TOS PROTOCOL AND PROCEDURE: SOIL BIOGEOCHEMICAL AND MICROBIAL SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/03/2011	ECO-00280	Initial Draft Release
B_DRAFT	01/13/2014	ECO-01140	Draft release. Will be finalized in next rev.
C	03/25/2014	ECO-01670	Production release, template change, and other changes as detailed in Appendix C
D	09/15/2014	ECO-02086	Minor updates to SOP B (Field Sampling) and SOP C (Lab Processing)
E	09/22/2014	ECO-02296	Migration to new protocol template
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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.

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1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling of soils and domain lab processing of soil samples for physical properties, nutrient stocks, nitrogen (N) transformations, and microbial biodiversity and function. These data will be used to quantify the stocks of soil carbon (C) and nutrients to understand ecosystem nutrient status, the isotopic (C and N) composition of the soil to gain a picture of integrated ecosystem processes, soil N transformations to understand the rates of microbially-mediated processes, and microbial biomass and community composition. NEON will characterize the soil properties, including pH and volumetric water content, which are some of the environmental controls on biogeochemical processes. As these datasets will be compared with one another, all analyses are performed on the same material when possible; however, due to differences in sampling frequencies for soil microbial communities, soil biogeochemical stocks, and soil N transformations, sometimes we collect samples separately. 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). 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), gives a picture of the integrated ecosystem processes occurring within soils or other media and possibly the source of that element. Commonly, it is 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 to multi-month incubation period (e.g., T0 to T7 days).

Microbial biomass provides an indication of microbial activity and correlates with numerous ecological processes. Biomass is measured as the difference between the total organic C measured in fumigated and non-fumigated samples. Microbial diversity and the change in microbial community dynamics 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. The sequences of the total DNA extracted from the soil (metagenome) will provide information on the functional potential of the microbial communities, while the sequence of the expressed mRNA genes (metatranscriptome) provides a snapshot of the active microbial processes occurring in the soil community.

Measurements of soil biogeochemistry and microbial community dynamics provide scientists, managers, and decision-makers with important information such as whether the ecosystem is retaining or losing

nutrients, how water and nutrients move through landscapes, and shifts in microbially-mediated ecosystem processes due to changes in nutrient concentrations. Comparing these data with those from adjacent systems, 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 redox behavior of the soil, and transport of chemical constituents from land to surface waters.

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 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 Brooks et al. (1985) and Vance et al. (1987).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000906	NEON Science Design for Terrestrial Biogeochemistry
AD[07]	NEON.DOC.000908	NEON Science Design for Terrestrial Microbial Ecology
AD[08]	NEON.DOC.014051	Field Audit Plan
AD[09]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control 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.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001577	Datasheets for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
RD[06]	NEON.DOC.001403	NEON Raw Data Ingest Workbook for TOS Terrestrial Biogeochemistry: Chemistry of Soils and Plants

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
^{2}H	Deuterium; the less common stable isotope of hydrogen
K^{+}	Potassium
m	Meter
M	Molar
mg	Milligram
ml	Milliliter
mRNA	Messenger Ribonucleic Acid
N	Nitrogen
^{15}N	Less common stable isotope of nitrogen
^{14}N	Common stable isotope of nitrogen
NH_4^{+}	Ammonium
NO_3^{-}	Nitrate
PDA	Personal Digital Assistant
PO_4^{3-}	Phosphate
P	Phosphorus
P&P	Procedure and Protocol
S	Sulfur
SO_4^{2-}	Sulfate
USDA	United States Department of Agriculture

2.4 Definitions

None given.

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 and Topp, 2002), as well as the Standard Soil Methods for Long-Term Ecological Research (Robertson et al., 1999). Soils are inherently spatially heterogeneous, and, thus, several samples must be collected in order to capture variability at multiple scales (e.g., soil core, sub-plot, plot, site). NEON scientists 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.

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).

In addition, the depth of soil to saprolite or bedrock will vary across domains. NEON soil sampling shall sample to a maximum depth of 30 ± 1 cm where possible. More detailed characterization of the

dominant soil type will occur during the construction period of NEON, including thorough description of soil pits dug from the surface to bedrock (where possible) at all core and relocatable sites. The Fundamental Instrument Unit (FIU) team will carry out this activity.

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 PDA 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 in the PDA and/or field data sheet. Once soil cores have been collected, extraction holes must be backfilled as per local permit regulations and the final sample location recorded so that subsequent samples are not collected in the same locations.

Soil Biogeochemical Stocks and Stable Isotopes. Soil samples collected for measurement of biogeochemical stocks (e.g., concentrations of C and N) and stable isotopes (e.g., ^{13}C and ^{15}N) undergo preliminary processing in the domain laboratory. This consists of sieving and drying soils according to the SOPs below. Subsamples of these soils are also analyzed for pH and moisture at the domain 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 below, 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 summer bout, composite samples of cores from the same sites will also be generated in the laboratory 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 to collect two companion soil cores at one location. One core goes back to the laboratory for immediate processing, while the other remains in a capped PVC tube (bottom left open) and is replaced in the soil. This “final”, incubated core remains in the ground for a specified period (one week to several months), 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, homogenizing the samples by hand, removing rocks and roots, and sieving to 2 mm. A subsample from the homogenized core is then placed in a cup with 2M KCl and shaken periodically for 18-24 hr. Simultaneously, subsamples of the soil core are prepared for moisture analysis and pH. At the conclusion of the 18-24 hr extraction period, the solution plus soil is filtered and the solution (i.e., liquid with soil filtered out) is poured into a tube and frozen prior to shipment to a laboratory for analysis of NH_4^+ and NO_3^-. These samples are also analyzed for soil pH and 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.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[08]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[09]).

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. The timing of sampling allows researchers to assess biogeochemical cycling under different environmental conditions or drivers. These drivers include, but are not limited to, climate forcing (e.g., solar radiation, air and soil temperature, and precipitation), disturbance (e.g., ice storms, wildfire, land use/land cover change), and plant phenology (e.g., dormancy, begin physiological activity, peak plant productivity, senescence). Sampling frequency will be set to allow researchers to investigate how microbial communities and nutrient dynamics change temporally. Finally, 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. Thus, at the different NEON sites, sampling frequency and spatial extent will vary depending on the climatic factors and landscape features, the biogeochemical context of the location (e.g., is this an area of high N deposition?), as well as logistical (e.g., site accessibility) and financial constraints.

Soil Biogeochemical Stocks and Stable Isotopes. Soil biogeochemical stocks and stable isotopes will be measured once every 10 years at 10-15 plots per site during the July-August window; during the initial years of Operations sampling, soils will be collected more frequently (e.g., each year) for these analyses as domains get up to speed. When soil cores for measurement of soil biogeochemical stocks and stable isotopes are collected, subsamples of the soil cores must also be analyzed for microbial community, microbial biomass, soil pH, and soil moisture.

Microbial Community Analysis. Microbial communities will change more frequently than the other soil properties that we measure. Hence, these collections occur in 8 plots three times per year: during the winter-spring and fall-winter transitions (when the ground is not frozen in temperate regions) and during the summer (July-August). Windows of snow-free time per domain are listed in Appendix E, and specific plots are provided in Appendix F). When sampling for soil biogeochemical stocks and stable isotopes occurs, soil for microbial analyses shall be collected at 10-15 plots instead of 8; soil for microbial analyses will be a subsample of the soil core collected for biogeochemical stocks and stable isotopes. This will count as the summer microbial sampling bout for that year. Microbial biomass sampling will be conducted 3 times per year every 5 years at each site. Finally, during a summer sampling bout, molecular –omics (metagenomics and metatranscriptomics) analyses will be conducted on soils that are composited at the plot scale.

Soil N Transformations. Soil N transformations tend to be variable both in space and time. These measurements will be made every 5 years at each NEON site. During a sampling year, three sampling events will occur. One sampling event will occur at all sites during July-August; this is the period of peak biomass in many temperate ecosystems, and it will also generate data from all sites at the same time. The two other sampling events will occur during expected "hot moments" of biogeochemical activity that cluster across groups of domains. In those that have a significant snow-covered and snowmelt season, we will make measurements over the winter (a multi-month incubation period) and during seasonal snowmelt (i.e., the seasonal transition). In those that have a dry/wet season, NEON will do one incubation during the length of the dry season, and one at the onset of the wet season (i.e., following the first rainfall).

