TOS PROTOCOL AND PROCEDURE: 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
(Continued on next page)			

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		• 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 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

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1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling and domain lab processing of soil samples 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 associated with NEON Soil Biogeochemical and Microbial Sampling

Measurement	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 5 yrs, 3x per year
16S/ITS rRNA qPCR	Measure microbial diversity, community composition, and microbial/fungal biomass	Every yr, 3x per year, core 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*
pH, moisture, temperature	Give context for biogeochemical and microbial measurements	Every soil sampling event

**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.

1.3 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]).

1.4 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 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	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry
AD[06]	NEON.DOC.000908	NEON Science Design for Terrestrial Microbial Ecology
AD[07]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON 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

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

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.

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.

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

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.

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.

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.

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.

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), as well as the Standard Soil Methods for Long-Term Ecological Research (Robertson et al., 1999). 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.

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 will not be separated (e.g., mineral sub-horizons A and B). Where possible, NEON will sample 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 will be employed, following consultation with NEON Science. A list of site-specific coring devices is available in Appendix E.

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 NEON data products NEON.DP1.00096, Soil physical properties (Megapit) and NEON.DP1.00097, Soil 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 products NEON.DP1.10008, Soil chemical properties (Distributed initial characterization) and NEON.DP1.10047, Soil physical 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 should note the impediment in the data entry application and/or field data sheet and seek a new location as close as possible to that of the predetermined sampling location. In some cases, an alternative 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 C. Subsamples of these soils are also analyzed for pH and moisture at the domain support facility; 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 SOP A, 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 support facility. During the peak greenness bout, 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 laboratory for processing. Processing of “initial” and “final” cores involves separating the organic and mineral horizons for analysis, removing rocks and roots, 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 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 these sampling procedures. To collect and process samples properly, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any issues associated with implementing this protocol.

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

Quality assurance will be performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[07]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

The timing, temporal frequency, and extent of soil sampling constitute "the science design" (see (AD [06]) and (AD [07]), 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 to enable deriving relationships amongst the physical, chemical and biological data. At the different NEON sites, sampling frequency may vary depending on climatic factors, such as length of the growing season. All of the soil analyses described individually are linked temporally, and these temporal linkages are described below. The timing of sample collection and types of samples collected during any particular bout are outlined in Table 2.

Microbial 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. 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 D. When sampling for soil BGC, soil for microbial

analyses is collected concurrently; soil for microbial analyses 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.

Table 2. Sampling requirements for different types of bouts.

	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	microbesBiomassBGC	microbes Biomass	fieldOnly
Field-generated Samples	Bulk -gen -gaX	Bulk -gen -gaX -comp*	Bulk -gen -gaX	Bulk -gen -gaX	Bulk -gen -gaX -comp*	Bulk -gen -gaX	Bulk
Lab-generated Samples	---	-comp*	---	-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.

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

Nitrogen Transformations and Microbial Biomass. Every 5 years, soil measurements of microbial biomass and N transformations 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 a also be analyzed for microbial genetic analysis and genetic archiving, microbial metagenomics, microbial biomass, N transformations, soil pH, and soil moisture.

Coordinated Plant-Soil Biogeochemical Measurements. Periodically, a suite of measurements are conducted to characterize plant and soil biogeochemical dynamics. Sampling for soil microbes of all types, microbial biomass, N transformations, and soil BGC should be completed during a coordinated year. As well, co-execution of all of the following protocols at a given site and in the same year is a high priority. The linked measurements include:

- Soil BGC, N transformations, and microbial biomass sampling (this protocol)
- Biogeochemistry component of TOS protocol: Litterfall and Fine Woody Debris (RD[07])
- TOS Protocol: Plant Belowground Biomass Sampling (RD[08])
- TOS Protocol: 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 SharePoint site.

4.2 Criteria for Determining Onset and Cessation of Sampling

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. As long as sampling does not commence prior to the sampling windows provided in Appendix D, a bout may be scheduled. However, it is recommended that domain staff designate a 14-day time period for sampling to allow for unanticipated delays that may push sampling outside of the designated window. This allows for schedule conflicts, weather, and other contingencies to occur without jeopardizing the timing of the sampling bout.

Completeness of a Sampling Bout. During a non-coordinated, off-year sampling bout (e.g. 'microbes,' Table 1), 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 issue tracking software 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 is intended to 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 1). 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.

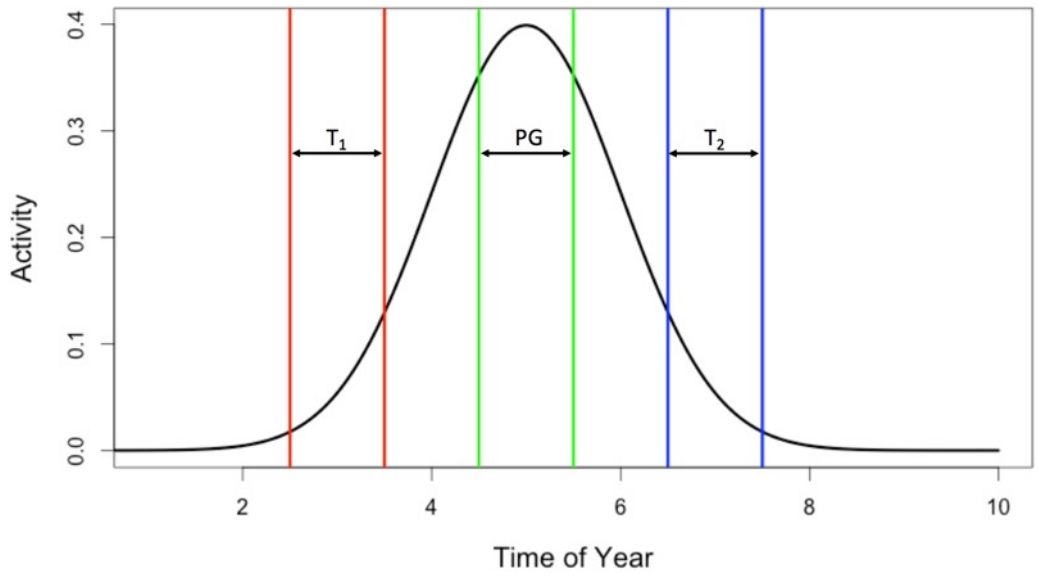


Figure 1. Generalized Timing of Soil Sampling. T1 captures the transition from dormancy/low activity to peak activity (PG), while T2 captures the transition from peak activity to dormancy/low activity. The time of year for each sampling period will vary by local geographic and climatic conditions.

Table 3. 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 receding
	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 • Ground freezing • Snow accumulating
	Wet-dry transition	3, 4, 11, 14, 17, 20	• Initiation of dry season
	Dry-wet transition	8, 16	• Initiation of wet season

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. High-latitude domains (Domains 18 and 19) only sample during the peak greenness period.

4.3 Timing for Laboratory Processing and Analysis

Table 4. Storage conditions and holding times for soil samples

Sample Type	Field Storage	Post-processing Lab Storage	Holding Time
Microbial Genetic Analysis (-gen), Genetic Archive (-gaX), Metagenomics (-comp)	Cooler with dry ice	Ultra-low temp freezer -80°C	Ship to contracted facilities as scheduled
N transformations (-kcl)	Cooler with ice packs	Refrigerator 4°C	Begin KCl extractions within **24 hours**
Microbial Biomass (-bm)	Cooler with ice packs	Ultra-low temp freezer -80°C	Process and freeze within **24 hrs** Ship to contracted facility within **14 days**
pH, moisture, soil BGC (-cn and -ba)	Cooler with ice packs	Refrigerator 4°C	Begin processing within **48 hours** (or Monday morning if collected Friday; max 72 hrs)

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 24 h 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 24 h, 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 24 hours 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 issue tracking software in order to discuss possible rescheduling of the sampling bout.

4.4 Sampling Timing Contingencies

Table 5. Contingency decisions for all soil measurements.

Delay/Situation	Action	Outcome for Data Products
Inability to finish sample bout	Communicate to staff scientists via problem ticket	Dataset may be incomplete or sampling bout delayed/redone. Latter may result in delay of data products delivery.
Partial completion of sample bout.	Communicate to staff scientists via problem ticket	Dataset may be incomplete or sampling bout redone. Latter may result in delay of data products delivery.
Delay in start of sampling bout after end of sampling window.	Communicate to staff scientists via problem ticket for further instruction.	Bout may be cancelled if it extends into a different sampling window; no data generated. If bout is rescheduled, samples may reflect different conditions.
Sampling is scheduled, but soil freezes.	Do not attempt to collect soils. Communicate to staff scientists via problem 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 the subplot area where soil sampling is to occur.	If the site is authorized in the Wetland SOP, use that protocol to conduct sampling. If not, contact staff scientists for further direction	Sampling methods will differ for affected locations.
There is standing water > 20 inches (50 cm) deep within the area where soil sampling is to occur.	Do not attempt to collect soils. Communicate to staff scientists via problem ticket for further instruction.	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 staff scientists via problem 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 data entry application and via a problem ticket	Data products not delivered for that bout.

4.5 Criteria for Reallocation of Sampling Within a Site

Soil sampling will occur on the schedule described above at **10 plots per site** and **3 locations per plot**. Ideally, sampling will occur at these plots for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project. However, circumstances may arise that require sampling within a site to be shifted from one particular plot to another. In general, sampling is considered to be compromised when sampling at a plot becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field staff to Science.

There are two main pathways by which sampling can be compromised. Sampling plots can become inappropriately suited to answer meaningful biological questions (e.g., landslide removes all topsoil from a steeply sloped plot). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

For soil sampling at sites that conduct 3 sampling bouts per year, a given plot must be sampled for at least two of the expected bouts in a year, and one of the completed bouts must be peak greenness. For soil sampling at sites with fewer than 3 sampling bouts per year, the peak greenness bout must be completed. Plots that fail to meet this criterion for 2 years in a row should be considered compromised and NEON Science will work to re-assign sampling to different plots.

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 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 7 CFR Part 301 Domestic Quarantine Notices of the Plant Protection Act (7 U.S.C. 7756). The NEON CLA sharepoint site provides instructions for preparing soil samples for shipment and resources for determining the quarantine status of NEON sites. Additional site-specific quarantine instructions can be found in Appendix D. 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 SOP I, with additional guidance on the CLA sharepoint site.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 6. General equipment list - Field sampling for all types of soil bouts.

Supplier	Supplier Number	R/S	Description	Purpose	Quantity	Special Handling
Durable Items						
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy, e.g. Garmin Etrex20x	Navigate to sampling location	1	N
Ben Meadows Forestry Suppliers	122731 40108 39943	R	Measuring tape, minimum 50 m	Locate coordinates for soil sampling locations	2	N
Forestry Supplier	89158	R	Digital soil thermometer	Measure soil surface temperature	2	N
		R	Cooler	Keep perishable samples chilled in field	2	N
VWR	15715152	R	Ice packs, -20° C	Chill perishable samples in field	16 (+)	N
		R	Deionized water	Rinse soil from equipment	2 liters	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity	Special Handling
		R	Chaining pin	Probe soil depth, find suitable sampling location	1	N
		R	Survey marking flag, pin flag, PVC or fiberglass stake	Flag soil sampling location	3	N
Forestry Supplier	91567	S	Laser Rangefinder, 0.3m accuracy	Locate X,Y coordinates in very steep plots	1	N
Grainger	5B317	S	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	1	N
Compass Tools Forestry Supplier	703512 90998	S	Foliage filter	Use with laser rangefinder in dense vegetation	1	N
Consumable Items						
		R	Weatherproof, adhesive barcode labels, Type I	Label homogenized sample bag with barcode-readable labels	1 sheet	N
Grainger Arrow Amazon	5NHH1 5520 B00006IBUV	R	Weatherproof adhesive labels	Label homogenized sample bag with human-readable labels	30-80	N
Ben Meadows Forestry Supplier	010510-1 49247	R	All weather copy paper	Print datasheets		N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity	Special Handling
		R	Permanent marker, fine tip	Label sample containers	3	N
		R	Batteries, AA and coin types	Spare batteries for GPS receiver and digital thermometer		N
		R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
		R	Paper towels or reusable cleaning cloth	Remove debris from soil sampling equipment	1 box or 2 cloths	N
		S	Trash bag	Dispose of consumables	2	N
Resources						
		R	Field tablet	Record data		N
RD[05]		R	Field datasheet	Backup to record data		N
		R	X,Y coordinates of sampling locations within each plot	Soil sampling locations	1	N

R/S=Required/Suggested

Table 7. Additional equipment list - Field sampling for bouts that include soil microbes at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
	EG07610000	S	Organic horizon cutter template (brownie frame)	Remove organic horizon	O horizons present	1	N
Amazon Grainger	41N620 41N620	R	Ruler, minimum 30 cm	Measure soil sample top and bottom depth	All	1	N
		R	Soil corer, 2 ± 0.5” diameter, minimum 30 cm long	Collect soil core	All	1	N
Ben Meadows Forestry Supplier	139303 33487	S	Soil knife (hori-hori)	Separate soil horizons, subsampling, etc.	All	1	N
Forestry Supplier	93012 93013	S	Spring scale (optional), 300g max	Weigh soil samples	For mass sampling approach	1	N
		S	Trowel	Remove soil core	All	1	N
		S	Strap wrench	Open stuck core barrels, only needed for certain coring devices	If required for coring device	1	N
		S	Toothbrush or bottle brush	Clean soil from core barrel and threads after sampling	All	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		R	Tablespoon or coffee scoop, sterilizable	Generate microbial subsamples	All	1	N
		Consumable items					
Fisher	14955182	R	Whirl-Pak bags, 2 oz	Contain soil for genetic analysis	All	30-40 per horizon	N
		R	Cryogenic, adhesive barcode labels, Type II	Label microbial analysis and metagenomics samples with barcode-readable labels	All microbe bouts	30-40 per horizon	N
Fisher	15-930-E	R	Cryogenic, adhesive labels	Label Whirl-pak bags for microbial analysis and metagenomics with human-readable labels	All microbe bouts	30-40 per horizon	N
Fisher Amazon	13-709-140 W985100	R	5.0 mL CryoElite tissue vials, sterile, Wheaton	Contain soil for microbial archive	All microbe bouts	150 per horizon	N
Fisher	13-709-141 to 13-709-146	R	CryoFILE tissue vial storage boxes, various colors, Wheaton	Store microbial archive vials	All microbe bouts	6 boxes per horizon	N
		R	Cryogenic, adhesive barcode labels, Type III	Label microbial archive vials with barcode-readable labels	All microbe bouts	150 per horizon	N
Fisher	15-930-E	R	Cryogenic, adhesive labels, cut horizontally in thirds	Label microbial archive sample containers with human-readable labels	All microbe bouts	1-2 sheets	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Domain dependent	Vendor dependent	R	Dry ice, pelletized	Freeze soil microbial subsamples	All	20 pounds	Y
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Collect homogenized soils	All	2 boxes	N
VWR	TWTX3044P	R	Sterile, 70% Ethanol pre-wetted wipes	Sterilize sampling equipment and gloves	All	10-20	N
SOS Clean Room	TX3215	S	Sterile, dry wipers	Alternative for sterilizing sampling equipment and gloves	All	10-20	N
		S	70% Ethanol made up in sterile, deionized water	Alternative for sterilizing sampling equipment and gloves. Required if using Sterile, dry wipers	All	1 bottle	Y

R/S=Required/Suggested

Table 8. Additional equipment list – Field sampling for bouts that include soil N transformations at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
		R	Hammer or rubber mallet	Insert cylinders into soil	1	N
Headquarters, email when resupply needed		R	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
		R	Loose-fitting caps for each cylinder (2.25” or 2.5” diameter).	Protect cylinder openings from debris and water	1/sampling location	N
		S	Wooden block (approx. 2” x 4” x 10”)	Use with mallet to pound cylinder into soil	2	N
		S	Monument stake installation strike plate	Use with mallet to pound cylinder into soil	1	N
		S	Extruder – long flathead screwdriver, soil knife, chaining pin, etc	Extrude soil sample from cylinder in clayey conditions	1	N
Fisher	1523911	S	Plastic tray	Separate soil core horizons in field	2	N
Ben Meadows Forestry Supplier	139303 33487	S	Soil knife (hori-hori)	Separate organic and mineral horizons	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
SpeeCo	161165TSC	S	T-Fence Post Puller (or similar)	Remove cylinder in high-clay soil	1	N
		S	1.0 chain	Use with post puller to remove cylinder in high-clay soil	1 foot	N
		S	4" x 3/8" hitch pin	Use with post puller to remove cylinder in high-clay soil	1	N
Consumable items						
		S	Plant wire	Use to secure caps to cylinders	30 feet	N
		S	8” Zip ties	Use to secure caps to cylinders	1/sampling location	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Contain soil samples	2 boxes	N
Grainger Forestry Supplier	9WKP4 57880	S	Orange flagging tape	Flag location of incubated soil core	1 roll	N
		S	Pin flag, survey marking flag, PVC or fiberglass stake	Flag location of incubated soil core (if permitted)	50	N

R/S=Required/Suggested

Table 9. Equipment list – Laboratory processing of soils for moisture content from one site.

