TOS PROTOCOL AND PROCEDURE: SOIL PIT SAMPLING FOR PLANT BELOWGROUND BIOMASS

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Change Record

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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 crucial to improving our understanding of how terrestrial ecosystems respond to environmental changes.

The Plant Biomass and Productivity Science Design document (AD[06]) prescribes two approaches by which NEON will improve understanding of plant belowground biomass stocks. First, NEON Tower Plots are sampled for plant fine root biomass every 3-5 years to a maximum depth of 30 cm, and these samples are gathered either with relatively large diameter cores (5-10 cm diameter), or by cutting monoliths with a soil knife (with approximate surface dimensions of 10 cm [W] x 10 cm [L]) (RD[05]). Due to the inverse relationship between root diameter and turnover rate, these routinely collected root samples are sorted by size category (< 0.5 mm, 0.5–1 mm, 1–2 mm, and 2–10 mm)(Burton and Pregitzer 2008), and also by status (live, dead). Second, NEON has performed a one-time characterization of the distribution of plant fine root biomass with depth in one soil pit per site, excavated to a maximum depth of 200 cm, and located near the NEON Tower in the dominant NLCD vegetation class at the site. For soil pit fine root biomass sampling, roots are also sorted by size category (≤ 2 mm, 2–30 mm) and by status (live, dead).

In combination with the Belowground Biomass Core sampling conducted as part of ongoing NEON Operations (RD[05]), the soil pit sampling for plant belowground biomass described here enables researchers to estimate total plant belowground biomass stocks at significantly greater soil depths than would otherwise be possible. Total root biomass (g m^{-2}) at depths greater than 30 cm can be substantial, and root data generated by the NEON soil pit sampling effort is a novel and important dataset at the continental scale.

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

We are indebted to multiple people from numerous institutions for providing facilities and equipment needed to carry out the soil pit root biomass sampling work.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Manual
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 Performance QA/QC Plan
AD[06]	NEON.DOC.000914	NEON TOS Science Design for Plant Biomass, Productivity, and Leaf Area Index

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	TOS Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.014038	TOS Protocol and Procedure: Core Sampling for Plant Belowground Biomass
RD[06]	NEON.DOC.002134	Datasheets for TOS Protocol and Procedure: Soil Pit Sampling for Plant Belowground Biomass
RD[07]	NEON.DOC.003691	Ingest workbook for Root sampling (Megapit) data product
RD[08]	NEON.DOC.001307	TIS Soil Pit Sampling Protocol

2.3 Acronyms

Acronym	Definition
OM	Soil organic material

2.4 Definitions

Term	Definition
organic material	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.

3 METHOD

To characterize soil physical and chemical properties with depth (RD[08]), and root biomass stocks with depth (this document), NEON samples soil depth profiles at one location per site, typically within the dominant vegetation and soil type(s), and adjacent to the NEON Tower. Unlike most NEON TOS protocols, the protocol described in this document differs in that it is implemented only once during the site construction phase, and is not implemented by Field Operations during the operations phase of the Observatory.

In this document, we describe the procedure for collecting plant fine root biomass samples from NEON soil pits. Briefly, contractors are enlisted to excavate one 1.5 m (W) x 2 m (L) soil pit per site, to a maximum depth of 2 meters, using a small track hoe or equivalent. Three of the four soil pit faces are secured with shoring, to ensure safety, and the fourth 1.5 m (W) x 2 m (H) face remains exposed to enable collection of soil samples. At a subset of sites characterized by permafrost (Alaska sites), soils are sampled with a CIPRE auger, rather than by soil pit excavation (see Appendix B.1).

Within each soil pit, samples are collected from 3 profiles, with all profiles coming from one exposed face (**Figure 1**). Typically, 10 cm (W) x 10 cm (L) x 10 cm (H) monoilith samples are collected at 10 cm depth increments down to 100 cm depth. From 100 cm depth down to the final pit depth, 10 cm (W) x 10 cm (L) x 20 cm (H) monolith samples are collected, resulting in a maximum of 45 monolith samples per soil pit. Soil samples are collected from most depth increments with a soil knife. However, for depth increments near the surface, a corer may be used to collect samples perpendicular to the soil surface. During the method development phase in 2012, , the following methods were also utilized at the OSBS, HARV and CPER sites: 1) a corer was used to collect samples from the exposed face parallel to the soil surface (coring perpendicular to the face); and 2) samples were collected perpendicular to the face using a drill bit attachment. In all cases the sampling method is recorded in the `sampleMethod` field as part of the NEON Megapit Root Data Product (NEON.DP1.10066).