4.2 Criteria for Determining Onset and Cessation of Sampling

Soil Biogeochemical Stocks and Stable Isotopes. Sampling of soil cores for biogeochemical and soil microbial community analysis (one large, combined bout) will occur during July-August. This period marks the timing of peak biomass in many NEON domains, and will create a synchronized dataset across all domains. As long as sampling does not commence prior to 1 July, or last longer than 31 August, the bout can be scheduled at the convenience of the domain staff.

Microbial Community Analysis. Sampling bouts will occur three times during the year in order to capture the prevailing conditions at the site during that season. Soil samples are collected during the summer months of July-August. [When soils for microbial analyses are collected as part of the soil biogeochemical stocks and stable isotopes bout in July-August, this counts as one of the three sampling periods per year]. For the two transitional sampling bouts, NEON staff scientists and domain Field Operations staff will determine the dates for soil microbial sampling on an annual basis following the guidelines in Table 1. The first bout of the year will take place when the soils are changing activity levels. In temperate zones, this would equate to the timing of soil thawing, which will be tested by the field team when the plots are free of snow and the sampling device can be pushed all the way into the ground. In wet/dry zones, this would be marked by the wet-dry season transition. The third bout of the

year will take place during the fall-winter transition, prior to the ground freezing. For wet/dry sites, this equates to the start of the wet season. Domain-specific guidelines for the timing of sampling bouts are provided in Table 1.

Table 1. Timing of Soil Microbial Sampling. The criteria listed below provide guidelines for determining the suitability of initiating a sampling bout. Not all criteria must be met. Note that Domains 18 and 19 are only sampled during the summer bout.

Bout	Sampling period	Domains	Timing of core sampling	Characteristics
Seasonal Transition #1	Winter-spring transition	1, 2, 5, 6, 7, 9, 10, 12, 13, 15	• Within 3 days of spring snowmelt initiation • No snow on ground • Within 2 weeks of ground thaw	Start of active period
	Wet-dry transition	3, 4, 8, 11, 14, 16, 17, 20	• ~1 week prior to end of wet season • No rainfall event for 1 week	Initiation of dry season
Summer	Summer	All	Between 1 July & 31 August	Timing of peak biomass
Seasonal Transition #2	Fall-winter transition	1, 2, 5, 6, 7, 9, 10, 12, 13, 15	• Within 1 week of the first snow • When mean air temperature is freezing, but ground is not frozen.	Start of quiescence period
	Dry-wet transition	3, 4, 8, 11, 14, 16, 17, 20	During or within 1 day of the first rains	Initiation of wet season

Soil N Transformations. Criteria for determining the onset and cessation of sampling for soil N transformations are summarized in Table 2. Those domains not doing the over-winter and snowmelt or over-dry season and first rains of wet season sampling periods (i.e., Domains 3, 4, 8, 11, 18, 19, and 20) will only do the July-August sampling period.

Table 2. Summary of timing criteria for measuring soil N transformations. Note that Domains 3, 4, 8, 11, 18, 19 and 20 are only sampled during the July-August collection period.

Sampling period	Domains	Timing of "initial" core sampling/prep of "final" core incubation	Length of incubation	Timing of "final" core collection
July-August	All	After 1 July	1 week	Before 31 August
Over-winter	1, 2, 5, 6, 7, 9, 10, 12, 13, 15	Within 1 week of the first snow	Variable (likely 4-5 months)	Upon initiation of snowmelt, when access via roads is permitted.
Snowmelt	1, 2, 5, 6, 7, 9, 10, 12, 13, 15	Within 3 days of spring snowmelt initiation	Variable (likely 1-5 weeks)	Within 1 week of no snow on the ground or when access via roads is permitted.
Over-dry season	14, 16, 17	Approximately 1 week prior to the end of the wet season and when there has not been rainfall for 1 week.	Variable (likely 4-5 months)	Approximately 1 month prior to expected first rains
First rains of wet season	14, 16, 17	During or within 1 day of the first rains	1 week	One week following collection of "initial"

4.3 Timing for Laboratory Processing and Analysis

Soil Biogeochemical Stocks and Stable Isotopes. Soil cores that are collected for microbial community analysis and biogeochemistry must have one subsample processed as for microbial analyses only, and the other subsample must be transferred to a cooler with ice packs and then processed within 24 hr (or immediately upon return to the laboratory, if field staff are working remotely). If soil core sample subsamples for biogeochemical analyses are not kept cold on ice packs in cooler for more than 4 hr, they may not be used. Field staff should be in communication with NEON staff via JIRA to reschedule the sampling bout.

Microbial Community Analysis. Soil cores (or subsamples from concurrent biogeochemical sampling) collected for microbial analyses only 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. Shipment instructions for these samples appear in SOP K. Soil subsamples to be used for biomass measurements should be stored field-moist and refrigerated at 4° C.

Soil N Transformations. Soil cores collected for this purpose should be processed within 24 h of field collection (applies to "initial" and "final" soil cores), and preferably immediately following collection.

Soil pH and moisture. Soil pH and moisture will be measured by domain staff whenever soils are collected for the above three groups of measurements. Processing of subsamples for soil pH and moisture must be done on soil kept cold (on ice packs, in a cooler) within 24 hr of collection (or immediately upon return to the laboratory, if field staff are working remotely; a maximum of three days).

4.4 Sampling Timing Contingencies

Table 3. Contingent decisions for all soil measurements

Delay/Situation	Action	Outcome for Data Products
Inability to finish sample bout	Communicate to staff scientists via problem ticket for further instruction.	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 for further instruction.	Dataset may be incomplete or sampling bout redone. Latter may result in delay of data products delivery.
Delay in start of sampling bout after 31 August.	Communicate to staff scientists via problem ticket for further instruction.	Samples may reflect different conditions.
Sampling for soil microbial community analysis or soil N transformations 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 products generated.
There is standing water within the entire plot area where soil sampling is to occur.	Do not attempt to collect soils. Communicate to staff scientists via problem ticket for further instruction.	Dataset may be incomplete or sampling bout delayed/redone. Latter may result in delay of data products delivery.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

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

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. Protocols for the handling of quarantined soils can be found in NEON's USDA Animal and Plant Health Inspection permit (RD[13]). Domains or sites with soils that require quarantine can be found in 7 CFR Part 301 Domestic Quarantine Notices of the Plant Protection Act (7 U.S.C. 7756). Quarantine soil samples that are being shipped to external laboratory facilities must include a copy of the USDA Soil Permit (and comply with outlined shipping guidelines) from the contracted facility. The protocol for including this permit is described in detail in this document.

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. In addition, 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 safety labels.

6 PERSONNEL RESOURCES

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. Equipment list - Preparation for field sampling

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Ice pack	Prepare for shipping of field-moist soils		N
Consumable items					
MX103942	R	All weather copy paper	Print datasheets		N

Table 5 Equipment list - Field sampling for soil microbe and biogeochemical stock at one site

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Cooler	Chill perishable samples in field	2	N
MX108279	R	Digital soil thermometer	Measure soil surface temperature	2	N
MX100703	S	GPS receiver, recreational accuracy	Navigate to sampling location		N
MX105086	R	Ice pack, -20° C	Chill perishable samples in field	16 (+)	N
MX100722	R	Measuring tape, minimum 30 m	Locate coordinates for soil sampling locations	2	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
EG07610000	S	Organic horizon cutter template	Remove organic horizon	1	N
MX100543	S	Ruler, minimum 30 cm	Measure soil core horizons	1	N
	S	Soil corer, 2-3" ID, minimum 30 cm long	Collect soil core	1	N
MX100721	S	Soil knife	Separate soil horizons, subsampling, etc.	1	N
	S	Trowel	Remove soil core	1	N
Consumable items					
	S	AA battery	Spare battery for GPS receiver		N
	R	Deionized water	Rinse soil from equipment	2 liters	N
MX100212	R	Dry ice, pelletized	Freeze soil microbial subsamples	20 pounds	Y
	R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
	S	Paper towel	Remove debris from soil sampling equipment	1 box or 2 cloths	N
	R	Permanent marker, fine tip	Label sample bag	3	N
	R	Resealable freezer bag, 1 pint	Contain soil for microbial biomass analysis	30	N
MX100592	R	Resealable plastic bag, 1 gal	Collect and homogenize soils, contain soil samples for soil pH, moisture, biogeochemical stocks and stable isotope analysis	2 boxes	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	S	Survey marking flag, PVC or fiberglass stake	Flag soil sampling location	3	N
	S	Trash bag	Dispose of consumables	2	N
MX108171	R	Whirl-Pak bags, 2 oz	Contain soil for microbial molecular analysis	100	N
Resources					
RD[05]	R	Field datasheet	Record data	---	N
MX106268	R	Weatherproof labels	Pre-label sample bags	100	Y
	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 (e.g. field datasheets unless PDA is available)

