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

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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. • 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. • 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 • 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

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 • 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* • 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 Relocatable 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

			• 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

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling and domain lab processing of soil samples in order 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
$\delta^{15}N$ and $\delta^{13}C$	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, relocatable sites
16S/ITS rRNA qPCR	Measure microbial diversity, community composition, and microbial/fungal biomass	Every yr, 3x per year, core sites; Every 5 yrs, 3x per year, relocatable sites*
16S rRNA sequencing		
ITS rRNA 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, relocatable sites*
pH, moisture, temperature	Give context for biogeochemical and microbial measurements	Every soil sampling event except N-trans Tfinal (no pH)
Soil biogeochemical archive	Provides community access to conduct measurements not being made by NEON	Every 5 yrs, 1x per year
Soil microbial archive	Provides community access to conduct measurements not being made by NEON	Every yr, 3x per year, all sites

*Long-term plan for frequency of microbial genetic and metagenomic analyses at non-core sites is still being determined.

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 integrity of soil samples collected during Field Operations. It includes detailed guidance for locating soil sampling sites, collecting soil cores, and recording field-associated metadata, field and 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 (Sparke 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.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan
AD[07]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry
AD[08]	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 Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: 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: Wetland Soil Sampling
RD[07]	NEON.DOC.001710	TOS Protocol and Procedure: Litterfall and Fine Woody Debris
RD[08]	NEON.DOC.014038	TOS Protocol and Procedure: Plant Belowground Biomass Sampling
RD[09]	NEON.DOC.001024	TOS Protocol and Procedure: 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: Site Management and Disturbance Data Collection
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples, Sensors and Equipment
RD[15]	NEON.DOC.002984	Standard Operating Procedure: Minimizing Site Disturbance During Aquatic and Terrestrial Observation System Sampling

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
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.

Coordinated Bout: Synchronized soil sampling bout that includes additional measurements of microbial biomass, soil nitrogen transformations, and soil biogeochemistry (during peak greenness). Occurs every 5 years at a 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 to create NEON electronic data entry applications.

Litter layer: Loose, unconsolidated plant material on top of the soil surface that is intact or partially shredded, but still easily recognizable as plant material. 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 (generally much less than half by weight). In general, decomposing plant material is poorly recognizable, except in high-latitude, high-altitude, or wetland sites where decomposition is very slow. Layer should be darker in color and friable (easily crumbled), and is sometimes greasy. If you feel more than a couple of mineral grains (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 for problem/incident tracking and resolution.

3 METHOD

The field protocol used by NEON for 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 40 x 40 m plots (**Figure 1**) that are distributed across each site. Soils are inherently spatially heterogeneous, thus several samples are collected in order to capture variability at multiple scales (e.g., soil core, plot, site). NEON Science supplies domain staff with a master list of plots where soil samples will be collected for the duration of Operations, as well as an additional list that contains randomly generated X,Y sampling coordinates originating from the southwest corner (i.e., the reference point) of each plot on the list. The latter are the within-plot locations for soil sampling. The within-plot locations for soil sampling are different for each sampling event in order to prevent repeat sampling of a given location. To keep track of sampling locations in the field and aid in navigation, marking the random

sampling locations on a laminated plot diagram (**Figure 1**, right) prior to a sampling bout is recommended.

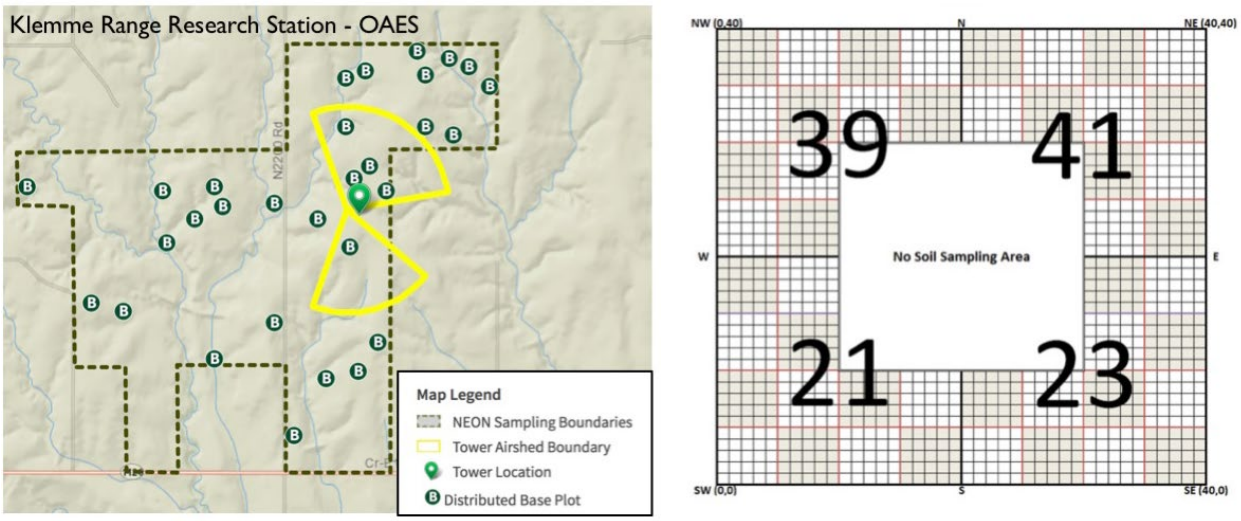

Figure 1. *Left*: Example site layout at the OAES field site. Soil sampling occurs at 10 base plots. Four plots are located in the tower airshed and 6 are distributed across the site. *Right*: Gridded plot map for a soil plot. Large numbers centered in each quadrat indicate the subplotID. For each sampling bout, one location is sampled in 3 of the 4 subplots.

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 sub-horizons are not separated (e.g., mineral sub-horizons A and B). Where possible, NEON samples mineral horizons using a 2 ± 0.5 inch 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.7.

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 tower location from the surface to 2 meters depth (or bedrock, whichever was shallower) at each core and relocatable 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) and the Natural Resources Conservation Service (NRCS) and characterized soil physical and chemical properties (including bulk density) to 1m 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, and contamination atypical of

the site (urban and agricultural sites). 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 Fulcrum application and/or field data sheet 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 for more details). Once soil cores have been collected, extraction holes must be backfilled as 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 drying soils according to SOP F and subsampling according to SOP J: Laboratory Subsampling During a Coordinated Bout. 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 archiving.

Microbial Analyses. Subsamples are either put on dry ice in the field (for microbial genetic analysis and archive samples), or kept field moist (for microbial biomass analysis), as described in field SOP's B and C, and shipped to the contracted laboratory 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. These composite samples are also kept on dry ice in the field.

Soil N Transformations. The general procedure for measuring rates of net N mineralization and net nitrification is via two companion soil cores taken from a given location. One core is collected for immediate processing (e.g. the "initial" core), 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), 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 separating the organic and mineral horizons for analysis, removing rocks and roots from organic soils and sieving mineral soils to 2 mm. A subsample of processed soil is then placed in a cup with 2M KCl and shaken. At the conclusion of the 1 hr extraction period, the soil extract solution is filtered and the filtrate is poured into a vial and frozen prior to shipment to a contracted laboratory for analysis of NH_4^+-N and NO_3^--N. Subsamples of initial soil samples are also 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 in NEON's ServiceNow tracking system.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

The timing, temporal frequency, and extent of soil sampling constitute "the science design" (see (AD[07]) and (AD[08]), and vary by NEON domain or site. Sampling frequency is set to allow researchers to investigate how microbial communities and nutrient dynamics change temporally. The extent of soil sampling allows researchers to evaluate the spatial heterogeneity of nutrient stocks 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. Sampling for all types of soil analyses is performed at 10 pre-selected plots (**Figure 1**). Many of the plots are spatially collocated with other NEON sampling protocols for examining the relationships between physical,

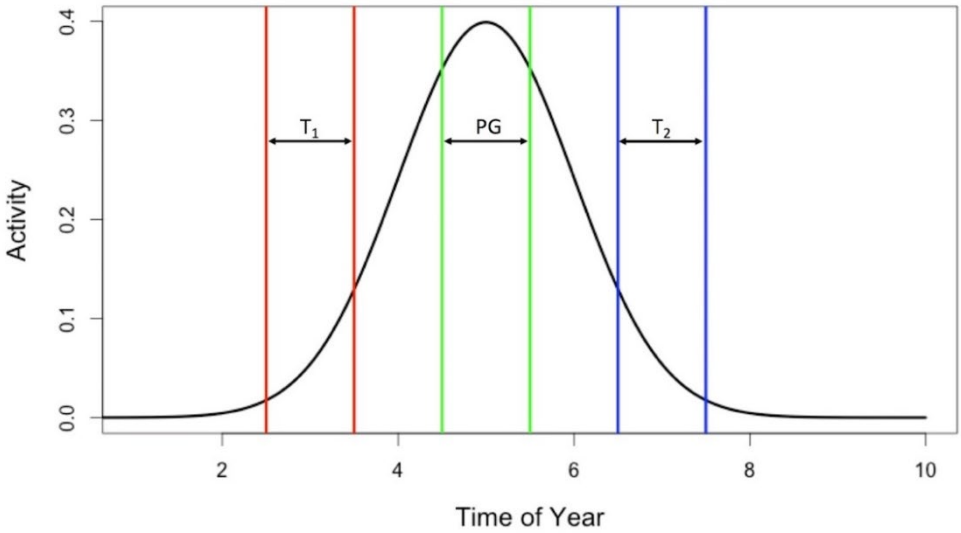

Figure 2. Generalized Timing of Soil Sampling. T1 captures the transition from dormancy/low activity to peak activity (PG), while T2 captures the transition from peak activity to dormancy/low activity. The time of year for each sampling period will vary by local geographic and climatic conditions.

chemical and biological data. At the different NEON sites, sampling frequency varies depending on climatic factors, such as length of the growing season. 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. Sampling requirements for different types of bouts. For detailed guidance on number of horizons to collect for each sample and bout type, refer to SOPs below and 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	microbes	microbes	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] -comp*	-bm[t]	-bm -kcl	-bm -kcl -cn -ba -comp*	-bm -kcl	kcl
Lab measurements	pH moisture	pH moisture	pH moisture	pH moisture	pH moisture	pH moisture	moisture

***Field generation of sample recommended, however sample can be created in lab if necessary.
[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. Sampling for microbial genetic analysis (-gen) and genetic archive (-gaX) occurs at 10 pre-determined plots per site. Microbial communities change more frequently than the other soil properties that we measure. Hence, these collections occur up to three times per year and are selected to capture windows in which microbial activity is ramping up or slowing down (**Figure 2**). All

sites conduct sampling during peak greenness, while the other two sampling events occur 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 are listed in Appendix C. When sampling for soil BGC, soil for all microbial analyses is collected concurrently and is a subsample of the soil core collected for BGC measurements.

Microbial Metagenomics Analysis. Samples for microbial metagenomics analysis (-comp) are collected along with soil microbial genetic samples at the same 10 plots. This sampling differs, however, in that it only occurs once per year, during the Peak Greenness window. Also, a single sample represents a composite of all sampling locations within a plot for a particular horizon.

Nitrogen Transformations and Microbial Biomass. Every 5 years, soil measurements of microbial biomass (-bm) and N transformations(-kcl) are conducted, along with soil microbial genetic and metagenomic measurements, at 10 plots per site. Microbial biomass and soil N transformations tend to be variable both in space and time. To account for seasonal variation, up to three sampling events occur during a sampling year, in conjunction with the microbial genetic analysis and genetic archive 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 metagenomics, microbial biomass, 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), and biogeochemical archiving (-ba) are collected alongside the other soil subsamples once every 5 years, during the Peak Greenness window. When soil BGC is measured, subsamples of the soil cores are also analyzed for microbial genetic analysis and genetic archiving, microbial metagenomics, microbial biomass, N transformations, soil pH, and soil moisture.

Coordinated Plant-Soil Biogeochemical Measurements. Soil sampling for soil BGC, N transformations, and microbial biomass (e.g., Coordinated bout) 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: Soil Biogeochemical and Microbial Sampling (this document)
- TOS Protocol and Procedure: Litterfall and Fine Woody Debris, litter chemistry SOP (RD[07])
- TOS Protocol and Procedure: Plant Belowground Biomass Sampling (RD[08])
- TOS Protocol and Procedure: Canopy Foliage Sampling (RD[09])

The inter-annual schedule lists the years when each site is scheduled to conduct Coordinated soil sampling along with other plant biogeochemistry measurements. This schedule can be found on the NEON Field Science SharePoint site.

Table 3. Sampling frequency 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 bout and plot types.

SOP	Plot Type	Plot Number	Bout Duration	Bouts Per Year	Yearly Interval	Remarks
SOP B, SOP D, SOP E, SOP F, SOP G, SOP H	All	All	2 weeks	1-3 per sampling year	NA	Suite of SOP's for Off-year bouts
SOP C, SOP D, SOP E, SOP F, SOP G, SOP H, SOP J, SOP K	All	All	2-4 weeks	1-3 per sampling year	5 y	Suite of SOP's for Coordinated bouts, synchronized microbial and biogeochemical measurements
SOP I	All	All	2 weeks	1 per sampling year	NA	SOP for metagenomics pooling carried out during peak greenness sampling window

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. Check cooler every 6 hours, refresh dry ice as needed.

c. When conducting an Off-year bout at a relocatable site, process collected soil samples in the laboratory:

    1) (Ideally) Within 1 day of collection.

    2) (Required) Within 72 h of collection.

d. When conducting a Coordinated bout or sampling at a core site, process collected soil samples in the laboratory:

    1) Within 1 day of collection.

4.2 Criteria for Determining Onset and Cessation of Sampling

Scheduling a Sampling Bout: Sampling typically takes 2-4 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, to the extent possible (**Table 4**). Adjacent bouts should be at least 14 days apart. It is recommended that domain staff schedule around a 14-day time period within the middle of the sampling window and adjust either earlier or later in the sampling window based on climate conditions as the bout approaches. This will enable capturing the relevant ecological conditions, and secondarily allows for schedule conflicts, weather, and other contingencies to occur without jeopardizing the timing of the sampling bout.

Important Note: The sampling windows defined in Appendix C are intended to guide scheduling, however ultimately on-the-ground conditions will 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 conditions (**Table 4**) or not suitable for sampling, the bout may be rescheduled any time within the sampling window, as long as there are 14 days between adjacent sampling bouts. Also, 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 managing 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.

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.4, 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 of a Sampling Bout.

- During a non-coordinated, Off-year sampling bout (e.g. 'microbes', **Table 2**), at least 50% of samples must be collected in order for the bout to be considered complete.
- During a Coordinated bout, at least 50% of the expected number of *tower* plot samples must be collected in order for the bout to be complete.
- If, prior to a scheduled bout, it becomes apparent that this level of effort will not be possible, contact Science to determine whether the bout should be cancelled, rescheduled, or continue as scheduled. If conditions occur during a bout that prevent sampling to the required level of effort, report the issue to Science using NEON's ServiceNow reporting system in order to determine whether the samples should be retained or discarded.

Soil BGC. Sampling of soil cores for biogeochemical and the full suite of microbial analyses (one large, coordinated bout) occurs during peak greenness. This will capture all sites at peak biological activity.

Microbial Genetic Analysis and Archiving. At most sites, sampling bouts occur three times during the year in order to capture the prevailing conditions at the site during different seasons. Soil samples are collected during peak greenness as well as two transitional periods. The sampling windows are determined on a per-site basis using historical remote sensing data 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, the transitional bouts will take place when the soils are expected to be changing activity levels (**Figure 2**). These broadly correspond with transitions to winter/spring, fall/winter, wet/dry, and dry/wet, depending on location and time of year. Prescribed sampling windows for each site 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.

Nitrogen Transformations and Microbial Biomass. The timing of sampling during a season corresponds with sampling for microbial genetic analyses (above) in order to capture similar seasonal characteristics and enable linkages between microbial and biogeochemical data. 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, 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. The soil sample collected for the initial core measurements is also subsampled for microbial (and other) analyses, in order to minimize the number of trips required to complete the protocol and maximize data linkages.

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 requirements 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 to reschedule the sampling bout.

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). If 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. Under normal conditions, processing begins immediately upon return to the laboratory, but when extenuating circumstances occur (e.g. working remotely or sampling occurred on a Friday), soil can be held up to 72 hours prior to processing. 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. This requirement should be followed even in cases when data QA and automated Refer to SOP L for more details on required shipping timing.

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	Up to 5 days at 4° C; up to 1 month at -20° C
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 sampling bout within required time frame	Communicate to NEON Science via ServiceNow ticket	Dataset may be incomplete or sampling bout delayed/redone. Latter may result in delay of data product delivery.
Incomplete sampling bout (defined in 4.2)	Communicate to NEON Science via ServiceNow ticket	Dataset may be incomplete or sampling bout redone. Latter may result in delay of data product delivery.

Delay/Situation	Action	Outcome for Data Products
Delay in scheduled start of sampling bout > 6 days but within sampling window	Submit schedule change request to reschedule bout	No adverse data outcome.
Delay in start of sampling bout after end of sampling window	Communicate to NEON Science via ServiceNow ticket: if delayed sampling approved, submit schedule change request.	Bout may be cancelled if it extends into a different sampling window; no data generated. If bout is rescheduled, samples may reflect different conditions.
Delays in sampling for one bout cause an adjacent bout to occur within 14 days	Communicate to NEON Science via ServiceNow ticket for further instruction	Delayed bout may be cancelled if adjacent bout cannot be rescheduled, no data generated.
Scheduled bout will not capture the intended biological conditions (e.g. no rain signaling onset of wet season)	Reschedule if possible, communicating as described in this Table. If schedule cannot be adjusted, follow guidance in Section 4.5 below	Bout may be rescheduled; may impact schedule of adjacent bout/s.
Sampling is scheduled, but soil freezes	Do not attempt to collect soils. Communicate to NEON Science via ServiceNow ticket for further instruction	Samples will not be collected for this time period; no data generated.
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	Sampling methods will differ for affected locations.
There is standing water > 20 inches (50 cm) deep within a plot where soil sampling is to occur.	Do not attempt to collect soils. Communicate to NEON Science via ServiceNow ticket.	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.	Do not attempt to collect soils. Communicate to NEON Science via ServiceNow ticket.	Bout may be cancelled if it extends into a different sampling window; no data generated. If bout is rescheduled, samples may reflect different conditions.
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.	Data products not delivered for that bout.

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

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (Appendix C).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, 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**).

To Report Missed or Incomplete Sampling:

1. Missed or incomplete sampling must be communicated to Science by an incident ticket.

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

b. The lead Field Ecologist should consult the Delayed or Cancelled Activities table to best determine when reporting is required.

2. Create a data record in the SLS: Field Sampling application for each Missed Sampling event in the field. A record must be made for each plot missed. For example, if an entire bout is missed, records for 10 plots are created.

a. Within each plot with missed or incomplete sampling, create a 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.

b. 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.

***NOTE**: This is in contrast to samples that were collected but were lost or compromised* while *conducting laboratory processing, resulting in no downstream data. 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 **samplingImpractical** field must be populated in the mobile collection device. There is a defined list of values that are possible, see **Table 7**.

4. For field data quality issues related to whether the sampling bout was carried out at the biologically relevant time, e.g. whether **sampleTiming** accurately reflects on-the-ground conditions, populate the **biophysicalCriteria** field. Such items include Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates or do not capture the desired biophysical conditions (**Table 8**).

