TOS PROTOCOL AND PROCEDURE:
SOIL PHYSICAL, CHEMICAL, AND MICROBIAL MEASUREMENTS

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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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1 OVERVIEW

1.1 Background

This document describes the required protocol for conducting field sampling of and domain lab processing for soil physical properties, nutrient stocks, soil nutrient transformations, and microbial biodiversity and function. We are interested in quantifying the stocks of soil carbon (C) and nutrients to understand ecosystem nutrient status, measuring the isotopic (C and N) composition of the soil to gain a picture of integrated ecosystem processes, quantifying soil nutrient transformations to understand the rates of microbially-mediated processes, and determining the microbial community composition. We will characterize the soil physical and chemical properties, including pH and volumetric water content, which are some of the environmental controls on microbially-mediated biogeochemical processes. As these datasets will be compared with one another, analyses for each component are performed on the same material; we do not collect separate soil cores for microbial measurements and nutrient stocks. 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. Soil nutrient transformations, such as nitrification, are calculated by comparing initial and final (i.e., T0 and T7 days) extractions of soil nitrate concentrations; they are expressed as mg NO_3^- per g dry soil per day.

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 sequence of the total DNA extracted from the soil (metagenome) will provide information on the functional potential of the microbial communities and the sequence of the expressed mRNA genes (metatranscriptome) informs on the active microbial processes occurring in the soil community.

Measurements of soil physical and biogeochemical properties 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 other ecosystem components, 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 activity, 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 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).

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 Physical, Chemical, and Microbial Measurements
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

Acronym	Definition
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
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 collecting soil cores to analyze physical properties, biogeochemistry, and microbial communities 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 length scales (e.g., soil core, sub-plot, plot, site). Plot locations in which soil samples will be collected vary for each NEON site; this information will be provided as a job ticket each year to domain managers and field personnel. In addition, soil core coordinates will be randomly generated within each plot. For each soil sampling location, staff scientists will provide field crews with x, y coordinates originating from the southwest corner (i.e., the reference point) of each plot. In many cases, multiple cores per sampling location must be collected and pooled into a composite sample, both to obtain enough material for analysis and to generate a representative sample at the soil core (i.e., centimeter to meter) scale.

Soil types and horizons differ throughout the 20 NEON domains. When an organic and mineral horizons are present within a single core they will be separated prior to analysis. However, other horizons will not be separated (e.g., 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 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.

No processing is required in the domain laboratory for microbial samples.

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

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure that quality standards are met.

