TOS PROTOCOL AND PROCEDURE: SOIL BIOGEOCHEMICAL AND MICROBIAL SAMPLING

PREPARED BY	ORGANIZATION	DATE
Lee Stanish	SCI	1/15/2018
Samantha Weintraub	SCI	1/15/2018
Eve-Lyn S. Hinckley	SCI	12/31/2014
Jacob Parnell	SCI	12/31/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Mike Stewart	SYE	01/17/2018
Dave Tazik	SCI	01/18/2018

RELEASED BY	ORGANIZATION	RELEASE DATE
Judy Salazar	CM	01/19/2018

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling and domain lab processing of soil samples in order to measure physical properties, carbon (C) and nitrogen (N) concentrations and stable isotopes, nitrogen transformation rates, and microbial biodiversity and function. These data can be used to quantify the stocks of soil C and N; they can also reveal ecosystem nutrient status, paint a picture of integrated ecosystem processes, and allow us to understand rates of key microbially-mediated processes in relation to microbial biomass and community composition. During each sampling, NEON will characterize 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.

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 will 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^- g^{-1} dry soil d^{-1}) or on an areal basis, normalized by bulk density (e.g., g NO_3^- m^{-2} d^{-1}). This calculation requires knowing the concentration (or amount) of NH_4^+ plus NO_3^- (net N mineralization) or NO_3^- (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.

Microbial measurements. Microbial biomass provides an indication of microbial activity and correlates with numerous ecological processes, such as soil productivity and N mineralization rates. Microbial biomass will be 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 will provide information on the functional potential of the microbial communities as well as changes in genomes and genome content through time. Finally, soil samples will be collected and preserved in a manner that should enable the community to use archive samples for RNA-based analyses.

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 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 archives.

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

This protocol is based closely on standard soil sampling methods as described by the Soil Science Society of America (Sparke et al., 1996; Dane et al., 2002), and methods published by the Long-term Ecological Research Network (Robertson et al., 1999). The latter reference reviews many studies on this topic that have compared different standard operating procedures. The protocol for 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.014051	NEON Science Performance QA/QC 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: Core Sampling for Plant Belowground Biomass
RD[09]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Sampling
RD[10]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration
RD[11]	NEON.DOC.004459	Incubation Cylinder assembly instructions
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
$CaCl_2$	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

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 or wetland sites, and layer should be darker in color and friable (easily crumbled). If you feel more than a couple of mineral grains (grit from sand, stickiness from clay, silt deposits on hands) it is most likely a mineral horizon high in organic matter (OM), not an organic soil.

Litter layer: Loose, unconsolidated plant material on top of the soil surface that is intact or partially shredded, but still easily recognizable as plant material. Not all sites will have a litter layer.

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

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 must be collected in order to capture

variability at multiple scales (e.g., soil core, plot, site). NEON Science will supply domain staff with a master list of plots where soil samples will be collected for the duration of Operations. The list will also contain randomly generated x,y coordinates originating from the southwest corner (i.e., the reference point) of each plot on the list; these 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 core they will be separated prior to analysis. However, other sub-horizons will not be separated (e.g., mineral sub-horizons A and Bw). 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, section E.4.

In addition, the depth of soil to saprolite or bedrock will vary across domains. NEON soil sampling shall be conducted to a maximum depth of 30 ± 1 cm where possible. More detailed characterization of the dominant soil types will occur during the construction period of NEON through two projects. One project will be led by the Terrestrial Instrumentation System (TIS) and includes thorough description of one large soil pit dug at the NEON tower location from the surface to 2 meters depth (or bedrock, whichever is shallower) at each core and relocatable site. The second project is carried out by the U.S. Department of Agriculture (USDA) and the Natural Resources Conservation Service (NRCS) and will characterize soil physical and chemical properties (including bulk density) to 1m depth at a subset of the Terrestrial Observation System (TOS) distributed soil plots.

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, seek a new location as close as possible to that of the predetermined sampling location, and note the new sampling location. 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 C and N Concentrations and Stable Isotopes (hereafter, Soil BGC Measurements). Soil samples collected for BGC measurements undergo preliminary processing in the domain laboratory. This consists of sieving and drying soils according to SOP D. Subsamples of these soils are also analyzed for pH and moisture at the domain support facility; another subsample is prepared for archiving.

Microbial Community Analysis. Subsamples are either put on dry ice in the field (for molecular analysis), or kept field moist (for biomass analysis), as described in SOP B, and shipped to the contracted laboratory facility for processing and analysis. These soils are also subsampled for measurement of soil pH and moisture at the domain facility. During the peak greenness bout, composite samples of cores

from the same plot will also be generated in the field for a series of molecular –omics analyses. These composite samples are treated the same as all other molecular samples.

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 a capped PVC or stainless steel cylinder (bottom left open) and is replaced in the soil. This "final", incubated core remains 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^+ and NO_3^-. Initial soil samples are also analyzed for both soil pH and moisture content, while final samples are only analyzed for moisture.

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

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

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC 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 will be 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 will affect the results, so these features are taken into account in the spatial component of the sampling design. At the different NEON sites, sampling frequency may vary depending on the 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.

Soil BGC. Soil BGC measurements will be made once every 5 years at 10 plots per site during the peak greenness window. When soil BGC is measured, subsamples of the soil cores must also be analyzed for microbial community, microbial biomass, N transformations, soil pH, and soil moisture.

Microbial Community Analysis. Microbial sampling will occur at the same plots that are designated for biogeochemical sampling (10 per site). Microbial communities will 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 will sample during peak greenness, while the other two sampling events will 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 onset and cessation of annual sampling per site are listed in Appendix D, and site-specific sampling windows per bout are provided in Appendix E. When sampling for soil BGC, soil for microbial analyses shall be collected concurrently; soil for microbial analyses will be a subsample of the soil core collected for BGC measurements.

Nitrogen Transformations and Microbial Biomass. Soil measurements of microbial biomass and N transformations will be conducted at all designated soil plots within a site every 5 years. Microbial biomass and soil N transformations tend to be variable both in space and time. To account for seasonal variation, three sampling events will occur during a sampling year in conjunction with the regular microbial sample collection. For N transformations, soil collected from the T-initial core should be used to generate the soil subsamples for moisture, pH, microbial and soil bgc analyses, when applicable.

Other Linked Biogeochemical Measurements. Periodically, a suite of synchronized measurements will be conducted aimed at characterizing plant and soil biogeochemical dynamics. Sampling for soil microbial community analysis, microbial biomass, N transformations, and BGC measurements should also be completed during a linked biogeochemistry year. 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: Core Sampling for Plant Belowground Biomass (RD[08])
- TOS Protocol: Canopy Foliage Sampling (RD[09])

Table 1. Target timing of coordinated measurements.

Off-Year			Coordinated 5 year		
T1	**PG**	**T2**	**T1**	**PG**	**T2**
P	P	P	P	P	P
M	M	M	M	M	M
	G			G	
			B	B	B
			N	N	N
				BGC	

For each type of sampling year, the three time periods are abbreviated as **T1**: Transition 1; **PG**: Peak Greenness; **T2**: Transition 2. Abbreviations: **P** – soil moisture and pH (physical attributes); **M** – microbes (excluding metagenomics); **G** metagenomics; **B** – microbial biomass; **N** – N transformations; **BGC** – C and N concentrations, stable isotopes, and archiving.

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 E, 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 sampling bout (e.g. microbes-only), at least 50% of samples must be collected in order for the bout to be considered complete. During a coordinated bout (e.g. microbes, N-transformation rates, and/or BGC sampling), 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, discard samples and report the issue to Science using NEON's issue tracking software.

Soil BGC Measurements. Sampling of soil cores for biogeochemical and soil microbial community analysis (one large, combined bout) will occur during peak greenness. This period is intended to capture all sites at peak biological activity.

Microbial Community Analysis. At most sites, sampling bouts will 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. [When soils for microbial analyses are collected as part of the soil BGC bout, this counts as one of the three sampling periods per year]. 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 E. Note that due to the short growing season, Domains 18 and 19 are only sampled during the peak greenness bout.

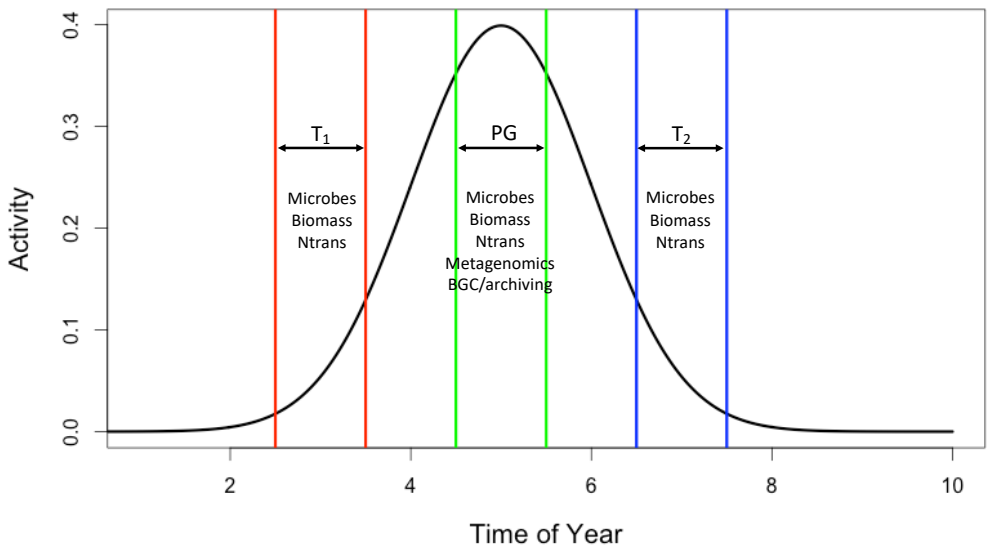


Figure 1. Generalized Timing of Soil Sampling

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 2. 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	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 (increasing activity) season
	Wet-dry transition	8, 16	• Initiation of dry (decreasing activity) season
Peak Greenness	Peak Greenness	All	• Timing of peak above-ground biomass
Seasonal Transition #2	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 (decreasing activity) 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 Community Analysis (above) in order to capture similar seasonal characteristics and enable linkages between microbial and biogeochemical data. An N transformation incubation should last 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 will 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 E. N transformation sampling should begin and end within the site-specific sampling periods defined in Appendix E, whenever possible. The end of the incubation may extend beyond the sampling window if 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 will also be 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) will only sample during the peak greenness period.

4.3 Timing for Laboratory Processing and Analysis

Soil BGC Measurements. Soil cores that are collected for BGC analyses must be placed in a cooler with ice packs, then be transferred to a 4°C refrigerator and processed within 48 hr. The exception is if samples are collected on a Friday, in which case they may be processed first thing Monday morning. Soil core subsamples destined for biogeochemical analyses that remain unchilled for more than 8 hours should be discarded, and Field staff should communicate with Science via NEON's issue tracking software to reschedule the sampling bout.