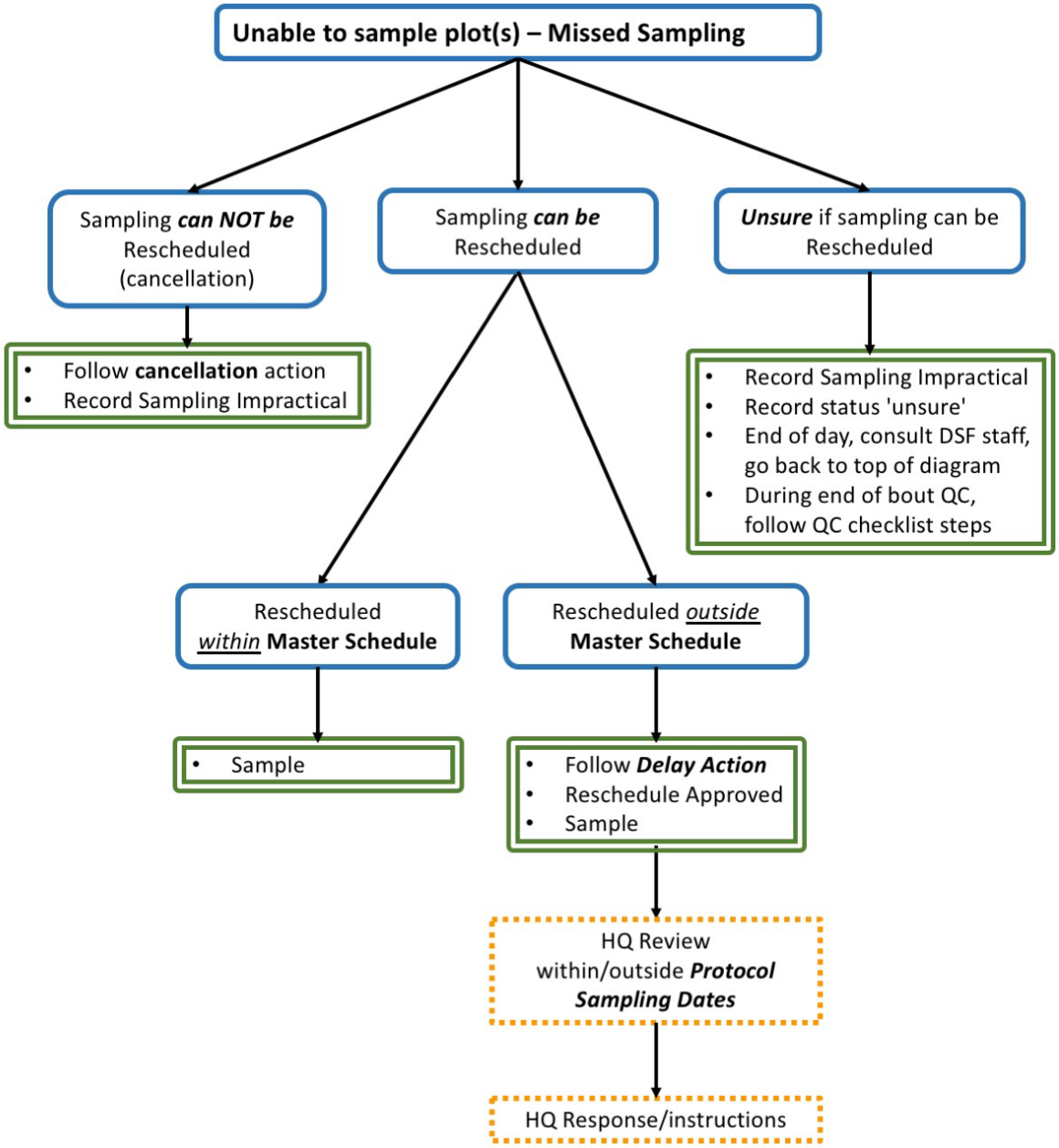

Figure 3. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Blue, rounded boxes represent contingencies: green double-lined boxes describe the required actions: orange dashed boxes indicate HQ actions. Required delay and cancellation actions are outlined for each protocol in the "Scheduled Field Activities – Delays and Cancellations" spreadsheet available on the SSL. Missed sampling events may also require a data quality flag and/or creation of a Site Management record.

Table 7. 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
Coordinates not suitable	Maximum number of attempts made to sample the randomly assigned X,Y coordinates within a subplot were unsuccessful
Location flooded	Standing or flowing water too deep to complete sampling
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Management	Management activities such as controlled burn, pesticide applications, etc.
Extreme weather	Events (e.g., thunderstorms, hurricanes) that compromise safety and access
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 location inaccessible due to other ecological reason described in the remarks

Table 8. 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 (default value)
OK – measurements outside intended sampling window but biophysical criteria met	Sampling occurred not within protocol sampling window but reflects the target conditions
Conditions do not meet biophysical criteria	Sampling does not reflect the target conditions
Other	Other potential sample timing inconsistencies described in the remarks

4.6 Estimated Time

The time required to complete the SOP's associated with this Protocol for a single sampling event/bout are listed in **Table 9**. It's important to note that the time required to implement a protocol will vary depending on a number of factors, such as experience level, site diversity, type of sampling bout, environmental conditions, and travel distances. 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 a framework for assessing progress. If a task is taking significantly longer than the estimated time, a ServiceNow ticket should be submitted.

Sampling should be scheduled at the beginning of the sampling window to allow time for contingencies to occur that could delay sampling.

Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 9. Estimated staff and labor hours 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	8 hrs	2	16 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	8 hrs	2	16 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 EHSS Policy, Program and Management Plan (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). Please 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.

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. Quarantined areas are updated annually in in 7 CFR Part 301 Domestic Quarantine Notices of the Plant Protection Act (7 U.S.C. 7756). The NEON Shipping Document (RD[14]) provides instructions for preparing soil samples for shipment. A list of quarantined sites as well as compliance instructions beyond those outlined in the Shipping Application can be found in Appendix D.1. Field Operations staff should check quarantine status annually for each site and be sure that they are complying with federal and location regulations.

Protocols for the handling of quarantined soils can be found in NEON's USDA Animal and Plant Health Inspection permit, posted on the CLA sharepoint site. General guidelines:

- While wearing gloves, remove any insects that are visible in the soil sample prior to field subsampling, especially if you are in an insect quarantine area.

- Also remove visible plant material (leaf litter, twigs, bark, and large roots) prior to field subsampling.

Quarantine soil samples that are being shipped to external laboratory facilities must include a copy of the receiving lab's USDA Soil Permit and comply with outlined shipping guidelines from the contracted facility. Additionally, all non-quarantine soils must be shipped with a USDA compliance agreement. The protocol for soil shipping is described in detail in RD[14], with additional guidance in the Shipping Application.

6 PERSONNEL

6.1 Training Requirements

All technicians must complete required safety training and protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

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 field and laboratory techniques, making salt solutions in the laboratory for pH and nitrogen transformation analyses, 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**). 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. Appendix D provides additional guidance for site-specific issues and protocol modifications for especially difficult sites. 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.

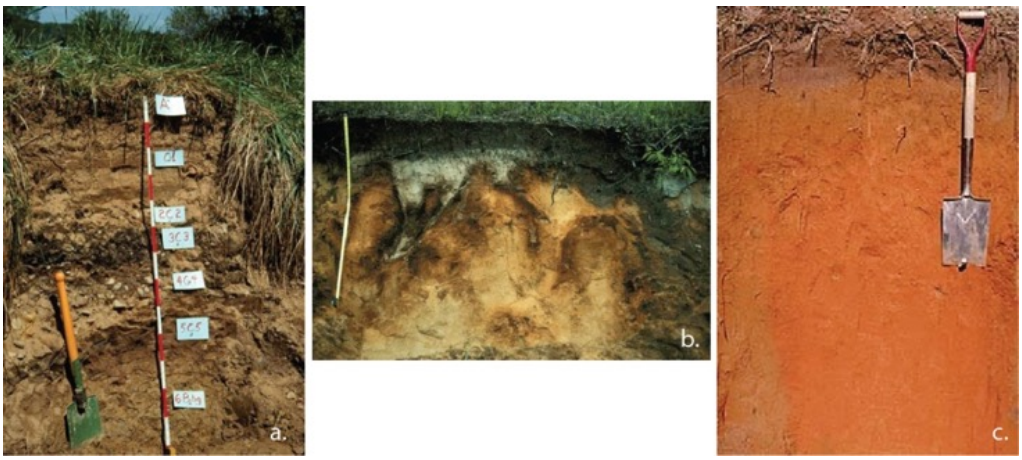

Figure 4. Soil Profiles from (a) Maryland, (b) Michigan, and (c) Florida. (Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

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 and clean sampling techniques 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 alcohol 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” square
- Gloves: these can be re-used at a sampling location if they are free of dirt/soil and have been sterilized thoroughly with an alcohol wipe or spray.

Finally, be aware of your activities, such as wiping your nose or eyes with a gloved hand, while sampling. You may employ a “clean-hand, dirty-hand” approach to managing the elements while maintaining clean samples.

7 STANDARD OPERATING PROCEDURES

SOP Overview

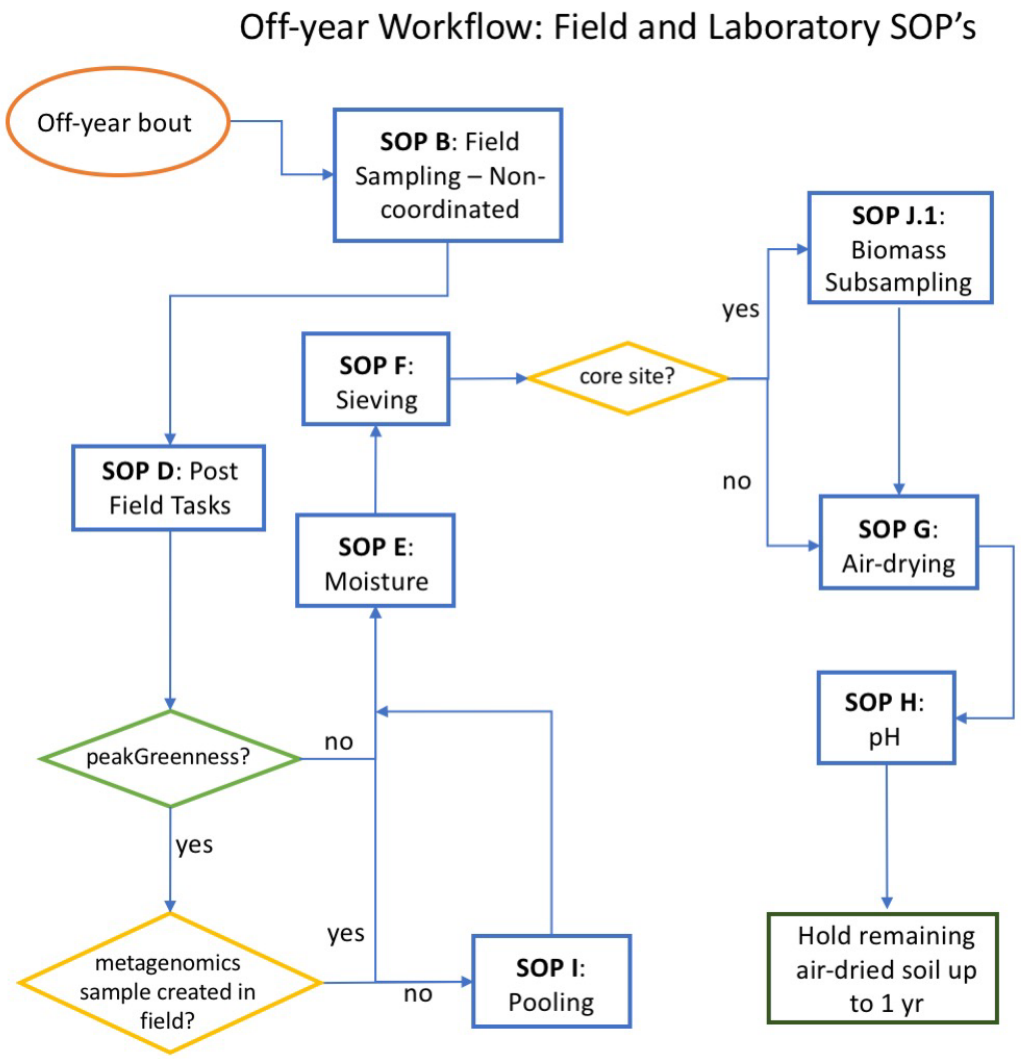

Figure 5. High-level workflow diagram showing the sequential organization of SOP's for field sampling and lab analysis/processing during an off-year, non-coordinated bout. Additional subsamples for metagenomics analysis are created during a peak greenness bout. Orange circle: start of workflow; diamonds: decision points; blue boxes: SOP's; green box: end of workflow. SOP's for data entry, QAQC, and shipment not included. Refer to SOP G: Air Drying Soils for guidance on holding air-dried soil.

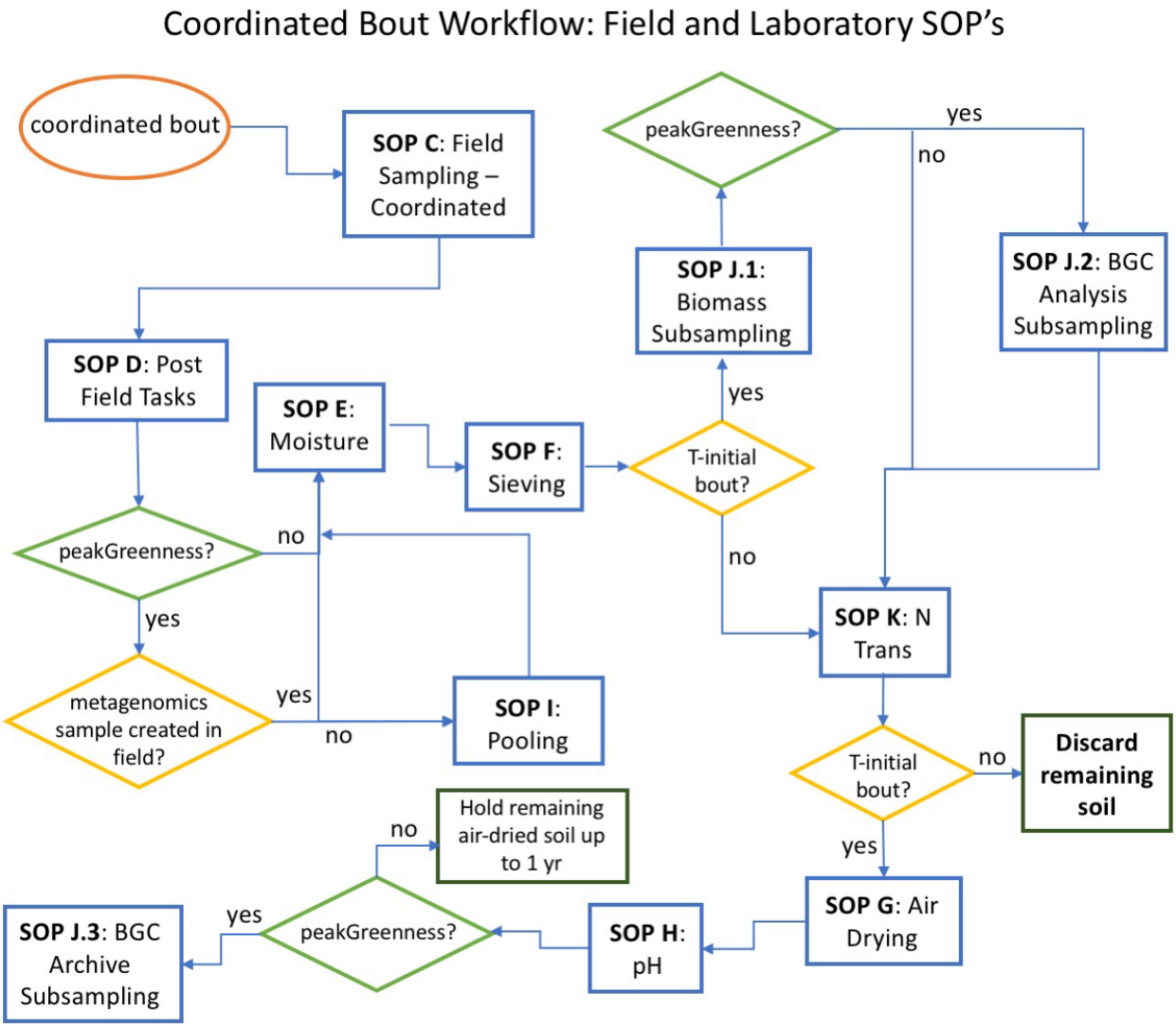

Figure 6. High-level workflow diagram showing the sequential organization of SOP's for field sampling and lab analysis/processing during a coordinated bout. Additional subsamples for metagenomics, BGC analysis, and BGC archiving are created during a peak greenness bout. Orange circle: start of workflow; diamonds: decision points; blue boxes: SOP's; green boxes: end of workflow SOP's for data entry, QAQC, and shipment not included. Refer to SOP G: Air Drying Soils for guidance on holding air-dried soil.

SOP A Preparing to Sample Soils

A.1 Prepare for Data Capture

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 fully charged at the beginning of each field day. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). Mobile devices should be synced at the end of each field day; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

1. Ensure that the mobile devices are fully charged at the beginning of each field day whenever possible. However, given the potential for mobile devices to fail in the field, **always** bring paper datasheets to **all** sampling locations at **all** times.
2. Fill out site information on field datasheet (RD [05]). Make sure to use proper formats, as detailed in datasheets.
3. 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. Ensure that all coordinates sampled from previous bouts are 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.
4. Gridded plot maps may be prepared prior to the bout: a full-sized printable map is located in the Supporting Documents section of the Sampling Support Library. Instructions on adding X,Y locations are in SOP B.1.

A.2 Prepare Sampling Equipment

5. Ensure all supplies listed in **Table 19** and **Table 20** are available.
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. Rub off rust with a sponge or towel, then rinse 3x with 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 inch) in coring device diameter over time. If planning to sample with a different diameter not listed in **Table 18**, 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 17 summarizes O-horizon presence and thickness in sites where NRCS has completed initial soil characterization.

8. Calibrate soil temperature probes. 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 bench top or in 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 7**. Equipment can be stored in the sand if desired.

    d. Wait for a five minutes, then take reading of 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.

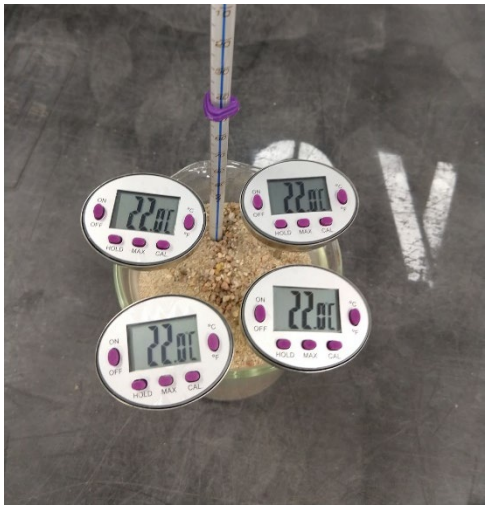

Figure 7. Temperature probe calibration setup

9. Obtain spatial and permitting information for the site:

    a. The GPS coordinates for the target plots that will be sampled. *Tip*: add these as waypoints in the GPS instrument to aid in plot navigation.

    b. Check Appendix D.1 for site-specific, federal permitting requirements. Note: other local permits may also be required.

10. Time-permitting, flag the southwest corner of each plot prior to sample collection to save time in the field.

11. If using the laser rangefinder, 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 19**) so that they can be applied to bulk sample bags in the field.

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 20**. 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 using higher strength ethanol than 70%, 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 20**) to a clean (non-sterile or sterile is OK) syringe. 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 and should only need one for filtering the entire 1 Liter of ethanol.