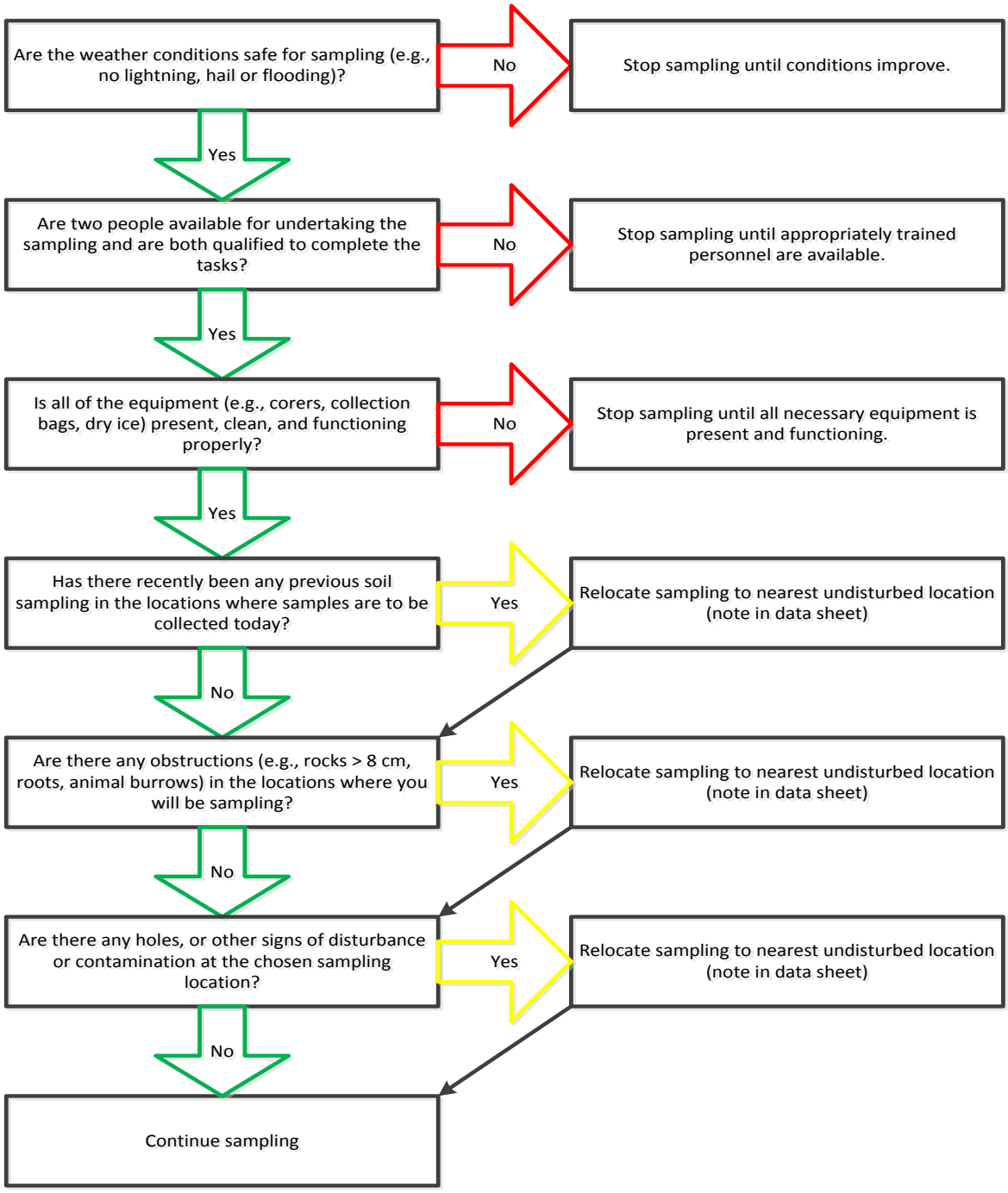


Figure 1. Decision tree

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

The timing, 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 terrestrial biogeochemical cycles within a particular window, and, therefore, timing depends on the dominant drivers that affect microbial communities, as well as nutrient stocks and fluxes in ecosystems. 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). The frequency of sampling allows researchers to investigate how microbial communities and nutrient dynamics change within and between seasons or years; for example, at Domain 1, soil respiration will be at its minimum during winter and maximum during summer, so one might choose to sample during the peak and trough of this cycle. 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 will occur more or less frequently, and to a greater or lesser extent, depending on the climatic factors and landscape features, the biogeochemical context of the location (e.g., is this an area of high N loading?), as well as logistical (e.g., site accessibility) and financial constraints.

Of the measurements made as part of NEON soil sampling efforts, nutrient and microbial dynamics will change more frequently than soil physical properties; hence, these collections follow different schedules. For the first 1-2 years, sampling will occur in a subset of four plots each month when the ground is not frozen to determine seasonal variation (specific plots provided in Appendix E). After 2 years, sampling will occur in up to 10 plots 3 times each year.

Sample once during the growing season (e.g., during July) for biogeochemical analyses, if these samples are being done in the sampling year. Sample monthly (Unless NEON staff scientist specifies differently, using a JIRA ticket), except when ground is frozen, for microbial analyses for the first 2 years. Following the first 2 years, sampling frequency will be 3 times per year with timing provided as domain-specific job tickets.

Sampling bouts should be approximately 25-35 days apart for soils collected for microbial analyses, unless NEON staff scientists specify differently at the beginning of the field season. Subsamples for microbial analyses and biogeochemical analyses are from the same sample (i.e., core or composite sample of multiple cores), so these collections occur simultaneously.

See Appendix D for domain-specific date estimates for onset and cessation of sampling.

4.2 Criteria for Determining Onset and Cessation of Sampling

NEON staff scientists and domain Field Operations staff will determine sampling dates for soil core collections pertaining to soil microbial community analysis for each sampling year. These sampling bouts will occur throughout the year to capture a range of conditions, but not when soils are frozen. Sampling of soil cores for biogeochemical and soil microbial community analysis (one large, simultaneous 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.