Microbial Community Analysis. Microbes respond rapidly to changes in their environment: these changes can influence microbial activity and community composition, and can lead to extensive RNA degradation. As such, soil samples collected for microbial community analyses must be put on dry ice immediately and then transferred to a -80°C freezer as soon as possible: failure to keep these samples frozen compromises the samples and they cannot be used. If this happens, notify NEON Science staff to reschedule the sampling bout.

Nitrogen Transformations. Soil cores collected for this purpose should be 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 should be stored refrigerated at 4°C. Due to the short shelf life of samples, it may be necessary to break up field work to ensure that processing begins within 24 h. Staff may split a bout into 'minibouts' within a sampling window or utilize multiple teams.

Microbial Biomass. The fatty acid composition of microbes in a soil sample can change within hours. Samples must be sealed well to avoid moisture gain/loss and kept in a cooler on ice packs as soon as possible. At the domain support facility, samples should be sieved within 24 hours of collection and then stored in a -80°C freezer until shipped. Samples should be shipped within 14 days of sample collection.

Soil pH and moisture. Soil pH and moisture will be measured by domain staff whenever soils are collected. Processing of subsamples for pH and moisture analysis must be conducted on soil kept cold (refrigerated or in a cooler with ice packs) and must begin within 48 hr of collection (or immediately upon return to the laboratory, if field staff are working remotely or sampling occurred on a Friday; a maximum of 72 hours).

4.4 Sampling Timing Contingencies

Table 3. Contingency decisions for all soil measurements.

Delay/Situation	Action	Outcome for Data Products
Inability to finish sample bout	Communicate to staff scientists via NEON's issue tracking mechanism	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 Soil 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.

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 (relocatable sites). 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 Operations 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.

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. If using a hacksaw to cut PVC incubation cylinders, (1) use new blades, (2) mark the pipe where it needs to be cut first, (3) seat the teeth into the PVC by holding handle of hacksaw firmly with one hand and placing other hand on top of saw, pushing firmly, and (4) saw carefully, do not to lose track of the line marker or go so fast that the saw jumps out of the cut.

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 samples for shipment and resources for determining the quarantine status of NEON sites. Additional site-specific quarantine instructions can be found in Appendix E. 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 (RD[13]). 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 J, with additional guidance on the CLA intranet 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 4. 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	S	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 30 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

Supplier	Supplier Number	R/S	Description	Purpose	Quantity	Special Handling
		R	Deionized/distilled water	Rinse soil from equipment	2 liters	N
		S	Survey marking 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						

Supplier	Supplier Number	R/S	Description	Purpose	Quantity	Special Handling
		R	Weatherproof, adhesive barcode labels	Labeling sample containers with barcode-readable labels	1 sheet	N
Grainger Arrow Amazon	5NHH1 5520 B00006IBUV	R	Weatherproof adhesive labels	Labeling sample containers with human-readable labels	30-80	N
Ben Meadows Forestry Supplier	010510-1 49247	R	All weather copy paper	Print datasheets		N
		R	Permanent marker, fine tip	Label sample containers	3	N
		S	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
		S	Paper towels	Remove debris from soil sampling equipment	1 box or 2 cloths	N
Resources						
RD[05]		R	Field datasheet	Record data		N
		R	X,Y coordinates of sampling locations within each plot	Soil sampling locations	1	N

R/S=Required/Suggested. Suggested indicates that a suitable alternative is acceptable.

Table 5. 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	Remove organic horizon	O horizons present	1	N
Amazon Grainger	41N620 41N620	R	Ruler, minimum 30 cm	Measure soil core horizons	All	1	N
		R	Soil corer, 2 ± 0.5 inch diameter, minimum 30 cm long	Collect soil core	All	1	N
Ben Meadows Forestry Supplier	139303 33487	S	Soil knife	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	Chaining pin (optional)	Probing soil depth	All	1	N
		S	Strap wrench	Opening stuck core barrels, only needed for certain coring devices	If required for coring device	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
		S	Toothbrush or toilet brush	Cleaning soil from core barrel and threads after sampling	All	1	N
		R	Tablespoon or coffee scoop, sterilizable	Generating microbial subsamples	All	1	N
		Consumable items					
Fisher Amazon	13-709-140 W985100	R	5.0 mL CryoElite tissue vials, sterile, Wheaton	Contain soil for microbial genetic archive	All microbe bouts	150-300	N
Fisher	15-930-E	R	Cryogenic, adhesive barcode labels, cut horizontally in thirds	Labeling cryogenic sample containers with barcode-readable labels	1 sheet	1-2 sheets	N
Domain dependent	Vendor dependent	R	Dry ice, pelletized	Freeze soil microbial subsamples	All	20 pounds	Y
Fisher	14955182	R	Whirl-Pak bags, 2 oz	Contain soil for microbial molecular analysis	All	30-75	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Collect homogenized soils	All	2 boxes	N
		S	Trash bag	Dispose of consumables	All	2	N
VWR	TWTX3044P	R	Sterile 70% Ethanol Wipes or 70% Ethanol/sterile deionized water	Sterilize sampling equipment and gloves	All	10-20 or 1 bottle	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
		S	Whirl-Pak bags, 8 oz or 13 oz	Contain all genetic archive subsamples from a plot	All	10-15	N

R/S=Required/Suggested. Suggested indicates that a suitable alternative is acceptable (e.g. field datasheets unless PDA is available)

Table 6. Additional equipment list – Field sampling for bouts that include soil N transformations at one site.

Item No.		R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
		R	Hammer or rubber mallet	Insert cylinders into soil	1	N
		R	Incubation cylinders, 2” inner diameter. Schedule 40 PVC tubing with a beveled edged or schedule 5 stainless steel (for rocky sites). See assembly, NEON.DOC.004459	Sample soil cores and store field-incubated soil cores	1/sampling location, plus 2 additional	N
		R	Loose-fitting caps for each cylinder. See assembly, NEON.DOC.004459	Protect cylinder openings from debris and water	1/sampling location	N
		S	Wooden block (approx. 2” x 4” x 10”)	Use with mallet to insert cylinder into soil	2	N
		S	Monument stake installation strike plate	Use with mallet to insert cylinder into soil	1	N
		S	Extruder (soil knife, chaining pin)	Extrude soil sample from cylinder in clayey conditions	1	N

Item No.		R/S	Description	Purpose	Quantity*	Special Handling
Fisher	1523911	S	Plastic tray	Separate soil core (horizons) in field	2	N
Ben Meadows Forestry Supplier	139303 33487	S	Soil knife	Separate organic and mineral horizons	1	N
		S	Post puller (similar to https://www.tractorsupply.com/tsc/product/speeco-t-fence-post-puller)	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

Item No.		R/S	Description	Purpose	Quantity*	Special Handling
		R	Deionized/distilled water	Rinse soil from equipment	2 liters	N
		S	Paper towel	Remove debris from soil sampling equipment	4 rags/1 box	N
		S	Permanent marker	Label sample bag	4	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	Survey marking flag, PVC or fiberglass stake	Flag location of incubated soil core	50	N
		S	Trash bag	Dispose of consumables	2	N
Resources						
RD[05]		R	Field datasheet	Record data	---	N

R/S=Required/Suggested

Table 7. 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	Record data	---	N

R/S=Required/Suggested

Table 8. Equipment list – Soil sieving, air-drying, and subsampling for microbial biomass, C-N measurements and biogeochemistry archive samples 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/distilled 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
Thomas	6258D80 6258H27	S	Lab tape, ethanol safe	Label samples	C-N and archive subsamples	30-60	N
		S	2.6” x 1” standard address labels	Label samples	C-N and archive subsamples	100	N
Fisher	033377	R	Scintillation vials, glass, 20 mL	Store C-N samples	C-N subsamples	30-60	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity *	Special Handling
Fisher	02911825	R	250 mL wide-mouth glass jars	Store biogeochemistry archive samples	Archive subsamples	30-60	N
		R	Cryogenic, adhesive barcode labels	Label frozen sample containers with barcode-readable labels	Microbial biomass samples	1-2 sheets	N
Fisher Thomas	0333723C 9718J20	R	Scintillation vials, plastic, 20 mL	Store microbial biomass samples	Microbial biomass samples	30-100	N
		R	Cryogenic adhesive labels	Label frozen sample containers with human-readable labels	Microbial biomass samples	1 sheet	N
Resources							
RD[05]		R	Lab datasheet	Record data		---	N

R/S=Required/Suggested. Suggested indicates that a suitable alternative is acceptable

Table 9. 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
ULINE	S-7630	R	Paper bag, #8	Hold soil subsamples for air-drying	All	50	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Fisher	C793	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	R	pH buffers (4, 7, 10)	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	Record data	All	---	N

R/S=Required/Suggested. Suggested indicates that a suitable alternative is acceptable (e.g. field datasheets unless PDA is available)

Table 10. 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	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. 2.5 kg size	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	35-65	N
		R	Ultra-pure deionized water	Prepare 2M KCl, clean	All	2-22 L	N
Fisher Thomas	0333723C 9718J20	R	Plastic scintillation vials with caps, 20 mL	Store filtered soil extracts for freezing and shipment	All	35-65	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
		S	2.6” x 1” standard address labels	Label samples	All	100	N
		R	Adhesive barcode labels	Label samples with barcode-readable labels	All	1 sheet	N
Resources							
RD[05]		R	Lab datasheet	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 problems procuring it.

Table 11. Equipment List - Shipping of oven-dried and air-dried samples for C-N 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 12. 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. For example, in Domain 1, this would include 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 sites in Domain 18 and 19 may not have a litter layer, but often have thick, partially decomposed organic horizons. Appendix E 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 should note any in-field decisions made that fall outside of the protocol guidelines.

The methods used to measure soil microbiology 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 other 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 bad weather conditions. Some general guidelines are:

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

- Coring device. This may be particularly difficult to clean, depending on your device. A wire brush wrapped in an ethanol wipe can help clean hard-to-reach spots. Technicians should sample as cleanly as is reasonably possible.
- Trowels or other digging tools
- “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.

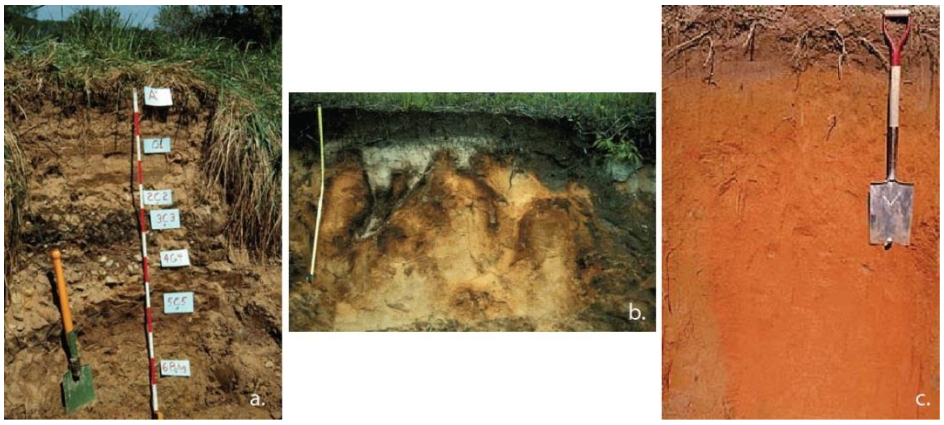


Figure 2. Soil Profiles from (a) Maryland, (b) Michigan, and (c) Florida. (Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

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 13. 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.