3. Gently drain excess ethanol out of the bag and discard as directed by your domain requirements (it's OK for some liquid to remain), re-seal, and place into a new, heavy-duty Ziploc 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 comes in handy in the field when hot or dry conditions can 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.3 Additional Prep 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 8** shows an example PVC incubation cylinder: the bottom edge has been beveled, which helps drive it in to soil, and two holes near the top aid in removal.

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.*

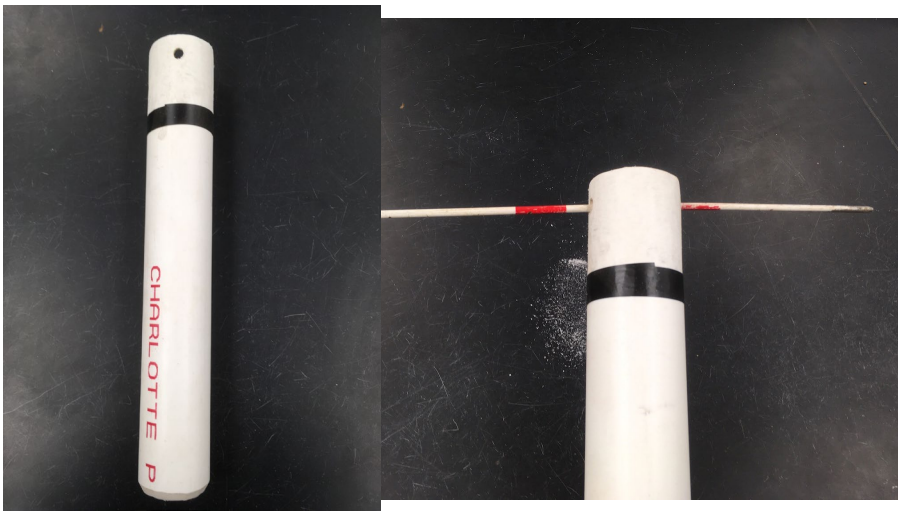

Figure 8. Example coring device used for soil N transformation sampling.

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.

    1) Ensure they are not overly damaged. Small chips on the beveled edge are ok, but if more than half of the beveled edge is missing due to chips and breakage, or if the cylinder has a large crack up the side, discard and contact NEON HQ to order more. In rocky soils, cylinders will require more frequent replacement.

2. Check that one loosely fitting (2.25” or 2.5” diameter) cap per sampling location is available. If mammal disturbance or strong winds are an issue at the site, 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 21**, verify that all laboratory supplies listed in **Table 25** 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. If possible, make 2M ultrapure potassium chloride for extractions in advance as it can take several hours to dissolve, see SOP K for instructions.

A.4 Label Sample Containers

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 10** 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 9**.

Figure 9. Barcode labels used for the various types of soil samples. Type I barcodes are weatherproof but are not cryo-safe; Type II and III barcodes are cryo-safe and are ideally sized for the sample containers.

About human-readable labels

The identifier convention for most soil sample types is:

plotID - horizon - coreCoordinateX - coreCoordinateY - collectDate
Ex: *CPER_001-M-0.5-30-20160130*

This is the **sampleID** for the bulk soil sample that generates all of 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-0.5-30-20160130-gen* is the microbial genetic analysis sample, appended with "-gen":

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

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

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

a. Whirl-paks 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 ziplock bag, and do not use any sample container that appears damaged or was previously opened.

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

2. Affix labels to all bags 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 10**. Apply all labels a minimum of 30 minutes prior to sampling on room-temperature containers to ensure they have time to adhere fully: they may also be applied at the start of the season. **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.

***Reminders**: The barcode scanner does not work on curved surfaces. This means the barcode should be aligned lengthwise along a vial, not horizontally wrapping around. Be sure that no wrinkles, folds, air bubbles or other obstructions are present that can impede reading of the barcode. Do not overlap labels.*

a. For the bulk, homogenized soil sample: Place the weatherproof Type I barcode labels (prefix 'A' plus 11 numbers) on each bag. Then, affix the pre-printed, human-readable labels on each bag that will hold the homogenized soil, leaving the coordinates field blank until you are at the plot and can confirm the X, Y location (example in **Figure 10**).

b. For the microbial genetic analysis samples, place the cryogenic Type II barcode labels (prefix 'B" plus 11 numbers) on each Whirl-pak. Each Whirl-pak is a unique sample and should have its own barcode label. Then, affix the pre-printed, human-readable labels on each, leaving the coordinates field blank until you are at the plot and can confirm the X, Y location.

c. For the microbial archive samples, place the cryogenic Type III barcode labels (prefix 'C' plus 11 numbers) on each vial. Each container is a unique sample and should have its own barcode label. Then, affix the human-readable labels on each microbial archive vial. These will be thin and may need to be hand-written.

d. **Off-year, non-coordinated bouts, Core sites only:** Prep microbial biomass vials. For each sample, affix a cryo-safe (Type II) barcode label to a new plastic scintillation vial. Orient the barcode from top to bottom. (not curving around). Also affix a pre-printed, cryogenic human-readable label with **biomassID**: sampleID + "-bm" (e.g. STEI_051-O-10.5-20.5-20160115-bm). Ensure that labels don't overlap (Figure 10).

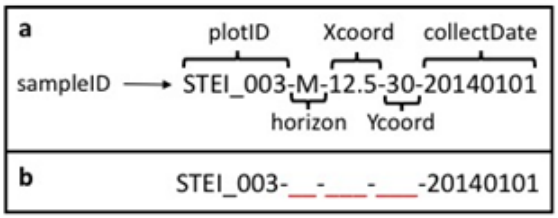

Figure 10. (a) Example of an annotated sampleID. (b) SampleIDs deployed in the field will typically have pre-filled plot and date information: depending on site, horizon may also be pre-filled.

3. **Coordinated Bouts:** Affix labels to all containers that will be used for lab generated samples. Apply labels as described in **Table 10**.

    a. For microbial biomass vials or KCl extracts: For each sample, affix a cryo-safe (Type II) barcode label to a new plastic scintillation vial. Orient the barcode from top to bottom (not curving around). Also affix a pre-printed, cryogenic human-readable label with correct ID:

    b. Biomass samples get a **biomassID** (sampleID + "-bm", e.g. STEI_051-O-10.5-20.5-20160115-bm).

    c. KCl samples get a **kclSampleID** (sampleID + "-kcl", e.g. ONAQ_005-M-10.5-20.5-20160115-kcl).

    d. Ensure that labels do not overlap, as shown in **Figure 11**.

Figure 11. Plastic scintillation vial showing proper orientation of labels. Applies to microbial biomass (-bm), KCl extraction (-kcl), and BGC analysis (-cn) vials.

4. For BGC analysis samples: For each sample, affix a weather-proof (Type I) barcode label to a new glass scintillation vial. Orient the barcode from top to bottom (not curving around) as shown in **Figure 11**. Additionally, affix a pre-printed human-readable label with the **cnSampleID** (sampleID + "-cn", e.g. CPER_001-O-10.5-10.5-20160101-cn).

5. For BGC archive samples: For each sample, affix a weather-proof (Type I) barcode label to a new glass bottle (**Table 23**), oriented top to bottom (not curved around). Additionally, affix a

pre-printed human-readable label with the **bgcArchiveID** (sampleID + "-ba", e.g. CPER_001-O-10.5-10.5-20160101-ba).

Table 10. 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 ziplock 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	Type III 1 per sample; 150-300 per bout	Cryogenic label cut horizontally into thirds	Top of vial (on the cap)
Microbial biomass (-bm)	SLS: Field Sampling	20 mL plastic scint vial	Type II 1 per sample; 30-60 per bout	Cryogenic label	Side of vial oriented vertically
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	250 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 upland soils with no more than 2.5 cm (1 inch) of standing water. For sites containing wetlands that have plots with > 2.5 cm of standing water, follow TOS SOP: Wetland Soil Sampling (RD[06]), if authorized to do so in that SOP. For sites with > 2.5 cm standing water that are not authorized to use the Wetland Soil Sampling SOP, contact NEON Science for additional guidance. Note that the Wetland SOP refers back to this SOP for various instructions.

Refer to **Table 2** and the **Quick References** section for reminders about which samples are produced by this SOP. Helpful instructional videos are also available in the NEON internal Training Center.

For some sites with thin organic layers or rocky soils, it may be difficult to obtain the soil volumes indicated in the SOP's without collecting additional cores. 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.

It is extremely important to note that 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 your soil masses for future reference.

Throughout the field protocol, it is essential to ensure clean sampling technique in order to reduce contamination and produce high-quality microbial data.

The majority of soil sampling bouts are collected following this SOP and will be of boutType = microbes or microbesBiomass. During these bouts, the following samples and measurements will be made:

- soil temperature
- litter depth
- microbial genetic analysis (-gen) and archiving (-gaX)
- core sites only: microbial biomass (-bm, in the laboratory)
- soil moisture (-sm, in the laboratory)
- soil pH (-pH, in the laboratory)

When sampling during peak greenness at Core sites, a plot-level composite sample also will be collected for microbial metagenomics analyses as part of the microbial sampling campaign. Instructions for generating these "-comp" samples in the field are provided in this SOP. 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. A list of Fulcrum applications for each sample type can be found in **Table 10**.

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] for further guidance.

1. Confirm with a handheld GPS that the GPS coordinates for the target plot match the GPS coordinates at your current location. Locate the southwest corner of the plot to verify that you are at the correct plot. Move along the perimeter of the plot as much as possible in order to minimize foot traffic within the plot.
2. You will collect soil at three randomly assigned locations within each plot, one in each randomly designated subplot (see Appendix F). You should have already identified the sample locations using the soil X,Y coordinate list for each plot/subplot combination.
3. Using a laminated, gridded plot map (as shown in Figure 1, print from the SSL), mark the X,Y coordinates you anticipate sampling at the plot. Keep in mind that rejected coordinates can occur, so have back-ups handy. For a particular X,Y coordinate, select the closest plot marker (e.g. SW 40, E 40, etc).
4. Next, calculate the distance and direction you need to navigate from each selected plot marker to a coordinate location.
    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, so for example moving 5 gridlines is the equivalent of 5 meters.
    b. For each X, Y coordinate, draw the distances and directions from the selected reference marker to the X,Y coordinate onto the map. Use these distances and directions to navigate from the selected plot marker to a sample location.
5. In relatively flat plots (<20% grade) where pulling a meter tape is practical, lay out a meter tape from the selected reference marker in the X (E/W) direction, using a compass to verify direction. Pull the tape to the measured distance to the target X coordinate and mark the point. From that point,

navigate in the Y (N/S) direction to the measured distance to the target Y coordinate. Place a marker at the location.

6. In very steep (>20% grade) plots or in heavily forested plots where pulling a tape is not possible, use a laser rangefinder set to HD (horizontal distance) mode to locate the 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] for details).
    d. Two technicians must work together. One stands at the selected reference marker of the plot 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 (If you are 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 location? In Tower plots, does the coordinate fall within 2 m of the perimeter of a litter trap? If any of these 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.
    - If five X,Y coordinates are rejected, do not sample within that subplot and notify Science. Make a record for that subplot and choose “Coordinates not suitable” in the **samplingImpractical** field, which will allow you to leave the X,Y coordinates and other

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

3. At an acceptable location, start near the exact location of the X,Y coordinate and carefully assess soil depth by probing the soil using a sterilized chaining pin or similar, moving outward (not more than 0.5 m away) until a suitable spot is found. Make an effort to avoid disturbing the litter layer while probing. Suitability varies from site to site and based on coring device, but in general a suitable spot will allow you to sample sufficient soil without requiring more than 2 brownies or cores. For sites with characteristically rocky or shallow soils, 3 brownies or cores can be considered as suitable. Mark the sampling area with flag or chaining pin.

4. 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. **Measure soil temperature.** At a spot adjacent to, but not directly on top of, the marked sampling location, remove the litter layer and carefully insert temperature probe (does not need to be sterilized) into the soil to 10 cm depth. Don't force the probe as it may break.
    - Allow probe to equilibrate for about 2 minutes, then record the **soilTemp** in degrees C in the Fulcrum application or field data sheet. ***Note***: Do not make measurement with the sun directly on probe (shade it with your body, if necessary).
2. **Measure litter layer depth**. Directly above the sampling location, use a ruler (clean, but does not need to be sterilized) to measure the depth of the undisturbed litter layer in cm (**litterDepth**, $\pm$ 0.1 cm) and record the value (or average value if more than one core/brownie is collected). Gently wiggle the ruler through the layer to the ground surface, minimizing disturbance to the litter profile as much as possible to ensure that you are not compacting it.

Reminder*: The litter layer is dead 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 greater than 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. Sterilize gloves and tools with 70% ethanol.
2. Cut out an organic horizon "brownie" using the square frame cutter tool and soil knife (hori-hori, **Figure 12**). With deep organic horizons, only 1 brownie may be needed; from many sites, two will be needed. At those sites, select two locations within 0.5 m of each other.

Figure 12. Example use of brownie cutter and hori-hori for O horizon sampling.

3. 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 in the field **sampleBottomDepth**.
4. Place all brownies collected at an individual location into a pre-labelled, 1-gallon resealable plastic bag. With a pre-sterilized, gloved hand, remove rocks, visible roots, insects, wood, moss, and other non-soil debris and homogenize. Or if it is difficult to pick the entire bag of homogenized soil in the field, it is acceptable to use a pair of pre-sterilized tweezers to pick rocks, roots, and non-soil debris from the subsamples while they are being generated in the field. Be sure to re-sterilize the 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.
5. With wet or saturated soils, dump out any excess water in the sample container after the soil has settled 10-15 seconds, if present.
6. **Ensure that you have sufficient soil for all subsampling and analyses:** In general, using the methods above should provide plenty of soil for all subsampling and lab processing. However, if site conditions raise concerns that you are low on soil, aim for a *minimum* 40 g of organic 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, 5-10 g target mass) and 5 labeled cryovials (fill to about 70% capacity).

Close whirl-pak such that the labels lay flat and are clearly visible. For collecting into the cryovials, scoop soil into the container using a sterile scoop. Any non-soil material that ends up in a container should be removed with sterilized tweezers or by hand using pre-sterilized gloves. The samples should appear as shown in **Figure 13**.

a. Complete the human-readable labels on the whirl-pak and the cryovials with horizon, X coordinate and Y coordinate.

The microbial genetic analysis sample label should appear as:
plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen
(ex. CPER_001-O-10-10.5-20160101-gen)

Note: The X, Y coordinates are labeled to the nearest 0.5 m, but do not show the decimal place for whole number coordinates (10, not 10.0).

The microbial genetic archive sample labels should appear as:
plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga*X* (*X*=1-5)
(ex. CPER_001-O-10-10.5-20160101-ga1)
Note: Be sure that the archive subsample number (e.g. 1-5) is appended to the end of the human-readable label.

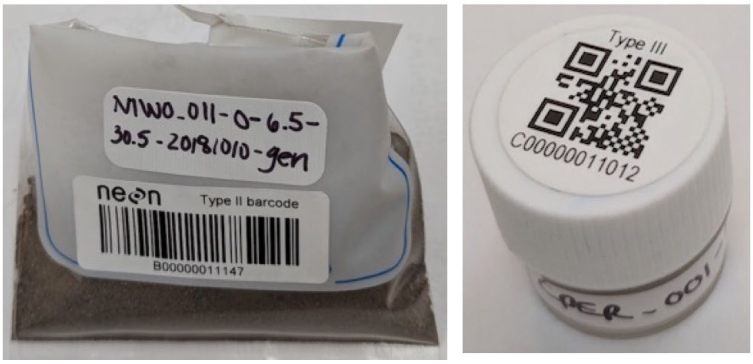

Figure 13. Example microbial genetic (-gen, left) and microbial archive (-gaX, right) samples. Microbial metagenomics (-comp) samples will look similar to -gen samples, except for content of the label.

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. To maximize data quality and 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 all newly added samples are in contact with dry ice so that they freeze quickly.

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. CPER_001-O-20160101-comp)

1) Place one scoop of homogenized soil in the whirl-pak, close the 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.

1) Place one scoop of homogenized soil in the whirl-pak and close the bag.

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

11. 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 (Figure 13), and record the **compositeSampleID** in the SLS: Metagenomics Pooling application or on a paper datasheet. Also scan the barcode label into the Fulcrum application if it is being used. 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 time does 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.

12. **Ensure that you have sufficient organic soil remaining for lab subsampling and analyses**:

- Microbes bout (off-year, any season): 30 g

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

13. 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**.
- Cryovials are labelled with the **geneticArchiveSampleID**.
- All barcode labels have been scanned and the values were populated correctly into the Fulcrum application. Perform a quick visual scan to catch any erroneously duplicated barcodes.

14. **Ensure that all samples are stored correctly.** Whirl-paks and cryovials should be in the cooler 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 all samples are frozen. Place the 1-gallon resealable bags in the cooler with the ice packs.

B.5 Collect mineral (M) horizon

Only collect an M horizon sample if you do not have an O horizon at your X, Y coordinate.

***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 14**).
2. Core to a total depth of 30 cm (± 1 cm) or saprolite, whichever is shallower. Total depth is the combined thickness of the O + M horizons. If an O horizon is not present, total depth should be the depth of the M horizon from the surface to a max depth of 30 cm. Always core vertically, not perpendicularly, when collecting on a slope.
    a. A piece of masking or lab tape can be placed on the outside of the corer to indicate the depth to stop driving the corer into the soil. It is also acceptable to core incrementally (e.g. in 10 cm increments) to reach the total depth, whichever works best.
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 as long as it does not contact non-sterile surfaces.

Figure 14. Field staff sterilizing a soil auger using chaining pin and 70% ethanol wipe. Gloves should be pre-sterilized.

4. Measure **sampleTopDepth** as distance from the top of the soil sample to the soil surface, and **sampleBottomDepth** as the depth from the soil surface to the bottom of the sample. When taking multiple cores, record the average depth values.
5. Record **horizon** (M), X, Y coordinates, and date on the sample bags and in the Fulcrum application or field datasheet.

Note: *O horizons with an average depth ≤ 1.0 cm are not sampled as a separate horizon. Samples with an O horizon ≤ 1.0 cm should be sampled with the M horizon material. If a thin O horizon is collected as part of the M horizon sample, select the value "Thin O horizon present" in the field* ***horizonDetails****.*

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 soil conditions, and to procure enough material for all samples and downstream analyses. In general, the volume of a *2" diameter x 30 cm depth core* should provide sufficient material for all samples and analyses. With a 1" diameter core, 2 cores should suffice. Plots with shallower soils and more coarse fragments (e.g. rocks and roots) may need to collect more cores or may have to use alternative methods (e.g. mass measurements) to ensure that sufficient material has been collected. Field technicians will have to exercise some judgment regarding number of cores needed to obtain sufficient soil for analyses. ***If you have questions or concerns related to sample quantities for a particular site, contact NEON Science by issuing a ServiceNow ticket.***

6. 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 15**). 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), 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 15. Dispensing soil core into bag for homogenization. Tapping the corer with a rubber mallet can help release soil from the barrel.

7. **Ensure that you have sufficient soil for all subsampling and analyses:** In general, using the methods above should provide plenty of soil for all subsampling and lab processing. However, if site conditions raise concerns that you are low on soil, aim for a *minimum* 100 g of mineral soil in the bulk homogenized bag.

8. **Create microbial genetic 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 5 labeled cryovials (fill to about 80% capacity). Close whirl-pak such that the labels lay flat and are clearly visible. 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.

    a. Complete the human-readable labels on the whirl-paks and the cryovials with horizon, coreCoordinateX and coreCoordinateY. **Figure 13** shows examples of properly labelled samples.