4.3 Timing for Laboratory Processing and Analysis

Soil cores collected for microbial community analysis only must be put on dry ice and then transferred to a -80°C freezer immediately after collection; failure to keep these samples frozen spoils the samples and they cannot be used. Shipment instructions for these samples appear in SOP E. Soil cores that are collected for microbial community analysis and biogeochemistry must have one split processed as for microbial analyses only, and the other split transferred to a cooler with ice and then processed within 24 hr (or immediately upon return to the laboratory, if field staff are working remotely). If soil core sample splits for biogeochemical analyses are not kept cold on ice in cooler for more than 4 hr, they may not be used and field staff should be in communication with NEON staff via JIRA to reschedule the sampling bout.

4.4 Sampling Timing Contingencies

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Inability to finish sample bout	Communicate to staff scientists via JIRA for further instruction.	Dataset may be incomplete or sampling bout redone. Latter may result in delay of data products delivery.
Partial completion of sample bout.	Communicate to staff scientists via JIRA 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 JIRA for further instruction.	Samples may reflect different conditions.
Sampling for soil microbial community analysis is scheduled, but soil freezes.	Do not attempt to collect soils. Communicate to staff scientists via JIRA for further instruction.	Samples will not be collected for this time period; no data products generated.

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, slide hammer corer). 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 2. Equipment list – Field sampling one site

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX105087	R	Ice packs, -20C	Sample preservation	All	16 (+)	N
	R	Coolers	Sample preservation	All	2	N
MX103483	R	Slide hammer corer (for bulk density)	Soil core collection for bulk density	If bulk density cores are being collected	1	N
	R	Meter tapes	Identification of random x, y coordinates for soil sampling locations	All	2	N
MX103206	R	Field thermometer	Measurement of soil surface temperature	All	2	N
MX102002	R	Permanent markers	Writing on sample bag	All	3	N
	S	Survey flags	Mark soil sampling location	All	3	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Bulb planter	Soil core collection	All	1	N
	S	Flexible trays for sample processing in field	Soil core division (horizons, subsamples, etc)	All	2	N
	S	Ruler	Measurement of horizon	All	1	N
	S	Trowel	Removal of soil core	All	1	N
	S	Ruler	Measurement of soil core horizons	All	1	N
	S	Hori hori knife	Cutting soil cores (organic horizon measurement, subsampling, etc)	All	1	N
	S	Organic horizon cutter template	Cut out organic horizon	If an organic horizon is present at the site	1	N
	S	PDA	Data entry	All	1	N
Consumable Items						
RD[05]	R	Field datasheets	Data entry	All	---	N
MX100212	R	Dry ice	Soil sampling preservation for microbial subsamples	All	20 pounds	Y

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100645 MX100646 MX100647 MX100644	R	Powderless gloves	Preventing contamination of soil samples	All	1 box	N
MX100592	R	Ziploc freezer resealable plastic bags (gallon)	Storing soil samples	All	2 boxes	N
	R	50 ml vials	Storing soil samples for microbial analysis	All	50	N
MX104432	R	Cryogenic vial labels	Labeling soil samples for microbial analysis	All	50	N
MX100308	R	DI water	Cleaning soil sampling equipment	All	2 liters	N
	S	Hard copy of job ticket for soil collection (i.e., information about plots to sample, random soil coring locations)	Soil sampling instructions	All	1	N
	S	Disposable paper tissues or cloth	Wiping soil sampling equipment	All	1 box or 2 cloths	N
	S	Pencils	Data entry	All	4	N
	S	Trash bag	Disposing of consumables	All	2	N

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

Table 3. Equipment list – Processing soils from 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
MX100592 MX100593	R	Resealable plastic bags (gallon and quart sizes)	Storing soil subsamples	All	1 box each	N
MX105089	R	Paper (e.g., “lunch”) bags	Air-drying soil subsamples	All	50	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX103233	R	Coin envelopes	Storing processed soil subsamples	All	1 box	N
	R	18 ml plastic scintillation vials	Storing processed soil subsamples for biogeochemical analysis	All	1 box	N
	R	Foil weighing boats	Soil moisture analysis	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 4. Equipment list – Shipping samples from one site

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX105087	R	Ice packs	Shipping soil samples	All	16 (+)	N
MX102297	R	Foam Cooler	Shipping soil samples	All	1	N
Consumable Items						
MX100212	R	Dry ice	Shipping soil samples for microbial analysis	All	20 pounds	Y
MAT111	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).

6.2 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 laboratory techniques, making salt solutions in the laboratory for pH analysis, and safe working practices for field sampling.