Soil samples are wet sieved to 2 mm to remove large roots and rocks, sieved to 250 µm to remove mineral soil, picked to separate roots from other organic material, and roots are then sorted to diameter size category (≤ 2 mm diameter, 2-30 mm) and status (live, dead). Picking and sorting roots is time consuming, and similar to other researchers, NEON employs 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, with the knowledge that fragments < 1 cm length may contribute meaningfully to total fine root biomass (Koteen and Baldocchi 2013). In contrast, root fragments < 1 cm length are quantified by the standard NEON Belowground Biomass Core protocol used in Operations (RD[05]). Sorted roots are then dried for a minimum of 48 h at 65 ˚C, and weighed to the nearest 0.0001 g using an analytical balance. Dried roots are then ground either with a mortar and pestle, or with a Wiley Mill (0.85 mm mesh), then analyzed for %C, %N, ^{13}C, and ^{15}N at the University of Wyoming Stable Isotope Facility. Residual root biomass not sent for analysis is archived at NEON for use by external members of the science community.

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

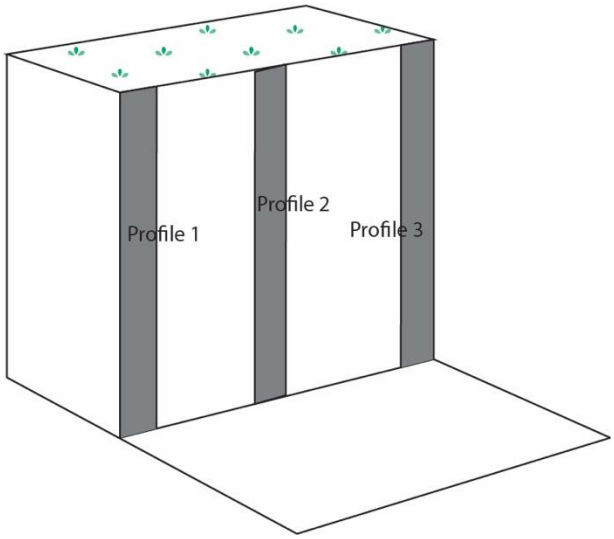

Figure 1. Illustration of the exposed face of a soil pit, showing where each of three vertical profiles are established for collection of soil samples by depth increment.

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Belowground fine root biomass sampling in soil pits is performed one time per site during the construction phase of the Observatory. For all soil pits, collection of samples from the pit is completed within 72 h.

4.2 Criteria for Determining Onset and Cessation of Sampling

The timing of soil pit excavation and sampling is tied to the NEON construction schedule, rather than biological cues: Broadly, northern latitude sites are sampled during the summer months, and southern latitude sites are sampled during the shoulder season and winter months. For Alaska sites, sampling is timed in the winter in order to minimize effects of transporting heavy CIPRE auger equipment on the landscape.

See the Megapit Root Data Product (NEON.DP1.10066) or NEON TOS Site Characterization Reports for exact sampling dates for a given site.

4.3 Timing for Laboratory Processing and Analysis

Following collection in the field, keep soil samples in cold storage prior to wet-sieving in the laboratory. Ideally, soil cores are processed in the laboratory within 24 h of collection in the field. However, it is acceptable to keep soil cores in cold storage (4-8 ˚C) for up to a maximum of 72 hours. Soil cores collected in Alaska during winter are an exception to this timing; if collected frozen, cores may be kept in cold storage (–20 ˚C) indefinitely prior to processing. Once laboratory processing is initiated on a given sample, processing should be carried all the way through without stopping.

4.4 Sampling Timing Contingencies

Table 1. Contingent decisions for Belowground Biomass Soilpit sampling, indicating how to respond to unanticipated delays in field or lab work, and the consequences of potential delays.

Delay/ Situation	Action	Outcome for Data Products
1-7 days	If delay prevents completion of soil pit sampling in the field: 1. Ensure all samples collected are labeled. 2. Place into cold storage (refrigerator or cooler with cold packs). 3. Resume and finish collecting samples ASAP. 4. If delay persists for > 1 day and the laboratory is nearby, begin sieving the available samples until it is possible to return to the field.	Increasing cold storage time > 72 h will lead to non-standard and unquantifiable mass loss from roots that continue to respire, resulting in greater uncertainty in fine root biomass estimates.

	If delay prevents completion of sieving: 1. Ensure all samples for which sieving is complete are labeled. 2. Place the samples into the drying oven as soon as possible. 3. Keep un-sieved samples in cold storage to minimize loss of biomass.	