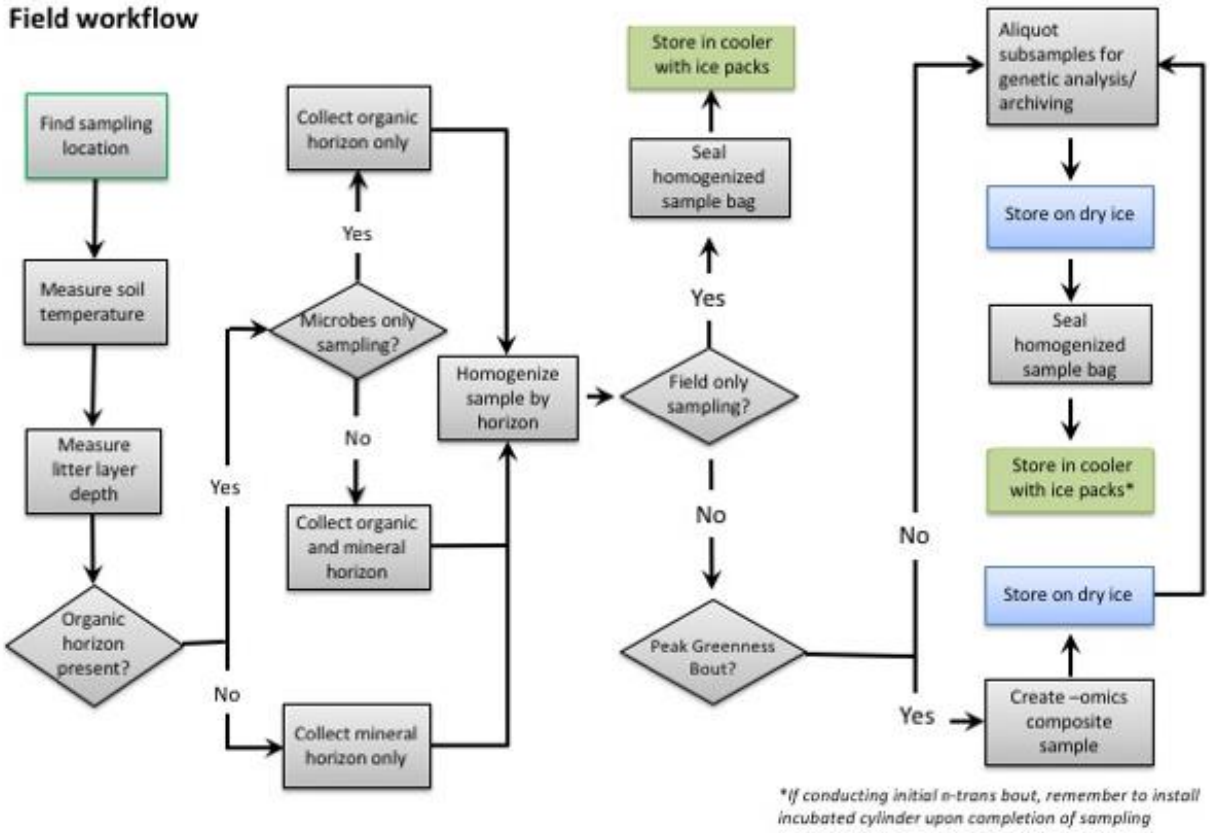
Sampling should be scheduled at the beginning of the sampling window to allow time for contingencies to occur that could delay sampling.

Table 13. 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

SOP	Estimated total time	Suggested # staff	Total person hours
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

7 STANDARD OPERATING PROCEDURES



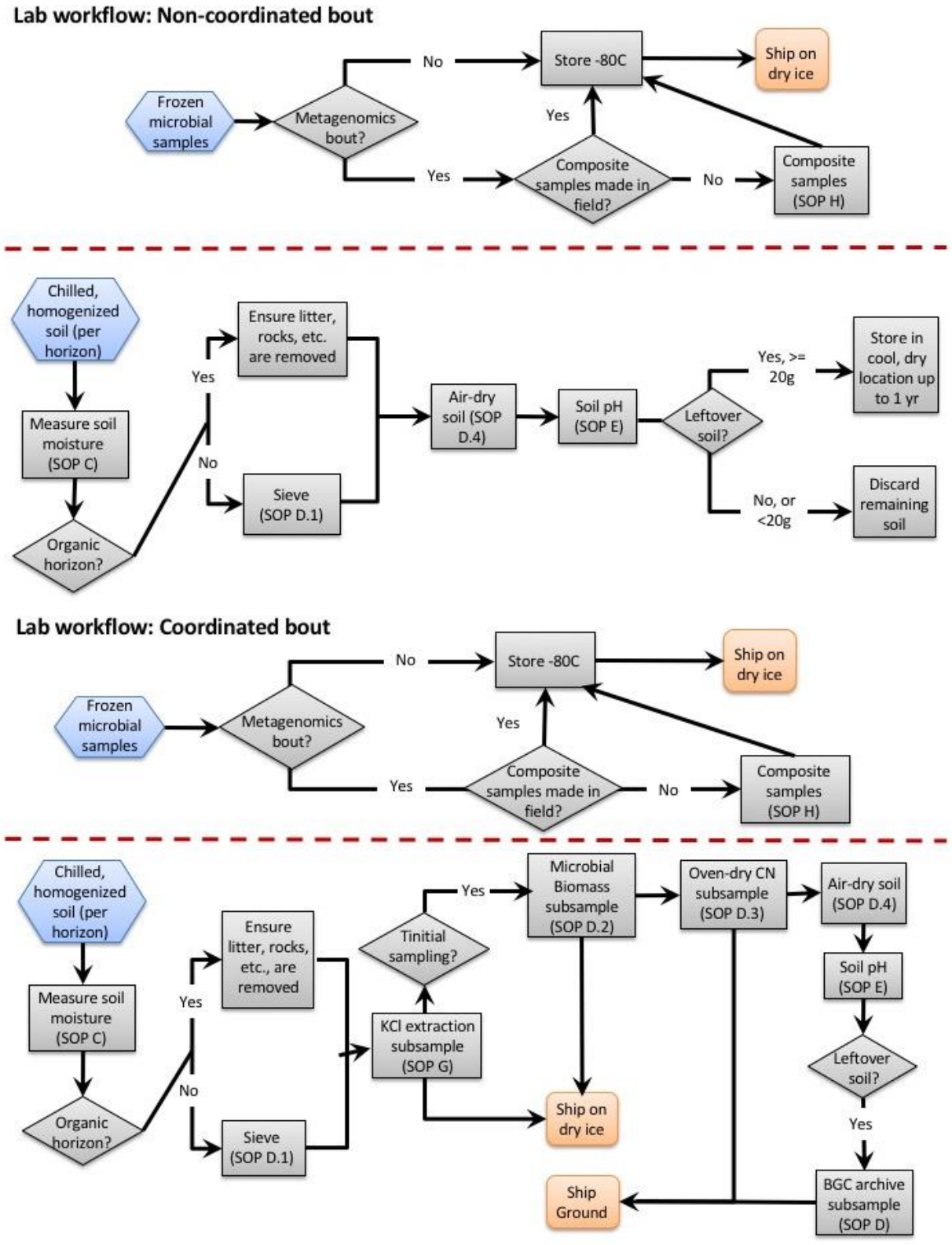


Figure 3. Workflows for soil sample collection, and lab processing and shipping.

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 for Sampling Soils

A.1 Preparing 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.

Mobile devices should be fully charged at the beginning of each field day whenever possible.

However, given the potential for mobile devices to fail in the field, paper datasheets should **always** be available for recording data. Paper datasheets should be brought to sampling locations at all times.

1. Ensure all supplies listed in the relevant tables above are available.

2. Fill out site information on field datasheet (RD [05]). Make sure to use proper formats, as detailed in datasheets.
3. Affix barcode labels to all bags that will be used for field sampling (note that barcodes are not initially associated with a particular sample, so it is fine to make these up in advance).
    a. Place the correct sized, weatherproof barcode labels on each bag that will hold the homogenized soil.
    b. Place the correct sized, cryogenic barcode labels on each container to be used for microbial genetic analyses. Each container is considered a unique sample and therefore should have a barcode label.
    c. Barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. Also, be sure that no wrinkles or folds are present in the label that can obscure the barcode.
4. Affix 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 X, Y location.
5. Affix pre-printed, cryo-safe human readable labels on each microbial whirlpak sample, for which there should be 30-80 (depending on bout type and number of soil horizons sampled) of the 2-oz whirlpaks per bout. For microbial archive vials in which the labels are too large, add a human-readable label to a 1-pint freezer-safe bag for storing archive vials collected from the same sample.

Important Note: adhesive labels can fall off after freezing. To reduce this risk, use only containers that are intended for frozen storage, and attach labels to the white area of the bag or container. When in doubt, hand-writing critical information such as plotID, X, Y coordinates, and collectDate on the container will help identify a sample without a label. While it is not necessary that a barcode label be fully wrapped in tape, clear packing tape may be added, however ensure that there are no air bubbles or other obstructions that would impede reading of the barcode. Avoid overlapping of labels.

6. Whirlpaks 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 an unused ziplock bag, and do not use any sample container that appears damaged or was previously opened.
7. Download and print soil X, Y coordinates for the plots 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.
8. Obtain the GPS coordinates for the target plots that will be sampled.
9. Time-permitting, flagging the southwest corner of each plot prior to sample collection may save time in the field.

SOP B Combined Field Sampling for Soil Biogeochemical Measurements and/or Microbes Soil Core Collection

This SOP is designed to sample well-drained upland soils with no more than 2.5 cm (1 inch) of standing water. For sites containing wetlands, follow TOS SOP: Wetland Soil Sampling (RD[06]) for sampling plots with > 2.5 cm of standing water, if authorized to do so in that SOP. For sites with > 2.5 cm standing water that are not explicitly 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 Figure 3 and the Quick References in Appendix B for guidance.

Sampling for microbial analyses involves field and laboratory components. Throughout the field protocol, it is essential to ensure clean sampling technique in order to reduce contamination. 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 microbes-only. During these bouts, the following samples and measurements will be made:

- soil temperature,
- litter depth,
- microbial genetic analysis and archiving,
- soil moisture,
- soil pH

During peak greenness bouts, additional –omics analyses will be conducted as part of the microbial sampling campaign. Instructions for generating these –omics 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.