    The microbial genetic analysis sample label should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen
(ex. CPER_001-M-10.5-10.5-20160101-gen)

Note: *The X, Y coordinates are labeled to the nearest 0.5 m, but do not show the decimal place for whole number coordinates (37, not 37.0).*

The microbial genetic archive sample labels should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga*X* (*X*=1-5)
(ex. CPER_001-M-10.5-10.5-20160101-ga1)

Note*: Be sure that the archive subsample number (e.g. 1-5) is appended to the end of the human-readable label*

9. Scan the barcode label for each sample into the mobile data recorder, if being used. Ensure that each barcode label is entered only once and is associated with the correct sample. To maximize data quality and reduce data entry errors, it is recommended that barcode labels are scanned *during* sample creation rather than in batches of multiple samples.

10. 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.

11. **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.

    - 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. CPER_001-M-20160101-comp)

      - Place one scoop of homogenized soil in the whirl-pak, close the bag, and place on ice packs.

    - If this not the first X,Y location to be sampled at a plot, obtain the whirl-pak created earlier from the wet ice 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.

      - 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.
      - If another X,Y location within the plot may be added to this bag, return the bag to cooler with ice packs.
      - 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 or on a paper datasheet. Also scan the barcode label, if using. 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 time does 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.*

12. **Ensure that you have sufficient soil remaining for lab subsampling and analyses**. In general, using the methods above should provide plenty of soil for lab processing. However, if site conditions raise concerns that you are low on soil after sub-sampling in the field, these are the **minimum** amounts of *mineral* soil that should remain in the bulk homogenized bag:
    - Microbes bout (off-year, any season): 60 g

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

13. 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**.
    - Cryovials are labelled with the **geneticArchiveSampleID**.
    - All field sampleID's have been recorded.
    - 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.

14. **Ensure that all samples are stored correctly.** Whirl-paks and cryovials should be in the cooler 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 all samples are frozen. Place the 1-gallon resealable bags in the 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 20**).

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
- biophysicalCriteria
- protocolVersion
- siteID
- plotID
- 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
- geneticArchiveSampleCount
- sampleExtent (Entire=entire horizon sampled, or to saprolite/bedrock; Obstruction=sampled to an obstruction; Maximum=sampled to maximum depth allowed by the protocol, horizon may extend deeper; Unknown=extent varied across cores or could not be determined)
- remarks
- measuredBy
- recordedBy

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

B.7 Field clean-up

1. Thoroughly rinse sampling equipment with deionized water (corer, thermometer, etc).
2. Between plots, wipe down reusable sampling equipment with deionized water and wipes or a scrub brush, followed by ethanol wipes or squirt bottle.
3. 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.

SOP C Field Sampling for Coordinated Bouts

This SOP is designed to sample upland soils with no more than 2.5 cm (1 inch) of standing water. For sites containing wetlands that have plots with > 2.5 cm of standing water, follow TOS SOP: Wetland Soil Sampling (RD[06]), if authorized to do so in that SOP and Appendix D. For sites with > 2.5 cm standing water that are not authorized to use the Wetland Soil Sampling SOP, contact NEON Science for additional guidance. Note that the Wetland SOP refers back to this SOP for various instructions.

Refer to **Table 2** and the Quick References for reminders about which samples are produced by this SOP. Helpful instructional videos are also available in the NEON internal Training Center.

Sampling soils involves several field and laboratory components. Throughout the field protocol, it is essential to ensure clean sampling technique in order to reduce contamination and produce high-quality microbial data. In the field, technicians measure soil temperature and litter depth, install an incubation cylinder, collect a soil core, subsample the soil core, and store subsamples for laboratory transport.

Soil sampling bouts collected following this SOP will be of boutType = microbesBiomass (T1/T2) or microbesBiomassBGC (PG). During these 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)
- 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)
- soil BGC analysis and archiving (-cn and -ba, in the laboratory)

When sampling during peak greenness, a plot-level composite sample will be collected for microbial metagenomics analyses. Instructions for generating these "-comp" samples in the field are provided in this SOP. 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. A list of Fulcrum applications for each sample type can be found in **Table 10**.

During Coordinated bouts, N transformation rate measurements are conducted. This involves collecting a T-initial sample for initial nitrogen concentration measurements, as well as deploying an incubated T-final core that will be left in the field for 2-4 weeks and collected for final 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.5**) . The Wetland SOP refers back to this SOP for various 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] for further guidance.

1. Confirm with a handheld GPS that the GPS coordinates for the target plot match the GPS coordinates at your current location. Locate the southwest corner of the plot to verify that you are at the correct plot. Move along the perimeter of the plot as much as possible in order to minimize foot traffic within the plot.
2. You will collect soil at three randomly assigned locations within each plot, one in each randomly designated subplot (see Appendix E). You should have already identified the sample locations using the soil X, Y coordinate list for each plot/subplot combination.
3. Using a laminated, gridded plot map (**Figure 1**), mark the X, Y coordinates you anticipate sampling at the plot (keeping in mind that rejected coordinates can occur, so have back-ups handy). For a particular X, Y coordinate, select the closest plot marker (e.g. SW 40, E 40, etc). Selecting the closest plot marker to an X, Y location also alleviates the need to walk through the inner 20x20 m plot: sensitive plant sampling occurs here and trampling should be avoided as much as possible.

4. Next, calculate the distance and direction you need to navigate from each selected plot marker to a coordinate location.
5. 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, so for example moving 5 gridlines is the equivalent of 5 meters.
6. For each X, Y coordinate, draw the distances and directions from the selected reference marker to the X, Y coordinate onto the map. Use these distances and directions to navigate from the selected plot marker to a sample location.
7. In relatively flat plots (<20% grade) where pulling a tape is practical, lay out a meter tape from the selected reference marker in the X (E/W) direction, using a compass to verify direction. Pull the tape to the measured distance to the target X coordinate and mark the point. From that point, navigate in the Y (N/S) direction to the measured distance to the target Y coordinate. Place a marker at the location.
8. In very steep (>20% grade) plots or in heavily forested plots where pulling a tape is not possible, use a laser rangefinder set to HD (horizontal distance) mode to locate the 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] for details).
    d. Two technicians must work together. One stands at the selected reference marker of the plot 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.
9. Place a marker at the X,Y location.

C.2 Assess sample location

1. Put on a clean pair of nitrile gloves (If you are 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:
   - Are there obvious disturbances, vegetation, large rocks or roots that would impede sampling within a 0.5 m radius of the location? In Tower plots, does the coordinate fall within 2 m of the perimeter of a litter trap? If any of these 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.
   - If five X,Y coordinates are rejected, do not sample within that subplot and notify Science. Make a record for that subplot and choose "Coordinates not suitable" in the **samplingImpractical** field, which will allow you to leave the X,Y coordinates and other metadata blank. Do not attempt to sample the fourth subplot that was not randomly selected for this bout.
3. At an acceptable location, start near the exact location of the X,Y coordinate and carefully assess soil depth by probing the soil using a sterilized chaining pin or similar, moving outward (not more than 0.5 m away) until **two** suitable spots are found. Make an effort to avoid disturbing the litter layer while probing. Suitability varies from site to site and based on coring device, but in general a suitable spot will allow you to sample sufficient soil without requiring more than 2 brownies or cores. For sites with characteristically rocky or shallow soils, 3 brownies or cores can be considered as suitable. Mark the two sub-locations with flags or chaining pins (**Figure 16**).

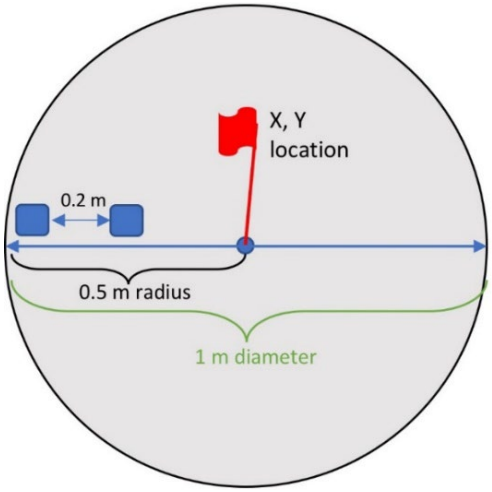

Figure 16. Area of acceptable sub-locations for sampling at an X, Y coordinate location. Example cores show ideal distance between initial and final core locations. Locations with rocky soils can use the entire 1 m area, as long as both sub-locations are within 0.5 m of the X, Y location.

- 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 should be within 0.5 m of the actual X, Y location.

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

5. 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. **Measure soil temperature.** At a spot adjacent to, but not directly on top of, the marked sampling location, remove the litter layer and carefully insert temperature probe (does not need to be sterilized) into the soil to 10 cm depth. Don't force the probe as it will break easily.
   - Allow probe to equilibrate for about 2 minutes, then record the **soilTemp** in degrees C in the Fulcrum application or field data sheet. ***Note***: Do not make measurement with the sun directly on probe (shade it with your body, if necessary).
2. **Measure litter layer depth**. Directly above the Tinitial sampling location, use a ruler (clean, but does not need to be sterilized) to measure the depth of the undisturbed litter layer in cm (**litterDepth**, $\pm$ 0.1 cm) and record the value (or average value if more than one core/brownie is collected). Gently wiggle the ruler through the layer to the ground surface, minimizing disturbance to the litter profile as much as possible to ensure that you are not compacting it.

***Reminder**: The litter layer is dead 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).*

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. Insert the PVC incubation 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 Appendix D*.

3. Estimate inserted depth of the PVC cylinder by measuring from top of the cylinder to 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, if needed.
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 X, Y coordinates on the 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.

1. At the second flagged sub-location, push the litter layer away from where you are going to core into the soil surface.
2. Sterilize sampling equipment, gloves, and other equipment that will come into contact with the sample with 70% ethanol.
3. **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 that is < 1.0 cm thick, skip this step and proceed directly to C.5.4.
    a. Push the litter layer away from where you are going to core into the soil surface.
    b. Using clean, sterile tools and equipment, cut out an organic horizon "brownie" using the square frame cutter tool and soil knife (hori-hori, **Figure 12**). With deep organic horizons, only 1 brownie may be needed; from most, two will be needed. At those sites, select two locations within the allowable distance of each other.

c. 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**.

d. Place all brownies collected at an individual location into a pre-labelled, 1-gallon resealable plastic bag. With a pre-sterilized, gloved hand, remove rocks, visible roots, insects, wood, moss, and other non-soil debris and homogenize. Or if it is difficult to pick the entire bag of homogenized soil in the field, it is acceptable to use a pair of pre-sterilized tweezers to pick rocks, roots, and non-soil debris from the subsamples while they are being generated in the field. Be sure to re-sterilize the 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.

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

e. **Ensure that you have sufficient soil for all subsampling and analyses:** Aim for the following *minimum* masses:
   i. Transitional (T1 or T2) bout: 75 g
   ii. Peak greenness bout: 100 g
   iii. If sample is below these masses, take another sample at the same X,Y location and re-homogenize.

f. **Generate microbial analysis 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). Close whirl-pak such that the labels lay flat and are clearly visible. 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. Any non-soil material that ends up in a container should be removed with sterilized tweezers or by hand using pre-sterilized gloves. The samples should appear as shown in **Figure 13**.
   i. Complete the human-readable labels on the whirl-pak and the cryovials with horizon, X coordinate and Y coordinate. The microbial genetic analysis sample

label should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen
(ex. CPER_001-O-10.5-10.5-20160101-gen)

Note: The X, Y coordinates are labeled to the nearest 0.5 m, but do not show the decimal place for whole number coordinates (37, not 37.0).

The microbial genetic archive sample labels should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga*X* (*X*=1-5)
(ex. CPER_001-O-10.5-10.5-20160101-ga1)

Note: Be sure that the archive subsample number (e.g. 1-5) is appended to the end of the human-readable label.

g. Scan the barcode label for each sample into the mobile data recorder, if being used. Ensure that each barcode label is entered only once and is associated with the correct sample. To maximize data quality and reduce data entry errors, it is recommended that barcode labels are scanned during sample creation, rather than in batches of multiple samples.

h. 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.

i. **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.

   i. 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. CPER_001-O-20160101-comp)

      Place one scoop of homogenized soil in the whirl-pak, close bag, and place on ice packs.

   ii. 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.

      1. Place one scoop of homogenized soil in the whirl-pak and close the bag.
      2. If another X,Y location within the plot might be added to this bag, return the bag to cooler with ice packs.

   iii. 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 (**Figure 13**), and record the **compositeSampleID** in

the SLS: Metagenomics Pooling application or on a paper datasheet. Also scan the barcode label, if one is used.

1. 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 time does 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.*

j. **Ensure that you have sufficient soil remaining** for lab subsampling and analyses. If sample size is below these masses, take another sample at the same X,Y location and re-homogenize. Minimum masses are:
   i. Coordinated, transition season bout (e.g. not peak greenness): 50 g
   ii. Coordinated, peak greenness bout: 75 g

4. **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 one of the O-horizon sample locations, if multiple brownies were taken. Only collect multiple cores if more material is needed.

   - 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.

5. Push the cylinder or coring device in to a total depth (from the soil surface) of 30 ± 1 cm, or to a depth comparable to the PVC cylinder if the incubated core was installed first. If your soil profile is shallow (you hit saprolite or bedrock at less than 30 cm), core to the depth of the saprolite or bedrock only.

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

6. Remove cylinder or coring device and empty soil directly into a new, pre-labeled 1-gallon bag. If you require a helper tool to extrude soil from the cylinder (e.g. long flat-head screwdriver, soil knife, chaining pin, 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 chaining pin threaded through the drill holes – may aid in removal.

7. 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), and insects using a sterilized, gloved hand.

8. 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 the bottom depth of the O layer. Measure the **sampleBottomDepth** as the depth from the soil surface to the bottom of the sample (**Figure 17**). When taking multiple cores, record the average depth values.

Figure 17. Example soil profile: O horizon sampleTopDepth = 0 cm, sampleBottomDepth = 3 cm; M horizon sampleTopDepth = 3 cm, sampleBottonDepth = 30 cm.

9. **Ensure that you have sufficient soil for all subsampling and analyses:** In general, using the methods above should provide plenty of soil for all subsampling and lab processing. However, if site conditions raise concerns that you are low on soil, aim for the following *minimum* masses. If below these masses, take another sample at the same X,Y location and re-homogenize.
    a. Transitional (T1 or T2) bout: 150 g
    b. Peak greenness bout: 210 g

10. **If there was no O horizon sampled <u>OR</u> it is a peak greenness bout: Create microbial genetic 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). Close whirl-pak such that the labels lay flat and are clearly visible. 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.
    a. Complete the human-readable labels on the whirl-paks and the cryovials with horizon, coreCoordinateX and coreCoordinateY. **Figure 13** shows examples of properly labelled samples.

        The microbial genetic analysis sample label should appear as:

        plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen

(ex. CPER_001-M-10.5-10.5-20160101-gen)

Note: The X, Y coordinates are labeled to the nearest 0.5 m, but do not show the decimal place for whole number coordinates (37, not 37.0).

The microbial genetic archive sample labels should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga*X* (*X*=1-5)

(ex. CPER_001-M-10.5-10.5-20160101-ga1)

Note: Be sure that the archive subsample number (e.g. 1-5) is appended to the end of the human-readable label.

b. Scan the barcode label for each sample into the mobile data recorder, if being used. Ensure that each barcode label is entered only once and is associated with the correct sample. To maximize data quality and reduce data entry errors, it is recommended that barcode labels are scanned *during* sample creation rather than in batches of multiple samples.

c. 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.

11. **During Peak Greenness: Generate microbial metagenomics (-comp) sample from M horizon.** 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. CPER_001-M-20160101-comp)

i. Place one scoop of homogenized soil in the whirl-pak, close the bag, and place on wet ice.

b. If this not the first X,Y location to be sampled at a plot, obtain the whirl-pak created earlier from the wet ice 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 or on a paper datasheet. Also scan the barcode label, if using.

d. 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 time does 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.*

12. **Ensure that you have sufficient mineral soil remaining for lab subsampling and analyses**:

- 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*

13. For labelling 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**.
- Cryovials are labelled with the **geneticArchiveSampleID**.
- All field sampleID's have been recorded.
- 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

14. Record metadata

- Update the paper soil X, Y coordinate list and subplot list.
- NtransBoutType = Tinitial
- boutType (microbesBiomass or microbesBiomassBGC), ensure that all of the sampleID's associated with that bout have been generated.
- sampleTiming
- biophysicalCriteria
- protocolVersion
- siteID
- plotID
- 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
- geneticArchiveSampleCount
- sampleExtent (Entire=entire horizon sampled, or to saprolite/bedrock; Obstruction=sampled to an obstruction; Maximum=sampled to maximum depth allowed by the protocol, horizon may extend deeper; Unknown=extent varied across cores or could not be determined)
- remarks
- measuredBy
- recordedBy

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

C.6 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.

***Note**: Soils being measured for N transformations MUST be processed and extracted in 2M KCl within 1 day of collection. 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, provided all necessary equipment (including ultra-pure Type I deionized water) is available. If laboratory facilities near the site are not available and sampling takes more than a day, alternate arrangements must be made. For example, a team can transport the 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.7 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 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 colonized it (e.g. made a nest), create a record in the Fulcrum application or datasheet but choose **sampleFate** = 'destroyed.' Only record minimal sample metadata and discard any sample material, if present.
3. Take off cap and remove cylinder from the ground.
    - If soil is dry or high in clay content, a helper tool – such as a post puller or a chaining pin threaded through the drill holes – may aid in removal.
    - 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.
    - Most cylinders should be in 'OK' condition.
    - If the cylinder has been disturbed – for example, an animal has removed it from the hole, or the water table has risen into the core – still collect but choose the appropriate **incubationCondition** choice and explain in remarks.
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 chaining pin 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 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 11**). Otherwise, discard material and do not include in the sample.

o 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-coreCoordinateX-coreCoordinateY-collectDate
   ex. ONAQ_001-M-8.5-21-20160721

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. 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 72 hours at relocatable 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 toilet-brush type 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. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable 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 terrestrial sampling plot is compromised after road-building activities. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful. If the criteria below are not met and thus locations are deemed unsuitable for sampling, Field Science should reach out to NEON Science staff to communicate the need for new sampling locations as soon as possible.

For soil sampling, there are numerous circumstances that might prevent sampling at a particular plot for a given bout or year. However, not all situations would warrant plot reassignment. These situations should be dealt with on a case-by-case basis. If it becomes apparent that a particular plot will be compromised either in the short or long-term (e.g. road now goes through plot), submit an incident ticket to discuss re-assigning sampling to a different plot.

If sampling at a given plot is not possible *during a bout* a ServiceNow ticket should be submitted by Field Science staff. Additionally, non-sampled locations must be documented in the SLS: Field application:

1. Review data records to determine if any plots/locations were scheduled for sampling but were not sampled: there should be 10 plots and 30 samples (minimum).
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'
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.
4. Create data records for missed sampling locations using the **samplingImpractica**l field in the SLS: Field application as described in Section 4.5.

D.3 Verification of Soil Temperature Probes – End of Season Only

Conduct this procedure following the domain's last T2 bout. The goal is to determine whether probes are still good or need to be replaced prior to the next field season.

1. Perform calibration of probes at room temperature following the steps listed in SOP A.
2. Place entire calibration set up in fridge (4°C or similar). Allow for overnight equilibration.
3. The next day, remove calibration set up from fridge 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 Fulcrum application or lab datasheet (RD [05]). Key reminders:

- Soil moisture is measured on soil that has not been sieved.
- For Off-year bouts, soil moisture analysis should begin within 48 h of field collection, or Monday morning for samples collected on Friday.
- For Coordinated bouts, 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 (8) below)*.
2. Weigh foil boat to nearest 0.01 g and record value in the Fulcrum application or datasheet (boatMass).
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 without taring the balance. **Ensure that any rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris have been removed, use clean forceps as needed.** Record weight 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.
5. Place all samples into drying oven, using 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 + weighing boat to nearest 0.01 g and record values in the Fulcrum application or datasheet. Record the date and time out of oven.
7. Dispose of soils according to permit requirements.
8. For weigh boats that are in good condition and can be re-used, clean in Alconox (or similar) 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?

