2	13.5
156	156	406	656	906	6.7	13.5
157	157	407	657	907	7.2	13.5
158	158	408	658	908	7.7	13.5
159	159	409	659	909	8.2	13.5
160	160	410	660	910	8.7	13.5
161	161	411	661	911	9.2	13.5
162	162	412	662	912	9.7	13.5

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
163	163	413	663	913	10.2	13.5
164	164	414	664	914	10.7	13.5
165	165	415	665	915	11.2	13.5
166	166	416	666	916	11.7	13.5
167	167	417	667	917	12.2	13.5
168	168	418	668	918	12.7	13.5
169	169	419	669	919	13.2	13.5
170	170	420	670	920	13.7	13.5
171	171	421	671	921	14.2	13.5
172	172	422	672	922	14.7	13.5
173	173	423	673	923	15.2	13.5
174	174	424	674	924	15.7	13.5
175	175	425	675	925	16.2	13.5
176	176	426	676	926	16.7	13.5
177	177	427	677	927	17.2	13.5
178	178	428	678	928	17.7	13.5
179	179	429	679	929	18.2	13.5
180	180	430	680	930	18.7	13.5
181	181	431	681	931	1.2	16.5
182	182	432	682	932	1.7	16.5
183	183	433	683	933	2.2	16.5
184	184	434	684	934	2.7	16.5
185	185	435	685	935	3.2	16.5
186	186	436	686	936	3.7	16.5
187	187	437	687	937	4.2	16.5
188	188	438	688	938	4.7	16.5
189	189	439	689	939	5.2	16.5
190	190	440	690	940	5.7	16.5
191	191	441	691	941	6.2	16.5
192	192	442	692	942	6.7	16.5
193	193	443	693	943	7.2	16.5
194	194	444	694	944	7.7	16.5
195	195	445	695	945	8.2	16.5
196	196	446	696	946	8.7	16.5
197	197	447	697	947	9.2	16.5
198	198	448	698	948	9.7	16.5
199	199	449	699	949	10.2	16.5
200	200	450	700	950	10.7	16.5
201	201	451	701	951	11.2	16.5
202	202	452	702	952	11.7	16.5
203	203	453	703	953	12.2	16.5
204	204	454	704	954	12.7	16.5

Cell Numbers subplotID = 31_400	Cell Numbers subplotID = 21_400	Cell Numbers subplotID = 23_400	Cell Numbers subplotID = 39_400	Cell Numbers subplotID = 41_400	easting offset	northing offset
205	205	455	705	955	13.2	16.5
206	206	456	706	956	13.7	16.5
207	207	457	707	957	14.2	16.5
208	208	458	708	958	14.7	16.5
209	209	459	709	959	15.2	16.5
210	210	460	710	960	15.7	16.5
211	211	461	711	961	16.2	16.5
212	212	462	712	962	16.7	16.5
213	213	463	713	963	17.2	16.5
214	214	464	714	964	17.7	16.5
215	215	465	715	965	18.2	16.5
216	216	466	716	966	18.7	16.5

APPENDIX G EQUIPMENT

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

Table 17. Equipment list – Sampling Plant Belowground Biomass in the field.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
	N	Mobile data collection device, tablet or equivalent	Record field sampling metadata	1 per team
Giddings Machine Co.; ST092R	Y	Soil core sampling tube, 36” length, 3” OD	Collect soil core sample	1
Giddings Machine Co.; HS114	Y	Soil core drive head assembly	Works with slide hammer to drive soil core tube into soil	1
Giddings Machine Co.; HS264	Y	Soil core drive head pin, 3” length	Attach drive head assembly to core tube	2
Giddings Machine Co.; ST236	Y	Soil core quick relief bit, 3” OD*	Attach to soil core sampling tube	1
Giddings Machine Co.; HS304	Y	Soil core slide hammer, 16#	Drive sampling tube into soil	1
Giddings Machine Co.; ST606	Y	Soil core basket retainer, 3” adapter	Attach basket retainer system to sampling tube; *for sandy soils that do not hold together*	1
Giddings Machine Co.; ST636	Y	Soil core basket retainer, 3” basket	Retain sandy soil in sampling tube; *for sandy soils that do not hold together*	2
Giddings Machine Co.; ST666	Y	Soil core basket retainer, 3” bit	Bit that works with basket retainer; *for sandy soils that do not hold together*	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Toothbrush	Clean soil corer threads in field, if changing bit is required	2
Target	N	Long-handled brush	Clean soil core tube between samples; *for soils that stick to core tube*	1
Amazon; N/A Cabela's; IK270217 REI; 895022	N	GPS unit, pre-loaded with plot locations	Navigate to plots or subplots	1
Forestry Suppliers; 91567	Y	TruPulse 360R laser rangefinder, current declination entered	Locate clip strip within a plot or subplot; *for slopes >20%, brushy vegetation*	1
CompassTools; 703512 Forestry Suppliers; 90998	Y	Foliage filter for laser rangefinder	Facilitates use of TruPulse in brushy conditions; *for brushy vegetation*	2
	N	Reflective surface (bicycle reflector or reflective tape on back of field notebook/clipboard)	Accurate location of clip strip with TruPulse in "FLT" mode	1
	N	Extra battery for TruPulse (CR123A type)	Battery backup for TruPulse	2
	N	Fiberglass meter tape (30m or longer)	Locate clip strip within plots or subplots; *for slopes <20%, grassland, savannah*	1
	N	Hand clippers, fine tip	Remove aboveground plant parts from soil coring location	1
	N	Large chest-style cooler, with frozen cold packs	Keep core samples cool, slow down root decomposition; one cooler per 8 cores sampled.	2+
Ben Meadows; 703512 Forestry Suppliers; 90998	N	Soil knife, hori-hori style	Loosen soil at surface to expose non-root plant parts, and collect monolith sample (when applicable)	1
	N	Sharpies	Label paper bags	2