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

- nitrogen transformations (SOP F and G)
- microbial biomass (SOP D)
- soil BGC measurements (peak greenness only, SOP D)
- soil biogeochemical archiving (peak greenness only, SOP D)

B.1 Identify the plot

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.
    a. 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).
    b. In very steep (>20% grade) plots, use a laser rangefinder set to HD (horizontal distance) mode to locate the X,Y coordinates.
        1) Check the battery and charge, if needed.
        2) Clean lenses with lens cloth or lens tissue, if needed.
        3) Check/set correct declination. See RD[10] for details.
        4) Calibrate the TruPulse tilt sensor (only needed after severe drop-shock; see RD[10] for details).
        5) 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.
        6) 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.
        7) Place a marker, such as a pin flag or stake, at the X-location.
        8) 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.
        9) Ensure that the azimuth is as close to 0° (True North) as possible and measure the Y-coordinate distance.
        10) Place a marker at the X,Y location.
4. 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).

B.2 Assess sample location

1. Navigate to the next X,Y coordinate location randomly assigned on the plot list. Visually assess the location for sampling suitability:
    a. Are there 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 plot list sheet. Move to next coordinate location on the list.
    b. Starting near the exact location of the X,Y coordinate, 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. Suitable 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.

B.3 Measure soil temperature

2. At each sampling location take one soil temperature reading.
    a. Remove the litter layer and carefully insert temperature probe into the soil (10 cm). Don't force the probe as it will break easily.

b. Allow probe to equilibrate (~2 min) before recording the value in degrees C in the field datasheets (**soilTemp**).
c. Do not make measurement with sun directly on probe (you can shade it with your body, if necessary).

B.4 Collect soil core

1. Identify soil core sampling location. All soil collected for a single sample should be located as close to the XY coordinates as possible, and should be no more than 0.5 m from the XY coordinates. Soil coordinates are provided in 0.5 m increments. Sampling outside the buffer region around the coordinates may cause future sampling locations to overlap.
2. Measure the depth of the litter layer in cm (**litterDepth**, $\pm$ 0.1 cm) above each core location and record the value (or average value if more than one core/brownie is collected). Litter layer depth can be measured using a ruler; remove litter layer and measure profile depth of undisturbed litter layer over soil. Be careful not to compact the litter layer where you are taking your measurement.
   - *The litter layer is dead but recognizable, in-tact plant material (i.e., leaves, wood, etc), whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition.*
3. Push the litter layer away from where you are going to core into the soil surface. Sterilize gloves with ethanol.
4. If an **organic horizon** is present, continue with the steps below. If not, skip to step 5.
   a. Using clean tools and equipment, cut out an organic horizon "brownie" using the square frame cutter tool and soil knife. 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. At all sites, record the **sampleTopDepth** as the depth from the soil surface (for O horizons this will be 0 cm). Measure the depth of each side of the brownie hole and determine the average value. Record in the field **sampleBottomDepth**.
      1) **Note #1:** rarely, a site could have an organic horizon that is > 30 cm. Only sample to 30 ± 1 cm depth.
      2) **Note #2:** organic horizons with an average depth < 1.0 cm do not need to be sampled as a separate horizon. Samples with an organic horizon less than 1.0 cm should be sampled according to Step 6. Note in the 'remarks' section that the sample contains a thin O horizon.
   b. Combine soils representing the same sample to form one composite sample of the O horizon, then place 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.**
   c. For microbial samples: Aliquot subsamples from the 1-gallon bag of homogenized mineral horizon into 1 labeled Whirlpak bag (fill about halfway, 5-10 g target mass) and 5 labeled cryovials (fill to about 70% capacity). Close whirlpak 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 pressing in the soil with the wall of the homogenized gallon bag. Pouring or scooping soil into the container using a sterile scoop is acceptable.
   d. Complete the human-readable labels on the whirlpak and the 1-pint combined archive freezer bag with horizon, X coordinate and Y coordinate. The whirlpak label should appear as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen

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

The archive bag label should appear as follows:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga

If hand-labelling vials, append the subsample number to the end of each geneticArchiveSampleID, starting at 1 and going up to 5. If recording on an electronic device, scan the barcode labels for each subsample.

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

1) During Peak Greenness: If you are conducting a peak greenness bout, generate a plot-level composite sample. This sample is generated from each X,Y coordinate sampled within a plot that is of the same horizon. Use a scooping device such as a coffee scoop or tablespoon when generating the composite sample, and pre-sterilize with an alcohol wipe prior to collecting each subsample.
   - <u>If this is the first X,Y location to be sampled at a plot:</u>
   a) Label a 2-oz whirlpak as follows:
      plotID-horizon-collectDate-comp (ex. CPER_001-M-20160101-comp)
   b) Place one scoop of homogenized soil in the whirlpak, close the bag, and place on <u>wet ice</u>. Record the sampleID on the datasheet and/or data entry application.
   - <u>If this not the first X,Y location to be sampled at a plot:</u>
   c) Obtain the whirlpak created earlier from the <u>wet ice</u> 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 whirlpak.
   d) Place one scoop of homogenized soil in the whirlpak and close the bag. If another X,Y location within the plot might be added to this bag, return the bag to <u>wet ice</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 whirlpak such that the labels lay flat and are clearly visible, and place the bag on <u>dry ice</u> to freeze it. Record the sampleID on the datasheet and/or data entry application.

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

e. Microbial Biomass Sampling: For bouts when microbial biomass will be sampled, ensure that there is approximately 10 g (field weight, accounting for rocks and roots) additional soil in the 1-gallon bag. This will be subsampled in the domain lab for microbial biomass analysis.

f. The remaining contents in the 1-gallon bag are for analysis of soil pH, moisture, and soil BGC measurements. If estimating soil masses, ensure a minimum of 75 g soil remains.

g. For labelling and data recording, be sure that:
   1) The gallon sample bag is labeled with the **sampleID**, measuredBy, and recordedBy
   2) Whirlpaks and cryovials are labelled with the **geneticSampleID** or the **geneticArchiveSampleID**.
   3) If available, scan the barcode label for each individual sample with the field tablet

   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).

h. Immediately place the Whirlpak and vials in the cooler with dry ice (microbial activity changes very quickly), ensuring that the newly added samples are in contact with dry ice. Place the 1-gallon resealable bags in the cooler with the ice packs.

5. Determine whether to collect mineral horizon (refer to Figure 3).
    a. When collecting samples for a microbes-only bout, collect mineral horizon **IF** no organic horizon is present.
    b. During a coordinated 5-year bout, collect mineral horizon from all sample locations, but when O-horizon is present, only subsample the M-horizon for microbial molecular analysis and genetic archiving during peak greenness (see Appendix B). During the BGC peak green sampling bout, M-horizon samples are always collected for microbial analyses, even if there is an organic horizon present.
6. If **mineral horizon** collection is required, core down so that the total depth of the soil core is 30 ± 1 cm. 'Total depth' means organic + mineral horizons, if an organic horizon is present. If an organic horizon is not present, total depth should be the depth of the mineral horizon to a max depth of 30 cm. Always core vertically, not perpendicularly, when collecting on a slope.

Note: the number of soil cores for ***mineral horizon soil sampling*** that you take per location depends on numerous factors including the soil corer being used, the type of sampling bout, and the specific physical properties of the soil. The goals are to collect a sample that broadly represents the local soil conditions and to collect sufficient material for all samples and downstream analyses. As a general guideline, the soil volume of a 2 inch diameter x 30 cm depth core should provide sufficient material for all samples and analyses. With a 1 inch diameter core, 2 cores should suffice. Plots with shallower soils and higher coarse fragment content may need to collect multiple cores per sample 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 per sample that are 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.***

    a. Take core(s) from locations where organic horizon was removed if organic horizon was present.
    b. Core to a total depth of 30 cm or to saprolite, whichever is shallower. If, in the unlikely event that a significant impediment to coring is encountered that is not representative of that location, replace soil back into 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. Measure **sampleTopDepth** as the distance from the top of the soil sample to the soil surface. If there is no O horizon, **sampleTopDepth** will be 0 cm. Measure **sampleBottomDepth** as the depth from the soil surface to the bottom of the sample.
    c. Record **horizon** (O or M) on the field data sheet and the sample bags.

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.

7. Place all mineral soil cores in one bag. Homogenize (mix) the soil thoroughly. When possible, it is preferred to avoid contact with the soil by closing the bag and mixing by inverting the bag and massaging. If this does not sufficiently homogenize the soil, then with a pre-sterilized, gloved hand, homogenize the soil. Remove any rocks, roots, insects and debris.
    a. Avoid contacting soil microbe samples as much as possible.

b. Avoid direct contact of gloved hands with the soil while mixing unless necessary to ensure adequate homogenization.

c. For microbial samples: Aliquot subsamples from the 1-gallon bag of homogenized mineral horizon into 1 labeled Whirlpak bag (fill about halfway, 10-20 g target mass) and 5 labeled cryovials (fill to about 80% capacity). 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 pressing 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 whirlpak and the 1-pint archive freezer bag with horizon, X coordinate and Y coordinate. The whirlpak label should appears as:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-gen

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

The archive bag label should appear as follows:

plotID-horizon-coreCoordX-coreCoordY-YYYYMMDD-ga

If hand-labelling vials, append the subsample number to the end of the **geneticArchiveSampleID**, starting at 1 and going up to 5. If recording on an electronic device, scan the barcode labels for each sample.

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

d. During Peak Greenness: If you are conducting a peak greenness bout, generate a plot-level composite sample. This sample is generated from each X,Y coordinate sampled within a plot that is of the same horizon. Use a scooping device such as a coffee scoop or tablespoon when generating the composite sample, and pre-sterilize with an alcohol wipe prior to collecting each subsample.

- <u>If this is the first X,Y location to be sampled at a plot:</u>

1) Obtain a pre-labeled, 2-oz whirlpak
2) Place one scoop of homogenized soil in the whirlpak, close the bag, and place on <u>wet ice</u>. Record the sampleID on the datasheet and/or data entry application.

- <u>If this not the first X,Y location to be sampled at a plot:</u>

(1) Obtain the whirlpak 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 whirlpak.

(2) Place one scoop of homogenized soil in the whirlpak and close the bag. If another X,Y location within the plot might be added to this bag, return to <u>wet ice</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 whirlpak such that the labels lay flat and are clearly visible.

b) When the composite sample is complete, record the **compositeSampleID** on the datasheet and/or data entry application, and scan the barcode label for each individual sample with the field tablet, if available. Also, complete the human-readable label with any missing information so that it appears as:

plotID-horizon-YYYYMMDD-comp

c) Place the bag on <u>dry ice</u> to freeze.

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

e. Microbial Biomass Sampling: For bouts when microbial biomass will be sampled, ensure that there is approximately 20 g (field weight, accounting for rocks and roots) of additional soil in the 1-gallon bag. This will be subsampled in the domain lab for microbial biomass analysis.