If oven space is limited and more than 1 day (Coordinated bouts) or 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.

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**. 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, air-dry, or subsample as directed. Key reminders:

- For relocatable sites conducting off-year, microbes-only bouts, sieving and subsampling should begin within 48 h of field collection, or Monday morning for samples collected on Friday.
- For core sites OR sites conducting a coordinated bout, sieving MUST begin within 1 day of field collection.

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 organic (O) horizon, do not sieve, but remove any rocks, coarse roots (> 2mm diameter), insects, wood, moss, and other non-soil debris and homogenize before proceeding.

Note: *The presence of non-soil material (e.g. roots) can dramatically impact the results of certain lab analyses, particularly microbial biomass measurements. This is because plant roots have unique lipid signatures that can overwhelm the microbial lipid signatures in the soil. Thorough removal of non-soil material is essential to generating high-quality data. If your samples have extensive coarse roots and other non-soil debris, allow up to 30 minutes per sample for adequate picking and soil homogenization. If picking takes a significant amount of time ensure that adequate staff are available to complete downstream processing in a timely manner.*

5. With a gloved hand, pass **M horizon** samples through a clean and dried, 2 mm screen diameter sieve. This will allow all particles ≤ 2 mm to be collected, 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 according to permit requirements.
7. Write 'sieved' on plastic gallon sample bags if adding soil back to them so there is no confusion as to which 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, just be sure they are cool to the touch prior to use.

NEXT STEPS: For a relocatable site conducting an off-year bout, proceed to SOP G: Air-drying Soils. For a core site, 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 During a 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 relocatable 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 material (organic horizon samples from field resealable plastic bags, and the mineral soil samples after sieving) into #8 paper bags labeled with the **sampleID**. The sample barcode label may also be transferred from the bulk soil bag to the paper bag if desired and may aid in populating data in the SLS: pH 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, as even though larger roots have been removed, fine roots maybe 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.
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 a subset of soil remaining 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. To store, select a sample from one X, Y location at each plot and for each horizon. 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 for up to one year. If paper bags have holes in them, transfer entire thing to plastic re-sealable bag first. Remaining samples from the same plot can be discarded after all analyses and subsampling have been completed. 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-homogenized), air-dried soil samples. Soil pH is measured potentiometrically in a saturated or supernatant liquid that is in equilibrium with a soil suspension of a 1:1 soil-to-H_2O or 1:2 soil-to-$CaCl_2$ (weight/volume) mixture for mineral soils and a ratio of 1:4 soil-to-H_2O and 1:8 soil-to-$CaCl_2$ ratio for organic soils. Samples are analyzed both in deionized (DI) water and 0.01 M calcium chloride ($CaCl_2$).

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 $CaCl_2 \cdot 2H20$ in just under 2 liters of house DI water.
3. Check pH of CaCl2 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.
5. Bring solution to final volume of 2L and label the container with your initials, '0.02M $CaCl_2$' and the creation date.

   Note: this 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 (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.
3. Using a clean tool, weigh out a subsample of air-dried organic or mineral soil and place into a 50 – 100 mL container. New or properly cleaned (see step 16 below) 50 mL conical tubes may be used and can facilitate processing of multiple samples simultaneously.
   a. For O-horizon samples, use 5 g (± 0.5 g) of soil that has been picked clean of rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris and air-dried.
      1) For fluffy, absorbent O horizons, the mass of soil may be decreased to as low as 1 g.
   b. For M horizons, use 10 g (± 0.5 g) of sieved, air-dried soil.
4. Record soil masses to 0.01 g accuracy in the SLS: pH Fulcrum application or lab datasheet.
5. Add DI H_2O to each sample:

a. For O horizon samples, add 20 mL of DI H_2O.

b. For M horizon samples, add 10 mL of DI H_2O.

c. Allow soil to absorb the H_2O. Thoroughly stir each sample with a clean stir rod for 20 seconds. Rinse stir rod with DI and dry with a kimwipe between samples.

6. Ensure that all soil is saturated, particularly when using conical tubes.

   a. Look for supernatant (a thin layer of liquid without soil particles) above the flocculated soil. If not present, add another aliquot (10 mL) of H_2O, stir and settle for 1 minute.

   b. Add as many aliquots as needed to ensure soil is completely saturated. Keep track of the total volume of solution added in the SLS: pH Fulcrum application or datasheet.

7. After letting the samples sit for 30 minutes, stir each sample with a clean stir rod for 20 seconds. Let the samples sit for another 30 minutes. Begin measurements 1 hour after the first sample in the batch was initially stirred (e.g. after 1 hour total has elapsed).

8. During the waiting period, calibrate the pH meter electrode using the buffer solutions that best encompass the ranges in soil pH encountered (either buffers 4, 7, and 10 for high pH soils, or 1.68, 4, and 7 for low pH soils). Follow calibration instructions in the manual for the probe. **NOTE**: For pH meters that auto-detect calibration solutions being used, ensure that the meter has selected the correct suite of calibration buffers (e.g. that the auto-selected pH values match the actual pH values used).

   a. Rinse the electrode with deionized water and gently shake off excess liquid between buffers. **Do not** touch the tip of the electrode with a wipe.

   b. Note: Check pH meter calibration using the middle buffer solution every 10-12 samples.

9. Begin measuring the **soilInWaterpH** in the batch of samples. Starting with the first sample, stir with a clean stir rod for 20 seconds, then let the sample settle for 1 minute.

10. Measure the pH of the sample by inserting the probe into the solution. Gently swirling the container while measuring pH can help the measurement stabilize but is not required. Only insert the probe far enough to completely immerse the probe tip.

    a. For M horizon samples, this will typically be ~2-3 cm.

    b. For O horizons the probe tip should be in the supernatant below the layer of floating organic material that will be present. It is OK if some flocculated soil is floating in the supernatant. If floating particles are adhering to the probe, give tube a gentle swirl to dislodge.

11. Allow reading to stabilize (tenths of pH unit not fluctuating, usually about 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.

12. Clean electrode: rinse thoroughly 2 to 3 times with deionized water and gently shake off excess liquid. Do not touch the tip of the electrode with a wipe. The *sides* of the probe only may be gently wiped with a kimwipe if soil residue is observed adhering to the probe.

    a. If both **soilInWaterpH** and **soilInCaClpH** have been measured and recorded for a sample, continue to Step 15.

13. After reading the **soilInWaterpH**, add $CaCl_2$ solution to the same container. Be sure to add the same volume of $CaCl_2$ as H_2O so that the final concentration of $CaCl_2$ remains 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 O horizon samples, add 20 mL of $CaCl_2$ (or however much H_2O was added).

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

    c. Stir sample with a clean stir rod for 20 seconds and let sample site for 1 minute. Rinse stir rod with DI H_2O and wipe with a kimwipe between samples.

14. Measure the **soilInCaClpH** of the sample by repeating steps 10-12 and record value in **soilInCaClpH** field.

15. Move on to the next sample in the batch. Repeat Steps 9-14 for all remaining samples, first measuring **soilInWaterpH** and then **soilInCaClpH**. Measure samples in the same order that the water was added so that samples have been sitting for similar amounts of time. With some practice, the next sample will have completed its 1 minute settling time and be ready for measurement as soon as the previous sample has completed its measurement.

16. 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 will be 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.

1. Obtain a pair of new nitrile gloves and pre-sterilize with 70% ethanol. Re-sterilize gloves between samples.
2. From the -80°C freezer, obtain 1 whirlpak from each core sample. Organize whirlpaks by placing those from the same collection date, plot, and horizon together. Double-check the labels to ensure that the sample collection dates, plot IDs, and soil horizons match. Typically, there will be 3 whirlpaks, but fewer than 3 is also possible.
3. At least 30 minutes prior to use, place a cryo-safe barcode label on each new whirlpak bag that will be used to store composite samples. Also, label each whirlpak bag with the plotID, horizon, collection date that matches a set of whirlpaks, and "-comp" for composite. These may be pre-printed cryo-safe labels or hand-written. *Ex. CPER_001-M-20140101-comp*
4. Repeat step 2 for every unique combination of plotID, horizon, and collection date. There should be 1 new whirlpak bag for every unique combination.
5. Thaw the material in a set of whirlpaks and transfer all material in each whirl-pak into the corresponding composite whirlpak bag. Homogenize the soil by gently kneading and/or shaking the outside of the closed whirlpak.
6. Return the sample bags to the -80°C freezer (or container of dry ice, if no freezer is accessible) immediately.
7. Repeat steps 4-5 for the remaining samples.
8. Complete the data sheet and/or the Fulcrum application by recording the sample information from the empty whirlpak bags. Ensure that the data sheet was completed correctly and completely, and discard empty whirlpaks. If using barcode labels, scan the barcode for each associated sample.
    a. When entering the sampleID's for each sample added to generate 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 During a Coordinated Bout

Follow this SOP for all Coordinated bouts, after completing SOP F.

- When **nTransBoutType** = Tinitial, subsamples are generated from sieved/picked, field-moist soils for microbial biomass analysis, bgc analysis and archive (peak green only), and KCl extraction.
- When **nTransBoutType** = Tfinal, only a KCl extraction sub-sample is generated.

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

J.1 Processing Microbial Biomass Samples

Microbial biomass samples are generated during coordinated bouts when **nTransBoutType** = Tinitial. Follow this SOP *immediately* after completing SOP F.

Note about processing organic soils: it is important to remove as much root material as possible. Remove roots with care, since crushing them during processing can release lipids that can interfere with microbial biomass estimates. Picking can take up to 30 minutes per sample to do sufficiently. If in doubt about a sample, contact Science. For Alaska sites, follow the site-specific guidance in Appendix 8D.3.

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 a new, clean scint vial on balance and record **biomassVialMass**, then tare/zero the balance.
3. For M horizons, transfer 10 g (± 0.5 g) of field-moist, sieved soil. Record **biomassFreshMass**. Close the vial.
4. For O horizons, transfer 5 g (± 0.5 g) of soil that has been picked clean of rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris. Record **biomassFreshMass**. Close the vial.
5. Ensure caps are on tightly. *Option*: wrap parafilm around cap to keep in place during shipment.

Note: If more than one vial is necessary to hold 5 g of soil, create a second scintillation vial. Place all vials for that sample into a pint-sized freezer bag and affix one barcode label for all vials associated with that sample. If more than 3 vials are required to obtain sufficient mass, contact Science.

6. Scan barcodes to associate each with the appropriate **biomassID** in the SLS: Field Sampling application. Human-readable labels should appear as:

   plotID-horizon-coreCoordX-coreCoordY-collectDate-bm

   ex. CPER_001-O-10.5-10.5-20160101-bm

7. For samples marked as containing *Toxicodendron spp*, ensure that this recorded in the shipping manifest.

8. Store vials at -80°C until ready for shipment.
9. 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 **sampleTiming** = peakGreenness, proceed to SOP J.2: Processing BGC Analysis Samples. Otherwise, proceed immediately 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 soil that has been picked clean of rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris.
    b. For M horizons, use field-moist, sieved soil.
2. For soils that are 100% saturated and have high clay content (ex: TOOL), 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 oven end date 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 Fulcrum application and that each barcode has been scanned.
    8. Store bottles 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 (non-sterile OK) gloves while handling samples.
2. Place a pre-labeled and barcoded archive jar on the balance 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**).
5. Ensure that all **bgcArchiveIDs** have been created in the SLS: BGC Sub-Sampling 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.

IMPORTANT REMINDERS:

- Subsampling MUST begin within 1 day of field collection in order to preserve sample integrity.
- 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 so 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.

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 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) 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 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.*

1. Wear clean (non-sterile ok) nitrile gloves.
2. For a large batch (20 L) of 2M KCl, measure 2,982 g ultrapure 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 ultrapure 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 a clean filter forceps, transfer ~ 20 new filters (don't need to count, ok to approximate) to a clean Buchner 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 a second time.
    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 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

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

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 labeled with a cupID, similar to the tinIDs used for soil moisture.

3. 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 application.
    - If sample mass is limiting, 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 remains in the sample, as described in SOP F.1: Sieving and Homogenizing Samples.
4. For each sample, measure 100 ± 2 ml of 2M KCl into a clean graduated cylinder (or, a volume scaled to the soil mass used, roughly 10:1) and add to the container of weighed soil. Record the **kclVolume** and the **extractionStartDate** (YYYY-MM-DD HH:MM).
5. 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 (not preferred) – 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:
        1) First, note the **kclReferenceID**, which describes the KCl batch used that day for extractions (format = siteID-extractionStartDate-'BRef1', example: CPER-20190418-BRef1). If a new batch of KCl is created in the midst of processing samples, it will have a new kclReferenceID (example: CPER-20190418-BRef2).
        2) In the Fulcrum application, records for all of the samples extracted on a single day should be nested under this **kclReferenceID**.
        3) 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).
6. Make sure caps on each extraction cup are on tightly, then shake each cup vigorously for ~15 seconds.
7. 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.
8. Place the box on its side so that samples shake end-to-end. **Shake for 1 hour at 150 rpm**.
9. 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.

10. 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 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.*

1. Obtain scintillation vials that have been pre-labeled as described in SOP A.
2. Set up the manifold (**Figure 18**) and attach to a vacuum pump. Check that all stopcocks are in the closed position (perpendicular to the tubing).

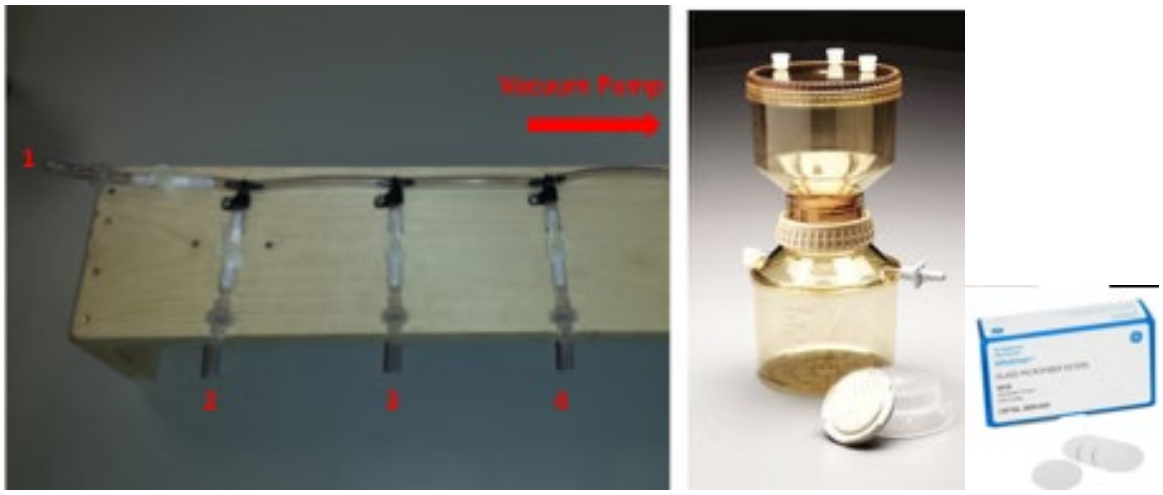

Figure 18. Filtration equipment for KCl extractions. Example of a sample manifold set-up (left) and other equipment used for filtration, including filtration unit (extraction funnel + collection cup) and filters (right).

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 set, 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 (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.
    a. Fill ~3/4-way two dishpans with house DI water.
    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
        1) 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.

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.

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. If samples are being lost due to a problem with sample processing that should be addressed, submit an issue ticket.

SOP L Data Entry and Verification

The importance of thorough, accurate data transcription cannot be overstated; the value of the efforts in the field is only manifested once the data are properly entered for delivery to NEON's end users.

Mobile data entry applications (e.g. Fulcrum) 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. As a best practice, field data collected on paper datasheets should be digitally transcribed according to the timelines described in the Data Management Protocol (RD[04]). 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. collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location.

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***. 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, -comp, -ga), other samples generated in field collection app (-bm): within 21 days of electronic data entry
- Lab metadata (pH, moisture), lab-generated subsample data (-cn, -kcl, -ba): within 30 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 things don't go as planned, horizon identification and separation in particular can be challenging. 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 11**).

Table 11. Protocol-specific horizon details values. In the event that 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.
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]). QC checklists are available in the SSL to streamline the data quality review process.

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 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. When preparing for an upcoming soil sampling bout, review the master site coordinate and subplot lists and ensure that they are up to date with records from the previous bout(s).

L.3 Sample Labels & Identifiers

By default, each sample or subsample produced by this protocol is assigned a human-readable sample identifier which contains information about the location, date, and horizon of the collected sample. Each

sample will also be associated with a scannable barcode, which will improve sample tracking and reduce transcription errors associated with writing sample identifiers by hand.

Barcode labels are adhesive and should be applied to dry, room temperature containers at least 30 minutes in advance of their use in the field, and it is encouraged to apply them well in advance of a sampling bout. Barcodes are unique, but are not initially associated with a particular sample. Use the appropriate barcode label type with each container (i.e., cryo-safe barcode labels only used for samples that are stored at -80°C, etc). Note that a barcode label is applied *in addition to* a human-readable label (hand-written or printed).

Barcodes are scanned into the Fulcrum application when the sample is placed into the container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. This is an important thing to check for when performing dataQA.

Data and sample IDs must be entered digitally and quality checked according to RD[04] prior to shipping samples to an external lab.

SOP M Sample Shipment

Refer to NEON Protocol and Procedure: Shipping Ecological Samples, Sensors and Equipment (RD[14]) for details on how, when and where to ship samples for analysis and archiving. Any discrepancies between the scientific requirements provided in the Protocol and the shipping instructions provided in the Shipping document should be communicated to Science.

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. Currently, it is only implemented at sites where problems have been encountered in implementing the current soil sampling protocol.

N.1 Identify the Plot

1. Navigate to the southwest corner of the plot. Using flags or some other marker, mark the locations that are approximately 5m from the corner of each plot, as shown in **Figure 19**. These locations do not have to be exact.

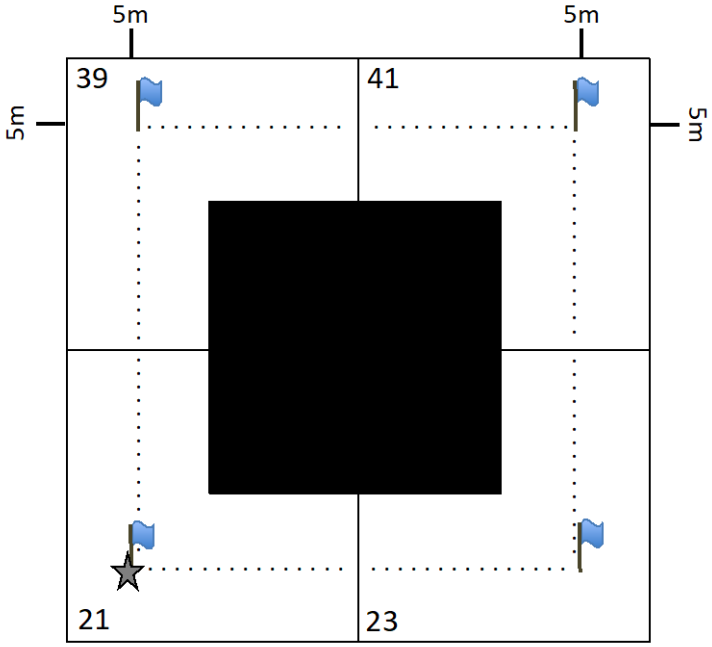

Figure 19. Schematic of TOS soil plot demonstrating the general layout of sample locations. Subplot ID's are noted in the left corner of each subplot. Flags denote the corners for the depth transect measuring area, star indicates the location to begin measurement. Dots indicate the general distribution of depth measurements.