Table 6. Equipment list – Field sampling soil N transformations at one site

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Cooler	Chill perishable samples in field	2	N
MX108279	R	Digital soil thermometer	Measure soil surface temperature	2	N
MX100703	S	GPS receiver, recreational accuracy	Navigate to sampling location	1	N
	S	Hammer or mallet	Insert cylinders into soil	1	N
MX105086	R	Ice pack, -20° C	Chill perishable samples in field	16 (+)	N
	R	Incubation cylinders (schedule 40 PVC or steel); 30 cm length x ≥ 5 cm diameter	Sample soil cores and store field-incubated soil cores	1/soil sampling location, plus 2 additional	N
	R	Loose-fitting caps for each cylinder	Protect cylinder openings from debris and water	1/soil sampling location	N
MX100722	R	Measuring tape, minimum 30 m	Locate coordinates for soil sampling locations	2	N
MX103931	S	Plastic tray	Separate soil core (horizons, subsamples, etc) in field	2	N
MX100721	S	Soil knife	Separate organic and mineral horizons	1	N
Consumable items					
	S	AA battery	Spare battery for GPS receiver	1	N
	R	Deionized water	Rinse soil from equipment	2 liters	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX103940	S	Flagging tape	Flag location of incubated soil core	1 roll	N
	R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
	S	Paper towel	Remove debris from soil sampling equipment	4 rags/1 box	N
	S	Permanent marker	Label sample bag	4	N
MX100592	R	Resealable plastic bag, 1 gal	Contain soil samples	2 boxes	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

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX104734		Centrifuge tube rack, for 20 mm tube	Preparing biogeochemical/stable isotope samples for oven drying		
MX100350		Sieve set	Sort mineral horizon soil particles to 2 mm		
MX103208	R	Sieve, 2 mm	Sort mineral horizon soil particles to 2 mm	1 set	N
Consumable items					
MX100634		Label tape, ethanol safe	Labeling scintillaiton vials		
	R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
MX105089	R	Paper bag, #8	Contain soil subsamples while air-drying	50	N
MX101278	R	Scintillation vials with caps, 20 mL	Preparing biogeochemical/stable isotope samples for oven drying; Securing samples for shipment		
Resources					
RD[05]	R	Lab datasheet	Record data	---	N

R/S=Required/Suggested

Table 8. Equipment list - soil pH measurement

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100267	R	pH meter	Measure pH value of samples	1	N
MX104770	S	Stir rod	Mix pH samples	1	N
MX100570	S	Volumetric flask, 1 L	Prepare calcium chloride solution for pH analysis	1	N
Consumable items					
MX105810	R	Calcium Chloride Dihydrate	pH analysis	2.94 g	N
MX105811	R	Calcium Hydroxide	Adjust pH of $CaCl_2$	1 ml	N
	S	Cup 50-100 mL	pH analysis	50 (+)	N
		Deionized water	Rinse pH meter electrode		
MX100213		Ethanol, 190 proof (95%)	Prepare work area		
MX105812	R	Hydrogen Chloride	Adjust pH of $CaCl_2$	1 ml	N
MX100642		Low lint wipe	Dry pH meter electrode		
	R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
MX100583 MX100584 MX100052	R	pH buffer (4, 7, 10)	Calibrate pH meter	1	N
MX100689 MX100690	R	Weigh boat, large or small	Weigh subsample for pH measurement	50 (+)	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
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 N transformations

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100639	S	Carboy, 20 L	Prepare and store 2M KCl	1	N
	R	Filtration flasks (at least 150 ml)	Contain discarded KCl solution	8	N
	R	Funnel	Filter samples	8	N
MX100391	R	Graduated cylinder, 250 mL plastic	Measure aliquot of KCl	1	N
	R	Manifold	Filter samples	1	N
	R	Vacuum pump	Filter samples	1	N
Consumable items					
	R	Deionized water	Prepare 2M KCl	20 liters	N
	R	Extraction cups and lids (e.g., urinalysis cups) or equivalent (120 ml capacity)	Extract KCl from soils	100	N
	R	Glass fiber filters	Filter samples	1 box	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Nitrile gloves, powderless	Prevent contamination of soil samples	1 box	N
	R	Potassium Chloride	Extract NH_4+ and NO_3- from soil		N
MX100592		Resealable plastic bag, 1 gallon	Organize sample tubes		
MX101278	R	Glass scintillation vials with caps, 20 mL	Contain soil extract sample for freezing and shipment to laboratory	100	N
Resources					
RD[05]	R	Lab datasheet	Record data	---	N

Table 10. Equipment list – Laboratory processing of soils for measuring pH at one site

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX103208	*R*	*Sieves*	Sorting soil particles to 2mm	All	*1 set*	*N*
	R	pH meter	Reading pH value of samples	All	1	N
	S	Cafeteria trays	Holding soil subsamples	All	4 (+)	N
	S	2 liter glass volumetric	Preparing solution calcium chloride solution for pH analysis	All	1	N
	S	Stir rod	Mixing pH samples	All	1	N
Consumable Items						
MX100645 MX100646 MX100647 MX100644	*R*	*Powderless gloves*	Preventing sample contamination	All	*1 box*	*N*

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX105089	R	Paper (e.g., "lunch") bags	Air-drying soil subsamples	All	50	N
MX105810	R	$CaCl_2 \cdot 2H_20$	pH analysis	All	2.94 g	N
MX100308	R	Deionized water	pH analysis	All	---	N
MX105812	R	HCl	Adjusting pH of $CaCl_2$	If solution is too basic	1 ml	N
MX105811	R	$Ca(OH)_2$	Adjusting pH of $CaCl_2$	If solution is too acidic	1 ml	N
MX100583 MX100584 MX100052	R	pH buffers (4, 7, 10)	Calibrating pH meter	All	1	N
RD[05]	R	Physical copy of datasheets	Data entry	All	---	N
	S	50-100 mL cups	pH analysis	All	50 (+)	N

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

Table 11. Equipment list - Laboratory processing of soils for N transformations at (one site)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Graduated cylinder (150 ml)	Measuring aliquot of KCl	All	1	N
	R	Funnels	Filtering samples	All	8	N
	R	Filtration flasks (at least 150 ml)	Filtering samples	All	8	N
	R	Vacuum pump	Filtering samples	All	1	N
	R	2 mm sieve	Sieving soils	All	1-2	N
	R	Manifold	Filtering samples	All	1	N
	S	Carboy (20 L)	Storing 2M KCl	All	1	N
	S	Cafeteria trays	Storing soil moisture subsamples in oven; storing soil extracts during extraction period	All	6	N
Consumable Items						

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	KCl	Extracting NH_4^+ and NO_3^- from soil	All		N
	R	Screw-cap polyethylene extraction cups and lids (e.g., urinalysis cups) or equivalent (120 ml capacity)	Extracting soils	All	100	N
	R	DI water	Preparing 2M KCl	All	20 liters	N
	R	Plastic tubes with screw tops (20 ml)	Storage of soil extract (sample) for freezing and shipment to laboratory	All	100	N
MX100645 MX100646 MX100647 MX100644	R	Powderless gloves	Preventing contamination of soil samples	All	1 box	N
	R	Glass fiber filters	Filtering samples	All	1 box	N

R/S=Required/Suggested

Table 12. Equipment list - oven-dried and air-dried samples

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items					

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	S	Cardboard box	Package samples for shipment	2 (+)	N
		Cushioning material (i.e. wadded newspaper)	Package samples for shipment		
	R	Packaging tape	Package samples for shipment	1	N
Resources					
	R	Shipping manifest	Inventory of specimens being shipped	1 per box	N
	S	USDA Permit to Receive Soils or Compliance Agreement	Comply with USDA regulations for quarantine soils	1 per box	N