Organize Whirlpak bags and archive cryovials into a larger freezer-safe bag for ease of sample tracking during storage and shipment.

f. If estimating soil masses, ensure a minimum of 100 g soil remains. Any homogenized soil in excess of 500 g can be dumped back into the borehole according to the site host agreement.

g. For labelling and data recording, be sure that:
   1) the gallon sample bag is labeled with sampleID, measuredBy, and recordedBy
   2) Whirlpaks and cryovials are labeled with the **geneticSampleID** or the **geneticArchiveSampleID**.
   3) If available, scan the barcode label for each individual sample with the field tablet.

   Note: The X, Y coordinates are labeled to the nearest 0.5 m.

8. Immediately place the Whirlpak and vials in the cooler with dry ice (microbial activity changes very quickly), and put the 1-gallon and 1-pint resealable bags in the cooler with the ice packs.
9. Update the soil X,Y coordinate list and 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.
   - Sampling season
   - siteID
   - plotID
   - collectDate (YYYYMMDD)
   - setDate (Tfinal samples only)
   - coreCoordinateX (as in X,Y coordinate list)
   - coreCoordinateY (as in X,Y coordinate list)
   - 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
   - numberMicrobeArchiveBags
   - 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 the mobile app, ensure that all barcodes have been scanned to the appropriate sample using a tablet

10. Thoroughly rinse sampling equipment with deionized or distilled water (corer, thermometer, etc).
11. Wipe down reusable sampling equipment with alcohol wipes or squirt bottle to the extent possible.
12. Discard gloves.

B.5 Sample preservation and transport

1. Keep homogenized bags of soils for BGC measurements, microbial biomass, soil pH, and soil moisture in the cooler with the ice packs and 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 molecular analysis and archiving in the cooler with dry ice and transfer to ultralow freezer upon return to domain lab. Soils for microbial molecular analysis and archive 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 collected horizons (e.g. organic, mineral) of each soil sample. Record the necessary metadata and values in lab datasheet (RD [05]). ***Soil moisture analysis should begin within 24-48 h of field collection for microbes-only bouts. Soil moisture analysis during an N transformations and microbial biomass bout MUST begin within 24 h of field collection*** **(see SOP G).** ***Soil moisture is measured on soil that has not been sieved.*** In cases where domain staff are working at remote sites, keep samples on ice packs in coolers and process within 24 hours of returning to the domain facility lab (72 hours max holding time for non N transformation bouts).

1. Weigh each horizon sample.
    a. Label foil weigh boats with unique tinIDs. Previously used weigh boats can be re-used after thorough cleaning. Record the **tinID**.
    b. Weigh foil boat to nearest 0.01 g and record value in datasheet or data entry application (boatMass).
    c. Wear clean nitrile gloves and clean or replace gloves between samples if gloves become soiled. 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).
        1) 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.

SOP D Laboratory Processing of Soils for BGC Measurements, Archiving, pH, and Microbial Biomass

D.1 Sieving Field Soils

For microbes-only bouts, process samples within 48 h of field collection or upon return to the domain facility if working remotely. In cases where technicians are working remotely, keep samples in coolers on cold ice packs until at the domain lab for up to 72 hours, and then process immediately. If samples are held for longer than 72 hours prior to processing, notify Science.

For initial and final n-transformation bouts and microbial biomass bouts, process samples within 24 h of field collection.

1. Wear nitrile gloves. 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. Rocks, roots, leaves, and debris can be discarded according to permit requirements.
3. If sample is **organic horizon**, do not sieve, but **remove any rocks, coarse roots (> 2mm diameter), insects, wood, moss, and other non-soil debris** and homogenize before proceeding.
4. With a gloved hand, pass **mineral horizon** samples through a 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 is taking longer than 30 minutes per sample, try one or more of the following tips and tricks:
    - If this is NOT an N transformation/microbial biomass 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 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 (email address)

D.2 Processing Microbial Biomass Samples

Follow this SOP immediately after completing SOP D.1 for a microbial biomass bout.

1. Affix cryo-safe barcode labels and human-readable labels to new plastic scintillation vials. Orient the barcodes long-wise, from top to bottom (not curving around). Ensure that labels do not overlap.
2. For mineral horizons, tare the labeled scint vial and transfer ~10 g of wet-sieved soil. Close the vial.
3. For organic horizons, tare the labeled scint vial and transfer ~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.
    a. **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 the barcode using a tablet to associate the barcode with the appropriate **biomassID**. Human-readable vials should be labeled with a biomassID as:

    plotID-horizon-coreCoordX-coreCoordY-collectDate-bm

    (ex. CPER_001-O-10.5-10.5-20160101-bm)
5. Store vials at -80C until ready for shipment.
6. Ship samples according to SOP Sample Shipment.

D.3 Oven-Drying Field Samples for BGC Measurements

1. Fill ¾ of a 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 wet-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.
    c. Every vial should be labeled with an appropriate size adhesive barcode label, placed long-wise from top to bottom (not curved around). Additionally, label the scintillation vial with a **cnSampleID** (sampleID + "-cn", e.g. ONAQ_005-M-10.5-20.5-20160115-cn) Suggestion: use a pre-printed label, or write neatly with lab tape, but do not cover barcode.
    d. 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 once dried. Without this, the soil cannot be removed for analysis.
2. Loosely cap vials.
3. Place open scintillation vials into the scintillation vial box, which holds 100 vials. Record oven start date and time in datasheet or mobile application. Oven-dry at 65°C for at least 48 hr. Record oven end date and time in datasheet or mobile application.
4. 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 mobile application and that each barcode has been scanned.
5. Store bottles at ambient temperature until shipping. Ship samples according to SOP J.2.
6. Air dry remaining soil as described in SOP D.4.

D.4 Air-Drying Field Samples

Follow this SOP when: 1) you are processing soils for pH; and/or 2) during a biogeochemistry (BGC) bout with remaining soil **after** subsampling for BGC measurements. 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 from sieving) into #8 paper bags labeled with the sampleID. With very wet or fine-grained soils that can leak out, it may be helpful to cover the seams along the bottom of the bag with masking tape. Break up large clumps and soil aggregates with a clean gloved hand and spread out soil to facilitate drying. Weigh the bagged sample and record initial mass on the sample bag.
2. 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.
3. Once a day, shake up soil to expose new surfaces.
4. 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. If change in weight between the current and previous measurement is less than 5 %, continue with sample processing. If change in weight is greater than 5%, then continue to weigh samples every 2 days until the change in weight is less than 5%.
5. At the conclusion of air-drying samples, record **airDryEndDate**. To continue with pH measurement, follow SOP E.
6. During a BGC bout, the remainder of the sample will be shipped to an archive facility.
    a. Wear clean gloves while handling samples.
    b. Transfer the soil to an archive bottle labeled with an appropriate size adhesive barcode label, placed long-wise from top to bottom (not curved around). Additionally, label the bottle with a bgcArchiveID (sampleID + "-ba", e.g. ONAQ_005-M-10.5-20.5-20160115-ba).
    c. Fill bottles up to, but not beyond the lip of the bottle.
    d. Ensure that all bgcArchiveIDs have been created in the mobile application and that each barcode has been scanned.
    e. Store bottles at ambient temperature until shipping. Ship samples according to SOP J.2.

Any soil remaining after all subsampling and analyses have been completed should 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 and values are recorded in the Lab Datasheet: Measuring Soil pH and Moisture (in RD[05]).

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.

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:
    a. dissolve 2.94 g of $CaCl_2 \cdot 2H_20$ in just under 2 liters of house deionized water in the volumetric flask.
    b. Check pH of $CaCl_2$ solution; it should be between 5.0 and 6.5.
    c. Adjust pH to desired value by adding concentrated 6N $Ca(OH)_2$ or 10N HCl one drop at a time
    d. Bring solution to final volume of 2L
    e. Note: this solution is stable for approximately 1 year, kept at room temperature out of direct sunlight.
4. Weigh out a subsample of air-dried organic or mineral (fraction ≤ 2 mm) 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.
    a. 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
    b. 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. DO NOT STIR.
7. 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.
8. Thoroughly stir by swirling samples for 10 seconds.
9. Further stir suspension (for 10 seconds) every 5 minutes for the next 30 minutes.
10. Determine if soil is completely saturated.
    a. Look for supernatant (a thin layer of liquid without precipitate) above the flocculated soil.
    b. If not present, add another aliquot (20 mL) of $CaCl_2$ solution and repeat stirring and settling.
    c. Keep track of the total volume of solution added in the datasheet or mobile application.
11. Calibrate the pH meter electrode using the buffer solutions that best encompass the ranges in soil pH encountered (either buffers 4, 7, and 10, or 1.68, 4, and 7). Follow calibration instructions in the manual for the probe.
    a. Rinse the electrode with deionized water and gently shake off excess liquid between buffers.
    b. Note: you only need to calibrate the pH probe one time for the group of samples.
12. Gently swirl the container while measuring pH of supernatant solution. It is OK if some flocculated soil is floating in the supernatant.
    a. Allow reading to stabilize (usually about 1 minute) and record pH value on datasheet.

b. Clean electrode: rinse thoroughly 2 to 3 times with deionized water and gently shake off excess liquid.
c. Measure each sample.

13. Repeat pH measurements with deionized water, analyzing subsamples in 20 mL deionized water instead of $CaCl_2$.
14. Discard remaining soil following any applicable soil permit guidelines.

SOP F Field Sampling for Soil Nitrogen Transformations

N transformation rate measurements are conducted once every five years during coordinated bouts. During these "on" years, N transformation sampling will occur three times, once during each of the windows specified in Appendix E. As these windows are the same as those used for microbial sampling, **material for N-transformation 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. For sampling of plots with a shallow water table (<30 cm below the soil surface) or standing water (<= 50 cm depth), follow the instructions in TOS SOP: Wetland Soil Sampling (RD[06]), if your site is authorized to do so in that SOP. Note that the Wetland SOP refers back to this SOP for various instructions.

N-transformation sampling should occur in batches 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 bouts, alternating between field and lab days (or using staggered field and lab teams). It is not critical that all cores are incubated for the exact same length of time, as long as the incubations begin and end within the designated sampling window.

F.1 Prepare for sampling

1. Ensure that you have the required number of clean incubation cylinders (one per soil sampling location plus a couple extra, typically 32). Figure 4 shows an example of a PVC incubation cylinder: note that the bottom edge has been shaved down or beveled, which helps drive it in to the soil. The two holes near the top aid in removal.
2. Check that one 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 (e.g. with plant wire).
3. Ensure that all field collection supplies listed in Tables 4, 5 (where appropriate), and 6 are available. Pre-label sample bags and containers as described in SOP A.

Verify that all laboratory supplies are also available. **It is very important that lab processing occurs within 24 hours of field collection**. Required equipment and supplies are listed in

4. **Table 10**. It is also advisable to make 2M potassium chloride in advance as it can take several hours to dissolve (see SOP G).