4.5 Criteria for Permanent Reallocation of Sampling Within a Site

Not applicable to the Belowground Biomass Soilpit protocol.

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 Manual (AD[02]) and EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. When executing this protocol, the Lead Field Technician or Staff Scientist maintains primary authority to stop work activities based on unsafe field conditions; however, all employees maintain the responsibility and right to stop their work in unsafe conditions.

Specific safety considerations for Belowground Biomass Soil pit sampling in the field:

- Use trench shoring, or equivalent, to secure the 3 out of 4 pit faces not used for sampling, and prevent injury from collapse.
    - Trench Shoring or equivalent data specifications and installation shall meet or exceed requirements from Battelle Ecology NEON Safety Department.
- Constantly monitor the exposed face used for sampling for signs of collapse (cracks, shifting).
- Use a secured ladder, or equivalent, for pit ingress/egress.
- Monitor oxygen/gas concentrations in the pit continuously.
- Wear close-toed work boots, safety vest, safety glasses and a hard hat at all times when working in the pit.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 2. Equipment list – Collecting soil pit samples in the field.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	GPS, hand-held, recreational accuracy	Measure lat/long of pit location	All	1	N
	R	Waterproof fine-point pen, Sharpie or equivalent	Sample labeling	All	2	N
	R	Soil knife, hori-hori or equivalent	Cut soil sample monoliths	All	2	N
	R	Scissors	Pre-cutting all-weather paper for labels	All	1	N
	R	Pruning clippers	Cutting thick roots encountered during sampling	All	1	N
	R	Cooler, large capacity, 110 L approximate volume	Sample cold storage	All	2	N
	R	Re-usable cold packs	Sample cold storage	All	20	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Ruler, metric units, 1 cm resolution, 30 cm minimum total length	Measure dimensions of soil monolith samples	All	2	N
	R	Meter tape or measuring stick, 2 meter minimum total length	Measure sampling depth increment	All	1	N
	S	Mallet, rubber	Aids inserting soil knife with precision	Hard soils	1	N
	S	Rock hammer	Aids with excavation	Hard soils, rocks	1	N
	S	Shovel	Aids with excavation	All	1	N
	R	Plastic trays, cement mixing style or equivalent, 18” (W) x 34” (L) x 6” (D) approximate dimensions, preferably light colored	Capture totality of monolith sample while sampling from face; same trays used for lab work (see table below)	All	2	N
Consumable items						
	R	Plastic freezer bags, gallon size, Ziploc or equivalent	Cold storage of soil samples prior to laboratory processing	All	60	N
	R	All-weather paper, pre-cut to 2” x 3” rectangles	Sample labeling	All	60	N
RD[06]	R	Datasheets	Recording field sampling data	All	2	N

R/S=Required/Suggested

Table 3. Equipment list – Processing soil pit samples in the laboratory.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	Plastic trays, cement mixing style or equivalent, 18" (W) x 34" (L) x 6" (D) approximate dimensions, preferably light colored	Aids in separating roots from mineral soil; dark roots show up clearly against light plastic background	All	2	N
	R	Water source with spray nozzle	Wet-sieving to separate roots from mineral soil	All	1	N
	R	Sieve, 2 mm mesh, 8 inch minimum diameter	Wet-sieving to separate roots from mineral soil	All	2	N
	S	Sieve, 250 µm mesh, 8 inch minimum diameter	Wet-sieving to separate roots from mineral soil	All	2	N
	R	Sieve pans, diameter to match sieve	Wet-sieving to separate roots from mineral soil	All	2	N
	R	Forceps, fine point	Separating roots from organic material, separating different root size categories	All	2	N
	R	Wire gauge, with 2 mm gap (12-gauge)	Sort roots into size classes during sieving and picking	All	1	N
	S	Plastic bucket, 5 gallon capacity	Soak soil monolith samples prior to sieving	Hard, dry soils	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Wiley Mill, with 0.85 mm mesh screen attachment	Grind weighed samples or chemical analysis	All	1	N
	R	Beaker, 250 mL, glass	Collect ground sample from Wiley Mill	All	2	N
Consumable items						
RD[06]	R	Datasheets	Recording lab root mass data	All	2	N
	R	Coin envelopes, 3” x 6” approximate dimensions	Storing root samples during drying	All	180	N
	S	Paper bags, 11” x 5” x 3” approximate dimensions, lunch sack style or equivalent	Grouping coin envelopes during drying	All	6	N
	R	Plastic weigh boats, small	Weighing samples	All	6	N
	R	Plastic scintillation vials, 25 mL	Storing and shipping dried, ground samples for chemical analysis	All	Up to 180 per pit	N
	R	Gloves, latex or nitrile	Prevent contamination of sample while weighing and grinding	All	12	N

R/S=Required/Suggested

6.2 Training Requirements

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

All NEON staff working at a soil pit must undergo the NEON Safety Department's Excavation & Trenching Awareness and Safety training (in accordance with Section 40, Excavation Safety, EHSS Policy, Program and Management Plan, AD[01]).