N.2 Measure Soil Depths

1. Beginning at the flag located in subplot 21, 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. 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, take a depth measurement approximately every 1 meter until you take 15 measurements. After 15 measurements, you should be in subplot 23. Take the next 15 measurements and record in the data sheet under subplot 23. 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) 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. There should be 30 measurements per subplot.
4. Remove markers once measurements are completed.
5. Enter completed Data Sheets electronically following the Manual Data Transcription Protocol, RD[04].

8 REFERENCES

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APPENDIX A QUICK REFERENCES

Table 12. 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)	✓	✓	✓

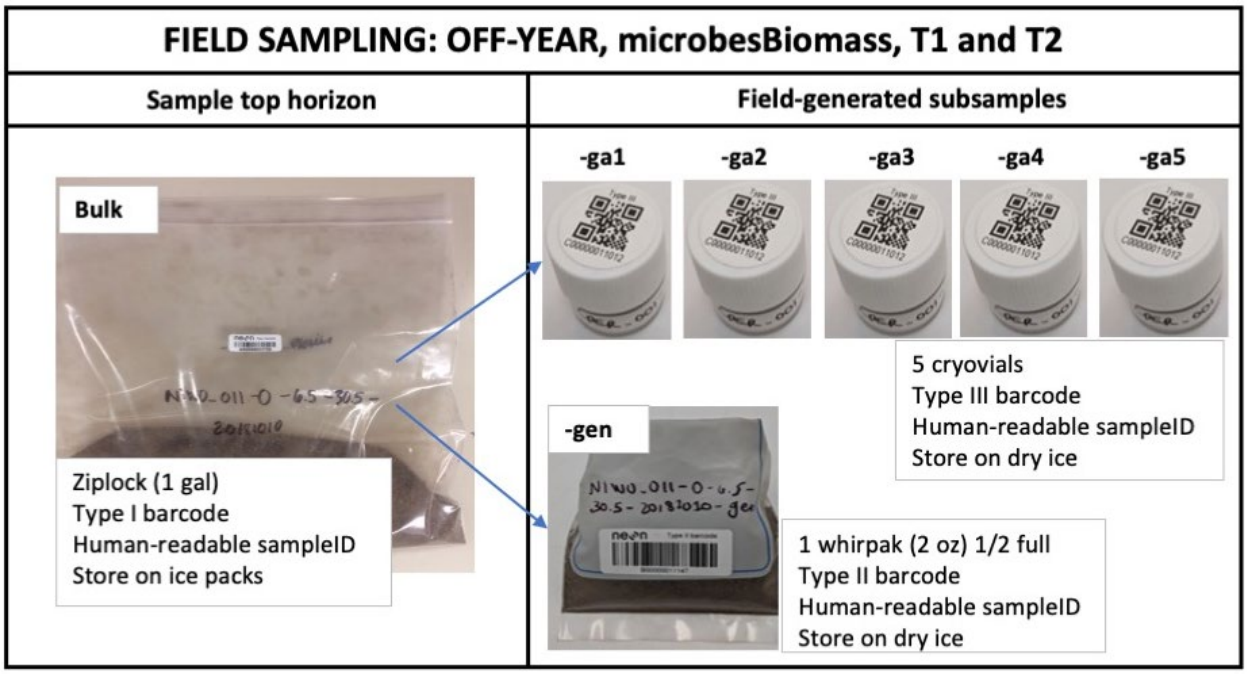

Figure 20. Field-generated samples at Core sites for boutType= microbesBiomass, sampleTiming= T1 or T2.

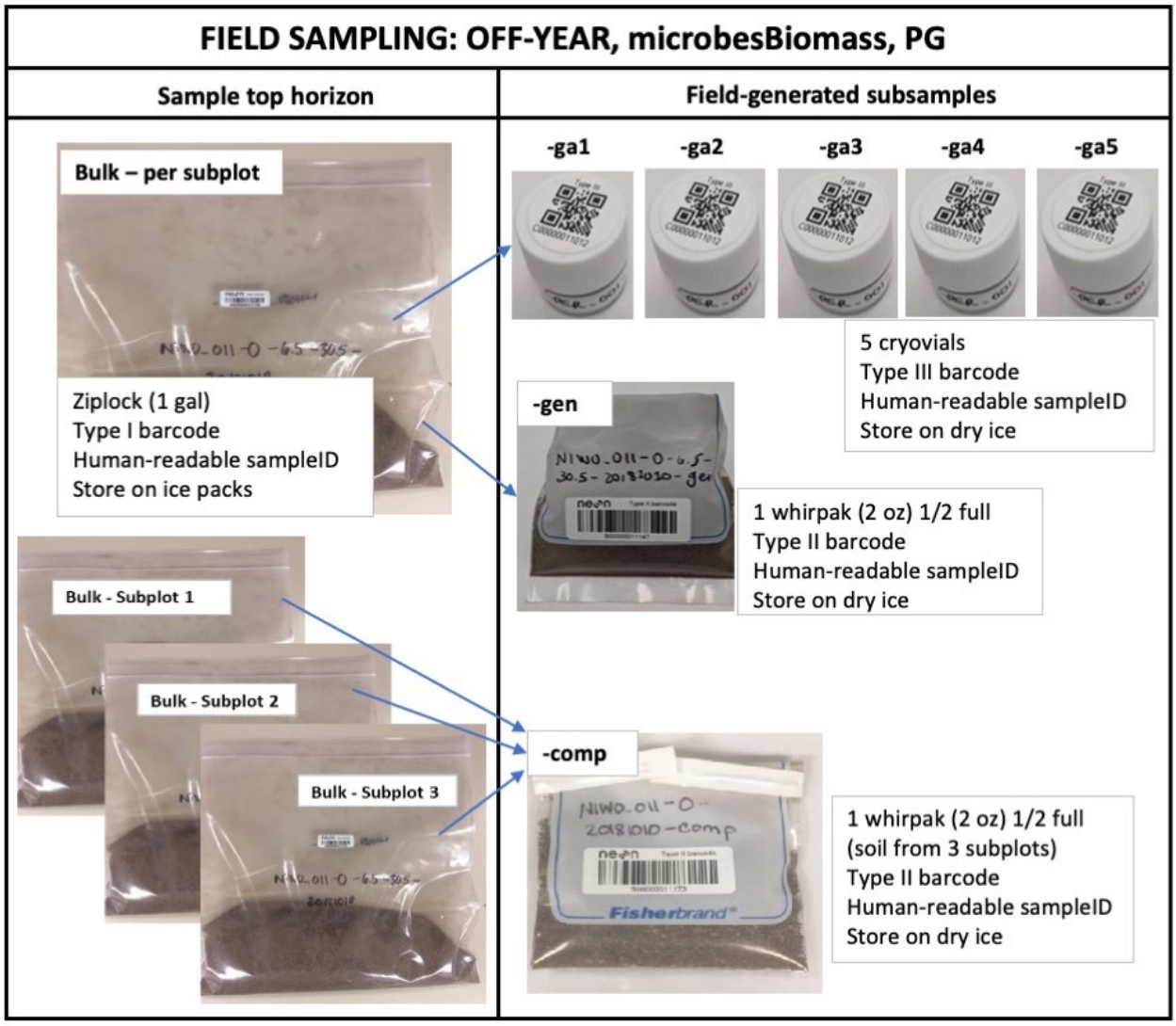

Figure 21. Field-generated samples at Core sites for boutType= microbesBiomass, sampleTiming= peak greenness.

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

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

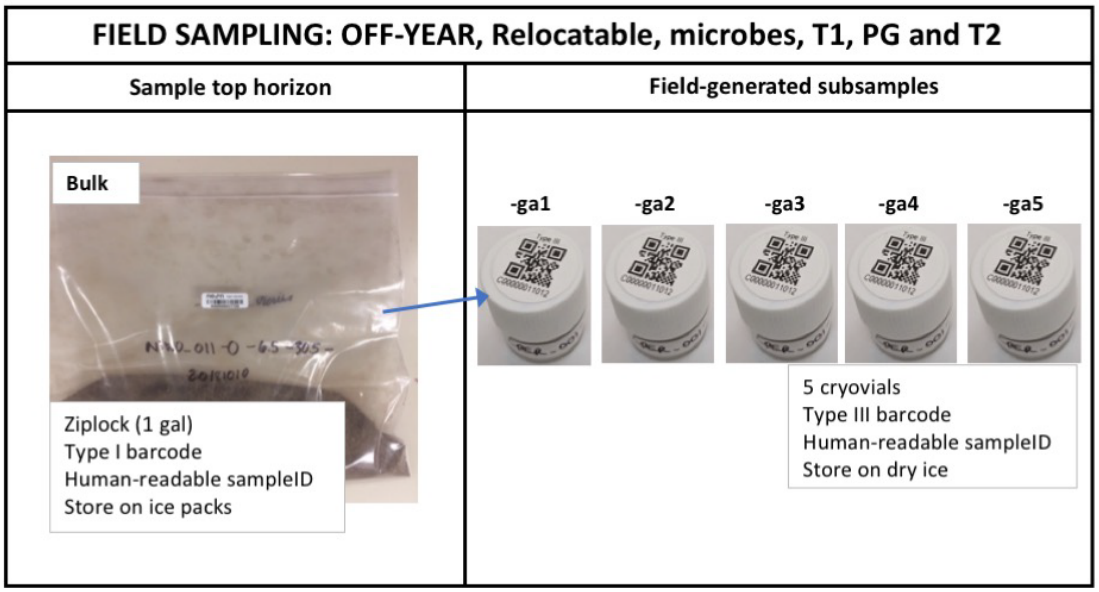

Figure 22. Field-generated samples at Relocatable sites for boutType= microbes, all bouts.

Table 14. 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)	Microbes 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	

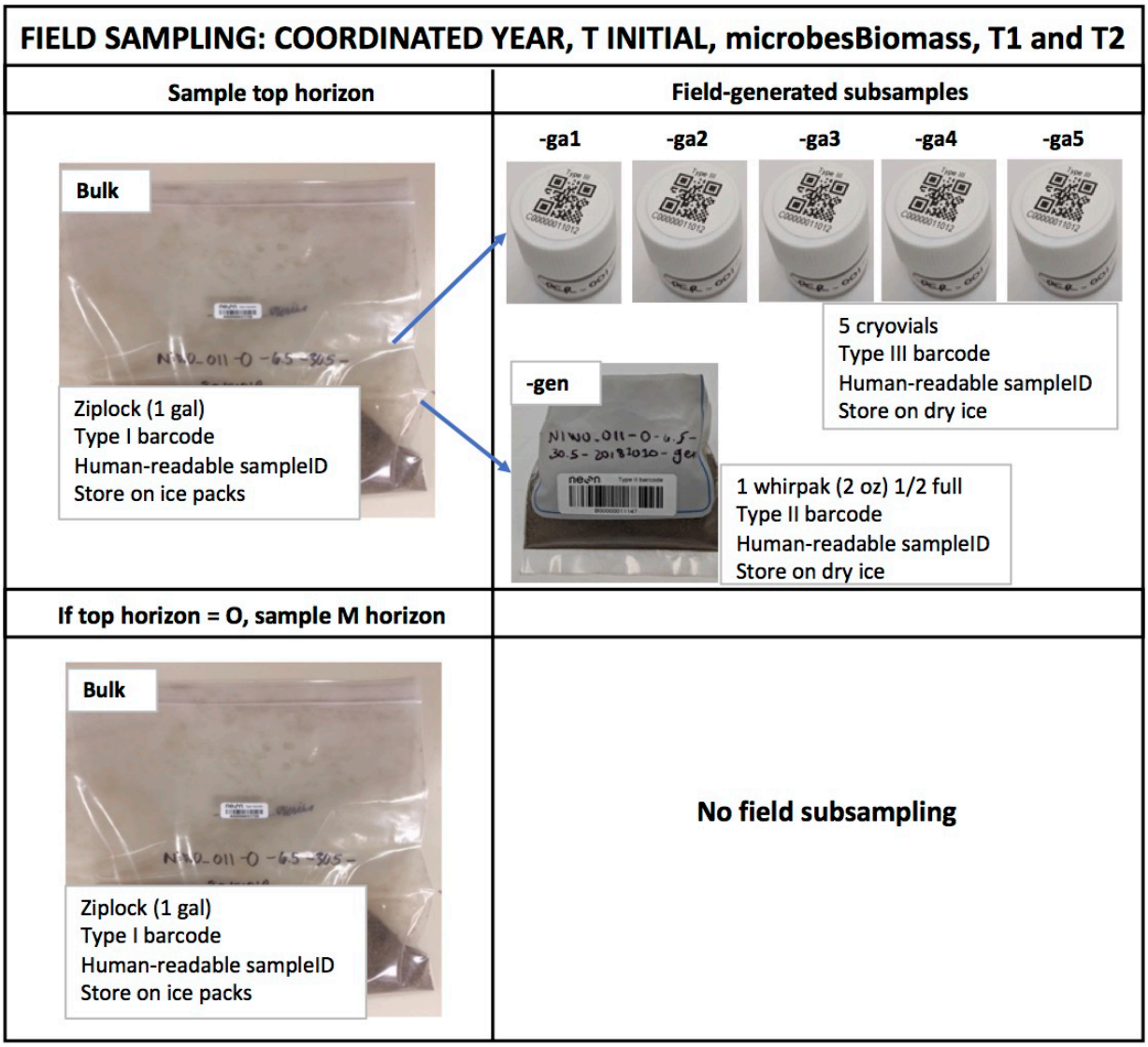

Figure 23. Field-generated samples during a Coordinated year for boutType= microbesBiomass, sampleTIming= T1 or T2.

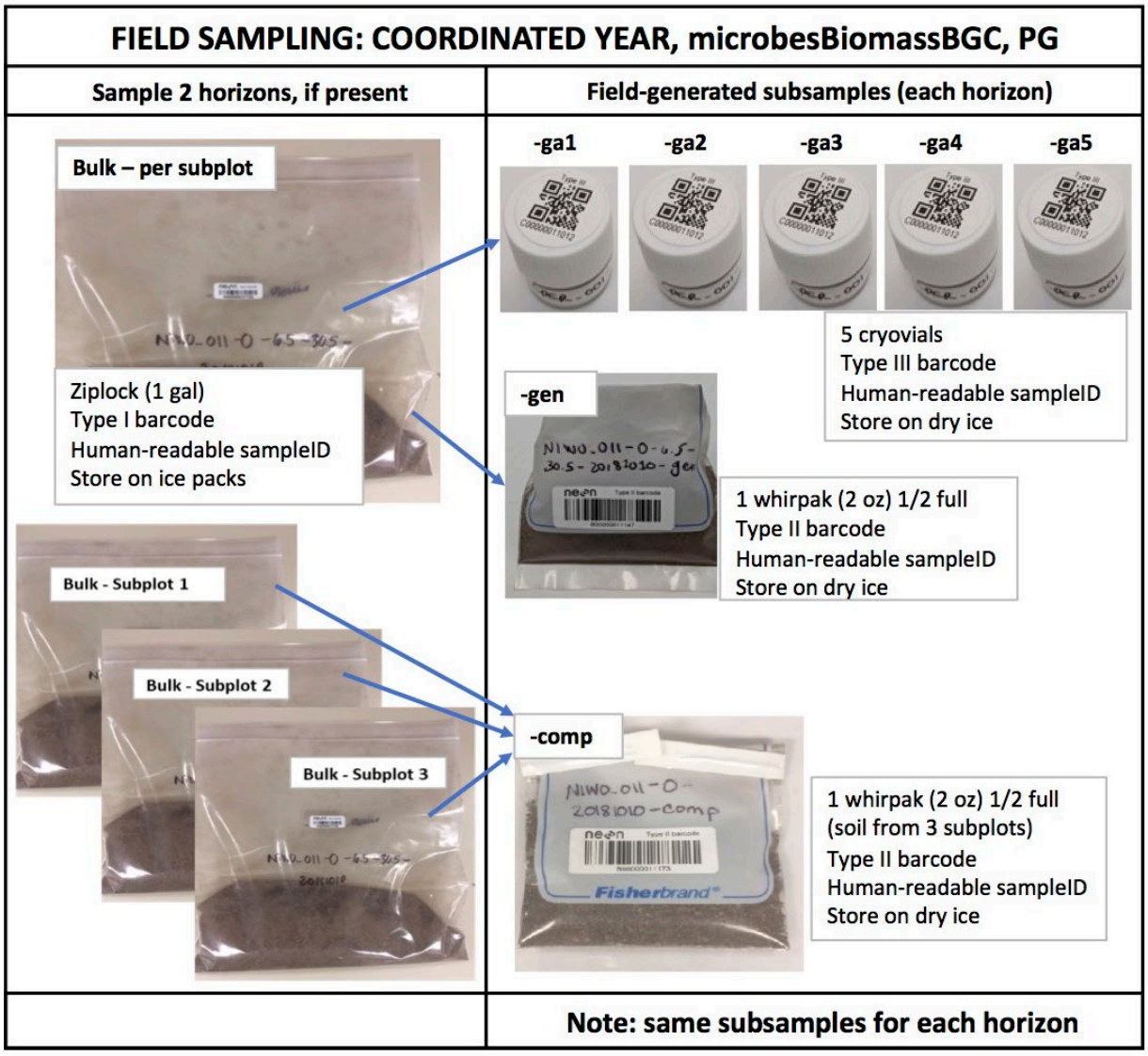

Figure 24. Field-generated samples during a Coordinated year for boutType= microbesBiomassBGC, sampleTiming = peak greenness.

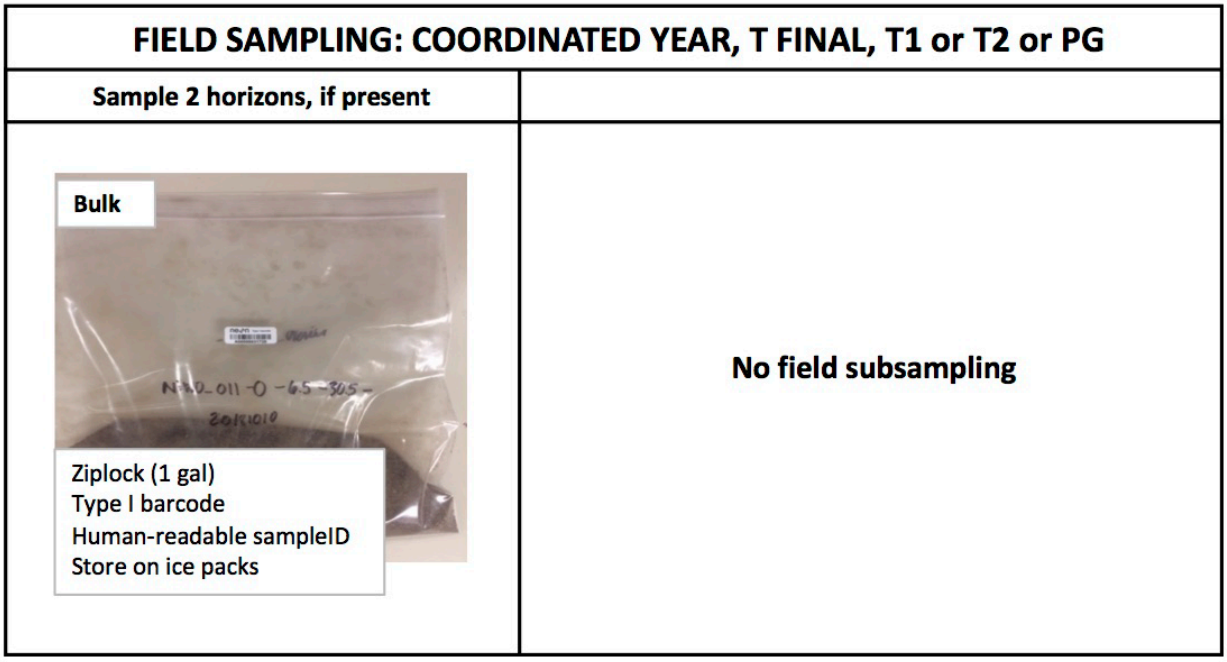

Figure 25. Field-generated samples during a Coordinated year for Tfinal sampling, boutType= fieldOnly, any time of year.

