TOS PROTOCOL AND PROCEDURE: SLS – SOIL BIOGEOCHEMICAL AND MICROBIAL SAMPLING

PREPARED BY	ORGANIZATION	DATE
Samantha Weintraub-Leff	SCI	02/08/2024
Lee Stanish	SCI	06/01/2020
Eve-Lyn S. Hinckley	SCI	12/31/2014
Jacob Parnell	SCI	12/31/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	02/08/2024

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	CM	02/08/2024

See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/03/2011	ECO-00280	Initial draft release
B_DRAFT	01/13/2014	ECO-01140	Draft release. Will be finalized in next rev.
C	03/25/2014	ECO-01670	Production release, template change, and other changes as detailed in Appendix C
D	09/15/2014	ECO-02086	Minor updates to SOP B (Field Sampling) and SOP C (Lab Processing)
E	09/22/2014	ECO-02296	Migration to new protocol template
F	02/23/2015	ECO-02538	• Changed title to reflect that protocol describes all soil biogeochemistry tasks • Improved organization of task parameters to promote clarity. • Added modules on sampling soils in the field and lab processing for N transformations. • Updated description of coring device specifications (JIRA ticket FOPS-1310, FOPS-1376, FOPS-1442, and FOPS-1501) because slide hammer corer is not useful in most domains. • "Composite" cores are no longer being collected; a targeted mineral soil sample volume is described, and individual domains are to collect the number of cores required to get that volume, given the coring device they are using. • Removed field and lab SOPs for sampling bulk density (JIRA ticket FOPS-1310). • Added contingency info for inundated plot conditions. • Updated soil pH SOP to reflect that mixing is okay if it is necessary (JIRA ticket FOPS-1374 and FOPS-1406). • Updated sampleID format to plotID_horizon_coreCoordinateX_coreCoordinateY_date (JIRA ticket FOPS-1067). • Separated SOPs for microbial sampling only and biogeochemistry/stable isotopes/microbial sampling (field and lab processing) in order to reduce confusion regarding what field staff should do for each type of effort. This action was in response to FOPs' end-of-season discussion with NEON staff scientists. • Updated soil microbial sampling frequency to three times per year and outlined timing in Table 1.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• Changed number of plots sampled at each site from four to eight. • Added sampling for microbial biomass to SOP B and SOP C, and created shipping instructions in SOP K; samples for microbial molecular and biomass analyses are now distinguished throughout. • Added in references for microbial biomass protocol. • Changed sample containers for microbial molecular analysis to whirlpaks rather than 50 mL vials. • Specified that during microbes only sampling bouts, only top horizon is sampled. • Updated timing of sampling in Appendix E to include domains 18-20.
G	1/29/2016	ECO-03071	• Specified timing for coordinated sampling for microbial biomass and soil N transformations. • Modified number of plots sampled for soil biogeochemistry from 10-15 to 10-12, to match science design. • Modified number of plots sampled for soil microbes from 8 to 10-12, to align with proposed change in Science Design, which matches microbial sampling spatial extent to BGC sampling extent. • Added distilled water as acceptable for rinsing instruments • Ensured all SOP's were numbered correctly: SOP K renumbered as SOP J • Removed Table 13, which was redundant with Table 17 (now Table 16). Formatted Table Captions to be consistent. • Removed redundant Table of Contents for Figure Captions. • Added in a recommendation for domain staff to designate a 30-day sampling period to avoid sampling outside of the acceptable window of July 1-Aug 31. • Table 5: Added MX number for optional spring scale to be used for weighing soils in the field. • Tables 7 and 9: Updated MX number for scintillation vials from HDPE to glass • Section 4.1: To match a change in the Science Design, updated number of plots for microbial sampling to match number of plots for BGC sampling. • Added to SOP A instructions to print x, y coordinates. • Added to SOP B soil masses for samples where needed. • Added a new SOP, SOP K, Soil Depth Survey Protocol. • Added ethanol wipes to consumable equipment list in Table 5 and link to example product. • Added section 7.1: How much soil to collect, to guide use of soil masses rather than soil volumes for sites that need it.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• Appendix C: Updated checklist for collecting quality soil samples to include cleaning equipment with ethanol wipes. • Appendix D: changed reminder that gloves can be re-used if properly sterilized. • Updated Appendix F – site specific information, with sampling modifications for GUAN. • Removed redundant table for lab processing of soils for N transformation. Updated remaining table (Now Table 11). • Added new table (Table 1) describing the target timing of coordinated soil measurements. • Modified Table 5 (previously 4) to become a general field equipment list to remove redundant information in more specific equipment lists in Tables 6 (formerly 5) and 7 (formerly 6). • Revised Figure 2: workflow, to reflect recent protocol updates and increase readability. • Added sections to SOP B and SOP F describing how to assess suitability of plot coordinates for sampling.
H	03/15/2017	ECO-04372	• Section 2.4: Added definitions for soil horizons • Section 4: Clarified descriptions for sample timing (4.1 and 4.2) and lab analysis timing (4.3) • Added Section 4.5, Plot Reallocation instructions • Clarified Table 2, characteristics associated with sample timing • Removed Table 3, onset and cessation of sampling for N transformations. Timing is consolidated with microbial/BGC sampling. • Added generalized figure demonstrating biologically relevant sample timing windows (Figure 1) • Table 4 (now Table 3): Updated sample contingency table • Created new Section 5.1 for plant protection and quarantine guidelines • Revised Table 7 (now Table 6): Field sampling equipment for N transformations • Revised Table 7 (formerly Table 8): Lab processing for soil moisture • Revised Table 8 (formerly Table 9): Sieving, air-drying and processing for BGC and archiving • Revised Table 9 (formerly Table 10): Equipment for pH measurement • Revised Table 10 (formerly Table 11): Lab processing of N transformation samples • Removed redundant Table 14: Shipping soils for BGC/isotopes

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• Revised Table 13 (formerly Table 15) to be shipping equipment list for microbial biomass samples • Removed redundant Table 16: Shipping equipment list for microbial biomass samples • Removed redundant Table 17: Shipping KCl extracts • SOP B: Modifications to microbial subsampling text and labeling instructions. Included instructions for plot-level pooling for metagenomics samples in the field • SOP C: Minor text modifications • SOP D: Added instructions for sieving difficult soils (D.1); Added details for archiving soil (D.3) • SOP E: Removed instructions to measure duplicates for pH • SOP F: Major revisions to field sampling for N transformations • SOP J: Minor reorganization of shipping instructions • Appendix B: Revised analysis checklist to match bout types (Table 15) and added new checklist describing analyses that are performed when N transformation sampling occurs (Table 16) • Appendix E: Added site-specific sampling windows • Appendix E.3: Added Table 18, site-specific sampling devices
J	04/07/2017	ECO-04602	• Section 4: Added clarification on scheduling N transformation incubations in relation to sampling windows; Added information regarding soil temperature requirements/holding times for microbial biomass samples • Section 5: Added safety tips for cutting PVC with a hacksaw • Table 6: Clarified incubation cylinder types • Table 10: Added shaker table information • Table 13: Removed, microbial biomass shipping equipment list now in Table 12. • SOP F: Updated instructions for scheduling field and lab work; revised Figure 4 caption • SOP G: Minor changes to filtering and storage instructions; added instruction for use of shaker table • SOP J: Removed J.3, shipping instructions for refrigerated microbial samples: samples ship frozen. • Appendix E.1: Revised table caption to describe the site-specific duration of N transformation incubations; updated T2 sampling window for STER; removed T1 and T2 sampling windows for BONA; Increased length of T2 sampling window for D04.

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
K	01/19/2018	ECO-05310	• Throughout SOP's: Added language for using barcode labels; inserted additional reminders to remove all rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris from unsieved samples • Section 4: Added sampling completeness criteria • Improved organization of equipment tables (Tables 5-10) • Table 10: Added Type I ultra-pure deionized water for KCl extractions, specified brand preference for KCl powder • Added new Table 13, Estimated time required for sampling • Figure 3: Broke out lab workflow based on bout type. • SOP A.1: revised generic language for mobile data entry • Revised microbial biomass sampling, processing, storage and shipment in SOP B, SOP D, and SOP J • SOP B: Revised container type for genetic archive samples • SOP F: Added instruction for incubationCondition field, how to record lost or destroyed incubated samples • SOP G: Added requirement to use Type I ultra-pure deionized water for KCl solution and final rinse, changed labeling convention for blanks, removed instruction to use soap during filtration equipment cleaning • SOP I: Added information about data QA steps in the Data Management Plan • SOP J: Revised instruction for packaging oven-dried bgc samples, added information about shipping applications • Appendix B: Improved quick reference checklists • Appendix E: Updated site-specific soil sampling devices, updated E.3 to provide sampling guidance for D18/19 • Added Appendix E.5: Site-specific instructions for quarantined sites • Overall document changes: Minor text clarifications.
L	03/11/2019	ECO-05980	• Revised timing of soil sampling windows based on 2005-2015 dataset of remote sensing and precipitation • Throughout protocol: harmonized references to sample types and bout types across Protocol and data entry applications; Added new table, figures and informational photos to increase protocol clarity • Section 2.4: Added definitions for Fulcrum and ServiceNow • Section 5: Added instruction to use NEON.DOC.001716 for safe sampling in areas with *Toxicodendron* spp • Tables 6 and 9: corrected error in label description • Tables 6, 7, 10, 11, 12: updated equipment lists, including suppliers and part numbers to be used for various supplies

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• SOP A: Re-organized and clarified language on use of barcode labels; moved instructions for coordinated bout (N-trans) sample prep into SOP A • SOP B: Updated formatting to reduce number of nested levels in lists • SOP C: Added contingencies for processing samples for soil moisture analysis • SOP E: Modified pH sample method to be more consistent with Robertson et al, 1999 SOP; added additional guidance for highly absorbent organic horizon samples • SOP F: Provided min and max distance for initial and final cores; added guidance to install final cylinder first if using normal coring device for initial sample (to make depths comparable); gave instruction to discard final sample if insects or animals nested in the core • SOP G: Reduced time requirements for hand-shaking and settling during extractions • SOP J: Added instruction to use cryogenic freezer boxes for shipment of microbial genetic archive samples • Appendix A: Removed non-standard bout types from reference checklists, added figures to display sample types. • Appendix E: Added note for DSNY to use wetland approach for N-transformation sampling; Generalized to 'peatland and permafrost' soil sampling guidance to encompass other sites outside AK; Updated tables for sample timing and site-specific soil sampling devices; created table of site-specific prevalence of organic horizons from NRCS initial characterization • Appendix F: added SOP for using randomized soil coordinate and subplot lists.
M	01/13/2020	ECO-06289	• Migrated to new protocol template (NEON.DOC.050006vJ) • Section 4.2: Clarified language on timing and duration of bouts • Section 4.4: Revised Contingencies table • Section 4.5: Added instructions on handling missed or incomplete bouts and field data quality issues • Section 7: Revised SOP overview workflow diagrams • SOP A: Moved preparation of containers for lab work to this SOP. • SOP A.2: Added instructions on making pre-sterilized ethanol for microbial sampling • Field and Lab SOPs: added instructions for how to collect and process samples in areas with heavy *Toxicodendron spp*

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• SOP B: Increased frequency of microbial biomass sampling at core sites to include sampling during off-year bouts. Revised SOP A, SOP B, Figure 5, SOP J, and Appendix A accordingly. • SOP B.1: Modified instructions for navigating to sample locations in plot • SOP C: replaces previous SOP F, field sampling for coordinated bouts. Added requirement to install the incubated core before taking T-initial sample. • Lab SOPs: Reorganized and split into smaller SOPs for discrete tasks. Added instructions on creating records when sample is lost and not analyzed • SOP G: Modified instructions for storing excess air-dried soil once all laboratory analyses are complete • SOP H: Modified pH method from LTER method in Robertson 1999 to NRCS method: only one soil sample used to measure water and $CaCl_2$ pH. No effect on data. • SOP J.1: Microbial biomass subsampling. Clarified instructions for subsampling organic horizons; added instructions for recording vial and soil masses • SOP J.3: Added instruction to record mass of BGC archive sample • SOP K: Provided more detail for equipment cleaning and storage; added new workflow to pre-leach filters with KCl using Buchner funnel; added guidance for order of blank extractions; added instruction to re-filter if solutions contain soil material • SOP L.1: Added requirements for timing of data QC to occur before sample shipment; Added horizonDetails field and instructions on entering data for it • Removed Shipping SOP: shipping information now located in separate Shipping document, NEON.DOC.005224 • Appendix A: Added quick reference for field samples collected at Gradient sites during off-year sampling • Appendix D: Added current list of USDA quarantined sites; provided additional guidance for alpine sites with variable onset of spring; added list of sites authorized to use the Wetland SOP • Appendix E: Updated specifications to soil temperature probe and sterile filters; changed table format • Minor text clarifications throughout.
N	06/19/2020	ECO-06464	• SOP A, SOP D, Table 19: Added instructions and equipment for temperature probe calibration and verification

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			• SOPs B and C: Added instruction not to sample within 2 m buffer area around litter traps in Tower plots • SOP L: Removed timelines for manual data transcription, instead referring to the Data Management protocol • Table 12: Updated boutType when sampling Core sites during Off-year bouts • Table 15: Updated sampling windows for SRER to accommodate variability in the monsoon • Table 25: Updated Potassium Chloride (KCl) part number to an ultrapure type with low nitrite/nitrate.
O	02/01/2022	ECO-06699	• Migrated to new protocol template (NEON.DOC.050006vK) • Clarified wording throughout • Section 4.4 – additional guidance for sample timing contingencies, including difficulties procuring dry ice • Section 4.5 – more detail around creating missed sampling records • SOPs A, B, C – removed instruction to add human-readable labels to archive cryovials • SOPs A and J.1 – changed from vials to centrifuge tubes for microbial biomass samples • SOPs B and C – added workflow diagrams • SOPs B and C – additional guidance for 1) not rejecting coordinates when disturbance is dominant condition of the subplot, 2) taking the average of several litter depth measurements 3) using a new ethanol wipe for equipment cleaning at each X,Y location, 4) preparation instructions for microbial subsamples if dry ice is not available • SOPs B, C, and Appendix D.6 – new guidance for sampling in drylands with biological soil crusts • SOP C – added guidance for > 25 cm organic horizons, no mineral horizon sampling should occur • SOP D – added back criteria from a previous revision to describe when a plot should be considered compromised, triggering the selection of a replacement plot • SOP H – several pH method clarifications including 1) instructions for creating acid and base solutions for adjusting pH of calcium chloride, 2) rationale for keeping samples open to the air and stirring by hand instead of cap and shake, and 3) guidance on when to add more solution and how deep to insert the pH probe for M horizons • SOP J.1 – removed need to record microbial biomass sample weights • Table 16: Updated T2 sampling window for BLAN

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			• Appendix D.2: Added site-specific guidance for PUUM to sample all horizons with the monolith method to minimize damage to Ohia roots and susceptibility to ROD • Table 19: Updated soil sampling devices for D06, D10/13, D20 • Table 25: Changed specifications for potassium chloride (KCl) back to ACS reagent grade. • Equipment list tables – added examples of metal 'brownie frames,' several part numbers updated due to supplier changes, exact brand adjusted to 'N' for a few consumables used in laboratory processing.
P	02/08/2024	ECO-07051	• Migrated to new protocol template (NEON.DOC.050006vL) • Updated NEON logo • Section 3 – updated Figure 1 to better display plot layout and new subplot naming (e.g., 21_400 instead of 21) • Section 4 – edited Table 3 to list SOPs for off-year bouts at gradient and core sites in order of use; Updated timing for lab processing for off-year bouts to 48 hrs preferred, 72 hrs maximum; more emphasis in scheduling section on importance of sampling target biophysical conditions; updated guidance for bout completeness and prioritization; clarified several entries in Table 6 (timing contingencies) • Section 5 – updated quarantine soils guidance • Section 6 – added text related to training for KCl and pH procedures • SOP A – included optional use of Soils QC application to prepare target sampling locations; added emphasis to review site-specific appendices; new steps for preparing to measure soil temperature; added option to prepare 2 mL cryovials for microbial archive if 5 mL not available • SOP B – use of pilot hole for measuring soil temperature; 2 mL cryovial instructions added; reference to the FRZ SOP as needed; added emphasis not to sample deeper than 30 cm • SOP C – all of the above for SOP B plus more emphasis on comparable depths for initial and final samples; updated to require (not optional) attached caps for PVC incubation cylinders; clarified use of sampleExtent; more scenarios added for incubationCondition • SOP D – clarified guidance for reporting incomplete bouts • SOP E – instruction to select 'used frozen soil' if moisture samples temporarily frozen due to lack of oven space or access • SOP G – added 5 day minimum for air drying

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
			• SOP H – added requirement to use soil-specific pH probe and 50 mL centrifuge tubes for all M horizon (1:1) samples; included more detail on mass for O horizons; added suggestion to process samples in batches/groups • SOP J – updated such that all horizon types use 50 mL centrifuge tubes to store microbial biomass samples • SOP K – added text here and Table 26 to only use purity-tested KCl powder distributed from HQ; added ultra-pure water rinse for specimen cups; instruction to shake vials if not freezing; included more detail on data entry for lost samples; noted option to create a second back-up scint vial if concerned about shipping • SOP N – Figure 23 revised to better display plot layout and new subplot naming (e.g., 21_400 instead of 21) • Appendix A – added option for 2 mL cryovials • Appendix C – updated protocol sampling windows using newer MODIS-EVI data (2012-2021) including description of general formulas for assigning those dates • Appendix D – updated D.1 quarantine section to include fruit fly guidance; added new section D.6 with instructions for woody horizons • Appendix E – updated to reflect new subplot naming • Appendix F – removed supplier/item number unless exact brand required; added 2 mL cryovials and boxes; added acceptable options for soil-specific pH probe

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1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling and domain lab processing of soil samples to measure physical properties, carbon (C) and nitrogen (N) concentrations and stable isotopes, nitrogen transformation rates, and microbial biodiversity and function. These data can be used to quantify the stocks of soil C and N; they can also reveal ecosystem nutrient status, paint a picture of integrated ecosystem processes, and allow us to understand rates of key microbially-mediated processes in relation to microbial biomass and community composition. During each sampling, NEON characterizes soil pH and gravimetric water content, as these are some of the dominant environmental controls on biogeochemical processes and microbes. As biogeochemical and microbial datasets will be compared with one another, all analyses are performed on the same material when possible. The goal is that NEON data will be used to address a variety of questions about biogeochemical cycling at multiple spatial and temporal scales (**Table 1**).

Typically, ecosystem stocks of C and N are expressed as mass per unit area (e.g., g C per m^2). For soil, this calculation requires knowing the dry mass of soil in a known volume (i.e., bulk density, g per cm^3), and the concentration (or amount) of the element per gram of dry soil (e.g., mg per g). Concentration measurements result from samples collected in this protocol, yet bulk density is not measured here. Instead, it is characterized via an extensive soil survey when each NEON site is established (more below). Isotopic ratios, the measure of a less common isotope (e.g., ^{15}N) relative to the most abundant isotope of an element (e.g., ^{14}N), give a picture of the integrated ecosystem processes occurring within soils or other media and possibly the source of that element. Commonly, they are expressed as per mil (‰) using the delta (δ) notation. Typically, rates of N transformations are expressed as mass of N per unit of dry soil per day (e.g., g NO_3^--N g^{-1} dry soil d^{-1}) or on an areal basis, normalized by bulk density (e.g., g NO_3^--N m^{-2} d^{-1}). This calculation requires knowing the concentration (or amount) of NH_4^+-N plus NO_3-N (net N mineralization) or NO_3-N (net nitrification) per gram of dry soil (e.g., mg per g) at the beginning and end of a multi-day incubation period (e.g., T0 to T14 days). The time of year and site characteristics (e.g. precipitation and temperature) will influence the background rates of nitrogen cycling activity.

A number of methods are employed to measure microbial diversity and abundances. Microbial biomass provides an indication of microbial activity and correlates with numerous ecological processes, such as soil productivity and N mineralization rates. Microbial biomass is measured using the Phospholipid Fatty Acid (PLFA) analysis. Using this method, biomass is estimated based on the fatty acid content of microbial cellular membranes. Microbial diversity and composition are measured by sequencing the 16S (Archaea and bacteria) and ITS (fungi) ribosomal DNA gene. This provides information on the members of the microbial community that are present as well as some indication of the relative abundance of each member of the community. Using shotgun metagenomics, the total DNA recovered from the soil samples is sequenced to capture total genomic content from the organisms present. This provides information on the functional potential of the microbial communities as well as changes in genomes and

genome content through time and space. Finally, soil samples are collected and preserved in a manner that should enable the external user community to use archive samples for RNA-based analyses.

Table 1. Summary of measurements and samples associated with NEON Soil Biogeochemical and Microbial Sampling.

Measurement/Sample	Rationale	Frequency
Total Organic C and Total N (%)	Insights into soil fertility, C and N stocks, N availability, decomposition	Every 5 yrs, 1x per year
δ^{15}N and δ^{13}C	Reveal integrated C and N cycling dynamics, sources of OM and nutrients, N loss pathways	
Inorganic N; Net nitrification & mineralization	Quantify N availability, N saturation, N loss potential	Every 5 yrs, 3x per year
Microbial biomass (PLFA)	Quantify microbial biomass and coarse-level functional groups	Every yr, 3x per year, core sites; Every 5 yrs, 3x per year, gradient sites
16S rRNA gene sequencing	Measure microbial diversity and community composition	Every yr, 3x per year, core sites; Every 5 yrs, 3x per year, gradient sites
ITS rRNA gene sequencing		
Shotgun metagenomics	Assess total genomic content, microbial community functional potential, aggregated traits	Every yr, 1x per year, core sites; Every 5 yrs, 1x per year, gradient sites
pH, moisture, temperature	Give context for biogeochemical and microbial measurements	Every soil sampling event except N-trans Tfinal (no pH)
Soil biogeochemical archive (air-dry)	Provides community access to conduct measurements not being made by NEON	Every 5 yrs, 1x per year
Soil microbial archive (ultralow temp frozen)	Provides community access to conduct measurements not being made by NEON	Every yr, 3x per year, all sites

Measurements of soil biogeochemistry and microbial community composition provide scientists, managers, and decision-makers with important information such as whether the ecosystem is retaining or losing carbon and nutrients, how water and nutrients move through landscapes, and shifts in microbially-mediated ecosystem processes. Comparing these data with other data collected by NEON, including atmospheric deposition, surface water transformations and transport, and above and belowground plant productivity, allows investigators to evaluate material fluxes across the landscape. Temporal and spatial considerations involved in sampling will provide data that can be used to address how the ecosystem is changing over time, as well as in response to climate shifts or land use/land cover change at local, regional, and continental scales. For example, changes in precipitation patterns can alter

wetting and drying cycles within the soil matrix. Such changes to the soil matrix will likely affect microbial process rates and functional potential.

The following protocol outlines the field and laboratory procedures required to collect, process, and maintain the integrity of soil samples. It includes detailed guidance for locating soil sampling sites, collecting soil cores, recording field-associated metadata, laboratory processing of soil cores, and storage and shipment of samples to analytical laboratories or archive facilities.

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

This protocol is based closely on standard soil sampling methods as described by the Soil Science Society of America (Sparks et al., 1996; Dane et al., 2002) and methods published by the Long-term Ecological Research Network (Robertson et al., 1999). The latter reference reviews many studies on this topic that have compared different standard operating procedures. The protocol for pH follows the USDA Natural Resources Conservation Service, standard method 4C1a2 (Burt, 2014). The protocol for microbial biomass was derived from Buyer and Sasser (2012) and Gomez et al. (2014).

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
AD[06]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry
AD[07]	NEON.DOC.000908	NEON Science Design for Terrestrial Microbial Ecology

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	OS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.001577	Datasheets for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
RD[06]	NEON.DOC.004130	TOS Standard Operating Procedure: SLS – Wetland Soil Sampling
RD[07]	NEON.DOC.001710	TOS Protocol and Procedure: LTR – Litterfall and Fine Woody Debris
RD[08]	NEON.DOC.014038	TOS Protocol and Procedure: BBC – Plant Belowground Biomass Sampling
RD[09]	NEON.DOC.001024	TOS Protocol and Procedure: CFC – Canopy Foliage Sampling
RD[10]	NEON.DOC.001716	TOS Standard Operating Procedure: *Toxicodendron* Biomass and Handling
RD[11]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration
RD[12]	NEON.DOC.004474	Manifold for Filtering KCl Extractions assembly instructions
RD[13]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples, Sensors and Equipment
RD[15]	NEON.DOC.002984	Standard Operating Procedure: Minimizing Site Disturbance During Aquatic and Terrestrial Observation System Sampling
RD[16]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[17]	NEON.DOC.014042	TOS Protocol and Procedure: DIV – Plant Diversity Sampling
RD[18]	NEON.DOC.005346	OS Standard Operating Procedure: FRZ – Preparation and Use of Dry Ice Alternative Freezing Materials

RD[19]	NEON.DOC.005108	NEON Terrestrial Observation System: Spatial and Temporal Sampling Strategy

2.3 Acronyms

Acronym	Definition
C	Carbon
^{12}C	Common stable isotope of carbon
^{13}C	Less common stable isotope of carbon
Ca^{2+}	Calcium cation
$CaCl_2$	Calcium chloride
cm	Centimeter
mm	Millimeter
DNA	Deoxyribonucleic Acid
DSF	Domain Support Facility (NEON regional office and lab facilities)
g	Grams
h	Hours
m	Meter
M	Molar
mg	Milligram
ml	Milliliter
N	Nitrogen
^{15}N	Less common stable isotope of nitrogen
^{14}N	Common stable isotope of nitrogen
NH_4^+	Ammonium
NO_3^-	Nitrate
USDA	United States Department of Agriculture

2.4 Definitions

A horizon: Mineral horizon formed at the surface from significant organic carbon accumulation. The horizon will be darker in color than the horizons below due to organic matter accumulation.

B horizon: Mineral horizon with accumulations of Iron, Manganese, secondary minerals, Aluminum-organic compounds, and/or clay, or development of soil structure. Can be higher in clay, may be brighter in color, or may contain more redoximorphic features (evidence of oxidation/reduction) than the horizons above it.

Biological soil crust: Community of organisms such as bacteria, fungi, lichen, and moss living on top of and stabilizing mineral soil.

Soil brownie: a square or block of soil excavated with a soil knife (hori-hori) using a cutting template, generally applies to sampling organic horizons.

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.

Coordinated Bout: Synchronized soil sampling bout that includes the full suite of microbial and biogeochemical measurements described in **Table 1**. Occurs on a 5-year interval at each site.

E horizon: Mineral horizon that exhibits significant loss of organic carbon, Iron, Manganese, Aluminum, and/or clays. The horizon is usually paler in color and lighter in texture (less clayey) than horizons below.

Fulcrum: Software tool used by NEON to create electronic data entry applications.

Litter layer: Loose, unconsolidated plant material (including leaves, needles, twigs, moss, and lichens) on top of the soil surface. Material may be largely intact or partially shredded, but still easily recognizable and has undergone minimal decomposition. Not all sites will have a litter layer.

Mineral horizon: A soil layer where accumulated minerals are the main components. Often feels gritty.

Organic horizon: A soil layer made of organic vegetal material in various states of decomposition, where the mineral fraction is only a small percentage of the layer (much less than half by weight). In general, decomposing plant material is poorly recognizable. Layer tends to be dark in color, friable (easily crumbled), and sometimes greasy. If more than a couple of mineral grains are detected (grit from sand, stickiness from clay) it is most likely a mineral horizon high in organic matter (OM), not an organic soil.

Saprolite: Porous mineral material formed in place by chemical weathering of igneous and metamorphic bedrock. It is often soft and friable and can be dug with hand tools.

ServiceNow: Software tool used by NEON 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 field protocol used by NEON for the collection of soil cores follows the protocols presented in the Soil Science Society of America Methods of Soil Analysis texts (Sparks et al., 1996; Dane et al., 2002), laboratory methods from the USDA Natural Resources Conservation Service (Burt, 2014), and the Standard Soil Methods for Long-Term Ecological Research (Robertson et al., 1999). Sampling is carried out at ten 40 m x 40 m base plots (**Figure 1**, left) that are distributed across each site. Soils are inherently spatially heterogeneous, thus several samples are collected within each plot in order to capture variability at multiple scales (e.g., profile, plot, site). NEON Science supplies domain staff with a list of plots where soil samples will be collected for the duration of field operations, plus additional lists that contain randomly generated X, Y sampling coordinates originating from the southwest corner of each plot (i.e., the coordinates are expressed as meters east and north relative to the southwest plot corner or point 21). These coordinates are the within-plot soil sampling locations, which are different for each sampling event to prevent repeat sampling of a given location. As such, plots and subplots are resampled but the precise locations within them are unique. To keep track of sampling locations in the field and aid in navigation, NEON technicians mark the random sampling locations on a laminated plot diagram (**Figure 1**, right) prior to each sampling bout.

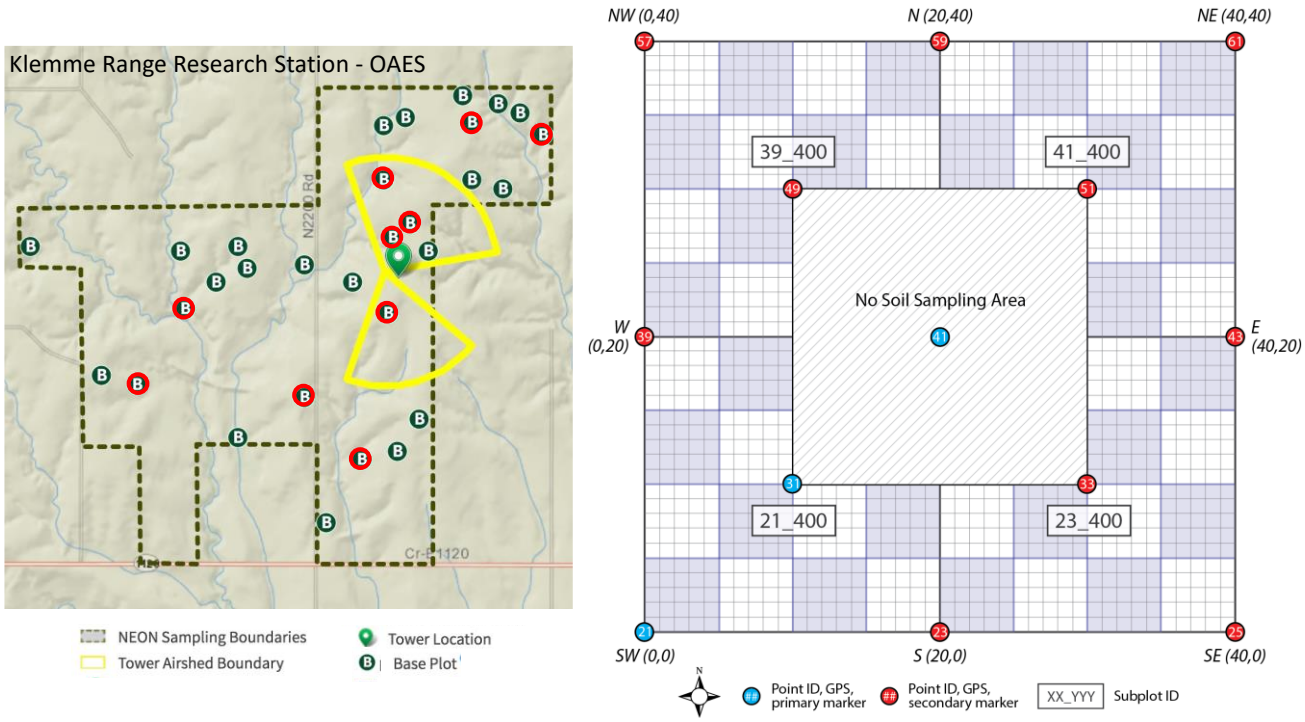

Figure 1. *Left*: Example site layout at OAES. Soil sampling occurs at 10 base plots (for example, those circled in red), 4 in the tower airshed and 6 distributed across the landscape. *Right*: Gridded map of a 40 m x 40 m soil plot. Plots are divided into four 20 m x 20 m subplots with a soil sampling exclusion zone in the plot center. Subplots are named for the southwest corner pointID (21, 23, 39, 41) and size (400 m^2), and three out of four are sampled per bout. PointIDs and markers for 23, 39, 43, and 59 are not present in small-stature Tower and Distributed plots.

For details on establishment of NEON base plots, plot internal layouts, and spatial co-location across protocols, see the NEON Terrestrial Observation System: Spatial and Temporal Sampling Strategy (Meier et al. 2023, RD[19]).

Soil types and horizons differ throughout the 20 NEON domains. When organic and mineral horizons are present within a single profile they are separated prior to analysis. However, other horizons are not separated (e.g., mineral horizons A and B). Where possible, NEON samples mineral horizons using a 2 ± 0.5 -inch inner diameter coring device. Where rockiness or other site soil characteristics make it difficult to use this diameter range, other diameters are employed, following consultation with NEON Science. A list of site-specific coring devices is available in Appendix D.9.

In addition, the depth of soil to saprolite or bedrock will vary across domains. NEON soil sampling is conducted to a maximum depth of 30 ± 1 cm where possible. More detailed characterization of the dominant soil types occurred during the construction period of NEON through two projects. One project was led by the Terrestrial Instrumentation System (TIS) unit and included a thorough description of one large soil pit ('megapit') dug at the NEON instrumented tower location from the surface to 2 meters depth (or bedrock, whichever was shallower) at each core and gradient site. These data are available in the NEON data product DP1.00096.001, "Soil physical and chemical properties, Megapit." The second project was carried out by the U.S. Department of Agriculture (USDA)- Natural Resources Conservation Service (NRCS) and characterized soil physical and chemical properties (including bulk density), by horizon, to 1 m depth at a subset of the Terrestrial Observation System (TOS) distributed soil plots, many of which overlap with ongoing NEON soil sampling. These data are available in NEON data product DP1.10047.001, "Soil physical and chemical properties, distributed initial characterization."

It is critical that the locations from which soil samples are collected have not been disturbed prior to sampling. Examples of disturbance include prior sampling, compaction from roads or trails, and contamination atypical of the site. Other factors that may necessitate relocation of sampling efforts include: obstruction by tree roots, large (i.e., > 8 cm) rocks, or holes (e.g., from small burrowing mammals). In any of the above scenarios, field personnel note the impediment in the soil coordinate lists and seek a new location within 0.5 m of the predetermined sampling location. In some cases, an alternative X, Y sampling location must be used instead (see SOP B and C for more details). Once soil cores have been collected, extraction holes must be backfilled per site host requirements and the final sample location recorded so that subsequent samples are not collected in the same locations.

Soil Biogeochemistry (hereafter, Soil BGC). Soil samples collected for C and N concentrations and stable isotope analysis undergo preliminary processing in the domain laboratory. This consists of sieving and air-drying soils according to SOP F and subsampling according to SOP J. After in-house preparation, samples are shipped to contracted laboratory facilities for analysis. Subsamples of these soils are also analyzed for pH and moisture at the domain laboratory; another subsample is prepared for archive.

Microbial Analyses. Subsamples are either put on dry ice in the field (for microbial genetic analysis and archive samples), or kept field moist and then prepped in the lab (for microbial biomass analysis), as

described in field SOP's B and C and lab SOP J.1. Then, samples are shipped to the contracted facilities for processing, analysis, or long-term storage. Subsamples of these soils are also analyzed for pH and moisture at the domain laboratory. During peak greenness bouts, composite samples of cores from the same plot are generated in the field for metagenomics analyses and kept on dry ice following collection.

Soil N Transformations. The procedure for measuring rates of net N mineralization and net nitrification involves two companion soil cores taken from a given location. One core is collected for immediate processing (e.g., the "initial" core) and is subsampled for microbial measurements, while the other remains in the soil, either capped in an open-bottom PVC cylinder, or in a buried bag for wetlands (see RD[06]). This "final", incubated core stays in the ground for a specified period (two to four weeks as in **Table 16**, shorter incubations for warmer periods and longer incubations in cooler periods), and is retrieved at the conclusion of that period and brought back to the domain laboratory for processing. Processing of "Tinitial" and "Tfinal" cores involves extracting soils with 2M KCl verified to be low in inorganic N contaminants, then filtering the extracts and shipping them to a contracted laboratory for analysis of NH_4^+-N and NO_3^--N. The name of the laboratory that processes each sample is recorded in the data and laboratory methods are available on the NEON data portal. Subsamples of initial soil samples are analyzed for both pH and moisture content, while final samples are only analyzed for moisture.

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 technicians **must** follow the protocol and associated SOPs. Use NEON's ServiceNow 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 into NEON's internal 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

The timing, temporal frequency, and extent of soil sampling constitute the Science Design (see AD[06] and AD[07]). The extent of soil sampling, at 10 plots per site (**Figure 1**), allows researchers to evaluate the spatial heterogeneity of nutrient pools and fluxes; differences in soil type, plant communities, or hillslope aspect should affect the results, so these features are taken into account in the spatial component of the sampling design. The sampling frequency allows researchers to investigate how microbial communities and nutrient dynamics change seasonally and over time. Soil samples are collected during peak greenness as well as two transitional periods (**Figure 2**). Sampling windows are determined on a per-site basis using satellite-based MODIS-EVI data averaged from 2012-2021 (Didan 2023) as an indicator of plant phenology, with the assumption that aboveground dynamics are a reasonable (though incomplete) proxy for belowground activity. Historical precipitation data are used instead at sites where remote sensing data demonstrate low temporal variance in greenness. In general, transitional bouts take place when soils are expected to be changing activity levels (**Figure 2**), such as during the transition from winter to spring or the dry to wet season.

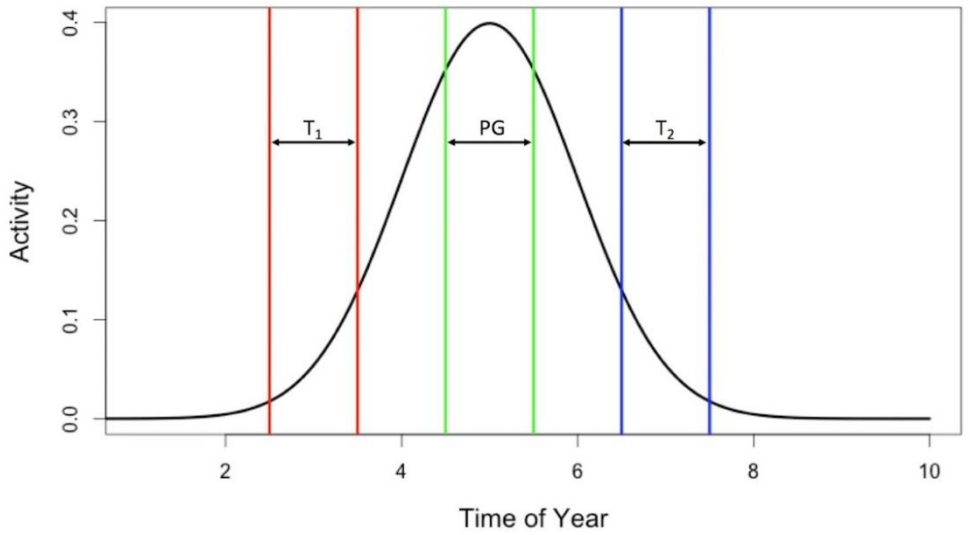

Figure 2. Generalized Timing of Soil Sampling. Transition 1 (T1) captures the transition from dormancy/low activity to a more active period, peak greenness (PG) is the period of peak MODIS-EVI, and Transition 2 (T2) captures the transition from the more active period to dormancy/low activity. The time of year for each sampling period will vary by local geographic and climatic conditions and is recorded in the data.