At least one NEON staff member working at a soil pit must have training certification from a Trench Shoring Services Safety in Competent Person Excavation Course as promulgated by 29 CFR Part 1926 Occupational Safety & Health Administration (OSHA).

For both the field and laboratory work, training must emphasize the importance of consistent, detailed labeling of all samples. ***Improper or inconsistent labeling is the most common and problematic error associated with this work!***

6.3 Specialized Skills

Demonstrated ability to distinguish between live and dead roots.

6.4 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.

An experienced two-person team will require between 1-3 days to complete soil monolith sample collection in the field, depending on soil conditions (e.g., height of water table, soil hardness, etc.). Lab work will require between 2-4 days per soil pit, with longer times required for sites with substantial O horizons.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. At least 30 days prior to arriving at the site for sampling, make sure that all consumables required for this field work are available for use (**Table 2** and **Table 3**).
    a. Re-order items as necessary prior to field work.
2. Coordinate lab space availability for wet-sieving and drying oven use with NEON Domain Manager or external facility. If using an external facility, requirements are:
    a. Space for sieving.
    b. Water source (indoor sink or outdoor faucet with hose attachment).
    c. Waste facility or outside location where soil separated from roots can be disposed.
    d. Drying oven that can maintain 65 ˚C for a minimum of 48 h, with capacity for up to 6 standard lunch bags containing root samples.
3. Coordinate with NEON Domain Manager or external facility manager to ship dried root samples back to NEON HQ after drying is complete.
    a. Generate pre-paid shipping labels, boxes, etc.
4. At least 14 days prior to field work, gather all field and laboratory supplies (**Table 2** and **Table 3**), and ship to the Domain Support facility, external facility, or hotel where staff are staying while completing the work. Ensure that:
    a. Batteries are charged on the gas monitor.
    b. Data sheets are printed on all-weather paper, and organized in a notebook or binder.
    c. All-weather paper has been cut into small approximately 2” x 3” pieces that can be labeled and put into re-closable plastic bags with soil samples.
    d. Soil core assembly is clean and in good working condition (if using).
    e. A 10 cm length is clearly marked on the soil core tube, measured from the bottom of the bit, not the bottom of the soil core tube.
        i. Use electrical tape to wrap the soil core tube and mark the correct depth for sampling.

SOP B Field Sampling

Once the soil pit has been excavated, the exposed sampling face has approximate dimensions of 1.5 m (W) x 2.0 m (H). Three sampling profiles, running from the soil surface to the final pit depth, are established on this face, resulting in approximately 0.75 m between each profile (**Figure 2**).

- Spacing of profiles may be micro-adjusted in order to accommodate simultaneous collection of soil blocks from the face that are needed to calibrate TIS soil sensors.

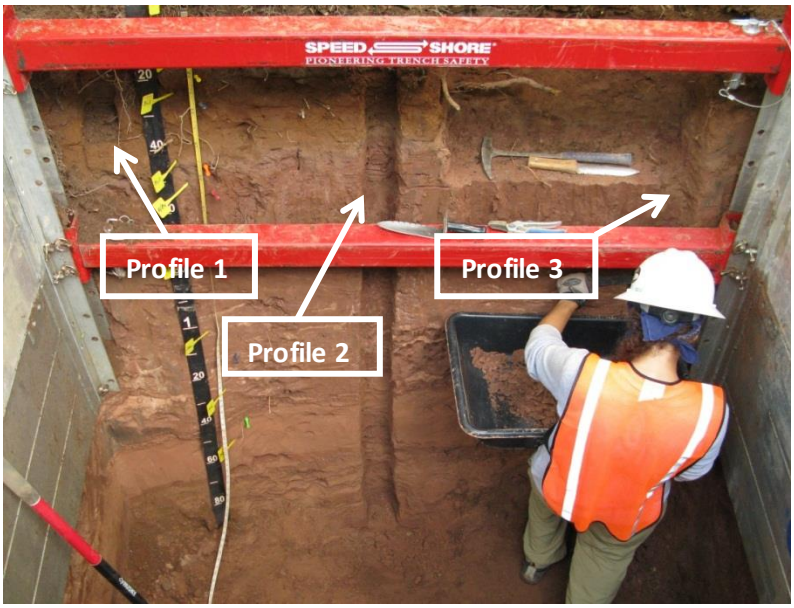

Figure 2. The exposed sampling face of a soil pit, showing: Profile 1 sampled to 30 cm depth (*left*), Profile 2 sampled to maximum pit depth (*center*), and Profile 3 sampled to approximately 100 cm depth (*right*). A large soil monolith used to calibrate TIS sensors was extracted from the face between Profile 2 and Profile 3.