R/S=Required/Suggested

Table 13. Equipment list – Shipment of refrigerated microbial biomass samples

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Ice pack	Chill perishable samples during shipment	20 pounds	Y
Consumable items					
MX102297	S	Insulated shipper	Package samples for shipment	1	N
	R	Packaging tape	Package samples for shipment	1	N
MX100592	S	Resealable plastic bag, 1 gal	Organize sample bags	~3	N
Resources					

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Shipping manifest	Inventory of specimens being shipped	1 per box	N
	S	USDA Permit to Receive Soils or Compliance Agreement	Comply with USDA regulations for quarantine soils	1 per box	N

R/S=Required/Suggested

Table 14. Equipment list - Shipment of microbial molecular analysis and N transformation samples

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items					
MX102297	R	Cardboard box or insulated shipper, UN packing group III	Package samples for shipment	1	N
		Cushioning material (i.e. wadded newspaper)			
	R	Dry ice shipping label	Label shipments containing dry ice	1	N
MX100212	R	Dry ice, pelletized	Keep samples frozen during shipment	20* pounds	Y
	R	Packaging tape	Package samples for shipment	1	N
MX100592	R	Resealable plastic bag, 1 gal, 4 mil	Organize sample bags	~3	N
		Styrofoam sheet	Insulate samples for shipment		
Resources					
	R	Shipping manifest	Inventory of specimens being shipped	1 per box	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	S	USDA Permit to Receive Soils or Compliance Agreement	Comply with USDA regulations for quarantine soils	1 per box	N

Table 15. Equipment list - Shipment of samples for analysis of soil biogeochemical stocks and stable isotopes from (one site)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Consumable Items						
	R	Packing tape	Shipping soil samples	All	1	N
	R	Cover letters and sample inventory spreadsheets for contracted facility(ies). These forms supplied by NEON Headquarters Staff to Domain managers.	Shipping soil samples	All	1 per box	N
	S	USDA Soil Permit from contracted facility(ies), including an necessary labels specified in the permit (see Safety section)	Shipping soil samples	All	1 per box	N
	S	Boxes	Shipping soil samples	All	2 (+)	N

Table 16. Equipment list – Shipping samples from one site

Table 17. Equipment list - Shipment of samples for molecular analysis of microbes from (one site)

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX105 087	R	Ice packs	Shipping soil samples	All	16 (+)	N
MX102 297	R	Foam Cooler	Shipping soil samples	All	1	N
Consumable Items						
MX100 212	R	Dry ice	Shipping soil samples for microbial analysis	All	20* pounds	Y
MAT11 1	R	Dry ice packing labels	Shipping soil samples for microbial analysis	All	1	N
	R	Packing tape	Shipping soil samples	All	1	N
	R	Cover letters and sample inventory spreadsheets for contracted facility(ies). These forms supplied by NEON Headquarters Staff to Domain managers.	Shipping soil samples	All	1 per box	N
	S	USDA Soil Permit from contracted facility(ies), including an necessary labels specified in the permit (see Safety section)	Shipping soil samples	All	1 per box	N
	S	Boxes	Shipping soil samples	All	2 (+)	N

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

* At sites with maximum shipping allowances less than 20 pounds, supplement with pre-chilled packing peanuts (or similar).

Table 18. Equipment list - Shipment of soil for microbial biomass analysis from one site

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX102297	R	Foam cooler	Shipping soil samples	All	1	N
Consumable Items						
	R	Cover letters and sample inventory spreadsheets for contracted facility(ies). These forms supplied by NEON Headquarters Staff to Domain managers.	Shipping soil samples	All	1 per box	N
		USDA Soil Permit from contracted facility(ies), including an necessary labels specified in the permit (see Safety section)	Shipping soil samples	All	1 per box	N
	S	1-gallon resealable storage bags	Holding sample bags	All	~3	N
MX100212	R	Ice packs	Keeping samples chilled	All	20 pounds	Y
	R	Packing tape	Shipping soil samples	All	1	N

R/S=Required/Suggested

Table 19. Equipment list - Shipment of soil extracts (soil N transformations) from one site

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
(None)						
Consumable Items						
	R	Cover letters and sample inventory spreadsheets for contracted facility(ies). These forms supplied by NEON Headquarters Staff to Domain managers.	Shipping soil samples	All	1 per box	N
MX100212	R	Dry ice	Keeping samples frozen.	All	Variable	Y
MX102297	R	Foam cooler	Shipping soil samples	All	1	N
	R	Packing tape	Shipping soil samples	All	1	N
	S	Sealable freezer bag (at least 1 qt size) that can withstand shipment with dry ice	Holding groups of test tubes.	All	20 pounds	N

6.3 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

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

6.4 Specialized Skills

Soil types and profile characteristics differ greatly across the NEON domains (see examples in Figure 3). 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 (loose vegetal matter that may be intact or partially shredded), organic horizon (often dark and slightly sticky, with pieces of vegetal matter in various stages of decomposition) and mineral horizons (little vegetal matter, primarily accumulated minerals). 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. Field personnel should be prepared to take extensive notes on any anomalous soil features that they observe when sampling, or in-field decisions that they make in order to carry out this protocol.

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. Field personnel should be familiar with basic microbiology and clean sampling techniques and use their best judgment to control for contamination from either themselves or from their surroundings, particularly during bad weather conditions. Some general guidelines are:

Any tool or instrument that is re-used should be cleaned with 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 new soil, it should first be cleaned. Examples of such tools include the coring devices, trowels or other digging tools, and the “brownie” square, to the extent possible. Coring devices may be particularly difficult to clean, therefore technicians should find a reasonable compromise between sample integrity and feasibility. Gloves can also be re-used if they have been thoroughly cleaned with an alcohol wipe and are free of dirt/soil. 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.

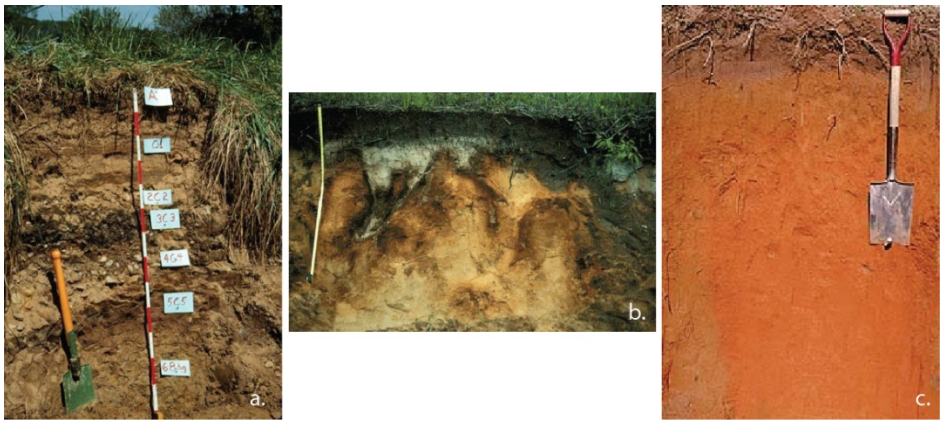

Figure 1. Soil profiles from (a) Maryland, (b) Michigan, and (c) Florida. (Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

6.5 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

We estimate that one soil sampling bout (i.e., microbial sampling, soil biogeochemical stocks and stable isotopes plus microbial sampling, or soil N transformations) per site requires 2 technicians for 1-5 days, plus travel to and from the site, and sample processing, including root and rock removal, sample homogenization, sieving (if required), and sample shipment.