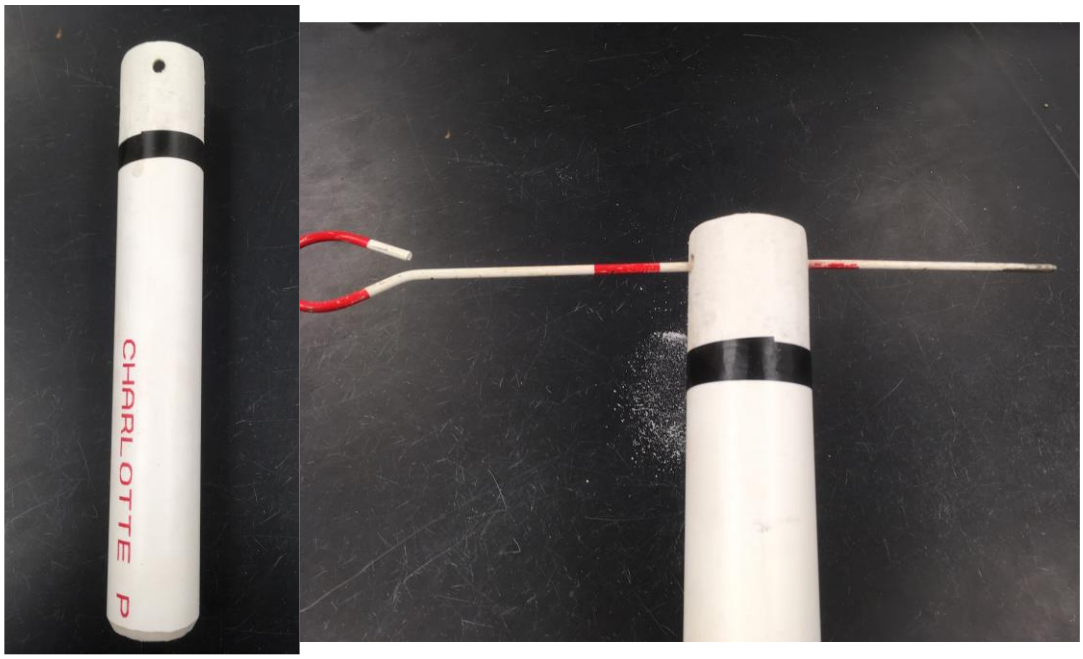


Figure 4. Example coring device used for soil N transformation sampling.

F.2 Identify the plot

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

F.3 Assess sample location

1. Navigate to the next X,Y coordinate location randomly assigned on the plot list. Visually assess the location for sampling ability:
   a. Are there 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 plot list sheet. Move to next coordinate location on the list.
   b. Starting near the exact location of the X,Y coordinate, carefully assess soil depth by probing the soil using a clean chaining pin or similar, moving outward (not more than 0.5 m away) until a suitable spot is found. A suitable spot will allow you to collect two core samples within 0.25 m of each other to the same depth. The target depth is 30cm, but a shallower depth is acceptable if that is the prevailing condition at the site.

F.4 Measure soil temperature

1. At each sampling location take one soil temperature reading.
   a. Remove the litter layer and carefully insert temperature probe into the soil (10 cm). Don't force the probe as they break easily.
   b. Allow probe to equilibrate (~2 min) before recording the value in degrees C in the field datasheets.
   c. Do not make measurement with sun directly onto probe (you can shade it with your body, if necessary).

F.5 Collect initial soil core

1. Identify soil core sampling location.
2. Put on a clean pair of nitrile gloves (do NOT reuse gloves between locations).
3. Measure the depth of the litter layer in cm (**litterDepth**, ± 0.1 cm) above each core location and record the value (or average value if more than one core/brownie is collected). Litter layer depth can be measured using a ruler; remove litter layer and measure profile depth of undisturbed litter layer over soil. Be careful not to compact the litter layer where you are taking your measurement.
   - *The litter layer is dead but recognizable, in-tact plant material (i.e., leaves, wood, etc), whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition.*
4. Push the litter layer away from where you are going to core into the soil surface.
5. Sterilize sampling equipment, gloves, and other equipment that will come into contact with the sample, as described in SOP B.4.
6. If an organic horizon is present, sample it by cutting an organic horizon "brownie" and record the depth, as described in SOP B.4. Place soil into a new, pre-labeled 1-gallon bag. If recording on an electronic device, scan the barcode label for each sample.
   a. **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.**
7. Label bag with:
   a. sampleID: plotID- horizon-coreCoordinateX-coreCoordinateY-collectDate (ex. ONAQ_001-O-8.5-21-20160721)
   b. measuredBy (technician name)
   c. recordedBy (technician name).
8. Sample the mineral horizon. Insert the bottom of the cylinder (section of pipe without holes, beveled edge of PVC) into the ground. If an organic sample was collected, insert cylinder into the footprint of the organic sample location. If soil is difficult to core, you can use a piece of wood or monument stake installation strike plate and mallet to pound the cylinder into the ground. If your soil is easy to core, you may simply be able to 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.
   a. If a site's normal coring device is 2 ± 0.5" in diameter, it is acceptable to use it for collection of the initial core instead of a cylinder.
9. Push the cylinder in to a total depth (from the soil surface) of 30 ± 1 cm. 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.

10. Remove cylinder and empty soil directly into a new, pre-labeled 1-gallon bag. Record depth. If you require a helper tool to extrude soil from the cylinder (e.g. soil knife, chaining pin), be sure to properly sterilize before use.
11. Label bag/s with:
    - sampleID: plotID- horizon-coreCoordinateX-coreCoordinateY-collectDate (ex. ONAQ_001-M-8.5-21-20160721)
    - measuredBy (technician name)

- recordedBy (technician name)

12. Refer to SOP B.4 for instructions on homogenizing, field subsampling, bag storage, data entry, equipment cleaning, and backfilling. Ensure that you have created all field subsamples that should be made for the designated boutType and sampleTiming.

F.6 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 E).

1. Locate a second soil coring location within 0.25 m of the collected soil core.
2. Push the litter layer away from where you are going to core into the soil surface.
3. Insert the incubation cylinder into the ground.
   a. If soil is difficult to core, use a piece of wood or monument stake installation strike plate and mallet to pound the cylinder into the ground; if soil is easy to core, you may simply be able to push it in.
   b. 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 D18 and D19 is provided in Appendix E.
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, in addition to placing the flag.

F.7 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.8 Collection of incubated soil core

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

1. Consult the soil coordinate list and navigate to plot where sampling for Tinitial soil N transformations occurred. Locate incubated core. Measure soil temperature within 10 cm of the incubated cylinder, as described in SOP F.4.

    a. If the core is missing, create a record in the datasheet or data entry application but choose **sampleFate =** 'destroyed' and only record minimal sample meta-data.
2. Take off cap and remove cylinder from the ground.
    a. If soil is dry or high in clay content, a helper tool – such as a post hole puller, or a chaining pin threaded through the drill holes – may aid in removal.
    b. If the soil is sandy, wet, or otherwise not well aggregated, soil within the cylinder may fall out during removal. 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.
    a. Most cylinders should be in 'OK' condition
    b. If the cylinder has been disturbed - for example, an animal has removed it from the hole, still collect but choose the appropriate **incubationCondition** choice and explain in remarks.
4. If an organic horizon is present, remove soil onto tray (or other surface for separating soil horizons), partition the organic and mineral horizons, and bag separately. It may be necessary to use a tool to push or scoop soil from the cylinder, such as a chaining pin or soil knife. It is not critical that the t-final sample remains sterile.
5. If only mineral soil is present, empty soil from corer directly into bag. If the soil remains stuck inside the core, a tool may be used to help push the soil out.
6. 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.
    a. For mineral horizons, if your site is known to have unconsolidated soil that may collapse when cylinder is removed, mark the soil surface on the outside of the cylinder prior to removal. Then, measure the **sampleBottomDepth** of the mineral horizon by taking the length (in cm) from this mark to the bottom of the cylinder.
7. For sites that have both M and O horizons, if you encounter a horizon in the incubated core that was not present in the initial core:
    a. If it's an O < 5 cm or an M < 2 cm, include it with the rest of the material and note in remarks
    b. If it's an O > 5 cm or an M > 2 cm, discard the material
8. Label bag with:
    a. sampleID: plotID- horizon-coreCoordinateX-coreCoordinateY-collectDate (ex. ONAQ_001-M-8.5-21-20160721)
    b. measuredBy (technician name)
    c. recordedBy (technician name).
9. Scan the barcode label for each sample, when applicable.
10. Place bag into cooler with ice packs.
11. Enter all required metadata in the field datasheet and/or mobile application, as listed in SOP B.
12. Backfill the bore hole according to site requirements.
13. 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 specifically for N transformation analyses. Note that for t-initial samples, downstream analyses associated with the type of sampling bout conducted (e.g. microbes, microbesBiomass, microbesBiomassBGC) must also be conducted. Refer to SOP's C, D and E for detailed instructions.

Reminder: N transformation sample processing MUST begin within 24 h after collecting the core

G.1 Preparing for KCl extraction

1. Prepare 2M KCl (149.1 g/L).
   a. 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 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.
   b. 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 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 3 below).

G.2 Measure soil moisture and prepare sample for KCl extraction

1. Soil moisture is a critical component for calculating N transformation rates. It is essential that this measurement be made. Subsample the collected soil samples for moisture analysis, according to SOP C. Remember to remove all rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris from subsample before weighing.
   a. 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 D.1. ***Field-moist mineral soil must be sieved and used for this analysis.*** You cannot wait to sieve.
   a. Begin with a 2 mm mesh sieve. If sieving is too difficult, a 4 mm mesh sieve may be used.
   b. 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. Use a new glove(s) for each sample. If you only handle the soil with one hand, you only have to replace one glove.
5. Do not sieve organic soil. Instead, ensure that the O-horizon 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 clean, gloved hand as needed.

6. ***For t-initial samples***, remember to set aside sieved material in order to subsample for microbial biomass, BGC measurements, biogeochemistry archive, and for pH. ***T-final samples do not require any of these measurements***.

G.3 Perform KCL extraction

1. Weigh 10 g ± 0.5 g subsamples of fresh sieved mineral or homogenized organic soil into a tared 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.
   a. If sample mass is limiting, it is acceptable to use less mass, but do not use less than 4 g per sample.
   b. If less than 4 g of O-horizon is available, combine it with the mineral matter and extract together. Record this in the **remarks**.
   c. For (un-sieved) O-horizons, ensure no rocks, large roots or any non-soil debris remains in the sample.
2. For each sample, measure 100 ± 2 ml of 2M KCl into the 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).
3. Every day that samples are extracted, three procedural blanks must be prepared - even if the KCl came from the same large carboy used to extract samples on a previous day.
   a. Add 100 ml KCl to each of three containers without soil and treat the same as samples containing soil. As stated above, if you have to make a new batch of KCl solution in the middle of processing a group of samples, you must prepare three *additional* blanks for the new solution. Label blanks as follows:
      1) First, create a unique **kclReferenceID** for the KCl batch from that day (format = siteID-extractionStartDate-'BRef1', example: CPER-20160418-BRef1). If a new batch of KCl is created in the midst of processing samples, create a new identifier (example: CPER-20160418-BRef2).
      2) For each of the three replicate blanks, create **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).
4. Make sure the caps on each extraction cup are on tightly, then shake the cups vigorously for ~30 seconds.
5. 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.
6. Place the box on its side so that samples shake end-to-end. **Shake for 1 hour at 150 rpm**.
7. Remove extraction cups and organize on benchtop. Record **extractionEndDate** (YYYY-MM-DD HH:MM) for each sample.
   a. 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.
8. Allow soil to settle without disturbance for ~ 30 minutes while setting up the filtering manifold. This will facilitate faster filtering.