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Thomas Fisher	1189J86 01910200	R	Balance, 0.01 g accuracy	Weigh fresh and dry soil moisture samples	1	N
		R	Spatula or scoopula	Transfer soil to weigh boat	1	N
Fisher	1523911	R	Plastic tray	Transport soil samples to and from oven	4	N
Consumable items						
Fisher	08732101	R	Aluminum foil weigh boat	Hold soil while drying	1 box	N
		R	Nitrile gloves, powderless	Prevent contamination of soil samples during handling	1 box	N
Thomas	1234Z63 2904F24	R	Lint-free wipes	Cleaning work area and equipment	1 box	N
		S	Ethanol, 70%	Clean work area	1 bottle	Y
Resources						
RD[05]		R	Lab datasheet	Backup to record data	---	N

R/S=Required/Suggested

Table 10. Equipment list – Soil sieving, air-drying, and subsampling for microbial biomass and soil BGC analysis and archive at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity *	Special Handling
Durable Items							
Fisher	04-881G	R	Sieve, 2 mm	Sorting soil particles to 2mm	All	1-2	N
Fisher	04 884 1AA	S	Sieve, 4 mm	Pre-sieving	High-clay, difficult to sieve soils	1-2	N
		R	Spatula or scoopula	Transfer soil between containers	All	2	N
Consumable items							
U-LINE	S-7630	R	Paper bag, #8	Hold soil subsamples for air-drying	All	30-60	N
		R	Deionized water	Clean work surfaces and equipment	All	1 bottle	N
		S	Ethanol, 70%	Prepare work area			
Thomas	1234Z63 2904F24	R	Low lint wipe	Clean and dry work area	All	1 box	N
		R	Nitrile gloves, powderless	Prevent contamination of soil samples during handling		1 box	N
Fisher	033377	R	Scintillation vials, glass, 20 mL	Store BGC analysis samples	BGC analysis subsamples	30-60	N
Fisher	02911825	R	250 mL wide-mouth glass jars	Store BGC archive samples	BGC archive subsamples	30-60	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity *	Special Handling
		R	Heat-resistant, adhesive barcode labels, Type I	Label BGC analysis and archive samples with barcode-readable labels	BGC analysis and archive samples	60-120	N
Avery	5520	S	2.6” x 1” address labels	Label BGC analysis and archive samples with human-readable labels	BGC analysis and archive samples	60-120	N
Fisher Thomas	0333723C 9718J20	R	Scintillation vials, plastic, 20 mL	Store microbial biomass samples	Microbial biomass samples	30-60	N
		R	Cryogenic, adhesive barcode labels, Type II	Label frozen sample containers with barcode-readable labels	Microbial biomass samples	30-60	N
Fisher	15-930-E	R	Cryogenic adhesive labels	Label frozen sample containers with human-readable labels	Microbial biomass samples	1 sheet	N
Resources							
RD[05]		R	Lab datasheet	Backup to record data		---	N

R/S=Required/Suggested

Table 11. Equipment List - Laboratory processing of soils for measuring pH at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Fisher	13636AB150B	R	pH meter	Reading pH value of samples	All	1	N
Fisher Thomas	02112300 0910200 1189J86	R	Balance, 0.01 g accuracy	Weigh soil samples	All	1	N
		S	Plastic tray	Holding soil subsamples	All	4 (+)	N
Fisher	10020F	S	Glass volumetric flask, 2L	Preparing calcium chloride solution for pH analysis	All	1	N
		S	Graduated cylinder, 50 mL capacity	Measure volumes of solutions for pH samples	All	2	N
		R	Spatula or scoopula	Transfer soil subsamples	All	2	N
Fisher	1451386	S	Stir rod	Mix pH samples	All	1	N
Consumable Items							
Fisher	191301597B 191301597C 191301597D 191301597E	R	Powderless gloves (s, m, l, xl)	Prevent sample contamination during handling, prevent bodily injury from hazardous chemicals	All	1 box	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
ULINE	S-7630	R	Paper bag, #8	Hold soil subsamples for air-drying	All	50	N
Fisher	AC423520250	R	Calcium Chloride	pH analysis	All	2.94 g	N
Fisher	0340910E	R	Deionized water wash bottle	Rinse equipment and pH electrode	All	1 bottle	N
Fisher	SA49	R	Hydrogen Chloride, HCl	Adjusting pH of $CaCl_2$	If solution is too basic	1 ml	Y
Fisher	C88500	R	Calcium Hydroxide, $Ca(OH)_2$	Adjusting pH of $CaCl_2$	If solution is too acidic	1 ml	Y
Fisher	13300154 13300153 13300152 13300155	R	pH buffers (4, 7, 10, 1.68)	Calibrating pH meter	All	1	N
		S	50-100 mL containers	pH analysis	All	50 (+)	N
Consumable Items							
Thomas	2904F24 1234Z63	R	Low lint wipe	Clean and dry work surfaces	All	1 box	N
Resources							
RD[05]		R	Lab datasheet	Backup to record data	All	---	N

R/S=Required/Suggested

Table 12. Equipment List – Laboratory processing of soils for N transformations at one site.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items							
Fisher	02112284	R	Balance, 0.01 g accuracy	Weigh soil samples	All	1	N
Fisher	0300742	R	Graduated cylinder (100-250 ml)	Measure aliquot of KCl	All	1	N
Fisher Global Equipment	SK-O330	R	Shaker table	Shake extracts	All	1	N
Thomas	4618N60	R	Reusable filter units	Filter samples and collect filtrate	All	4	N
		R	Large beaker (at least 500 ml)	Collect discarded KCl filtrate	All	1	N
		R	Vacuum pump	Filter samples	All	1	N
Fisher	04-881G	R	2 mm sieve	Sieve soils	All	1-2	N
		R	Manifold. See assembly, NEON.DOC. 004474 (RD[12])	Filter samples	All	1	N
Fisher	10020F	S	Volumetric flask, 1 L	Prepare 2M KCl solution	Small batch of samples	1	N
Fisher	2319-0050	S	Carboy (20 L)	Prepare and store 2M KCl solution	Large batch of samples	1	N
		R	Spatula or scoopula	Transfer soil between containers	All	2	N
		S	Plastic dishpan, 3-gallon capacity (e.g. Rubbermaid #2951, or similar)	Wash filtering equipment	All	2	N

Consumable Items							
Sigma-Aldrich	P3911*	R	KCl, ACS grade. Larger size container (2.5 or 5 kg)	Extract NH_4^+ and NO_3^- from soil	All		N
Thomas	6186M96	R	Screw-cap polyethylene extraction cups and lids (e.g., urinalysis cups) or equivalent (120 ml capacity)	Extract NH_4^+ and NO_3^- from soil	All	33-75	N
Fisher (LabChem)	LC267405	R	Ultra-pure Type I deionized water	Prepare 2M KCl, final rinse of filtering equipment	All	1-2 20L carboys	N
Fisher Thomas	0333723C 9718J20	R	Plastic scintillation vials with caps, 20 mL	Store filtered soil extracts for freezing and shipment	All	33-75	N
Fisher	191301597B 191301597C 191301597D 191301597E	R	Powderless gloves (s, m, l, xl)	Prevent contamination of soil samples	All	1 box	N
Fisher	0987414A	R	Glass fiber filters, 47 mm diameter, GF/A type	Filter samples	All	1 box	N
Grainger	5CNK5 8YAT5	S	Resealable plastic bag, 1 gallon	Organize vials containing sample extracts	All	1 box	N
Fisher	15-930-E	R	Cryogenic adhesive labels	Label vials with human-readable labels	All	1 sheet	N
		R	Cryogenic, adhesive barcode labels, Type II	Label samples with barcode-readable labels	All	1 sheet	N
Resources							
RD[05]		R	Lab datasheet	Backup to record data	---	N	

R/S=Required/Suggested

* Substitute products have been tested and caused problems. Only this product should be used - contact NEON Science if there are issues procuring it.

Table 13. Equipment List - Shipping of oven-dried and air-dried samples for BGC analysis and archiving

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
	Consumable items					
		R	Cardboard box	Package samples for shipment	2 (+)	N
		R	Cushioning material (i.e. wadded newspaper)	Package samples for shipment	As needed	
		R	Packaging tape	Package samples for shipment	1	N
	Resources					
		R	Shipping manifest	Inventory of specimens being shipped	1 per box	N
		R	USDA Permit to Receive Soils or Compliance Agreement	Comply with USDA regulations for receiving soils	1 per box	N

R/S=Required/Suggested

Table 14. Equipment List - Shipping of samples for microbial molecular analysis, microbial biomass, and N transformations.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
Consumable items							
ULINE	S-16478	R	Cardboard box or insulated shipper	Package samples for shipment	All	1+	N
		R	Cushioning material (i.e. newspaper)	Protect samples from damage during shipment	All	As needed	N
		R	Dry ice shipping label	Label shipments containing dry ice	All	1	N
Varies by Domain	Varies by Vendor	R	Dry ice, pelletized	Keep samples frozen during shipment	All	20* lbs	Y
		R	Packaging tape	Package samples for shipment	All	1 roll	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal, 4 mil	Organize sample bags	All	~3	N
		R	Styrofoam sheet	Insulate samples for shipment	All	As needed	N
Resources							
		R	Shipping manifest	Inventory of specimens being shipped	All	1 per box	N
		R/S	USDA Permit or Compliance Agreement	Comply with USDA regulations for quarantine soils	Soils (not extracts)	1 per box	N

R/S=Required/Suggested. *At sites with maximum shipping allowances less than 20 pounds, supplement with pre-chilled packing peanuts (or similar).

6.2 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[04]).

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.

6.3 Specialized Skills

Soil types and profile characteristics differ greatly across the NEON domains (see examples in Figure 2). 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.

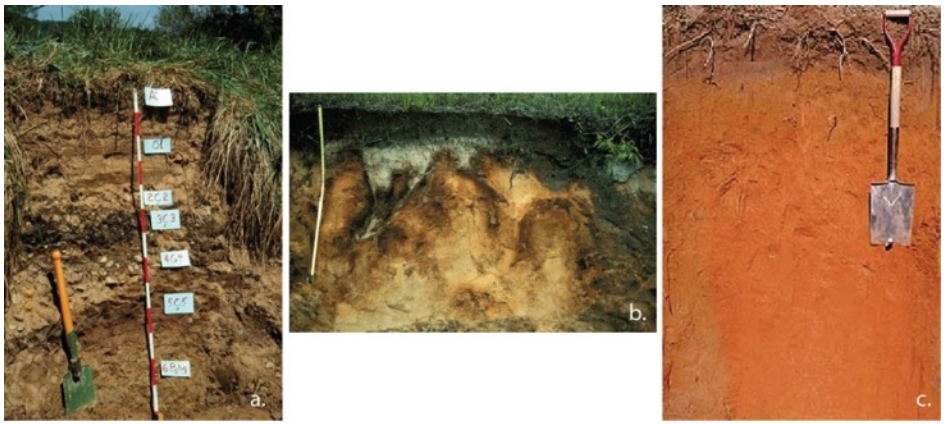


Figure 2. Soil Profiles from (a) Maryland, (b) Michigan, and (c) Florida. (Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

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% 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 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.

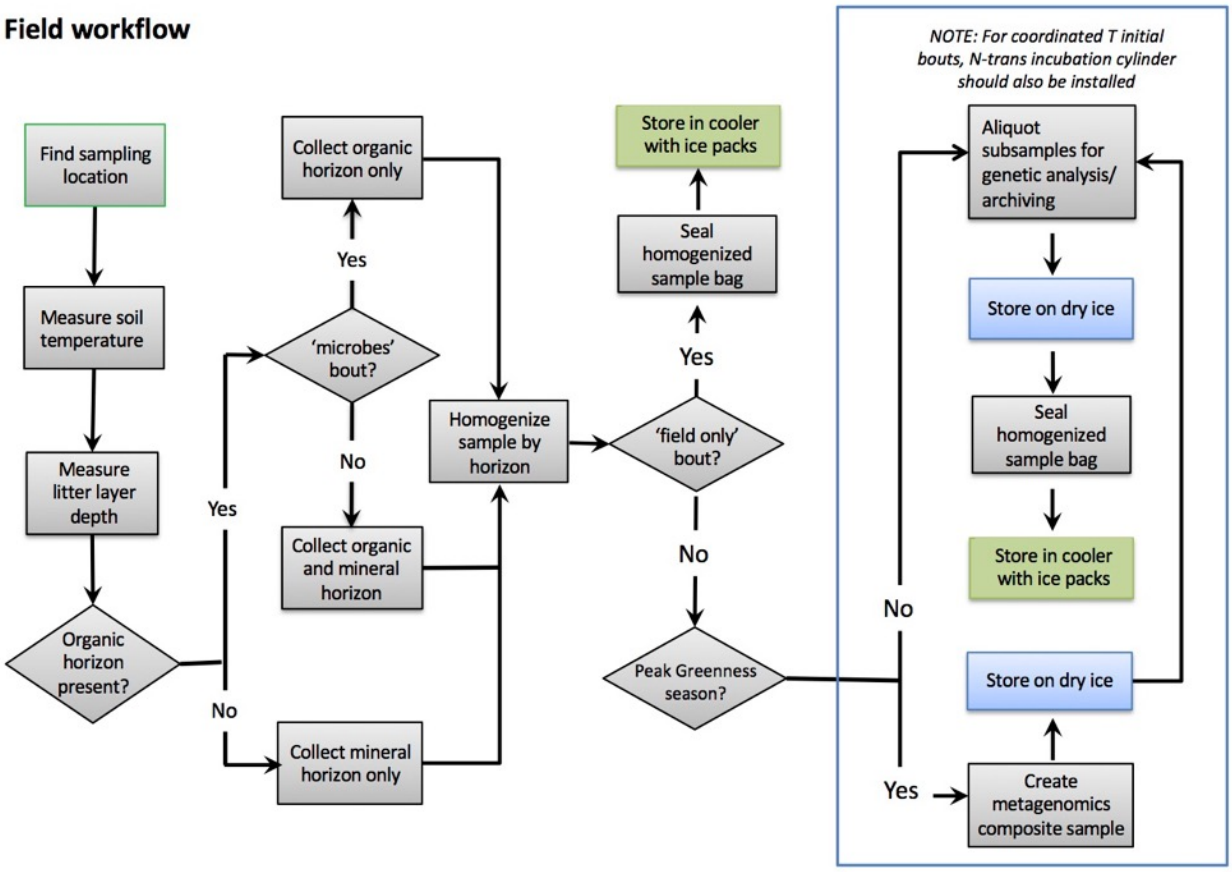
6.4 Estimated Time

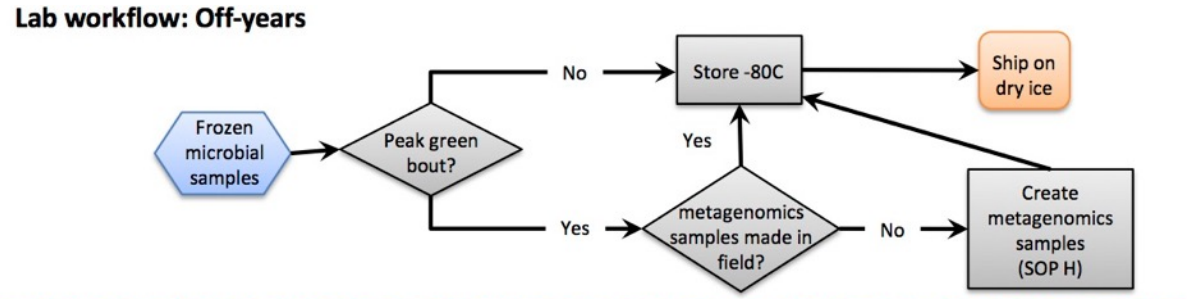
The time required to complete the SOP’s associated with this Protocol for a single sampling event/bout are listed in **Table 15**. It’s important to note that the time required to implement a protocol will vary depending on a number of factors, such as experience level, site diversity, type of sampling 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 problem ticket should be submitted.