7 STANDARD OPERATING PROCEDURES

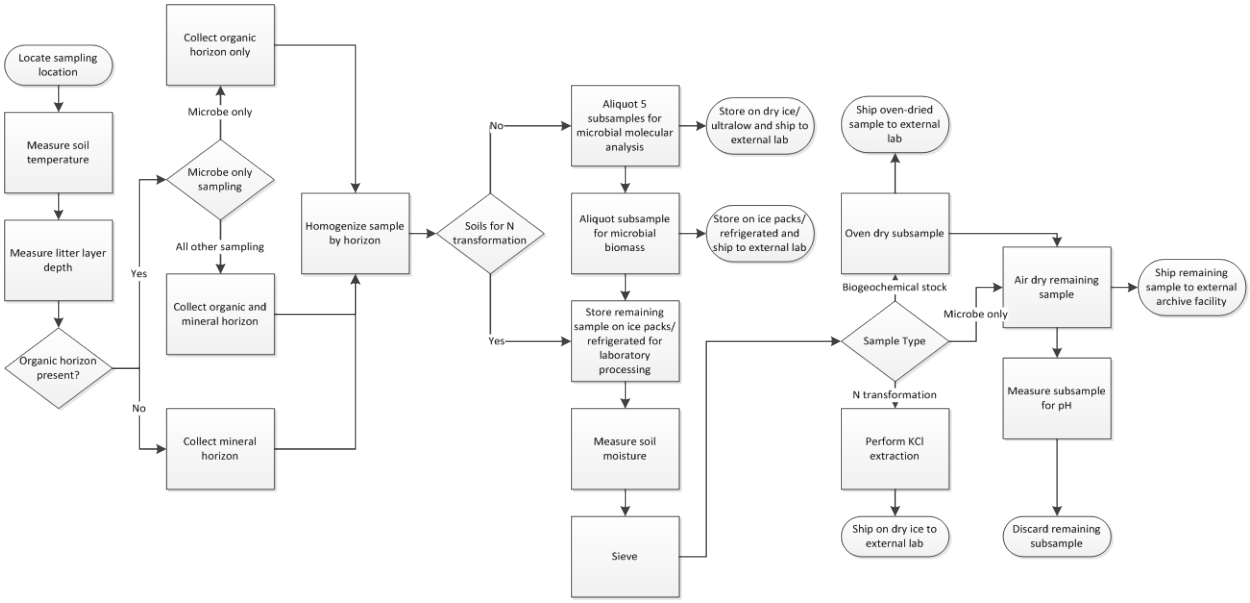

Figure 2 Soil collection, processing and shipping workflow

SOP A Preparing for Sampling Soils (All Types of Soil Field Collections)

1. Fill out site information on field datasheet (RD [05]). Make sure to use proper formats, as detailed in datasheets.
2. Print cryovial labels (leave coordinates field blank until you confirm core x, y location).
3. Prior to sample collection, plots where soil samples will be collected should be identified and flagged.

SOP B Combined Field Sampling for Soil Biogeochemical Stocks, and Stable Isotopes, and/or Microbes Soil Core Collection

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, collect a soil core, subsample the soil core, and store subsamples for laboratory transport.

When sampling for soil biogeochemical and stable isotope analyses occurs, soils are also subsampled for microbial analysis. This "major" sampling bout includes field measurement of soil temperature, field subsampling for microbial analysis and archiving, field subsampling for soil moisture, soil pH, soil biogeochemical stocks and stable isotope analyses, and archiving.

During a summer bout, additional –omics analyses will be conducted as part of a microbial sampling campaign. This does not involve changes to the field sampling; however, in the lab, technicians should follow SOP H ("Generation of composite samples") to process samples for these analyses.

B.1 Identify the plot

1. Navigate to the southwest corner of the plot.
2. Lay out meter tapes on the west and south sides of the plot and locate x, y coordinates (i.e. sampling location). You will collect soil at three random locations within each plot.
3. Put on a clean pair of nitrile gloves (1 pair per random sampling location, put on a new pair at each location; do NOT reuse gloves between locations).

B.2 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 it will 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).

B.3 Collect soil core

2. Identify soil core sampling location.
1. Measure the depth of the litter layer (cm) above each core location and record the value. The litter layer is generally composed of undecomposed plant material (i.e., leaves are still recognizable), whereas an organic horizon will contain organic material in various states of decomposition.. This 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.

2. Push the litter layer away from where you are going to core into the soil surface.
3. If an **organic horizon** is present,
    a. Using clean tools and equipment, cut out an organic horizon "brownie" from two locations within 0.5 m of each other using the square frame cutter tool. Measure the depth of each side and record the average value in cm at both locations. Note: in the rare case, a site could have an organic horizon that is > 30 cm. In that instance, only sample to 30 ± 1 cm depth.
    b. Combine soils from the two locations to form one composite sample of the organic horizon. Put the organic horizon samples into a 1-gallon resealable plastic bag and homogenize by hand.
    c. Aliquot subsamples from the 1-gallon bag of homogenized organic horizon material into 6 labeled 2 oz. Whirlpak bags for microbial molecular analysis. Fill bags at least halfway. Number the bags 1-6 (the order is not important).
    d. Place approximately 20 g from the 1-gallon bag into a labeled, 1-pint resealable freezer bag for the microbial biomass sample.
    e. The remaining contents in the 1-gallon bag are for analysis of soil pH, moisture, and biogeochemical stocks and stable isotopes.
    f. Label all sample bags with sampleID (plotID-horizon-coreCoordinateX-coreCoordinateY-date), measuredBy, and recordedBy). The X, Y coordinates are labeled to the nearest 0.1 m.
    g. Place the Whirlpaks in the cooler with dry ice, and the remaining bagged soils into a cooler with ice packs.
4. Determine whether to collect mineral horizon.
    a. When collecting soil microbe samples only, collect mineral horizon **IF** no organic horizon is present.
    b. When collecting biogeochemical stocks and soil microbes, collect mineral horizon from all sample locations. During the biogeochemistry sampling bout, mineral horizon samples are always collected for microbial analyses, even if there is an organic horizon present.
5. 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, the whole mineral soil core should be 30 ± 1 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 the soil corer being used; in order to accommodate site-specific challenges related to soil sampling (e.g., rocks and roots). Target the soil volume of a 6 cm diameter x 30 cm depth core in order to obtain sufficient material for laboratory analyses. In some cases, multiple cores per sample are required. For example, if coring device is 2.5 cm diameter and bedrock is at 20 cm, take two cores per sample. However, if coring device is 2.5 cm diameter and soils are 15 cm deep, three cores per sample may be

needed to collect sufficient material. Field technicians will have to exercise some judgment regarding number of cores per sample that are needed to obtain sufficient soil for analyses. ***In cases where more than two cores per sample are required, verify the approach with NEON Science Staff by issuing a problem ticket.***

a. Take core(s) from locations where organic horizon was removed if organic horizon was present.
b. If the total soil depth is < 30 cm or there are significant impediments to coring (e.g., roots and rocks throughout the site or depth to saprolite is < 30 cm), core to the depth you are able and make a note in the 'remarks' field of the datasheet. If you have to move the x,y coordinate, due to an impediment (e.g., large root, rock, or previous sampling in that area), write the original location in the 'remarks' field and note that you had to change it and why.
c. Record horizon type 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.

6. Place all mineral soil cores in one bag and homogenize (mix) by hand.
   a. Avoid contacting soil microbe samples as much as possible.
   b. Mixing by hand should be avoided unless necessary to ensure adequate homogenization.
7. Aliquot subsamples from the 1-gallon bag of homogenized mineral horizon material into 6 labeled Whirlpak bags for soil microbe molecular analysis. Fill bags at least halfway. Number the bags 1-6 (the order is not important).
8. Place approximately 50 g from the 1-gallon bag into a labeled, 1-pint resealable freezer bag for the microbial biomass sample.

Organize Whirlpak bags from the same sample using rubber bands, clips, or a larger freezer bag for ease of sample tracking during storage and shipment.

9. Complete the labels on all sample bags with the sampleID (plotID-horizon-coreCoordinateX-coreCoordinateY-date), measuredBy (technician name), and recordedBy (technician name). The x,y coordinates are labeled to the nearest 0.1 m.
10. Immediately place the Whirlpaks in the cooler with dry ice (mRNA changes very quickly), and put the 1-gallon and 1-pint resealable bags in the cooler with the ice packs.
11. Enter metadata in field datasheet.
12. Thoroughly rinse sampling equipment with deionized water (corer, thermometer, etc).
13. Wipe down reusable sampling equipment with alcohol wipes or squirt bottle to the extent possible.
14. Discard gloves.

B.4 Sample preservation and transport

1. Keep soils for microbial biomass, biogeochemistry stocks and stable isotopes, soil pH, and soil moisture in the cooler with the ice packs and transfer to 4°C refrigerator upon return to domain lab. Soils for microbial biomass are shipped according to I.2 with no additional laboratory processing.
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 I.3 with no additional laboratory processing.

SOP C Laboratory Measurement of Soil Moisture Content

Analysis of the moisture present in the 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 be done within 24 h of field collection for both soil biogeochemistry and stable Isotope sampling bouts and microbial sampling only bouts. Soil moisture is measured on soil that has not been sieved.*** In cases where domain staff are working at remote sites, keep samples on fresh ice packs in coolers and process within 24 hours of return to the domain facility lab.