Target sampling windows for each site, based on historical data plus site-specific adjustments, are provided in Appendix C. Note that due to the short growing season, Domains 18 and 19 are only sampled during the peak greenness bout. Temporal linkages between the different soil analyses are described below. The timing and types of sample collection for particular bouts are outlined in **Table 2**, while a general outline of the timing of SOP implementation is in **Table 3**.

Table 2. Sample types for the different soil bouts. For detailed guidance on the number of horizons to collect for each sample and bout type, refer to the field SOPs as well as Appendix A.

	Off-Year			Coordinated bout			
N-trans Bout Type	**No**			**T initial**			**T final**
Sample Timing	T1	PG	T2	T1	PG	T2	T1, PG, T2
Bout Type	microbes or microbes Biomass[t]	microbes or microbes Biomass[t]	microbes or microbes Biomass[t]	microbes Biomass	microbes BiomassBGC	microbes Biomass	fieldOnly
Field-generated Samples	Bulk -gen[t] -gaX	Bulk -gen[t] -gaX -comp[t]	Bulk -gen[t] -gaX	Bulk -gen -gaX	Bulk -gen -gaX -comp	Bulk -gen -gaX	Bulk
Lab-generated Samples	-bm[t]	-bm[t]	-bm[t]	-bm -kcl	-bm -kcl -cn -ba	-bm -kcl	kcl
Lab measurements	pH moisture	pH moisture	pH moisture	pH moisture	pH moisture	pH moisture	moisture

[t] *Core sites only*

Abbreviations

Sample Timing:
- **T1:** Transition 1
- **PG:** Peak Greenness
- **T2:** Transition 2

Sample:
- **Bulk:** Homogenized soil used for all subsamples and analyses

Subsamples:
- **-gen:** soil microbial genetic analysis subsample
- **-gaX:** soil microbial genetic archive subsample, X denotes subsample number of 1-5 (for up to 5 vials)
- **-comp:** composited soil microbial metagenomics subsample
- **-bm:** soil microbial biomass subsample
- **-kcl:** soil KCl extraction sample
- **-cn:** Soil BGC analysis subsample
- **-ba:** Soil BGC archive subsample

Microbial Genetic Analyses and Biomass. Microbial communities can change frequently in response to environmental shifts. Thus, sampling for microbial genetic analysis (-gen), genetic archive (-gaX), and microbial biomass (-bm) occurs up to three times per year (depending on site type) and is aimed at capturing windows in which microbial activity is ramping up, at peak, or slowing down (**Figure 2**). All sites conduct sampling during peak greenness, and most sample at two other times during seasonal transitions. At 'temperature-driven' sites, these transitional windows are intended to capture

snowmelt/ground thaw in the spring and plant senescence in the fall. At 'precipitation-driven' sites, the transitional windows are intended to capture the onset of the wet and dry seasons. The estimated dates for onset and cessation of annual sampling per site based on historical data are listed in Appendix C. When sampling for soil BGC and nitrogen transformations, soil for all microbial analyses is collected concurrently and is subsampled from the same homogenized T-initial soil sample.

Microbial Metagenomics Analysis. Samples for microbial metagenomics analysis (-comp) are collected up to once per year (depending on site type), during the Peak Greenness window. A single sample represents a composite of all sampling locations within a plot for a particular horizon.

Nitrogen Transformations. Every 5 years, soil measurements of N transformations (-kcl) are conducted. To account for seasonal variation, up to three sampling events occur during a sampling year, in conjunction with the microbial sampling described above. Soil collected from the N transformation T-initial core is used to generate subsamples for moisture, pH, microbial genetic analysis and genetic archive, microbial biomass, microbial metagenomics, and soil BGC (when applicable). Soil collected from the incubated T-final core is used solely for moisture and N transformation measurements.

Soil BGC. Samples used to measure soil carbon and nitrogen concentrations and stable isotopes (-cn) as well as those created for biogeochemical archiving (-ba) are collected alongside the other soil subsamples once every 5 years, during the Peak Greenness window. Because these pools are expected to change slowly, they are measured less frequently.

Coordinated Plant-Soil Biogeochemical Measurements. Soil sampling during Coordinated bouts, which includes microbial measurements, N transformations, and soil BGC, occurs according to the schedule in **Table 3**. Implementation of these components of the protocol are scheduled on an inter-annual basis as part of the suite of synchronized TOS measurements aimed at characterizing plant and soil biogeochemical dynamics. Synchronized protocols and SOPs include:

- TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Sampling (this document)
- TOS Protocol and Procedure: LTR – Litterfall and Fine Woody Debris, litter chemistry SOP (RD[07])
- TOS Protocol and Procedure: BBC – Plant Belowground Biomass Sampling (RD[08])
- TOS Protocol and Procedure: CFC – Canopy Foliage Sampling (RD[09])

Table 3. Sampling frequency and SOP order for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling procedures on a per SOP, per bout type basis. Note that SOP's L (Data Entry) and M (Shipping) apply to all bouts.

Type of Sampling	SOPs in the order performed	Plot Type	Plot Number	Bout Duration	Bouts Per Year	Yearly Interval
Off-year bouts at gradient sites	A, B, D, E, F, G, H	All	10	up to 2 weeks	1-3 per sampling year	All years except for coordinated (4 of 5)
Off-year bouts at core sites	A, B, D, E, F, J.1, G, H	All	10	up to 2 weeks	1-3 per sampling year	All years except for coordinated (4 of 5)
Coordinated bouts, any site type	A, C, D, E, F, J.1, J.2, K, G, J.3, H	All	10	2-4 weeks	1-3 per sampling year	Every 5 years
Metagenomics pooling if not conducted in the field	SOP I	All	10	NA	1 per sampling year	Annual for core sites, every five years for gradient sites

Scheduling Considerations

1. **Field Work and Laboratory Processing**: After soil samples are collected from a given sampling location, the following points are critical with respect to timing:
    a. Keep bulk soil samples cold until they are processed in the laboratory. Change cooler ice packs every 12 h if not able to transfer to a 4°C refrigerator upon return from the field.
    b. Keep frozen samples on dry ice until they are processed or stored in an ultra-low temperature freezer (-80°C). Check cooler every 6 hours, refresh dry ice as needed.
    c. When conducting an Off-year bout at a gradient site, process collected soil samples in the laboratory:
        i. Within 48 hr of collection (preferred), or 72 hrs (maximum)
    d. When conducting a Coordinated bout or sampling at a core site, process collected soil samples in the laboratory:
        i. Within 1 day of collection.

4.2 Criteria for Determining Onset and Cessation of Sampling

Scheduling a Sampling Bout: Sampling for one bout typically takes 2-4 field days to complete. Bouts should be scheduled according to the site-specific sampling windows provided in Appendix C. For T1

(early season) bouts, sampling should occur within 10 days after observing the first signs of the target seasonal event (**Table 4**), to the extent possible. Adjacent bouts should be at least 14 days apart, meaning the first day of sampling of a new bout is at least 2 weeks after the last sampling day of the previous bout. It is recommended that domain staff schedule a 14-day time period within the middle of the sampling window and adjust either earlier or later in the window based on climate conditions that year as the bout approaches. This will enable capturing the relevant biophysical conditions while allowing for some flexibility without jeopardizing the timing of the sampling bout.

Important Note: The sampling windows defined in Appendix C are intended to guide scheduling based on historical data; however, on-the-ground conditions must ultimately dictate the timing of a bout, which may deviate from historical data. If conditions at the time of a scheduled bout are either not representative of the target biophysical conditions (**Table 4**) or are not suitable for sampling, the bout should be rescheduled, ideally for a different time within the sampling window or even beyond it if conditions dictate. Keep in mind the need for 14 days between adjacent sampling bouts, and that Coordinated bouts may not extend so late that the incubations for N-transformations overlap with the start of the next scheduled bout. Refer to the contingencies table (**Table 6**) and the detailed workflow in **Figure 3** for further guidance on scheduling issues.

Table 4. Summary of Timing for Soil Sampling. Note that Domains 18 and 19 are only sampled during the Peak Greenness collection period.

Bout	Sample Timing	Domains	Characteristics
Seasonal Transition #1 (T1)	Winter-spring transition	1, 2, 5, 6, 7, 9, 10, 12‡, 13, 15, 17	• Start of active period • Ground thawed • Snow melt*
	Dry-wet transition	3, 4, 11, 14, 17, 20	• Initiation of wet season* • Changing microbial activity levels
	Wet-dry transition	8, 16	• Initiation of dry season* • Changing microbial activity levels
Peak Greenness	Peak Greenness	All	• Timing of peak above-ground biomass
Seasonal Transition #2 (T2)	Fall-winter transition	1, 2, 5, 6, 7, 9, 10, 12, 13, 15, 17	• Start of quiescent period • Frost on ground • Snow accumulating
	Wet-dry transition	3, 4, 11, 14, 17, 20	• Initiation of dry season
	Dry-wet transition	8, 16	• Initiation of wet season

* Ideally sampling should occur within 10 days after observing a signal of these transitional events.

‡ At YELL, Tower plots cannot be sampled in spring due to a bear management closure, see Appendix C

Duration of a Sampling Bout. Field sampling for a particular bout should be completed as quickly as possible, but ***should not take longer than 14 calendar days*** to complete. Some sites, specified in Appendix D, demonstrate significant within-site variability in seasonality that may cause plots to reach optimal sampling at times spanning > 14 days. For these sites, a bout may take longer than 14 days, however all plots experiencing similar on-the-ground conditions should be sampled as close in time as possible, but at least within 14 days.

Completeness and Prioritizing a Sampling Bout. For a soil bout to be considered complete, all designated sampling locations (three subplots in each of the 10 designated soil plots, or 30 X, Y locations) must be sampled. Anything less than this, due to logistical or ecological reasons, is an incomplete or partial bout. Details on how to record missed sampling are given in Section 4.5. If staffing shortages or other issues mean that at the outset, it is known that a complete bout will be impossible or unlikely, Field Science should use the Plot Prioritization list and Prioritization Matrix to guide the allocation of sampling effort.

Special Considerations for Nitrogen Transformations: An N transformation incubation lasts 2-4 weeks, the length of which depends on the time of year and conditions at a site. For instance, the prevalence of cold and/or dry conditions result in lower activity rates, thus requiring longer incubations, while warm and wet conditions promote higher activity rates and make shorter incubations preferable. The NEON Science staff have estimated appropriate target incubation lengths on a per-site basis, which can be found in Appendix C. N transformation sampling begins and ends within the site-specific sampling periods defined in Appendix C whenever possible. The end of the incubation may extend beyond the sampling window when required by logistics or weather, but the majority (more than half) of the incubation length should fall within the window whenever possible.

4.3 Timing for Laboratory Processing and Analysis

Many analyses performed on soil samples are time and temperature sensitive and may be compromised by not following the appropriate storage conditions described below and in **Table 5**. For more detail on storage conditions, refer to the SOP's below. For requirements on the timing and conditions of shipping, refer to NEON Protocol and Procedure: Shipping Ecological Samples, Sensors and Equipment (RD[14]).

Microbial Genetic and Metagenomics Analyses and Archiving (-gen, -gaX, -comp). Microbes respond rapidly to changes in their environment. In order to preserve samples for potential future analyses (e.g., RNA and/or protein analysis), soil samples collected for microbial genetic and metagenomic analysis and archive are frozen as quickly as possible and must remain frozen: failure to do so may render the samples unusable. If this happens, notify NEON Science staff immediately via ServiceNow.

Nitrogen Transformations (-kcl). Soil cores collected for this purpose are transferred to a cooler with ice packs. Samples must be processed within 1 day of field collection (applies to "initial" and "final" soil cores). For example, samples collected on a Tuesday must complete the KCl extraction procedure by end of the day Wednesday. When held overnight, soils are stored refrigerated at 4°C. Due to the short shelf

life of samples, it is sometimes necessary to break up field work to ensure that processing begins within 1 day, e.g., staff may split a bout into 'minibouts' within a sampling window or utilize multiple teams.

Microbial Biomass (-bm). The fatty acid composition of microbes in a soil sample can change within hours. Sample bags are sealed well to avoid moisture gain/loss and stored in a cooler on ice packs as soon as possible. At the domain support facility, samples are sieved (or, for O horizons, picked free of coarse roots) within 1 day of collection and then stored in a -80°C freezer.

pH, Moisture and soil BGC (-cn, -ba). Processing of subsamples for pH, moisture and soil BGC is conducted on soil kept cold. For coordinated bouts, processing must begin within one day of collection. For off-year bouts soil should be processed within 48 hours of collection (preferred), or within 72 hours (maximum). Soil cores destined for BGC analyses that remain un-chilled for more than 8 hours may need to be discarded, and Field staff should notify Science staff via NEON's ServiceNow reporting system in order to discuss possible rescheduling of the sampling bout.

Table 5. Storage conditions and holding times for soil samples. For samples that require shipping, the hold times represent the maximum amount of time samples may remain at the domain before they **must** be shipped.

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
Microbial Genetic Analysis (-gen), Genetic Archive (-gaX), Metagenomics (-comp)	Cooler with dry ice	Ultra-low temp freezer -80° C	Up to 3 months when stored accordingly
Bulk sample	Cooler with ice packs	Refrigerator until further subsampling and processing 4° C	Process within 1 day (core sites and coordinated bouts) or 48-72 hrs (off-year, gradient sites) Hold up to 7 days at 4° C or 1 month at -20° C in case of sub-sample loss
Microbial Biomass (-bm)	--	Ultra-low temp freezer -80° C	Up to 6 weeks when stored accordingly
KCl extracts (-kcl)	--	Freezer -20° C	Up to 8 weeks, ship initial and final samples together.
Biogeochemistry (-cn)	--	Oven-dry at 65°C, then room temperature	Samples do not expire but should be shipped in a timely manner, max 2 months following collection
Soil BGC archive (-ba)	--	Air-dry, then room temperature	Samples do not expire but should be shipped in a timely manner, max 4 months following collection

4.4 Sampling Timing Contingencies

Table 6. Contingency decisions for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling. Figure 3 provides a flow chart of the process.

Delay/Situation	Action	Outcome for Data Products
Inability to finish all sampling within required time frame, e.g, incomplete bout	Communicate to NEON Science via ServiceNow ticket.	Dataset may be incomplete or delayed.
Delay in scheduled start of sampling bout > 7 days	Submit schedule change request to reschedule bout.	If target biophysical criteria present, no adverse data outcome. If target conditions no longer present, bout may be cancelled with no data generated, or samples may reflect non-target biophysical conditions.
Delays in sampling for one bout cause adjacent bout to occur within 14 days	If possible, submit schedule change request to push back adjacent bout. If not, reach out to NEON Science via ServiceNow ticket for discussion.	Delayed bout may be cancelled if adjacent bout cannot be rescheduled, no data generated.
Scheduled bout will not capture the intended biophysical criteria (e.g., no rain signaling onset of wet season)	If possible, submit schedule change request to sample during target conditions. If not, sample and follow guidance to flag data as in Section 4.5 below.	Bouts may be rescheduled, and if not, data may not be as useful for time series aimed at capturing similar seasonal dynamics.
Inclement weather prevents or disrupts sampling	Light rain, wind and snow are no issue. For sampling in precipitation, consider bringing an umbrella to the field. But if weather conditions make cleaning and sterilizing tools impossible, stop sampling and return later.	No adverse data outcome as long as sampling can resume within the 14 day bout window.
Sampling all 3 subplots from a given soil plot is not possible in the same day	Return to the plot and collect remaining subplot(s) **asap**, within 3 days max, then create -comp sample in the lab (if needed). If this is not possible, follow missed sampling guidance in Section 4.5	Within-plot replicates may not be as similar to each other as when sampling occurs all on the same day.
It is not possible to procure dry ice in time for the bout, all reasonable options explored but unsuccessful	If possible, submit schedule change request to wait for dry ice. If not, sample and store microbial subsamples following instructions in the FRZ SOP [RD18].	Microbial data or archive samples may be of lower quality due to difference in sample preservation method.

Delay/Situation	Action	Outcome for Data Products
There is standing water 1-20 inches (2.5-50 cm) deep within a plot where soil sampling is to occur.	If the site is authorized in the Wetland SOP, use that protocol to conduct sampling. If not, contact NEON Science via ServiceNow for further direction.	Samples may not be collected, or sampling methods may differ for affected locations.
There is standing water > 20 inches (50 cm) deep within a plot or subplot where soil sampling is to occur.	Do not attempt to collect soils. Create missed sampling records as in Section 4.5 and communicate incomplete sampling to NEON Science via ServiceNow ticket.	Samples will not be collected for this time period; no data generated.
Sampling is scheduled, but soil freezes	Do not attempt to collect soils. Communicate to NEON Science via ServiceNow ticket and create missed sampling records.	Samples will not be collected for this time period; no data generated.
Dusting of snow present, but ground not frozen and snow easily removed.	Brush away snow and sample according to appropriate SOP.	No adverse data outcome.
Impenetrable snow is present on the majority of the plot.	If possible, submit schedule change request to sample after snow melts. If not, do not collect soils, communicate to NEON Science via ServiceNow ticket, and create missed sampling records.	Bout may be cancelled if it extends into a different sampling window or the field season is over; no data generated.
Entire bout is missed or cancelled due to a site-level incident and cannot be rescheduled	Record issue in the Site Management Fulcrum application and via a ServiceNow ticket.	Samples will not be collected for this time period; no data generated.

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. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather, or
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above 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[13]).

Missed or Incomplete Sampling Terms

There are several standard terms that inform Missed or Incomplete Sampling. These are used internally for NEON scheduling and externally to help researchers understand the context for missing values or protocol timing deviations in the dataset. These terms include:

- **Protocol Sampling Dates**: Bout-specific sampling dates based on historical data (Appendix C).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by NEON 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 3**, 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 3**).

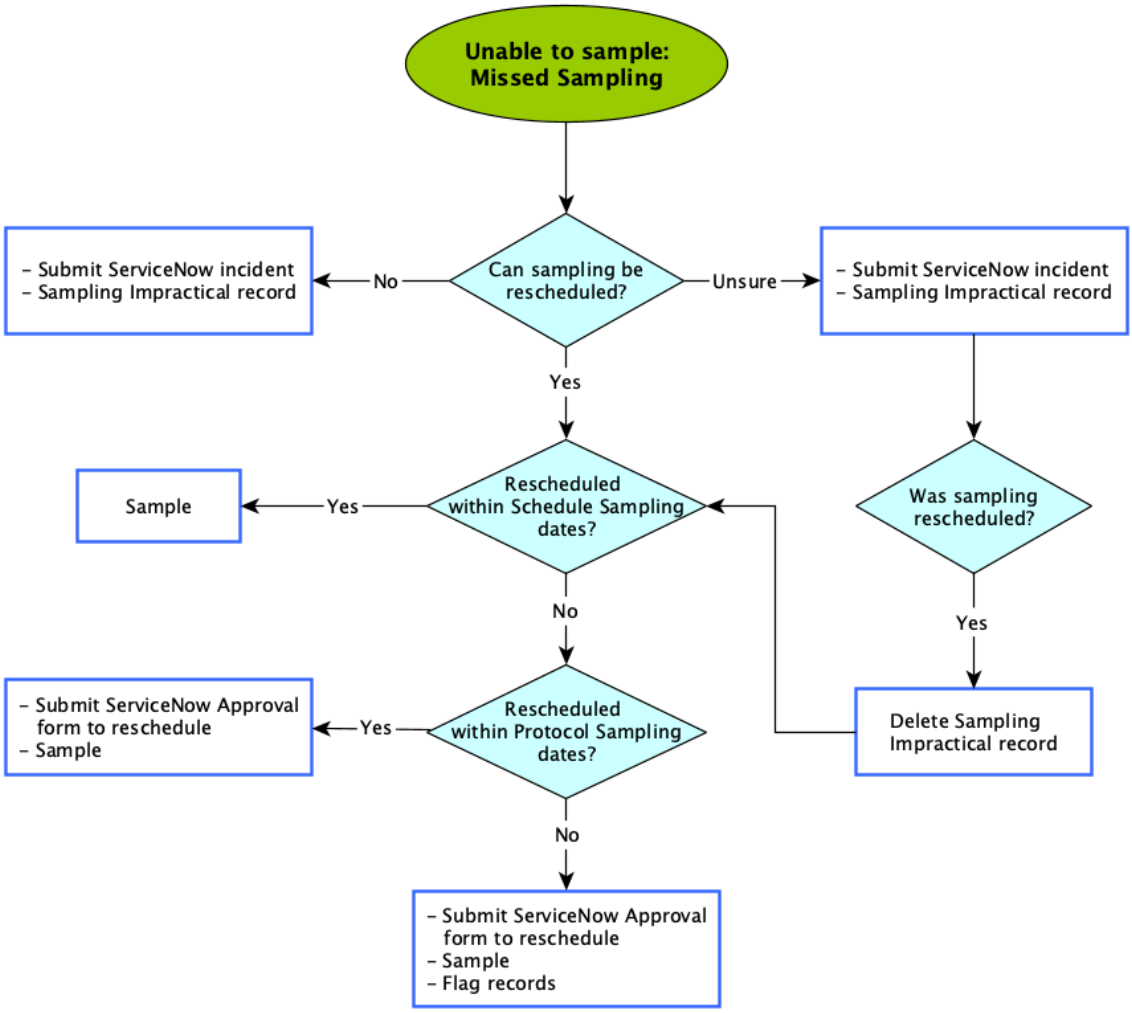

Figure 3. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action.

To Report Missed or Incomplete Sampling:

1. *Internal reporting (for NEON staff only):* Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to NEON Science by a ServiceNow Incident.

    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 3**).

    b. Consult **Table 7** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science Sharepoint library. However, this protocol is the ultimate source of information should any discrepancy exist.

Table 7. Guidance for responding to delays and cancellations encountered during implementation of TOS Protocol and Procedure: SLS - Soil Biogeochemical and Microbial Sampling.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
TOS Soil Sampling, Off-year or Coordinated Tinitial Bouts	> 7 days	IS/OS Schedule Change request. Indicate if target biophysical criteria still present.	Submit Incident ticket for canceled or reduced sampling at any soil plots.
TOS Soil Sampling, Coordinated Tfinal Bouts	> 4 days	IS/OS Schedule Change request. Indicate how long cores have been incubating.	Submit Incident ticket for any plots where Tfinal cores cannot be retrieved.

2. *External reporting (capture in the data):* Create a Sampling Impractical Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. This includes cases where missed sampling is anticipated, for example YELL Tower plots in spring due to the bear management closure.
    a. Create a parent data record in the SLS: Field Sampling application for each Missed Sampling event in the field. A parent record must be made for each plot missed. For example, if an entire bout is missed, records for 10 plots are created.
    b. Within each plot with missed or incomplete sampling, create a child record for each randomly assigned subplot that was not able to be sampled; there should be 3 records per plot, which is the same as for a complete bout.
        i. These subplots can be sampled for the next bout.
        ii. Only create 'Tfinal' sampling impractical records if 'Tinitial' N transformations samples were collected.
    c. For Missed Sampling records, data in downstream Fulcrum applications (e.g., Lab apps) are not recorded. For example, if no soil samples were collected for a particular subplot, then no entries are made in the SLS: Moisture application.
        i. NOTE: This contrasts with samples that were collected but were lost or compromised *during* laboratory processing. In these instances, records in downstream applications should be recorded. See details in specific SOP's for further guidance.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 8**).
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific 'flag' must also be recorded (**Figure 3**). For soil sampling, populate the appropriate choice in the **biophysicalCriteria** field (**Table 9**).

5. Status field work as 'partial' if any sampling impractical records were created (as in OS Standard Operating Procedure: Monthly Activity Status Tracking) and submit an Incident to notify NEON Science that the bout was incomplete.

Table 8. Protocol-specific Sampling Impractical reasons to record. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
OK	No known issue (default value)
Location frozen	Location frozen
Location snow covered	Location snow covered
Location burned	Plot recently burned and not safe to access
Location flooded	Standing or flowing water too deep to complete sampling
Logistical	Site or plot access compromised (e.g., downed tree blocking road to plot), or insufficient staffing or equipment to complete sampling
Management	Management activities such as controlled burn, pesticide applications, etc.
Extreme weather	Events (e.g., thunderstorms, hurricanes) that compromise safety and access
Wildfire	Sampling location inaccessible due to active wildfire or post fire safety hazards
Wildlife Hazard	Wildlife hazard, specific hazard described in remarks
Coordinates not suitable	Maximum number of attempts made to sample the randomly assigned X, Y coordinates within a subplot were unsuccessful
Horizon not present	Applicable only to T-final cores, use if a horizon was present in the T-initial sample but not found in the incubated sampled.
Other	Sampling not possible for other ecological reason described in the remarks

Table 9. Values for **biophysicalCriteria**, a qualifier field for **sampleTiming**. In the event that more than one is applicable, choose the dominant value.

biophysicalCriteria entry	Description
OK - no known exceptions	Sampling occurred on schedule, no known issues capturing the intended biophysical conditions
OK - schedule change but conditions met	Sampling occurred not within protocol sampling windows (Appendix C) but did still capture the target biophysical conditions
Conditions not met	Sampling did not capture the target conditions
Other	Other potential sample timing inconsistencies described in the remarks

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 a particular location requires significantly more time than expected, NEON Science may propose to move these sampling locations.

Table 10. Estimated staff and labor hours per bout required for implementation of TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling.

SOP	Estimated time	Suggested staff	Total person hours
A: Preparing for Sampling	3 hrs	2	6 hrs
B: Field Sampling, Off-year	16 hrs	4	64 hrs
C: Field Sampling, Coordinated	20 hrs	4	80 hrs
D: Post-field Tasks	2 hrs	1	2 hrs
E: Soil Moisture	4 hrs	2	8 hrs
F: Sieving and Picking	8-16 hrs	2	16-32 hrs
G: Air Drying	3 hrs spread over multiple days	1	3 hrs
H: Soil pH	6 hrs	2	12 hrs
I: Composite Sample Generation	2 hrs	2	4 hrs
J: Lab Subsampling, Coordinated Bout	4-8 hrs	2	8-16 hrs
K: N transformation lab processing	10-15 hrs	2	20-30 hrs
L: Data Entry and Verification	2 hrs/app (6 total)	2	24 hrs
M: Sample Shipment	2 hrs/shipment (up to 4 total)	2	8 hrs

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.

Work that involves disturbance of soils or plant litter may increase the concentration of fungal spores and bacterial pathogens in the air. Take precautions to prevent inhalation of dust from soils and plant litter. Review zoonotic diseases in AD[02] for information on areas of high risk and symptoms of fungal infection.

If *Toxicodendron spp* are present at a given site, Field Operations should utilize the procedures outlined in TOS Standard Operating Procedure: Toxicodendron Biomass and Handling (RD[10]) in order to minimize exposure while sampling and to properly clean equipment that came in contact with toxic oils. Soil samples taken from areas with heavy *Toxicodendron* should be collected and processed with additional care. Further instructions are provided in the SOPs that follow.

Soil sampling equipment can be sharp and/or heavy (i.e., hori hori knife, coring device). Take precautions to handle these tools with appropriate care. Dry ice used for preserving microbial samples must be handled with appropriate safety protection and must never be stored in airtight containers. Shipment of samples to external laboratory facilities on dry ice requires additional safe handling techniques, the availability of a Safety Data Sheet, and additional safety labels.

Measurement of pH involves use of strong acids and bases for calibration purposes. Refer to the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]) for instructions on safe handling and disposal of these types of chemicals.

5.1 Plant Protection and Quarantine

Shipment of plants and soils are regulated by USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine Office under 7 CFR 330. In order to protect against the spread of potential plant pathogens or unwanted pests, transportation of quarantined soils requires a USDA soil permit and special treatment of stored or discarded soils. This applies in particular to soil samples being transported from outside the continental U.S., which are all considered quarantined, and from a quarantined county to a non-quarantined one. USDA soil quarantines are somewhat fluid, especially where the quarantine is for fruit flies. As such, all domains, but especially those in the southern U.S. and California, should regularly check the current USDA soil quarantine map to determine if their county is under quarantine.

A list of sites that are commonly quarantined can be found in Appendix D.1. Field Science must be aware of each site's quarantine status to be sure they are complying with federal and location regulations.

Detailed protocols for the handling and shipment of quarantined soils can be found in the USDA permit and compliance agreements for each laboratory where soils are shipped. These permits and compliance agreements are posted on the Collections and Laboratory Analysis page of the NEON Sharepoint Library, and summary notes on how to treat samples are included in the "laboratories_CLA" Fulcrum application under the "Permits" column.

General guidelines for field collection of quarantine soils includes:

- While wearing gloves, remove any insects that are visible in the soil sample prior to field subsampling and transport, especially if you are in an insect quarantine area.
- Remove visible plant material (leaf litter, twigs, bark, and large roots) prior to field subsampling.

To minimize confusion on preparing soil shipments, all soil samples will be shipped following the regulations below, whether from a USDA quarantine area or not. The protocol for soil shipping is described in detail in RD[14] - here are some of the key points as relates to USDA permit compliance:

- Pack samples in double leak-proof containment, with a layer of absorbent padding between the primary and secondary container.
- Address package to the named permit holder listed on the receiving laboratory's permit.
- Print and include in the shipment a copy of the receiving lab's USDA Soil Permit and/or compliance agreements, as outlined in the "laboratories_CLA" Fulcrum application ("Permits" column).

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

Field personnel are to be trained in use of the soil corer, identifying and differentiating local soil horizons, using dry ice for sample preservation and transport, practicing clean and sterile field and laboratory techniques, making salt solutions in the laboratory, conducting pH measurements and soil nitrogen transformation extractions, and safe working practices for field sampling.

6.2 Specialized Skills

Soil types and profile characteristics differ greatly across the NEON domains (see examples in **Figure 4**).

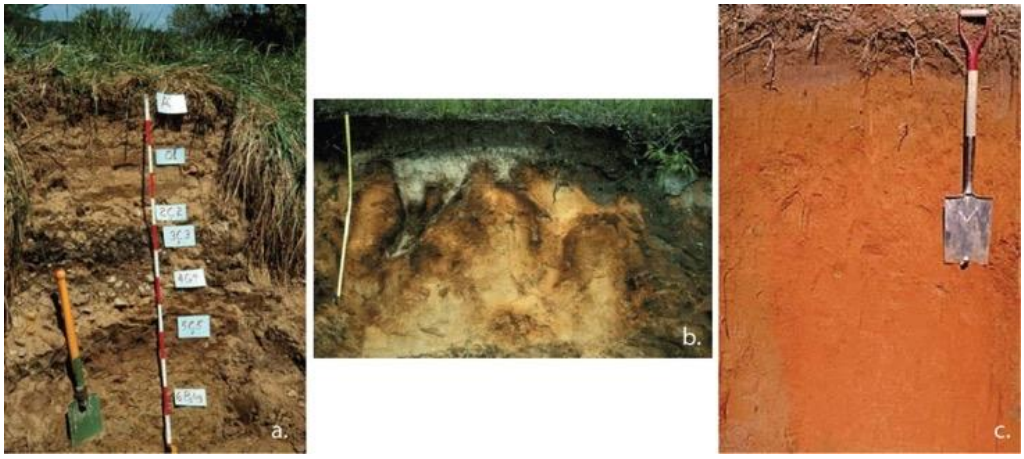

Figure 4. Soil Profiles from (a) Maryland, (b) Michigan, and (c) Florida. (Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

When sampling soil, field personnel must be familiar with the basic characteristics of a typical soil profile at the local NEON site, such as ability to differentiate between organic and mineral horizons and be familiar with typical soil depth. Personnel should review the site-specific horizon resources posted on the NEON Internal Sampling Support Library (SSL) to familiarize themselves with local soil conditions. For example, in Domain 1, this includes understanding differences among the **leaf litter**, **organic horizon,** and **mineral horizons** (see Definitions section). The NEON protocol requires removing the litter layer, and sampling the organic and mineral soil horizons separately. In other locations, such as Domain 10, an organic horizon may not exist, but other features (e.g., a plow horizon, shallow soils) may be present. Likewise, permafrost and peatland sites such as those found in Domains 5, 18 and 19 may not have a litter layer, but often have thick, partially decomposed organic horizons. In drylands, biological soil crusts are an important feature of the landscape that soil samplers must be able to recognize, while in

some forest sites organic horizons can be very woody. Appendix D provides guidance for site-specific issues and protocol modifications for challenging sites and conditions. Field personnel should contact Science regarding any anomalous soil features that they observe when sampling and note any in-field decisions made that fall outside of the protocol guidelines.

The methods used to measure presence and relative abundance of soil microbes are extremely sensitive: less than 10 copies of a single gene can be detected, meaning that human and environmental contamination can occur very easily. Care must be taken to ensure that all samples and sampling equipment remain free of contamination to the extent possible. Conducting lab work for N transformations similarly requires attention to details in order to prevent contamination of equipment and samples with exogenous N sources. Field personnel should be familiar with basic microbiology as well as sterile and clean sampling techniques (see Definitions section), and use their best judgment to control for contamination from themselves and from their surroundings, particularly during field sampling in bad weather conditions. Some general guidelines are:

Any field sampling tool or instrument that is re-used should be cleaned with deionized water and sterilized with either 70% ethanol wipes or 70% sterilized ethanol from a squirt bottle and wiped down prior to re-use. Basically, if a tool touches a new soil sample or other source of contamination, it should first be cleaned. Examples of such tools include:

- Coring device. This may be particularly difficult to clean, depending on your device. A bottle brush wrapped in an ethanol wipe can help clean hard-to-reach spots. Technicians should sample as cleanly as is reasonable.
- Trowels, hori-hori, or other digging tools
- Organic horizon “brownie” frame
- Gloves: these can be re-used at a sampling location if they are free of dirt/soil and have been sterilized thoroughly with an ethanol wipe or spray.

Be aware of activities such as wiping nose or eyes with a gloved hand while sampling. A “clean-hand, dirty-hand” approach may be employed to manage the elements while maintaining clean samples.

Finally, the laboratory procedures involved with measuring pH and extracting soils for inorganic nitrogen content have many details and must be followed precisely and in the order specified to assure quality data. Especially when creating reagents and combing soil and solution, these SOPs should be treated like ‘recipes.’ In addition, the domain must have at least one staff member who works on the soil protocol that is trained in proper storage and routine maintenance of the pH probe.

7 STANDARD OPERATING PROCEDURES

The following workflow diagrams display which Standard Operating Procedures (SOPs) are conducted and in what order depending on bout type. Off-year sampling occurs the majority of years while coordinated sampling is once every five years per site. Also see **Table 3** for a list of SOPs per bout type. The SOPs are generally presented in the order needed for an off-year, gradient site bout.

SOP Overview

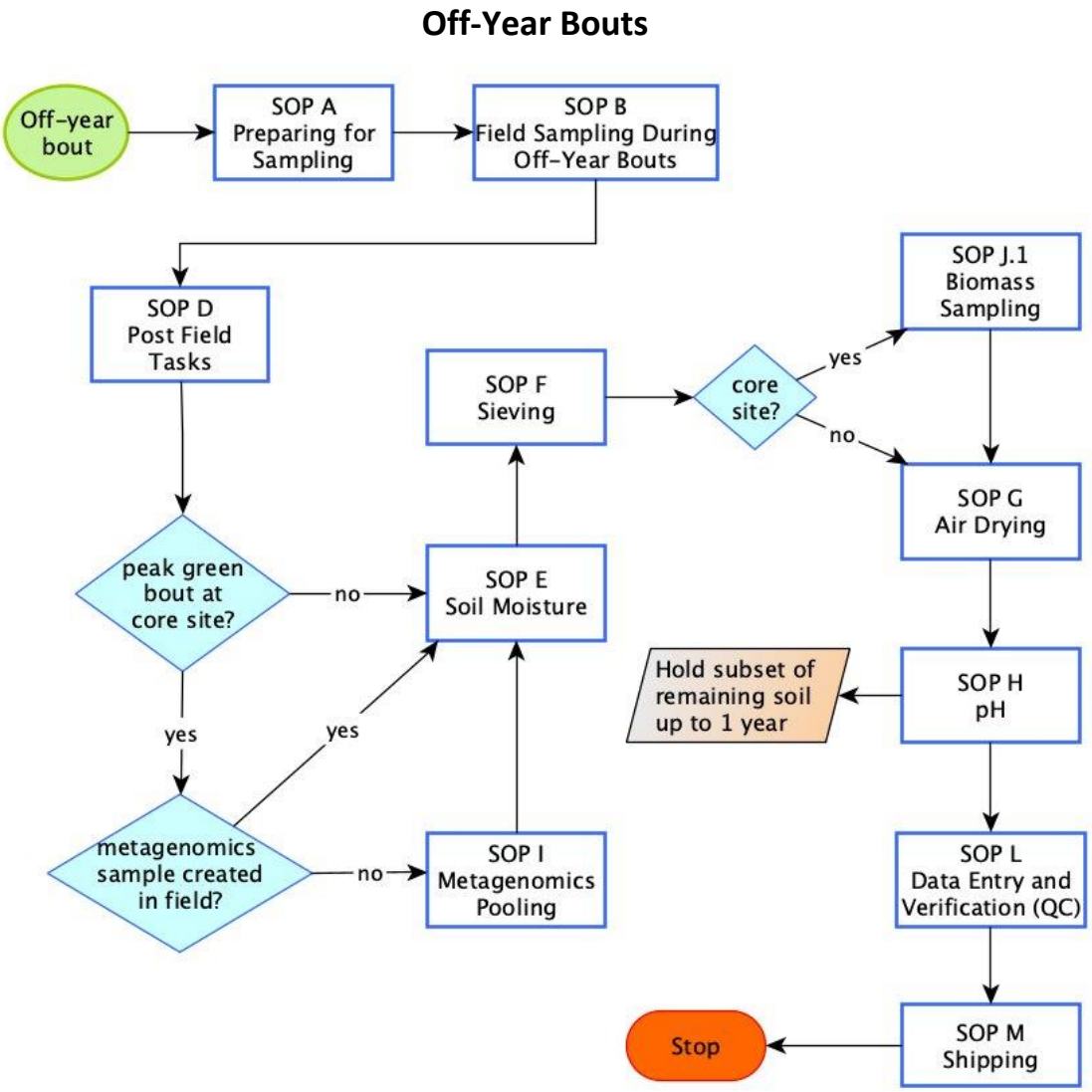

Figure 5. High-level workflow diagram showing the sequential organization of SOP's for field sampling and lab analysis/processing during an off-year, non-coordinated bout. Data verification should be completed as soon as possible after each SOP but is included only once in the diagram for simplicity. Refer to SOP G: Air Drying Soils for guidance on holding air-dried soil.