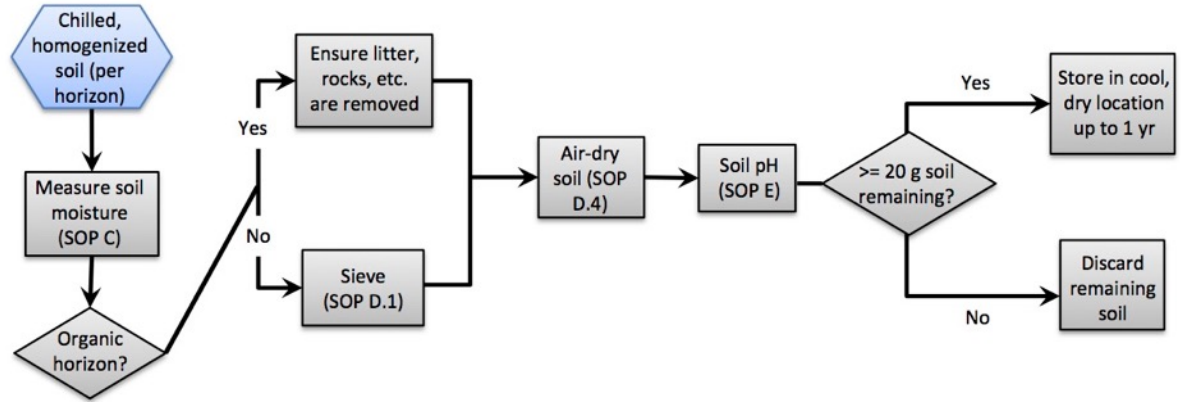
Sampling should be scheduled at the beginning of the sampling window to allow time for contingencies to occur that could delay sampling.

Table 15. Estimated time required to complete the Standard Operating Procedures for a sampling event.

SOP	Estimated total time	Suggested # staff	Total person hours
A: Preparing for Sampling	2 hrs	2	4 hrs
B: General Field Sampling	16 hrs	4	64 hrs
C: Soil Moisture	8 hrs	2	16 hrs
D: General Soil Lab Processing	8-16 hrs	2	16-32 hrs
E: Soil pH	8 hrs	2	16 hrs
F: N transformation field sampling	20 hrs	4	80 hrs
G: N transformation lab processing	10-15 hrs	2	20-30 hrs
H: Composite Sample Generation	2 hrs	2	4 hrs
I: Data Entry and Verification	2 hrs/app (6 total)	2	24 hrs
J: Sample Shipment	2 hrs/shipment (up to 4 total)	2	8 hrs


Field workflow
NOTE: For coordinated T initial bouts, N-trans incubation cylinder should also be installed
Find sampling location
Measure soil temperature
Measure litter layer depth
Organic horizon present?
Yes
No
Collect organic horizon only
'microbes' bout?
Yes
No
Collect organic and mineral horizon
Collect mineral horizon only
Homogenize sample by horizon
'field only' bout?
Yes
No
Store in cooler with ice packs
Seal homogenized sample bag
Peak Greenness season?
No
Yes
Aliquot subsamples for genetic analysis/ archiving
Store on dry ice
Seal homogenized sample bag
Store in cooler with ice packs
Store on dry ice
Create metagenomics composite sample


Lab workflow: Off-years
Frozen microbial samples
Peak green bout?
No
Yes
Store -80C
Ship on dry ice
Yes
metagenomics samples made in field?
No
Create metagenomics samples (SOP H)


Chilled, homogenized soil (per horizon)
Measure soil moisture (SOP C)
Organic horizon?
Yes
No
Ensure litter, rocks, etc. are removed
Sieve (SOP D.1)
Air-dry soil (SOP D.4)
Soil pH (SOP E)
>= 20 g soil remaining?
Yes
Store in cool, dry location up to 1 yr
No
Discard remaining soil

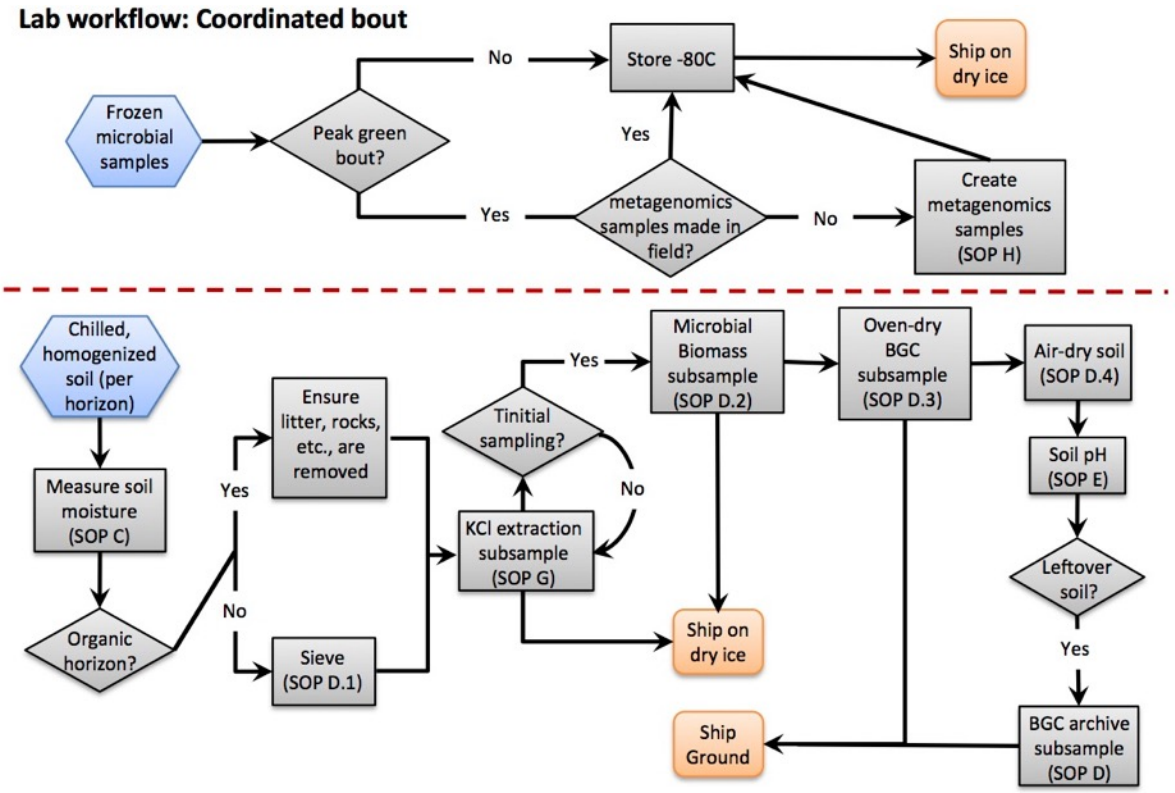


Figure 3. Workflows for field sampling (top), and lab processing and shipping for off-year bouts (middle) and coordinated bouts (bottom).

7.1 How much soil to collect?

For simplicity, the amount of soil to collect for each analysis is indicated by volume. For most sites, this approach to measuring amounts of soil will be sufficient. However, 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 sites with these soil types, 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 SOP B.

It is extremely important to recognize the limitation with the mass approach: 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. Unfortunately, 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.

SOP A Preparing to Sample Soils

A.1 Prepare Sampling Equipment

1. Ensure all supplies listed in the relevant tables above are available.
    - When using a metal coring device, 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 the rust with a sponge or towel, then rinse 3 times with deionized water and dry with a clean cloth or paper towels. For severe rust, a metal scrubber may be necessary.
2. 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 21** summarizes O-horizon presence and thickness in sites where NRCS has completed initial soil characterization (table will be updated as more data becomes available).
3. Obtain 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.
4. Time-permitting, flag the southwest corner of each plot prior to sample collection to save time in the field.
5. 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.
6. **For coordinated bouts**, follow the additional sample preparation instructions below. If not, skip to A.2.
7. Ensure that the required number of incubation cylinders are available (one per soil sampling location plus 2 extra, e.g., 32). **Error! Reference source not found.** shows an example PVC incubation cylinder:

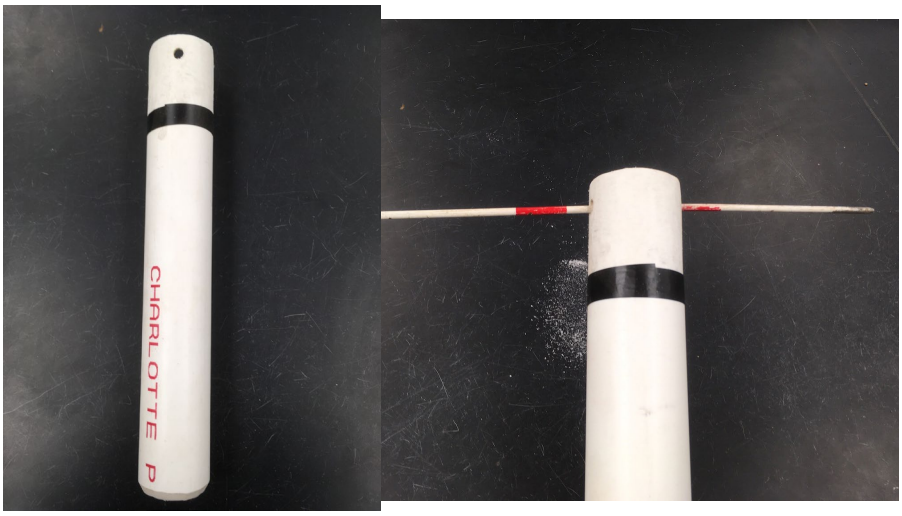


Figure 4 Example coring device used for soil N transformation sampling.

note the bottom edge has been shaved down or beveled, which helps drive it in to the soil. Two holes near the top aid in removal.

- 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.*

- If cylinders have been used before, ensure they have been cleaned with soap and water and rinsed with deionized water prior to re-use. Staining and small chips on the beveled edge are ok.

8. Check that one loosely fitting 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.

9. Along with all field supplies listed in Table 8, verify that all laboratory supplies listed in Table 12 are also available. **Lab processing must occur within 24 hours of field collection**, thus all supplies must be in hand. If possible, make 2M potassium chloride for extractions in advance as it can take several hours to dissolve, see SOP G for instructions.

A.2 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. 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, where possible; 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 Error! Reference source not found. for instructions on using and maintaining the soil coordinate and subplot lists.

A.3 Label sample containers

1. Acquire sample containers that will be required for the upcoming bout (**Table 16**). Always bring extra containers to the field in case a container becomes damaged or contaminated.

- 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.

- New 1-gallon freezer-safe bags should be used for collecting all 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). Use the sample containers and labels referred to in Equipment Tables 5 and 6 and apply as described in Table 15. Apply all labels a minimum of 30 minutes prior to sampling to ensure they have time to adhere fully. **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. Also, be sure that no wrinkles, folds, air bubbles or other obstructions are present in the label that can impede reading of the barcode. Do not overlap labels.

   - For the bulk, homogenized soil sample: Place the weatherproof Type I barcode labels 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.
   - For the microbial genetic analysis samples, place the cryogenic Type II barcode labels 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.
   - For the microbial archive samples, place the cryogenic Type III barcode labels 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.

Table 16. Sample containers and barcode labels to use for field-generated samples.

Sample Type	Container Type	Barcode Type	Human-readable Label Type	Location of Barcode
Homogenized field sample	1-gallon ziplock bag	Type I 	Weatherproof address label	Can vary, should be flat area on gallon bag
Microbial genetic analysis, microbial metagenomics (-gen, -comp)	2-oz. Whirl-pak	Type II 	Cryogenic label	White area of whirl-pak

Microbial archive (-gaX)	5 ml tissue vial	Type III 	Cryogenic label cut horizontally into thirds	Top of vial (on the cap)

Important Note: adhesive labels can fall off after freezing. To reduce this risk, use only containers intended for frozen storage, and attach labels to the white area of the bag or container. When in doubt, hand-writing critical info such as plotID, X, Y coordinates, and collectDate on the container will help identify a sample without a label. Notify Science of any problems that arise with labels not adhering.

SOP B Field Sampling

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.

There are numerous types of sampling bouts and samples produced by this protocol: refer to Table 1, Figure 3 and the Quick References in Appendix B for guidance. 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, collect a soil core, subsample the soil core, and store subsamples for laboratory transport.

The majority of soil sampling bouts will be of boutType = microbes. During these bouts, the following samples and measurements will be made:

- soil temperature
- litter depth
- microbial genetic analysis (-gen) and archiving (-gaX)
- soil moisture (-sm)
- soil pH (-pH)

When sampling during peak greenness, 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 H ("Generation of composite samples") to generate samples for these analyses in the lab.

During coordinated bouts, other samples and measurements are made ***in addition*** to those made for a microbes bout. These include:

- nitrogen transformations (-kcl, SOP F and G)
- microbial biomass (-bm, SOP D)
- soil BGC analysis and archiving (-cn and -ba, peak greenness only, SOP D)

B.1 Identify the plot and sampling location

1. Confirm with a handheld GPS that the GPS coordinates for the target plot match the GPS coordinates at your current location.