QUICK GUIDE TO SOIL COLLECTION DURING A MICROBES BOUT

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

STEP 1 – Obtain dry ice. If needed, cold soak coolers before going into field.

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.

STEP 5 – Measure litter layer.

STEP 6 – If organic horizon is present, collect with clean "brownie cutter", 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. Sterilly remove rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris. Fill 1 pre-labeled whirl-pak (2 oz.) ~1/2-way and 5 pre-labeled cryo vials ¾ of the way. Complete sample labels, close whirl-paks (labels clearly visible), and store on dry ice. Record barcode labels.
>
> **STEP 6b –** For peak green bouts, sample for metagenomics. Use sterile scoop to place soil in a 2 oz. whirl-pak. Add a scoop of homogenized soil at next X,Y location within the same plot - horizon combination, storing on wet ice between X,Y locations. When all X, Y locations have been added, close whirl-pak(s) (labels clearly visible), and store on dry ice. Scan barcodes.
>
> **STEP 6c** –Ensure that at least 25g of homogenized organic soil or 50g 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, collect mineral horizon core(s) with approved coring device for your site. Follow steps **6a** – **6c** for microbial subsampling.

STEP 8 – Measure sample depth in brownie square or bore hole. Remember: For samples collected from the ground surface, **sampleTopDepth**= 0 cm.

STEP 9 – **Data QA**. Ensure all data have been recorded on datasheets and/or Fulcrum application. If using barcode labels, ensure all labels have been scanned to the correct sample ID's.

STEP 10 - Backfill boreholes in accordance with permit.

STEP 11 – Rinse equipment using deionized water and clean towels. Sterilize immediately before re-use.

QUICK GUIDE TO SOIL COLLECTION DURING A COORDINATED BOUT

STEP 1 – Prepare sampling equipment before going into field.

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.

STEP 4 - Measure litter layer.

STEP 5 – If present, collect organic horizon with "brownie cutter", hori-hori, or similar.

STEP 6 – 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, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris.

STEP 7 - Collect mineral horizon core(s) with incubation cylinder or similar-diameter coring device for your domain, place in bag and homogenize. With a sterilized, gloved hand, remove rocks, coarse roots. Insects, wood, moss, and other non-soil debris.

STEP 8 - Subsample for microbial analysis, archive, and metagenomics following **STEPS 6a** and **6b** of 'Microbes' Quick Guide.

STEP 9 – Ensure that at least 75g of homogenized organic soil or 160g mineral soil is available for analyses at the domain lab.

STEP 10 – Label bag/s. Store homogenized bags in cooler on ice packs and store all microbial samples on dry ice.

STEP 11 – Measure sample depth/s in brownie square or bore hole and record. Remember: For samples collected from the ground surface, **sampleTopDepth** = 0 cm.

STEP 12 – If conducting an N-transformations T initial bout, set up the incubated core. You will return to retrieve and extract this core in 2-4 weeks.

STEP 13 – **DATA QA**. Ensure all data have been recorded on datasheets and/or Fulcrum application. If using barcode labels, ensure all labels 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.

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, collectDate, etc).
- ☑ Obtain dry ice, and cold soak coolers if needed.
- ☑ Upload GPS coordinates for plots and review job ticket.
- ☑ Know any special permit requirements for the site.

At soil sample location: Check…

- ☑ Does a handheld GPS confirm that you are indeed at the correct plot?
- ☑ Is designated sampling area disturbed?
- ☑ Did you probe area within 0.5 m of X,Y coordinates to find a good sampling location?
- ☑ If a location was rejected, did you record why on the datasheet?
- ☑ Did you record metadata on datasheet and/or Fulcrum application (plotID, 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 touch a microbial sample.
- ☑ Wear clean gloves. Either change or clean gloves between samples.
- ☑ Measure soil temperature at each sample location.
- ☑ Measure depth and remove leaf litter before coring.
- ☑ Homogenize samples prior to field subsampling and ensure that rocks, coarse roots (> 2 mm diameter), insects, wood, moss, 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, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris out of organic soils.
- ☑ Transfer to labeled, barcoded scintillation vials. *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 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.
- ☑ If applicable, 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 estimates for the start and stop dates of sampling. Sampling occurs when soil activity theoretically increases from its annual minimum and continues until activity returns to its annual minimum, as described in Section 4.2. The dates are estimated from satellite MODIS-EVI phenology data averaged from 2005-2014 (Didan 2015), or from historic precipitation data for sites that are driven by precipitation regime (**Table 4**). Estimated dates 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 of this protocol.

Table 15. 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 15 – May 15 (24)	July 1 – Aug 31 (18)	Oct 8 – Nov 7 (24)
	BART	Apr 21 – May 20 (24)	June 1 – July 31 (18)	Sept 28 – Oct 28 (24)
02	SCBI	Mar 27 – Apr 26 (24)	July 1 – Aug 31 (18)	Oct 18 – Nov 17 (24)
	SERC	Mar 15 – Apr 15 (24)	July 1 – Aug 31 (18)	Oct 15 – Nov 15 (24)
	BLAN	Mar 10 – Apr 10 (24)	July 1 – Aug 31 (18)	Oct 15 – Nov 15 (24)
03	JERC	Mar 15 – Apr 15 (18)	July 15 – Sept 1 (14)	Oct 15 – Nov 15 (18)
	DSNY	Mar 2 – Apr 1 (18)	Aug 15 – Oct 15 (14)	Oct 18 – Nov 17 (18)
	OSBS	Mar 12 – Apr 11 (18)	July 1 – Sept 15 (14)	Oct 13 – Nov 12 (18)
04	GUAN	July 1 – Aug 1 (14)	Sept 1 – Nov 15 (14)	Dec 1 – Mar 1 (18)*
	LAJA	July 1 – Aug 1 (14)	Oct 15 – Nov 30 (14)	Dec 1 – Mar 1 (18)*
05	UNDE	May 6 – June 5 (24)	June 15 – Aug 31 (21)	Sept 13 – Oct 13 (24)
	TREE	Apr 15 – May 15 (24)	June 15 – Aug 31 (21)	Oct 1 – Oct 31 (24)
	STEI	Apr 15 – May 15 (24)	June 15 – Aug 31 (21)	Oct 1 – Oct 31 (24)
06	UKFS	Mar 17 – Apr 16 (24)	June 15 – Aug 31 (18)	Oct 15 – Nov 15 (24)
	KONZ	Apr 1 – May 1 (24)	June 15 – Aug 31 (18)	Oct 1 – Oct 31 (24)
	KONA	Apr 1 – May 1 (24)	June 15 – Aug 31 (18)	Sept 28 – Oct 28 (24)
07	ORNL	Mar 15 - Apr 15 (21)	May 1 – July 31 (18)	Oct 13 – Nov 12 (21)
	MLBS	Apr 21 – May 21 (24)	June 1 – Aug 31 (18)	Oct 8 – Nov 7 (24)
	GRSM	Apr 1 – May 1 (21)	June 1 – Aug 31 (18)	Oct 15 – Nov 15 (21)
08	TALL	Mar 17 – Apr 16 (21)	May 1 – July 31 (18)	Oct 28 – Nov 27 (21)
	DELA	Mar 15 – May 1 (21)*	June 1 – July 31 (18)	Oct 28 – Nov 27 (21)
	LENO	Mar 30 – May 1 (21)*	June 1 – July 31 (18)	Nov 2 – Dec 2 (21)

09	WOOD	May 1 – May 31 (24)	July 1 – Aug 31 (18)	Oct 1 – Nov 15 (24)
	DCFS	May 1 – May 31 (24)	July 1 – Aug 31 (18)	Oct 1 – Nov 1 (24)
	NOGP	Apr 15 – May 15 (24)	July 1 – Aug 31 (18)	Oct 1 – Nov 1 (24)
10	CPER	Apr 1 – May 1 (24)	May 15 – July 15 (18)	Nov 1 – Dec 1 (24)
	STER	Apr 1 – May 1 (24)	June 1 – July 31 (18)	Oct 15 – Nov 15 (24)
	RMNP	May 1 – May 31 (28)	June 15 – Aug 31 (21)	Sept 15 – Oct 15 (28)
11	CLBJ	Mar 2 – Apr 1 (18)	Apr 1 – May 15 (14)	Oct 23 – Nov 22 (18)
	OAES	Mar 1 – Apr 1 (18)	May 1 – June 30 (14)	Aug 1 – Aug 31 (18)
12	YELL	May 1 – May 31 (24)*	July 1 – Aug 31 (21)	Sept 8 – Oct 8 (24)
13	NIWO	May 21 – Jun 20 (28)*	July 1 – Aug 31 (21)	Aug 29 – Sept 28 (28)
	MOAB	Mar 1 – Apr 1 (18)	May 15 – July 31 (24)	Oct 15 – Nov 15 (24)
14	JORN	June 15 – July 15 (18)	Aug 1 – Sept 15 (21)	Oct 18 – Nov 17 (24)
	SRER	May 31 – July 15 (18)	Aug 1 – Sept 1 (21)	Oct 15 – Nov 27 (24)
15	ONAQ	Mar 1 – Apr 15 (18)	May 15 – July 15 (24)	Oct 8 – Nov 8 (24)
16	ABBY	Apr 15 – May 15 (21)	June 1 – July 31 (24)	Oct 8 – Nov 8 (24)
	WREF	Apr 26 – May 26 (21)	June 1 – July 31 (24)	Oct 1 – Oct 31 (24)
17	SJER	Oct 15 – Nov 15 (24)	Feb 15 – Apr 1 (18)	May 6 – June 5 (24)
	SOAP	Mar 15 – May 1 (24)	May 15 – July 15 (21)	Oct 15 – Nov 15 (24)
	TEAK	Apr 15 – May 15 (24)	July 1 – Aug 15 (21)	Oct 1 – Nov 1 (24)
18	TOOL	NA	July 1 – Aug 15 (28)	NA
	BARR	NA	July 1 – Aug 15 (28)	NA
19	HEAL	NA	June 15 – Aug 15 (21)	NA
	DEJU	NA	June 1 – July 31 (21)	NA
	BONA	NA	July 1 – Sept 30 (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 bout should be rescheduled if wet season rains occur within 72 hours of scheduled bout	Unpredictable transition from wet to dry season
D08	DELA	T1: If flooding prevents a scheduled sampling event, then the bout may be rescheduled through no later than May 31	Unpredictable spring flooding
	LENO		
D10 D13 D17	RMNP NIWO TEAK	T1: If sampling occurs late in the sampling window due to a persistent snowpack, incubation may last approximately 21 days instead of 28	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 fall under the CFR 301 – Domestic Quarantine Notices and are required 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 APHIS quarantine document for the most updated information on quarantine status.

Domain	Site	Quarantined Materials	Containment Action
03	OSBS	Soil and plant material	Refer to NEON Protocol and Procedure: Shipping Ecological Samples, Sensors and Equipment and the notes in the Shipping Application for details on sample shipment.
	DSNY		
	JERC		
04	GUAN		
	LAJA		
07	GRSM		
	ORNL		
11	CLBJ		
14	JORN		
20	PUUM		
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.

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, 4 subplots were rejected: 23 in GUAN_001, 39 in GUAN_004, and 21 and 41 in GUAN_005. It is recommended that: - GUAN_005 be replaced with a plot that has a minimum of 3 subplots that meet the soil volume criteria; - All sampling in plot GUAN_001 occur within subplots 21, 39, and 41;

	- All sampling in plot GUAN_004 occur within subplots 21, 23, and 41.
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 outlined in TOS SOP: Wetland Soil Sampling (RD[06]), e.g., using buried bags.

D.3 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 26** 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 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 lead to much more soil than needed, depending on the horizon thickness. If you find yourself with much more soil than needed and want to remove some of the soil before homogenizing and sub-sampling, do so by cutting the monolith lengthwise from top to bottom. This will ensure that 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. In general, litter depths will be 0 cm, unless there was visible, 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.
   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.
      1) 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).

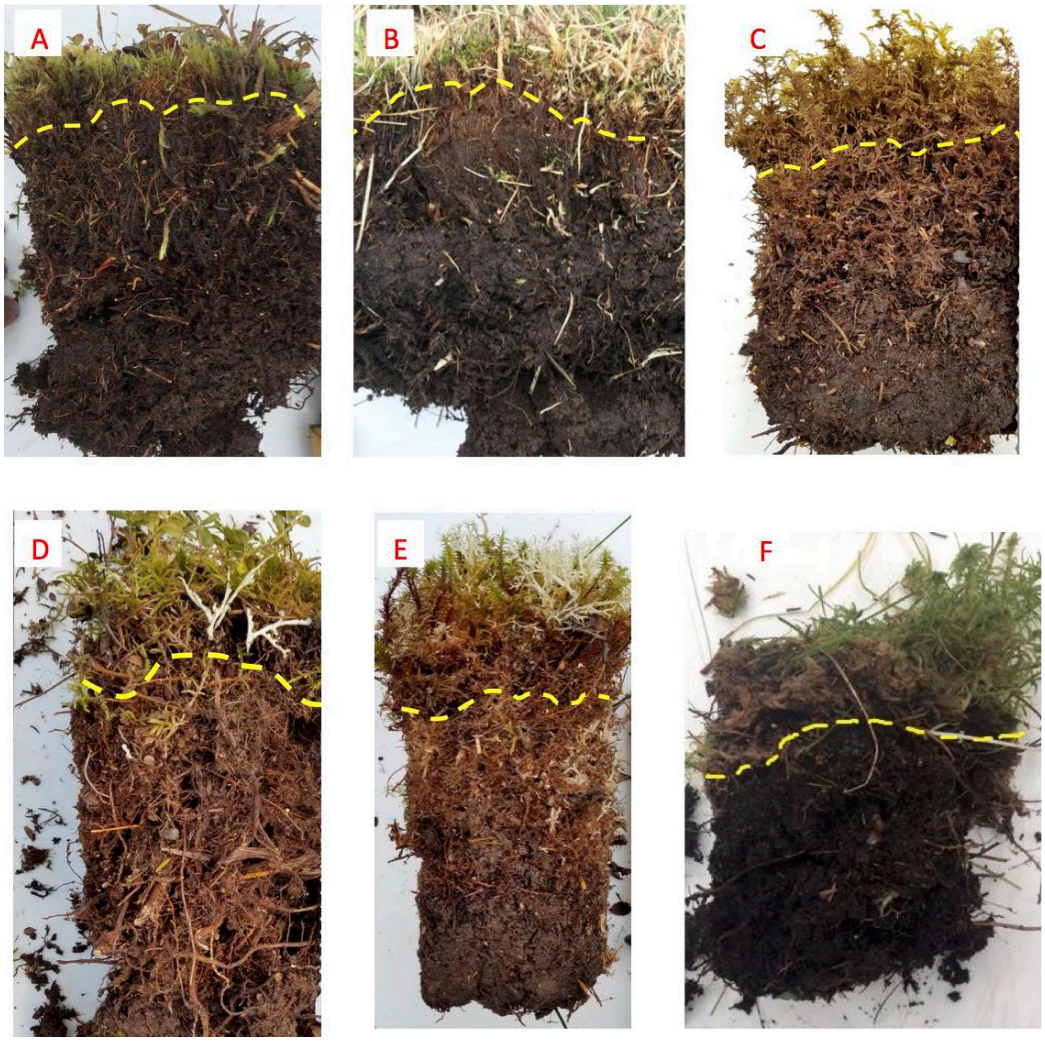

Figure 26. Images demonstrating the start of the soil surface (yellow dashed line) at TOOL (A), BARR (B), DEJU (C), HEAL (D), BONA (E), and TREE (F). Source: NEON Terrestrial Instrument System (TIS), in collaboration with NRCS.

D.4 Sites with Variable Seasonality Across Plots

Table 16. 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.5 Sites authorized to use the Wetland SOP

Domain	Sites

01	HARV
D03	DSNY, OSBS
05	TREE, UNDE, STEI
08	DELA, LENO
09	WOOD, NOGP, DCFS
18/19	HEAL, TOOL, BARR, BONA

D.6 Soil horizons from NRCS Initial Characterization

Table 17. 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	--	--	--

Domain	Site	# plots	# plots with O horizon	Median O horizon depth (cm)	Min O horizon depth (cm)	Max O horizon depth (cm)
10	STER	11	0	--	--	--
10	RMNP	14	14	5	2	11
11	CLBJ	11	0	--	--	--
11	OAES	15	0	--	--	--
13	NIWO	13	8	4.5	2	8
13	MOAB	11	0	--	--	--
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
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.7 Site-specific soil sampling devices

Table 18. Soil types and sampling devices for each site.

Domain	Site	Soil Type(s)	Sampling Device(s)
01	HARV	Soils mostly organic. Loamy and rocky mineral soils	AMS auger, part# 400.09 2 inch diameter
	BART		
02	SCBI	Rocky soils	AMS auger, part# 400.08, 2.25 inch diameter AMS hammer-head replaceable tip soil probe kit, part# 425.501, 1 inch diameter
	SERC		
	BLAN		
03	JERC	Relatively deep organic and mineral soils, few rocks	AMS auger 2 ¼ inch diameter
	DSNY		
	OSBS		
04	GUAN	Extremely shallow, rocky soil	AMS soil probe, part# 401.17 1 1/8 inch diameter
	LAJA	High-clay soil	Soil auger, 2 inch diameter

05	UNDE		AMS slide hammer corer, part# 404.50 2 inch diameter
	TREE		
	STEI		
06	UKFS	High-clay soil	AMS soil auger, part# 402.36 2 ¼ inch diameter. Alt: JMC Backsaver, handle (part# PN001) plus sample tube (part# PN012) 12 x 1 ¼ inch diameter
	KONZ	Very rocky, shallow soils	
	KONA		
07	ORNL	Variable	Forestry Suppliers Carbon Steel Auger Mud/Clay, 2 inch diameter Part# 78400
	MLBS		
	GRSM		
08	TALL	Sandy soils	AMS auger, part # 400.08 Maximo #110504 2 ¼ inch diameter
	DELA	Moist, sticky clay soil	AMS mud auger, part# 350.20 2 ¼ inch diameter
	LENO	Moist, sticky clay soil	
09	WOOD	Moist, wet, sticky clay soil	AMS auger, part# 400.54 2 ½ inch diameter; or AMS auger, part# 415.23 2 ¼ inch diameter
	DCFS	Moist, sticky clay soil	
	NOGP	Dry, rocky soil	
10	CPER		JMC auger, part# 072, 2 inch diameter or AMS auger, part# 402.36 2 ¼ inch diameter
	STER	High clay soil	
	RMNP	Rocky soil	
11	CLBJ	Sandy soils	AMS auger, part# 400.08 2 ¼ inch diameter
	OAES		
12	YELL		AMS Auger, part# 400.08, 2 1/4 inch diameter
13	NIWO	Rocky soil	see D10 entries
	MOAB	Sandy soil	AMS Auger, part# 400.08 2 ¼ inch diameter
14	JORN	Sandy soil	AMS Hex QP Sand Auger, part# 58536 2 ¼ inch diameter
	SRER	Sandy soil	AMS Hex QP Sand Auger, part# 58536 2 ¼ inch diameter
15	ONAQ	Rocky soil	AMS Auger, part# 400.06 3 ¼ inch diameter
16	ABBY	Organic and Mineral Soils	Forestry Suppliers Carbon Steel Auger Mud/Clay, 2 inch diameter Part# 78400
	WREF		
17	SJER		AMS auger, part# 400.08 2 ¼ inch diameter
	SOAP		
	TEAK		
18	TOOL	Gelisols: thick organic horizon, cryoturbation	Soil monoliths cut with a hori-hori 4 x 4 inch square template
	BARR		

19	HEAL		
	DEJU		
	BONA		
20	PUUM		Forestry Suppliers Carbon Steel Auger 2 inch diameter Part# 78401

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 27**):

- 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 Science.