1. Weigh each horizon samples.
   a. 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.).
   b. Label weigh boat with sampleID and weigh foil boat to nearest 0.01 g and record value in the datasheet.
   c. Place 5 ± 0.1 g of a field moist organic horizon sample (not sieved) or 10 ± 0.1 g of a field moist mineral horizon sample (not sieved) into the weighed foil weighing boat. Record weight to nearest 0.01 g.
2. Place all samples into drying oven (organize samples on a tray to quickly transfer all samples into oven) at 105°C for 48 h. Record time in oven on datasheet.
3. At conclusion of drying period, immediately weigh dried sample + weighing boat to nearest 0.01 g and record values in the datasheet. Record the date and time out of oven.
4. Dispose of soils according to permit requirements and keep all weigh boats that are clean and undamaged for reuse.

SOP D Laboratory Processing of Soils for Biogeochemical Stocks and Stable Isotopes, Archiving, and pH

D.1 Sieving Field Soils

1. Process samples within 24 h of field collection or 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, and then process immediately.
2. 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.).
3. 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.
4. If sample is **organic horizon**, do not sieve.
5. Shake **mineral horizon** samples through a series of sieves, the smallest being 2 mm screen diameter sieve (this will allow all particles ≤ 2 mm to pass through to the collection pan). If the sample is unable to pass through the sieve, submit a problem ticket to receive further instruction.
6. Discard particles > 2 mm according to permit requirements.
7. Record metadata on the lab datasheet (RD[**]); site, collectDate, measuredBy, plotID, coreCoordinateX and coreCoordinateY, and horizonType) and the processingDate and processingTime.

D.2 Oven-Drying Field Samples from Biogeochemical Stocks and Stable Isotopes

1. **F**ill a scintillation vial with each unique sample. Transfer sampleID to scintillation vial (Suggestion: write sample information on lab tape and wrap tape completely around middle of scintillation vial.). Do not cap vials.
2. Place open scintillation vials into the scintillation vial box, which holds 100 vials. Oven-dry at 60°C for 48 hr. Record start and end time in lab processing datasheet. When drying period is complete, cap vials and ship to contracted laboratory for analysis (see SOP K).
3. Air dry remaining soil as described in D.3.
4. Ship oven-dried samples as described in I.1.

D.3 Air-Drying Field Samples

Follow this SOP if you are processing soils for pH as part of a sampling bout for microbial analysis and with remaining soil samples **after** subsampling soils for oven drying from the biogeochemical stocks and stable isotopes.

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 metadata above.
2. Leave the bag open to air-dry on a clean lab bench or table, away from other activities that might disturb samples. Shake up soil to expose new surfaces once or twice each day. Record startDate and startTime of air-drying in the lab datasheet.
3. Weigh at the startDate, then after one week, and daily thereafter to ensure samples have dried completely. Air-drying soil can take several days depending on the initial moisture content. Do not continue with processing until change in weight is less than 5 % over a 48 h period.
4. At the conclusion of air-drying samples, a subsample will be analyzed in the domain facility for pH (SOP E). The remainder of the sample will be shipped to archive facility according to I.1 (biogeochemistry bout only).

SOP E Laboratory Measurement of pH

Soil pH is measured on sieved, 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]).

1. Wipe lab benchtop with ethanol prior to processing samples.
2. Wear gloves throughout this procedure. If you do not touch the soil samples directly, you do not need to change gloves between samples.
3. Make the 0.01 M $CaCl_2$ solution: dissolve 2.94 g of $CaCl_2 \cdot 2H_20$ in 2 liters of DI water. Note: this solution is stable for approximately 1 year, kept at room temperature out of direct sunlight.
4. Check pH of $CaCl_2$ solution; it should be between 5.0 and 6.5.
5. Adjust one drop at a time with concentrated 6N $Ca(OH)_2$ or 10N HCl if needed (rarely is).
6. Weigh out a subsample of air-dried organic or mineral (fraction ≤ 2 mm) soil and place into 50 – 100 mL cup. Use 5 ± 0.1 g for organic soil and 10 ± 0.1 g for mineral soil.
7. Add 20 mL of $CaCl_2$ solution. DO NOT STIR.
8. 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.
9. Thoroughly stir for 10 seconds with a glass rod or plastic stir stick.
10. Further stir suspension (for 10 seconds) every 5 minutes for the next 30 minutes.
11. Allow suspension (i.e., the flocculated soil) to settle undisturbed for 30 – 60 minutes. Time required will vary by soil type.
12. Determine if soil is completely saturated.
    a. Look for supernatant (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.
13. Calibrate the pH meter electrode with pH buffers 4, 7, and 10 according to the manual for the probe. Note: some domains may need the 1.68 buffer.
    a. Rinse the electrode with deionized water and dry it between buffers.
14. Measure pH of supernatant solution, taking care to NOT disturb the flocculated soil.
    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 dry with a fresh lab tissue.
    c. Measure each sample. Note: you only need to calibrate the pH probe one time for the group of samples.
15. Repeat preparation and pH measurement of 5 samples.
    a. Select 5 soil samples for duplicate (i.e., “Dup”) pH measurement. (Choose soil samples that have ample leftover material.)
    b. Measure pH.

c. If the original and duplicate subsamples differ by ≥ 0.5 in their pH reading, take a third pH reading.
d. Record all original and duplicate values as separate entries in the data ingest.

16. Repeat pH measurements and 5 duplicate measurements with deionized water, analyzing subsamples in 20 mL deionized water instead of $CaCl_2$.
17. Discard remaining soil (following soil permit guidelines where applicable).

SOP F Field Sampling for Soil Nitrogen Transformations

F.1 Identify the plot

1. Navigate to the plot.
2. Lay out meter tapes on 2 adjoining sides of the plot and locate sampling location(s) using the tapes as guides for the given x, y coordinates.
3. Put on a clean pair of nitrile gloves (1 pair per random sampling location, put on a new pair at each location; do NOT reuse gloves between locations).

F.2 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.3 Collect soil core

1. Identify soil core sampling location.
2. Measure the depth of the litter layer (cm) above each core location and record the value. This 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.
3. Push the litter layer away from where you are going to core into the soil surface. The litter layer is generally composed of undecomposed plant material (e.g. leaves are still recognizable), whereas an organic horizon will contain organic material in various states of decomposition.
4. Insert the corer (section of pipe with beveled edge) into the ground. If your soil is difficult to core, you can use the wooden circle and mallet; if your soil is easy to core, you may just need to push in pipe. Always core vertically, not perpendicular, when collecting on a slope.
5. Push the corer in to a total depth 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 corer to indicate the depth to stop driving the corer into the mineral soil horizon.

6. 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.
7. If only mineral soil is present, empty soil from corer directly into bag.

8. Label bag with the plotID, coreCoordinateX, coreCoordinateY, collectDate, horizonType, coreType, measuredBy (technician name), and recordedBy (technician name).
9. Break up the soil core and homogenize (mix) in the bag with your gloved hand.
10. Place bag into cooler with ice packs.
11. Enter the metadata in field datasheet.
12. Thoroughly rinse sampling equipment with deionized water (corer, thermometer, bulb planter, etc).
13. Discard gloves.
14. Backfill soil core location according to site requirements.

F.4 Set up incubated soil core

Note: this core will remain in the ground for the duration of the incubation period (one week to a few months, see Table 2. Length of incubation is dependent on which collection you are doing (e.g., the July-August incubation or the snowmelt season incubation).

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 the wooden circle and mallet; if soil is easy to core, you may just need to push in pipe.
4. Leave corer in the ground and loosely place a cap over the top of the corer
5. Cover the cap with any litter that was pushed away.
6. Mark the location of the core with a 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.5 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: collected soils MUST be processed and extracted in KCl within 24 h, and, preferably, immediately after collecting the core in the field. If travel to/from a domain facility is not possible within this timeframe, it may be necessary to prepare KCl prior to the field trip, weigh and extract the soils in the field.

F.6 Collection of incubated soil core

Note: collection of the incubated soil core marks the end of the sampling bout, following the specified incubation peri**od i**n Table 2.

Navigate to plot where sampling for soil N transformations occurred and locate incubated core.