2. Navigate to the southwest corner of the plot.

3. Identify the sample location using the soil X,Y coordinate list for the particular plot/subplot combination. You will collect soil at three randomly assigned locations within each plot, one in each randomly designated subplot (see Appendix F).

    - In relatively flat plots (<20% grade), lay out meter tapes on the west and south sides of the plot and locate X, Y coordinates (i.e. sampling location).

    - In very steep (>20% grade) plots, use a laser rangefinder set to HD (horizontal distance) mode to locate the X,Y coordinates.

        - Clean lenses with lens cloth or lens tissue, if needed.
        - Check/set correct declination. See RD[11] for details.
        - Calibrate the TruPulse tilt sensor (only needed after severe drop-shock; see RD[11] for details).
        - Two technicians must work together. One stands at the SW corner 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.
        - 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.
        - Place a marker, such as a pin flag or stake, at the X-location.
        - 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.
        - Ensure that the azimuth is as close to 0° (True North) as possible and measure the Y-coordinate distance.
        - 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? If so, 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 <u>five</u> X,Y coordinates are rejected, do not sample within that subplot and notify Science.

    - 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.

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 will break easily.

    - Allow probe to equilibrate for about 2 minutes, then record the **soilTemp** in degrees C on the field data sheet and/or data entry application.

    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). To ensure that you are not compacting the litter layer, remove litter layer and measure the depth of the undisturbed litter profile.

    Reminder*: The litter layer is dead but recognizable, intact plant material (i.e., leaves, wood, etc), whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition.*

B.4 Collect Organic (O) Horizon

Only follow step B.4 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 step B.4 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 around the coordinates 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. Using clean tools and equipment, cut out an organic horizon "brownie" using the square frame cutter tool and soil knife (hori-hori, Figure 5). 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.

3. At all sites, 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, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris and homogenize.**

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

- **<u>Generate microbial analysis (-gen) and archive (-gaX) samples</u>**: 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.

- Complete the human-readable labels on the whirl-pak and the cryovials with horizon, X coordinate and Y coordinate. shows examples of properly labelled -gen and -gaX samples.

  The microbial genetic <u>analysis</u> 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 <u>archive</u> 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*

- 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.

- 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.

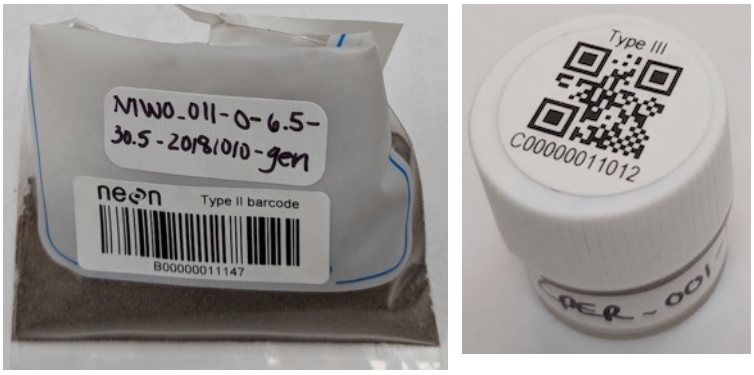


Figure 6. Example microbial genetic (-gen, left) and microbial archive (-gaX, right) samples. Microbial metagenomics (-comp) samples will look similar to -gen samples, except for content of the label.

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

  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 the bag, and place on <u>ice packs</u>. Record the **compositeSampleID** on the datasheet and/or data entry application. Scan the barcode label into the data entry application, if using.

  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 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.
    - If another X,Y location within the plot might be added to this bag, return the bag to cooler with <u>ice packs</u>.
    - 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 6), and record the **compositeSampleID** on the datasheet and/or data entry application. As soon as the bag is closed and the data have been entered, place the bag on <u>dry ice</u> 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 H for generating a composite sample in the DSF.*

- **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 organic soil that should remain in the bulk homogenized bag:

  - Microbes bout (off-year, any season): 25 g
  - Coordinated, transition season bout (e.g. not peak greenness): 50 g
  - Coordinated, peak greenness bout: 75 g

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

- For labelling and data recording, be sure that:

  - The homogenized sample bag is labeled with the **sampleID**, **measuredBy**, and **recordedBy**
  - 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 data entry application. Perform a quick scan to catch any erroneously duplicated barcodes.

- **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.

5. **Determine whether to also collect mineral (M) horizon** (refer to **Figure 3**).

   - For an off-year, **microbes** bout, do not collect the mineral horizon if an organic horizon sample was collected at that X,Y location. Skip to Step B.6.

   - During a **coordinated, peak greenness** bout, collect M horizon (if present) from all sample locations. All analyses and subsamples will be completed. Continue on to Step B.5.

   - During a **coordinated, transition season** bout, collect M horizon (if present) from all sample locations. Field sub-sampling for microbial genetic analysis and archive will <u>not</u> be completed on the M horizon; however, the bulk, homogenized soil will be used in the lab for microbial biomass and N-transformation analyses, pH, and moisture analysis. Continue on to Step B.5.

B.5 Collect mineral (M) horizon

Only collect M horizon if a) you do not have an O horizon at your X, Y coordinate; or b) your bout type and sample timing dictate that M horizon should also be collected (B.4.5 above).

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 around the coordinates may cause future sampling locations to overlap.

1. Pre-sterilize sampling equipment (Figure 7).

Figure 7. Sterilizing soil auger using chaining pin and 70% ethanol wipe. Gloves should be pre-sterilized.

2. If the O horizon was already collected, core at the same location(s) where O horizon was removed.

3. 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.

    - If a significant impediment to coring is encountered that is not representative of that location, replace 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 any non-sterile surfaces.

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 mineral soil horizon. You can also core incrementally (e.g., 10 cm increments) to reach the total depth, if that works best with your site-specific coring device.

4. Measure **sampleTopDepth** as distance from the top of the soil sample to the soil surface. If there is no O horizon, the top depth will be 0 cm. Measure **sampleBottomDepth** as the depth from the soil surface to the bottom of the sample (Figure 8).

5. Record **horizon** (M), X,Y coordinates and date on the field data sheet and the sample bags.

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, add a note in 'remarks' field that the sample contains a thin O horizon.

Figure 8 Example soil profile: O horizon sampleTopDepth = 0 cm, sampleBottomDepth = 3 cm; M horizon sampleTopDepth = 3 cm, sampleBottonDepth = 30 cm.

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 8). 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.

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

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 problem ticket***

8. **Create microbial genetic analysis (-gen) and archive (-gaX) samples**: *Reminder: do not create these subsamples if there is an O-horizon and it is a transition season coordinated bout.* 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.

9. Complete the human-readable labels on the whirl-paks and the cryovials with horizon, coreCoordinateX and coreCoordinateY. Figure 9 shows examples of properly labelled -gen and -ga samples.

Figure 9. Dispensing soil core into bag for homogenization. Tapping the corer with a rubber mallet can help release soil from the barrel.

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*

10. 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.

11. 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.

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

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 whirlpak, close the bag, and place on wet ice. Record the **compositeSampleID** on the datasheet and/or data entry application. Scan the barcode label into the data entry application, if using.

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.

  - If another X,Y location within the plot may be added to this bag, return the bag to wet ice.

  - 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 6), and record the **compositeSampleID** on the datasheet and/or data entry application. As soon as the bag is closed and the data have been entered, place the bag on dry ice and ensure that newly added whirl-paks are in contact with dry ice. Microbial activities will change rapidly until frozen.

***Note**: If time does not permit a composite sample to be created in the field, simply collect an additional whirl-pak and follow SOP H for generating a composite sample in the domain lab.*

13. **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): 50 g
    - Coordinated, transitional bout (e.g. not peak greenness): 100 g
    - Coordinated, 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.

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

    - The bulk, homogenized sample bag is labeled with **sampleID**, **measuredBy**, and **recordedBy**
    - 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 data entry application. Perform a quick scan to catch any erroneously duplicated barcodes.

- **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.

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 5).

B.6 Data Entry

1. Update the soil X,Y coordinate list and subplot list
2. Enter metadata in field datasheet or data entry application:
    - NtransBoutType (None, Tinitial, Tfinal)
    - boutType (microbes, microbesBiomass, microbesBiomassBGC, field only). For the selected boutType, ensure that all of the sampleID's associated with that bout have been generated.
    - sampleTIming
    - protocolVersion
    - siteID
    - plotID
    - collectDate (YYYYMMDD)
    - setDate (Tfinal samples only)
    - coreCoordinateX
    - coreCoordinateY
    - standingWaterDepth (nearest 0.1 cm)
    - time (HH:MM)
    - soilTemp (nearest 0.1 degree)
    - litterDepth (nearest 0.1 cm)
    - sampleTopDepth (nearest 0.1 cm)
    - sampleBottomDepth (nearest 0.1 cm)
    - samplingDevice
    - numberCores
    - horizon
    - 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 using a tablet

B.7 Field clean-up

1. Thoroughly rinse sampling equipment with deionized water (corer, thermometer, etc).

2. Wipe down reusable sampling equipment with alcohol wipes or squirt bottle to the extent possible.

3. Discard gloves.

B.8 Sample preservation and transport

1. 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.

2. 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 J.4 with no additional laboratory processing.

SOP C 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 data entry 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 24 h of field collection* (see SOP F).**

1. Weigh each horizon sample.
    - 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 (5) below).*
    - Weigh foil boat to nearest 0.01 g and record value in datasheet or data entry application (boatMass).
    - Wear un-soiled (e.g. clean, but sterile not required) nitrile gloves. Gloves may be re-used between samples as long as they are rinsed with DI to remove soil and dried well. Place approximately 5 g of a field moist organic horizon sample (not sieved) or approximately 10 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.** Record weight to nearest 0.01 g (freshMassBoatMass).
        - It is acceptable to use less mass if sample quantity is limited: 2 g minimum for O-horizons, 5 g minimum for M-horizons
2. 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 on datasheet or data entry application.
3. Immediately after removing samples from oven, weigh dried sample + weighing boat to nearest 0.01 g and record values in the datasheet or data entry application (**dryMassBoatMass**). Record the date and time out of oven.
4. Dispose of soils according to permit requirements.
5. For weigh boats that are in good condition and can be re-used, clean in soap and water and rinse with deionized water. Dry weigh boats either in the oven or at ambient temperature, and store in a dry location.

NO OVEN SPACE?

If oven space is limited and more than 24 hours (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.

SOP D Sieving of Field Soils and Laboratory Processing for Microbial Biomass and BGC Analysis and Archiving

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 or air-dry and subsample as directed. Record the necessary metadata and values in the data entry application or lab datasheet (RD [05]). Key reminders:

- ***For off-year microbes bouts, sieving and subsampling should begin within 48 h of field collection, or Monday morning for samples collected on Friday.***
- ***For coordinated bouts, sieving MUST begin within 24 h of field collection*** **(see SOP F).**

D.1 Sieving and Homogenizing Samples

1. Wear nitrile gloves (non-sterile but clean). Use a **new** glove for each soil sample (Suggestion: use one hand to handle the sample so that you only have to replace one glove. If you use two hands, replace both gloves).
2. With gloved hand, stir soil sample to homogenize (mix), breaking up any soil clods completely. At the same time, remove rocks, roots, leaves, and debris and discard according to permit requirements (see section 5.1 Plant Protection and Quarantine).
3. 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 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.
4. 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 E), 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 problem ticket to receive further instruction.
5. Discard particles > 2 mm according to permit requirements.

6. Record metadata on the lab datasheet:
    - siteID (ex. DSNY)
    - plotID (ex. ONAQ_010)
    - horizon (O or M)
    - coreCoordinateX
    - coreCoordinateY
    - collectDate (format: YYYYMMDD)
    - measuredBy
7. Clean sieve using deionized 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.

D.2 Processing Microbial Biomass Samples

Microbial biomass samples are generated during coordinated bouts. Follow this SOP **immediately** after completing section D.1.

1. Affix cryo-safe (Type II) barcode labels and human-readable labels to new plastic scintillation vials. Orient the barcodes from top to bottom (not curving around vial). Ensure that labels do not overlap (Figure 10).

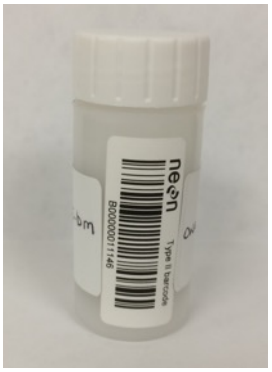


Figure 10. Properly labeled microbial biomass vial with Type II barcode and human-readable label.

2. For M horizons, tare the labeled scint vial and transfer ~10 g (± 1 g) of wet-sieved soil. Close the vial.
3. For O horizons, tare the labeled scint vial and 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. Close the vial.
    - **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. Only use one barcode to represent all vials. If more than 3 vials are required to obtain sufficient mass, contact Science for guidance.
4. If using barcodes, scan to associate each with the appropriate **biomassID** in the soil core collection data entry application. Human-readable labels should appear as:

    plotID-horizon-coreCoordX-coreCoordY-collectDate-bm

    (ex. CPER_001-O-10.5-10.5-20160101-bm)
5. Store vials at -80°C until ready for shipment.

6. Ship samples according to SOP A.

D.3 Processing BGC Analysis Samples

1. Fill ¾ 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 M horizons, use field-moist, sieved soil.
    b. 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.
2. Every vial should be labeled with a Type I adhesive barcode label, placed long-wise from top to bottom (not curved around, see Figure 10). Additionally, label the scintillation vial with a human-readable **cnSampleID** as follows:

    plotID-horizon-coreCoordX-coreCoordY-collectDate-cn

    (ex. CPER_001-O-10.5-10.5-20160101-cn)

    a. 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.
4. Place scintillation vials into the scintillation vial box, which holds 100 vials. Record ovenStartDate and time in datasheet or data entry application. Oven-dry at 65°C for at least 48 hr. Record oven end date and time in datasheet or mobile application.
5. 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 data entry application and that each barcode has been scanned.
6. Store bottles at ambient temperature until shipping. Ship samples according to SOP J.2.
7. Air dry remaining soil as described in SOP D.4.

D.4 Air-Drying and Processing BGC Archive Samples

Follow this SOP when: 1) you are processing soils for pH; and/or 2) during a coordinated, peakGreenness bout with soil remaining **after** subsampling for BGC analysis. Refer to **Figure 3** and the Quick References in Appendix B for guidance.

1. 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 pH data entry application. With very wet or fine-grained soils that can leak out, cover the seams along the bottom of the bag with masking tape.
2. Break up large clumps and soil aggregates with a clean gloved hand and spread out soil to facilitate drying.
3. Weigh the bagged sample and record initial mass on the sample bag. This initial mass is used to track the completion of sample drying.

4. Loosely close bag and place on a clean lab bench or table, away from other activities that might disturb samples. Record **airDryStartDate** on a paper datasheet.
5. Once a day, shake up soil to expose new surfaces.
6. Weigh samples again when they appear dry, which may vary from days to weeks depending on soil moisture content, climate and soil type. It is crucial that samples have dried completely.
7. 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%.
8. At the conclusion of air-drying samples, record **airDryEndDate**. To continue with pH measurement, follow SOP E.
9. During a coordinated, peakGreenness bout, generate a BGC archive sample.
    - Wear clean (non-sterile OK) gloves while handling samples.
    - Transfer the soil to an archive bottle labeled with a Type I adhesive barcode label, oriented top to bottom (not curved around). Additionally, label the bottle with a human-readable **bgcArchiveID** as:

      plotID-horizon-coreCoordX-coreCoordY-collectDate-ba

      (ex. CPER_001-O-10.5-10.5-20160101-ba)
    - Fill bottles up to, but not beyond the lip of the bottle.
    - Ensure that all bgcArchiveIDs have been created in the data entry application and that each barcode has been scanned.
    - Store bottles at ambient temperature until shipping. Ship samples according to SOP J.2.
10. Any soil remaining after all subsampling and analyses have been completed should be held in the same paper bags with the tops folded and stored in a safe location for up to one 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. After one year, any soil left can be discarded according to permit requirements.