1. Record pit-level sampling metadata:
    a. **siteID**: e.g., *ABBY*
    b. **samplingProtocolVersion**: The current version of the protocol.
    c. **latitude** and **longitude**: Use recreational grade GPS unit, and record in decimal degrees to nearest 0.001
    d. **measuredBy**: Person collecting samples.
    e. **recordedBy**: Person recording sampling metadata.

To sample each depth profile:

2. Place a meter tape or measuring stick along the entire depth of the sampling face in order to accurately determine required sampling depth increments.
3. At the surface of the profile, use pruners/clippers to clip and remove vegetation rooted into the top surface of the profile. Trim vegetation until it is flush with the soil surface.
4. Pre-label 2" x 3" pieces of all-weather paper with required information:
    a. **siteID**: e.g., *ABBY*
    b. **pitProfileID**: 1 = left, 2 = center, 3 = right, when standing looking at the sampling face.
    c. **depthIncrement**: use '**topDepth – bottomDepth'** notation, e.g., *10–20 cm,* where:
        i. **topDepth**: Depth from the soil surface to the top of the sample to be cut (cm).
        ii. **bottomDepth**: Depth from the soil surface to the bottom of the sample to be cut (cm).
5. With the soil surface defined as depth = 0 cm, use the soil knife and begin to collect 10 cm (W) x 10 cm (L) x 10 cm (H) monoliths at 10 cm depth increments ***down to 100 cm depth.***
    a. Use the shovel or soil knife to make the sampling face as vertical and flat as possible. This will minimize uncertainty in the calculated sample volume.
    b. Use a soil knife and a ruler to measure and cut soil sample monoliths as precisely as possible from the sampling face.
        i. A soil corer, oriented perpendicularly to the soil surface, may be used instead for depth increments near the soil surface. If using a soil corer, collect 3 core samples per depth increment, and sum the total length to generate the **sampleHeight** needed in step (7).
    c. Use a rubber mallet to drive the soil knife (if necessary).
    d. Place a plastic tray directly underneath where the sample is cut, and carefully collect the entirety of the soil from the sampled volume into the tray (see **Figure 2**).
    e. Transfer the soil sample from the tray to a re-sealable gallon freezer bag, place the corresponding label in the bag, and place the bag in cold storage (i.e., cooler with cold packs).
6. Use a ruler to measure the exact dimensions of the volume from which the soil monolith sample was collected (L x W x H). Do NOT measure the monolith itself.
    a. If the shape of the volume from which the sample was extracted is not made from perfect right angles, make multiple measurements and calculate an average.
    b. Skip this step if a soil corer was used to collect the sample.

7. Record required soil sample information on the field datasheet:
    a. **date**: *YYYY-MM-DD* format.
    b. **pitProfileID**: Defined as in step (4).
    c. **sampleMethod**: Enter either **SK** (soil knife) or **BDC** (bulk density corer).
    d. **topDepth**: Defined as in step (4).
    e. **bottomDepth**: Defined as in step (4)
    f. **depthIncrementID**: An incrementing identifier for the depth increment within a profile; *e.g., 1, 2, 3*, etc. with '1' being the depthIncrement nearest the soil surface.
    g. **sampleHeight**: The vertical dimension of the soil sample, which should be approximately 10 cm for samples collected above 100 cm pit depth, nearest 0.1 cm.
    h. **sampleArea**:
        i. For samples collected with **sampleMethod = SK**, record the average dimensions of the top and bottom surface of the sample (L x W), nearest 0.1 cm; e.g., *10.1 x 9.8 cm.* These data are used to automatically calculate the sample volume.
        ii. For samples collected with **sampleMethod = BDC**, record the area of the soil core aperture. For example, a soil corer with 5 cm inside diameter → 19.63 cm^2.
    i. **remarks**: Free-form remarks about sample collection germane to end-users.
8. Repeat steps (6) – (8) for all depth increments from the surface down to 100 cm depth.
9. ***Below 100 cm depth,*** use the soil knife to collect 10 cm (W) x 10 cm (L) x 20 cm (H) monoliths at 20 cm depth increments, down to the deepest extent of the pit (maximum 200 cm).
    a. Repeat steps (6) – (8) for all depth increments between 100 cm depth and the bottom of the pit.
10. Continue sampling Profiles 2 and 3 as described above. Sampling may span more than one day if necessary.
11. Maintain all samples in cold storage until they can be wet-sieved in the laboratory. Cold storage prevents mass loss due to respiration, and decomposition of very fine root structures.