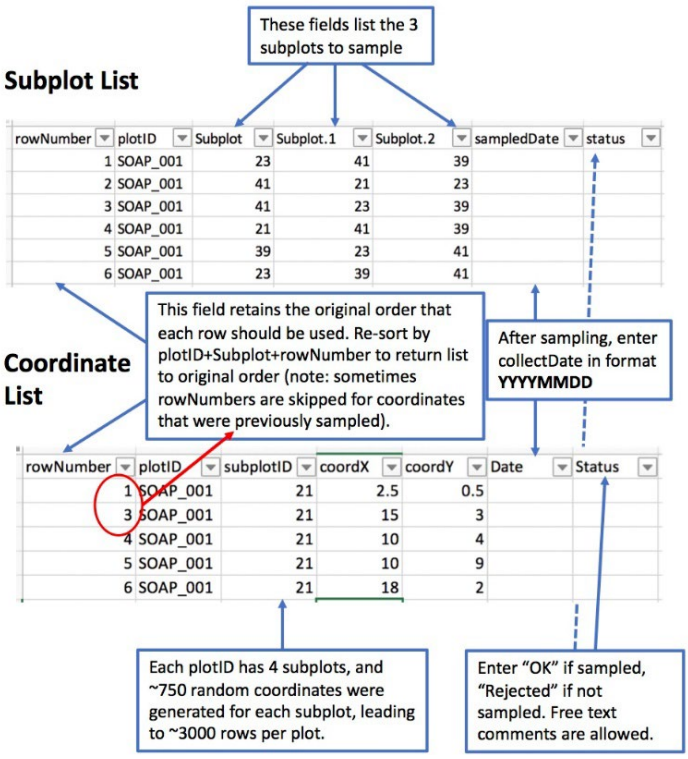

Figure 27. Anatomy of the soil subplot and coordinate lists.

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 your data entry and QA procedure.
2. Prior to commencing a bout, open up the **subplot** list.
    a. 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 "sampledate" 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 2:

	rowNum	plotID	Subplot	Subplot.1	Subplot.2	sampledD	status
2	1	NOGP_001	23	41	39	20160101	OK
3	2	NOGP_001	41	21	23		
4	3	NOGP_001	41	23	39		

3. Open up the **plot** list.
    a. Filter the spreadsheet for the target plot. For this example, the plot is NOGP_001.
        1) Next, filter the plot list for the target subplot, starting with the first subplot in the row. For this example, we'd start by filtering to subplot41.
        2) Working your way down the list, select the first 20 or so 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 always bring extras in case coordinates must be rejected in the field.
        3) 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=27, y=34.5.
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.

1	rowNum	plotID	subplotID	coordX	coordY	Date	Status
2266	3	NOGP_001	41	27	35.5	20160101	OK
2267	4	NOGP_001	41	20	32.5	20160101	OK
2268	5	NOGP_001	41	24	37	20160101	OK
2269	6	NOGP_001	41	27	34.5		
2270	7	NOGP_001	41	26	36.5		
2271	8	NOGP_001	41	38.5	37.5		
2272	9	NOGP_001	41	21	33.5		
2273	10	NOGP_001	41	32	38.5		

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, please add freeform comments in the Status field as to why location was rejected. Ex: 'presence of large boulder'; 'standing water >50cm in subplot 23'
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: 'location disturbed by burrowing animals'

Important Reminders

- Always record the exact coordinates provided in the list: do not shift coordinates for any reason. 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 plot list.
- Do not modify any of the other columns in the subplot or plot lists. Only enter information into the "Date" and "Status" fields. If any other fields are accidentally overwritten, contact Science to make corrections.
- 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 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 19. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
Cabela's IK270217; REI 895022	N	GPS receiver, recreational accuracy, e.g. Garmin Etrex20x	Navigate to sampling location	1	N
Ben Meadows Forestry Suppliers 122731; 40108; 39943	N	Measuring tape, minimum 50 m	Locate coordinates for soil sampling locations	2	N
Forestry Suppliers 89158	N	Digital soil temperature probe, ±1° accuracy, ability to calibrate	Measure soil temperature	2	N
	N	Glass beaker, 1000 mL	Calibrate soil temperature probes	1	N
Hardware store, any kind	N	Sand	Calibrate soil temperature probes	1 L	N
Shipped from HQ CalVal, contact if need replacement	Y	NIST traceable thermometer	Calibrate soil temperature probes	1	N
	N	Cooler	Keep perishable samples chilled in field	2	N
VWR 15715152	N	Ice packs, -20° C	Chill perishable samples in field	16 (+)	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
	N	Deionized water	Rinse soil from equipment	2 liters	N
Paragon Spring Company 'pig-tail stakes'	N	Metal stake, not painted, stainless steel, min 30 cm length	Probe soil depth, find suitable sampling location, remove soil from coring device	1	N
	N	Survey marking flag, pin flag, PVC or fiberglass stake	Flag soil sampling location	3	N
Forestry Supplier 91567	Y	Laser Rangefinder, 0.3m accuracy	Locate X,Y coordinates in very steep plots	1	N
Grainger 5B317	N	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	1	N
Compass Tools 703512; Forestry Suppliers 90998	Y	Foliage filter	Use with laser rangefinder in dense vegetation	1	N
Consumable Items					
	Y	Weatherproof, adhesive barcode labels, Type I	Label homogenized sample bag with barcode-readable labels	1 sheet	N
Grainger 5NHH1; Amazon B00006IBUV	Y	Avery weatherproof adhesive labels, 2 5/8" x 1"	Label homogenized sample bag with human-readable labels	30-80	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
ULINE S-21339	Y	Warning pictogram label for samples containing *Toxicodendron spp*	Alert handlers to use care when processing sample	1 roll	N
Ben Meadows 010510-1; Forestry Suppliers 49247	N	All weather copy paper	Print datasheets		N
	N	Permanent marker, fine tip	Label sample containers	3	N
	N	Batteries, AA and coin types	Spare batteries for GPS receiver and digital thermometer		N
	N	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
	N	Paper towels or reusable cleaning cloth	Remove debris from soil sampling equipment	1 box or 2 cloths	N
	N	Trash bag	Dispose of consumables	2	N
	N	Field tablet	Record data		N
RD[05]	Y	Field datasheet	Backup to record data		N
	Y	X,Y coordinates of sampling locations within each plot	Soil sampling locations	1	N
	N	Laboratory soap, e.g. Alconox, Contrex	General cleaning of reusable field and laboratory equipment	1	N

Table 20. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
EG07610000	Y	Organic horizon cutter template (brownie frame)	Remove organic horizon	1	N
Amazon 41N620; Grainger 41N620	N	Ruler, minimum 30 cm	Measure soil sample top and bottom depth	1	N
Varies by Domain	Y	Soil corer, 2 ± 0.5" diameter, minimum 30 cm long	Collect soil core	1	N
Forestry Suppliers 33489	Y	Soil knife (hori-hori)	Separate soil horizons, subsampling, etc.	1	N
Forestry Suppliers 93012; 93013	N	Spring scale (optional), 300g max	Weigh soil samples (when using mass sampling approach)	1	N
	N	Trowel	Remove soil core	1	N
	N	Strap wrench	Open stuck core barrels, only needed for certain coring devices	1	N
	N	Toothbrush or bottle brush	Clean soil from core barrel and threads after sampling	1	N
	N	Stainless steel tweezers, fine-tipped	Removing non-soil material from field subsamples	1	N
	N	Tablespoon or coffee scoop, sterilizable	Generate microbial subsamples	1	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
	N	Glass bottle, 1 L	Hold extra sterilized ethanol, if prepared in house	1	Y
Consumable Items					
Fisher 14955182, Nasco B01064	Y	Whirl-Pak bags, sterile, write-on, 2 oz	Contain soil for genetic analysis	30-40 per horizon	N
	Y	Cryogenic, adhesive barcode labels, Type II	Label microbial analysis and metagenomics samples with barcode-readable labels	30-40 per horizon	N
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	N
Fisher 13-709-140; Amazon W985100	Y	5.0 mL CryoElite tissue vials, sterile, Wheaton	Contain soil for microbial archive	150 per horizon	N
Fisher 13-709-141 to 13-709-146	Y	CryoFILE tissue vial storage boxes, various colors, Wheaton	Store microbial archive vials	6 boxes per horizon	N
	Y	Cryogenic, adhesive barcode labels, Type III	Label microbial archive vials with barcode-readable labels	150 per horizon	N
Fisher 15-930-E	Y	Cryogenic, adhesive labels, cut horizontally in thirds	Label microbial archive sample containers with human-readable labels	1-2 sheets	N
Domain and Vendor dependent	N	Dry ice, pelletized	Freeze soil microbial subsamples	20 pounds	Y

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Grainger 5CNK5; 8YAT5	N	Resealable plastic bag, 1 gal	Collect homogenized soils	2 boxes	N
VWR TWTX3044P	N	Sterile, 70% Ethanol pre-wetted wipes	Sterilize sampling equipment and gloves	10-20	N
SOS Clean Room TX3215	Y	Sterile, dry wipers	Alternative for sterilizing sampling equipment and gloves	10-20	N
	N	70% Ethanol made up in sterile, deionized water	Alternative for sterilizing sampling equipment and gloves. Required if using Sterile, dry wipers	1 bottle	Y
FisherSci SVGPL10RC; 0974103	Y	0.2 micron filtration unit, sterile, polyethersulfone	Alternative for creating sterilized ethanol	1	N
	N	Bleach, standard grade	Sterilizing containers for holding in-house made sterile water or ethanol	1 bottle	Y

Table 21. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
	N	Hammer or rubber mallet	Insert cylinders into soil	1	N
Headquarters, email when resupply needed	N	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
	N	Loose-fitting caps for each cylinder (2.25” or 2.5” diameter)	Protect cylinder openings from debris and water	1/sampling location	N
	N	Wooden block (approx. 2” x 4” x 10”)	Use with mallet to pound cylinder into soil	2	N
	N	Monument stake installation strike plate	Use with mallet to pound cylinder into soil	1	N
	N	Extruder – long flathead screwdriver, soil knife, chaining pin, etc	Extrude soil sample from cylinder in clayey conditions	1	N
Fisher 1523911	N	Plastic tray	Separate soil core horizons in field	2	N
Forestry Suppliers 33489	N	Soil knife (hori-hori)	Separate organic and mineral horizons	1	N
SpeeCo 161165TSC	N	T-Fence Post Puller (or similar)	Remove cylinder in high-clay soil	1	N
	N	1.0 chain	Use with post puller to remove cylinder in high-clay soil	1 foot	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
	N	4" x 3/8" hitch pin	Use with post puller to remove cylinder in high-clay soil	1	N
Consumable items					
	N	Plant wire	Use to secure caps to cylinders	30 feet	N
	N	8” Zip ties	Use to secure caps to cylinders	1/sampling location	N
Grainger 5CNK5; 8YAT5	N	Resealable plastic bag, 1 gal	Contain soil samples	30 per horizon	N
Grainger 9WKP4; Forestry Suppliers 57880	N	Orange flagging tape	Flag location of incubated soil core	1 roll	N
	N	Pin flag, survey marking flag, PVC or fiberglass stake	Flag location of incubated soil core (if permitted)	50	N

Table 22. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
Fisher 01910200; Mettler Toledo 11144914	N	Balance, 0.01 g accuracy	Weigh fresh and dry soil moisture samples	1	N
	N	Spatula or scoopula	Transfer soil to weigh boat	1	N
Fisher 1523911	N	Plastic tray	Transport soil samples to and from oven	4	N
Consumable items					
Fisher 08732101	N	Aluminum foil weigh boat	Hold soil while drying	1 box	N
	N	Nitrile gloves, powderless	Prevent contamination of soil samples during handling	1 box	N
Thomas 1234Z63; 2904F24	N	Lint-free wipes	Cleaning work area and equipment	1 box	N
	N	Ethanol, 70%	Clean work area	1 bottle	Y
Resources					
RD[05]	Y	Lab datasheet	Backup to record data	---	N

Table 23. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
Fisher 04-881G	N	Sieve, 2 mm	Sorting soil particles to 2mm	1-2	N
Fisher 04 884 1AA	N	Sieve, 4 mm	Pre-sieving for high-clay, difficult to sieve soils	1-2	N
	N	Spoon, spatula or scoopula	Transfer soil between containers	2	N
Consumable items					
U-LINE S-7630	N	Paper bag, #8	Hold soil subsamples for air-drying	30-60	N
	N	Deionized water	Clean work surfaces and equipment	1 bottle	N
	N	Ethanol, 70%	Prepare work area		
Thomas 1234Z63; 2904F24	N	Low lint wipe	Clean and dry work area	1 box	N
	N	Nitrile gloves, powderless	Prevent contamination of soil samples during handling	1 box	N
Fisher 0333723C; Thomas 9718J20	Y	Scintillation vials, plastic, 20 mL	Store microbial biomass samples	30-60	N
	Y	Cryogenic, adhesive barcode labels, Type II	Label microbial biomass sample containers with scanable barcode	30-60	N
Fisher 15-930-E	Y	Cryogenic adhesive labels	Label microbial biomass sample containers with human-readable labels	1 sheet	N
Fisher 033377	Y	Scintillation vials, glass, 20 mL	Store BGC analysis samples	30-60	N
Fisher 02911825	Y	250 mL wide-mouth glass jars	Store BGC archive samples	30-60	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
	Y	Heat-resistant, adhesive barcode labels, Type I	Label BGC analysis and archive samples with scanable barcode	60-120	N
Avery 5520	N	2.6” x 1” address labels	Label BGC analysis and archive samples with human-readable labels	60-120	N
ULINE S-21339	Y	Warning pictogram label for samples containing *Toxicodendron spp*	Alert handlers to use care when processing sample	1 roll	N
Resources					
RD[05]	Y	Lab datasheet	Backup to record data	---	N

Table 24. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
Fisher 13636AB150B	Y	pH meter, 0.01 unit accuracy	Reading pH value of samples	1	N
Fisher 02112300; Thomas 0910200	N	Balance, 0.01 g accuracy	Weigh soil samples	1	N
Fisher 10020F	N	Glass volumetric flask, 2L	Preparing calcium chloride solution for pH analysis	1	N
	N	Graduated cylinder, 50-100 mL capacity	Measure volumes of solutions for pH samples	2	N
	N	Spoon, spatula or scoopula	Transfer soil subsamples	2	N
Fisher 1451386	N	Stir rod	Mix pH samples	1-2	N
Consumable Items					
Fisher 191301597B; 191301597C; 191301597D; 191301597E	N	Powderless gloves (s, m, l, xl)	Prevent sample contamination during handling, prevent bodily injury from hazardous chemicals	1 box	N
U-LINE S-7630	N	Paper bag, #8	Hold soil subsamples for air-drying	50	N
Fisher AC423520250	Y	Calcium Chloride Dihydrate, $CaCl_2 \cdot 2H_20$	pH analysis	2.94 g	N

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Fisher 0340910E	N	Deionized water wash squeeze bottle	Rinse equipment and pH electrode	1 bottle	N
Fisher SA49	N	Hydrogen Chloride, HCl, 1N	Adjusting pH of $CaCl_2$ when solution is too basic	1 ml	Y
Fisher C88500	Y	Calcium Hydroxide, $Ca(OH)_2$	Adjusting pH of $CaCl_2$ when solution is too acidic	1 ml	Y
	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
	N	50-100 mL containers	pH analysis	50 (+)	N
Thomas 2904F24; 1234Z63	N	Low lint wipe	Clean and dry work surfaces	1 box	N
Resources					
RD[05]	Y	Lab datasheet	Backup to record data	---	N

Table 25. 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.

Item Number	Exact Brand	Description	Purpose	Quantity	Special Handling
Durable Items					
Fisher 02112300; Thomas 0910200	N	Balance, 0.01 g accuracy	Weigh soil samples	1	N
Fisher 10020F	N	Volumetric flask, 1 L	Prepare 2M KCl solution for small batch of samples	1	N
Fisher 2319-0050	N	Carboy (20 L), Nalgene brand or similar	Prepare and store 2M KCl solution for large batch of samples	1	N
Fisher FB966C	Y	Ceramic Buchner funnel	Pre-leach GF/A filters	1	N
Fisher S15464	N	Side-arm flask, 500 mL	Pre-leach GF/A filters	1	N
Amazon, various	N	#8 Stopper, 1 hole, 3/8”	Pre-leach GF/A filters	1	N
Home Depot, various	N	Clear vinyl tubing, 3/8” ID, 1/2”OD	Pre-leach GF/A filters	2-3 ft	N
	N	Spoon, spatula or scoopula	Transfer soil between containers	2	N
Fisher 0300742	N	Graduated cylinder (100-250 ml)	Measure aliquot of KCl	1	N
Thomas 4618N60	Y	Reusable filtration units	Filter samples and collect filtrate	4	N
Thomas 5117G80	N	Filter forceps	Handle GF/A filters	1	N
Fisher SK-O330	Y	Global Equipment Shaker table	Shake extracts	1	N
RD[12]	Y	Manifold. See assembly document	Filter samples	1	N
	N	Vacuum pump	Filter samples	1	N

	N	Large beaker (at least 500 ml)	Collect discarded KCl filtrate	1	N
Rubbermaid #2951	N	Plastic dishpan, 3-gallon capacity	Wash filtering equipment	2	N
Consumable Items					
VWR JT4001-1	Y	JT Baker Potassium Chloride (KCl), Ultrapure Bioreagent, Item # 4001-01	Extract NH_4^+ and NO_3^- from soil with low NO_2^- and NO_3^- contamination	several kgs	N
Thomas 6186M89 Fisher 13-711-56	Y	Screw-cap polyethylene specimen cups and lids, sterile, 120 ml capacity	Extract NH_4^+ and NO_3^- from soil	33-75	N
Fisher LC267405	Y	Ultra-pure Type I deionized water, LabChem brand	Prepare 2M KCl, rinse filtering equipment	1-2 20L carboys	N
Fisher 0333723C; Thomas 9718J20	Y	Plastic scintillation vials with caps, 20 mL	Store filtered soil extracts for freezing and shipment	33-75	N
Fisher 191301597B; 191301597C; 191301597D; 191301597E	N	Powderless gloves (s, m, l, xl)	Prevent contamination of soil samples	1 box	N
Fisher 0987414A	Y	Glass fiber filters, 47 mm diameter, GF/A type	Filter samples	1 box	N
Grainger 5CNK5; 8YAT5	N	Resealable plastic bag, 1 gallon	Organize scint vials containing sample extracts	1 box	N
Fisher 15-930-E	Y	Cryogenic adhesive labels	Label vials with human-readable labels	1 sheet	N
	Y	Cryogenic, adhesive barcode labels, Type II	Label samples with scannable barcodes	1 sheet	N

Resources					
RD[05]	Y	Lab datasheet	Backup to record data	N	