SOP E Laboratory Measurement of pH

Soil pH is measured on sieved (or hand-homogenized), air-dried soil samples. Soil pH is measured potentiometrically in a supernatant liquid that is in equilibrium with a soil suspension of a 1:2 soil-to-liquid (weight/weight) mixture for mineral soils and a ratio of 1:4 for organic soils. Samples are analyzed both in 0.01 M calcium chloride ($CaCl_2$) and deionized (DI) water.

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.

Sterility requirements: Clean instruments and gloves using DI water and dry thoroughly between samples. Sterility is <u>not</u> required.

1. Clean lab benchtop prior to processing samples.
2. Put on a clean pair of gloves. If you do not touch the soil samples directly, you do not need to change gloves between samples.
3. Make the 0.01 M $CaCl_2$ solution:
   - dissolve 2.94 g of $CaCl_2 \cdot 2H_20$ in just under 2 liters of house deionized water in the volumetric flask.
   - Check pH of $CaCl_2$ solution; it should be between 5.0 and 6.5.
   - Adjust pH to desired value by adding concentrated 6N $Ca(OH)_2$ or 10N HCl one drop at a time
   - Bring solution to final volume of 2L
   - Note: this solution is stable for approximately 1 year, kept at room temperature out of direct sunlight.
4. Using a clean tool, weigh out a subsample of air-dried organic or mineral soil and place into a 50 – 100 mL container. Clean 50 mL conical tubes may be used and can facilitate processing of multiple samples simultaneously.
   - For O-horizon samples, use 5 ± 0.1 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. For very fluffy, absorbent O horizons, the mass of soil may be decreased to as low as 1 g soil per 20 mL solution.
   - For M horizons, use 10 ± 0.1 g of sieved, air-dried soil.
5. Record weights in the datasheet or mobile application.
6. Add 20 mL of $CaCl_2$ solution. Allow soil to absorb $CaCl_2$ solution. If it has not fully absorbed solution within 10 min, you may gently swirl the soil plus solution to mix.
7. Thoroughly mix by stirring/swirling samples for 20 seconds.
8. Let sample sit for 30 minutes and then stir the suspension for 20 seconds.
9. Determine if soil is completely saturated.
   - Look for supernatant (a thin layer of liquid without precipitate) above the flocculated soil.
   - If not present, add another aliquot (20 mL) of $CaCl_2$ solution and repeat stirring and settling.
   - Keep track of the total volume of solution added in the datasheet or mobile application.
10. 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.

- Rinse the electrode with deionized water and gently shake off excess liquid between buffers. Do not use a wipe to dry the electrode.
- Note: you only need to calibrate the pH probe one time for the group of samples.

11. Gently swirl the container while measuring pH of supernatant solution. It is OK if some flocculated soil is floating in the supernatant.
    - Allow reading to stabilize (usually about 1 minute) and record pH value on datasheet. If reading still fluctuates slightly after 1 minute, read pH every 5 seconds for 15 seconds, then record the average.
    - Clean electrode: rinse thoroughly 2 to 3 times with deionized water and gently shake off excess liquid. Do not use a wipe to dry the electrode.
    - Measure each sample.
12. Repeat pH measurements with deionized water, analyzing subsamples in 20 mL deionized water instead of $CaCl_2$.
13. Discard remaining soil following any applicable soil permit guidelines.

SOP F Field Sampling for Soil Nitrogen Transformations

N transformation rate measurements are conducted every five years during coordinated bouts. During these "on" years, N transformation sampling will occur three times, once during peak greenness and twice during seasonal transitions, during the specific windows listed in Appendix E. As these windows are the same as those used for microbial sampling, **material for N-transformation T-initial analysis should be subsampled for microbial and all downstream laboratory analyses**. Soil material to 30 cm maximum depth is sampled. 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 (frequently <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 in that SOP. The Wetland SOP refers back to this SOP for various instructions.

N-transformation sampling should occur in reasonably sized groups/batches of samples - generally at least 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, and this consumes time and resources. If possible, all field sampling should occur in one long field day. A team of 2 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, use 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 within the designated sampling windows.

F.1 Identify the plot and sampling location

1. Navigate to the plot and sampling location according to the instructions in SOP B.1.

F.2 Assess sample location

1. Assess the X, Y location for sampling suitability following the instructions in SOP B.2. Then, flag **two** sub-locations that are within 0.1-0.5 m of each other.
2. In sites/plots with rocky soils:
    a. Probe thoroughly for rocks as they will impede installation of the incubation cylinders
    b. The two sub-locations may be further apart than 0.5 m if needed, as long as they are within 0.5 m of the X, Y location

F.3 Measure soil temperature and litter depth

1. Take soil temperature and litter depth readings according to the instructions in SOP B.3.

F.4 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, proceed to Section F.5 and install the PVC incubation cylinder *first, then* collect the initial sample following the guidelines below. Initial and final cores should have depths within ± 5 cm of each other; since PVC sometimes cannot penetrate as deep as coring devices, PVC should be installed first and the depth used to guide the initial sample.
- If a site's normal coring device is not 2 ± 0.5" in diameter, or there are other logistical concerns, the PVC cylinders can be used to collect both the initial and final sample. Follow the instructions below.

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. 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:
    - Sample it by cutting an O horizon "brownie" (or several for thin O-horizons), record the depth, and place material in a new, pre-labeled 1-gallon bag as described in SOP B.4.2-B.4.4.
        - **Remember to use a pre-sterilized, gloved hand to remove rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris and homogenize.**
    - Generate microbial analysis (-gen) and archive (gaX) subsamples. Label, scan, and store them as described in SOP B.4.4.
    - During peak greenness, generate a microbial metagenomics sample (-comp) following SOP B.4.4.
    - Make sure there is sufficient soil remaining for lab subsampling and analyses, as in SOP B.4.4.
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 the O-horizon sample location.
    - 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.
    - If soil is easy to core, simply push it in.
    - Always core vertically, not perpendicular, when collecting on a slope.
    - If you are unable to install the cylinder after multiple attempts, notify Science.
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), be sure to properly sterilize before use.
    - Record depth and homogenize as in SOP B.5.4-SOP B.5.6.
    - Generate microbial genetic analysis (-gen) and archive (-gaX) subsamples. Label, scan, and store them as described in SOP B.5.8-SOP B.5.11.
    - During peak greenness, generate a microbial metagenomics sample (-comp) following SOP B.5.12.
    - Make sure there is sufficient soil remaining for lab subsampling and analyses, as in SOP B.5.13.
7. Record key metadata as described in SOP B.6.
    - NTransBoutType = Tinitial.

F.5 Set up incubated soil core

Note: this core will remain in the ground for the duration of the incubation period (two to four weeks, see Appendix D).

Sterility requirements: If used to take the initial sample, incubation cylinder should be cleaned using DI and then dried before re-use. Sterility is not required.

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.
    - 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
    - If soil is easy to core, you may simply be able to push it in.
    - 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 E.*
3. If using the site's normal coring device to collect initial core, 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 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. Mark the location of the core with a non-metallic pin flag. If there is overhanging vegetation, consider tying a piece of flagging to the nearest tree/branch/bunchgrass/etc, site host permitting.
    - Write the X, Y coordinates on the flag to aid with data entry upon sample retrieval

F.6 Sample preservation and transport

1. Keep collected soil cores in cooler with ice packs and transfer to 4° C refrigerator upon return to domain lab.

Note: Soils being measured for N transformations MUST be processed and extracted in 2M KCl within 24 h 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.

F.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 D.

1. Consult the soil coordinate list and navigate to the plot and X, Y location where sampling for t-initial soil N transformations occurred. Locate incubated core. Measure soil temperature and litter depth within 10 cm of the incubated cylinder, according to the instructions in SOP B.3.
    - If the core is missing, or if insects or animals have colonized it (e.g., they've made a nest): create a record in the datasheet or data entry application but choose **sampleFate =** 'destroyed,' only record minimal sample meta-data, discard any sample material if present.
2. 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 hole 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. If this occurs, use a clean trowel or gloved hand to collect fallen soil from the bore hole. It is also acceptable to dig down next to the core and insert a knife or gloved hand under the core while removing.
3. 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.
4. 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.
5. 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 works well.

6. 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 note in remarks
    - If it's an O > 5 cm or an M > 2 cm, discard the material

8. Record the approximate depth of each horizon from the bore hole. It is acceptable to excavate the hole further if needed to accurately read borehole depth, or the boundary between horizons.

    - 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:

    plotID- horizon-coreCoordX-coreCoordY-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 B.6.

    - NTransBoutType = Tfinal, incubationMethod = coveredCore.

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 24 hours.

SOP G Laboratory Processing of Soils for N Transformations

This SOP describes the instructions for processing samples for N transformation analyses. Helpful instructional videos are also available in the NEON internal Training Center. Both initial and final samples are processed in the exact same way, using potassium chloride (KCl) to extract inorganic N. Note that for t-initial samples, downstream analyses associated with the type of sampling bout conducted (e.g. microbesBiomass, microbesBiomassBGC, Table 1) must also be conducted. Refer to SOP's C, D and E for detailed instructions.

Note: N transformation sample processing must begin within 24 h after collecting the core

G.1 Prepare for KCl extraction

1. Prepare 2M KCl (149.1 g/L).
    - Prepare a large batch (20 L) of 2M KCl. Wearing nitrile gloves, measure 2,982 g KCl into a clean receptacle and add to a clean (DI rinsed) 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.
    - If you require a small volume of KCl, prepare the solution in a 1 L volumetric flask. Wearing nitrile gloves, measure 149.1 g KCl into a weigh boat. Transfer to a clean (DI rinsed) 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.

Note: KCl in solution is good for ~1 year, so ideally a large batch is made at the beginning of the sampling year and then used for initial and final extractions of each of the 1-3 sampling bouts. Remake solution as necessary. If you have to remake solution in the middle of extracting soil samples, you must prepare an additional set of three blanks for the new batch of KCl (see Step G.3 below).

2. Pre-label extraction cups and 20 mL plastic scintillation vials with **kclSampleID** (sampleID + "-kcl", e.g. ONAQ_005-M-10.5-20.5-20160115-kcl), one each per soil sample. Scintillation vials should also be labeled lengthwise with a Type II cryogenic scannable barcode label (Figure 11).

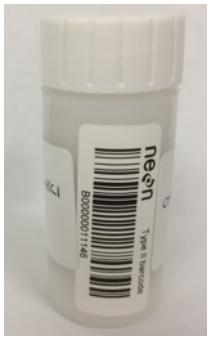


Figure 11. Properly labeled scintillation vial for KCl Samples, no label overlap.

G.2 Measure soil moisture and prepare sample for extraction

1. Soil moisture is a critical component for calculating N transformation rates, thus it is essential that this measurement be made. Subsample the collected soil samples for moisture analysis, according to SOP A. Remember that non-sieved soil is used, but remove all rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris from subsample before weighing.
    - If sample mass is limiting, it is acceptable to use a smaller mass of soil than specified in SOP C. The minimum sample size is 1.0 g.
2. For mineral horizons, sieve the collected soil samples as instructed in SOP **Error! Reference source not found.**. ***Field-moist mineral soil must be sieved and used for this analysis.*** You cannot wait to sieve.
    - Use a new glove(s) for each sample. If you only handle the soil with one hand, you only have to replace one glove.
    - Begin with a 2 mm mesh sieve. If sieving is too difficult, a 4 mm mesh sieve may be used.
    - If sieving is slow, it is acceptable to estimate how much soil will be needed for all downstream analyses, then sieve roughly double that amount. Make sure that the sample is well-homogenized prior to sieving and that sieved material is representative.
3. Place sieved material in a labeled, resealable plastic bag.
4. Do not sieve organic soil. Instead, ensure that the O-horizon material has been picked clean of rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris and is very well homogenized. Break up clumps and ensure samples are well-mixed, using a gloved hand as needed.
5. ***For t-initial samples***, remember to set aside sieved material in order to subsample for microbial biomass, BGC measurements and archive (during peak greenness), and for pH. ***T-final samples do not require any of these measurements***.

G.3 Perform KCl extraction

1. Put on a new pair of nitrile gloves. Use the same pair of gloves until they become visibly dirty. When that happens, change gloves.
2. Weigh 10 g ± 0.5 g subsamples of fresh sieved mineral or homogenized organic soil into a tared, labeled extraction cup (i.e., "zero-out" the extraction cup on the scale before putting the soil into it so you get the weight of the soil, not including the cup). Record the **soilFreshMass** to the nearest 0.01 g.
    - If sample mass is limiting, it is acceptable to use less mass, but do not use less than 4 g per sample.
    - If less than 4 g of O-horizon is available, combine it with the mineral matter and extract together. Record this in the **remarks**.
    - For (un-sieved) O-horizons, ensure no rocks, large roots or non-soil debris remains in the sample.
3. For each sample, measure 100 ± 2 ml of 2M KCl into a clean (DI rinsed) 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).
4. Every day that samples are extracted, 3 procedural blanks must be prepared - even if the KCl came from the same large carboy used to extract samples on a previous day.
    - Add 100 ml KCl to each of 3 extraction cups without soil and treat the same as samples containing soil. As above, 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. Label blanks as follows:

- First, note the **kclReferenceID**, which describes the KCl batch used that day for extractions (format = siteID-extractionStartDate-'BRef1', example: CPER-20160418-BRef1). If a new batch of KCl is created in the midst of processing samples, it will have a new kclReferenceID (example: CPER-20160418-BRef2).
    - In the data entry application, records for all of the samples extracted on a single day should be nested under this kclReferenceID
- For each of the 3 replicate blanks, record **kclBlankID**'s by appending the **kclReferenceID** with a dash followed by the letters A-C (ex: CPER-20160418-BRef1-A, CPER-20160418-BRef1-B, CPER-20160418-BRef1-C).

5. Make sure caps on each extraction cup are on tightly, then shake each cup vigorously for ~15 seconds.
6. 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.
7. Place the box on its side so that samples shake end-to-end. **Shake for 1 hour at 150 rpm**.
8. Remove extraction cups and organize on benchtop. Record **extractionEndDate** (YYYY-MM-DD HH:MM) for each sample.
    - 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.
9. Allow soil to settle without disturbance for ~ 15 minutes while setting up the filtering manifold. This will facilitate faster filtering.

G.4 Filter Samples

Note: samples are filtered in batches – the size of the batch will depend on the number of filtration set-ups that can go on the manifold at one time, 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, pre-leaching a new filter, and then filtering another sample.