B.1 Troubleshooting

Table 4. Common problems encountered during soil pit sample collection, and resolution.

Problem	Resolution
One or more rocks is/are entirely contained within the sample volume.	Accept the rock as part of the sample. The rock is part of the representative sample volume.

Problem	Resolution
One or more rocks is/are part of the sample volume, but extend into the soil outside of the sample volume.	• Sample the soil from around the rock within the prescribed sample volume. • The recorded **sampleArea** and **sampleHeight** should include the portion of the rock that was part of the sample.
A rock is large enough that the entire intended sample volume would be rock.	Choose one of two options: • Micro-adjust the sampling volume to the right or left of the existing profile up to 10 cm, and sample as normal. • If the previous option will not yield a sample, record '**targetTaxaPresent = N,** no roots due to rock' in the **remarks** field.

B.2 Developmental Methods

Three pits were sampled during the method development phase with methods that differed from those described above (pits excavated at OSBS, HARV, and CPER sites).

Soil corer method at depth:

1. The corer is oriented perpendicular to the sampling face, and placed so that it is vertically centered within the given depth increment.
2. Drive the corer into the sampling face to collect a 30 cm length sample (if possible).
    a. A rubber mallet may be used to drive the corer into the sampling face. Be aware that this may cause the sampling face to collapse.
    b. If the corer cannot be inserted 30 cm into the sampling face, multiple samples may be sampled and pooled. In this case, record the sum of the length of all cores sampled in the **sampleHeight** field.
3. Record required sampling information, and place the sample in cold storage, as above.

Drillbit method at depth:

At the CPER site, a circular drill bit was used to collect soil similar to the soil corer at depth method. The drill bit utilized is typically employed to cut a hole in a door for a door latch assembly, and creates a small cylindrical sample of approximately 7 cm length. Multiple samples were pooled per depth increment, as described for the soil corer method at depth.

SOP C Laboratory Processing

The objective of the laboratory processing procedure is to separate roots from mineral and organic soil particles, sort roots to sizeCategory (≤ 2 mm, 2–30 mm diameter) and rootStatus (live, dead), and obtain dry mass values for sampled roots. The data produced via this procedure are sufficient to calculate fine root density per depth increment (mg cm^{-3}), and fine root mass per unit area per depth increment (g m^{-2}), as reported in the NEON Megapit Roots data product.

C.1 Wet-Sieving and Drying

Processing steps for a soil monolith sample:

1. Remove samples from cold storage one at a time for processing via the wet-sieving procedure.
    - Wear gloves to prevent contaminating root samples that will later be analyzed for C and N isotopes.
2. For hard, dry and/or clay rich soils:
    a. Place the sample in a 5 gallon plastic bucket and cover with water.
    b. Plan ahead and soak for a minimum of 1 h, and up to 12 h (i.e., overnight) prior to sieving, in order to facilitate breaking up soil particles without damaging roots.
    c. After soaking, gently massage the soil sample to help separate roots from mineral soil.
    d. Clip the sample label to the side of the bucket to track critical sample information.
3. Pre-label four coin envelopes with the following sample information:
    a. **siteID**: e.g., *ABBY*
    b. **pitProfileID**: 1 = left, 2 = center, 3 = right
    c. **date**: use *YYYY-MM-DD* format
    d. **topDepth** and **bottomDepth**: e.g., *10-20 cm*
    e. **sizeCategory**: choose either *'≤ 2 mm'* or *'2 – 30 mm'*
    f. **rootStatus**: choose either '*live*' or '*dead*'
4. Remove rocks and portions of roots > 30 mm diameter from the sample.
5. Pass the sample through the 2 mm sieve, and then the 250 µm sieve. A 1 mm mesh sieve may be more appropriate than a 2 mm sieve, depending on soil type and root diameter. Sieve a portion of the sample at a time, until the entire sample is processed.
    a. Place the 2 mm sieve over a large plastic tray (light colored is best), and while spraying gently with water, apply gentle manual pressure to break up soil particles and wash mineral soil through the sieve. Most roots, and some soil organic material, will be retained on the sieve.