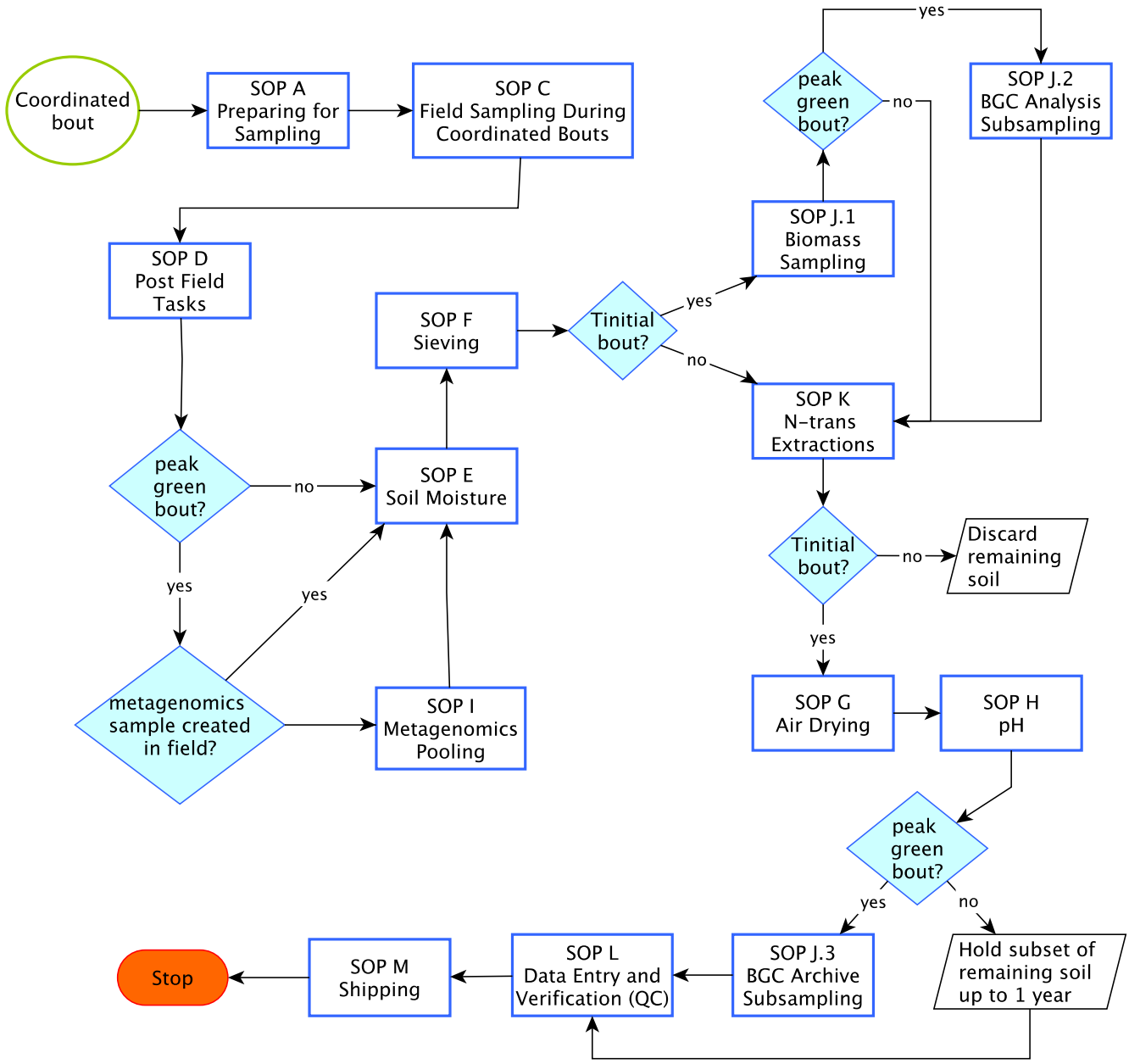

Figure 6. High-level workflow diagram showing the sequential organization of SOP's for field sampling and lab analysis/processing during a coordinated bout. Data verification should be completed as soon as possible after each SOP but is included only once in the diagram for simplicity. Refer to SOP G: Air Drying Soils for guidance on holding air-dried soil.

SOP A Preparing to Sample Soils

A.1 Preparing for Data Capture

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

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

Additionally, prior to a soil sampling bout:

1. Download and print soil X, Y coordinates for the subplots from each plot that will be sampled. Soil coordinate and subplot lists are available from the Sampling Support Library (SSL). Ensure that all coordinates sampled from previous bouts are accurately recorded on the coordinate lists to prevent repeat sampling. Refer to SOP L.2 and Appendix E for instructions on using and maintaining the soil coordinate and subplot lists.
2. Gridded plot maps may be pre-marked with target sampling locations prior to the bout. There are two options to do this:
    a. Use a full-sized printable map located in the Supporting Documents section of the SSL. Instructions on adding X, Y locations are in SOPs B.1 and C.1.
    b. If available, use the Soils QC application that is maintained by Field Science and linked in the SSL. The tool enables automated mapping of target X, Y locations using the coordinate and subplot lists, including directions to navigate to them from nearby plot markers.

A.2 Preparing for Field Sampling

Leave enough time to complete all of these steps, some items may require more than 1 day.

1. Ensure all supplies listed in **Table 20** and **Table 21** are available.
    a. For microbial genetic archive sampling, the 5 mL tissue vials listed in **Table 21** are preferred. If these are not available, 2 mL cryovials may be used instead. Vial size must be consistent within a bout and site, ensure there is enough of one kind available.
2. Check expiration dates on cryovials used for microbial genetic archive. Vials that are in open, expired packages should be discarded, but vials in expired sealed bags may still be used.
3. Plan and save sampling routes for field teams using standard site navigation procedures (RD[16]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots.

4. Check Appendix D.1 for site-specific, federal soil quarantine and permitting requirements. Note: other local permits may also be required.
5. **Be familiar with relevant site-specific instructions in Appendix D**. There is something for every domain in this site-specific section, ensure all samplers have reviewed it.
6. When using a metal coring device:
    a. Check that rust isn't present on parts of the coring device that will contact the soil. To remove rust, soak affected areas in over-the-counter white vinegar for a few hours or overnight. Rub off rust with a sponge or towel, then rinse 3x with deionized (DI) water and dry with a clean cloth or paper towel. For severe rust, a metal scrubber may be necessary.
    b. It is best to maintain consistency (± 0.5 ") in coring device diameter over time. If planning to sample with a different diameter not listed in **Table 19**, contact Science.
7. Review site-specific horizon resources linked in the NEON SSL to get a sense for types of soils likely to be encountered and whether there may be organic (O) horizons present. In addition, **Table 18** summarizes O-horizon presence and thickness from NRCS initial soil characterization.
8. Prepare soil temperature probe and pilot hole creation tool for field use. This is needed to prevent damage of the probes and ensure measurement at a standard depth.
    a. Measuring from the tip of the temperature probe, add electrical tape to the metal stem at the 10 cm mark (**Figure 7**, panel a). This marks the measurement depth and serves as a reference for where to handle the probe.
    b. On the probe, measure distance from the bottom of the 10 cm mark to the point of increasing diameter (**Figure 7**, panel b).
    c. Measuring from the end of the pilot hole device, add electrical tape at this distance (**Figure 7**, panel c)

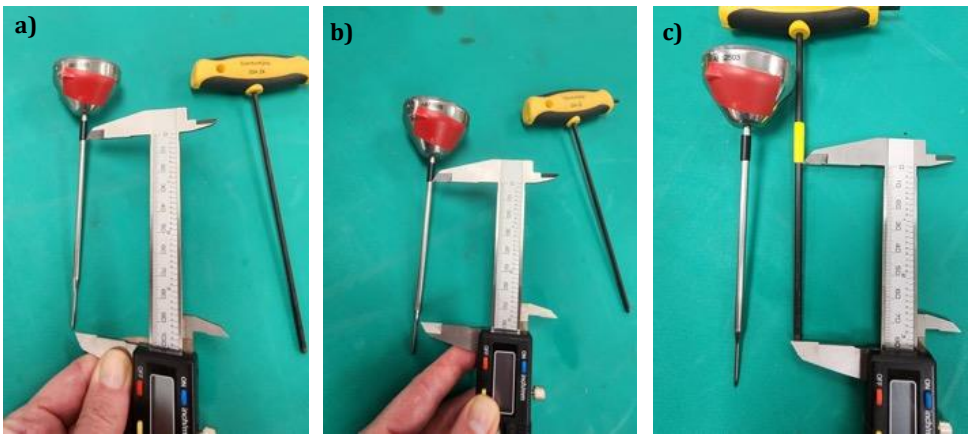

Figure 7. Preparing temperature probe and pilot hole tool to take soil temperature recordings at 10 cm depth.

9. If using a user-calibratable soil temperature probe (preferred), calibrate the unit. Time permitting, do this within 7 days of the start of each bout. At minimum, do this 3x/year, before the first T1, PG, and T2 bout for the domain.
    a. Fill a 1000 mL beaker with 800-1000 mL of sand. This setup can be re-used indefinitely once created, simply store on a bench top or in a cabinet.
    b. If freshly created, leave beaker out on the benchtop overnight to equilibrate with the room temperature.
    c. Place calibratable probes and NIST-traceable thermometer into the sand, as in **Figure 8**. Equipment can be stored in the sand if desired.
    d. Wait for five minutes, then take a reading from the NIST-traceable thermometer to the nearest 0.1 °C, understanding that temperature gradations on the thermometer are in ~0.5 °C. This is an acceptable level of precision for soil measurements.
    e. Calibrate the digital thermometer to match this temperature following the manufacturers' instructions.

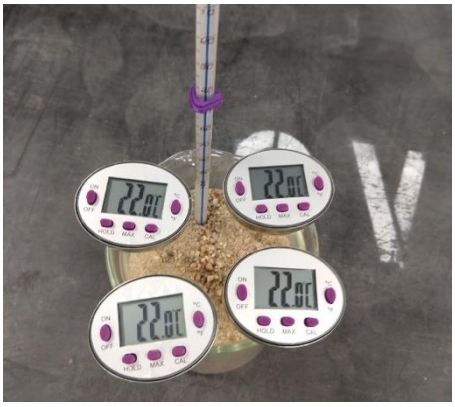

Figure 8. Temperature probe calibration setup.

10. If not using a user-calibratable temperature probe, conduct the verification procedure outlined in **Section D.3,** but at the same frequency as the calibration procedure above, namely:
    a. Time permitting, do this within 7 days of the start of each bout. At minimum, do this 3x/year, before the first T1, PG, and T2 bout for the domain.
    b. Once temperatures can no longer be verified from a given unit as specified in **Section D.3**, the item must be discarded and a new unit ordered.
11. If planning to use the laser rangefinder to navigate to X, Y locations, check the battery and charge, if needed. Also be sure to check declination at least annually and make sure the value stored in the rangefinder is updated.

12. If samples are likely to be collected from areas with heavy *Toxicodendron spp*, pack sample warning labels (**Table 20**) so that they can be applied to bulk sample bags in the field.

A.3 Preparing Sterilized Ethanol for Microbial Sampling:

In general, the simplest way to ensure sterility while collecting microbial samples is to use ethanol wipes such as those listed in **Table 21**. These wipes come pre-sterilized with ethanol and are ready-to-use. For sites that must use dry, pre-sterilized wipers, wet the wipers as follows to ensure that you are collecting high-quality microbial samples.

1. Wear clean nitrile gloves. If you are starting with ethanol that is greater than 70% strength, first make up a 70% ethanol solution by diluting your high-strength ethanol with DI water. Make up 1-Liter and store in a clean, glass (preferred over plastic if possible) bottle for up to 1 year.
2. Filter-sterilize the 70% ethanol as follows: attach a 0.2 micron sterile filter (**Table 21**) to a clean syringe (non-sterile or sterile is OK). Open a new bag of wipers and hold the filter setup above the bag. Allow the filtered ethanol to drain directly into the bag. Add enough ethanol to completely saturate the wipers - this may take a minute. You can re-use the filter multiple times in a single day and should only need one for filtering the entire 1 Liter of ethanol. However, do not re-use filters again, they should be discarded after preparing a batch of wipes.
3. Gently drain excess ethanol out of the bag and discard according to domain lab requirements. It is OK for some liquid to remain in the bag. Re-seal and place into a new, heavy-duty resealable plastic bag. As much as possible, do not touch the wipers or the interior of the bag.
4. NOTE: These directions also apply when making pre-sterilized ethanol for squirt bottles, which may be needed in the field when very hot and dry conditions cause the wipes to dry out too quickly (< 10 seconds). Before putting sterilized ethanol into the bottle, soak the clean bottle in a 1:10 bleach: DI H_2O solution for 10 minutes, then rinse with 0.2 micron sterilized DI water until no bleach odor remains (at least 6x). *Caution*: bleach is caustic: handle appropriately.
5. Store at room temperature for up to 2 months.

A.4 Additional Preparation for Coordinated Bouts

1. Ensure that the required number of incubation cylinders are available (one per soil sampling location plus 2 extra, e.g., 32). **Figure 9** shows an example PVC incubation cylinder (Schedule 40, 2" inner diameter): the bottom edge has been beveled, which helps drive it in to soil, two holes near the top aid in removal, and a piece of tape marks the 30 cm target sampling depth.
    a. Most sites use 35 cm long cylinders, but the following sites use 20 cm long ones due to thin, rocky soils: GUAN, KONZ, MLBS, RMNP, NIWO, YELL, SRER, and PUUM. *Contact NEON Headquarters if a sufficient number of appropriate length cylinders are not available at the Domain Support Facility.*

b. If cylinders have been used before: Ensure they have been scrubbed clean with laboratory soap (Alconox, Contrex, or similar detergent) and rinsed thoroughly with DI water prior to re-use. Some residual staining is expected even after this cleaning and is not a problem.

c. Ensure cylinders are not overly damaged. Small chips on the beveled edge are ok, but if more than half of the beveled edge is missing/broken, or if the cylinder has a large crack up the side, discard and request replacements. Rocky soils are notably hard on the cylinders.

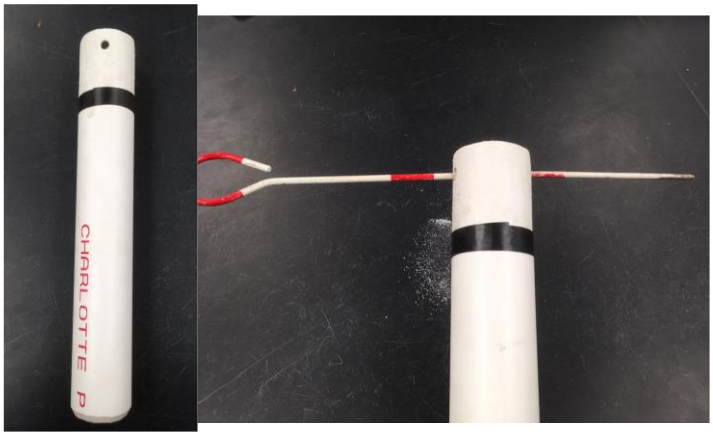

Figure 9. Example PVC cylinder used for soil N transformation sampling.

2. Check that one loosely fitting cap (e.g., designed for use with 2.25” or 2.5” or larger inner diameter PVC) per sampling location is available. Drill holes in the caps to allow them to be attached to the cylinders with plant wire or zip-ties.

3. Along with all field supplies listed in **Table 22**, verify that all laboratory supplies listed in **Table 26** are available and that equipment has been properly cleaned and/or stored according to SOP K. *Lab processing must occur within 1 day of field collection*, thus all supplies must be on hand.

4. Check expiration date on ultrapure water used to make potassium chloride solution. Water in open, expired packages should not be used, although ‘expired’ water in a sealed carboy is ok if the expiration date is within 2 years.

5. If possible, make 2M potassium chloride for extractions in advance as it can take several hours to dissolve, see SOP K for instructions.

A.5 Labels and Identifiers

All sample types generated during soil sampling require some type of label, and most require a scannable barcode in addition to a human-readable label. Barcodes improve sample traceability and improve data quality. **Table 11** provides a quick reference to the types of samples that require barcodes, as well as the container types and locations of barcodes on containers for each soil sample type.

About barcode uses and placement

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future samples. Likewise, all samples destined for the Biorepository or an external laboratory must have a barcode affixed to assist in the shipping and receipt of samples. The barcodes that are used for the various soil sample types are shown in **Figure 10**.

Figure 10. Barcode labels used for the various types of soil samples. Type I barcodes are weatherproof but are not cryo-safe; Type II, III, and IV barcodes are cryo-safe and are ideally sized for specific sample containers.

About human-readable labels

The identifier convention for most soil sample types is:

plotID - horizon - subplotID - collectDate
Ex: *CPER_001-M-21-20210630*

This is the **sampleID** for the bulk soil sample that generates all the downstream sub-samples. Identifiers for these downstream samples simply append a character string to the end of the **sampleID** that defines the sample type. For example, *CPER_001-M-21-20210630-gen* is the microbial genetic analysis sample, appended with "-gen":

For the pooled microbial metagenomics (-comp) samples, the only difference is the lack of subplotID, since samples are composited for the plot. The convention is:

plotID + horizon + collectDate + "-comp"
Ex: *CPER_001-M-20160130-comp*

Preparing Sample Containers

1. Acquire sample containers for the upcoming bout (e.g. equipment tables in Appendix F). Always bring a few extra containers to the field in case a container becomes damaged or contaminated.

a. Whirl-Pak bags and cryovials are sterile until opened: to reduce contamination, do not open containers until immediately before use. Keep new containers in a clean location such as a new resealable plastic bag, and do not use any sample container that appears damaged or was previously opened.

b. New 1-gallon freezer-safe resealable plastic bags should be used for collecting bulk homogenized soil samples. Do not re-use bags for field sampling. Clean, used bags may be suitable for other purposes, such as organizing Whirl-Pak bags after sample collection.

2. Affix barcodes and pre-printed labels to all containers that will be used for field sampling. Barcodes are not initially associated with a particular sample, so it is fine to add these in advance. Apply labels as described in **Table 11** a minimum of 30 minutes prior to sampling on room-temperature containers so they have time to adhere fully: they may also be applied at the start of the season.

3. **Only** use the labels listed in the equipment tables: do not substitute different labels as they may not meet the specifications and can fall off, thereby leading to sample loss.

a. Bulk, homogenized soil sample: Place a weatherproof Type I barcode label (prefix 'A' plus 11 numbers) on each bag. Then, affix pre-printed, human-readable labels on each bag that will hold the homogenized soil. SampleIDs can be generated ahead of time.

b. Microbial genetic analysis samples: place a cryogenic Type II barcode label (prefix 'B" plus 11 numbers) on each Whirl-Pak. Each Whirl-Pak is a unique sample and should have its own barcode label. Also affix a pre-printed, cryogenic human-readable label with geneticSampleID: sampleID + "-gen."

c. Microbial archive samples: barcode type depends on the container size. Place a cryogenic Type III barcode label (prefix 'C' plus 11 numbers) on each 5 mL vial (preferred container), or a cryogenic Type IV barcode label (prefix 'D' plus 11 numbers) on each 2 mL vial. Each container is a unique sample and should have its own barcode label. There is no human-readable label for this sample type.

d. Microbial biomass vials**:** place a cryogenic Type II barcode label (prefix 'B" plus 11 numbers) on each new plastic 50 mL centrifuge tube. Orient the barcode from top to bottom so it can be scanned (not curving around). Also affix a pre-printed, cryogenic human-readable label with biomassID: sampleID + "-bm." Ensure no label overlap (**Figure 11**, left).

e. KCl extraction vials**:** place a cryogenic Type II barcode label (prefix 'B" plus 11 numbers) to a new plastic scintillation vial. Orient the barcode from top to bottom so it can be scanned (not curving around). Also affix a pre-printed, cryogenic human-readable label with kclSampleID: sample + "-kcl." Ensure no label overlap (**Figure 11**, right).

f. BGC analysis samples: Place a weatherproof Type I barcode label (prefix 'A' plus 11 numbers) on each new glass scintillation vial. Orient the barcode from top to bottom so it can be scanned (not curving around). Additionally, affix a pre-printed human-readable label with the cnSampleID: sampleID + "-cn".

g. BGC archive samples: Place a weatherproof Type I barcode label (prefix 'A' plus 11 numbers) on each new glass bottle (**Table 24**), oriented top to bottom so it can be scanned (not curving around). Additionally, affix a pre-printed human-readable label with the bgcArchiveID: sampleID + "-ba".

Figure 11. Sample containers illustrating correct orientation of labels. Left: Microbial biomass. Right: KCl extract.

Table 11. Details on labeling and data entry for each sample type. A barcode is required for all sample types listed.

Sample Type	Data Entry App	Container Type	Required Barcode Used and Quantity	Human-readable Label Type	Location of Barcode
Homogenized field sample	SLS: Field Sampling	1-gallon resealable plastic bag	Type I 1 per sample; 30-60 per bout	Weatherproof address label	Can vary, should be flat area on gallon bag
Microbial genetic analysis (-gen)	SLS: Field Sampling	2-oz. Whirl-pak	Type II 1 per sample; 30-60 per bout	Cryogenic label	White area of whirl-pak
Microbial metagenomics (-comp)	SLS: Metagenomics Pooling	2-oz. Whirl-pak	Type II 1 per plot x horizon; 10-20 per bout	Cryogenic label	White area of whirl-pak
Microbial archive (-gaX)	SLS: Field Sampling	5 ml tissue vial (preferred) or 2 mL cryovial	Type III (for 5 mL) or Type IV (for 2 mL) 1 per sample; 150-300 per bout	None	Top of vial on the cap for 5 mL; Side of vial oriented vertically for 2 mL
Microbial biomass (-bm)	SLS: Field Sampling	50 mL centrifuge tube	Type II 1 per sample; 30-60 per bout	Cryogenic label	Side of tube oriented vertically

Sample Type	Data Entry App	Container Type	Required Barcode Used and Quantity	Human-readable Label Type	Location of Barcode
KCl extracts (-kcl)	SLS: Nitrogen Transformations	20 mL plastic scint vial	Type II 1 per sample + blanks; 33-70 per bout	Cryogenic label	Side of vial oriented vertically
BGC analysis (-cn)	SLS: BGC Sub-sampling	20 mL glass scint vial	Type I 1 per sample; 30-60 per bout	Weatherproof address label	Side of vial oriented vertically
BGC archive (-ba)	SLS: BGC Sub-sampling	240 mL glass jar	Type I 1 per sample; 30-60 per bout	Weatherproof address label	Side of vial oriented vertically

SOP B Field Sampling During Off Year Bouts

This SOP is designed to sample the top horizon only of upland soils with ≤ 2.5 cm (1 inch) of standing water. For sites containing wetlands that encounter > 2.5 cm (1 Inch) of standing water, follow TOS SOP: Wetland Soil Sampling (RD[06]), if authorized to do so in that SOP and Appendix D. For sites where > 2.5 cm (1 Inch) of standing water is present but they are not authorized to use the Wetland SOP, submit a ServiceNow ticket for additional guidance. The Wetland SOP refers back to this SOP for instructions.

An overview of the process is shown in **Figure 12**. Refer to **Table 2** and the **Quick References** section for additional reminders about which samples are produced by this SOP. Instructional videos are also available in the NEON internal Training Center. Throughout the field protocol, use sterile sampling technique as much as possible to reduce contamination and produce high-quality microbial data.

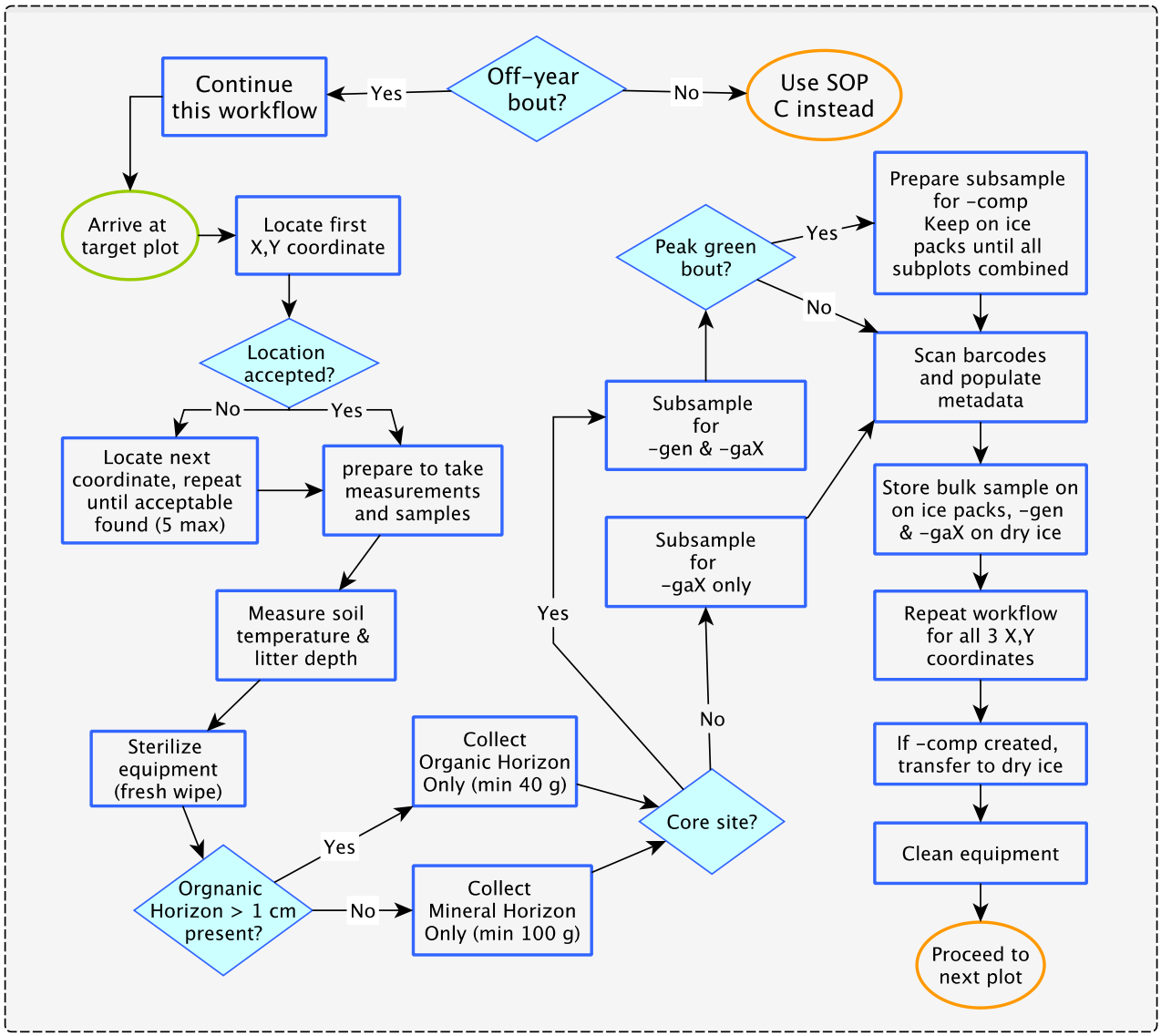

Figure 12. Workflow for SOP B, field sampling during off-year bouts.

The majority of soil sampling bouts are collected following this SOP and will be of boutType = microbes (gradient sites) or microbesBiomass (core sites). Only the top horizon is collected, meaning either an organic horizon (if present) or mineral horizon sample will be collected per X,Y location but not both.

For some sites with thin organic layers or rocky soils, it may be difficult to obtain the soil volumes indicated in the SOP's for these situations, it is recommended that field crews estimate soil masses in the field using a spring scale (or similar) to determine whether they have collected sufficient soil material. The estimated mass of soil material required for each analysis is noted for each sample type in SOPs B and C.

The presence of rocks, roots, and moisture will *drastically* affect soil mass values. Field crews must account for these factors when weighing soil samples: if not, insufficient amounts of soil will be collected. There is no hard and fast rule for estimating the mass contributions of rocks, roots and soil moisture: field crews will have to use their best judgment. Here are some suggestions:

a) Remove as much root and rock material as possible prior to weighing. Estimate the percentage of rock and root material remaining and add that to the target soil mass;
b) Estimate soil moisture and add that to the target soil mass. For soil that appears dry, add 20% to the required mass; for saturated soils, double the required mass;
c) Be conservative; assume that you need more material than you estimate, rather than less;
d) Keep a record of soil masses for future reference.

During off-year bouts, the following samples and measurements will be made:

- soil temperature
- litter depth
- microbial archive (-gaX)
- core sites only: microbial genetic analysis (-gen) and microbial biomass (-bm, in the laboratory)
- core sites only, peak green: microbial metagenomics composite (-comp)
- soil moisture (-sm, in the laboratory)
- soil pH (-pH, in the laboratory)
- Fulcrum applications related to each sample type are listed in **Table 11**.

Instructions for generating the plot-level composite microbial metagenomics sample ("-comp") in the field are provided in this SOP. Generating "-comp" samples in the field is the preferred method, but if field generation is not possible (due to bad weather, loss of daylight, etc.), technicians should follow SOP I, "Generation of composite samples" to generate samples for these analyses in the lab.

Reminder: When conducting a Coordinated bout, use SOP C.

B.1 Identify the Plot and Sampling Location

REMINDER: Always practice care when navigating to and within sample plots. Follow best practices to minimize disturbances within the plots. Refer to RD[15] and RD[16] for further guidance.

1. Upon arrival at a plot, use a navigation device to confirm that you are in the correct location. Locate one of the plot markers such as the southwest corner (point 21, **Figure 1**) as an additional source of verification. Move along the plot perimeter as much as possible, minimize foot traffic within the plot.
2. Soil will be collected at three randomly assigned locations within each plot, one in each randomly assigned subplot (Appendix E).
3. If not already done, use a laminated, gridded plot map (as shown in **Figure 1**, printed from the SSL) to mark the X, Y coordinates that are the targets for sampling. Recall that the X coordinate is the number of meters east, and the Y coordinate is the number of meters north, in relation to the southwest plot corner (point 21).
    a. Keep in mind that rejected coordinates can occur, so have back-ups ready.
4. For a particular X, Y coordinate, select the closest plot marker that is available in the field.
5. Next, calculate the distance and direction that must be navigated from each selected plot marker to a coordinate location. This step may be skipped if using pre-printed maps from the Soils QC application as those include distance and direction from nearby plot markers.
    a. Using the map, measure the distance you must traverse by counting the number of gridlines from your chosen plot marker to the X, Y coordinate drawn onto the map. Start with the longest distance first. Each gridline corresponds to 1 meter.
    b. For each X, Y coordinate, note the distances and directions from the selected plot marker to the X, Y coordinate on the map. Use these distances and directions to navigate from the selected plot marker to a sample location.
6. In relatively flat plots (<20% grade) where using a meter tape gives accurate distances:
    a. Lay out a meter tape from the selected plot marker in the X (E/W) direction. Use a compass to verify direction.
    b. Pull the tape to the measured distance to the target X coordinate and mark the point.
    c. From that point, navigate in the Y (N/S) direction to the measured distance to the target Y coordinate. Use a compass to verify direction.
    d. Place a marker at the X, Y location.
7. In plots with steeper slope (>20% grade), or in heavily forested plots where pulling a tape is not possible, use a laser rangefinder set to HD (horizontal distance) mode to locate X, Y coordinates.

a. Clean lenses with lens cloth or lens tissue, if needed.

b. Check/set correct declination. See RD[11] for details.

c. Calibrate the TruPulse tilt sensor (only needed after severe drop-shock; see RD[11]).

d. Two technicians must work together. One stands at the selected plot marker and operates the rangefinder. The second person navigates to the first potential X-location, following the directions of the rangefinder operator and using the reflective tape so that an accurate horizontal distance measurement can be obtained.

e. The rangefinder operator must ensure that the angle (azimuth) is as close to 90° as possible from True North when measuring the X-coordinate distance.

f. Place a marker, such as a pin flag or stake, at the X-location.

g. The rangefinder operator then moves to stand directly over the marker. Using either a measuring tape or the TruPulse with a reflective surface, work with the second person to locate the Y-coordinate location.

h. Ensure that the azimuth is as close to 0° (True North) as possible and measure the Y-coordinate distance.

i. Place a marker at the X, Y location.

B.2 Assess Sample Location

1. Put on a clean pair of nitrile gloves. At the same plot, gloves can be re-used after rinsing with DI water to remove coarse debris and drying thoroughly. Do NOT reuse gloves between plots.
2. Assess the location for sampling suitability:
    - Are there obvious disturbances, vegetation, large rocks, or roots that would impede sampling within a 0.5 m radius of the X, Y location?
    - In Tower plots, does the coordinate fall within 2 m of the perimeter of a ground or elevated litter trap?
3. If any of the above conditions are met, reject the location and record why on the soil coordinate list. Move to the next coordinate location on the subplot list until an acceptable one is found.
    - If five X, Y coordinates are rejected, do not sample within that subplot and submit a ServiceNow ticket. Make a record for that subplot and choose “Coordinates not suitable” in the **samplingImpractical** field, with X, Y coordinates and other metadata blank. Do not attempt to sample the fourth subplot that was not randomly selected for this bout.
    - If a disturbance is the dominant condition of a subplot (e.g., more than half the area is covered in debris from logging), do collect a sample but use the **horizonDetails** field along with **remarks** to provide more information on this condition.

- In drylands, do not reject locations due to presence of mature biological soil crust (see Appendix D.7).

4. At an accepted location, start near the exact location of the X, Y coordinate and assess soil depth by probing the soil using a sterilized pigtail stake (or other poking device free of paint and rust). Move outward up to 0.5 m from the X, Y location until a suitable spot is found where the probing device does not hit an impediment.

    a. The target depth for soil sampling is 30 cm, or the entire horizon if thinner. Some sites have characteristically shallow and rocky soils. At these sites, a suitable location may not allow coring to 30 cm depth - this is acceptable.

    b. Suitability varies from site to site and based on coring device, but in general will allow sufficient sample collection with one to three brownies or cores.

5. Mark the exact target sampling area identified via probing with a flag or pigtail stake.

6. Assess whether there is an O horizon present that is > 1 cm in thickness. If so, B.4 will be used to collect a sample. If not, B.5 will be used. Only the top horizon is collected during this SOP.

7. If the surface soil is mixed (e.g., from logging, tilling) and horizon type is difficult to determine, classify as O or M based on whether mineral or organic material dominates.

    a. Take a small mass of soil in your hand from near the sampling area and assess its texture. If it feels grainy or gritty, treat it as an M horizon. If smooth, friable, and a bit greasy, treat as an O horizon. Ensure that handled soil is not included in the sample collection. Note 'Horizon indeterminate' in the **horizonDetails** field if still uncertain.

8. If there is heavy *Toxicodendron spp* in the location you will core, take steps to avoid exposure to toxic oils as described in RD [10]. Be prepared to record *Tox* presence both in the SLS: Field data entry application as well as on the physical sample bag by applying a warning label sticker.

B.3 Measure Soil Temperature and Litter Depth

1. **Create pilot hole.** At a spot adjacent to but not directly on top of the marked sampling location, push aside the litter layer and create a pilot hole with the pilot hole tool (**Figure 13**, panel a). Try multiple locations until little resistance is found. Insert pilot hole tool to the bottom of electrical tape and spin.

2. **Measure soil temperature.** Insert temperature probe into the pilot hole to a depth of 10 cm, which is indicated by the bottom of the electrical tape on the probe stem (**Figure 13**, panel b).

    a. When handling the probe, do not push or pull on the digital display as this may cause the unit to break. Instead, handle at the top of metal stem under the digital display, at or near the electrical tape marking (**Figure 13**, panel c). Do not touch the tip of the probe.

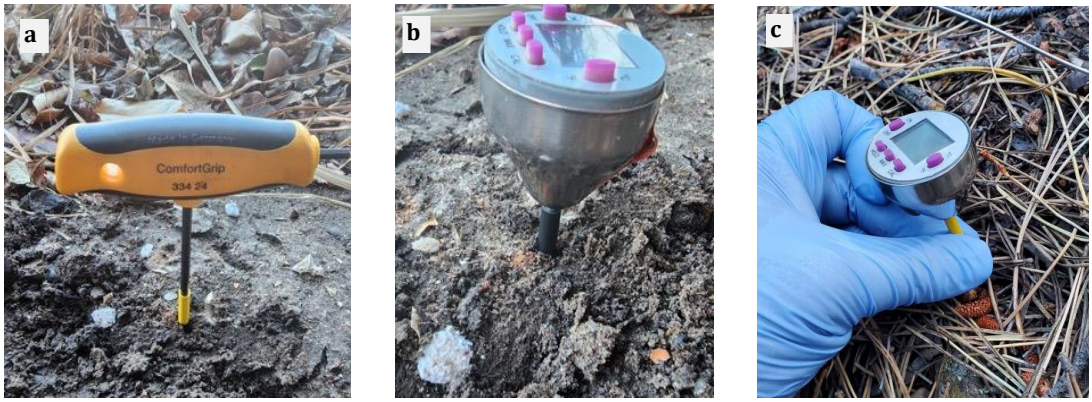

Figure 13. Creating a pilot hole, then taking a soil temperature reading at 10 cm depth.

b. Allow probe to equilibrate for about 2 minutes, then record **soilTemp** in degrees C. *Important:* Do not make measurement with the sun directly on probe, if needed shade it with your body or a piece of field equipment (clipboard, backpack, etc.)

3. **Measure litter layer depth**. In the area around the marked sampling location, use a ruler to measure the depth of the undisturbed litter layer in cm (**litterDepth**, ± 0.1 cm). Take a few readings as this parameter is likely to be spatially variable at a fine scale, then record the average value – the Fulcrum application has a calculator to help with this.

    - Gently wiggle the ruler through the layer to the ground surface, minimizing disturbance to the litter profile to minimize compaction.

***Reminder**: The litter layer is dead and detached but recognizable, intact plant material (i.e., leaves, wood, etc) that is not consolidated, whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition that is consolidated as a layer.*

B.4 Collect Organic (O) Horizon

Only follow this step if the sampling location has an O horizon with a depth > 1.0 cm. If a location has no O horizon or one that is ≤ 1.0 cm, skip this step and proceed directly to B.5.

***Reminder**: All soil collected for a single sample should be located as close to the X,Y coordinate as possible, and should be no more than 0.5 m from the X, Y coordinate. Soil coordinates are provided in 0.5 m increments. Sampling outside the buffer region may cause future sampling locations to overlap.*

1. Push the litter layer away from where you are going to core into the soil surface.
2. Sterilize gloves and tools with 70% ethanol. Use new ethanol wipes for each X, Y location. Within a location, fold wipe after sterilizing each tool to expose a fresh/unsoiled surface.

3. Cut out an organic horizon "brownie" using a frame cutter tool and soil knife (hori-hori, **Figure 14**). With deep organic horizons, only 1 brownie may be needed; from many sites, two or three will be needed. At those sites, select multiple locations within 0.5 m of each other.

Figure 14. Example use of brownie cutter and hori-hori for O horizon sampling.

4. Record the **sampleTopDepth** as the depth from the soil surface (0 cm). Measure the depth of each side of the brownie hole(s) and determine the average value. Only sample to 30 ± 1 cm maximum depth. Record in the field **sampleBottomDepth**.
5. Record appropriate **horizonDetails** entries if applicable, for example 'Woody horizon' (8D.6) or 'Thin M horizon present' plus additional **remarks** as needed.
6. Place all brownies collected at an X, Y location into a pre-labelled, 1-gallon resealable plastic bag. With a pre-sterilized, gloved hand, remove rocks, insects, recognizable and undecomposed plant material, and other non-soil debris and homogenize. Shake large root masses to dislodge soil, then discard. Break up large clods to facilitate subsampling.
    a. If it is difficult to pick the entire bag of homogenized soil in the field, plan to use a pair of pre-sterilized tweezers to pick rocks, roots, and non-soil debris from the microbial subsamples while generating them. Re-sterilize tweezers with ethanol and a sterile wipe in between samples. Regardless of approach, it is *imperative* that field subsamples are picked clean of non-soil material: failure to do so can render the samples useless.
7. With wet or saturated soils, dump out any excess water after the soil has settled for 10-15 secs.
8. **Ensure that you have sufficient soil for all subsampling and analyses:** In general, using the methods described above should provide plenty of soil for all subsampling and lab processing. However, if concerned, aim for a *minimum* 40 g of organic soil in the bulk homogenized bag.
9. **Generate microbial analysis (-gen, Core sites only) and archive (-gaX, all sites) samples**: Aliquot subsamples from the 1-gallon bag of homogenized soil into 1 labeled Whirl-Pak bag (fill about halfway, 5-10 g target mass) and/or 5 labeled cryovials (fill to about 70% capacity).
    a. Close Whirl-Pak such that the labels lay flat and are clearly visible, then complete the label. Final samples should appear as shown in **Figure 15**.