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Ben Meadows; 100952	N	Chaining pins, steel, unpainted	Stretching tapes to enable location of target clip strip; *for slopes <20%; grassland, savannah*	2
	N	Measuring device, with 1 cm demarcations (e.g., tape, ruler, collapsible measuring stick, etc.)	Measure depth of the litter layer and depth of soil core bore hole	1
	N	Length of dowel, 1” PVC or equivalent (36” total length)	Push soil core sample out of soil core sampling tube; *for soil cores that stick to tube*	1
	N	Heavy duty work gloves	Protect hands during soil core sampling	1 pair/ person
	N	Rubber mallet	Drive soil knife into soil to collect sample; *for monolith sampling*	1
Consumable items				
	N	4”x 5” pin flags with PVC stakes	Accurate location of clip strip; PVC stakes avoid magnetic interference with compass or TruPulse	6
	N	Heavy duty freezer bags, 1.5 or 2 gallon	Store and organize soil core samples	40+
	N	Hearing protection	Prevent hearing damage from use of slide hammer	As needed
	N	Pencils	Record sampling metadata	2
	N	Waterproof paper, Rite-in-the-Rain or equivalent	Datasheet printing, and material for making labels to record soil core metadata in the field	10+ Sheets
	N	All-weather address labels, 1” x 2 5/8”	Moveable label to track sample from field through lab processing	25 sheets
		Sampling Cell Lists (Clip Lists)	Identify sampling cell for soil collection associated with peak biomass clip harvest	Varies
		Random Tower Subplot Lists	Identify subplots for soil sampling	Varies

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
		Belowground biomass "Field Sampling Datasheets"	Backup to record sampling metadata in the event of tablet failure	Varies
	N	Horticultural grade sand	Backfill soil sampling holes at sites where specified by site host	4-5 lbs per core
Request from NEON HQ	Y	Adhesive barcode labels (Type I)	Label field-collected soil samples with barcode readable labels	1 per sample
		Sample warning pictogram label	Identify possible presence of acute toxins that may cause serious eye or skin irritation	1 per sample

Table 18. Equipment list – Processing Plant Belowground Biomass in the lab.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
		Root washing station	Remove mineral soil from organic material	1
	N	Plastic bucket, bin, or equivalent (5 gallon, 20 L, etc.)	Soak core sample prior to sieving to break up cohesive clays and rehydrate roots; *suggested*	6
Fisher; 04-881-10G 04-884-1AE	N	Soil sieve, 2 mm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material	6
Fisher; 04-881-10L 04-884-1AJ	N	Soil sieve, 1 mm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material; *for sandy soil sieving*	6
Fisher; 04-881-10U 04-884-1AS	N	Soil sieve, 250 µm stainless mesh, 8" or 12" diameter	Remove mineral soil from organic material	6
	N	Rubber or silicone spatula	Transfer soil and roots from bucket to sieve(s)	3
	N	Rectangular plastic bin, enamel pan or equivalent, with lid; clear or white (app. 30 cm x 20 cm, or 13"x 9")†	Facilitates separating roots (which float) from mineral particles; allows secure storage in refrigerator	6+

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Bioquip; 4731, 4732, 4734, 4735	N	Forceps, blunt tip, stainless steel	Separate roots from organic material, sort root fragments from OM for dilution sampling	3
Amazon; B011W5LEJC Grainger; 5C735	N	* Wire gauge with openings approx. 2mm and 1mm	Sort roots into size classes during sieving and picking	3-10
	N	Small wire clippers	Clip and separate smaller diameter roots that emerge or fork from bigger roots; *if multiple Size Categories* exist	2
Thomas Scientific; 1711H10	Y	Grinding mill, Wiley, 20 mesh	Grind larger fine root sample volumes; *for sample masses > 750 mg*	1
	N	Porcelain mortar, 65 mL capacity, with pestle	Grind smaller fine root sample volumes, avoid loss of small samples in mill; *for sample masses < 750 mg*	1 set
Sepor; 040G-000	N	Sample microsplitter, 1/8”	Creates identical sub-samples from ground sample	1
Sepor; 040G-001	N	Hi-back pans for sample microsplitter	Creates identical sub-samples from ground sample	1
	N	Sharpie, extra fine tip	Labeling envelopes and scint vials	2
	N	Balance, 0.001 g accuracy or better	Weigh very light root samples	1
	N	Desiccator	Keep oven-dried samples moisture free before weighing	1
Consumable items				
	N	Pencils	Record dry weight of root samples	2
		Lab Weighing Datasheet	Record dry weight of root samples	Variable
Fisher 0333723C; Thomas 9718J20	N	Plastic scintillation vials with caps, 20 mL volume	Containers for ground CN-samples. Must be new/unused and stored to minimize dust or other contamination. Do not use if stored otherwise.	Up to 3 per sample cell