b. Let mineral soil in the tray settle, then decant small roots and organic material that passed through the 2 mm sieve through the 250 µm sieve. Set the 250 µm sieve aside.

   i. If there is a considerable amount of mineral soil that may be masking small, fine roots, you may decant through the 250 µm sieve multiple times, using both plastic trays.

c. Use forceps and the wire gauge to pick and sort roots from the surface of the 2 mm sieve. Place sorted roots into their respective labeled coin envelopes from step (3).

   i. Ignore root fragments < 1 cm total length, regardless of diameter.

   ii. Live roots may be distinguished from dead roots on the basis of color and friability. Dead roots are often darker in color (brown or black) and brittle, whereas live roots are lighter and can typically be bent into a 'U' shape without breaking. If status for a given root is unclear, classify as **rootStatus = live**.

   iii. For larger roots that may have more than one sizeCategory due to smaller branching roots, use clippers or scissors to separate at the branch point.

   iv. Do not separate individual roots with a taper into different sizeCategories. For tapering roots, measure diameter at the largest point on the root.

a. Use forceps and the wire gauge to pick and sort roots from the surface of the 250 µm sieve, and from the surface of the water in the plastic tray. Use the same sorting guidelines as in (c) above.

6. Once all roots are picked and sorted from the sample, dispose of mineral and organic soil and water effluent per site host requirements and USDA regulations.

7. Thoroughly clean sieves and plastic trays with water between samples.

8. Coin envelopes with roots may be organized in large brown paper bags (lunch sack type, or equivalent) before being placed in the drying oven.

9. Proceed to the next sample, and return to step (1).

10. At the end of each day's sorting, place samples in a 65 ˚C drying oven for a minimum of 48 h.

    a. Wet freshly sorted and picked root samples may be returned to cold storage for up to 72 h post-field collection if it is not possible to place directly into a drying oven.

    b. To avoid extending travel to wait for samples to dry, make arrangements with the laboratory facility manager to ship roots back to NEON HQ using pre-paid labels (see SOP E for shipping requirements).

C.2 Weighing and Grinding

It is assumed that sample weighing and grinding takes place at the NEON HQ facility, and that dried roots have been shipped according to SOP E.

1. Carefully remove dried root mass from the coin envelope, and weigh with an analytical balance.
    - Wear gloves to prevent contaminating root samples that will later be analyzed for C and N isotopes.
2. Record required sample information:
    a. **siteID**: e.g., *ABBY*
    b. **date**: *YYYY-MM-DD* format
    c. **pitProfileID**: 1 = left, 2 = center, 3 = right
    d. **depthIncrementID**: An incrementing identifier for the depth increment within a profile; *e.g., 1, 2, 3*, etc. with '1' being the depthIncrement nearest the soil surface.
    e. **topDepth**: e.g., *10 cm*
    f. **rootStatus**: either *live* or *dead*
    g. **sizeCategory**: choose from '*≤ 2 mm*' or '*2 – 30 mm*'
    h. **rootDryMass**: Record to the nearest 0.0001 g
3. Return the sample to its labeled coin envelope to await grinding for chemical analysis, and store in a cool, dry location.
4. Grind sorted samples one at a time in with a Wiley Mill (0.85 mm mesh) for chemical analysis. Wear latex or nitrile gloves while grinding samples. Do NOT grind samples < 0.0200 g mass; the analytical laboratory will grind these samples.
    a. Pre-label 20 mL plastic scintillation vials with the **sampleID**.
        i. sampleID = siteID.pitProfileID.topDepth.rootStatus.sizeCategory (e.g., '*ABBY.1.20.live.0-2*' or '*BART.3.120.dead.2-30*')
    b. Pass sample through the Wiley Mill and collect ground sample into a clean 250 mL glass beaker, then transfer to the appropriately labeled scint vial, and seal.
    c. Clean the beaker and the Wiley Mill with compressed air between samples.
5. Collect ground samples in the tray in which the scint vials were originally packaged, and store in a cool, dry place until samples are shipped to the analytical laboratory according to SOP E.

C.3 Equipment Maintenance

1. Analytical balances should be calibrated with a standard calibration weight set:
    a. After initial installation.
    b. Any time the balance is moved.
    c. Every 6 months or if you suspect readings are inaccurate for any reason.