The microbial genetic analysis sample label should appear as:

plotID-horizon-subplotID-YYYYMMDD-gen
(ex. HARV_001-O-21-20210415-gen)

b. For collecting into the cryovials, scoop soil into the container using a scoop that has been sterilized with ethanol. Any non-soil material that ends up in a container should be removed with sterilized tweezers or by hand using pre-sterilized gloves. Keep caps face-down on a sterilized surface.

   i. If using 2 mL vials with a smaller opening, sterilized tweezers and/or a small ethanol-sterilized 'ramrod' device may be used to load soil into vials.

c. For organizational purposes, it is best to keep all 5 cryovials from a given subplot in a bag labeled with plotID and subplotID, or keep them in a single row of a cryovial box.

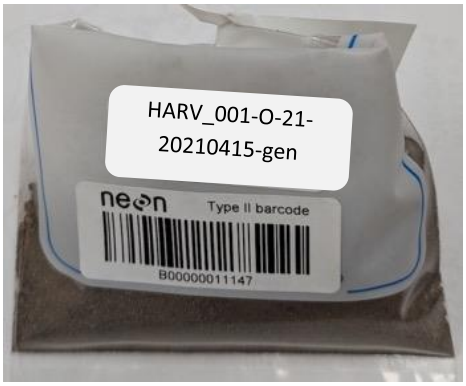

Figure 15. Example microbial genetic (-gen) sample with completed label. Microbial metagenomics (-comp) samples will look similar except for the content of the label.

10. Scan the barcode label for each sample into the mobile data recorder. Ensure that each barcode label is entered only once and is associated with the correct sample, although for -gaX samples, the barcodes are interchangeable. To reduce data entry errors, it is recommended that barcode labels are scanned *during* sample creation, rather than in batches of multiple samples.

11. Place microbial samples on dry ice immediately after verifying labels are correct and complete to retain sample integrity. Ensure all newly added samples are in contact with dry ice so that they freeze quickly. *If dry ice could not be procured, follow instructions in the FRZ SOP [RD18] to freeze and store samples using ultra-cold ice packs.*

12. **During Peak Greenness, Core Sites: Generate microbial metagenomics (-comp) sample**: generate a plot-level composite sample by subsampling material from each X, Y coordinate within a plot that is of the same horizon. Use a pre-sterilized (with 70% ethanol) scooping device such as a coffee scoop or tablespoon when generating the composite sample. Approximately equal amounts of soil should be composited from each X, Y location.

a. If this is the first X, Y location to be sampled at a plot, label a 2-oz Whirl-Pak as follows:

plotID-horizon-collectDate-comp
(ex. HARV_001-O-20210715-comp)

i. Place one scoop of homogenized soil in the Whirl-Pak, close bag, and place on ice packs.

b. If this not the first X, Y location to be sampled at a plot, obtain the Whirl-Pak created earlier from the cooler with ice packs. Check that horizon ID matches the horizon ID for the sample you want to add. If this is a new horizon for this plot, create a new Whirl-Pak.

i. Place one scoop of homogenized soil in the Whirl-Pak and close the bag.

ii. If another X, Y location within the plot might be added to this bag, return the bag to cooler with ice packs.

c. If this is the last X, Y location for this plot, mix the soil by gently massaging the outside of the bag and/or inverting and shaking. Close Whirl-Pak such that the labels lay flat and are clearly visible (**Figure 15**), and record the **compositeSampleID** in the SLS: Metagenomics Pooling application. Also scan the barcode label into the application. As soon as the bag is closed and the data has been entered, place the bag on dry ice and ensure that newly added Whirl-Paks are in contact with dry ice. Microbial activities will change rapidly until frozen.

Note: If conditions do not permit a composite sample to be created in the field, simply collect an additional Whirl-Pak at each sample coordinate and follow SOP I for generating a composite sample in the Domain Support Facility (DSF).

13. **Ensure that sufficient organic soil remains for lab subsampling and analyses**: below is the minimum but more soil is acceptable.

- microbes or microbesBiomass bout (off-year, any season): 30 g

Note: Any homogenized soil in excess of 250 g can be dumped back into the borehole according to the site host agreement.

14. For labelling and data recording, be sure that:

- The homogenized sample bag is labeled with the **sampleID**, **measuredBy**, and **recordedBy.**
- If a *Toxicodendron spp* was abundant, apply a sample warning label sticker to the homogenized bag of soil.
- Whirl-Paks are labelled with the **geneticSampleID** or the **compositeSampleID**.
- All barcode labels have been scanned and the values populated correctly into the Fulcrum application. Perform a quick visual scan to catch errors or duplicated barcodes.

15. **Ensure that all samples are stored correctly.** Whirl-Paks and cryovials should be in the cooler in contact with dry ice (microbial activity changes very quickly). Double check that all samples are frozen, and shift samples/dry ice as needed to ensure that they stay frozen. Ensure 1-gallon bags containing homogenized samples are well sealed and place in the cooler with the ice packs.

B.5 Collect Mineral (M) Horizon

For off-year bouts, only collect an M horizon sample if you do not have an O horizon at your X, Y coordinate, or if the O horizon is ≤ 1.0 cm, in which case it gets sampled along with the M. Dryland sites, refer to Appendix D.7 for instructions related to biological soil crusts.

***Reminder**: All soil collected for a single sample should be located as close to the X, Y coordinate as possible, and should be no more than 0.5 m from the X, Y coordinate. Soil coordinates are provided in 0.5 m increments. Sampling outside the buffer region may cause future sampling locations to overlap.*

1. Pre-sterilize sampling equipment (**Figure 16**). Use new ethanol wipes for each X, Y location. Within a location, fold wipe after sterilizing each tool to expose a fresh/unsoiled surface.
2. Core to a depth of 30 cm (± 1 cm) or saprolite, whichever is shallower. Always core vertically, not perpendicularly, when collecting on a slope.
    a. A piece of masking or lab tape should be placed on the outside of the corer to indicate the depth to stop driving the corer into the soil (**Figure 16**). It is also acceptable to core incrementally (e.g., in 10 cm increments) to reach 30 cm, whichever works best.
    b. Do not sample past 30 cm. Deeper cores should be discarded, collect a new 30 (± 1) cm core.

Figure 16. Field staff sterilizing a soil corer using a 70% ethanol wipe. Gloves should be pre-sterilized.

3. If a significant impediment to coring is encountered that is not representative of that location, replace the soil back in the borehole and move to another location within the 0.5 m radius. It is not necessary to re-sterilize the coring device.

4. Record the **sampleTopDepth** as depth from the soil surface (0 cm). Measure **sampleBottomDepth** as the depth from the soil surface to the bottom of the sample, measured in the borehole. When taking multiple cores (see info box below), record average depth values.

Remember: *O horizons ≤ 1.0 cm are not sampled as a separate horizon. If a thin O horizon is collected as part of the M horizon sample, select 'Thin O horizon present' in the* ***horizonDetails*** *field.*

> HOW MANY SOIL CORES?
>
> The number of M horizon cores needed per location depends on factors including the soil corer used, type of sampling bout, and local soil conditions. The goals are to collect a sample that represents the local soils and procure enough material for all samples and downstream analyses. If you can core to 30 cm depth and are using a 2" diameter or greater coring device, one core should provide sufficient material for all samples and analyses. If using a smaller diameter coring device, or working in plots with shallower soils or more coarse fragments (e.g. rocks and roots), 2 or 3 cores may be needed; ensure that each is taken to the max depth possible at that X, Y location. Mass measurements can help to ensure that sufficient material has been collected. ***For questions related to sample quantities for a particular site, contact NEON Science via a ServiceNow ticket.***

5. If multiple cores were collected at a location, combine cores to create one composite sample of the M horizon, then place into a pre-labelled, 1-gallon resealable plastic bag (**Figure 17**). Homogenize (mix) the soil thoroughly. When possible, avoid contact with the soil by closing the bag and mixing by inverting and massaging. If this does not sufficiently homogenize the soil, then with a pre-sterilized, gloved hand, homogenize the soil. Remove any large rocks, coarse roots (> 2 mm diameter), other plant material, and insects using a sterilized, gloved hand.

    a. With wet or saturated soils, dump out any excess water in the sample container after the soil has settled 10-15 seconds, if present.

Figure 17. Dispensing soil core into bag for homogenization. For certain soils and sampling devices, tapping the corer with a rubber mallet may be very helpful in releasing soil from the barrel.

6. **Ensure sufficient soil for all subsampling and analyses:** In general, using the methods described above should provide plenty of soil for all subsampling and lab processing. However, if concerned, aim for a *minimum* of 100 g of mineral soil in the bulk homogenized bag.

7. **Generate microbial analysis (-gen, Core sites only) and archive (-gaX, all sites) samples**: Aliquot subsamples from the 1-gallon bag of homogenized soil into 1 labeled Whirl-pak bag (fill about halfway, 10-20 g target mass) and/or 5 labeled cryovials (fill to about 80% capacity).

    a. Close whirl-pak such that the labels lay flat and are clearly visible, then complete the label. **Figure 15** shows examples of properly labelled samples.

        The microbial genetic analysis sample label should appear as:

        plotID-horizon-subplotID-YYYYMMDD-gen
        (ex. HARV_001-M-21-20210415-gen)

    b. For collecting into the cryovials, the simplest approach may be to insert the open container into the bag of homogenized soil with a pre-sterilized, gloved hand, and press in the soil with the wall of the homogenized gallon bag. Pouring or scooping soil into the container using a sterile scoop is also acceptable. Keep cryovial caps face-down on a sterilized surface.

        i. If using 2 mL vials with a smaller opening, a small ethanol-sterilized 'ramrod' device may be helpful to load soil into vials.

    c. For organizational purposes, it is best to keep all 5 cryovials from a given subplot in a bag labeled with plotID and subplotID, or keep them in a single row of a cryovial box.

8. Scan the barcode label for each sample into the mobile data recorder. Ensure that each barcode label is entered only once and is associated with the correct sample, although for -gaX samples, the barcodes are interchangeable. To reduce data entry errors, it is recommended that barcode labels are scanned *during* sample creation rather than in batches of multiple samples.

9. Place microbial samples on dry ice immediately after verifying labels are correct and complete to retain sample integrity. Ensure that all newly added samples are in contact with dry ice so that they freeze quickly. *If dry ice could not be procured, follow instructions in the FRZ SOP [RD18] to freeze and store samples using ultra-cold ice packs.*

10. **During Peak Greenness: Generate microbial metagenomics (-comp, Core sites only) sample**: generate a plot-level composite sample by subsampling material from each X, Y coordinate sampled within a plot that is of the same horizon. Use a pre-sterilized (with 70% ethanol) scooping device such as a coffee scoop or tablespoon when generating the composite sample. Approximately equal amounts of soil should be composited from each X, Y location.

    a. If this is the first X, Y location to be sampled at a plot, label a 2-oz Whirl-Pak as follows:

        plotID-horizon-collectDate-comp
        (ex. HARV_001-M-20210715-comp)

        i. Place one scoop of homogenized soil in the Whirl-Pak, close bag, and place on ice packs.

    b. If this not the first X, Y location to be sampled at a plot, obtain the Whirl-Pak created earlier from the ice packs cooler. Check that the horizon ID matches the horizon ID for the sample you want to add. If this is a new horizon for this plot, create a new Whirl-Pak.

        i. Place one scoop of homogenized soil in the Whirl-Pak and close the bag. Aim for the same volume of soil from each X, Y location.

        ii. If another X, Y location within the plot may be added to this bag, return the bag to cooler with ice packs.

    c. If this is the last X, Y location for this plot, mix the soil by gently massaging the outside of the bag and/or inverting/shaking. Close Whirl-Pak such that the labels lay flat and are clearly visible, and record the **compositeSampleID** in the SLS: Metagenomics Pooling application. Also scan the barcode label. As soon as the bag is closed and the data have been entered, place the bag on dry ice and ensure that newly added Whirl-Paks are in contact with dry ice so that it freezes as quickly as possible. Microbial activities will change rapidly until frozen.

***Note**: If conditions do not permit a composite sample to be created in the field, simply collect an additional Whirl-Pak at each X, Y location and follow SOP I for generating a composite sample in the domain lab.*

11. **Ensure that sufficient mineral soil remains for lab subsampling and analyses**: below is the minimum but more soil is acceptable

    - microbes or microbesBiomass bout (off-year, any season): 60 g

    Note: Any homogenized soil in excess of 250 g can be dumped back into the borehole according to the site host agreement.

12. For labelling and data recording, be sure that:

    - The bulk, homogenized sample bag is labeled with **sampleID**, **measuredBy**, and **recordedBy**
    - If a *Toxicodendron spp* was abundant, also apply a sample warning label sticker to the homogenized soil bag.
    - Whirl-Paks are labelled with the **geneticSampleID** or the **compositeSampleID**.
    - All barcode labels have been scanned and the values were populated correctly into the Fulcrum application. Perform a quick scan to catch any erroneously duplicated barcodes.

13. **Ensure that all samples are stored correctly.** Whirl-Paks and cryovials should be in the cooler in contact with dry ice (microbial activity changes very quickly). Double check that all samples are frozen, and shift samples/dry ice as needed to ensure that they stay frozen. Ensure 1-gallon bags containing homogenized samples are well sealed and place in cooler with the ice packs.

TIPS & TRICKS For ease of sample tracking during storage and shipment, organize Whirl-Pak bags into a larger freezer-safe bag. Cryovials can be stored in cryogenic storage boxes (**Table 21**).

B.6 Data Entry

1. Update the paper soil X, Y coordinate list and subplot list
2. Enter metadata in the SLS: Field Sampling application or field datasheet:

    - NtransBoutType = None
    - boutType (microbes or microbesBiomass), ensure that all sampleID's have been appropriately generated.
    - sampleTiming – as specified in **Table 4**
    - biophysicalCriteria – as specified in **Table 9**
    - samplingProtocolVersion
    - siteID
    - plotID
    - subplotID
    - collectDate (YYYYMMDD)
    - coreCoordinateX
    - coreCoordinateY
    - standingWaterDepth (nearest 0.1 cm)

- time (HH:MM)
- soilTemp (nearest 0.1 degree)
- litterDepth (nearest 0.1 cm)
- toxicodendronPossible (choice of Y/N)
- sampleTopDepth (nearest 0.1 cm)
- sampleBottomDepth (nearest 0.1 cm)
- samplingDevice
- numberCores
- horizon
- horizonDetails – as specified in **Table 12**
- genetic sample and/or archive sample preparation method (default is dry ice, select 'ultra-cold ice packs' if following the FRZ SOP [RD18])
- geneticArchiveSampleCount
- geneticArchiveContainer (5 mL or 2 mL vial)
- sampleExtent (Entire = entire horizon sampled, or to saprolite/bedrock; Obstruction = sampled to an obstruction such as rock, root, etc; Maximum = sampled to maximum depth allowed by the protocol (30 cm), horizon may extend deeper; Unknown = extent varied across cores or could not be determined)
- remarks - *only those relevant to an end user and not already captured in the data, use standardized remarks where possible*
- measuredBy
- recordedBy

When using a mobile data recorder, ensure barcodes have been scanned to the appropriate sample.

B.7 Field Clean-up

1. Between plots, thoroughly rinse or wipe down reusable sampling equipment (corer, thermometer, hori hori, etc) with deionized water. For very dirty equipment, use a scrub brush or wipe. Then sterilize using 70% ethanol wipes or an ethanol squirt bottle.
2. Discard gloves between plots.

B.8 Sample Preservation and Transport

1. For transport, ensure that samples comply with any quarantine and/or site host requirements, such as double bagging samples, ensuring that all insect pests have been removed, required permits are on-hand, etc.
2. Keep bulk, homogenized bags of soil in the cooler with the ice packs; transfer to 4°C refrigerator upon return to domain lab. Ensure that sample bags are well sealed to prohibit moisture loss.

3. Keep soils for microbial genetic analysis (-gen), genetic archiving (-gaX), and microbial metagenomics (-comp) in the cooler with dry ice; transfer to ultralow freezer upon return to domain lab. All of these sample types are shipped according to SOP M with no additional laboratory processing, though labels and barcodes must be QC'd before shipment (SOP L).

SOP C Field Sampling for Coordinated Bouts

This SOP is designed to sample both organic and mineral horizons if present in upland soils with ≤ 2.5 cm (1 inch) of standing water. For sites containing wetlands that encounter > 2.5 cm (1 Inch) of standing water, follow TOS SOP: Wetland Soil Sampling (RD[06]), if authorized to do so in that SOP and Appendix D. For sites where > 2.5 cm (1 Inch) of standing water is present but they are not authorized to use the Wetland Soil Sampling SOP, submit a ServiceNow ticket for additional guidance. The Wetland SOP refers back to this SOP for instructions.

An overview of the process is shown in **Figure 18** and **Figure 19**. Refer to **Table 2** and the **Quick References** section for additional reminders about which samples are produced by this SOP. Helpful instructional videos are also available in the NEON internal Training Center. Throughout the field protocol, it is essential to ensure clean and sterile sampling techniques as much as possible in order to reduce contamination and produce high-quality microbial and biogeochemical data.

Soil sampling bouts collected following this SOP will occur every five years and be of boutType = 'microbesBiomass' (T1/T2) or 'microbesBiomassBGC' (PG). If both an organic horizon > 1 cm deep and a mineral horizon are present, both should be collected, although field microbial subsamples are only collected from the top (organic) horizon unless it is a PG bout.

During coordinated bouts, the following samples and measurements will be made:

- soil temperature
- litter depth
- N-trans incubation core deployed
- microbial genetic analysis (-gen) and archiving (-gaX)
- peak green: microbial metagenomics analysis (-comp)
- soil moisture (-sm, in the laboratory)
- soil pH (-pH, in the laboratory)
- N-trans extractions for inorganic N (-kcl, in the laboratory)
- microbial biomass (-bm, in the laboratory)
- peak green: soil BGC analysis and archiving (-cn and -ba, in the laboratory)
- A list of Fulcrum applications for each sample type can be found in **Table 11**

Instructions for generating the plot-level composite sample ("-comp") in the field are provided in this SOP. Field generation of the "-comp" sample is the preferred method, but if field generation is not possible (due to bad weather, loss of daylight, etc.), technicians should follow SOP I to generate samples for these analyses in the lab.

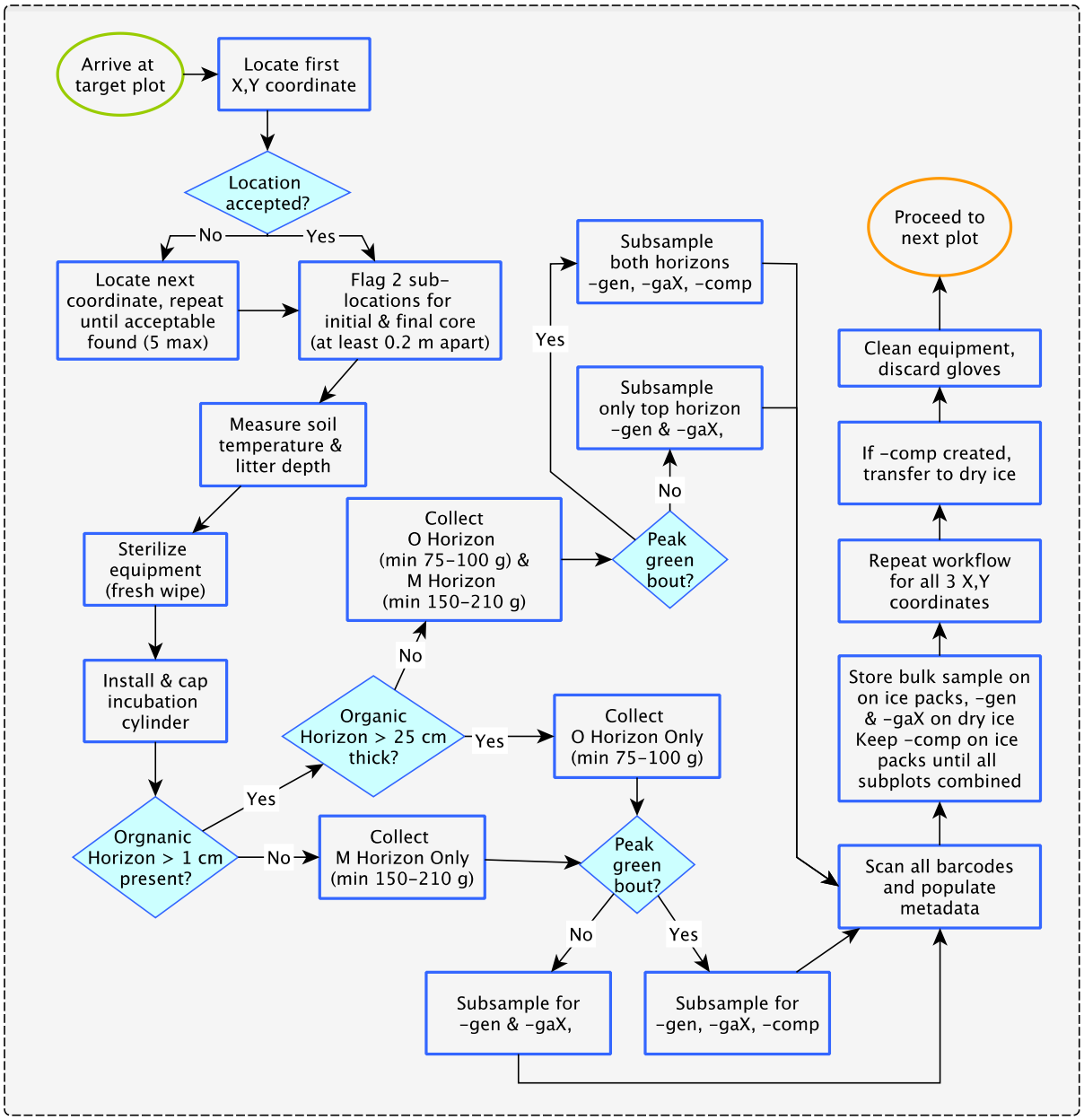

Figure 18. Workflow for SOP C, field sampling for Tinitial during coordinated bouts. The lower bound for soil mass ranges is for transition season bouts, whereas the upper bound is for Peak Green bouts.

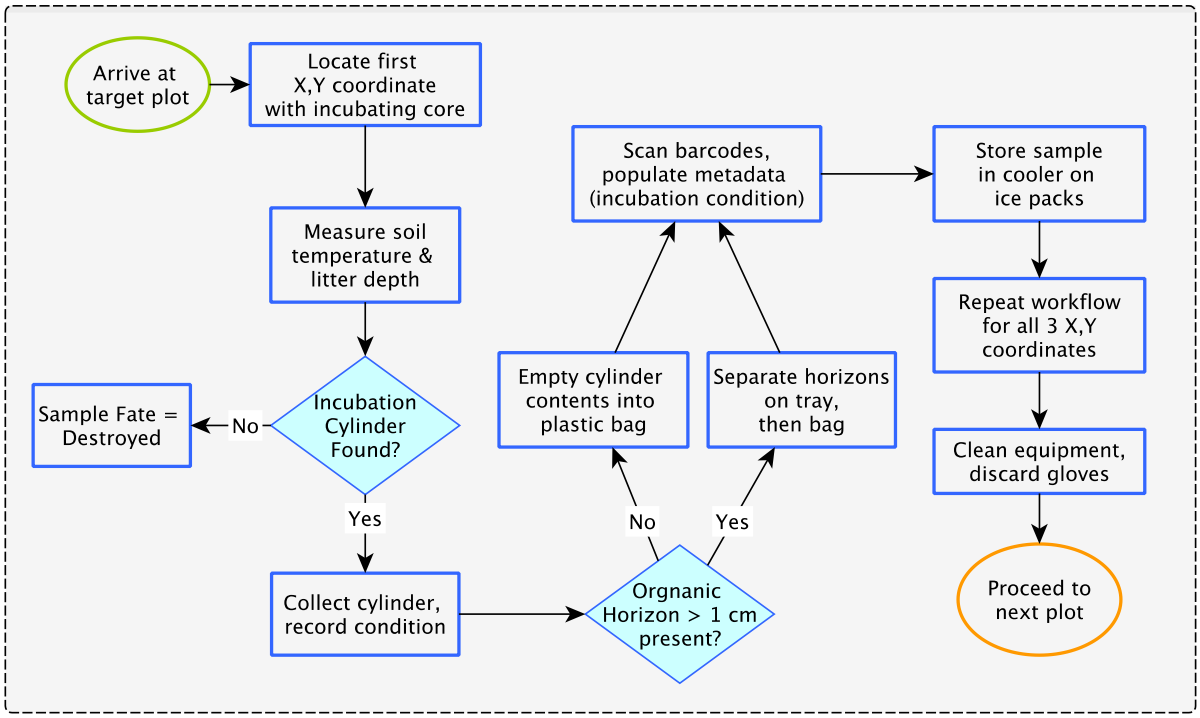

Figure 19. Workflow for SOP C, field sampling for Tfinal during coordinated bouts.

During Coordinated bouts, N transformation rate measurements are conducted. This involves collecting a T-initial sample for initial inorganic nitrogen concentration measurements, as well as deploying an incubated T-final core that will be left in the field for 2-4 weeks (duration depends on temperature, **Table 16**) and collected for final inorganic nitrogen concentration measurements. As these measurements are coordinated with microbial and biogeochemical sampling, **the same soil material used for N-transformation T-initial analysis is subsampled for microbial and all other downstream analyses**. If both O and M horizons are present, both are sampled and processed separately.

Note: For wetland sampling where plots have a shallow water table (<30 cm below the soil surface) or standing water (<= 50 cm depth), a modified method is needed because water table fluctuations can cause nitrogen leaching from the covered core, compromising the integrity of N transformation estimates. Instead of using incubated covered cores, the 'buried bag' method must be used. See TOS SOP: Wetland Soil Sampling (RD[06]) and follow the instructions therein if your site is authorized to do so (Appendix D, section D.4). The Wetland SOP refers back to this SOP for instructions.

Coordinated bout field sampling should occur in reasonably sized groups/batches of samples – minimum 2-3 plots per day but ideally more, in order to maximize efficiency of laboratory processing. Each day that samples are processed in the lab requires creation of several procedural blanks for N-transformation samples, and this consumes time and resources. If possible, all field sampling should occur in one long field day. A team of 2 or 3 can then conduct the required lab procedures the following day. Experience shows that this is most feasible for the T-final incubated sample collection. For T-initial

collections, it is acceptable to split sample collection and processing into two or three 'mini-bouts,' alternating between field and lab days (or, using staggered field and lab teams). It is not critical that all cores incubate for the exact same number of days, as long as the incubation length is recorded and incubations begin and end according to the guidelines in Section 4.2 and Appendix C.

C.1 Identify the Plot and Sampling Location

REMINDER: Always practice care when navigating to and within sample plots. Follow best practices to minimize disturbances within the plots. Refer to RD[15] and RD[16] for further guidance.

1. Upon arrival at a plot, use a navigation device to confirm that you are in the correct location. Locate one of the plot markers such as the southwest corner (point 21, **Figure 1**) as an additional source of verification. Move along the plot perimeter as much as possible, minimize foot traffic within the plot.
2. Soil will be collected at three randomly assigned locations within each plot, one in each randomly designated subplot (see Appendix E).
3. If not already done, use a laminated, gridded plot map (as shown in **Figure 1**, printed from the SSL) to mark the X, Y coordinates that are the targets for sampling. Recall that the X coordinate is the number of meters east, and the Y coordinate is the number of meters north, in relation to the southwest plot corner (point 21).
    d. Keep in mind that rejected coordinates can occur, so have back-ups handy.
4. For a particular X, Y coordinate, select the closest plot marker that is available in the field
5. Next, calculate the distance and direction you need to navigate from each selected plot marker to a coordinate location. This step may be skipped if using pre-printed maps from the Soils QC application as those include distance and direction from nearby plot markers.
    a. Using the map, measure the distance you must traverse by counting the number of gridlines from your chosen plot marker to the X, Y coordinate drawn onto the map. Start with the longest distance first. Each gridline corresponds to 1 meter.
    b. For each X, Y coordinate, note the distances and directions from the selected plot marker to the X, Y coordinate on the map. Use these distances and directions to navigate from the selected plot marker to a sample location.
6. In relatively flat plots (<20% grade) where using a meter tape gives accurate distances:
    a. Lay out a meter tape from the selected plot marker in the X (E/W) direction. Use a compass to verify direction.
    b. Pull the tape to the measured distance to the target X coordinate and mark the point.
    c. From that point, navigate in the Y (N/S) direction to the measured distance to the target Y coordinate. Use a compass to verify direction.

d. Place a marker at the X, Y location.

7. In plots with steeper slope (>20% grade), or in heavily forested plots where pulling a tape is not possible, use a laser rangefinder set to HD (horizontal distance) mode to locate X, Y coordinates.

    a. Clean lenses with lens cloth or lens tissue, if needed.

    b. Check/set correct declination. See RD[11] for details.

    c. Calibrate the TruPulse tilt sensor (only needed after severe drop-shock; see RD[11]).

    d. Two technicians must work together. One stands at the selected plot marker and operates the rangefinder. The second person navigates to the first potential X-location, following the directions of the rangefinder operator and using the reflective tape so that an accurate horizontal distance measurement can be obtained.

    e. The rangefinder operator must ensure that the angle (azimuth) is as close to 90° as possible from True North when measuring the X-coordinate distance.

    f. Place a marker, such as a pin flag or stake, at the X-location.

    g. The rangefinder operator then moves to stand directly over the marker. Using either a measuring tape or the TruPulse with a reflective surface, work with the second person to locate the Y-coordinate location.

    h. Ensure that the azimuth is as close to 0° (True North) as possible and measure the Y-coordinate distance.

    i. Place a marker at the X, Y location.

C.2 Assess Sample Location

1. Put on a clean pair of nitrile gloves. At the same plot, gloves can be re-used after rinsing with DI water to remove coarse debris and drying thoroughly. Do NOT re-use gloves between plots.

2. Assess the location for sampling suitability:

    a. Are there obvious disturbances, vegetation, large rocks or roots that would impede sampling within a 0.5 m radius of the X, Y location?

    b. In Tower plots, does the coordinate fall within 2 m of the perimeter of a ground or elevated litter trap?

3. If any of the above conditions are met, reject the location and record why on the soil coordinate list. Move to next coordinate location on the subplot list until an acceptable one is found.

    a. If five X, Y coordinates are rejected, do not sample within that subplot and submit a ServiceNow ticket. Make a record for that subplot and choose “Coordinates not suitable” in the **samplingImpractical** field, leaving X ,Y coordinates and other metadata

blank. Do not attempt to sample the fourth subplot that was not randomly selected for this bout.

b. If a disturbance is the dominant condition of a subplot (e.g., more than half the area is covered in debris from logging), do collect a sample but use the **horizonDetails** field along with **remarks** to provide more information on this condition.

c. In drylands, do not reject locations due to presence of mature biological soil crust (see Appendix D.7)

4. At an accepted location, start near the exact location of the X, Y coordinate and assess soil depth by probing the soil using a sterilized pigtail stake (or other poking device free of paint and rust). Move outward up to 0.5 m from the X, Y location until **two** suitable spots are found (**Figure 20**).

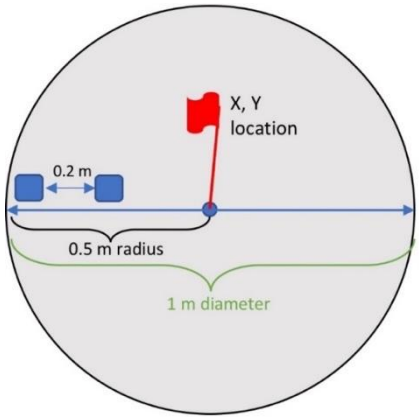

Figure 20. Two suitable locations for T-initial sampling at an X, Y coordinate location, with ideal distance between initial and final core locations shown (0.2 m). Locations with rocky soils can use the entire 1 m diameter area, as long as both sub-locations are within 0.5 m of the X, Y coordinate.

a. The target depth for soil sampling is 30 cm. However, some sites have characteristically shallow and rocky soils. At these sites, a suitable location may not allow coring to 30 cm depth, especially given the need to install a PVC cylinder - this is acceptable.

b. Suitability varies from site to site and based on coring device, but in general will allow sufficient sample collection with one to three brownies or cores.

5. Mark the two precise target sampling areas identified via probing with flags or pigtail stakes.

d. Sub-locations should be no closer than 0.2 m of each other and ideally within 0.5 m of each other, and both core locations must be within 0.5 m of the actual X, Y location.

6. In sites/plots with rocky soils, probe thoroughly for rocks as they will impede installation of the incubation cylinders.

7. If there is heavy *Toxicoddendron spp* in the location you will core, take steps to avoid exposure to toxic oils as described in RD [10]. Be prepared to record *Tox* presence both in the SLS: Field data entry application as well as on the physical sample bag by applying a warning label sticker.

C.3 Measure Soil Temperature and Litter Depth

1. **Create pilot hole.** At a spot adjacent to but not directly on top of the marked sampling location, push aside the litter layer and create a pilot hole with the pilot hole tool (**Figure 13**, panel a). Try multiple locations until little resistance is found. Insert pilot hole tool to the bottom of electrical tape and spin.
2. **Measure soil temperature.** Insert temperature probe into the pilot hole to a depth of 10 cm, which is indicated by the bottom of the electrical tape on the probe stem (**Figure 13**, panel b).
    a. When handling the probe, do not to push or pull on the digital display as this may cause the unit to break. Instead, handle at the top of metal stem under the digital display, at or near the electrical tape marking (**Figure 13**, panel c). Do not touch the tip of the probe.
    b. Allow probe to equilibrate for about 2 minutes, then record **soilTemp** in degrees C. *Important:* Do not make measurement with the sun directly on probe, if needed shade it with your body or a piece of field equipment (clipboard, backpack, etc.)
3. **Measure litter layer depth**. In the area around the marked sampling locations, use a ruler to measure the depth of the undisturbed litter layer in cm (**litterDepth**, $\pm$ 0.1 cm). Take a few readings as this parameter is likely to be spatially variable at a fine scale, then record the average value – the Fulcrum application has a calculator to help with this.
    - Gently wiggle the ruler through the layer to the ground surface, minimizing disturbance to the litter profile to minimize compaction.

***Reminder**: The litter layer is dead and detached but recognizable, intact plant material (i.e., leaves, wood, etc) that is not consolidated, whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition that is consolidated as a layer.*

C.4 Set up Incubated Core

***Note:** this core will remain in the ground for the duration of the incubation period (two to four weeks, see Appendix C). We install it first so that the T-initial and T-final cores will have similar depths (**± 5 cm**). Note that if PVC is used to collect the initial core as well, it maybe be best to install both cylinders simultaneously. Pound them in a few cm at a time, stop when one can no longer go any deeper.*

1. At one of the two flagged sub-locations, push the litter layer away from where you are going to core into the soil surface.
2. If PVC incubation cylinder is not already marked at 30 cm (**Figure 9**), consider adding such a marking. Then, push the cylinder into the ground.
    a. If soil is difficult to core, use a piece of wood or monument stake installation strike plate and mallet to pound the cylinder into the ground.

b. If soil is easy to core, simply push it in.

c. If the soil has a thick, fluffy organic layer, use a soil knife to cut around the cylinder as you insert it. This will help avoid compaction. *Additional guidance for peatland and permafrost sites is provided in 8D.5*.

3. Estimate inserted depth of the PVC cylinder. If it does not reach the 30 cm mark, measure from top of the cylinder to the soil surface, then subtract this number from the total length (35 cm for most sites, 20 cm for rocky sites). **Use this depth ± 5 cm to guide sampling for the initial core.**

4. Leave cylinder in the ground and place a loose-fitting cap over the top so that air exchange can occur but detritus and water do not fall in. Secure cap to cylinder using zip tie or plant wire.

5. Cover the cap with any litter that was pushed away.

6. Site host permitting, mark the location of the core with a non-metallic pin flag. Or, if there is overhanging vegetation, consider tying a piece of flagging to the nearest tree/bunchgrass/etc.

a. Write the subplot and X, Y coordinates on flag to aid with data entry upon sample retrieval.

C.5 Collect Initial Soil Core

There are two options for collecting the initial soil core - choose whichever works best.

- If a site's normal coring device is 2 ± 0.5" in diameter, it can be used to collect the initial core. However, make sure that T-initial and T-final cores have bottom depths within ± 5 cm of each other, even if the coring device can penetrate deeper than the PVC. This is why the T-final core is established first.
- If a site's normal coring device is not 2 ± 0.5" in diameter, or there are other logistical concerns, PVC cylinders can be used to collect both initial and final samples. See note above about installing initial and final cylinders simultaneously to help ensure similar bottom depths.

1. At the second flagged sub-location, push the litter layer away from where you are going to core into the soil surface. Sterilize glove and tools with 70% ethanol, use a new ethanol wipe for each X, Y location.

2. **If an O horizon > 1 cm is present, collect an O horizon sample by following these steps**. If a location has no O horizon or one < 1 cm thick, skip these steps and proceed directly to Step 14.

> If the surface soil has been mixed (e.g., from logging) and horizon type is difficult to determine, classify as O or M based on whether mineral or organic material dominates. Take a small mass of soil in your hand from near the sampling area and assess its texture. If it feels grainy or gritty, treat as an M horizon. If smooth, friable, and a bit greasy, treat as an O horizon. Ensure that handled soil is not included in the sample collection. Note 'Horizon indeterminate' in the **horizonDetails** field if uncertain even after this test.

3. Cut out an organic horizon "brownie" using the frame cutter tool and soil knife (hori-hori, **Figure 14**). With deep organic horizons, only 1 brownie may be needed; from many sites, two or three will be needed. At those sites, select multiple locations within 0.5 m of each other.

4. Record the **sampleTopDepth** as the depth from the soil surface (0 cm). Measure the depth of each side of the brownie hole and determine the average value. Only sample to 30 ± 1 cm maximum depth. Record the average in the field **sampleBottomDepth**.

5. Record appropriate **horizonDetails** entries if applicable, for example 'Woody horizon' (Appendix D.6) or 'Water table encountered' plus additional **remarks** as needed.

6. Place all brownies collected at an X, Y location into a pre-labelled, 1-gallon resealable plastic bag. With a pre-sterilized, gloved hand, remove rocks, insects, recognizable and undecomposed plant material, and other non-soil debris and homogenize. Shake large root masses to dislodge soil, then discard. Break up large clods to facilitate subsampling.

    a. If it is difficult to pick the entire bag of homogenized soil in the field, plan to use a pair of ethanol-sterilized tweezers to pick rocks, roots, and non-soil debris from the microbial subsamples while generating them. Re-sterilize tweezers with ethanol and a sterile wipe in between samples. Regardless of approach, it is *imperative* that field subsamples are picked clean of non-soil material: failure to do so can render the samples useless.

7. With wet or saturated soils, dump out any excess water after the soil has settled 10-15 seconds.

8. **Ensure that you have sufficient soil for all subsampling and analyses:** Ensure the following *minimum* masses are met, although more soil is preferable:

    a. Transitional (T1 or T2) bout: 75 g

    b. Peak greenness bout: 100 g

    c. If sample is below these masses, take another sample at the same X, Y location and re-homogenize.

9. **Generate microbial analysis (-gen) and archive (-gaX) samples**: Aliquot subsamples from the 1-gallon bag of homogenized soil into 1 labeled Whirl-Pak bag (fill about halfway, 5-10 g target mass) and 5 labeled cryovials (fill to about 70% capacity).

    a. Close Whirl-Pak such that the labels lay flat and are clearly visible, then complete the label. Final samples should appear as shown in **Figure 15**.

    The microbial genetic analysis sample label should appear as:

    plotID-horizon-subplotID-YYYYMMDD-gen
    (ex. HARV_001-O-21-20210415-gen)

    b. For collecting into the cryovials, scoop soil into the container using a scoop that has been sterilized with ethanol. Any non-soil material that ends up in a container should be

removed with sterilized tweezers or by hand using pre-sterilized gloves. Keep caps face-down on a sterilized surface.

i. If using 2 mL vials with a smaller opening, sterilized tweezers and/or a small ethanol-sterilized 'ramrod' device may be used to load soil into vials.

c. For organizational purposes, it is best to keep all 5 cryovials from a given subplot in a bag labeled with plotID and subplotID, or keep them in a single row of a cryovial box.