1. Set up the manifold (Figure 12, left) and attach to a vacuum pump. Check that all stopcocks are in the closed position (i.e. perpendicular to tubing).
2. 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 gloves and put on a new pair.
3. 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.
4. *Add the filter*: Open filtration units. Using clean forceps, place a 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 become folded in the process.
5. Attach the filter units to each port of the manifold.
6. Prime each filter with KCl solution as follows:

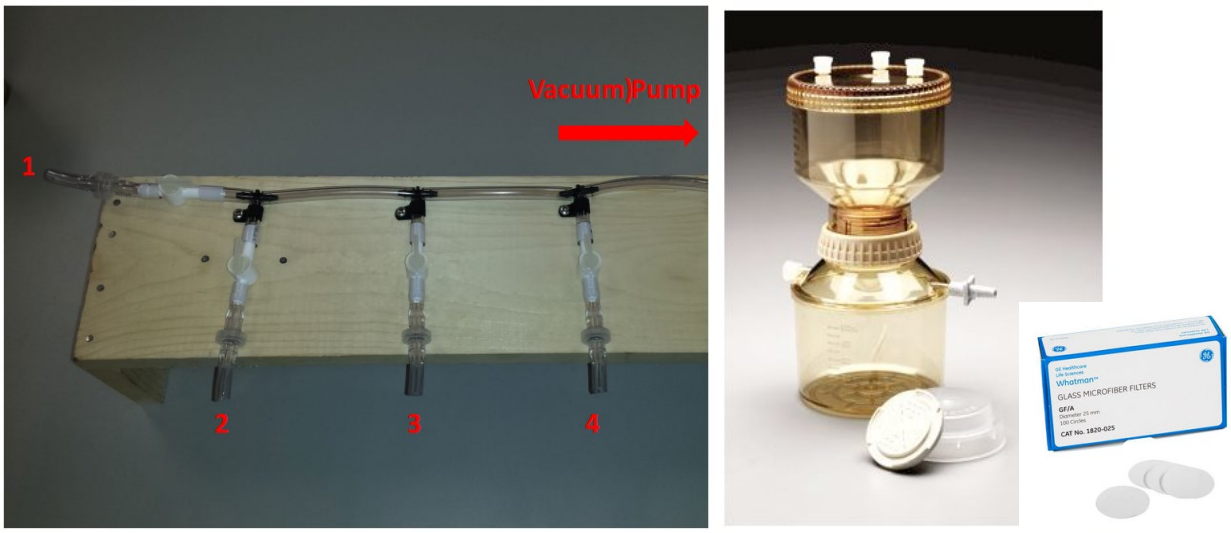


Figure 12 Filtration equipment for KCl extractions. Example of a sample manifold set-up (left) and other equipment used for filtration, including filtration unit (extraction funnel + collection cup) and filters (right).

- Saturate the filter with KCl solution
- Turn on the pump. Open the stopcocks until KCl flowthrough is complete. Close the stopcock/s.
- Remove filtration unit from the tubing, open it, and pour out the filtered KCl into a waste vessel. Use care not to contaminate the filter during this process.
- Ensure collection cup is empty, then replace filter and reassemble filtration unit.

7. Turn on the pump (if turned off) 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. Wait for sample to filter completely, then transfer the filtrate from the collection cup into a labeled 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.
9. 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.
10. Clean filtration units thoroughly prior to re-use.
    - Fill ~3/4-way two dishpans with house deionized water.
    - While wearing gloves, immerse filter holder and cup in the first water basin and swirl. This 'dirty basin' will remove most particulates and soil residue
    - Transfer filter unit to the second, 'rinse' basin and swirl.
    - 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
    - Conduct a final rinse with Type 1 ultra-pure deionized water, either directly from the container or using a squirt bottle
    - Shake to remove excess water, then re-assemble. Equipment is ready to use.

11. When filtering is complete, freeze extracts at -20° C. If freezer space allows, freeze them in the cardboard trays in which the vials are packaged: this ensures the frozen filtrate is at the bottom of the vial, where it is less prone to expand and crack the vial. If freezer space does not allow, instead place sample vials in a resealable plastic bag and label it with siteID and date and place that in the freezer. Store frozen until shipment to the contracted laboratory facility (see SOP J).

G.5 Sample Storage

Samples can be stored frozen at the Domain Support Facility for several weeks prior to shipping, but ideally not longer than 6 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. Refer to the NEON internal CLA site for shipping details.

SOP H 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. From the -80°C freezer, obtain 1 whirl-pak from each core sample. Organize whirl-paks by placing those from the same collection date, plot, and same horizon together. Double-check the labels to ensure that the sample collection dates, plot IDs, and soil horizons match. Typically, there will be 3 whirl-paks, but fewer than 3 is also possible.
2. 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 whirl-pak bag with the plotID, horizon, collection date that matches a set of whirl-paks, and "-comp" for composite. These may be pre-printed cryo-safe labels or hand-written. *Ex. CPER_001-M-20140101-comp*

   Place that bag with the corresponding whirl-paks.
3. Repeat step 2 for every unique combination of plotID, horizon, and collection date. There should be 1 new whirl-pak bag for every unique combination.
4. Thaw the material in a set of whirl-paks and transfer all material in each whirl-pak into the corresponding composite whirl-pak bag. Homogenize the soil by gently kneading and/or shaking the outside of the closed whirl-pak.
5. Return the sample bags to the -80°C freezer (or container of dry ice, if no freezer is accessible) immediately.
6. Repeat steps 4-5 for the remaining samples.
7. Complete the data sheet and/or the data entry application by recording the sample information from the empty whirl-pak bags. Ensure that the data sheet was completed correctly and completely, and discard empty whirl-paks. If using barcode labels, scan the barcode for each associated sample.
   - 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 data entry application.
8. Ship samples to contract facility as outlined in SOP J.

SOP I 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 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. 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, 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 on hand to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

If an entire bout is missed, no data need to be entered for protocol-specific apps; however, plot- or site-level events that prevent sampling must be tracked in the Site Management data entry application (and also with a problem ticket). Refer to the domain manager and to the Science Team for additional guidance on recording such events.

I.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 (typically within a week of collection). 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***.

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

I.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 F. 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). 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).

I.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 data entry 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 J Sample Shipment

Information included in this SOP typically conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA intranet site (available through the Sampling Support Library) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

Reminder: All required field and domain lab data for a batch of samples that are being shipped should be entered electronically *before* shipping can occur. Any expected delays in data entry for samples that must be shipped should be communicated to Science and to CLA as soon as possible and prior to shipping.

J.1 Handling Quarantine Material

In order to protect against the spread of potential plant pathogens or unwanted pests, USDA regulates the transport of soil – especially from outside the continental US and from quarantine areas. Details of these regulations are provided in section 5.1 and explicit soil shipment instructions are provided below. Note that quarantine shipping regulations do not apply to shipping KCl extracts from the soil N transformations SOP.

J.2 Preparing shipping documentation

Creating a shipping inventory: Whenever samples are shipped, they must be accompanied by a hard-copy inventory enclosed within each shipping container. In addition, a corresponding electronic version of the file must be emailed to the laboratory and the NEON CLA contact as soon as possible after the samples have been shipped.

1. Navigate to the "Shipping Information for External Facilities" document on the CLA intranet site. There, you will find instructions on which items (Permit or Compliance Agreement, cover letters, etc) are required to include in the shipment. Check this document each time a new shipment is prepared as it is subject to change.
2. Print out required documents as needed to include in shipment box. Affix any labels (e.g., PPQ) required by the Compliance Agreement/Permit(s).
3. Prepare a shipping inventory detailing the contents of the shipment, using the appropriate shipping applications (this requires the use of the Shipping: Shipment Creation, Shipping: Shipment Review, and the Stork Shipment Verification Tool). Print a copy of the inventory (which can be downloaded from the Stork Shipment Verification Tool) to include in the shipment box.

J.3 Preparing oven-dried and air-dried samples for shipment

Oven-dried and **air-dried** samples are shipped at ambient temperatures and do not require rush delivery. No hazardous or dangerous DOT regulated materials are shipped with these soils; however, receiving of quarantine soils is regulated by the USDA. Receiving labs must have either a Permit to Receive Soils or a Compliance Agreement in order to receive soils.

1. Place **oven-dried BGC soil sample vials** into one of the cardboard trays that the scintillation vials come in, then place tray inside a small garbage bag and close snugly with a twist tie or overhand knot so that vials are held in place and the bag will not leak in case of sample breakage. Line a corrugated cardboard box with a large trash bag, then place smaller bag inside. Make sure air is out of the bags, then close outer trash bag with twist tie or overhand knot.
2. For **air-dried BGC archive soil sample jars**, line box with large trash bag and pack samples within bag. Make sure that air is out of all the bags, then close outer trash bag with a twist tie or overhand knot.
3. Fill empty space in shipping box with abundant cushioning material (i.e. peanuts, newspaper) to prevent glass containers from shifting and breaking in transport.
4. Insert the hard copy shipping manifest, along with any other required agreements and permits, into a resealable plastic bag and add to each shipping container prior to sealing.

J.4 Preparing microbial genetic samples, KCl extracts, and microbial biomass samples for shipment

Samples for **microbial molecular analysis**, **microbial genetic archiving**, **KCl analysis, and microbial biomass** analysis are shipped on dry ice via overnight delivery. Note that dry ice is a Class 9 regulated material and must be shipped according to CFR 49 Subchapter C, Hazardous Materials Regulations.

Dry ice releases carbon dioxide gas which can build up pressure and rupture packaging. Ensure the packaging used allows the release of this pressure to prevent rupturing the package. Dry ice must be packaged using **UN packing group III** compliant materials. The maximum amount of dry ice per package is **200 kg**. Refer to Chemical Hygiene Plan and Biosafety Manual (AD[03]) for additional requirements on commercial shipment of hazardous or dangerous materials.

1. Organize samples.
    - Microbial genetic analysis and archive: Genetic archive samples may need to be shipped to a different facility than genetic analysis samples, or be treated differently when arriving at the same facility. Organizing the samples prior to shipment ensures that the receiving lab will process the samples correctly. Also, please use the recommended bag sizes in order to reduce precious freezer space.
        - Separate the subsamples destined for genetic archive (sample ID's ending with "-ga") from samples destined for genetic analysis (ending with "-gen" or "-comp"). The subsamples should already be organized together into labeled, cryogenic freezer boxes(e.g.Figure 13). If not, organize the vials from the same plot/site into cryo boxes with cryo-safe labels containing information on the site, collect date(s), and plotID(s). Ensure that all cryovial sample ID's and barcode numbers have been entered electronically. Ensure that each vial has a unique geneticArchiveID (ex. *BLAN_001-13.5-20 20160415-ga3*).

Figure 13. Example of a labelled cryo-box containing genetic archive samples.

- Place genetic analysis samples (ending in "-gen") from the same bout into a resealable freezer bag of sufficient size (e.g. 1-gallon). Label the bag with the plotID(s) + collectDate(s) + 'gen' (ex. *BLAN_001 20160415 gen*). Multiple plotID's may be placed in the same bag. If more than 1 bag is required to contain all samples from a bout, label the bags sequentially (e.g. "Bag 1 of 2").
- Place metagenomics samples (ending with "-comp") from the same bout into a resealable freezer bag of sufficient size (e.g. 1-gallon). Label the bag with the siteID + collectDate(s) + 'comp' (ex. "BLAN 20160415 comp"). If more than 1 bag is required to contain all samples from a bout, label the bags sequentially (e.g. "Bag 1 of 2").

- All other samples: Place frozen samples from the freezer into 1-gallon resealable freezer bags.

2. Use corrugated cardboard boxes which meet UN packing group III requirements. Add Styrofoam along the walls of the box as insulation. Ensure the Styrofoam IS NOT sealed to be airtight. Styrofoam must not be used as an outer packaging.
3. Weigh samples, either by placing into bags or in a pre-weighed box (not the shipping container). Record the weight for later use. If samples will be left out > 5 minutes, replace samples in freezer.
4. Place a large trash bag open inside the shipping container. This serves as secondary containment for soil samples in case of breakage. All dry ice and samples will be placed within the trash bag.
5. Weigh the empty shipping container and record the weight for later use.
6. Place a layer of dry ice to the bottom of the pre-weighed shipping container. Add the samples, then surround the samples on all sides with more dry ice. Add a layer of dry ice to the top of the samples, then re-weigh the container and its contents. Subtract the weight of the empty container and samples from the total: this is the total weight of dry ice.

- IMPORTANT: Use an appropriate box size for the samples: Samples should occupy at least 25% of the volume of a box, but not more than 50%.
- NOTE: Some local carriers limit the weight of dry ice per package to 2.5kg. Check with your local shipping carrier to check weight limits.
- If weight restrictions apply, use cold-soaked packing peanuts, or similar, to keep samples frozen.

7. When packing items in the container, put dry ice and specimens as close together as possible and add more dry ice on top. Between 40-60% of the volume of the box should be occupied by dry ice, but do not overload the box with dry ice. Fill any remaining empty space with wadded newspaper, Styrofoam peanuts, or bubble wrap. Empty space will cause the dry ice to sublimate faster. As dry ice sublimates, specimens will move around in packaging; cushioning provides additional protection for samples during shipment.
8. Complete packaging and labeling for Class 9 dry ice hazard shipment.
9. Insert the hard copy shipping manifest, along with any other required agreements and permits, into a resealable plastic bag and add to each shipping container prior to sealing.

J.5 Shipping samples

1. Submit the shipment on the Stork Verification Tool (linked from the SSL) to email the shipment manifest and receipt forms to all parties.
2. Complete packing slip, address shipment, and ship using the delivery method required for the sample type to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.

J.6 Timelines

Ship samples according to the sample-specific conditions described in SOP J. Microbial molecular samples and samples that have been air-dried or oven-dried prior to shipment do not "expire"; however, to decrease build-up of samples in the domain facility and avoid data product delays, it is better to ship quickly (e.g., within 6 weeks of collection) so that samples are not lost or damaged. If there is an issue with the ability of a receiving laboratory to accept samples (e.g., contract not established, problem with soil permit), the shipment may have to be held back. In this case, please submit a problem ticket; ***never discard samples without consulting NEON HQ Staff***.

J.7 Return of Materials or Containers

If using insulated shipper kits or other reusable containers, include return ground shipping forms and instructions for the laboratory to return shipping materials.

J.8 Laboratory Contact Information and Shipping/Receipt Days

For laboratory contact information and allowable shipping/receipt days, see CLA's NEON intranet site, available through the Sampling Support Library. In general, frozen samples being shipped overnight should only be sent Monday-Wednesday.

SOP K 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.

K.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 14. These locations do not have to be exact.

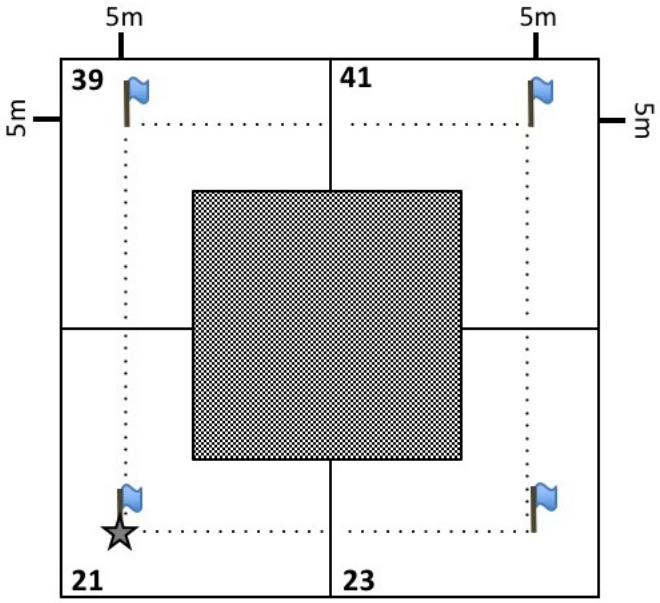


Figure 14. Schematic of TOS soil plot demonstrating the general layout of sample locations. Subplot ID's are noted in the left corner of each subplot. Flags denote the corners for the depth transect measuring area, star indicates the location to begin measurements. Dots indicate the general distribution of depth measurements.