G.4 Filtering 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 four. 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 5) 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. 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:
    a. Saturate the filter with KCl solution
    b. Turn on the pump. Open the stopcocks until KCl flowthrough is complete. Close the stopcock/s.
    c. Remove filtration unit, open, and dispose of filtrate in a waste vessel. Use care not to contaminate the filter during this process.
    d. 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 into each funnel.
8. Wait for sample to filter completely, then transfer the filtrate from the collection tube into a 20 ml scintillation vial, leaving enough room for the liquid to expand when the sample freezes (1-2 mL of headspace should suffice). Cap sample tightly.
    a. If a sample takes longer than 10 minutes to filter, but a sufficient volume of sample has already been filtered (minimum 15 mL), then it is acceptable to stop filtering the sample. Transfer the filtrate as instructed above and discard the rest of the unfiltered extract.
    b. Every vial should be labeled with an appropriate size adhesive barcode label, placed long-wise from top to bottom (not curved around the vial).
    c. Additionally, label each vial with a **kclSampleID** (sampleID + "-kcl", e.g. ONAQ_005-M-10.5-20.5-20160115-kcl), using a pre-printed label or write neatly on laboratory tape. Do not cover barcode.
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 collection cups.
10. Clean filter holders thoroughly prior to re-use.
    a. Fill ~3/4-way two dishpans with house deionized water.
    b. 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
    c. Transfer filter unit to the second, 'rinse' basin and swirl.
    d. Rinse filter holder and cup 3X with fresh house deionized water - can be directly from the wall unit, or from a carboy or squirt bottle
    e. Conduct a final rinse with Type 1 ultra-pure deionized water, either directly from the container or using a squirt bottle
    f. Shake to remove excess water, then re-assemble. Equipment is ready to use.

11. When filtering is complete, place sample vials in a resealable plastic bag. Label the bag with siteID and package date and place in the -20° C freezer. Alternatively, if freezer space allows, freeze the vials in the cardboard trays in which they 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. Store frozen until shipment to the contracted laboratory facility (see SOP J).

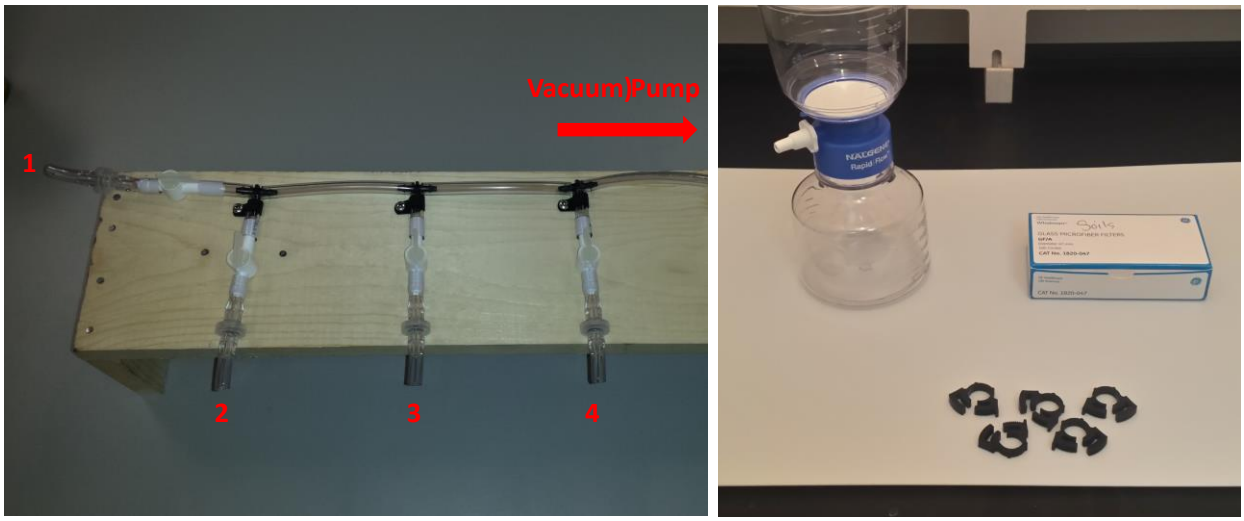


Figure 5. Filtration equipment for KCl extractions. Example of a sample manifold set-up (left) and other equipment used for filtration, including filtration unit, filters, and clamps to secure tubing (right).

G.5 Sample Storage

Samples can be stored frozen at the domain lab 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 NEON CLA for shipping details.

SOP H Generation of Composite Soil Samples for Microbial –omics Analyses

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 -80C freezer, obtain 1 whirlpak from each core sample. Organize whirlpaks 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 whirlpaks, but fewer than 3 is also possible.
2. Place a cryo-safe barcode label on each new whirlpak bag that will be used to store composite samples. Also, label each whirlpak bag with the plotID, horizon, collection date that matches a set of whirlpaks, and "-comp" for composite. These may be pre-printed cryo-safe labels or hand-written. *Ex. CPER_001-M-20140101-comp*
   Place that bag with the corresponding whirlpaks.
3. Repeat step 2 for every unique combination of plotID, horizon, and collection date. There should be 1 new whirlpak bag for every unique combination.
4. Thaw the material in a set of whirlpaks and transfer all material in each whirlpak into the corresponding composite whirlpak bag. Homogenize the soil by gently kneading and/or shaking the outside of the closed whirlpak.
5. Return the sample bags to the -80C freezer (or container of dry ice, if no freezer is accessible) immediately.
6. Repeat step 4 for the remaining samples.
7. Complete the "composite sample inventory" sheet and/or the data entry application by taking the sample information from the empty whirlpak bags. Ensure that the sample inventory sheet was completed correctly and completely, and discard empty whirlpaks. If using barcode labels, scan the barcode for each associated sample using a field tablet.
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 may be tracked in other ways for a site. Refer to the domain manager and to the Science Team for guidance on recording such events.

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]).

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. 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).

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 scan-able barcode, which will not contain information specific to sample provenance, but will improve sample tracking and reduce transcription errors associated with writing sample identifiers by hand.

If available, adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample; it is encouraged to apply these in advance. 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 mobile 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.

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 Hazardous 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 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 soil samples**, 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.
    a. Microbial genetic analysis and archive: Genetic archive samples may need to be shipped to a different facility than molecular 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.
        1) 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 in labeled, pint-sized freezer bags. If not, organize the vials from the same sample into a freezer bag with cryo-safe labels (human-readable and barcode labels, if both are used). **Tip**: Affix labels within the freezer bag facing outward toward the front of the bag, ensuring that the barcode can be scanned and that the label can be read. If hand-writing, label the bag with plotID-X,Y coordinate-collectDate-ga (ex. *BLAN_001-13.5-20 20160415-ga*).
        2) Place all non-composite 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 + collectDate(s) + 'gen' (ex. *BLAN_001 20160415 gen*). If more than 1 bag is required to contain all samples from a bout, label the bags sequentially (e.g. "Bag 1 of 2").

3) Place the metagenomics subsamples (ending with "-comp") from the same sampling 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").

b. 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. Put samples to be shipped into insulated shipper, then weigh the box containing samples. Add dry ice under and around the samples and reweigh the box to determine the amount of dry ice in each package.
   a. 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.
   b. If weight restrictions apply, use cold-soaked packing peanuts, or similar, to keep samples frozen.
4. When packing items in the container, put dry ice and specimens as close together as possible and add more dry ice on top. Fill 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.
5. Note that this must be done quickly as it requires the samples be initially placed into the box without dry ice. Samples can thaw quickly and must remain frozen at all times.
6. Complete packaging and labeling for Class 9 dry ice hazard shipment.
7. 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.

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

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 3. These locations do not have to be exact.

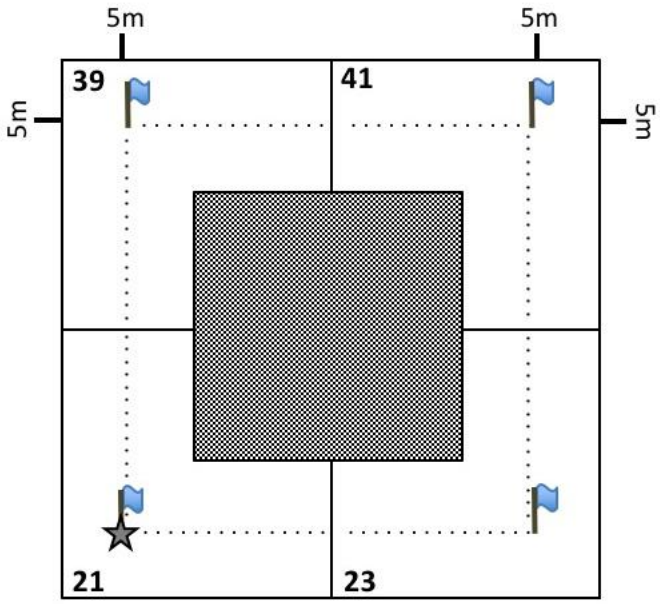


Figure 6. Schematic of TOS soil plot demonstrating the general layout of sample locations.
Subplot ID's are noted in the left corner of each subplot. Flags denote the corners for the depth transect measuring area. The star indicates the location to begin measurements. Dots indicate the general distribution 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.

COMMENT: 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

Buyer, J.S., and M. Sasser. 2012. High throughput phospholipid fatty acid analysis of soils. Applied Soil Ecology 61: 127-130.

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 14. 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 15. Checklist of analyses associated with a type of soil sampling bout, not including nitrogen transformation rate sampling. 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)	Soil moisture (lab)	Soil pH (lab)	Microbial biomass (lab)	BGC measure-ments	BGC archive (lab)
Microbes Only (microbes)	Transition	✓	**X** whirlpaks (top horizon)		✓	✓			
	Peak greenness	✓	**X** whirlpaks (top horizon)	**X** 1 plot-level whirlpak (top horizon)	✓	✓			
Microbes, Biomass, and BGC (microbesBiomass BGC)	Peak greenness	✓	**X** whirlpaks 2 horizons)	**X** 1 plot-level whirlpak (2 horizons)	✓	✓	**X** sieved soil (2 horizons)	✓	**X** air-dried soil (2 horizons)
Microbes and Biomass (microbesBiomass)	Transition	✓	**X** whirlpaks (top horizon)		✓	✓	**X** sieved soil (2 horizons)		
	Peak greenness*	✓	**X** whirlpaks (top horizon)	**X** 1 plot-level whirlpak (top horizon)	✓	✓	**X** sieved soil (2 horizons)		
Microbes and BGC* (microbesBGC)	Peak greenness	✓	**X** whirlpaks (2 horizons)	**X** 1 plot-level whirlpak (2 horizons)	✓	✓		✓	**X** air-dried soil (2 horizons)
Biogeochemistry Only* (BGC)	Peak greenness	✓			✓	✓		✓	**X** air-dried soil (2 horizons)

* Non-standard bout type, conducted only if directed by Field Operations leadership or Science.