10. Scan the barcode label for each sample into the mobile data recorder. Ensure that each barcode label is entered only once and is associated with the correct sample, although for -gaX samples, the barcodes are interchangeable. To reduce data entry errors, it is recommended that barcode labels are scanned during sample creation, rather than in batches of multiple samples.

11. To retain sample integrity, place microbial samples on dry ice immediately after verifying labels are correct and complete. Ensure all newly added samples are in contact with dry ice so that they freeze quickly. *If dry ice could not be procured, follow instructions in the FRZ SOP [RD18] to freeze and store samples using ultra-cold ice packs.*

12. **During Peak Greenness: Generate microbial metagenomics (-comp) sample**: generate a plot-level composite sample by subsampling material from each X, Y coordinate sampled within a plot that is of the same horizon. Use a pre-sterilized (with 70% ethanol) scooping device such as a coffee scoop or tablespoon when generating the composite sample. Approximately equal amounts of soil should be composited from each X, Y location.

a. If this is the first X, Y location to be sampled at a plot, label a 2-oz Whirl-Pak as follows:

plotID-horizon-collectDate-comp
(ex. HARV_001-O-20210715-comp)

i. Place one scoop of homogenized soil in the Whirl-Pak, close bag, and place on ice packs.

b. If this not the first X, Y location to be sampled at a plot, obtain the Whirl-Pak created earlier from the cooler with ice packs. Check that horizon ID matches the horizon ID for the sample you want to add. If this is a new horizon for this plot, create a new Whirl-Pak.

i. Place one scoop of homogenized soil in the Whirl-Pak and close the bag.

ii. If another X, Y location within the plot might be added to this bag, return the bag to cooler with ice packs.

c. If this is the last X, Y location for this plot, mix the soil by gently massaging the outside of the bag and/or inverting and shaking. Close whirl-pak such that the labels lay flat and are clearly visible (**Figure 15**), and record the **compositeSampleID** in the SLS: Metagenomics Pooling application. Also scan the barcode label into the application. As soon as the bag is closed and the data have been entered, place the bag on dry ice and ensure that newly added Whirl-Paks are in contact with dry ice. Microbial activities will change rapidly until frozen.

Note: If conditions do not permit a composite sample to be created in the field, simply collect an additional whirl-pak at each sample coordinate and follow SOP I for generating a composite sample in the DSF.

13. **Ensure sufficient O horizon soil remains for lab subsampling and analyses**: below is the minimum but more soil is preferable:
    - Coordinated, transition season bout (e.g. not peak greenness): 50 g
    - Coordinated, peak greenness bout: 75 g
14. **Sample the mineral (M) horizon.** Insert the bottom of the cylinder (beveled edge of PVC) or the site's normal coring device into the ground. If an O-horizon sample was collected, insert cylinder or coring device into the footprint of an O-horizon sample location.
    - If using the PVC cylinder and soil is difficult to core, use a piece of wood or monument stake installation strike plate and mallet to pound the cylinder into the ground. If soil is easy to core, simply push it in.
    - Always core vertically, not perpendicular, when collecting on a slope.
15. Push the cylinder or coring device in to a total depth (from the soil surface) of 30 ± 1 cm, or to a depth comparable (± 5 cm) to the incubated cylinder if it did not reach 30 cm depth.
    a. For most sites, if < 30 cm is sampled because the t-final cylinder was installed to a shallower depth, choose **sampleExtent** = 'obstruction' for the initial core. For rocky sites using short cylinders, the same applies but the max depth in this case is 15 cm (dictated by PVC length).
    b. If a ≤ 1 cm O horizon is collected as part of the M horizon sample, select the value 'Thin O horizon present' for **horizonDetails**.
    c. If the O horizon is very thick, e.g., ≥ 25 cm, do not collect the M horizon as it will be too difficult to get enough material for downstream analyses.
    d. Do not sample past 30 cm. Deeper cores should be discarded, collect a new 30 (± 1) cm core.

A piece of masking or lab tape should be placed on the outside of the cylinder or corer to indicate the depth to stop driving it into the mineral soil horizon.

16. Remove cylinder or coring device and empty soil directly into a new, pre-labeled 1-gallon bag. If a helper tool is needed to extrude soil from the cylinder (e.g. long flat-head screwdriver, soil knife, pigtail stake, pry bar), be sure to properly sterilize before use.

    a. If soil is dry or high in clay content, a helper tool – such as a post puller or a pigtail stake threaded through the drill holes – may aid in removal of the cylinder from the ground.

17. Homogenize (mix) the soil thoroughly. When possible, avoid contact with the soil by closing the bag and mixing by inverting and massaging. If this does not sufficiently homogenize the soil, then with a pre-sterilized, gloved hand, homogenize the soil. Remove any large rocks, coarse roots (> 2 mm diameter), other plant material, and insects using a sterilized, gloved hand.

18. With wet or saturated soils, dump out any excess water in the sample container after the soil has settled 10-15 seconds, if present.

19. **Ensure sufficient M horizon soil remains for all subsampling and analyses:** Make sure the following *minimum* masses are met, although more soil is preferable:

    a. Transitional (T1 or T2) bout: 150 g

    b. Peak greenness bout: 210 g

20. *If more material is needed*: collect another core (or two), each to 30 cm total depth from the surface, or a depth comparable (± 5 cm) to the incubated cylinder if it did not reach 30 cm.

21. Measure **sampleTopDepth** as distance from the top of the soil sample to the soil surface. When there is no O horizon, **sampleTopDepth** = 0 cm. When there is an O-horizon, it will be bottom depth of the O layer. Measure the **sampleBottomDepth** as depth from the soil surface to the bottom of the borehole (**Figure 21**). When taking multiple cores, record average depth values.

Figure 21. Example soil profile: O horizon sampleTopDepth = 0 cm, sampleBottomDepth = 3 cm; M horizon sampleTopDepth = 3 cm, sampleBottonDepth = 30 cm.

22. **If there was no O horizon sampled <u>OR</u> it is a peak greenness bout: Create microbial analysis (-gen) and archive (-gaX) samples from M horizon**. Aliquot subsamples from the 1-gallon bag of homogenized soil into 1 labeled Whirl-Pak bag (fill about halfway, 10-20 g target mass) and 5 labeled cryovials (fill to about 80% capacity).

    a. Close Whirl-Pak such that the labels lay flat and are clearly visible, then complete the label. **Figure 15** shows examples of properly labelled samples.

        The microbial genetic analysis sample label should appear as:

        plotID-horizon-subplotID-YYYYMMDD-gen
        (ex. HARV_001-M-21-20210415-gen)

    b. For collecting into the cryovials, the simplest approach may be to insert the open container into the bag of homogenized soil with a pre-sterilized, gloved hand, and press in soil with the wall of the homogenized gallon bag. Pouring or scooping soil into the container using a sterile scoop is also acceptable. Keep cryovial caps face-down on a sterilized surface.

        i. If using 2 mL vials with a smaller opening, a small ethanol-sterilized 'ramrod' device may be helpful to load soil into vials.

    c. Any non-soil material that ends up in a container should be removed with sterilized tweezers or by hand using pre-sterilized gloves.

    d. For organizational purposes, it is best to keep all 5 cryovials from a given subplot in a bag labeled with plotID and subplotID, or keep them in a single row of a cryovial box.

23. Scan the barcode label for each sample into the mobile data recorder. Ensure that each barcode label is entered only once and is associated with the correct sample, although for -gaX samples, the barcodes are interchangeable. To reduce data entry errors, it is recommended that barcode labels are scanned *during* sample creation rather than in batches of multiple samples.

24. Place microbial samples on dry ice immediately after verifying labels are correct and complete to retain sample integrity. Ensure that all newly added samples are in contact with dry ice so that they freeze quickly. *If dry ice could not be procured, follow instructions in the FRZ SOP [RD18] to freeze and store samples using ultra-cold ice packs.*

25. **<u>During Peak Greenness: Generate microbial metagenomics (-comp) sample from M horizon.</u>** Generate a plot-level composite sample by subsampling material from each X, Y coordinate sampled within a plot that is of the same horizon. Use a pre-sterilized (with 70% ethanol) scooping device such as a coffee scoop or tablespoon when generating the composite sample. Approximately equal amounts of soil should be composited from each X, Y location.

    a. If this is the first X, Y location to be sampled at a plot, label a 2-oz Whirl-Pak as follows:

        plotID-horizon-collectDate-comp
        (ex. HARV_001-M-20210715-comp)

i. Place one scoop of homogenized soil in the Whirl-Pak, close bag, and place on ice packs.

b. If this not the first X, Y location to be sampled at a plot, obtain the Whirl-Pak created earlier from the ice packs cooler. Check that the horizon ID matches the horizon ID for the sample you want to add. If this is a new horizon for this plot, create a new Whirl-Pak.

i. Place one scoop of homogenized soil in the Whirl-Pak and close the bag.

ii. If another X, Y location within the plot may be added to this bag, return the bag to cooler with ice packs.

c. If this is the last X, Y location for this plot, mix the soil by gently massaging the outside of the bag and/or inverting/shaking. Close Whirl-Pak such that the labels lay flat and are clearly visible, and record the **compositeSampleID** in the SLS: Metagenomics Pooling application. Also scan the barcode label. As soon as the bag is closed and the data have been entered, place the bag on dry ice and ensure that newly added Whirl-Paks are in contact with dry ice so that it freezes as quickly as possible. Microbial activities will change rapidly until frozen.

***Note**: If conditions do not permit a composite sample to be created in the field, simply collect an additional Whirl-Pak at each X, Y location and follow SOP I for generating a composite sample in the domain lab.*

26. **Ensure that sufficient mineral soil remains for lab subsampling and analyses**: below are the minimums but more soil is preferable.

- Transitional (T1 or T2) bout: 100 g
- Peak greenness bout: 160 g

***Note**: Any homogenized soil in excess of 500 g can be dumped back into the borehole according to the site host agreement*

27. For labeling and data recording, be sure that:

- Bulk, homogenized sample bags are labeled with **sampleID**, **measuredBy**, and **recordedBy**
- If a *Toxicodendron spp* was abundant, also apply a sample warning label sticker to the homogenized bag of soil.
- Whirl-Paks are labelled with the **geneticSampleID** or the **compositeSampleID**.
- All barcode labels have been scanned and the values were populated correctly into the Fulcrum application. Perform a quick scan to catch any erroneously duplicated barcodes.

28. Update the paper soil X, Y coordinate list and subplot list.

29. Enter metadata in the SLS: Field Sampling application or field datasheet:

- NtransBoutType = Tinitial
- boutType (microbesBiomass or microbesBiomassBGC), ensure that all of the sampleID's associated with that bout have been generated.
- sampleTiming – as specified in **Table 4**
- biophysicalCriteria – as specified in **Table 9**
- samplingProtocolVersion
- siteID
- plotID
- subplotID
- collectDate (YYYYMMDD)
- coreCoordinateX
- coreCoordinateY
- standingWaterDepth (nearest 0.1 cm)
- time (HH:MM)
- soilTemp (nearest 0.1 degree)
- litterDepth (nearest 0.1 cm)
- toxicodendronPossible (choice of Y/N)
- sampleTopDepth (nearest 0.1 cm)
- sampleBottomDepth (nearest 0.1 cm)
- samplingDevice
- numberCores
- horizon
- horizonDetails – as specified in **Table 12**
- genetic sample and/or archive sample preparation method (default is dry ice, select 'ultra-cold ice packs' if following the FRZ SOP [RD18])
- geneticArchiveSampleCount
- geneticArchiveContainer (5 mL or 2 mL vial)
- sampleExtent (Entire = entire horizon sampled, or to saprolite/bedrock; Obstruction = sampled to an obstruction such as rock, root, etc, includes t-initial when t-final encountered an obstruction; Maximum = sampled to maximum depth allowed by the protocol, horizon may extend deeper (for short cylinders, maximum ~ 15 cm); Unknown = extent varied across cores or could not be determined)
- remarks – *only those relevant to an end user and not already captured in the data, use standardized remarks where possible*
- measuredBy
- recordedBy

When using a mobile data recorder, ensure all barcodes have been scanned to the appropriate sample.

C.6 Field Clean-up

1. Between plots, thoroughly rinse or wipe down reusable sampling equipment (corer, thermometer, hori hori, etc) with deionized water. For very dirty equipment, use a scrub brush or wipe. Then sterilize using 70% ethanol wipes or an ethanol squirt bottle.
2. Discard gloves between plots.

C.7 Sample Preservation and Transport

1. For transport, ensure that samples comply with any quarantine and/or site host requirements, such as double bagging samples, ensuring that all insect pests have been removed, required permits are on-hand, etc.
2. Keep bulk, homogenized bags of soil in the cooler with the ice packs; transfer to 4°C refrigerator upon return to domain lab. Ensure that sample bags are well sealed to prohibit moisture loss.
3. Keep soils for microbial genetic analysis (-gen), genetic archiving (-gaX), and microbial metagenomics (-comp) in the cooler with dry ice; transfer to ultralow freezer upon return to domain lab. All of these sample types are shipped according to SOP M with no additional laboratory processing, though labels and barcodes must be QC'd before shipment (SOP L).

***Note**: Soils being measured for N transformations MUST be processed and extracted in 2M KCl within 1 day of collection. For example, samples collected on a Monday must complete SOP K by end of the day Tuesday. If the Domain Support Facility is far from the sampling site and sampling requires multiple days, processing and extraction may occur in a local laboratory facility or field house, provided all necessary equipment and supplies are available. If there are no facilities near the site and sampling takes > 1 day, alternate arrangements must be made. For example, a team can transport soils back to the Domain Support Facility for extraction while another team finishes sampling. Alternatively, the soil team can split the field collection bout into two sampling periods, with laboratory processing in between.*

C.8 Collection of Incubated Soil Core

***Note:** Collection of the incubated soil core marks the end of the sampling bout, following the incubation length guidelines specified in Appendix C.*

1. Consult the soil coordinate list or previous Fulcrum records and navigate to the plot and X, Y location where sampling for T-initial soil N transformations occurred.
2. Locate incubated core. Measure soil temperature and litter depth within 10 cm of the incubated cylinder, according to the instructions in SOP C.3.
    - If the core is missing, or if insects or animals have completely colonized it (e.g. made a nest), create a record in the Fulcrum application or datasheet but choose **sampleFate** = 'lost' and select the appropriate **incubationCondition** value. Discard any sample material, if present.

3. The person who will handle the cylinder should put on a pair of nitrile gloves. Then take off cap and remove cylinder from the ground.

    a. If soil is dry or high in clay content, a helper tool – such as a post puller or a pigtail stake threaded through the drill holes – may aid in removal.

    b. If the soil is sandy, wet, or otherwise not well aggregated, soil within the cylinder may fall out during removal. To avoid this, dig down next to the core and insert a knife or gloved hand under the core, then remove. If the core is pulled and soil falls out, use a clean trowel or gloved hand to collect soil that clearly fell from the borehole.

4. Record the condition of the incubation cylinder using the **incubationCondition** field.

    - We hope the majority of cylinders are in 'OK' condition.
    - If a cylinder has been disturbed – for example, an animal has removed it from the hole, the water table has risen into the core from below or flooded it from above, there is moderate insect activity – still collect but choose the appropriate **incubationCondition** choice and explain further in remarks if needed.
    - If > 10 cylinders have condition other than OK, communicate this via a ServiceNow Incident.

5. If an O horizon is present, remove soil onto tray, partition the O and M horizons, and bag separately. It may be necessary to use a tool to push or scoop soil from cylinder, such as a pigtail stake or soil knife, but take care not to mix the O and M material. It is not critical that the t-final sample remains sterile since no microbial subsamples are collected but use clean technique. If you end up needing to handle the soil material directly, wear gloves.

6. If only M-horizon soil is present, empty soil from PVC cylinder directly into bag. If the soil remains stuck inside the core, a tool may be used to help push the soil out. In clayey conditions, a long flathead screwdriver or pry bar works well.

    a. For high-clay soils, it is also acceptable to place the entire PVC tube into a bag, place in a cooler on ice packs, and transport to the DSF for removal of soil later.

7. For sites that have both O and M horizons:

    - If you encounter a horizon in the incubated core that was not present in the initial core:
        - If it's an O < 5 cm or an M < 2 cm, include it with the rest of the material and select the appropriate value for **horizonDetails** (**Table 12**). Otherwise, discard material and do not include in the sample.
    - If you *do not* encounter a horizon in the incubated core that was present in the initial core:
        - Make a record for this missing horizon but choose 'Horizon not present' for **samplingImpractical** and include minimal sample metadata.

8. Record the approximate depth of each horizon from the bore hole. It is ok to excavate the hole further if needed to accurately read borehole depth or the boundary between horizons.
    a. For M horizons, if your site is known to have unconsolidated or sandy soil that may collapse when cylinder is removed, mark the soil surface on the outside of the cylinder prior to removal. Then measure **sampleBottomDepth** by taking the length (in cm) from mark to bottom of the cylinder.

9. Label bag with sampleID, and add a warning label if a *Toxicodendron spp* was present:

    plotID-horizon-subplotID-collectDate
    ex. HARV_001-M-21-20210415

10. Scan the barcode label for each sample if using a mobile data recorder.
11. Place sample into cooler with ice packs.
12. Record key metadata as described in SOP C.4.
    - nTransBoutType = Tfinal, incubationMethod = covered core
13. Backfill the bore hole according to site requirements.
14. The same pair of nitrile gloves may be worn to collect multiple cylinders. Change when they rip or become too dirty to clean with DI water.
15. Keep collected soil cores in cooler with ice packs and transfer to 4° C refrigerator upon return to domain lab. Process within 1 day of sample collection.

SOP D Post-Field Sampling Tasks

D.1 Sample Storage and Equipment Cleaning

1. Store samples at the correct temperatures.
    - The following sample types should be kept on dry ice in the field and be transferred to a -80˚ C freezer at domain lab. Samples must remain frozen:
        - Microbial genetic analysis (-gen),
        - Microbial metagenomics analysis (-comp),
        - Microbial genetic archive (-gaX)
    - The following sample types should be kept on ice packs in the field and be transferred to a 4˚ C fridge at domain lab:
        - Homogenized, bulk soil sample
    - Samples should be processed within 1 day of collection for a coordinated bout and at core sites for an off-year bout, and within 48 hrs preferred, 72 hrs maximum at gradient sites for an off-year bout.

2. Clean field equipment.
    - Metal soil sampling devices (e.g. auger, hori-hori blade): clean with tap water and round scrub brush, then dry with a clean rag. Store dry to minimize rust development. Check between bouts for rust and remove as needed.
    - Brownie cutter: clean with tap water and scrubber as needed to remove residual soil material. Store dry.
    - Re-usable sub-sampling equipment (e.g. tweezers, scoopulas, sterile work surface): Clean with soap and water, rinse thoroughly with tap water, then a final rinse with DI water, then air-dry. Store dry.

D.2 Document Incomplete Sampling Within a Site

Soil sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4.2 and Appendix C. Any sampling that does not occur according to these guidelines must be documented, as described in Section 4.5.

Ideally, sampling will occur at the same plot locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced. There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a road gets installed through a terrestrial plot. Second, sampling locations may be located in areas that become logistically impossible to sample on a schedule that is biologically meaningful.

For soil sampling, there are numerous circumstances that might prevent sampling at a particular plot for a given bout or year. However, most do not warrant plot reassignment. For sites that conduct 3 sampling bouts per year, a given plot should be sampled for at least two of the expected bouts, and one must be peak greenness. For soil sampling in Alaska, the peak greenness bout must be completed. Plots that fail to meet these criteria for 2 years in a row for reasons other than staff or resource shortages are considered compromised and NEON Science will work to re-assign sampling to a different plot.

If it becomes apparent that a particular plot will be compromised in the long-term before these criteria are met (e.g. plot turns into a permanent cattle holding pen), submit a ServiceNow Incident. Science and Field Science will discuss the best course of action and likely move sampling to a different plot.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled. There should be 10 plots and 30 samples (minimum) per soil bout.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]'
    a. Example: 'TOS Sampling Incomplete: SLS – Could not access plot due to closed road'
    b. In the ticket, please mention whether missed sampling was due to staff/resource shortages, issues within or navigating to the plot, or a combination.
3. Science reviews incident tickets periodically to assess if any plots have become compromised.

D.3 Verification of Soil Temperature Probes

If using a user-calibratable temperature probe, conduct this procedure following the domain's last T2 bout of the field season. If the probe is not user-calibratable, conduct as specified in Section A.2. The goal is to determine whether probes are still good or need to be replaced prior to the next bouts.

1. Perform calibration of probes at room temperature following the steps listed in Section A.2.
2. Place entire calibration set up in refrigerator (4 °C or similar). Allow for overnight equilibration.
3. The next day, remove calibration set up from refrigerator and record temperatures of soil probes and thermometer immediately after. Data are for internal purposes only and do not need to be transmitted to Science, record wherever is convenient.
4. Next, place calibration set up in oven set to 50-60 °C. Allow for overnight equilibration.
5. The next day, remove calibration set up from oven and record temperatures on soil probes and thermometer immediately after removal.
6. Ensure temperatures on the probe are within 1 °C of NIST-traceable thermometer at both cool and warm temps. If outside this range, take probe(s) out of circulation and replace.

SOP E Laboratory Measurement of Soil Moisture Content

Analysis of the moisture present in soil is important for understanding the field conditions experienced by soil microbial communities and constraints on soil biogeochemical processes. Conduct the following steps to generate soil moisture data for each soil sample. Record the necessary metadata and values in the *SLS: Moisture [PROD]* Fulcrum application or lab datasheet (RD [05]). Key reminders:

- Soil moisture is measured on soil that has not been sieved.
- The weigh boat is not tared before adding soil, the combined weight (boat + soil) is recorded.
- For Off-year bouts at gradient sites, soil moisture analysis should begin within 48 hrs preferred, 72 hrs maximum of field collection.
- For Coordinated bouts or off-year bouts at core sites, soil moisture analysis MUST begin within 1 day of field collection.

1. Label foil weigh boats with unique tinIDs (any combination of letters and numbers, as long as they are unique). Record the **tinID**. *Can re-use previous weigh boats as long as they are thoroughly cleaned (see Step 9 below)*.
2. Weigh foil boat to nearest 0.01 g and record value in the Fulcrum application or datasheet (boatMass). Do not tare the balance.
3. Wear un-soiled (e.g., clean, but sterile not required) nitrile gloves. Gloves may be re-used between samples as long as any visible dirt is rinsed off with DI and gloves are dried well.
4. Place 5 ± 0.5 g of a field moist organic horizon sample (not sieved) or 10 ± 0.5 g of a field moist mineral horizon sample (not sieved) into the weighed foil boat. **Ensure that any rocks, large roots (> 2 mm diameter), insects, recognizable and undecomposed plant material, and other non-soil debris have been removed, use forceps as needed.** Record weight of boat + soil to nearest 0.01 g (freshMassBoatMass).
    a. It is acceptable to use less mass if sample quantity is limited: 2 g minimum for O-horizons, 5 g minimum for M-horizons.
    b. It is not a data quality concern if more mass is used, but it is best to stay within these guidelines to help ensure sufficient soil remains for all downstream analyses.
5. Place all samples into the drying oven, taking care not to spill material while moving weigh boats. Tip: organize samples on a tray to quickly transfer all samples into oven. Dry samples at 105°C for at least 48 h. Record time in oven in the Fulcrum application or datasheet.
6. After removing samples from oven, wait until they are cool to the touch, then weigh dried sample + weigh boat to nearest 0.01 g and record values in the Fulcrum application or datasheet. Record the date and time out of oven.
7. If any issues are encountered while weighing soils (weigh boat spills, tinID mistakenly associated with 2 unique samples, etc), note via an appropriate selection in the 'sampleCondition' field.

8. Dispose of soils according to permit requirements.
9. For weigh boats that are in good condition and can be re-used, clean in Alconox (or similar laboratory soap) and water and rinse with deionized water. Dry weigh boats either in the oven or at ambient temperature, and store in a dry location.

> NO OVEN SPACE OR ACCESS?
>
> If oven space is limited or ovens are not available and more than 1 day (Coordinated bouts) or 48-72 hours (Off-year bouts) will elapse before soil moisture measurements can begin, subsample the unsieved, bulk homogenized soil into Whirl-Paks and store subsamples in the freezer at -20°C for up to two weeks before initiating soil moisture measurements. Make sure to note 'used frozen samples' in the **sampleCondition** field.

What to do when a sample is lost

- Create a record for the sample.
- Enter the sampleID and any other metadata measured for that sample.
- Select 'lost' for **moistureSampleFate,** which will populate 'tin lost' for **sampleCondition**. This allows you to leave other required fields empty.

NEXT STEPS: Continue to SOP F: Sieving and Picking Field Samples.

SOP F Sieving and Picking Field Soils for Lab Processing

Soils are inherently heterogeneous and must be thoroughly mixed prior to conducting downstream analyses. For mineral (M) horizons, sieving accomplishes this purpose. It also allows for the exclusion of non-soil material (particles > 2mm). For organic (O) horizons, sieving is not effective for homogenizing; instead, manual removal of non-soil particles is required. Conduct the following steps to homogenize/sieve samples, then oven-dry, air-dry, or subsample as directed. Key reminders:

- For gradient sites conducting off-year, microbes-only bouts, sieving and subsampling should begin within 48 hrs of field collection (preferred), or 72 hrs (maximum)
- For core sites OR sites conducting a coordinated bout, sieving MUST begin within 1 day of field collection.

TIPS & TRICKS

For large-mass samples, the entire soil quantity may not be needed for downstream analyses. If this is the case and sieved soils will go straight into paper bags to air-dry (following any required subsampling), consider holding back a bit of unsieved, homogenized soil in the original gallon bag in case soil moisture needs to be re-measured. Make sure the bag is well-sealed and store for up to 1 week at 4 °C.

F.1 Sieving and Homogenizing Samples

1. Wear un-soiled (e.g. clean, but sterile not required) nitrile gloves. Gloves may be re-used between samples as long as any visible dirt is rinsed off with DI and gloves are dried well (ethanol suggested to quicken drying).
2. If the sample is marked as containing *Toxicodendron spp*, take additional precautions to reduce exposure to toxic oils by: 1) changing gloves after handling each sample, 2) not touching one's skin or clothes while sieving or picking, 3) throwing all plant material caught by the sieve directly into the trash, and 4) immediately cleaning up any sieving-related debris.
3. With gloved hand, stir soil sample to homogenize (mix), breaking up any soil clods completely.
4. If sample is an **O horizon**, do not sieve but break up large clumps of soil and remove any rocks, insects, large roots (> 2 mm diameter), recognizable and undecomposed plant material, and other non-soil debris by hand and homogenize before proceeding. Some soils are difficult to hand-pick, particularly those with many live fine roots; allow up to 30 minutes per sample. This is critical to ensure high-quality data, especially for microbial biomass analyses as plant roots have unique lipid signatures that can overwhelm microbial lipid signatures in soil. If hand-picking takes > 30 minutes per sample:
    - Only hand-pick as much material as required to get a representative subsample for the analyses to be conducted. For example, if sieved soil is destined for pH measurement only (SOP H), 15-20 g of moist organic soil post-processing should suffice.
5. With a gloved hand, pass **M horizon** samples through a clean and dried, 2 mm mesh sieve. This will allow all particles ≤ 2 mm to be collected (i.e., pass through the sieve), while larger particles

are discarded. Certain soils can be difficult to sieve, particularly those with high clay content. If sieving sufficient soil quantities for downstream processing takes > 30 minutes per sample, try one or more of the following tips and tricks:

- If this is NOT a coordinated bout, partially air-dry the sample prior to sieving. Break up soil clumps with a gloved hand and place in a paper bag 24-48 hours. Resume sieving.
- Only sieve as much material as required to get a representative subsample for the analyses to be conducted. For example, if sieved soil is destined for pH measurement only (SOP H), 30 g of mineral soil post-processing should suffice. Any sieved material that sticks to the underside of the sieve can be scraped off with your hand or a scoopula.
- "Pre-sieve" the soil by passing it first through a 4 mm mesh sieve.
- If the sample is still unable to pass through the sieve, submit a ServiceNow ticket to receive further instruction.

6. Discard particles > 2 mm or anything hand-picked from O horizons according to permit requirements.
7. If adding sieved soil back to plastic gallon sample bags, write 'sieved' on them so there is no confusion as to whether they have been processed.
8. Clean sieve using DI water and pat dry with a clean paper towel before re-use. Sieves can be placed in a drying oven to help speed up drying between uses, but ensure they are cool to the touch prior to use.

NEXT STEPS:

- For a gradient site conducting an off-year bout, proceed to SOP G: Air-drying Soils.
- For a core site conducting an off-year bout, proceed *immediately* to SOP J.1: Processing Microbial Biomass Samples, and then continue to air-drying soils in SOP G.
- For any site conducting a coordinated bout, proceed *immediately* to SOP J: Laboratory Subsampling for Core Sites and Coordinated Bout.

SOP G Air-drying Soils

Air-dried soil is used for measuring soil pH (all bouts *except* Coordinated, Tfinal) and for generating a BGC archive sub-sample (Coordinated, peakGreenness). Follow this SOP after all other sub-sampling has been completed.

- For a gradient site conducting an off-year, non-coordinated bout, follow this SOP after completing SOP F. Aim for 100 g of soil to air dry.
- For a core site conducting an off-year, non-coordinated bout, follow this SOP after completing SOP J.1. Aim for 100 g of soil to air-dry.
- For any site conducting a coordinated, Tinitial bout, follow this SOP after completing SOP K: Laboratory Processing of Soils for N Transformations. Refer to **Figure 6** and the Quick References for guidance. Aim for 400 g of soil to air dry.

1. Wear un-soiled (e.g. clean, but sterile not required) nitrile gloves. Gloves may be re-used between samples as long as any visible dirt is rinsed off with DI and gloves are dried well.

2. Place all remaining hand-picked (O) or sieved (M) soil material into #8 paper bags labeled with the **sampleID**. The sample barcode label may be transferred from the bulk soil bag to the paper bag if desired and may aid in populating data in the *SLS: pH [PROD]* application. With very wet or fine-grained soils that can leak out, cover the seams along the bottom of the bag with masking tape.

   For samples marked as containing *Toxicodendron spp*, take precautions to reduce exposure to toxic oils; even though larger roots have been removed, fine roots may still be present. Touch these sample as little as possible, and change gloves if the sample is handled directly. Make sure to either transfer the sample warning label to the paper bag, or add a new one.

3. Break up large clumps and soil aggregates with a gloved hand (non-sterile but clean) and spread out soil to facilitate drying.

4. Weigh the bagged sample and record in the air-drying tracking spreadsheet available on the SSL; initial mass may also be recorded on the sample bag. This initial mass is used to track the completion of sample drying.

5. If the mass is significantly greater than the maximum target air-drying mass (100 g for off-year bouts, 400 g for coordinated bouts), then remove soil from the bag to get closer to the target mass; the initial mass should be re-measured and recorded.

6. Loosely close bag and place on a clean lab bench or table, away from other activities that might disturb samples. Record **airDryStartDate** on the air-drying tracking spreadsheet, if desired.

7. Once every few days, shake up soil to expose new surfaces.

8. Weigh samples again when they appear dry, which may be days to weeks depending on soil moisture content, climate and soil type. Record the mass in the air-drying tracking spreadsheet. It is crucial that samples have dried completely.
    a. Do not take a second weight until at least 5 days have passed since **airDryStartDate**, this is the minimum air-drying duration.
9. If change in weight between the current and previous measurement is < 5 %, continue with sample processing. If change in weight is > 5%, then begin to weigh samples every 2 days until the change in weight is < 5%.
10. At the conclusion of air-drying samples, record **airDryEndDate**.

NEXT STEPS:

- For an off-year, non-coordinated bout, proceed to SOP H: Laboratory Measurement of pH. Any air-dried material remaining after completion of pH analysis should be stored or discarded following the 'Storing excess soils' guidance below.
- For a coordinated, peakGreenness bout, proceed to J.3: Processing BGC Archive Samples.

Storing excess soils. During a non-coordinated bout or a coordinated T1 or T2 bout, hold remaining soil after all subsampling and analyses have been completed, up to 1 year. This holding time will allow for dry soil re-measurement or re-subsampling if needed, and may also allow external community members to opportunistically request excess soil material. If space is not an issue, store all samples for the 1-year period. If space is limited, select a sample from one subplot in each plot and for each horizon per bout, discarding the others. Fold the tops of the paper bags, and place in a large box or action packer (36 or 48 gallons) and store in safe location. If paper bags have holes in them, transfer entire thing to a plastic re-sealable bag first. After one year, stored soil can be discarded according to permit requirements. If storage space in the container runs out before the 1 year time point, discard the oldest samples first to make room for newer samples.

SOP H Laboratory Measurement of pH

Soil pH is measured on sieved (or hand-picked), air-dried soil samples. Soil pH is measured potentiometrically in a saturated or supernatant liquid that is in equilibrium with a soil suspension. Each domain should have a dedicated, soil-specific pH probe for use in this procedure. In general, M-horizon soils use a 1:1 soil-to-H_2O and 1:2 soil-to-$CaCl_2$ (weight/volume) mixture, whereas O-horizon soils use a 1:4 soil-to-H_2O and 1:8 soil-to-$CaCl_2$ ratio. Samples are analyzed both in deionized (DI) water and 0.01 M calcium chloride ($CaCl_2$). Helpful instructional videos are available in the NEON internal Training Center.

- For all sites, pH measurements should be completed within 60 days of sample collection. If this is not possible, advise NEON Science of the delay via ServiceNow.

Safety advisory: This SOP involves handling strong acids and bases. Handle hazardous materials carefully and according to NEON EHSS guidelines. Always wear gloves for your protection.

H.1 Prepare the 0.02 M $CaCl_2$ Solution

1. Put on a new pair of nitrile gloves.
2. In a 2-L volumetric flask, dissolve 5.88 g of $CaCl_2 \cdot 2H_20$ in just under 2 liters of house DI water.
3. Check pH of $CaCl_2$ solution; it should be between 5.0 and 6.5.
4. Adjust pH to desired value by adding concentrated 6N $Ca(OH)_2$ or 1N HCl one drop at a time.
    a. To create 1N HCl, dilute 10N HCl by a factor of 10 (for example, 10 mL 10N HCl in 90 mL DI water), adding acid to water and wearing appropriate protective lab equipment.
    b. To create 6N $Ca(OH)_2$, dissolve 22.23 g of powder in DI water using a 100 mL volumetric flask. The flask may warm, handle wearing appropriate protective lab equipment.
5. Bring $CaCl_2$ solution to final volume of 2L and label the container with your initials, '0.02M $CaCl_2$' and the creation date.

Note: The $CaCl_2$ solution is stable for approximately 1 year, kept at room temperature out of direct sunlight.

H.2 Measure pH

1. Clean lab benchtop with DI water prior to processing samples.
2. Wear un-soiled nitrile gloves (i.e., clean, but sterile not required). Gloves may be re-used between samples as long as any visible dirt is rinsed off with DI and gloves are dried well.
3. Gather and label vessels to weigh soil and measure pH
    a. For O horizons, use either 50 mL conical tubes or 100 mL glass beakers.

b. For M horizons (1:1 ratio), only use 50 mL conical tubes. This shape of container is needed to have enough solution to submerse the reference junction (small ceramic plug) on the pH probe.

4. Using a clean tool, weigh out a subsample of air-dried soil.
    a. For M horizons, use 10 g (± 0.5 g) of sieved, air-dried soil.
    b. For O-horizon samples, use up to 5 g (± 0.5 g) of picked clean soil.
        i. For horizons that are very fluffy and absorbent, or if using conical tubes, O horizon mass may need to be lower. When using conical tubes, try 2 g of soil. If that proves too much soil to wet without overflowing the container, decrease to as low as 1 g without decreasing the amount of solution added.
5. Record soil mass to 0.01 g accuracy in the *SLS: pH [PROD]* Fulcrum application or lab datasheet.
6. Identify a group of samples that will be processed as a batch (suggested: 5 to 15 samples)
7. One at a time, add DI H_2O to each sample in the batch using a graduated cylinder or equivalent volumetric device with precision to ± 1 mL:
    a. For M horizon samples, add 10 mL DI H_2O – this will be a 1:1 soil to solution ratio.
    b. For O horizon samples, add 20 mL DI H_2O – this will be a 1:4 (or greater) soil to solution ratio.
8. Allow soil to absorb the H_2O. Thoroughly stir each sample with a clean stir rod for 20 seconds. Clean stir rod with a Kimwipe and DI water between samples.
9. Ensure that all soil is saturated, particularly when using conical tubes.
    a. For M horizons, look for supernatant, a thin layer of liquid above the settled soil (**Figure 22**).
    b. For O horizons, the particles will not settle, but observe that all soil material is wetted. There should be a thin liquid layer beneath the floating particulate matter
    c. If needed, add another aliquot (10 mL) of H_2O, stir and settle. *For the majority of sites, few M horizons will need this extra volume. It is more commonly needed for O horizons.*
    d. Add as many 10 mL aliquots as needed to ensure soil is completely saturated. Keep track of the total volume of solution added in the *SLS: pH [PROD]* Fulcrum application or datasheet.
        i. The application may warn when adding > 10 mL DI H_2O for an M horizons sample. If more solution is needed to fully wet the soil, simply accept the message and proceed.

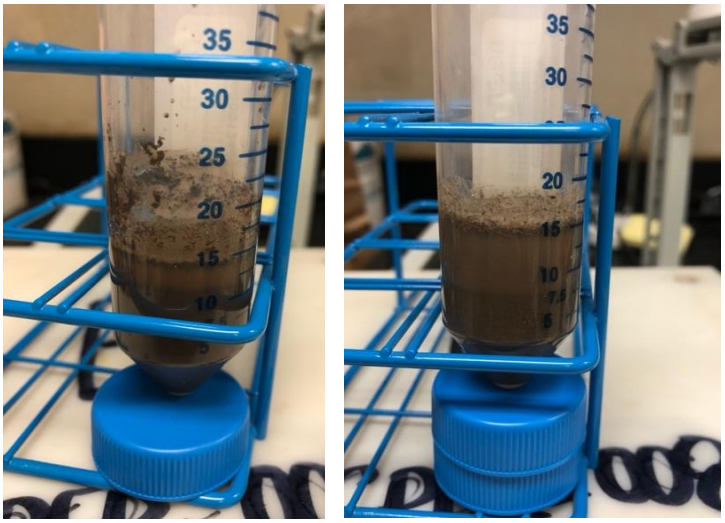

Figure 22. Two M horizon samples ready for pH measurement. The thickness of the supernatant varies with soil texture and chemistry, but in both cases the samples are fully wetted with only 10 mL of water added.