K.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. 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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Dane, J.H., and G.C. Topp (Eds). 2002. *Methods of Soil Analysis, Part 4: Physical Methods*. Soil Science Society of America, Madison, WI. 1692 pp.

Gomez, J.D., K. Denef, C.E. Stewart, J. Zheng, and M.F. Cotrufo. 2014. Biochar addition rate influences soil microbial abundance and activity in temperate soils. European Journal of Soil Science 65: 28-39.

Robertson, G.P., D.C. Coleman, C.S. Bledsoe, and P. Sollins (Eds). 1999. *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, New York, NY. 480 pp.

Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, and M.E. Sumner (Eds). 1996. *Methods of Soil Analysis, Part 3: Chemical Methods*. Soil Science Society of America, Madison, WI. 1390 pp.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 17. Datasheets associated with this protocol.

NEON Doc. #	Title
NEON.DOC.001577	Datasheets for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling

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

APPENDIX B QUICK REFERENCES

Table 18. Checklist of samples and analyses associated with an off-year soil sampling bout. ✓ = measurement; X = physical sample.

Bout Type	Sample Timing	Soil temp (field)	Microbes and archive (field)	Metagenomics (field)	Soil moisture (lab)	Soil pH (lab)
microbes	Transition	✓	**X** whirlpaks (top horizon)		✓	✓
	Peak greenness	✓	**X** whirlpaks (top horizon)	**X** 1 plot-level whirl-pak (top horizon)	✓	✓

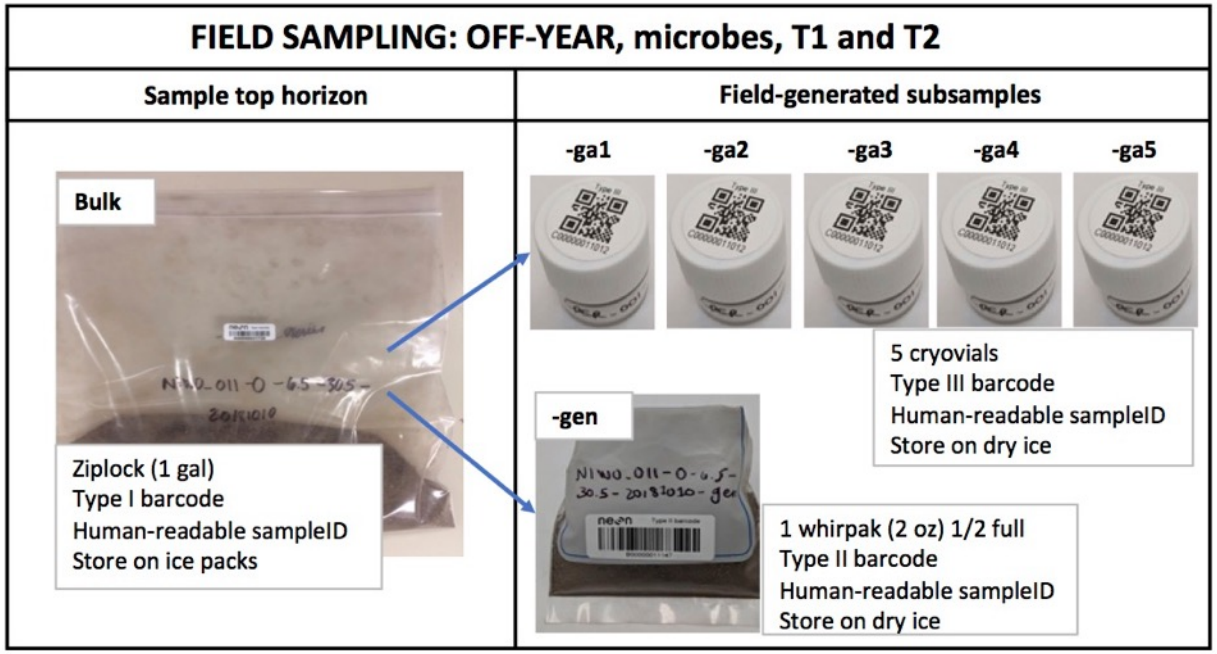


Figure 15. Field-generated samples for boutType= microbes, sampleTiming= T1 or T2.

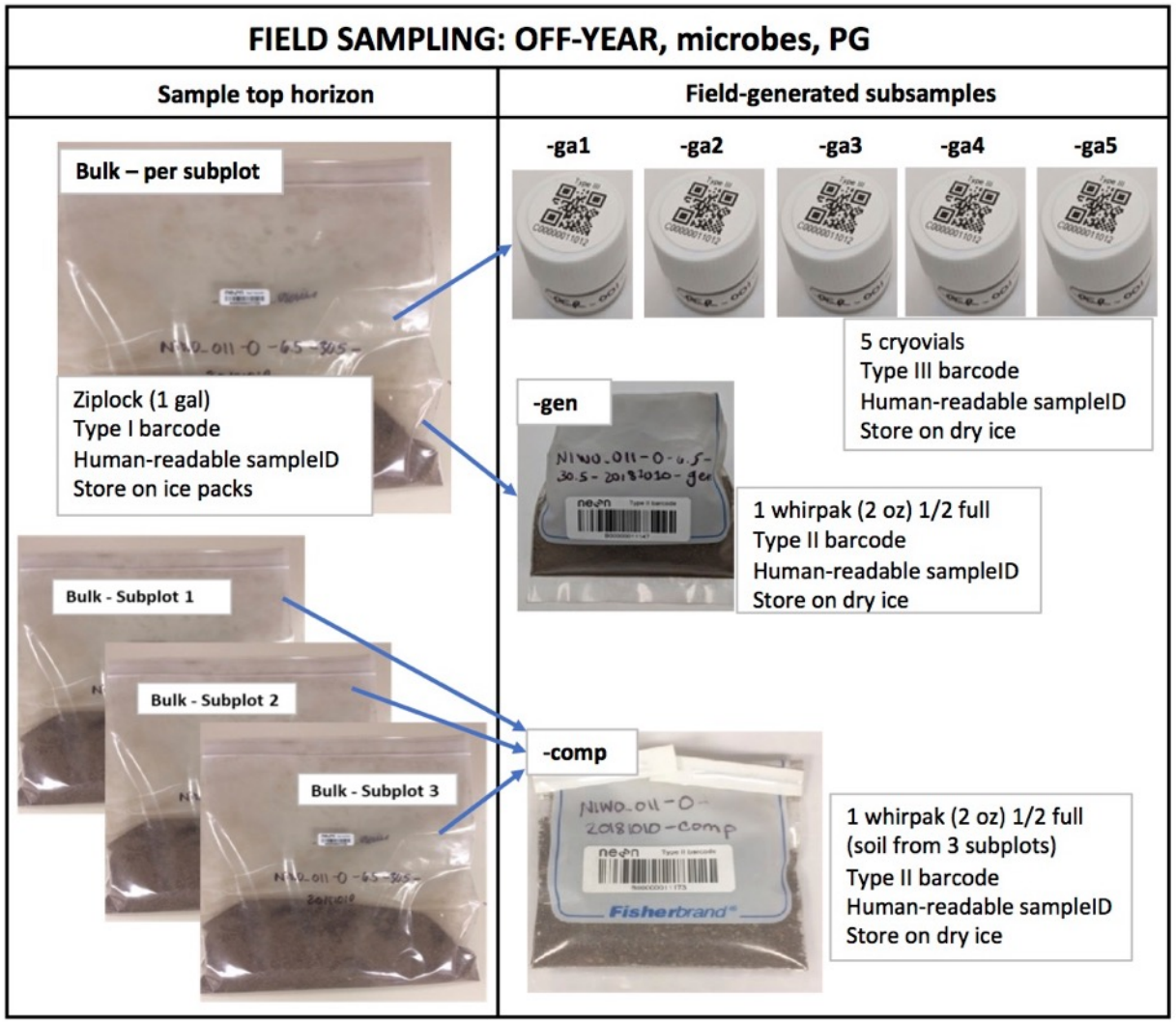


Figure 16. Field-generated samples for boutType= microbes, sampleTiming= peak greenness.

Table 19. 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 moisture (lab)	Soil pH (lab)	KCl extraction (lab)	BGC measure and archive (lab)
$T_{initial}$ sampling									
microbesBiomass	Transition	✓	**X** Whirl-paks (top horizon)		**X** sieved soil (2 horizons)	✓	✓	**X**	
microbesBiomassBGC	Peak greenness	✓	**X** Whirl-paks (2 horizons)	**X** plot-level whirl-pak (2 horizons)	**X** sieved soil (2 horizons)	✓	✓	**X**	**X** air-dried soil (2 horizons)
T_{final} sampling									
Field only	All	✓				✓		**X**	

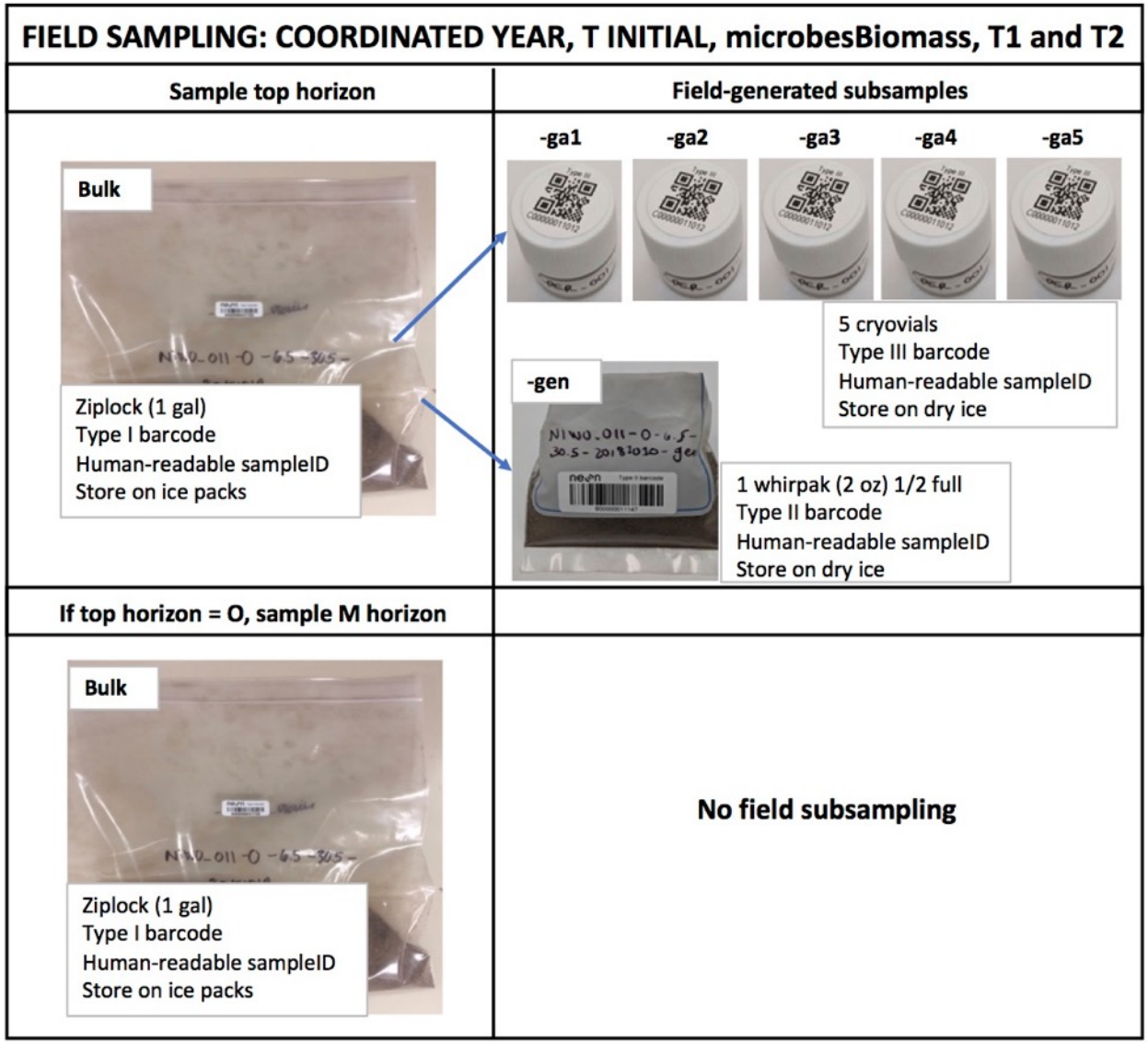


Figure 17. Field-generated samples for boutType= microbesBiomass, sampleTIming= T1 or T2.

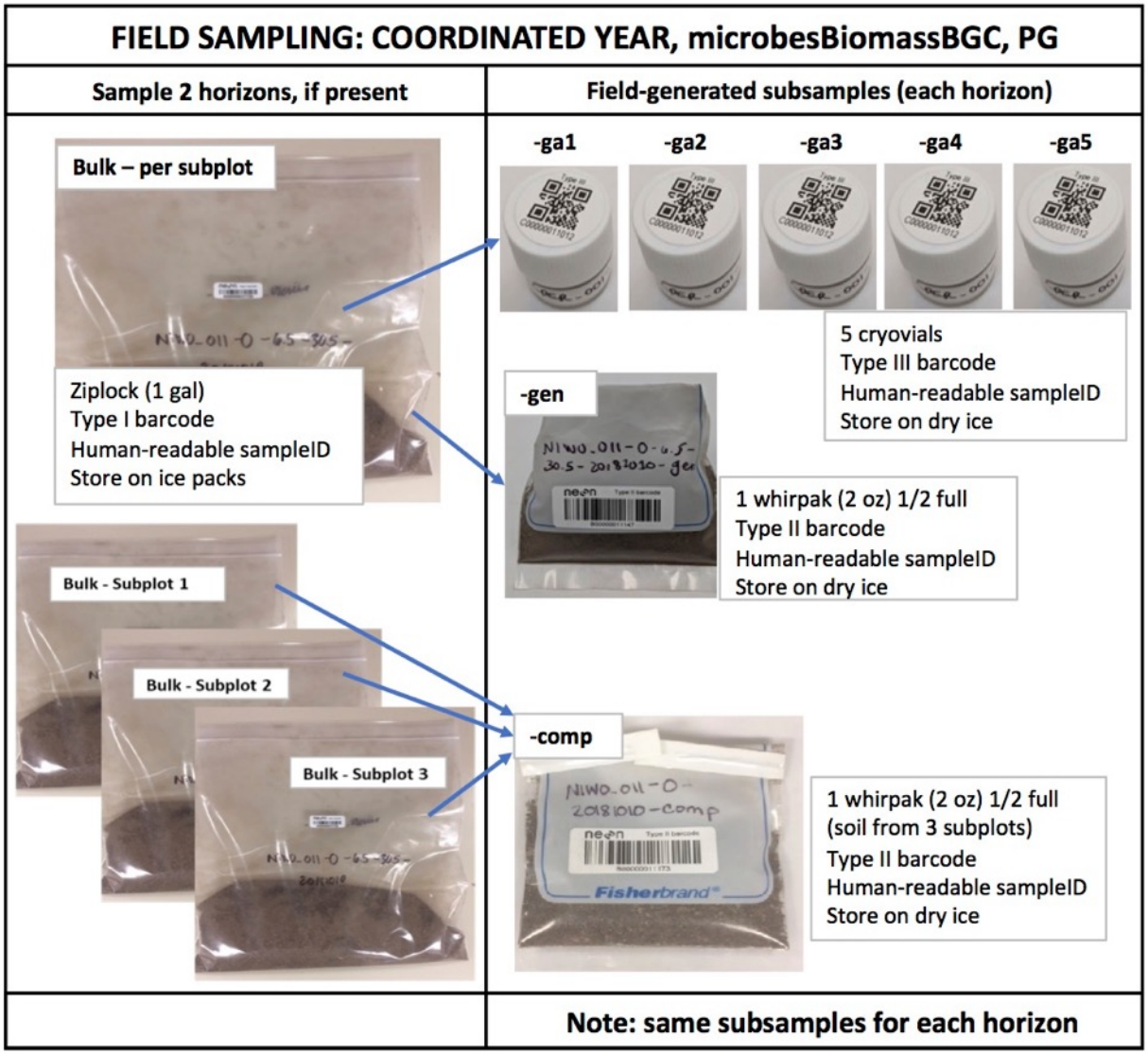


Figure 18. Field-generated samples for boutType= microbesBiomassBG, sampleTiming = peak greenness.