Table 16. Checklist of analyses associated with a type of soil sampling bout when sampling for nitrogen transformation rates (NtransBoutType= Tinitial or Tfinal). 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)	Metagenomics (field)	Microbes and archive (field)	Microbial biomass (lab)	Soil moisture (lab)	Soil pH (lab)	KCl extraction (lab)	BGC measure and archive (lab)
$T_{initial}$ sampling									
Microbes Only* (microbes)	Transition	✓		**X** whirlpaks (top horizon)		✓	✓	✓	
	Peak greenness	✓	**X** plot-level whirlpak (top horizon)	**X** whirlpaks (top horizon)		✓	✓	✓	
Microbes, Biomass, and BGC (microbesBiomass BGC)	Peak greenness	✓	**X** plot-level whirlpak (2 horizons)	**X** whirlpaks (2 horizons)	**X** sieved soil (2 horizons)	✓	✓	✓	**X** air-dried soil (2 horizons)
Microbes and Biomass (microbesBiomass)	Transition	✓		**X** whirlpaks (top horizon)	**X** sieved soil (2 horizons)	✓	✓	✓	
	Peak greenness*	✓	**X** plot-level whirlpak (top horizon)	**X** whirlpaks (top horizon)	**X** sieved soil (2 horizons)	✓	✓	✓	
Microbes and BGC (microbesBGC)*	Peak greenness	✓		**X** whirlpaks (2 horizons)		✓	✓	✓	**X** air-dried soil (2 horizons)
T_{final} sampling									
Field only	All	✓				✓		✓	

* Non-standard bout type, conducted only if directed by Field Operations leadership or Science.

QUICK GUIDE TO SOIL MICROBIAL SAMPLING

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 relative (x, y) coordinates to locate pre-determined sample locations.

STEP 3 – Sterilize any equipment or consumables that will contact the sample by wiping with 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. Remove rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris. Fill 1 pre-labeled whirlpak (2 oz.) ~1/2-way and 5 pre-labeled cryo vials ¾ of the way. If sampling for –omics, use sterile scoop to place soil in a 2 oz. whirlpak. Complete sample labels, close whirlpaks (labels clearly visible), and store on dry ice.
>
> **STEP 6b** – If sampling for microbial biomass, ensure an additional ~10 g homogenized soil is available for subsampling at the domain lab. Store the homogenized bag on ice packs.

STEP 7 – If organic horizon is not present, collect mineral horizon core(s) with approved coring device for your domain. Follow steps **6a** and **6b** for microbial subsampling.

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

STEP 9 – 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 COLLECTING BIOGEOCHEMISTRY AND N-TRANSFORMATION SAMPLES

STEP 1 – Prepare sampling equipment before going into field.

STEP 2 - Use plot ID and relative (x, y) coordinates to locate pre-determined sample locations.

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

STEP 4 - Measure soil temperature.

STEP 4 - Measure litter layer.

STEP 5 - Collect organic horizon with "brownie cutter", hori-hori, or similar.

STEP 6 – Put organic samples into 1 bag and homogenize. With a 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 gloved hand, remove rocks and roots.

STEP 8 – Label bag/s and store in cooler on ice packs.

STEP 9 – Measure sample depth/s in bore hole and record. Remember: For samples collected from the ground surface, **sampleTopDepth** = 0 cm.

STEP 10 – If collecting an N-transformations initial core, set up the incubated core.

STEP 11 – Ensure all data have been recorded on datasheets and/or data entry application.

STEP 12 - Backfill boreholes in accordance with permit.

STEP 13 – 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 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
- ☑ Core to 30 ± 1 cm and measure sample top and bottom depths in borehole (not the corer).
- ☑ 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.
- ☑ Store microbial molecular 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 bulk refrigerated sample.
- ☑ Measure pH on 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 genetic analysis and archive samples in ultralow freezer (-80° C).
- ☑ Ship analysis samples separately from archive samples.
- ☑ Ship samples on dry ice in to external lab/s according to the schedule provided by NEON CLA. Do not ship on Fridays.

Microbial Biomass Samples: Be sure to…

- ☑ Wet-sieve mineral or remove rocks, coarse roots (> 2 mm diameter), insects, wood, moss, and other non-soil debris out of organic soils and transfer to scintillation vials.
- ☑ 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.

Biogeochemistry Samples: Be sure to…

- ☑ Create oven-dried subsamples for BGC measurements.
- ☑ Subsample air-dried material for biogeochemistry archive.
- ☑ Ship BGC and archive samples to the appropriate lab/s at ambient temperature according to the Domain schedule.

Nitrogen Transformation Samples: Don't forget to…

- ☑ Extract soil using 2M potassium chloride within 24 h of collection.
- ☑ Filter extracts and store at -20° C.
- ☑ Ship KCl extracts on dry ice to the appropriate labs according to the Domain schedule. Do not ship on Fridays.

Data Entry: Did you…

- ☑ Track and record the dates and times of specimen processing, including drying times?
- ☑ 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 17. Approximate sampling date ranges for soil core sampling at NEON sites. Logistical constraints may prevent sampling from lasting the entire time period at certain sites. Note: soil biogeochemical and stable isotope analyses will be conducted on the soil cores taken within the Peak Greenness window during years when these analyses are scheduled.

Domain	Site	Approx. Start Date	Approx. End Date
01	HARV	April 15	Dec 1
	BART	April 21	Nov 15
02	SCBI	March 15	Dec 1
	SERC	March 15	Dec 1
	BLAN	March 10	Dec 1
03	JERC	March 15	Dec 1
	DSNY	March 1	Dec 1
	OSBS	March 10	Dec 1
04	GUAN	July 1	Jan 1
	LAJA	July 1	Jan 1
05	UNDE	May 1	Nov 1
	TREE	April 15	Nov 15
	STEI	April 15	Nov 15
06	UKFS	March 15	Dec 15
	KONZ	Apr 1	Nov 15
	KONA	Apr 1	Nov 15
07	ORNL	March 15	Dec 1
	MLBS	Apr 15	Dec 1
	GRSM	Apr 1	Dec 1
08	TALL	Mar 15	Dec 15
	DELA	Mar 1	Dec 15
	LENO	Mar 10	Dec 15
09	WOOD	May 1	Nov 1
	DCFS	May 1	Nov 1
	NOGP	Apr 15	Nov 1
10	CPER	Apr 1	Jan 1
	STER	Apr 1	Oct 15
	RMNP	May 1	Nov 15
11	CLBJ	Mar 1	Nov 15
	OAES	Mar 15	Dec 1
12	YELL	May 1	Nov 1
13	NIWO	May 21	Nov 15
	MOAB	Mar 27	Nov 15

14	JORN	Mar 22	Dec 1
	SRER	May 31	Dec 15
15	ONAQ	Mar 17	Nov 1
16	ABBY	Apr 15	Dec 1
	WREF	Apr 26	Nov 1
17	SJER	Feb 15	Nov 15
	SOAP	Mar 15	Nov 15
	TEAK	Apr 15	Dec 1
18	TOOL	July 1	Sept 15
	BARR	July 1	Sept 15
19	HEAL	July 1	Sept 15
	DEJU	July 1	Sept 15
	BONA	July 1	Sept 15
20	PUUM	Nov 1	Jun 30

APPENDIX E SITE-SPECIFIC INFORMATION

E.1 Targeted site-specific sampling windows. 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 half 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 23 – Nov 22 (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 28 – 27 Nov (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)	Sept 18 – Oct 18 (24)
	DCFS	May 1 – May 31 (24)	July 1 – Aug 31 (18)	Sept 18 – Oct 18 (24)
	NOGP	Apr 15 – May 15 (24)	July 1 – Aug 31 (18)	Sept 18 – Oct 18 (24)
10	CPER	Apr 1 – May 1 (24)	May 15 – July 15 (18)	Nov 17 – Dec 17 (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)	Oct 1 – Oct 31 (28)
11	CLBJ	Mar 2 – Apr 1 (18)	Apr 1 – May 15 (14)	Oct 23 – Nov 22 (18)
	OAES	Mar 17 – Apr 16 (18)	May 1 – June 30 (14)	Oct 15 – Nov 15 (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 27 – Apr 26 (18)	May 15 – July 31 (24)	Sept 28 – Oct 28 (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 17 – Apr 16 (18)	May 15 – July 15 (24)	Oct 8 – Nov 8 (24)
16	ABBY	Apr 15 – May 15 (21)	June 1 – July 31 (24)	Oct 15 – Nov 15 (24)
	WREF	Apr 26 – May 26 (21)	June 1 – July 31 (24)	Sept 18 – Oct 18 (24)
17	SJER	Oct 1 – Oct 31 (24)	Feb 15 – Apr 1 (18)	May 6 – June 5 (24)

	SOAP	Mar 15 – Apr 15 (24)	May 15 – July 15 (21)	Oct 15 – Nov 15 (24)
	TEAK	Apr 15 – May 15 (24)	July 1 – Aug 15 (21)	Oct 15 – Nov 15 (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)

* NOTE: Sampling in YELL tower plots cannot occur earlier than July 1 of each field season due to closure of a Bear Management Area.

E.2 Sites with extremely rocky or low volume soils

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.

E.3 Site-specific soil sampling guidance for D18 and D19

High-latitude soils are different from their low-latitude counterparts. The presence of permafrost, a very short growing season, the predominance of moss, and slow rates of decomposition make these soils unique. Therefore, the definitions of, and manner of delineating between, soil horizons requires specialized instructions. Specifically:

- The surface of the soil is 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 high-latitude sites. In Alaska, material may still be very 'fibric,' e.g. have recognizable plant parts that are 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 D18 and 19

1. At a suitable X, Y location, use clippers (or equivalent) to remove live surface vegetation 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).
2. Place coring device in the brownie footprint and drive 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. 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)
    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

E.4 Site-specific soil sampling devices

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

Domain	Site	Soil Type(s)	Sampling Device(s)
01	HARV	Soils mostly organic. Loamy and rocky mineral soils	AMS auger, part# 400.09 2 inch diameter
	BART		
02	SCBI	Rocky soils	AMS 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	JMC Backsaver, handle (part# PN001) plus sample tube (part# PN012) 12 inch x 1 1/4 inch diameter
	KONZ	Very rocky, shallow soils	AMS soil auger, part# 402.36 2 1/4 inch diameter
	KONA	NA	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	
	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	NA
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	AMS slide hammer corer, part# 404.49 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

E.5 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.