10. Let the samples sit for 30 minutes, then stir each sample in the batch with a clean stir rod for 20 seconds. Let the samples sit for another 30 minutes.
    a. Soils need to be open to the atmosphere during the waiting period so they can equilibrate with the air. If using conical tubes, do not cap.
    b. Samples must be stirred with a stir rod, they cannot be capped and shaken as this may entrain carbon dioxide and influence pH readings.
11. During the waiting period, calibrate the pH meter electrode using the buffer solutions that best encompass the ranges in soil pH encountered. Follow calibration instructions in the manual for the probe and ensure that only the soil-specific pH probe is used. **NOTE**: For pH meters that auto-detect calibration solutions being used, ensure that the meter selects the correct suite of calibration buffers (e.g. that the auto-selected pH values match the actual pH values used).
    a. For high pH soils, use buffers 4, 7, and 10
    b. For low pH soils, use buffers 1.68, 4, and 7
    c. Rinse the electrode with deionized water into a waste beaker and gently shake off excess liquid between buffers. **Do not** touch the tip of the electrode with a wipe.
12. Begin measuring **soilInWaterpH** 60 minutes after the first sample in the batch was initially stirred (e.g. after 1 hour total since adding solution has elapsed).
13. Starting with the first sample, stir with a clean stir rod for 20 seconds, then let the sample settle for 1 minute.
14. Measure the pH of that sample by inserting the probe into the soil suspension. The probe should be inserted deep enough that the refence junction (small ceramic plug) near the tip of the probe

is in the solution. Gently swirling the container while measuring pH can help the measurement stabilize but is not required. Ensure the probe porthole is open.

a. How deep to insert the probe tip depends on the soil horizon type:

i. For M horizon samples, measure in the top 1/3 of the slurry, e.g., only submerse probe tip ~ 1.5 cm. This more dilute part of the slurry will avoid most settled particles.

ii. For O horizons, submerse probe tip into the solution *below* the layer of floating organic material that will be present. It is OK if some flocculated soil is floating in this region.

iii. If the pH meter has automated temperature correct, ensure the temperature probe is inserted into the solution alongside the pH probe.

15. Allow reading to stabilize (tenths of pH unit not fluctuating, usually < 1 minute) and record pH value to 0.01 accuracy. If reading still fluctuates slightly after 1 minute, read pH every 5 seconds for 15 seconds, then record the average.

16. Clean electrode: rinse thoroughly with deionized water into a waste beaker and gently shake off excess liquid. Do not touch the tip of the electrode with a wipe, although the *sides* of the probe may be gently wiped with a Kimwipe if soil residue is observed.

a. If both **soilInWaterpH** and **soilInCaClpH** have been measured and recorded for a sample, continue to Step 20.

17. After reading the **soilInWaterpH**, immediately add $CaCl_2$ solution to the same container. Add the same volume of 0.02 M $CaCl_2$ as DI H_2O so that the final concentration of $CaCl_2$ is 0.01 M, and enter this value into the **vol CaCl2 added** field. The entire sample may be transferred to a larger container if adding more volume will cause sample to overflow.

a. For M horizon samples, add 10 mL of $CaCl_2$ (or however much H_2O was added).

b. For O horizon samples, add 20 mL of $CaCl_2$ (or however much H_2O was added).

18. Stir sample with a stir rod for 20 seconds and let sample sit for 1 minute. Rinse stir rod with DI H_2O and wipe with a Kimwipe between samples.

19. Immediately after 1 minute of settling, measure **soilInCaClpH** of the sample by repeating Steps 14-16 and record value in **soilInCaClpH** field. *There is no additional waiting period*.

20. Move on to the next sample in the batch. Repeat for all remaining samples, first measuring **soilInWaterpH** and then **soilInCaClpH** in sequence. Measure samples in the same order that the water was added so that samples will sit for similar amounts of time.

a. Check pH meter calibration using the middle buffer solution every 10-12 samples. Recalibrate if drift is observed.

21. Discard remaining soil slurries following applicable soil permit guidelines. Any containers that will be re-used should be washed with laboratory soap (Alconox, Contrex, etc) and water, rinsed thoroughly with tap water, and given a final DI water rinse.

What to do when a sample is lost:

- Create a record for the sample.
- Enter the **sampleID** and any other metadata measured for that sample.
- Select “lost” for **phSampleFate**. This allows you to leave other required fields empty.

SOP I Generation of Composite Soil Samples for Microbial Metagenomics Analysis

While most of the microbial molecular analyses are conducted at the scale of a core sample, metagenomic analyses are conducted on soil at the plot scale. This SOP describes the laboratory procedure for generating and labeling a composite soil sample during instances when the composite sample was not generated in the field.

Note: Metagenomic samples are only collected during the peak greenness bout at Core sites or Gradient sites during coordinated sampling years.

1. At least 30 minutes prior to use, place a cryo-safe barcode label on each new Whirl-Pak bag that will be used to store composite samples. Also, label each Whirl-Pak bag with the **compositeSampleID** composed of the plotID, horizon, collection date, and "-comp" for composite. These should be pre-printed cryo-safe labels. *Ex. HARV_001-M-20210715-comp*
2. Repeat Step 1 for every unique combination of plotID, horizon, and collection date. There should be 1 new Whirl-Pak bag for every unique combination.
3. Obtain a pair of new nitrile gloves and pre-sterilize with 70% ethanol. Re-sterilize gloves between samples.
4. From the -80 °C freezer, obtain 1 Whirl-Pak from each core sample. Organize Whirl-Pak bags by placing those from the same collection date, plot, and horizon together. Double-check the labels to ensure that the sample collection dates, plotIDs, and soil horizons match. Typically, there will be 3 Whirl-Paks, but fewer than 3 is also possible.
5. Thaw the material in a set of Whirl-Pak bags and transfer all material in each into the corresponding composite Whirl-Pak bag. Homogenize the soil by gently kneading and/or shaking the outside of the closed Whirl-Pak.
6. Return the sample bags to the -80 °C freezer (or container of dry ice, if no freezer is accessible) immediately.
7. Repeat for the remaining samples.
8. Complete data entry by recording which samples were composited. Ensure that all metadata have been recorded, then discard empty Whirl-Paks. Scan the barcode for each -comp sample.
    a. When entering the sampleID's for each sample added to the composite sample, the order of each sampleID *must* match the order of the sample barcode ID's in the Fulcrum application.

SOP J Laboratory Subsampling for Core Sites and Coordinated Bouts

Follow this SOP for core site bouts and Coordinated bouts where **nTransBoutType** = Tinitial, immediately after completing SOP F Sieving and Picking Field Soils for Lab Processing.

IMPORTANT: Subsampling MUST begin within 1 day of field collection to preserve sample integrity.

J.1 Processing Microbial Biomass Samples

Reminder about processing organic soils: recall that it is critical to remove as much root material as possible. If samples still contain a lot of non-friable root material, spend a few more minutes removing roots with forceps before proceeding. This is most likely for O-horizon samples. If in doubt, contact Science. For Alaska sites, follow site-specific guidance in Appendix D.5.

1. Wear un-soiled nitrile gloves (i.e., clean, but sterile not required). Gloves may be re-used between samples as long as any visible dirt is rinsed off with DI and gloves are dried well.
2. Place a centrifuge tube holder or rack on the balance, then add a new, clean 50 mL centrifuge tube. With the cap off, tare the balance.
3. For M horizons, transfer 10 g (± 0.5 g) of field-moist, sieved soil.
4. For O horizons, transfer 5 g (± 0.5 g) of field-moist soil that has been picked clean.
    a. It is OK if > 1 tube is needed to hold 5 g of O horizon soil. Place all tubes for that sample into a pint-sized freezer bag and affix a Type II barcode label to the bag to serve as **biomassSampleCode** for the sample. If > 3 tubes are required, contact Science.
5. Ensure caps are on tightly. Place tubes in centrifuge tube rack if it helps with organization.
6. Scan barcodes to associate each with the appropriate **biomassID** in the *SLS: Field Sampling [PROD]* application. Human-readable labels should appear as:

    plotID-horizon-subplotID-collectDate-bm
    ex. HARV_001-O-21-20210415-bm

7. Store vials at -80 °C until ready for shipment.
8. Ship samples according to SOP M.

What to do when a sample is lost:

- Create a **biomassID** for the sample.
- Select “lost” for **biomassSampleFate**.

NEXT STEPS:

- If this is *not* a coordinated bout, proceed to **SOP G**, air-drying soils.
- If conducting a Tinitial N-trans bout *and* **sampleTiming** = peakGreenness, proceed to **SOP J.2**: Processing BGC Analysis Samples.
- Otherwise, proceed to **SOP K**: Laboratory Processing of Soils for N Transformations.

J.2 Processing BGC Analysis Samples

Biogeochemical analysis of soil for C and N contents and stable isotopes is performed during coordinated bouts when **sampleTiming** = peakGreenness.

1. Fill ½ of a glass scintillation vial with each unique sample. If sample quantity is limiting, it is ok to put less soil in the vial, approximately ¼ full minimum.
    a. For O horizons, use field-moist soil that has been picked clean.
    b. For M horizons, use field-moist, sieved soil.
2. For soils that are 100% saturated and have high clay content (ex: TOOL, wetlands), first dry the sample in a tin, then transfer it to the vial. Without this, the soil cannot be removed for analysis.
3. Loosely cap vials, such that the cap is only resting on top (not threaded).
4. For samples marked as containing *Toxicodendron spp*, add a sample warning label to the cap.
5. Place scintillation vials containing samples into the scintillation vial box, which holds up to 100 vials. Record **ovenStartDate** and time in the SLS: BGC Sub-Sampling Fulcrum application or datasheet.
6. Oven-dry at 65 °C for at least 48 hr. More time may be needed: ensure samples look visibly dry before removing. Record **ovenEndDate** and time in datasheet or Fulcrum application.
    a. If not started already, begin processing N transformation samples (SOP K) immediately after CN samples are generated.
7. When drying period is complete, tighten caps on vials (or transfer to vials for saturated soils). Ensure that all **cnSampleIDs** have been created in the *SLS: BGC Sub-Sampling [PROD]* Fulcrum application and that each barcode has been scanned.
8. Store vials at ambient temperature until shipping. Ship samples according to SOP M.

NEXT STEPS: Proceed *immediately* to SOP K: Laboratory Processing of Soils for N Transformations. Return to complete section J.3: Processing BGC Archive Samples once air-drying is completed.

J.3 Processing BGC Archive Samples

During coordinated bouts when **sampleTiming** = peakGreenness, an archive BGC soil sample is created from any air-dried soil remaining after all laboratory subsampling and analyses have been completed.

1. Wear clean gloves while handling samples (non-sterile OK).
2. Place a pre-labeled and barcoded archive jar on the balance with no lid and tare it.
3. Transfer any remaining air-dried soil from the #8 paper bag. Fill bottles up to, but not beyond the lip of the bottle.
4. Record soil mass added to the jar to nearest 0.01 g (**bgcArchiveMass**).
    a. Ideally at least 10 g O horizon soil or 50 g M horizon soil are archived per sampling location. If masses are below these thresholds, take note so that future peak-green coordinated bouts conducted in the Domain can sample more soil.
5. Ensure that all **bgcArchiveIDs** have been created in the *SLS: BGC Sub-Sampling [PROD]* Fulcrum application and that each barcode has been scanned.
6. For samples marked as containing *Toxicodendron spp*, add a sample warning label to the cap.
7. Store bottles at ambient temperature until shipping. Ship samples according to SOP M.

What to do when a BGC analysis or archive sample is lost:

- Create a record for the sample.
- Enter the **sampleID** and any other metadata measured for that sample.
- Select “lost” for **cnSampleFate or bgcArchiveFate**. This allows you to leave other required fields empty.

SOP K Laboratory Processing of Soils for N Transformations

This SOP describes the instructions for processing samples for N transformation analyses and is used for all coordinated bouts. Helpful instructional videos are also available in the NEON internal Training Center. Both Tinitial and Tfinal samples are processed in the exact same way, using potassium chloride (KCl) to extract inorganic N.

Note: *There have been rare instances of entire bouts of N-trans extracts getting destroyed or lost during shipment. If this has happened in the Domain and/or there are shipping concerns, it is acceptable to create a second back-up scintillation vial when collecting filtered extracts (K.4). Label these with kclSampleID but no barcode and discard after samples are successfully shipped to the external lab.*

IMPORTANT REMINDERS:

- Subsampling MUST begin within 1 day of field collection in order to preserve sample integrity. Ex: For samples collected on a Monday, SOP K must be completed by the end of Tuesday.
- Soil moisture is a critical component for calculating N transformation rates, thus it is essential that this measurement be made. Follow SOP E, if not done already.
- In order to measure concentrations of soil inorganic N in diverse ecosystems, care must be taken to use clean lab procedures (as defined below) and not introduce contaminant N.
- KCl used for extractions is first purity-tested to ensure low inorganic N, then shipped to each DSF. Field Science should not purchase KCl directly unless advised by NEON Science.

K.1 Cleaning and Storage of Equipment and Supplies

1. Cleaning and storage of durable equipment:
    a. Wash all equipment that will be used for extractions (Nalgene filtration units, beakers, flasks, carboys, graduated cylinder, scoopulas, funnel, etc) as follows: 1x wash with lab soap (Alconox, Contrex, etc), 5x rinse with house DI, 2x final rinse with ultrapure DI.
    b. Once the equipment is dry, place in sealed, new plastic bags or in a closed cabinet with tops covered in parafilm.
    c. If unsure whether equipment has been cleaned and stored properly, wash immediately prior to use. It is ok to use items while wet but shake off excess water prior to use.
2. Storing and handling of consumable equipment:
    a. Once opened, packages of scintillation vials, extraction cups and filters must be kept in sealed plastic bags or another clean, closed environment. This will keep out dust, which contains ammonium and nitrate.
    b. Wear clean nitrile gloves while pulling vials and cups out of bulk packaging and cap them immediately upon removal.

c. Handle filters with clean (house DI-rinsed) filter forceps only.

3. Plan to conduct extractions in a 'clean lab' environment. Lab spaces with a lot of dirt/dust/organic particles may introduce contaminant N to the samples.

    a. At a minimum, clean benchtop areas where extractions will take place using house DI to remove dust and dirt, and keep this workspace clean while conducting extractions.

K.2 Prepare 2M KCl Solution and Pre-leach Filters

Note: *KCl in solution is good for ~1 year, so ideally a large batch (20 L) is made at the beginning of the sampling year and then used for filter prep and initial/final extractions of each of the 1-3 sampling bouts. Remake solution as necessary. If the solution must be remade in the middle of a set of extractions, prepare an additional set of three blanks for the new batch of KCl, as in section K.3.*

Reminder: Only use KCl that has been purity tested then sent to the DSF. Use care when making solutions and follow all instructions exactly as written below.

1. Wear clean nitrile gloves (non-sterile ok).
2. For a large batch (20 L) of 2M KCl, measure 2,982 g KCl into a clean receptacle and add to a clean 20 L carboy. Add **Type I ultra-pure deionized water** to just below the 20 L mark. Cover and swirl carboy, allowing KCl to dissolve (may take several hours). Once dissolved, top off the carboy to the 20 L mark with Type I ultra-pure deionized water.
3. For a small batch of KCl, measure 149.1 g KCl into a new weigh boat. Transfer to a clean 1 L volumetric flask and fill with **Type I ultra-pure deionized water** to below the 1 L mark. Cover and swirl flask, allowing the KCl to dissolve (may take up to 1 h). Once dissolved, top off the flask to 1-L with Type I ultra-pure deionized water.
4. Pre-leach GF/A filters using 2M KCl and a Buchner funnel. <u>These are good for 1 week</u>.
    a. Using clean filter forceps, transfer ~ 20 new filters (don't need to count, ok to approximate) to a clean Buchner funnel. To make the filters easier to separate after leaching, stack them in groups of ~ 5 that are slightly offset in the funnel.
    b. Place #8 stopper with hole onto Buchner funnel stem and insert stopper into side-arm flask. Attached side-arm flask to pump with plastic tubing.
    c. Add 2 M KCl until the stack of GF/A filters is saturated (20-30 mL), then turn on the pump to leach filters.
    d. Repeat (c) to leach the filters two more times for a total of 3 rinses.
    e. Use clean filter forceps to transfer pre-leached filters to a new, pint-size clean plastic bag. Write 'GF/A filters, leached with 2M KCl' and the date. Make sure the bag is sealed and place it in the fridge (4°C).

f. Repeat steps above for as many filters as may be needed in the next week.

K.3 Perform KCl Extraction

Reminder: KCl extraction is performed on sieved/picked soil. If not already completed, follow instructions in SOP F before extracting samples.

1. Put on a new pair of nitrile gloves. Use the same pair of gloves until they become visibly soiled. When that happens, either clean them with DI water and dry, or change gloves.
2. Obtain extraction cups that have either been pre-labeled with a **kclSampleID** as described in SOP A, or use a cupID labeling system, similar to the tinIDs used for soil moisture.
3. Add 10 mL of **Type I ultra-pure deionized water** to each specimen cup. Screw on the cap, then shake vigorously for a few seconds, ensuring the entire cup and cap are wetted.
4. Open the cap and discard water. Shake out droplets.
5. Repeat Steps 3 & 4 for a second rinse. This ensures removal of any contaminant N in the cups.
6. Tare a cup, then add 10 ± 0.5 g of field-moist, sieved M or homogenized O horizon soil. Record the **soilFreshMass** to the nearest 0.01 g in the *SLS: Nitrogen Transformations [PROD]* application.
    - If sample mass is limiting or KCl solution is running low and more cannot be made, it is acceptable to use less mass, but do not use less than 4 g per sample.
    - If < 4 g O horizon is available, combine it with the mineral matter and extract together. Record this in the **remarks**.
    - Ensure that for O horizons, no rocks, large roots or non-soil debris remain in the sample, as described in SOP F.1: Sieving and Homogenizing Samples.
7. Measure 100 ± 2 ml of 2M KCl into a clean graduated cylinder and add to the container of weighed soil. If less soil mass was used, measure out a volume scaled to the soil mass, roughly 10:1. Record the **kclVolume** and **extractionStartDate** (YYYY-MM-DD HH:MM). The latter should reflect the approximate time that soil and solution are combined.
8. Make sure cap on the extraction cup is on tightly, then shake vigorously for ~15 seconds.
9. Repeat for all samples that need to be extracted. If this is a large number of samples, it may make sense to split them into two groups – for example, weigh soil and add solution to the second sample group while the first sample group is on the shaker table.
10. Every day that samples are extracted, procedural blanks must be prepared - even if the KCl came from the same large carboy used to extract samples on a previous day. Create 3 procedural blanks, unless you are extracting ≤6 samples in a day (not preferred) or KCl solution is running low – in which case create 2 blanks.

a. Add 100 ml KCl to each of the blank extraction cups and treat the same as samples containing soil. Label blanks as follows:

   i. First, note the **kclReferenceID**, which describes the KCl batch used that day for extractions (format = siteID-extractionStartDate-'BRef1', example: CPER-20190418-BRef1). *Blanks are labeled with the date of extraction, not the date of field collection*. If a new batch of KCl is created in the midst of processing samples, it will have a new kclReferenceID (example: CPER-20190418-BRef2).

   ii. In the Fulcrum application, records for all of the samples extracted on a single day should be nested under this **kclReferenceID**.

   iii. For each of the replicate blanks, record **kclBlankID**'s by appending the **kclReferenceID** with a dash followed by the letters A-C (ex: CPER-20190418-BRef1-A, CPER-20190418-BRef1-B, CPER-20190418-BRef1-C).

b. If you have to make a new batch of KCl solution in the middle of processing a group of samples, you must prepare 3 *additional* blanks for the new solution (BRef2).

c. In the Fulcrum application, all samples extracted with a set of procedural blanks should be nested in that 'parent' record, this ensures the data from samples and blanks can be linked.

11. Place all samples and blanks in a box or similar container that fits on the shaker table. Use padding to fill empty space to ensure that cups do not shift while shaking.
12. Place the box on its side so that samples shake end-to-end. **Shake for 1 hour at 150 rpm**.
13. Remove extraction cups and organize on benchtop.
    - If a substantial amount of KCl leaked out of any sample cup during shaking, estimate how much using the cup gradations and adjust **kclVolume** accordingly, noting the leak in the **sampleCondition** field.
14. Allow soil to settle without disturbance for ~ 15 minutes while setting up the filtering manifold. This will facilitate faster filtering.

K.4 Filter Samples

Note: *Samples are generally filtered in batches, the size of which will depend on the number of filtration set-ups available - generally 4. Soil samples within a batch may finish filtering at different times. New samples can be added by closing the stopcock on the vacuum line that has finished, cleaning and replacing the filtration apparatus, applying a new pre-leached filter, and then filtering another sample.*

Reminder: Samples should be filtered soon after they are done extracting, within minutes to a few hours and certainly on the same day. Do not allow extracts to sit overnight before filtering.

1. Obtain scintillation vials that have been pre-labeled as described in SOP A.
2. Set up the manifold (**Figure 23**) and attach to a vacuum pump. Check that all stopcocks are in the closed position (perpendicular to the tubing).

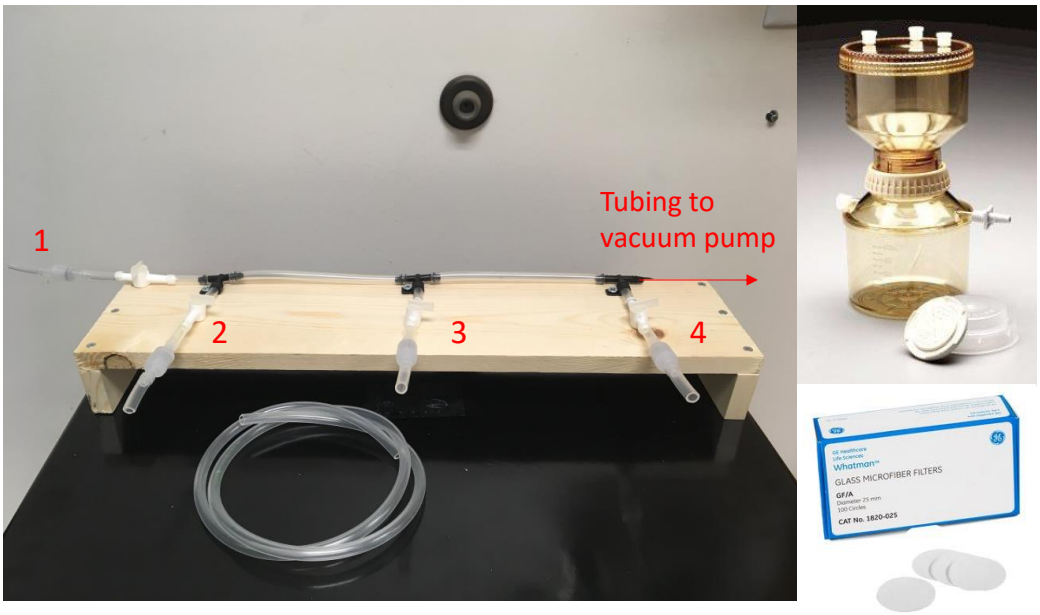

Figure 23. Filtration equipment for KCl extractions. Example of a manifold set-up (left) and other equipment including filtration unit (extraction funnel + collection cup) and filters (right).

3. Put on a new pair of nitrile gloves. Use the same pair of gloves throughout this procedure as long as they do not get splashed with sample. If that occurs, discard and put on a new pair.
4. Plan to spread blank extractions throughout, such that Blank A is filtered near the beginning of the day, Blank B in the middle, and Blank C near the end. This may help with troubleshooting if any of the blanks have high nitrogen.
5. Turn on the pump. Open one of the stopcocks (turn parallel to tubing) and check that a vacuum is created on that port by placing a gloved hand on the open end of the tubing. If there is no suction, close the stopcock, then look for clogs or leaks between connections in the tubing. Open the stopcock and test again until a vacuum is detected. Repeat for all ports, testing one at a time, then turn off the pump.
6. *Add the filter*: Open filtration units. Using clean filter forceps, place a pre-leached filter onto each filter holder (it does not matter which side of the filter faces up or down, they are equivalent). Close filtration units, making sure filters do not fold or tear in the process.
7. Turn on the pump and open the stopcock(s). Pour 20-30 mL of soil solution from one sample into an extraction funnel. Repeat for all filtration units.

8. While samples are filtering, record **extractionEndDate** (YYYY-MM-DD HH:MM) for each sample, and scan the **kclSampleCode** for each scint vial.

9. Once sample has filtered completely, transfer the filtrate from the collection cup into the-labeled, barcoded 20 ml scintillation vial. Leave enough room for the liquid to expand when the sample freezes (at least 1-2 mL of headspace). Cap sample tightly.

    - If a sample takes longer than 10 minutes to filter, but a sufficient volume of sample has already been produced (minimum 15 mL), it is acceptable to stop filtering the sample. Transfer the filtrate as instructed above and discard the rest of the unfiltered extract.
    - If soil particles are observed in the scint vial, this means the filter tore or was not seated properly in the holder. Replace filter with a new one and filter sample again.

10. Discard remaining filtrate from the collection cup into a waste vessel. KCl is a neutral salt and can be disposed of down the drain. Discard used extraction cups in the trash.

11. Clean filtration units thoroughly prior to re-use between samples.

    a. Fill two dishpans ~3/4-way with house DI water. These should be relatively clean dishpans used only for washing laboratory equipment.

    b. While still wearing gloves, immerse filter holder and cup in the first water basin and swirl. This 'dirty basin' will remove most particulates and soil residue.

        i. When dirty basin water becomes excessively dirty (very brown in color, full of large soil particles), change out for fresh house DI water.

    c. Transfer filter unit to the second, 'rinse' basin and swirl.

    d. Rinse filter holder and cup 3X with fresh house deionized water - can be directly from the wall unit, or from a carboy or squirt bottle.

    e. Conduct a final rinse with Type 1 ultra-pure deionized water, either directly from the container or using a squirt bottle.

    f. Shake to remove excess water, then re-assemble. Equipment is ready to use.

12. When filtering is complete, freeze extracts ***upright*** at -20°C – either in the cardboard trays in which the vials come, or some other tray or box. This ensures frozen filtrate will remain at the bottom of the vial, where it is less prone to expand and crack the vial or push off the cap. Once samples are frozen and prior to shipping, transfer vials to a resealable plastic bag labeled with siteID and date, as this will be more space-efficient for shipment.

    a. If vials do not freeze after some time in the -20°C freezer, try shaking them. If this is ineffective and vials never freeze, note this in the **sampleCondition** field.

13. Store frozen until shipment to contracted lab facility. Ship samples according to SOP M.

K.5 Sample Storage

Samples can be stored frozen at the Domain Support Facility for several weeks prior to shipping, but ideally not longer than 8 weeks. Over time, ammonium can convert back into ammonia, which is volatile and escapes from the vial, causing underestimates of mineralization rates. It is desirable to ship T-initial and T-final extracts from a given bout at the same time, soon after the T-final samples are collected, and once data QA is complete. If there are any issues with the cold chain during sample storage, note this using the appropriate **sampleCondition** value and also a remark.

What to do when a sample is lost:

- Create a record for the sample.
- Enter the **sampleID** and any other metadata measured for that sample.
- Select “lost” for **kclSampleFate**. This allows you to leave other required fields empty. Additionally, select **sampleCondition** = “sample lost” and make a note in remarks explaining why no data are expected. If samples are lost due to a problem with sample processing or shipping that should be addressed with NEON Science, submit an issue ticket.

SOP L Data Entry and Verification

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. Data collected on paper data sheets must be transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

L.1 Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data are accurate and complete. This protocol requires that certain QA checks be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office. Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before a sampling window closes. Invalid metadata (e.g. soil temperature, plotIDs) are difficult to correct when field crews are no longer at a sampling location.

QC checklists are available in the SSL to guide the data quality review process once back at the office. Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess inadvertently duplicated data and transcription errors to maintain data ***validity*** and ***integrity***. QC activities should occur as soon as possible following data collection since errors will be easier to fix; this is especially true for the field collection data. The latest that data QA should be completed is determined by the timing of automated data upload to the NEON database, which occurs at a pre-set time after data are electronically entered. For the following types of data, ensure that QA is completed within the following timeframes:

- Field metadata, field-generated subsample data (-gen, -ga), other samples generated in field collection app (-bm): within 21 days of electronic data entry
- Certain lab-generated subsamples that need to be shipped for analysis plus associated metadata (-kcl, -comp): within 30 days of electronic data entry
- Other lab-generated subsamples that take longer to process (-cn, -ba), plus measurements that do not involve sample shipping (pH, moisture): within 45 days of electronic data entry

Data QA and automated data upload should always occur *before* samples are shipped in order to prevent errors from affecting shipped samples. The shipment hold times listed in **Table 5** are designed to provide sufficient time for data QA and automated data upload.

Sometimes when performing soil sampling, horizon identification and separation can be challenging, or other noteworthy situations are encountered that are likely to impact the data. Communicate these situations in the **horizonDetails** field. This would include, for example, instances when a thin O horizon was mixed with an M horizon sample, or vice versa for T-final samples (**Table 12**).

Table 12. Protocol-specific horizon details values. If more than one is applicable, choose the dominant option.

horizonDetails	Description
OK	No known issues (default value)
Thin O horizon present	Thin O horizon collected as part of M horizon sample
Thin M horizon present	For Tfinal samples, thin M horizon collected as part of O horizon sample
Horizon indeterminate	Soil horizon was mis-classified or could not be classified in the field
Water table encountered	Standing water was not present, but borehole was flooded due to high water table.
Mature biological soil crust present	Mature biological soil crust was present and homogenized into the soil sample
Woody horizon	The horizon contained a large amount of decayed wood, see Appendix D.6
Other	Horizon not fitting Protocol definitions due to other reason described in the remarks

In addition to the QA measures described in this section, QA measures needed for this protocol are described in the Data Management Protocol (RD[04]).

L.2 Soil Coordinate Lists

Every soil coordinate location should only be sampled once during a site's lifetime. Master lists of unique coordinates and subplots are generated for each site and are available in the SSL, with usage instructions in Appendix Appendix E. After completing a sampling bout, update the master soil coordinate and subplot lists for the site with the date and status (e.g. sampled, rejected due to rock, etc). If you discover that one or more X, Y locations were sampled but were not the next coordinates designated for sampling, or if a set of coordinates were re-sampled, document the discrepancy in a ServiceNow Incident. Data from resampled coordinates will likely be discarded, or at minimum flagged. When preparing for an upcoming soil sampling bout, review the master site coordinate and subplot lists and ensure that they are up to date in terms of noting X, Y sampling locations from the previous bout(s).

L.3 Sample Labels & Identifiers

By default, each sample and subsample produced by this protocol, with the exception of the genetic archive samples, is assigned a human-readable sample identifier which contains information about the location, date, and horizon of the collected sample. Each sample is also associated with a scannable barcode, which improves sample tracking and reduces transcription errors associated with writing sample identifiers by hand.

Data and sample IDs must be entered digitally and quality checked according to RD[04] prior to shipping samples to an external lab. This is especially important for subsamples that are created in the field, namely -gen and -comp, as these sample ID labels are often completed by hand and are thus prone to error. Before these types of samples are shipped, QC the identifiers as follows:

1. Prepare a cooler or other insulated container and fill with dry ice.
2. Remove as many -gen or -comp samples from the ultra-low freezer as can fit in the container while keeping each partially submerged in the ice. Samples should have contact with dry ice as much as possible while QC checks occur, but it is OK if some condensation is observed during this process.
3. Verify that the sample ID and barcode on each bag matches identically the sample ID and barcode listed in Fulcrum. The inventory application may be helpful for this.
4. Make corrections to sample ID labels as needed, making sure that all notations are legible.
5. Return samples to the ultralow freezer until it is time to ship them (RD[14]). Continue this QC process until all -gen and -comp samples have been checked.

SOP M Sample Shipment

1. Follow sample shipping timelines listed in **Table 5** to maintain appropriate sample hold times and storage conditions.
    a. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment in order to ship samples to external laboratories or the Biorepository (RD[14]).

SOP N Soil Depth Surveys of Plots

This SOP is intended to collect information on soil quantities and distributions in sampling plots to determine the need for site-specific modifications based on limited soil quantities, extremely rocky soils, etc. It is only implemented at sites where problems have been encountered in implementing the current soil sampling protocol. Field Science should reach out to Science via ServiceNow if they believe a depth survey is required for one of their plots.

N.1 Identify the Plot

1. Navigate to the southwest corner of the plot (point 21). Using flags or some other marker, mark the locations that are approximately 5m from the corner of each plot, as shown in **Figure 24**. These locations do not have to be exact.

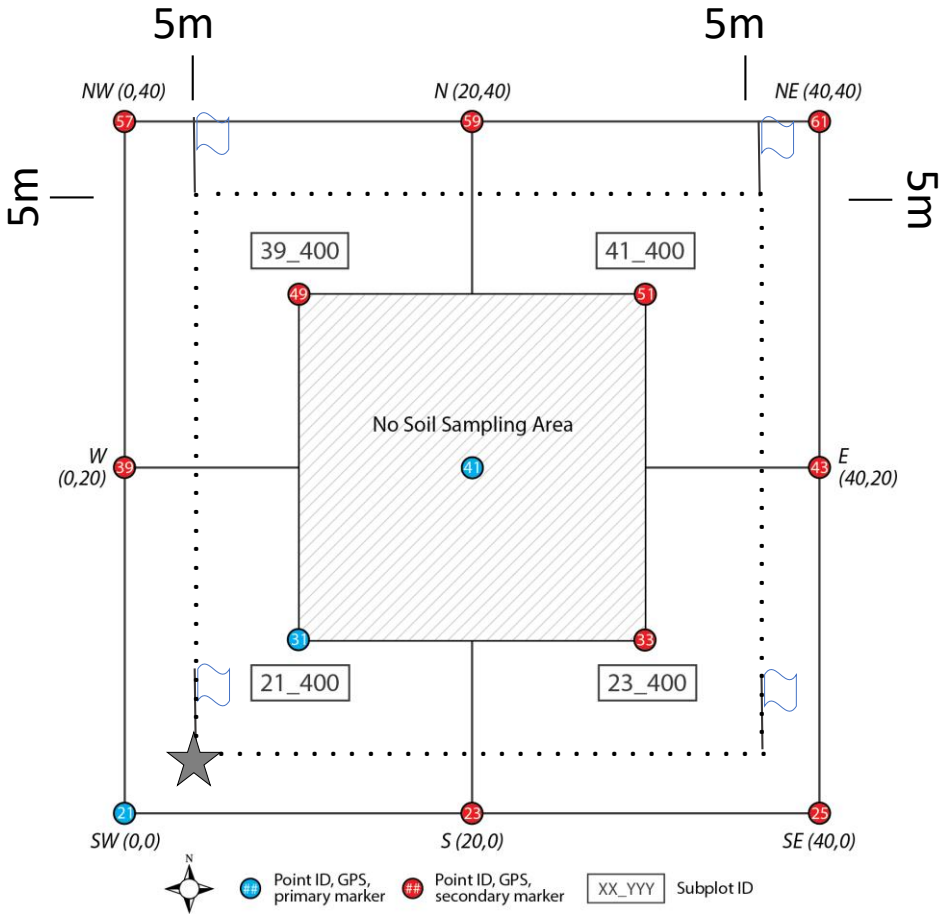

Figure 24. Schematic of TOS soil plot demonstrating the general layout of sample locations. Subplot ID's are noted in the center of each quadrat. Flags denote the corners for the depth transect measuring area, star indicates the location to begin measurement. Dots indicate the general distribution of depth measurements.

N.2 Measure Soil Depths

1. Beginning at the flag located in subplot 21_400, insert soil depth measuring device vertically into the ground and measure depth to the nearest 0.1 cm. Sterility is not required for this survey, although equipment should be cleaned with water and dried before and after use. Record in the data sheet **Field Datasheet: NEON Soil Depth Survey**, under Subplot 21_400. Enter important observations or issues encountered in the remarks section for these and all other measurements.

Note: If any of the selected points fall within an obstruction, such as plant roots, trees, etc., record the depth as zero and note the obstruction. Do not attempt to make a physical measurement within such obstructions.

2. Moving due east toward subplot 23_400, take a depth measurement approximately every 1 meter until you take 15 measurements. After 15 measurements, you should be in subplot 23_400. Take the next 15 measurements and record in the data sheet under subplot 23_400. When you reach a flag, turn 90 degrees to the left and continue measuring approximately every 1 meter. Again, after 15 measurements you should be in the next subplot (41_400) and should record measurements in the appropriate subplot column.
3. Continue moving counterclockwise through the subplots until you reach the beginning. Note that the final 15 measurements will be in Subplot 21_400. There should be 30 measurements per subplot.
4. Remove markers once measurements are completed.
5. Enter completed Data Sheets electronically following the instructions in the Data Management Protocol, RD[04]. NEON Science will review these data and determine whether there is sufficient soil to conduct the sampling protocol or if any modifications are required.

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APPENDIX A QUICK REFERENCES

Table 13. Checklist of samples and analyses associated with an **off-year** soil sampling bout at Core sites. ✓ = measurement; X = physical sample.

Bout Type	Sample Timing	Soil temp (field)	Microbial analysis and archive (field)	Metagenomics (field)	Microbial biomass (lab)	Soil moisture (lab)	Soil pH (lab)
microbes Biomass	Transition	✓	X Whirlpaks/ cryovials (top horizon)		✓	✓	✓
	Peak greenness	✓	X Whirlpaks/ cryovials (top horizon)	X 1 plot-level whirl-pak (top horizon)	✓	✓	✓

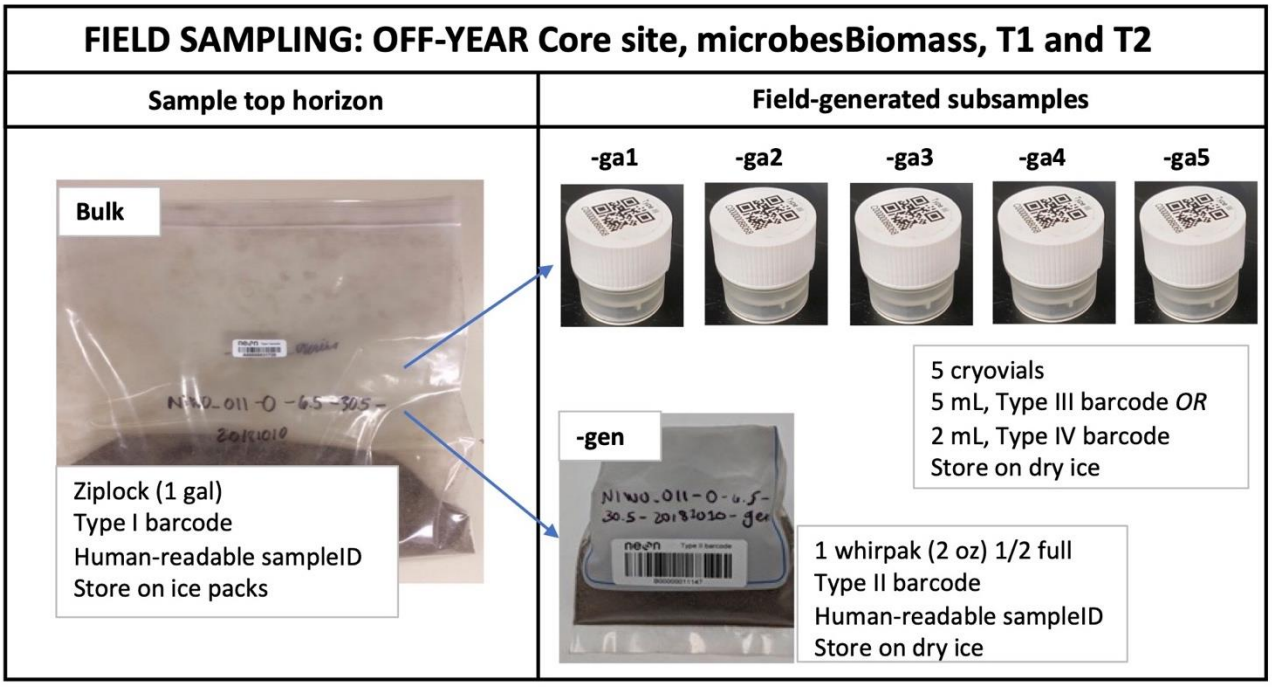

Figure 25. Field-generated samples at Core sites for boutType= microbesBiomass, sampleTiming= T1 or T2.