SOP D Data Entry and Verification

Data are recorded manually into field and lab data sheets for the Belowground Biomass Soilpit protocol. These data must be transcribed into 3 tables in order to be ingested into the NEON Cyberinfrastructure:

- **mpr_perpitprofile_in**: Data collected and calculated per megapit sampling profile.
- **mpr_perdepthincrement_in**: Data collected and calculated per depth increment per profile.
- **mpr_perrootsample_in**: Data collected and calculated per root sample per depth increment per profile.

Table structure is described as part of the Megapit Root ingest workbook RD[07].

D.1 Field Data

1. Transcribe Field Datasheets within 14 days of collection.
2. Field data are transcribed into the **mpr_perpitprofile_in** and **mpr_perdepthincrement_in** ingest tables.
    a. Consult RD[07] to determine appropriate values and formats for each field.
3. Once all data have been collected and transcribed for a given soil pit, save only the data from this most recent pit to a .csv file for ingest by NEON CYI.

D.2 Lab Data

1. Transcribe Lab Datasheets within 14 days of collection.
2. Lab data are transcribed into the **mpr_perrootsample_in** ingest table.
    a. Consult RD[07] to determine appropriate values and formats for each field.
3. Once all data have been collected and transcribed for a given soil pit, save only the data from this most recent pit to a .csv file for ingest by NEON CYI.

SOP E Sample Shipment

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

E.1 Handling Hazardous Material

Not applicable.

E.2 Supplies/Containers

For shipping dried roots from site to HQ:

- NEON technicians performing the sampling work should provide their laboratory contact with a shipping box (if desired), a pre-paid shipping label, and a copy of the USDA letter authorizing NEON to ship over-dried plant material.
- The pre-paid shipping label can be purchased using the NEON account through UPS or FedEx, according to whichever is most convenient for the laboratory contact.
- Place dried roots, contained in labeled coin envelopes inside paper lunch bags, into the shipping box. Use newspaper, packing paper, or equivalent to prevent sample movement within the box.

For shipping ground samples from HQ to the Analytical Laboratory:

- Ground samples stored in sealed scint vials and arranged in original flats used to ship vials may be stacked into boxes with dimension 18" (W) x 18" (L) x 18" (H).
- Use packing paper or equivalent to ensure flats of vials cannot tip and spill sealed vials into the box.

E.3 Timelines

Dried, ground samples can be stored indefinitely in dry conditions at room temperature before being shipped to the external Analytical Laboratory.

E.4 Conditions

Samples may be shipped at room temperature.

E.5 Grouping/Splitting Samples

Group and ship samples from the same soil pit for analysis at the external Analytical Laboratory.

E.6 Return of Materials or Containers

Not applicable.

E.7 Shipping Inventory

Prepare an electronic and paper inventory of all sampleIDs included in each shipment to the Analytical Laboratory. Send a paper copy with the physical samples.

E.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

Burton, A. J., and K. S. Pregitzer. 2008. Measuring forest floor, mineral soil, and root carbon stocks. Pages 129-142 *in* C. M. Hoover, editor. Field measurements for forest carbon monitoring. Springer-Verlag, New York.

Koteen, L., and D. Baldocchi. 2013. A randomization method for efficiently and accurately processing fine roots, and separating them from debris, in the laboratory. Plant and Soil **363**:383-398.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 5. Datasheets associated with this protocol.

NEON Doc. #	Title
NEON.DOC.002134	Datasheets for TOS Protocol and Procedure: Soil Pit Sampling for Plant Belowground Biomass

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

APPENDIX B SITE SPECIFIC MODIFICATIONS

B.1 Domains 18/19 (BARR, TOOL, BONA, DEJU, HEAL)

Modifications to the standard soil pit excavation approach are required for sites in Domains 18 and 19 (Alaska), due to the fact that soils are characterized by permafrost layers, and vegetation is highly sensitive to disturbance. To mitigate the impact of sampling, cores are extracted during the winter months using a sled-mounted CIPRE auger, rather than soil pit excavation.

1. Extract a minimum of three large-diameter cores per site using a CIPRE auger.
2. Record the **sampleArea**, based on the cross-sectional area of the auger.
3. Record the total length of the core, this is the equivalent of the total pit depth.
4. Store cores in –20 ˚C cold storage until they are processed for fine root biomass.
5. Working from the end of the core that was the soil surface, cut 10 cm increments down to a total length of 100 cm, and process each increment via the wet-sieving method (SOP C.1).
6. From the 100 cm mark on the core (relative to what was the soil surface), cut 20 cm increments down to the final length of the core, and process via the wet-sieving method.
7. Dry, weigh, and grind roots as in SOP C.2, and record all required information in the 'Lab Datasheet'.