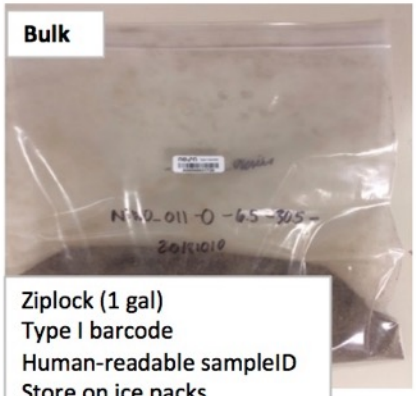
FIELD SAMPLING: COORDINATED YEAR, T FINAL, T1 or T2 or PG	
Sample 2 horizons, if present	
	**No field subsampling**

Figure 19. Field-generated samples 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 a peak greenness bout, 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. Record barcode label(s).
>
> **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 data entry 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 data entry 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 C 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 data entry 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 data entry 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 Fridays.

Microbial Biomass Samples: Be sure to…

- ☑ Within 24 hours 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 24 h of collection.
- ☑ Filter extracts and store in labeled, barcoded scintillation vials at -20° C.
- ☑ Ship KCl extracts on dry ice to the appropriate labs according to the Domain schedule. 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 electronic data entry application?

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 D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are based on historic records and 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. Estimated dates provide general guidance of when each domain can expect ground to be suitable for sampling.

Table 20. 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 – June 30 (18)	Aug 1 – Sept 1 (21)	Oct 28 – Nov 27 (24)
15	ONAQ	Mar 1 – Apr 1 (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 LENO	T1: If flooding prevents a scheduled sampling event, then the bout may be rescheduled through no later than May 31	Unpredictable spring flooding
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.
D13	NIWO	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

APPENDIX E SITE-SPECIFIC INFORMATION

E.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.

Site	Quarantined Materials	Containment Action
Mountain Lake Biological Station	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.

E.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.

E.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: 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 20 for site-specific photos that can help guide where the soil starts.
2. Place coring device in the brownie footprint and insert it into the 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
4. Process the material following the rest of the instructions in SOP B. 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 nitrogen transformation bout**, install incubation cylinder close to initial core location.
    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 F
    d. Return cut-away surface vegetation so that it covers/buries the cylinder.
        i. Use a pin flag, flagging tape, or some other marker (site host permitting) to assist in relocating the core.

e. Follow all instructions for collecting the incubated cylinder as described in SOP F. 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).

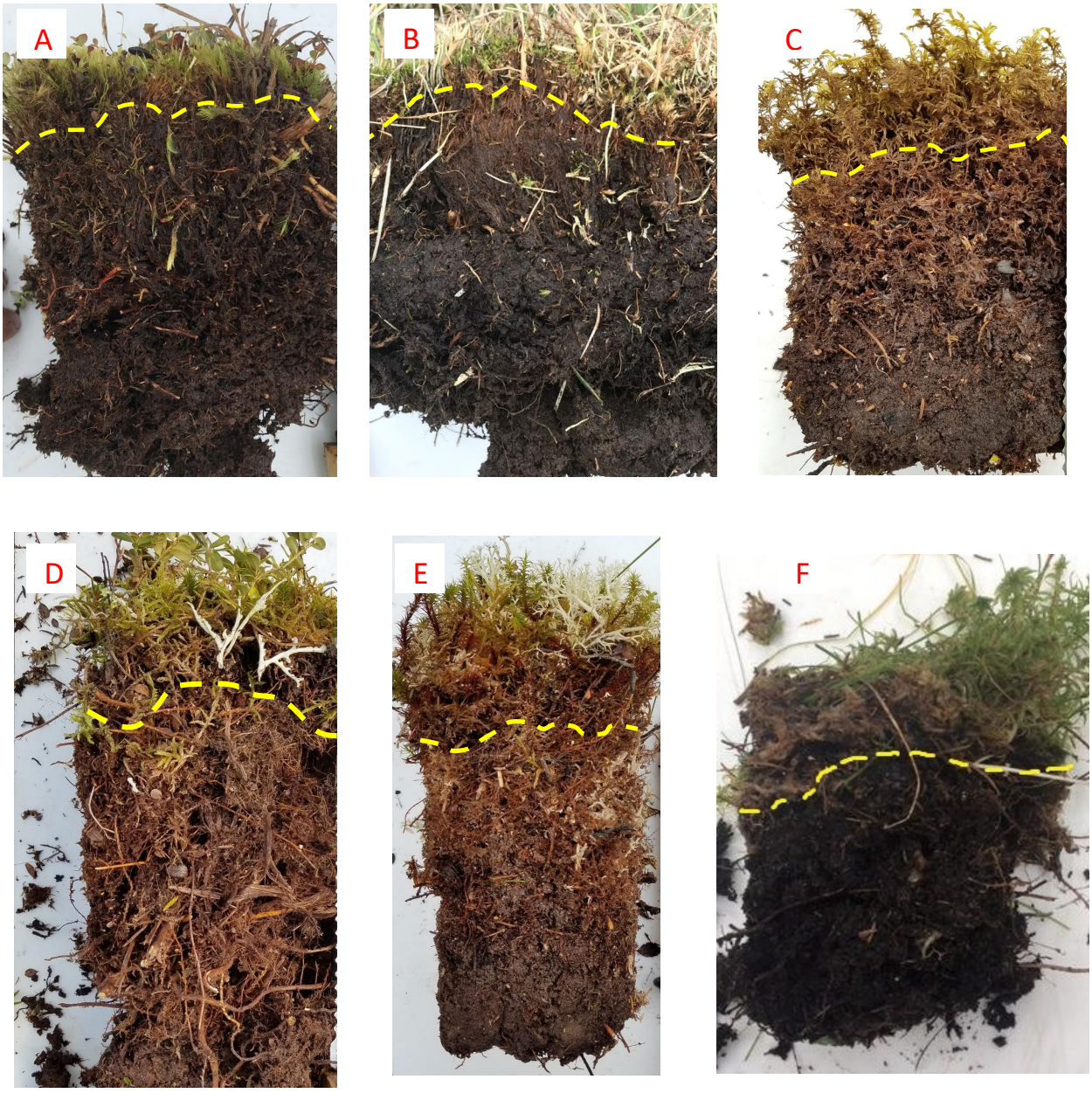


Figure 20 Images demonstrating the start of the soil surface (yellow dashed line) at TOOL (A), BARR (B), DEJU (C), HEAL (D), BONA (E), and TREE (F). Source: NEON Terrestrial Instrument System (TIS), in collaboration with NRCS.

E.4 Soil horizons from NRCS Initial Characterization

Table 21. 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)
1	BART	15	15	14	3	30
1	HARV	18	18	5.5	1	140
2	SCBI	18	3	4	4	5
2	SERC	20	0	--	--	--
3	DSNY	16	0	--	--	--
3	JERC	23	0	--	--	--
3	OSBS	23	8	10	5	40
4	GUAN	18	0	--	--	--
5	STEI	14	2	62.5	25	100
5	TREE	11	6	6.5	3	100
5	UNDE	13	7	76	3	200
6	KONZ	13	0	--	--	--
6	UKFS	17	0	--	--	--
6	KONA	19	0	--	--	--
7	GRSM	13	10	5	2	9
7	ORNL	20	5	4	2	5
8	DELA	21	1	2	2	2
8	LENO	18	0	--	--	--
8	TALL	26	1	10	10	10
9	DCFS	14	0	--	--	--
9	NOGP	18	1	4	4	4
9	WOOD	15	6	10	1	18
10	CPER	17	0	--	--	--
10	STER	11	0	--	--	--
11	CLBJ	11	0	--	--	--
11	OAES	15	0	--	--	--
13	MOAB	11	0	--	--	--
14	JORN	16	0	--	--	--
14	SRER	12	0	--	--	--
15	ONAQ	11	0	--	--	--
16	ABBY	15	9	3	1	9
17	SJER	10	1	1	1	1
17	SOAP	12	6	6.5	2	12
18	BARR	20	21	80	10	120
19	DEJU	18	16	8.5	4	48
19	HEAL	15	15	17	5	60
20	PUUM	10	9	5	1	30

E.5 Site-specific soil sampling devices

Table 22. 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 1/4 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 1/4 inch diameter. Alt: JMC Backsaver, handle (part# PN001) plus sample tube (part# PN012) 12 inch x 1 1/4 inch diameter
	KONZ	Very rocky, shallow soils	
	KONA	NA	
07	ORNL	Variable	AMS auger 2 inch diameter
	MLBS		
	GRSM		
08	TALL	Sandy soils	AMS auger, part # 400.08 Maximo #110504 2 1/4 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	JMC Backsaver, handle (part# PN001) plus sample tube (part# PN012) 1 1/4 inch diameter
	DCFS	NA	NA
	NOGP	Dry, rocky soil	AMS auger, 3 1/4 inch diameter
10	CPER	NA	JMC auger, part# 072, 2 inch diameter or AMS auger, part# 402.36 2 1/4 inch diameter
	STER	NA	
	RMNP	Rocky soil	
11	CLBJ	Sandy soils	AMS sand auger, part# 400.42 2 1/4 inch diameter
	OAES		AMS auger, part# 400.08 2 1/4 inch diameter

12	YELL	NA	AMS auger, 2 inch diameter
13	NIWO	Rocky soil	see D10 entries
	MOAB	Sandy soil	AMS Auger, part# 400.08 2 1/4 inch diameter
14	JORN	Sandy soil	AMS Hex QP Sand Auger, part# 58536 2 1/4 inch diameter
	SRER	Sandy soil	
15	ONAQ	Rocky soil	AMS Auger, part# 400.06 3 1/4 inch diameter
16	ABBY	Organic and Mineral Soils	Forestry Suppliers carbon steel auger, mud/clay, part# 78400, 2 inch diameter
	WREF		
17	SJER	NA	AMS auger, part# 400.08 2 1/4 inch diameter
	SOAP	NA	
	TEAK	NA	
18	TOOL	Gelisols: thick organic horizon, cryoturbation	Soil monoliths cut with a hori-hori 4 x 4 inch square template
	BARR		
19	HEAL	NA	
	DEJU	NA	
	BONA	NA	
20	PUUM	NA	NA

APPENDIX F 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:

- 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 the X, Y coordinates for each 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.

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**.

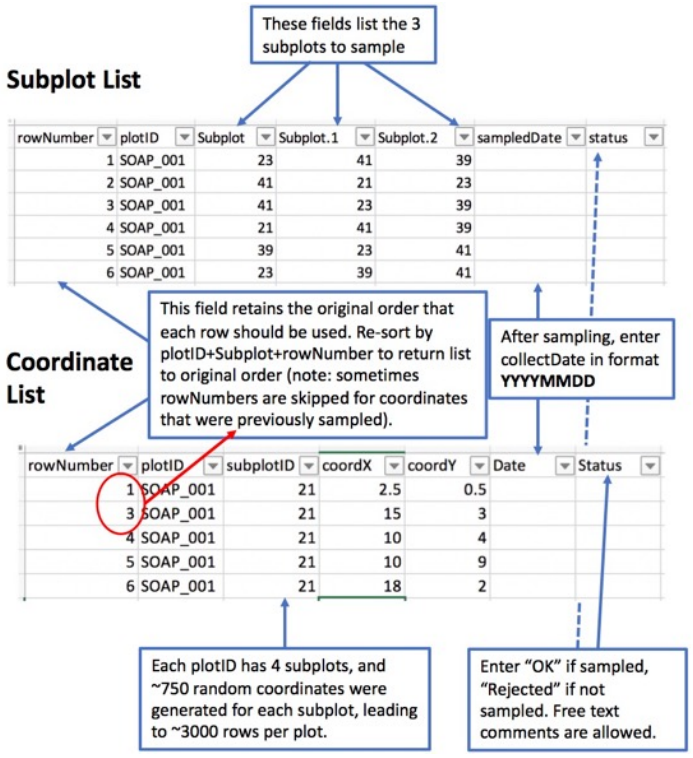
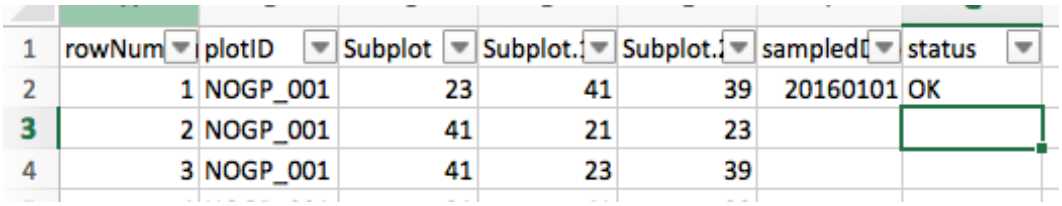


Figure 21. Anatomy of the subplot and coordinate lists

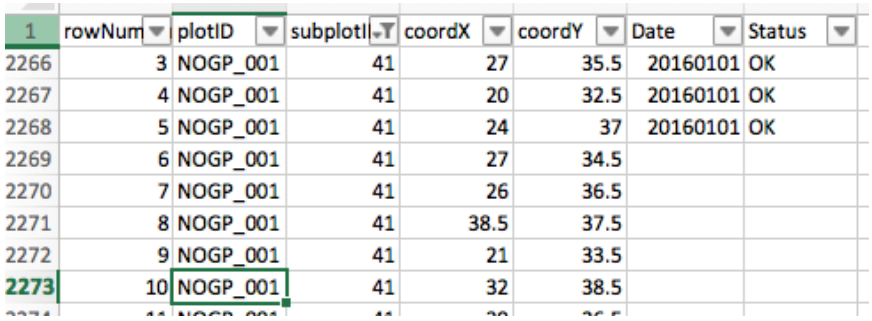
F.1 Preparing for a bout

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.
        i. 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.
        ii. 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.
        iii. Either print these rows or copy and paste into a separate spreadsheet for printing later. Be sure to copy the plotID, subplotID, coordX, coordY, Date, and Status fields. In the following example, you would start with coordinates x=27, y=34.5.



	rowNum	plotID	subplotI	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		

        iv. Now that you have one subplot completed, repeat the steps outlined above for the remaining 2 subplots designated for sampling.
    b. Once you have obtained the coordinates for each subplot for a given plotID, move onto the next plotID. Repeat steps 3a.i-3a.iv 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.

F.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'

F.3 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.