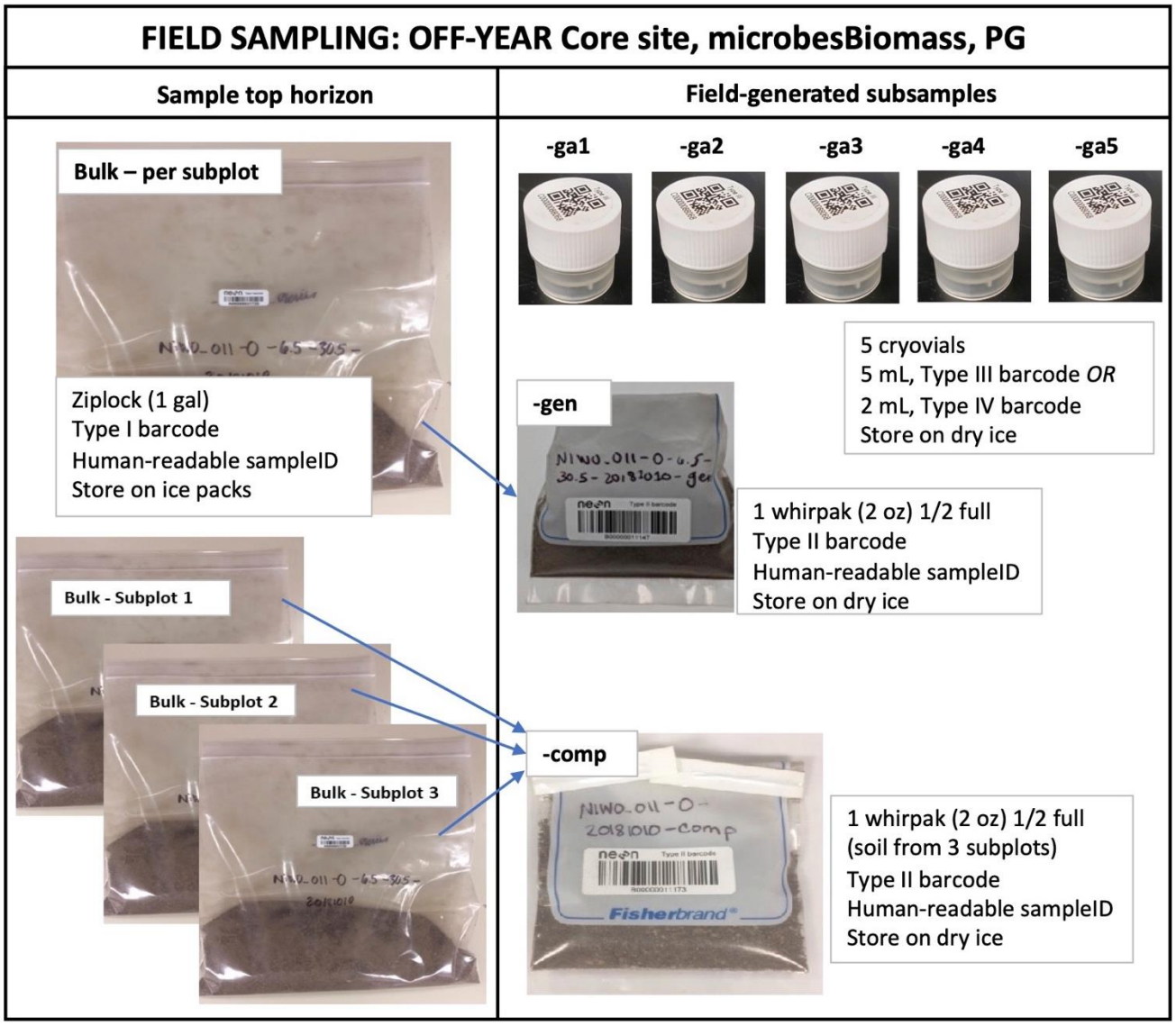

Figure 26. Field-generated samples at Core sites for boutType= microbesBiomass, sampleTiming= peak greenness.

Table 14. Checklist of samples and analyses associated with an **off-year** soil sampling bout at Gradient sites. ✓ = measurement; X = physical sample.

Bout Type	Sample Timing	Soil temp (field)	Microbial archive (field)	Metagenomics (field)	Soil moisture (lab)	Soil pH (lab)
microbes	Transition	✓	X cryovials (top horizon)	None	✓	✓
	Peak greenness	✓	X cryovials (top horizon)	None	✓	✓

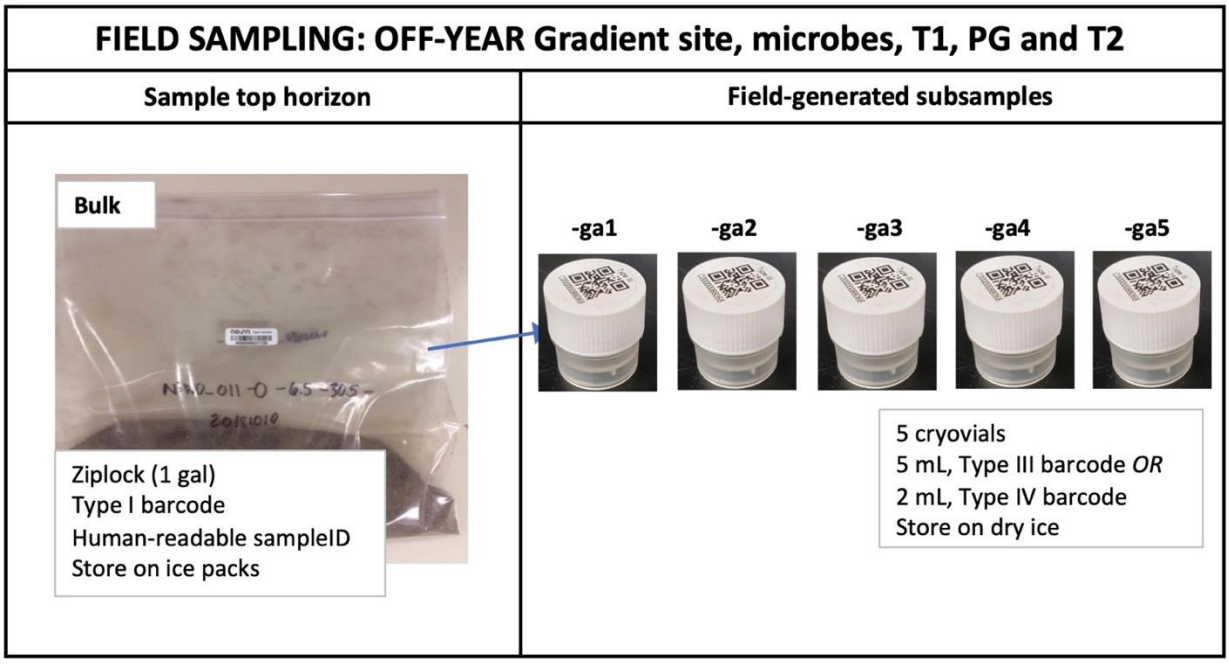

Figure 27. Field-generated samples at Gradient sites for boutType= microbes, all bouts.

Table 15. Checklist of samples and analyses associated with a **coordinated** soil sampling bout (NtransBoutType= Tinitial or Tfinal). ✓ = measurement; X = physical sample. For sites with both O and M horizons, the number of horizons to collect per sampling location is indicated.

Bout Type	Sample Timing	Soil temp (field)	Microbial analysis and archive (field)	Metagenomics (field)	Microbial biomass (lab)	Soil moist (lab)	Soil pH (lab)	KCl extract (lab)	BGC measure and archive (lab)
$T_{initial}$ sampling									
microbes Biomass	Transition	✓	X Whirlpaks/ cryovials (top horizon)		X sieved soil (2 horizons)	✓	✓	X	
microbes Biomass BGC	Peak greenness	✓	X Whirlpaks/ cryovials (2 horizons)	X plot-level whirl-pak (2 horizons)	X sieved soil (2 horizons)	✓	✓	X	X dried soil (2 horizons)
T_{final} sampling									
Field only	All	✓				✓		X	

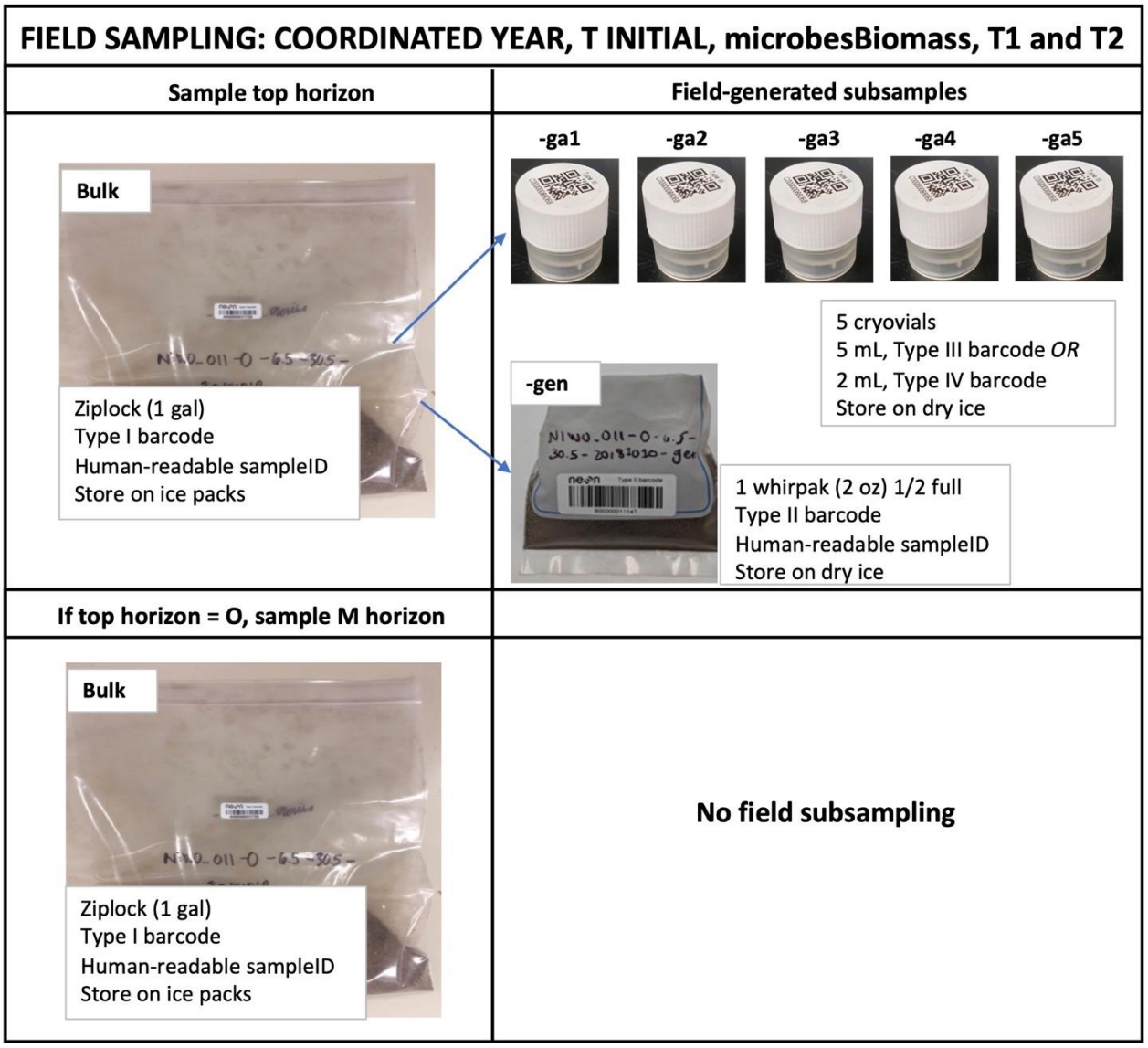

Figure 28. Field-generated samples during a Coordinated year for boutType= microbesBiomass, sampleTiming= T1 or T2.

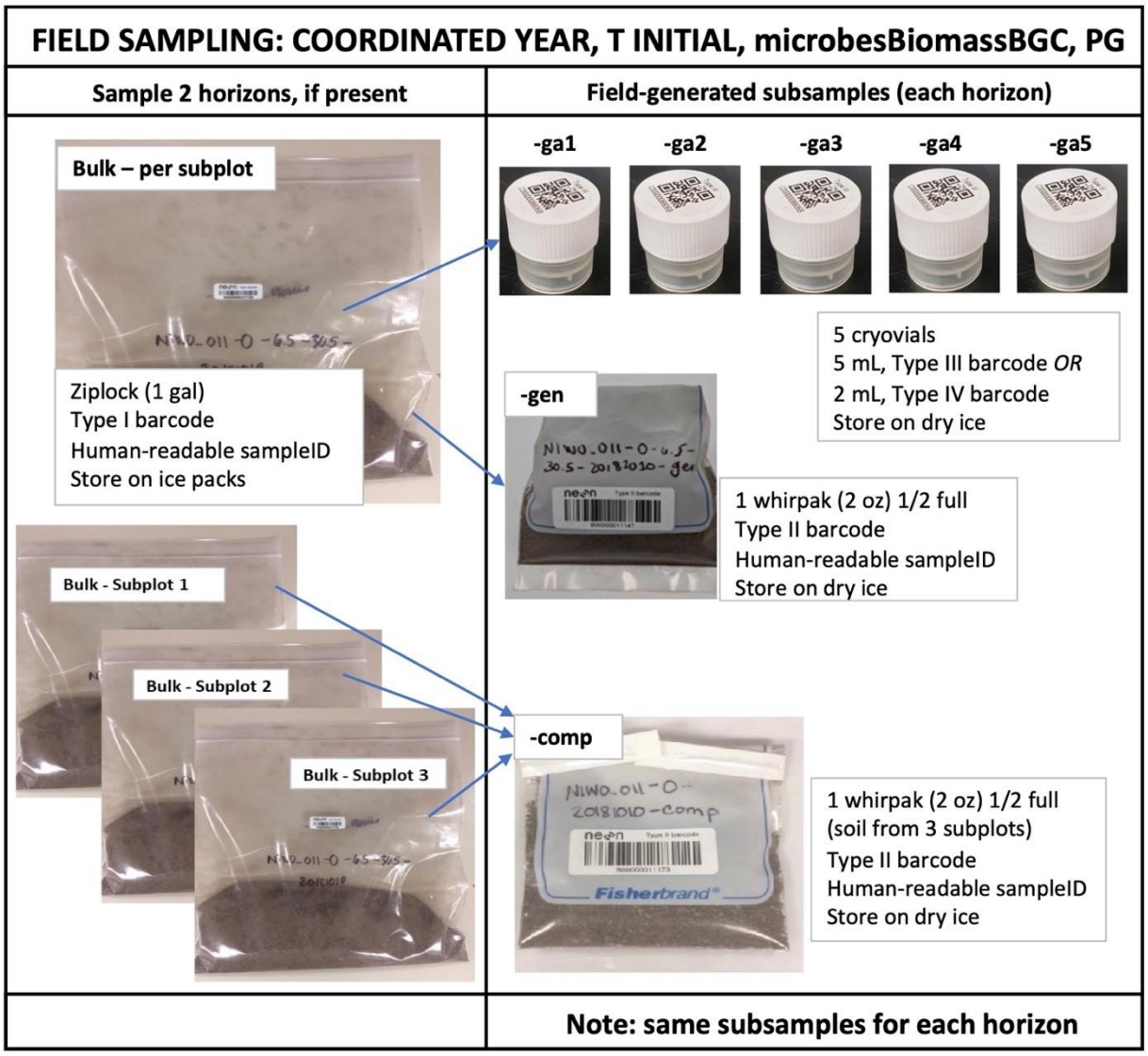

Figure 29. Field-generated samples during a Coordinated year for boutType= microbesBiomassBGC, sampleTiming = peak greenness.

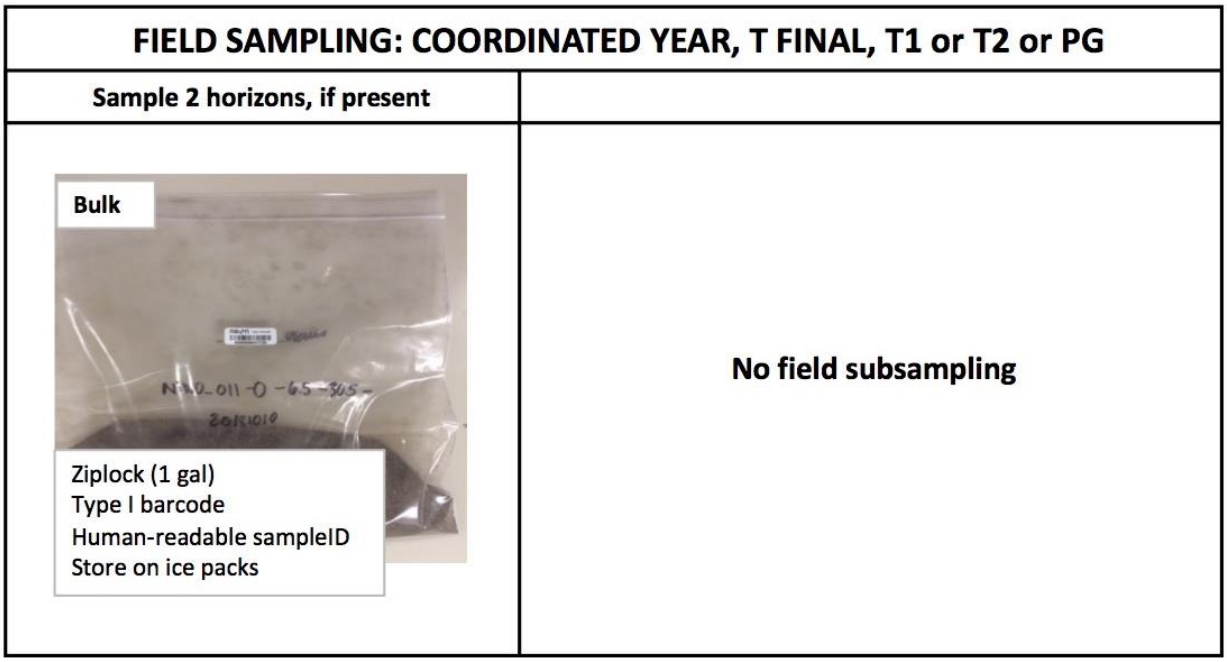

Figure 30. Field-generated samples during a Coordinated year for Tfinal sampling, boutType= fieldOnly, any time of year.

QUICK GUIDE TO SOIL COLLECTION DURING OFF-YEAR BOUTS

REMINDER: *Use sterile technique as much as reasonably possible.*

STEP 1 – Obtain dry ice, ice packs, coolers, and all sampling supplies.

STEP 2 - Use plot ID and X, Y coordinate lists to locate pre-determined sample locations.

STEP 3 – Sterilize any equipment or consumables that will contact the sample by wiping with 70% ethanol.

STEP 4 - Measure soil temperature at 10 cm depth.

STEP 5 – Measure litter layer.

STEP 6 – If organic horizon is present, collect with clean "brownie frame" plus hori-hori, or similar.

> **STEP 6a** – Put organic samples into new 1-gallon bag and homogenize well by shaking bag and crushing aggregates with your hands on the outside of the bag. Sterilely remove rocks, insects, coarse roots (> 2 mm diameter), recognizable and undecomposed plant matter, and other non-soil debris. Fill 1 pre-labeled Whirl-Pak (2 oz.) ~1/2-way (Core sites) and 5 pre-labeled cryovials ¾ of the way (all sites). Complete -gen sample label, close Whirl-Pak (label clearly visible), scan barcodes, and store on dry ice.
>
> **STEP 6b –** For peak green bouts at Core sites, sample for metagenomics. Use sterile scoop to place soil in a 2 oz. Whirl-Pak. Add a scoop of homogenized soil at the next X, Y location within the same plot-horizon combination, storing on ice packs between X, Y locations. When all X, Y locations have been added, close Whirl-Pak(s) (labels clearly visible), scan barcodes, and store on dry ice.
>
> **STEP 6c** –Ensure that at least 30g of homogenized organic soil or 60g mineral soil is available for analyses at the domain lab. Store the bag of homogenized soil on ice packs.

STEP 7 – If organic horizon is not present or < 1 cm thick, collect mineral horizon core(s) with approved coring device for your site. Follow steps **6a** – **6c** for microbial subsampling.

STEP 8 – Label bag/s. Store homogenized bags in cooler on ice packs and store all microbial samples on dry ice.

STEP 9 – Measure sample depth in brownie square or bore hole. Remember: For samples collected from the ground surface, **sampleTopDepth**= 0 cm.

STEP 10 – **Data QA**. Ensure all data have been recorded on datasheets and/or Fulcrum application. Ensure all barcodes have been scanned to the correct sample ID's.

STEP 11 - Backfill boreholes in accordance with permit.

STEP 12 – Rinse equipment using deionized water and clean towels. Sterilize immediately before re-use.

QUICK GUIDE TO SOIL COLLECTION DURING A COORDINATED BOUT

STEP 1 – Obtain dry ice, ice packs, coolers, and all sampling supplies including incubation cylinders.

STEP 2 - Use plot ID and X, Y coordinate lists to locate pre-determined sample locations.

STEP 3 – Sterilize any equipment or consumables that will contact the sample by wiping with 70% ethanol.

STEP 4 - Measure soil temperature at 10 cm depth.

STEP 4 - Measure litter layer.

STEP 5 – Set up the incubated core. You will return to retrieve and extract this core in 2-4 weeks.

STEP 6 – If present, collect organic horizon with "brownie frame" plus hori-hori, or similar.

STEP 7 – Put organic samples into a 1-gallon bag and homogenize either by closing bag and inverting or mixing with a pre-sterilized gloved hand. With a sterilized, gloved hand, remove rocks, insects, coarse roots (> 2 mm diameter), recognizable and undecomposed plant matter, and other non-soil debris.

STEP 8 - Collect mineral horizon core(s) using a spare incubation cylinder or similar-diameter coring device for your domain, place in bag and homogenize. Bottom depth should be similar (± 5 cm) to the incubated core. With a sterilized, gloved hand, remove any large rocks, coarse roots (> 2 mm diameter), and insects.

STEP 9 – During T1/T2 bouts, subsample only the top horizon for microbial analysis, archive, and metagenomics following **STEPS 6a** and **6b** of 'Off-year' Quick Guide. For PG bouts, sample both horizons if present.

STEP 10 – Ensure that at least 50 g (T1/T2) or 75g (PG) of homogenized organic soil and 100 (T1/T2) or 160g (PG) mineral soil is available for analyses at the domain lab. These are minima, more soil is better.

STEP 11 – Label bag/s. Store homogenized bags in cooler on ice packs and store all microbial samples on dry ice.

STEP 12 – Measure sample depth/s in brownie square or bore hole and record. Remember: For samples collected from the ground surface, **sampleTopDepth** = 0 cm.

STEP 13 – **DATA QA**. Ensure all data have been recorded on datasheets and/or Fulcrum application. Ensure all barcodes have been scanned to the correct sample ID's.

STEP 14 - Backfill boreholes in accordance with permit.

STEP 15 – Rinse equipment using deionized water and clean rag.

STEP 16 – return to collect incubating cylinders 2-4 weeks later. No field subsamples are created.

APPENDIX B REMINDERS

COLLECTING QUALITY SOIL SAMPLES

Pre-sampling: Be sure to…

- ☑ Prepare soil coordinate lists for each sampling location.
- ☑ Ensure all sampling equipment is available, operational, and ready for use.
- ☑ Pre-label sample containers (printed labels recommended) with information that will not change (e.g. plotID, subplotID, collectDate, etc).
- ☑ Obtain dry ice and ice packs plus appropriately sized coolers.
- ☑ Ensure samplers have a navigation device that will enable navigating to the plot.
- ☑ Check the sampling schedule.
- ☑ Know any special permit requirements for the site plus any site-specific instructions.

At soil sample location: Check…

- ☑ Does a navigation device confirm that you are indeed at the correct plot?
- ☑ Is designated sampling area disturbed or does it have an object such as large rock, tree, or litter trap that would prevent sampling there?
- ☑ Did you probe area within 0.5 m of X, Y coordinate to find a suitable sampling location?
- ☑ If a location was rejected, did you record why on the coordinate list?
- ☑ Did you record metadata (plotID, subplotID, collectDate, etc.)?

Coring: Remember to…

- ☑ When sampling for microbes, always sterilize gloves and equipment before use and at every sample location! Do not allow a 'dirty' object to touch a microbial sample.
- ☑ Wear clean gloves. Either change or clean gloves between samples.
- ☑ Measure soil temperature at 10 cm depth each sample location.
- ☑ Measure litter depth and then brush aside leaf litter before coring.
- ☑ Homogenize samples prior to field subsampling and ensure that rocks, insects, coarse roots (> 2 mm diameter), and other non-soil debris have been removed.
- ☑ Core to 30 ± 1 cm and measure sample top and bottom depths in borehole (not the core).
- ☑ Backfill hole with appropriate material when you are done.
- ☑ Decontaminate equipment (e.g., corer, tray, brownie cutter, etc.) between sample locations.

Sample Handling: Be sure to…

- ☑ Label sample bags and double check labels against datasheets and/or Fulcrum application.
- ☑ Store microbial genetic analysis, archive, and metagenomics samples in cooler with dry ice.
- ☑ Store bulk soil samples in cooler with ice packs.

PROCESSING SOIL SAMPLES IN THE LAB

All Bouts: Remember to…

- ☑ Transfer bulk soil sample bag to refrigerator (4° C).
- ☑ Prepare to sieve, dry, subsample, and conduct other processing as required for the type of bout.
- ☑ Measure soil moisture on unsieved bulk soil sample.
- ☑ Measure pH on sieved, air-dried sample (except for Tfinal bout of N-transformation sampling). When measuring pH, rinse electrode with DI water between samples.

Microbial Genetic Samples: Be sure to…

- ☑ Store microbial genetic analysis, archive, and metagenomics samples in ultralow freezer (-80° C).
- ☑ Ship analysis samples separately from archive samples.
- ☑ Ship samples on dry ice to external lab/s according to the schedule provided by NEON CLA. Do not ship on Thursdays or Fridays.

Microbial Biomass Samples: Be sure to…

- ☑ Within 1 day of collection, sieve mineral soil or remove rocks, insects, coarse roots (> 2 mm diameter), recognizable and undecomposed plant matter, and other non-soil debris out of organic soils.
- ☑ Transfer to labeled, barcoded centrifuge tubes. *Scan barcodes.*
- ☑ Store in ultralow freezer (-80° C).
- ☑ Ship samples on dry ice to external lab according to the schedule provided by NEON CLA. Do not ship on Fridays.

Nitrogen Transformation Samples: Don't forget to…

- ☑ Extract sieved soil using 2M potassium chloride within 1 day of collection.
- ☑ Filter extracts and store in labeled, barcoded scintillation vials at -20° C.
- ☑ Ship extracts on dry ice to external lab/s according to the schedule provided by NEON CLA. Do not ship on Fridays.

Biogeochemistry Samples: Be sure to…

- ☑ Create subsamples for BGC analysis used oven-dried (65° C), sieved or hand-picked soil.
- ☑ Use remaining air-dried and sieved or hand-picked soil for the BGC archive.
- ☑ Ship BGC analysis and archive samples to the appropriate lab/s at ambient temperature according to the Domain schedule.

Data Entry: Did you…

- ☑ Track and record the dates and times of sample collection and processing?
- ☑ Describe irregularities or deviations from protocol?

- ☑ Enter all information from datasheets (if used) into Fulcrum application?
- ☑ Complete the Data QC Checklists for all relevant Fulcrum applications?

Preserve Sample Integrity: Make sure…

- ☑ All sample label information is correctly transcribed.
- ☑ All barcodes have been scanned and are associated with the correct sample ID's.
- ☑ Gloves are changed and/or cleaned and sieves cleaned between samples.

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are the Protocol Sampling Dates and estimate the start and stop dates of sampling. Sampling is designed to occur when ecosystem productivity (as a proxy for belowground activity) is increasing from its annual minimum, at peak, and returning to annual minimum, as described in Section 4.2. The dates in **Table 16** are estimated from satellite MODIS-EVI phenology data averaged from 2012-2021 (Didan 2023). The following formulas are used to generate each sampling window:

- Transition 1 = Greenup date + 30 days
- Peak Green = Maturity date to Senescence date
- Transition 2 = Dormancy date – 30 days

These formulas are used in general, however site-specific modifications have been applied in some cases either for logistical reasons or to better capture the intended biophysical criteria (**Table 4**). Moreover, historic precipitation data are used to derive the Protocol Sampling Dates instead of greenness for tropical sites (D04 and D20). Estimated dates are used for advanced bout scheduling and to provide general guidance of when each domain can expect the site to be suitable for soil sampling. 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.

Table 16. Sampling windows for each site. The number in parentheses is the recommended number of days for N transformation incubations (± 4 days is acceptable). The majority of the incubation period (more than 50% of the days) should fall within the sampling window.

Domain	Site	Transition 1 Window	Peak Green Window	Transition 2 Window
01	HARV	Apr 21 – May 21 (24)	June 15 – Aug 13 (18)	Oct 8 – Nov 7 (24)
	BART	Apr 21 – May 20 (24)	June 18 – Aug 16 (18)	Oct 3 – Nov 2 (24)
02	SCBI	Apr 2 – May 2 (24)	May 30 – Aug 4 (18)	Oct 16 – Nov 15 (24)
	SERC	Mar 27 – Apr 26 (24)	June 2 – Aug 11 (18)	Oct 22 – Nov 21 (24)
	BLAN	Mar 23 – Apr 22 (24)	June 1 – Aug 19 (18)	Oct 10 – Nov 9 (24)
03	JERC	Apr 5 – May 5 (18)	June 24 – Aug 18 (14)	Oct 12 – Nov 11 (18)
	DSNY	Mar 7 – Apr 6 (18)	May 24 – July 28 (14)	Oct 20 – Nov 19 (18)
	OSBS	Mar 12 – Apr 11 (18)	May 23 – July 25 (14)	Oct 10 – Nov 9 (18)
04	GUAN	May 1 – July 1 (14)	Sept 1 – Nov 15 (14)	Dec 1 – Mar 1 (18)*
	LAJA	May 1 – July 1 (14)	Sept 1 – Nov 15 (14)	Dec 1 – Mar 1 (18)*
05	UNDE	Apr 29 – May 29 (24)	June 22 – Aug 13 (21)	Sept 23 – Oct 23 (24)
	TREE	Apr 26 – May 26 (24)	June 21 – Aug 12 (21)	Sept 28 – Oct 28 (24)
	STEI	Apr 26 – May 26 (24)	June 21 – Aug 12 (21)	Sept 28 – Oct 28 (24)
06	UKFS	Mar 28 – Apr 27 (24)	June 10 – Aug 11 (18)	Oct 13 – Nov 12 (24)
	KONZ	Apr 14 – May 14 (24)	June 17 – Aug 11 (18)	Oct 8 – Nov 7 (24)
	KONA	Apr 5 – May 5 (24)	June 11 – Aug 11 (18)	Oct 17 – Nov 16 (24)
07	ORNL	Mar 24 - Apr 23 (21)	May 18 – July 23 (18)	Oct 14 – Nov 13 (21)

Domain	Site	Transition 1 Window	Peak Green Window	Transition 2 Window
	MLBS	Apr 15 – May 15 (24)	June 10 – Aug 11 (18)	Oct 9 – Nov 8 (24)
	GRSM	Apr 3 – May 3 (21)	June 6 – Aug 9 (18)	Oct 12 – Nov 11 (21)
08	TALL	Mar 24 – Apr 23 (21)	May 19 – July 20 (18)	Oct 19 – Nov 18 (21)
	DELA	Mar 15 – May 1 (21)*	June 1 – July 31 (18)	Oct 12 – Nov 11 (21)
	LENO	Mar 30 – May 1 (21)*	June 1 – July 31 (18)	Oct 18 – Nov 17 (21)
09	WOOD	May 12 – June 11 (24)	July 12 – Aug 24 (18)	Oct 3 – Nov 2 (24)
	DCFS	May 4 –June 3 (24)	July 5 – Aug 19 (18)	Oct 6 – Nov 5 (24)
	NOGP	Apr 20 – May 20 (24)	June 27 – Aug 14 (18)	Oct 7 – Nov 6 (24)
10	CPER	Apr 6 – May 6 (24)	June 16 – July 22 (18)	Sept 25 – Oct 25 (24)
	STER	Mar 27 – Apr 26 (24)	May 26 – Aug 14 (18)	Sept 24 – Oct 24 (24)
	RMNP	May 1 – May 31 (28)*	June 22 – Aug 6 (21)	Sept 24 – Oct 24 (28)
11	CLBJ	Mar 13 – Apr 12 (18)	May 15 – July 21 (14)	Nov 7 – Dec 7 (18)
	OAES	Mar 8 – Apr 7 (18)	May 6 – July 9 (14)	Aug 14 – Sept 13 (18)
12	YELL	May 1 – May 31 (24)*	July 1 – Aug 7 (21)	Sept 25 – Oct 25 (24)
13	NIWO	May 4 – Jun 20 (28)*	July 10 – Aug 17 (21)	Sept 2 – Oct 2 (28)
	MOAB	Apr 15 – May 15 (18)	June 1 – July 31 (24)	Oct 17 – Nov 16 (24)
14	JORN	June 15 – July 15 (18)	Aug 1 – Sept 15 (21)	Oct 16 – Nov 15 (24)
	SRER	May 31 – July 15 (18)	Aug 1 – Sept 15 (21)	Oct 18 – Nov 17 (24)
15	ONAQ	Mar 31 – Apr 30 (18)	May 6 – June 28 (24)	Sept 14 – Oct 14 (24)
16	ABBY	Apr 15 – May 29 (21)	June 12 – July 29 (24)	Oct 6 – Nov 5 (24)
	WREF	Apr 26 – June 9 (21)	June 23 – Aug 1 (24)	Oct 6 – Nov 5 (24)
17	SJER	Oct 5 – Nov 5 (24)	Mar 3 – Apr 10 (18)	May 12 – June 11 (24)
	SOAP	Mar 1 – Mar 31 (24)	May 13 – June 27 (21)	Sept 27 – Oct 27 (24)
	TEAK	May 1 – May 31 (24)*	June 22 – Aug 8 (21)	Oct 1 – Oct 31 (24)
18	TOOL	NA	July 8 – Aug 7 (28)	NA
	BARR	NA	July 22 – Aug 20 (28)	NA
19	HEAL	NA	June 28 – Aug 7 (21)	NA
	DEJU	NA	June 25 – Aug 5 (21)	NA
	BONA	NA	June 23 – Aug 3 (21)	NA
20	PUUM	Nov 1 – Nov 30 (18)	Dec 15 – Jan 15 (14)	June 1 – June 30 (18)

*Allowable deviations from sampling windows or incubation lengths:

Domain	Site	Deviation	Rationale
D04	LAJA GUAN	T2: Bout should be scheduled early in window, but should be cancelled and rescheduled if wet season rains occur within 72 hours of scheduled bout	Unpredictable transition from wet to dry season

Domain	Site	Deviation	Rationale
D08	DELA LENO	T1: If flooding prevents a scheduled sampling event, the bout may be rescheduled through no later than May 31	Unpredictable spring flooding
D10 D13 D17	RMNP NIWO TEAK	T1: If sampling occurs late in the sampling window due to a persistent snowpack, incubation may be shortened to approximately 21 days	Unpredictable timing of snowmelt
D12	YELL	T1: Anticipate cancellation of sampling at Tower plots into foreseeable future	Per the Yellowstone National Park Bear Management Plan, the YELL tower plots cannot be sampled between March 10 - June 30.

APPENDIX D SITE-SPECIFIC INFORMATION

D.1 Quarantined Sites

The following sites are commonly under USDA soil quarantine and should plan to follow additional containment measures in order to prevent the spread of nuisance and/or invasive species. Note that quarantine status may change at a site, and this may not be reflected in the table below. Refer to the USDA soil quarantine map for the most updated information on quarantine status and contact Science via a Service Now ticket if aware of quarantine status changes.

- Domain 03: OSBS, DSNY, JERC
- Domain 04: GUAN, LAJA
- Domain 07: GRSM, ORNL
- Domain 08: TALL, DELA, LENO
- Domain 11: CLBJ
- Domain 14: JORN
- Domain 20: PUUM

Some sites have additional soil quarantine requirements, which are detailed below. Regarding fruit flies, the three sites in D17 are listed, but any sites in counties identified as previously quarantined for fruit fly (red hatch marks on the USDA soil quarantine map) should follow the fruit flies containment action.

Domain	Site	Quarantined Materials	Containment Action
07	MLBS	Soil and plant material	Secondary leak-proof containment required before transporting soils from MLBS to DSF in Tennessee. Place a trash bag inside each cooler prior to loading samples. Place ice packs or dry ice inside the trash bag. Place ziplock bags/whirlpaks within the trash bag. When cooler is full, close trash bag with a metal twist tie, or similar, then close cooler.
D17	SJER SOAP TEAK	Soil and plant material	Check the quarantine map prior to each soil bout. If a site is under active quarantine for fruit flies at the time of sampling (orange on the quarantine map): 1. Reach out to the local USDA/APHIS office and ask for permission to proceed with sampling. Start the conversation early as additional requirements such as inspection of the shipment or additional limited permits or certificates may be necessary.

Domain	Site	Quarantined Materials	Containment Action
			8. Submit a ServiceNow request for Science to update the quarantine status in the Shipping Fulcrum application. As a general practice, carry the most recent quarantine map with permit documents during soils bouts, and keep a record of quarantine status around soils bouts in case there are any concerns about soil samples.

D.2 Sites with Known Issues that Require Sampling Modifications

GUAN	
Issue: Extremely rocky soils (as quantified in SOP K).	Solution: Current soil plots were evaluated at the subplot level for ability to conduct long-term sampling. Based on the defined criteria, 2 subplots were permanently rejected: 23 in GUAN_001 and 39 in GUAN_004. • All sampling in GUAN_001 will occur within subplots 21, 39, and 41 • All sampling in GUAN_004 occur within subplots 21, 23, and 41. Additionally, 30-40% of the area of subplot 21 is covered by a large slab. Any coordinates that fall on that slab can be skipped outright (e.g., not counted as rejection) to make sampling more efficient.
DSNY	
Issue: Water table can rise very quickly and unpredictably	Solution: During coordinated years, all bouts and plots at DSNY should be measured for N-transformation rates using the modified method in TOS SOP: Wetland Soil Sampling (RD[06]), e.g., using buried bags.
PUUM	
Issue: Coring with an auger damages the root system of *Metrosideros polymorpha.* Cuts of large diameter roots (2-5 cm) make trees susceptible to attack from *Ceratocystis spp*. fungi and increase susceptibility to Rapid Ohia Death (ROD)	Solution: All soil samples at PUUM should be collected using the monolith method, including both O and M horizons. This allows samplers to excavate/cut around large roots, leading to less damage to the trees while still collecting a standard, consistent soil volume. If very large monoliths are collected, follow the instructions for 'Sub-sectioning soil monoliths' in Appendix D.5.

D.3 Sites with Variable Seasonality Across Plots

Table 17. List of sites with variable seasonality across plots, and allowable duration of bouts.

Domain	Site	Key Characteristics	Duration of sampling bout
10	RMNP	Large differences in elevation, slope, aspect, vegetation cover, impact timing of seasonal changes	Plots with different characteristics may be sampled > 14 days apart; Sample plots with similar characteristics as close as possible, but no more than 14 days apart
13	NIWO		
17	TEAK		

D.4 Sites Authorized to Use the Wetland SOP

Domain	Sites
01	HARV
03	DSNY, OSBS
05	TREE, UNDE, STEI
08	DELA, LENO
09	WOOD, NOGP, DCFS
18/19	HEAL, TOOL, BARR, BONA

D.5 Sites with Permafrost and Peatland Soils

Soils that develop in cold, wet places have unique features due to a combination of the presence of permafrost, a very short growing season, the predominance of moss, and slow rates of decomposition. Therefore, the definitions of, and manner of delineating between, soil horizons requires specialized instructions. Specifically:

- The surface of the soil is generally guided by the plants: where roots are growing, and there is predominantly dead instead of live plant material, that is where the soil begins (**sampleTopDepth** = 0 cm).
- This is a functional definition specific to peatland and permafrost-type sites. For instance, in Alaska, material may still be very 'fibric,' e.g. have recognizable plant parts slowly decomposing, but it is still considered organic soil, since roots grow in it, and should be sampled as such.
- Finding the top/start of this soil can be difficult because live and dead plant material will be a continuum from the surface downward. To help, technicians should use other guides:
    i. Color – shift from green/white to brown
    ii. Texture – the material will become soft and friable if mostly dead
    iii. Presence of live roots growing among dead organic material.