2. Remove core (within corer) from the ground. Take off top.
3. 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.
4. If only mineral soil is present, empty soil from corer directly into bag.
5. Label bag with the plotID, coreCoordinateX, coreCoordinateY, collectDate, horizonType, coreType, measuredBy (technician name), and recordedBy (technician name).
6. Break up the soil core and homogenize (mix) in the bag with your gloved hand.
7. Place bag into cooler with ice packs.
8. Enter the metadata in field datasheet.

SOP G Laboratory Processing of Soils for N Transformations

Note: these soils MUST be processed and extracted in 2M KCl within 24 h, and, preferably, immediately after collecting the core in the field. If travel to/from a domain facility is not possible within this timeframe, it may be necessary to prepare cups with KCl prior to field sampling, weigh and extract the soils in the field for both initial core and incubated soil collection.

G.1 Preparing for KCl extraction

1. Prepare 2M KCl (149.2 g/L).
   a. Wearing nitrile gloves, measure KCl and add to carboy.
   b. Add deionized water to carboy with KCl in the appropriate ratio (i.e. 1L deionized water per 149.2 g KCl)
   c. If preparing in 20 L carboy, 2984 g KCl should be added to the carboy, then add deionized water to the 20 L mark.

Note: KCl can take a long time to dissolve. It is best to prepare the solution prior to going to the field to collect samples. The KCl can be left dissolving in the carboy and periodically shaken to aid the process. KCl in solution is good for ~1 year, so it can be made at the beginning of the sampling year and then used for initial and final extractions of each of the 2-3 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 7 below).

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

1. Subsample the collected soil samples for moisture analysis, according to SOP C.
2. Sieve the collected soil samples according to D.1. ***Field-moist soil must be sieved and used for this analysis.*** You cannot sieve air-dried soil and analyze it for N transformations.
3. Place sieved material in a labeled freezer 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.

G.3 Perform KCL extraction

5. Weigh 10 g ± 0.1 g subsamples of fresh sieved 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). Enter the exact weight (10 ± 0.1 g is acceptable error around the measurement; enter weight to four significant figures, for example, 10.08 g) into the datasheet.
6. For every group of 10 soil samples, choose one soil sample to analyze in triplicate (i.e., prepare three subsamples of weighed soil to extract and analyze). In the datasheet, indicate that this is

a triplicate analysis by entering 'y' into the 'triplicate' column. For all other samples that are only analyzed once, enter 'n' into this column.

7. For each sample, measure 100 ml of 2M KCl into the graduated cylinder and add to the container of weighed soil.
8. For the entire group of samples, prepare three "blanks". Add 100 ml extractant 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.
9. Shake all of the cups for ~30 seconds each and place on trays.
10. The samples should extract for 18-24 h. During this period, shake every 8 h for ~30 seconds.

G.4 Filtering Samples

Note: samples are filtered in batches of 5 (the number of filtration set-ups that can go on the manifold at one time). Between batches, wash the filtration set-ups (funnel plus flask) with detergent and deionized water. Soil samples within a batch may finish filtering at different times. New samples can be filtered individually 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, filtration funnels and flasks, and vacuum pump.
   a. Open the stopcocks of all filtration lines to allow vacuum to pull from each funnel plus flask set-up.
   b. Test vacuum to make sure it is working properly.
2. Put on a new pair of nitrile gloves. Use the same pair of gloves on 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. Place a glass fiber filter into the bottom of each funnel.
4. Leach each filter with KCl solution by pouring 50 ml of KCl solution into each funnel, running the pump; dispose of KCl solution in flask.
5. Pour a sample into each funnel and turn pump on.
6. Wait for sample to filter completely then pour the filtrate from the flask into a 20 ml plastic tube and cap tightly.
7. Label the sample tube with the sampleID.
8. Discard remaining filtrate into flask.
9. Place tubes containing the sample filtrate in a resealable plastic bag (i.e., grouped together in the bag). Position the tubes vertically, **cap end up**, in the -20° C freezer. Store frozen until shipment to the contracted laboratory facility (see SOP K).

G.5 Sample Storage

Samples can be stored frozen at the domain lab for up to two weeks prior to shipping. During the July-Aug sampling and incubation period, initial and incubated samples can be shipped together, but all other bouts (e.g., snowmelt, or firstRains) will have initial and incubated samples shipped separately.

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, metagenomics and metatranscriptomic analyses will be conducted on soil at the plot scale. This SOP describes the laboratory procedure for generating and labeling a composite soil sample. NOTE: Metagenomics and metatranscriptomics are only measures on samples collected during the summer 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. Label a 1-pint resealable freezer bag with the plotID, horizon, and collection date that matches a set of whirlpaks and place that bag with the corresponding whirlpaks.
3. Repeat step 2 for every unique plotID, horizon, and collection date. There should 1 resealable freezer bag for every set of whirlpaks.
4. With the soil remaining frozen, transfer all material from the set of whirlpaks into the corresponding 1-pint bag. The soil should not be thawed and homogenization is not required.
5. Repeat step 4 for the remaining samples.
6. Return the 1-pint freezer bags to the -80C freezer (or container of dry ice, if no freezer is accessible) immediately.
7. Complete the “composite sample inventory” sheet 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.
8. Ship samples to contract facility as outlined in SOP J.

SOP J Data Entry and Verification

Field data collected on paper datasheets must be digitally transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Data ingest file pertaining to this protocol is the NEON Raw Data Ingest Workbook for TOS Terrestrial Biogeochemistry: Chemistry of Soils and Plants (RD [06]).

SOP K 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 shipping document on CLA's NEON intranet site.

K.1 Handling Hazardous Material

Shipment of plants and soils are regulated by USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine Office under 7 CFR 330. Soil from Puerto Rico and Hawaii are regulated as foreign soil, soil from areas within CONUS may be regulated as quarantined soils. Foreign soils may only be shipped to facilities holding a valid Permit to Receive Soil while domestic soils from quarantine areas may be shipped to facilities holding either a valid Permit to Receive Soil or a valid Compliance Agreement.

For more information on which domestic soils are regulated, contact the local Plant Protection and Quarantine office or Permit Services in Riverdale, Maryland at (301) 734-8645; fax (301) 734-5786, or the State Plant Regulatory Officials of destination state (i.e., state in which the contracted lab facility(ies) is/are located).

Quarantine shipping regulations do not apply to shipping KCl extracts from the soil N transformations SOP.

K.2 Shipping oven-dried and air-dried soils

Oven-dried and **air-dried** samples are shipped at ambient temperatures. No hazardous or dangerous DOT regulated materials are shipped with these soils, however, receiving of quarantine soils is regulated by USDA. Receiving labs must have either a Permit to Receive Soils or a Compliance Agreement in order to receive soils from quarantined areas.

1. Place **oven-dried soil sample vials** containing soils in 1-gallon resealable plastic bags (not more than 10 samples per bag), then place in a corrugated cardboard box for shipment. If uncertain whether vials are watertight, double bag samples for shipment.
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.
3. Fill empty space in shipping box with cushioning material (i.e. peanuts, newspaper) to prevent shifting.
4. If soils are being shipped from quarantine area:
    a. Include a copy of required permits and affix any labels (e.g., PPQ) required by the permit.
    b. Include address and cover letter explaining shipment, along with shipping manifest inside the shipping box.
5. Address shipment and ship ground.

K.3 Shipping microbial biomass analysis

Refrigerated **microbial biomass** samples are shipped at cold temperatures. No hazardous or dangerous DOT regulated materials are shipped with these soils, however, receiving of quarantine soils is regulated by USDA. Receiving labs must have either a Permit to Receive Soils or a Compliance Agreement in order to receive soils from quarantined areas.

To ensure minimal changes to biomass during storage, samples should be shipped for analysis as soon as possible, and **no more than 1 week** following sample collection.

1. Place refrigerated **microbial biomass samples** in 1-pint resealable bags inside a second 1-gallon resealable plastic bags.
2. Pack samples in insulated shipping container with ice packs to keep samples chilled during shipment.
3. Fill empty space in shipping box with cushioning material (i.e. peanuts, newspaper) to prevent shifting.
4. If soils are being shipped from quarantine area:
    a. Include a copy of required permits and affix any labels (e.g., PPQ) required by the permit.
    b. Include address and cover letter explaining shipment, along with shipping manifest inside the shipping box.
5. Address shipment and ship samples standard overnight. ***Do NOT ship on Friday or the day before a holiday***.