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Glass scintillation vials with caps, 20 mL volume	Containers for ground archive samples. Must be new/unused and stored to minimize dust or other contamination. Do not use if stored otherwise.	Up to 3 per sample cell
	N	Large weigh boats or aluminum weigh pans (metal may be best if static is problematic)	Weigh relatively large quantities of dried root samples	50+
	N	Clasp envelopes, 6”x 9”, Kraft paper	Store and organize sieved roots during and after drying	480-640
	N	Coin envelopes, 3⅜”x6”, Kraft paper	Store and organize sieved roots during and after drying	50
	N	Paper bag, 8# Kraft	Organize root samples in the drying ovens	20
	N	Small weigh boats or aluminum weigh pans (metal may be best if static is problematic)	Weigh relatively small quantities of dried root samples	50+
Fisher; 15-930-C	Y	Cryogenic-type adhesive labels, 0.5” x 1.25”	Label scintillation vials; exact brand required to ensure adhesion to sample vials.	1 per sample vial
Request from HQ	Y	Adhesive barcode labels (Type I)	Label samples with barcode readable labels	As needed
	N	Desiccant	Keep oven-dried samples moisture free before weighing	As needed
ULINE; S-21339	N	Sample warning pictogram label 	Label scintillation vials to identify possible presence of acute toxins that may cause serious eye or skin irritation	1 per sample vial

* Gauge 12 = 2.05 mm, and gauge 18 = 1.02 mm; while not *exactly* the diameters desired, the gauges listed here are acceptable for this protocol.

† Note: the exact dimensions of the pan/tub are not critical, it serves as an aid for more easily spotting roots suspended in water. The only requirement is that it can safely contain liquid; a white material also makes identifying roots easier.

Table 19. Equipment list – Dilution sampling for fine root biomass fragments < 1cm.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
Fisher; 04-881-10-DD 04-884-1BC	N	Soil sieve, 53 µm stainless mesh, 8" or 12" diameter	Consolidate residual fraction from both samples per clip strip, rinse, and transfer to beaker for dilution	2
Fisher; S88857200 S07978S	N	Magnetic mixing plate, stir range 150-2500; or, 50-1500 rpm, minimum 4 x 4 inch stirring surface	Randomize aqueous suspended residual fraction	1
	N	Magnetic stir bar, 2" to 3" length	Randomize aqueous suspended residual fraction	2
	N	Beaker, 1 L	Hold smaller volumes of aqueous suspended residual fraction	2
	N	Beaker, 2 L	Hold large volumes of aqueous suspended residual fraction	2
	N	Beaker, 4 L	Hold very large volumes of aqueous suspended residual fraction; e.g., for soils with thick O horizon	2
	N	Plunger, diameter approx. 1 cm less than beaker diameter	Stop mixing vortex, randomize aqueous suspended residual fraction	1 per beaker size
	N	Syringe, 40 – 60 mL, with tip cut off to make a 1 cm diameter aperture	Aspirate sub-sample from randomized aqueous residual fraction	2
	N	Plastic laboratory squirt bottle, filled with DI water	Rinse syringe following sub-sampling	1
Fisher; 08-732-102	N	Aluminum weighing dishes, 65 mL	Hold and dry root and organic material from sub-samples.	200
	N	Forceps, fine tip	Pick small root fragments apart from organic material	10-15
	N	Heavy duty sheet tray, baking or equivalent	Transfer aqueous samples in aluminum dishes to drying ovens; hold and protect samples throughout oven drying procedure.	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Balance, 0.001 g or 0.0001 g (preferred) accuracy	Weigh extremely light dried dilution samples	1
	N	Threaded rod or bolt, long enough to fit beaker, ¼" diameter recommended	Plunger device for dilution sampling, rod	1
	N	Semi-rigid or rigid waterproof material (e.g., vinyl laminate wall base moulding, polycarbonate), circular cut-out, with diameter ~1cm less than beaker diameter	Plunger device for dilution sampling, plunger base	1
	N	Wood Dowel, 12" by ¾" diameter, optional	Plunger device for dilution sampling, plunger handle	1
	N	Hex Nuts, ¼" (or diameter that fits threaded rod)	Plunger device for dilution sampling, fastening	4
	N	Desiccator	Keep oven-dried samples moisture free before weighing	1
Consumable Items				
	N	Distilled or filtered water (18.2 MOhm not required, lesser purity acceptable)	Suspend residual fraction for dilution method; avoid mineral build-up on weighing tins used for very light samples	As needed
	N	Desiccant	Keep oven-dried samples moisture free before weighing	As needed