Specialized Equipment Needed for Sampling: Hand clippers

Follow these step-by-step instructions to obtain soil samples in permafrost and peatland sites, such as those found in D5, D18 and D19.

1. At a suitable X, Y location, use clippers (or equivalent) to remove live surface vegetation, including lichens, from a 'brownie' area until roots are apparent and the material transitions from being mostly live to mostly dead. Pay attention to where fibrous material becomes friable, use a sterilized, gloved hand as needed. This is the surface of the soil (**sampleTopDepth** = 0 cm). See **Figure 29** for site-specific photos that can help guide where the soil starts.
2. Place coring device in brownie footprint and insert into ground to 30 cm depth (or refusal).
    a. If soil is very 'fluffy', use a soil knife to cut around the perimeter of the coring device while inserting. This may help avoid compaction.
    b. It is also acceptable to use a brownie-type square to collect soil monoliths with a knife.
3. Extrude or collect material onto a plastic tray and separate O and M horizons.
    a. If an M horizon is present, it will have a grainy/gritty feel. If it's an O horizon, almost no mineral grains will be present, it will instead feel like friable, smooth plant material (may also feel greasy). Note that high-latitude M-horizons can be very dark and organic rich.

    Sub-sectioning soil monoliths. The diameter of the brownie frame (10 cm, or 4 in) is much larger than the diameter of a coring device (2-2 ½ in), and when used to collect soil can result in more soil than needed, depending on bout type and horizon thickness. If much more soil is collected than needed, remove some before homogenizing and sub-sampling by cutting the monolith lengthwise from top to bottom. This will ensure soil from the entire depth profile will be evenly represented in the final soil sample.

4. Process the material following the rest of the instructions in SOP B or C, depending on bout type. Litter depths will be 0 cm in most tundra samples, unless there was a measurable layer of visible, detached, dead leaf litter material on top of the soil surface.
5. **If conducting a Coordinated bout**, install incubation cylinder before taking the initial core.
    a. Remove live surface vegetation and find the soil surface as described above.
    b. Install the cylinder to 30 cm depth (or refusal). Use a soil knife to cut around the perimeter of the cylinder while inserting to help avoid compaction.
        i. Or, if there is standing water or a high water table, follow the Wetland SOP [RD(06)]
    c. Place cap on the cylinder and attach cap to cylinder as described in SOP C
    d. Return cut-away surface vegetation so that it covers/buries the cylinder.

i. Use a pin flag, flagging tape, or some other marker (site host permitting) to assist in relocating the core.

e. Follow all instructions for collecting the incubated cylinder as described in SOP C. Do not push down or otherwise attempt to change the positioning of the cylinder before collecting the sample when you return, even if the active layer has thickened in sites with permafrost.

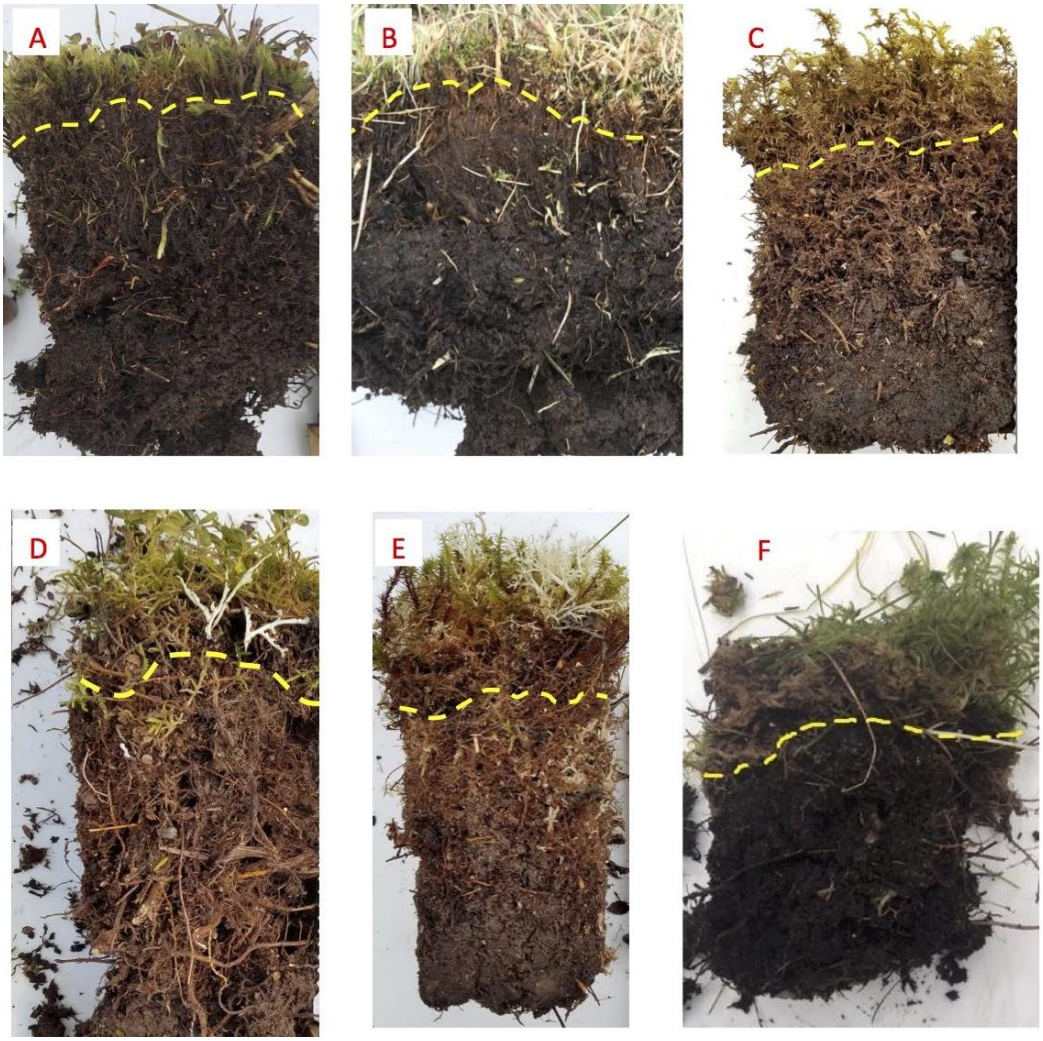

Figure 31. Images demonstrating the start of the soil surface (yellow dashed line) at TOOL (A), BARR (B), DEJU (C), HEAL (D), BONA (E), and TREE (F). Source: NEON Terrestrial Instrument System (TIS), in collaboration with NRCS.

D.6 Forest Sites with Woody Horizons

While sampling in forest ecosystems, soil horizons that are very woody may be encountered (**Figure 32**). In these situations, highly decayed wood may still be recognizable as such, but there are key indicators that this material should be sampled as soil:

- Roots are growing throughout the material.
- The material is extremely soft and friable, crumbles easily upon handling.

When such material is encountered, it should be sampled as soil following the instructions in SOPs B and C. Ensure to select 'Woody horizon' in the **horizonDetails** field since the chemistry and microbial community of this material may be quite different due to its woody nature. The material is considered part of the O horizon if found on the surface (**Figure 32**), or part of the M horizon soil if highly decayed logs or woody matter are buried. Break up large clumps while homogenizing and before subsampling.

Figure 32. Woody organic soil from Domain 16.

D.7 Dryland Sites with Biological Soil Crusts

Soils that develop in areas with very low precipitation and few vascular plants are often overlain by biological soil crusts. These communities of bacteria, fungi, moss, and lichens help to stabilize the soil surface and prevent erosion. They also play a very important role in biogeochemical cycling. As such, it is important for NEON soil sampling to include biocrusts when encountered at randomly assigned coordinates. However, training and sampling procedures require slight modification as described below.

Applicable Sites: MOAB, ONAQ, JORN, SRER

Specialized Equipment Needed: 2mm sieve + tray plus a few liters of DI water

Additional Training

1. Review the training powerpoint associated with the Plant Diversity protocol (RD[17]) for identification of biological soil crusts.
2. Use the training to learn how to recognize **mature, darkly pigmented biocrust (Figure 33)**. There are several different types of mature, darkly pigmented biocrusts, including those dominated by moss, lichens, or cyanobateria. Review the training slides for example photos, but note that soil sampling does not require separating these different biocrust types. It only requires being able to tell mature crust apart from bare soil or immature/lightly pigmented crust.

Figure 33. Example of darkly pigmented mature biological soil crust.

Sampling

1. Upon arriving at an X,Y location, determine if there is mature, darkly pigmented biocrust present. Either way, do not reject the location.
2. If mature biocrust is present:
    a. Collect a sample as normal (e.g., core through the crust to 30 cm or max depth possible)
    b. Record '*Mature biological soil crust present*' in the **horizonDetails** field of the fulcrum application
    c. Before taking any microbial subsamples, sieve the bulk sample to 2mm. This will break up the biocrust, ensuring that the sample is adequately homogenized, and the subsamples are representative of the entire soil community.
    d. Aliquot microbial subsamples following the rest of the instructions in SOP B or C, depending on bout type. Collect all other relevant metadata as described in the SOPs.
    a. When finished sampling at that X, Y location, clean both the sieve and tray.
        i. Rinse with DI water, then shake gently to remove excess water drops.
        ii. Wipe tray and sieve (both sides) with a sterile ethanol wipe.
3. If mature biocrust is not present, sample as normal.

4. Repeat for all X, Y locations sampled.

D.8 Soil Horizons from NRCS Initial Characterization

Table 18. Prevalence and depth of organic (O) horizons by site, based on NRCS initial soil characterization. Note that exact plots analyzed by the NRCS may differ from the plots currently being sampled, and conditions may vary.

Domain	Site	# plots	# plots with O horizon	Median O horizon depth (cm)	Min O horizon depth (cm)	Max O horizon depth (cm)
01	BART	15	15	14	3	30
01	HARV	18	18	5.5	1	140
02	SCBI	18	3	4	4	5
02	SERC	20	0	--	--	--
02	BLAN	15	0	--	--	--
03	DSNY	16	0	--	--	--
03	JERC	23	0	--	--	--
03	OSBS	23	8	10	5	40
04	GUAN	18	0	--	--	--
04	LAJA	19	0	--	--	--
05	STEI	14	2	62.5	25	100
05	TREE	11	6	6.5	3	100
05	UNDE	13	7	76	3	200
06	KONZ	13	0	--	--	--
06	UKFS	17	0	--	--	--
06	KONA	19	0	--	--	--
07	GRSM	13	10	5	2	9
07	ORNL	20	5	4	2	5
07	MLBS	10	7	2	1	8
08	DELA	21	1	2	2	2
08	LENO	18	0	--	--	--
08	TALL	26	1	10	10	10
09	DCFS	14	0	--	--	--
09	NOGP	18	1	4	4	4
09	WOOD	15	6	10	1	18
10	CPER	17	0	--	--	--
10	STER	11	0	--	--	--
10	RMNP	14	14	5	2	11
11	CLBJ	11	0	--	--	--
11	OAES	15	0	--	--	--
12	YELL	11	3	5	3	7
13	NIWO	13	8	4.5	2	8
13	MOAB	11	0	--	--	--

Domain	Site	# plots	# plots with O horizon	Median O horizon depth (cm)	Min O horizon depth (cm)	Max O horizon depth (cm)
14	JORN	16	0	--	--	--
14	SRER	12	0	--	--	--
15	ONAQ	11	0	--	--	--
16	ABBY	15	9	3	1	9
16	WREF	11	11	6	2	10
17	SJER	10	1	1	1	1
17	SOAP	12	6	6.5	2	12
17	TEAK	12	8	3	1	11
18	BARR	20	21	80	10	120
18	TOOL	19	19	22	1	100
19	DEJU	18	16	8.5	4	48
19	HEAL	15	15	17	5	60
19	BONA	16	16	9.5	5	80
20	PUUM	10	9	5	1	30

D.9 Site-specific Soil Sampling Devices

Table 19. Soil types and sampling devices for M horizons for each site.

Domain	Site	Soil Type(s)	Sampling Device(s)
01	HARV	Organic horizons + loamy and rocky mineral soils	AMS auger, part# 400.09, 2” diameter
	BART		
02	SCBI	Rocky soils	AMS auger, part# 400.08, 2.25” diameter AMS hammer-head replaceable tip soil probe kit, part# 425.501, 1” diameter
	SERC		
	BLAN		
03	JERC	Relatively deep organic and mineral soils, few rocks	AMS auger, 2 ¼” diameter
	DSNY		
	OSBS		
04	GUAN	Extremely shallow, rocky soil	AMS soil probe, part# 401.17, 1 1/8” diameter
	LAJA	High-clay soil	AMS, part# 418.03, 2" Stainless Steel Mud Auger, 5/8" Thread
05	UNDE	Variable organic and mineral soils	AMS slide hammer corer, part# 404.50, 2” diameter
	TREE		
	STEI		
06	UKFS	High-clay soil	AMS, part# 402.37, 2¼” diameter, mud auger
	KONZ	Very rocky, shallow soils	
	KONA	High-clay soil	
07	ORNL		

Domain	Site	Soil Type(s)	Sampling Device(s)
	MLBS	Variable organic and mineral soils	Forestry Suppliers Carbon Steel Auger Mud/Clay, 2” diameter Part# 78400
	GRSM		
08	TALL	Sandy soils	AMS auger, part # 400.08, 2 ¼” diameter
	DELA	Moist, sticky clay soil	AMS mud auger, part# 350.20, 2 ¼” diameter
	LENO	Moist, sticky clay soil	
09	WOOD	Moist, wet, sticky clay soil	AMS auger, part# 400.54, 2 ½” diameter; or AMS auger, part# 415.23, 2 ¼” diameter
	DCFS	Moist, sticky clay soil	
	NOGP	Dry, rocky soil	
10	CPER	Sandy soil	AMS, part# 400.08, 2¼" diameter, regular auger AMS, part# 400.42, 2 ¼" diameter, sand auger AMS auger, part# 402.36, 2 ¼” diameter
	STER	High clay soil	
	RMNP	Rocky soil	
11	CLBJ	Sandy soils	AMS auger, part# 400.08, 2 ¼” diameter
	OAES	Rocky high clay soil	
12	YELL	Rocky, shallow soil	AMS Auger, part# 400.08, 2 ¼” diameter
13	NIWO	Rocky soil	see D10 entries
	MOAB	Sandy soil	AMS Auger, part# 400.08, 2 ¼” diameter
14	JORN	Sandy soil	AMS Hex QP Sand Auger, part# 58536, 2 ¼” diameter
	SRER	Sandy soil	AMS Hex QP Sand Auger, part# 58536 2 ¼” diameter
15	ONAQ	Rocky soil	AMS Auger, part# 400.06, 3 ¼” diameter
16	ABBY	Forest soils, organic and mineral	Forestry Suppliers Carbon Steel Auger Mud/Clay, 2” diameter Part# 78400
	WREF		
17	SJER	Sandy soil	AMS auger, part# 400.08, 2 ¼” diameter Bulb corer (2” diameter) used at certain locations with very shallow soil
	SOAP	Mostly sandy with sporadic areas of high clay	
	TEAK	Rocky, sandy soil	
18	TOOL	Gelisols: thick organic horizon, cryoturbation	Soil monoliths cut with a hori-hori & 10 x 10 cm square template
	BARR		
19	HEAL	thick organic horizon, roots	
	DEJU	Thin organic + rocky mineral soil	
	BONA	thick organic horizon, roots	
20	PUUM	Volcanic soils with high organic matter	Soil monoliths cut with a hori-hori & 10 x 10 cm square template

APPENDIX E USING SOIL COORDINATE LISTS

The soil coordinate and subplot lists provide randomly-generated X, Y locations at which soil sampling should occur. The lists are generated programmatically to ensure that there are no repeat sample locations, to prevent sampling within the inner 20x20 non-destructive sampling area in the plots, and to ensure that there are enough locations to enable sampling at each plot for at least 30 years. In order to retain the random design of the protocol and to ensure that no location is sampled more than once, it is critical that the order of the subplot and coordinate lists provided are **not changed**.

The format of the soil lists is as follows (refer to **Figure 34**):

- Soil subplot lists - 1 .xlsx formatted spreadsheet per site. Lists the 3 subplots to sample.
  - Example file: DSNY_sls_subplots_allPlots.xlsx
- Soil coordinate lists – 1 .xlsx formatted spreadsheet per site. Lists X, Y coordinates per subplot.
  - Example file: DSNY_soilCoordList_noDuplicates.xlsx

All files are posted in the Sampling Support Library (SSL) under the category, SoilXYLists. If you are unable to locate these files, please contact NEON Science.

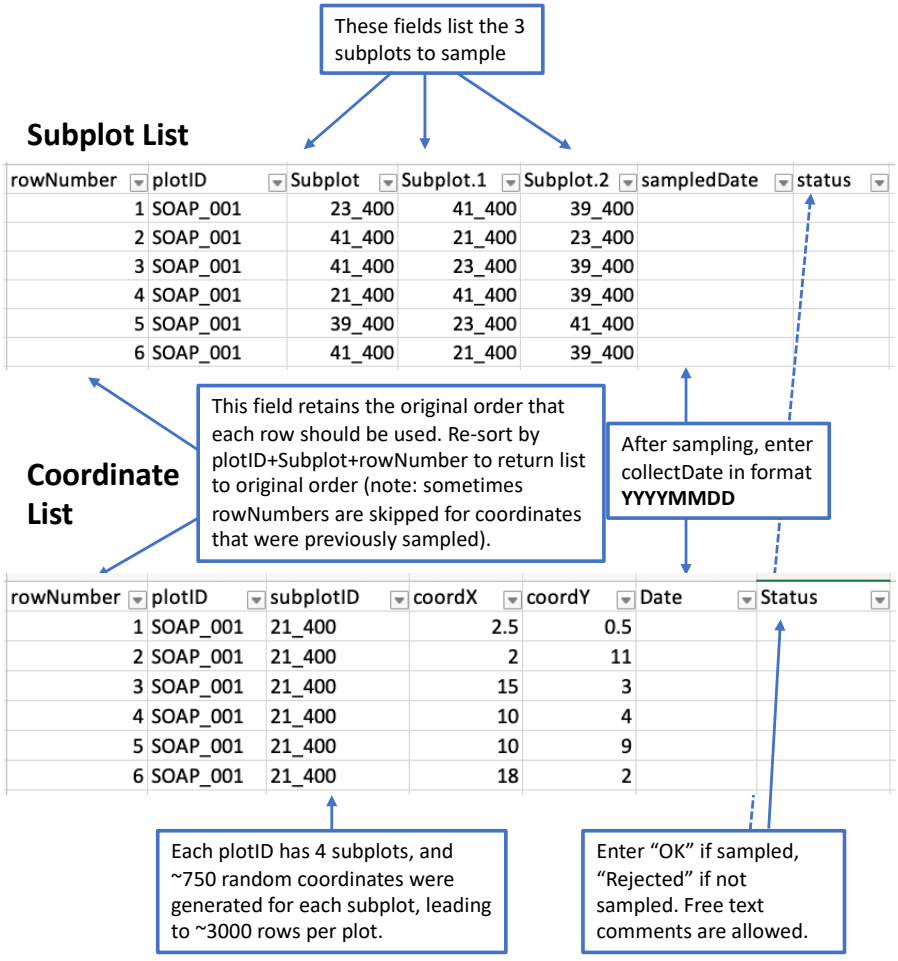

Figure 34. Anatomy of the soil subplot and coordinate lists.

The system for tracking and recording soil coordinates is not fool-proof, and requires attention to detail and a bit of upkeep. If you do not update the list with previously sampled locations, then it is easy to accidentally resample a coordinate. If you resample a coordinate, **YOU MUST DISCARD THE SAMPLE**.

E.1 Preparing for a Bout

1. Ensure that the coordinates and subplots used in previous bouts have been entered into the list. It is best to update your lists at the same time as electronic data entry occurs; make it a part of data entry and QA routines.
    a. It is insufficient to update coordinate lists using only notes from the Field teams. It is critical to also check Fulcrum data to ensure that actually sampled locations are recorded.
    b. If available, the Soils QC application can help to cross-check and update coordinate lists
2. Prior to commencing a bout, open up the **subplot** list.
    c. Start with the first plot you need to sample. Working your way down the list, select the first row that hasn't already been used (e.g. the row does not have a "sampledDate" and "status" recorded). These will be the 3 subplots that you should sample for that plot for the upcoming bout. In the following example, for NOGP_001 you would use the subplots in rowNumber 19:

	A	B	C	D	E	F	G
1	rowNumber	plotID	Subplot	Subplot.1	Subplot.2	sampledDate	status
17	16	NOGP_001	41_400	21_400	39_400	20221011	OK
18	17	NOGP_001	39_400	41_400	21_400	20230424	OK
19	18	NOGP_001	41_400	23_400	21_400	20230709	OK
20	19	NOGP_001	21_400	41_400	39_400		

3. Open up the **plot** list.
    a. Filter the spreadsheet for the target plot. For this example, the plot is NOGP_001.
        i. Next, filter the coordinate list for the target subplot, starting with the first subplot in the row. For this example, we'd start by filtering to subplot 21_400.
        ii. Working your way down the list, select the next 5 rows that haven't already been used (e.g. the row does not have a "Date" and "Status" recorded). Only one coordinate location per subplot is sampled, however bring extras in case coordinates must be rejected in the field (up to 5 coordinates are attempted before skipping a subplot).
        iii. Either print these rows or copy and paste into a separate spreadsheet for printing later. Be sure to copy the plotID, subplotID, coordX, coordY, Date, and Status fields. In the following example, you would start with coordinates X = 14, Y = 2.5.

4. Now that you have one subplot completed, repeat the steps outlined above for the remaining 2 subplots designated for sampling.

    a. Once you have obtained the coordinates for each subplot for a given plotID, move onto the next plotID. Repeat steps 1-4 above until all plots for a given bout have a list of possible X, Y coordinates for the upcoming bout. Be sure to print these coordinates and bring to the field.

	A	B	C	D	E	F	G	H
1	rowNumber	plotID	subplotID	coordX	coordY	Date	Status	
10	10	NOGP_001	21_400	4	19.5	20220427	OK	
11	11	NOGP_001	21_400	7	2	20220713	OK	
12	12	NOGP_001	21_400	7.5	3.5	20221011	OK	
13	13	NOGP_001	21_400	10	3.5	20230424	rejected, boulder	
14	14	NOGP_001	21_400	19	4	20230424	OK	
15	15	NOGP_001	21_400	4	6.5	20230709	OK	
16	16	NOGP_001	21_400	14	2.5			
17	17	NOGP_001	21_400	10.5	4.5			
18	18	NOGP_001	21_400	7	2.5			
19	19	NOGP_001	21_400	19.5	5			
20	20	NOGP_001	21_400	7	17.5			

E.2 After Completing a Bout

1. Update the subplot list. Record the Date and Status as follows:

    - "Date" = collectDate (YYYYMMDD)
    - "Status" = OK, or rejected.
        - If subplot(s) are rejected or skipped for logistical reasons, please add freeform comments in the Status field as to why location was not sampled. Ex: 'Maximum number of coordinates rejected in subplot 21_400'; 'standing water > 50cm in subplot 23_400', 'Insufficient staff, could not sample in subplot 23_400'

2. Update the soil coordinate list. Record the Date and Status as follows:

    - "Date" = collectDate (YYYYMMDD)
    - "Status" = OK or rejected
        - If coordinates are rejected, please add free-form comments in the Status field as to why the location was rejected. Ex: 'rejected, location disturbed by burrowing animals'

3. If available, the Soils QC application can help with coordinate and subplot list updating.

Important Reminders

- Always record the exact coordinates provided in the list. If you are unable to sample within 0.5m of the set of coordinates, reject that row and move onto the next row in the coordinate list.
- Do not modify any other columns in the subplot or coordinate list files. Only enter info into the "Date" and "Status" fields. If any other fields are accidentally edited, contact NEON Science.
- Always turn off any temporary filters you have placed on the spreadsheets before closing the file. All rows should be visible when the file is opened later on.
- If, after completing a bout, you realize that coordinates were accidentally re-sampled, contact NEON Science immediately. If caught early enough, it may be possible to re-sample the plot.

APPENDIX F 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 20. General Equipment List – Field sampling for all types of soil bouts. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the listed item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	GPS receiver, recreational accuracy, e.g. Garmin Etrex20x	Navigate to sampling location	1
	N	Measuring tape, minimum 50 m	Locate coordinates for soil sampling locations	2
	N	Digital soil temperature probe, ±1° accuracy, ability to calibrate, minimum 11.5 cm long stem	Preferred tool to measure soil temperature, calibrate prior to each bout	2
		Digital soil temperature probe, ±1° accuracy, traceable, at least 11.5 cm long stem	Tool for measuring soil temperature if calibratable option not available	2
	N	1/8” diameter x 5” long shaft hex key or similar tool	Create a pilot hole before inserting soil temperature probe	1
	N	Glass beaker, 1000 mL	Calibrate soil temperature probes	1
	N	Sand	Calibrate soil temperature probes	1 L
Fisher; 13-201-502	Y	NIST traceable thermometer	Calibrate soil temperature probes	1
	N	Cooler	Keep perishable samples chilled in field	2
	N	Ice packs, -20° C	Chill perishable samples in field	16 (+)
	N	Deionized water	Rinse soil from equipment	2 liters
	N	Metallic stake or other poking device, not painted, stainless steel, min 30 cm length	Probe soil depth, find suitable sampling location, help remove soil from coring device	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Survey marking flag, pin flag, PVC or fiberglass stake	Flag soil sampling location	3
Forestry Supplier; 91567	Y	Laser Rangefinder, 0.3m accuracy	Locate X,Y coordinates in very steep plots	1
	N	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	1
Compass Tools; 703512 Forestry Suppliers; 90998	Y	Foliage filter	Use with laser rangefinder in dense vegetation	1
Consumable Items				
Supplied by HQ	Y	Weatherproof, adhesive barcode labels, Type I	Label homogenized sample bag with barcode-readable labels	1 sheet
Grainger; 5NHH1; Amazon; B00006IBUV	Y	Avery weatherproof adhesive labels, 2 5/8” x 1”	Label homogenized sample bag with human-readable labels	30-80
ULINE; S-21339	Y	Warning pictogram label for samples containing *Toxicodendron spp*	Alert handlers to use care when processing sample	1 roll
	N	All weather copy paper	Print backup datasheets	
	N	Permanent marker, fine tip	Label sample containers	3
	N	Batteries, AA and coin types	Spare batteries for GPS receiver and digital thermometer	
	N	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box
	N	Paper towels, reusable cleaning cloth, bottle brush	Remove debris from soil sampling equipment	
	N	Trash bag	Dispose of consumables	2

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Field tablet	Record data	
RD[05]	Y	Field datasheet	Backup to record data	
See coordinate lists	Y	X,Y coordinates of sampling locations within each plot	Soil sampling locations	1
	N	Laboratory soap, e.g. Alconox, Contrex	General cleaning of reusable field and laboratory equipment	1

Table 21. Additional Equipment List – Field sampling for bouts that include soil microbes at one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the listed item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	Organic horizon cutter template (brownie frame): plastic or stainless steel preferred, 100 ± 10 cm^2 area, rust-free.	Sample organic horizon	1
	N	Ruler, minimum 30 cm	Measure soil sample top and bottom depth	1
Varies by Domain, see **Table 19**	Y	Soil corer, 2 ± 0.5” diameter, minimum 30 cm long	Collect soil core	1
Forestry Suppliers; 33489	Y	Soil knife (hori-hori)	Separate soil horizons, subsampling, etc.	1
	N	Spring scale (optional), 300g max	Weigh soil samples (when using mass sampling approach)	1
	N	Trowel	Remove soil core	1
	N	Strap wrench	Open stuck core barrels, only needed for certain coring devices	1
	N	Stainless steel tweezers, fine-tipped	Removing non-soil material from field subsamples	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Tablespoon or coffee scoop, sterilizable	Generate microbial subsamples	1
	N	Glass bottle, 1 L	Hold extra sterilized ethanol, if prepared in house	1
Consumable Items				
Fisher; 14955182 Nasco; B01064	Y	Whirl-Pak bags, sterile, write-on, 2 oz	Contain soil for genetic analysis	30-40 per horizon
Supplied by HQ	Y	Cryogenic, adhesive barcode labels, Type II	Label microbial analysis and metagenomics samples with barcode-readable labels	30-40 per horizon
Fisher; 15-930-E	Y	Cryogenic, adhesive labels	Label Whirl-pak bags for microbial analysis and metagenomics with human-readable labels	30-40 per horizon
Fisher; 13-709-140 Amazon; W985100	Y	5.0 mL CryoElite tissue vials, sterile, Wheaton	Preferred container for frozen soil for microbial archive	150 per horizon
Fisher; 13-709-141 to 13-709-146	Y	CryoFILE tissue vial storage boxes, various colors, Wheaton	Store microbial archive vials if using 5 mL Wheaton vials	6 boxes per horizon
Supplied by HQ	Y	Cryogenic, adhesive barcode labels, Type III	Label 5 mL microbial archive vials with barcode-readable labels	150 per horizon
	N	2 mL liquid nitrogen safe cryovials	Container for frozen soil for microbial archive if 5 mL vials not available	150 per horizon
	N	Cryovial freezer storage box for 2 mL vials, fiberboard/cardboard type only	Store microbial archive vials if using 2 mL vials	Several boxes
Supplied by HQ	Y	Cryogenic, adhesive barcode labels, Type IV	Label 2 mL microbial archive vials with barcode-readable labels	150 per horizon
	N	Dry ice, pelletized	Freeze soil microbial subsamples	20 pounds

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Resealable plastic bag, 1 gal	Collect homogenized soils	2 boxes
	N	Sterile, 70% Ethanol pre-wetted wipes	Sterilize sampling equipment and gloves	30-60
SOS Clean Room; TX3215	Y	Sterile, dry wipers	Alternative for sterilizing sampling equipment and gloves	30-60
	N	70% Ethanol made up in sterile, deionized water	Alternative for sterilizing sampling equipment and gloves. Required if using Sterile, dry wipers	1 bottle
FisherSci; SVGPL10RC, 0974103	Y	0.2 micron filtration unit, sterile, polyethersulfone	Alternative for creating sterilized ethanol	1
	N	Bleach, standard grade	Sterilizing containers for holding in-house made sterile water or ethanol	1 bottle

Table 22. Additional Equipment List – Field sampling for bouts that include soil N transformations at one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	Hammer or rubber mallet	Insert cylinders into soil	1
Headquarters, email when resupply needed	Y	Incubation cylinders, 2” inner diameter. Schedule 40 PVC tubing with a beveled edged	Sample soil cores and store field-incubated soil cores	1/ sampling location, plus 2 extras
	N	Loose-fitting caps for each cylinder (designed for use with 2.25” or 2.5” inner diameter PVC)	Protect cylinder openings from debris and water	1/ sampling location
	N	Wooden block (approx. 2” x 4” x 10”)	Use with mallet to pound cylinder into soil	2
	N	Monument stake installation strike plate	Use with mallet to pound cylinder into soil	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Extruder – long flathead screwdriver, soil knife, etc	Extrude soil sample from cylinder in clayey conditions	1
	N	Plastic tray	Separate soil core horizons in field	2
	N	Soil knife (hori-hori)	Separate organic and mineral horizons	1
	N	T-Fence Post Puller (or similar)	Remove cylinder in high-clay soil	1
	N	1.0 chain	Use with post puller to remove cylinder in high-clay soil	1 foot
	N	4" x 3/8" hitch pin	Use with post puller to remove cylinder in high-clay soil	1
Consumable items				
	N	Plant wire	Use to secure caps to cylinders	30 feet
	N	8” Zip ties	Use to secure caps to cylinders	1/ sampling location
	N	Resealable plastic bag, 1 gal	Contain soil samples	30 per horizon
	N	Orange flagging tape	Flag location of incubated soil core	1 roll
	N	Pin flag, survey marking flag, PVC or fiberglass stake	Flag location of incubated soil core (if permitted)	50

Table 23. Equipment List – Laboratory processing of soils for moisture content from one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	Balance, 0.01 g accuracy	Weigh fresh and dry soil moisture samples	1
	N	Spatula or scoopula	Transfer soil to weigh boat	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Plastic tray	Transport soil samples to and from oven	4
Consumable items				
	N	Aluminum foil weigh boat	Hold soil while drying	1 box
	N	Nitrile gloves, powderless	Prevent contamination of soil samples during handling	1 box
	N	Lint-free wipes	Cleaning work area and equipment	1 box
	N	Ethanol, 70%	Clean work area	1 bottle
Resources				
RD[05]	Y	Lab datasheet	Backup to record data	---

Table 24. Equipment List – Soil sieving, air-drying, and subsampling for microbial biomass and soil BGC analysis and archive at one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	Sieve, 2 mm	Sorting soil particles to 2mm	1-2
	N	Sieve, 4 mm	Pre-sieving for high-clay, difficult to sieve soils	1-2
	N	Spoon, spatula or scoopula	Transfer soil between containers	2
Consumable items				
	N	Paper bag, #8	Hold soil subsamples for air-drying	30-60
	N	Deionized water	Clean work surfaces and equipment	1 bottle
	N	Ethanol, 70%	Prepare work area	
	N	Low lint wipe (KimWipes or similar)	Clean and dry work area	1 box

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Nitrile gloves, powderless	Prevent contamination of soil samples during handling	1 box
	N	50 mL centrifuge tubes, polypropylene	Store and ship microbial biomass samples	30-60
	N	Centrifuge tube holder	Hold microbial biomass sample tube during subsampling (optional)	1-2
Supplied by HQ	Y	Cryogenic, adhesive barcode labels, Type II	Label microbial biomass sample containers with scanable barcode	30-60
Fisher; 15-930-E	Y	Cryogenic adhesive labels	Label microbial biomass sample containers with human-readable labels	1 sheet
	N	Scintillation vials, glass, 20 mL	Store BGC analysis samples	30-60
Fisher; FB02911825	Y	240 mL wide-mouth glass jars	Store BGC archive samples	30-60
Supplied by HQ	Y	Heat-resistant, adhesive barcode labels, Type I	Label BGC analysis and archive samples with scanable barcode	60-120
	N	2.6” x 1” address labels	Label BGC analysis and archive samples with human-readable labels	60-120
ULINE; S-21339	Y	Warning pictogram label for samples containing *Toxicodendron spp*	Alert handlers to use care when processing sample	1 roll
Resources				
RD[05]	Y	Lab datasheet	Backup to record data	---

Table 25. Equipment List – Laboratory processing of soils for measuring pH at one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
Fisher; 13636AB150B	Y	pH meter	Reading pH value of samples	1
Fisher; 13-620-183A 13-642-243	Y	pH probe, dedicated to soil-only measurements	Measuring pH of samples	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Balance, 0.01 g accuracy	Weigh soil samples	1
	N	Glass volumetric flask, 2L	Preparing calcium chloride solution for pH analysis	1
	N	Graduated cylinder, 25-50 mL capacity	Measure volumes of solutions for pH samples	2
	N	Spoon, spatula or scoopula	Transfer soil subsamples	2
	N	Stir rod	Mix pH samples	1-2
Consumable Items				
	N	Powderless gloves (s, m, l, xl)	Prevent sample contamination during handling, prevent bodily injury from hazardous chemicals	1 box
	N	Paper bag, #8	Hold soil subsamples for air-drying	50
Fisher; AC423520250	Y	Calcium Chloride Dihydrate, $CaCl_2 \cdot 2H_20$	pH analysis	2.94 g
	N	Deionized water wash squeeze bottle	Rinse equipment and pH electrode	1 bottle
	N	Hydrochloric acid (HCl), 10N	To make a 1N solution used to adjust pH of $CaCl_2$ when solution is too basic	10 mL
	N	Calcium Hydroxide, $Ca(OH)_2$	To make a 6N solution used to adjust pH of $CaCl_2$ when solution is too acidic	25 g
	N	pH buffers (4.01, 7.00, 10.01, 1.68), NIST- or USA-traceable	Calibrating pH meter. Use the 3 buffers that most closely bracket expected pH values	1
	N	50 mL centrifuge tubes, polypropylene	pH analysis – required for M horizons, optional for O horizons	30-60
		100 mL glass beakers	pH analysis, optional for O horizons	30
	N	Low lint wipe (KimWipes or similar)	Clean and dry work surfaces	1 box

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Resources				
RD[05]	Y	Lab datasheet	Backup to record data	---

Table 26. Equipment List – Laboratory processing of soils for N transformations at one site. Exact Brand indicates circumstances when only the listed product number(s) meets the specifications for the item.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable Items				
	N	Balance, 0.01 g accuracy	Weigh soil samples	1
	N	Volumetric flask, 1 L	Prepare 2M KCl solution for small batch of samples	1
	N	Carboy (20 L), Nalgene brand or similar	Prepare and store 2M KCl solution for large batch of samples	1
Fisher; FB966C	Y	Ceramic Buchner funnel	Pre-leach GF/A filters	1
	N	Side-arm flask, 500 mL	Pre-leach GF/A filters	1
	N	#8 Stopper, 1 hole, 3/8”	Pre-leach GF/A filters	1
	N	Clear vinyl tubing, 3/8” ID, 1/2”OD	Pre-leach GF/A filters	2-3 ft
	N	Spoon, spatula or scoopula	Transfer soil between containers	2
	N	Graduated cylinder (100-250 ml)	Measure aliquot of KCl	1
Thomas; 4618N60	Y	Reusable filtration units	Filter samples and collect filtrate	4
	N	Filter forceps	Handle GF/A filters	1
Fisher; SK-O330	Y	Global Equipment Shaker table	Shake extracts	1
RD[12]	Y	Manifold. See assembly document	Filter samples	1
	N	Vacuum pump	Filter samples	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Large beaker (at least 500 ml)	Collect discarded KCl filtrate	1
	N	Plastic dishpan, 3-gallon capacity	Wash filtering equipment	2
Consumable Items				
Shipped to domains after purity testing	Y	ACS Reagent Grade Potassium Chloride (KCl) – purity tested to ensure low inorganic N contaminants	Extract NH_4^+ and NO_3^- from soil	several kgs
	N	Screw-cap polypropylene specimen cups and lids, sterile, 120 ml capacity	Extract NH_4^+ and NO_3^- from soil	33-75
	N	Ultra-pure Type I deionized water, certified	Prepare 2M KCl, rinse filtering equipment	1-2 20L carboys
Fisher; 0333723C Thomas; 9718J20	Y	Plastic scintillation vials with caps, 20 mL	Store filtered soil extracts for freezing and shipment	33-75
	N	Powderless gloves (s, m, l, xl)	Prevent contamination of soil samples	1 box
Fisher; 0987414A	Y	Glass fiber filters, 47 mm diameter, GF/A type	Filter samples	1 box
	N	Resealable plastic bag, 1 gallon	Organize scint vials containing sample extracts	1 box
Fisher; 15-930-E	Y	Cryogenic adhesive labels	Label vials with human-readable labels	1 sheet
Supplied by HQ	Y	Cryogenic, adhesive barcode labels, Type II	Label samples with scannable barcodes	1 sheet
Resources				
RD[05]	Y	Lab datasheet	Backup to record data	---