K.4 Shipping microbial molecular analysis and KCl extraction

Samples for **microbial molecular analysis** and **KCl extraction** are shipped on dry ice. 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. Place frozen samples from the ultralow freezer for shipment in 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 to surround 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 with 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. If soils are being shipped from quarantine area:
   a. Include a copy of required permits and affix any labels (e.g., PPQ) required by the permit.
   b. Include address and cover letter explaining shipment, along with shipping manifest inside the shipping box.
8. Address shipment and send the samples standard overnight (or priority overnight, if dry ice weight limits apply). ***Do NOT ship on Friday or the day before holiday***.

K.5 Timelines

Ship samples immediately following processing steps (i.e., within 24 h). Samples that have been air-dried or oven-dried prior to shipment do not “expire”, but to decrease build-up of samples in the domain facility, it is better to ship quickly so that samples are not lost or damaged. However, if there is an issue with receiving contracted laboratory being able 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***.

See sections I.1-I.4 above for sample-specific guidelines.

K.6 Grouping/Splitting Samples

These details are TBD, and may be added in a later revision of this protocol.

K.7 Return of Materials or Containers

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

K.8 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping manifest enclosed within the shipping container AND a corresponding electronic version of the manifest (Excel file) emailed to the laboratory or archive.

Place the hard copy shipping manifest in resealable plastic bag on top of packing materials and send electronic manifest and shipper tracking information to CLA contact **and** the receiving laboratory.

K.9 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 20. 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

WHAT ANALYSES DO I DO? SOIL BIOGEOCHEMICAL AND STABLE ISOTOPE BOUT VS. MICROBES SAMPLING ONLY

Table 21. Soil Biogeochemical and Stable Isotope Sampling Bout vs. Microbial Sampling Only

Bout Type	Soil Temp (field)	Soil moisture (lab)	Soil pH (lab)	Archiving (field/lab)
Microbial Sampling Only	☐	☐	☐	☐ In field: whirlpacks; no archiving of dried soil in lab
Soil Biogeochemistry, stable isotopes (includes microbes)	☐	▱	☐	☐ In field: whirlpacks; archive air-dried soil in lab

APPENDIX C QUICK REFERENCES

COLLECTING QUALITY SOIL SAMPLES FOR BIOGEOCHEMICAL ANALYSIS

STEP 1 - Cold soak coolers for microbial samples before going into field.

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

STEP 3 - Measure litter layer.

STEP 4 - Collect 2 organic horizon areas per sample with "brownie cutter"

STEP 5 – Put organic samples into 1 bag, homogenize, and label. Fill 5 x 2 oz. whirlpaks ~1/2-way, label, and store on dry ice. Fill 1-pint bag ~1/2 way and label. Store both bagged samples on ice packs.

STEP 6 - Collect mineral horizon core(s) with approved coring device for your domain, place in bag and homogenize. Fill 5 x 2 oz. whirlpacks ~1/2-way. Fill 1-pint freezer bag ~ ½ way.

STEP 7 – Label bag and whirlpaks: store whirlpaks on dry ice, bag on ice packs.

STEP 8 - Backfill boreholes in accordance with permit.

STEP 9 – Thoroughly clean equipment using deionized water and clean rag.

COLLECTING QUALITY SOIL SAMPLES FOR MICROBIAL ANALYSIS

REMINDER: Use sterile technique as much as reasonably possible.

STEP 1 - Cold soak coolers for molecular samples before going into field.

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

STEP 3 - Measure litter layer.

STEP 4 – If present, collect 2 organic horizon areas per sample with “brownie cutter”

STEP 5 – If present, put organic samples into 1 bag and homogenize. Fill 5 x 2 oz. whirlpaks ~1/2-way, label, and store on dry ice. Fill a 1-pint freezer bag ~1/2 way and label. Store the two bagged samples on ice packs.

STEP 6 – If organic horizon is not present, collect mineral horizon core(s) with approved coring device for your domain, place in bag and homogenize. Fill 5 x 2oz. whirlpaks ~1/2-way, label, and store on dry ice. Fill a 1-pint freezer bag ~1/2 way and label. Store the two bagged samples on ice packs.

STEP 8 - Backfill boreholes in accordance with permit.

STEP 9 – Thoroughly clean equipment using deionized water and clean rag.

APPENDIX D REMINDERS

COLLECTING QUALITY SOIL SAMPLES FOR BIOGEOCHEMICAL AND MICROBIAL ANALYSES

Pre-sampling: Be sure to…

- ☑ Cold soak coolers for shipping "field-moist" samples (if required).
- ☑ Upload GPS coordinates for sample locations and review job ticket.
- ☑ Know any special permit requirements for target site.

At soil sample location: Check…

- ☑ Is designated sampling area disturbed?
- ☑ If location moved more than 1 m, did you record reasons and new (x, y) coordinates on datasheet?
- ☑ Did you record metadata on datasheet (i.e., plot ID, date, etc…)?

Coring: Remember to…

- ☑ Change gloves between pre-determined sample locations.
- ☑ Measure soil temperature at each sample location.
- ☑ Measure and remove leaf litter before coring.
- ☑ Homogenize samples for microbial and chemical analyses.
- ☑ Core to 30 ± 1 cm and measure core depth in borehole (not the corer).
- ☑ Backfill hole with appropriate material when you are done.
- ☑ Decontaminate equipment between sample locations. (e.g., corer, tray, brownie cutter, etc…)

Sample Handling: Be sure to…

- ☑ Label sample bags.
- ☑ Store microbial molecular samples in cooler with dry ice.
- ☑ Store subsamples for soil biogeochemistry/stable isotopes/soil pH/soil moisture and microbial biomass in cooler with ice packs.

Processing: At end of day…

- ☑ Transfer microbial molecular samples to ultralow freezer in lab.
- ☑ Transfer samples for biogeochemistry/stable isotopes/soil pH/soil moisture and microbial biomass to refrigerator.
- ☑ Use different glove for each sample.
- ☑ Homogenize, sieve, dry, and store soil as required.

PROCESSING SOIL SAMPLES IN THE LAB

Microbial Samples: Be sure to…

- ☑ Store molecular samples in ultralow freezer (-80° C).
- ☑ Store biomass samples refrigerated (4° C).
- ☑ Ship molecular samples on dry ice to external lab via FedEx, standard (or priority, in select locations) overnight.
- ☑ Ship biomass samples on ice packs as soon as possible to external lab via FedEx, standard overnight.
- ☑ Inform external lab and NEON HQ about Friday shipments/Saturday deliveries.
- ☑ Measure soil pH and moisture using refrigerated subsample.

Preserve Sample Integrity: Make sure…

- ☑ Samples are sieved the same day they are collected.
- ☑ All sample label information is correctly transcribed.
- ☑ Gloves are changed and sieves cleaned between samples.
- ☑ Air- and oven-drying times are tracked appropriate datasheets.
- ☑ pH electrodes are cleaned between samples.

Avoid cross-contamination. Be sure to change gloves between samples!

Data Entry: Did you…

- ☑ Record the date and time of specimen processing?
- ☑ Describe irregularities or deviations from protocol?
- ☑ Enter all information from datasheets into computer?

APPENDIX E 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 monthly when ground is not frozen/snow-covered. Estimated dates provide general guidance of when each domain can expect ground to be suitable for sampling. Verify whether ground is frozen or not each month based on local conditions.

Table 22. Approximate sampling dates for soil core sampling at NEON sites Note: soil biogeochemical and stable isotope analyses will be conducted on the soil cores taken within the July-August window *during years when these analyses are scheduled*.

Domain	Approx. Start Date	Approx. End Date
01	April 1	Jan 1
02	March 1	Jan 1
03	Jan 1	Dec 31
04	Jan 1	Dec 31
05	April 1	Jan 1
06	March 1	Jan 1
07	March 1	Jan 1
08	Jan 1	Dec 31
09	April 1	Jan 1
10	March 1	Jan 1
11	Jan 1	Dec 31
12	April 1	Jan 1
13	March 1	Jan 1
14	Jan 1	Dec 31
15	March 1	Jan 1
16	Jan 1	Dec 31
17	Jan 1	Dec 31
18	June 1	Sept 30
19	June 1	Sept 30
20	Jan 1	Dec 31

APPENDIX F SITE-SPECIFIC INFORMATION

None given. This appendix will be updated with site-specific information once soil characterization work has been completed (as of Rev F).