6.3 Specialized Skills

Soil types and profile characteristics differ greatly across the NEON domains (see examples in Figure 2). When sampling soil, field personnel must be familiar with the basic characteristics of a typical soil profile at the local NEON site, such as ability to differentiate between organic and mineral horizons and be familiar with typical soil depth. For example, in Domain 1, this would include understanding differences among the leaf litter (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.

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

6.4 Estimated Time

The time required to 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 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

SOP A Preparing for Sampling

1. Fill out site information on field datasheet, located in Datasheets for TOS Protocol an Procedure: Soil Physical, Chemical, and Microbial Measurements (RD [05]). Make sure to use proper formats, as detailed in datasheets and described by domain managers. For example, eventDate (YYYYMMDD) and eventTime fields must be recorded based on 24 h with no colon (e.g. 1830). Print cryovial labels with sampleID and eventDate (changes in coordinates should be reprinted and applied as a new label upon return to domain laboratory) for 50 ml vial microbe samples.
2. If applicable, based on job ticket: “Cold-soak” coolers for shipment: 24 h prior to anticipated shipment time, place open shipping coolers with ice packs into a freezer. These will be used for shipping field-moist soils.
3. Prior to sample collection, plots and subplots where soil samples will be collected should be identified and marked.

SOP B Field Sampling

B.1 Identify the plot

1. Navigate to the southwest corner of the plot where sampling is to occur.
2. Lay out meter tapes on the west and south sides of the plot.
3. Find the sampling location(s) using the tapes as guides for the given x, y coordinates.
4. Put on a clean pair of powderless gloves (you only need 1 pair per random sampling location to take the cores that constitute a sample, but you MUST put on a new pair at each location—between samples—do not reuse gloves).

B.2 Measure soil temperature

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

B.3 Collect soil core

1. Identify the first soil core sampling location. Always core vertically, not perpendicular, when collecting on a slope. 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.
2. Push the litter layer away from where you are going to core into the soil surface. The litter layer is general composed of undecomposed plant material (e.g. leaves are still recognizable), whereas an organic horizon will contain organic material in various states of decomposition.
3. If an **organic horizon** is present, conduct steps 4-5. If not, skip to step 9.
4. Cut out an organic horizon "brownie" using the square frame cutter tool. Measure the depth of each side and record the average value in cm.
5. Repeat steps 2-4 at up to two other locations within 0.5 m of the first location if more material is needed (this will be specified in the current year's job ticket/sample list). The three locations form one composite sample. Put the organic horizon samples into a 1 gallon resealable plastic bag and homogenize by hand.
6. Fill a 50 ml vial with homogenized organic horizon material from the resealable plastic bag for microbial analysis. The remaining contents in the resealable plastic bag are for physical/elemental/isotopic analysis.

7. Label each bag/vial with the sampleID, plotID, subplotID, coreCoordinateX, coreCoordinateY, eventDate, horizon, measuredBy (technician name), and recordedBy (technician name).
8. Place the microbial sample in the cooler with dry ice, and the physical/elemental/isotopic sample into a cooler with ice packs.
9. Collect up to three **mineral horizon** cores per sample location using the slide hammer corer or other coring device. If significant obstructions such as rocks are present, another approved device may be used to get the soil out of the ground.
    a. If collecting solely for microbial diversity measurements (and pH), collect one core. Can be collected using a device other than slide hammer corer as long as the sample is a representative core to 30 cm.
    b. If collecting for soil microbial diversity and biogeochemistry, collect up to three cores in order to get enough material to complete all domain and contract laboratory measurements.
    c. The slide hammer corer **MUST** be used for the bulk density core.
10. If organic horizon was present, take cores from the locations where you collected organic horizon brownies. Core down so that the total depth of the soil core (organic + mineral soil) is maximum of 30 cm as measured in the soil core hole, not the extracted soil core, which can become compacted.

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.

11. If an organic horizon is not present, core 30 cm of mineral soil. If the 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 'commentsCollection' field of the datasheet.
12. Place all mineral soil cores collected from the sample coordinates into the same resealable plastic bag.
13. Break up the cores and homogenize (mix) in the bag with your gloved hand.
14. Fill a 50 ml vial to the top with homogenized mineral soil.
15. Label the vial and the resealable plastic bag with the sampleID, plotID, subplotID, coreCoordinateX, coreCoordinateY, eventDate, horizon, measuredBy (technician name), and recordedBy (technician name).
16. Put the bag in the cooler with the ice packs, and immediately place the vial in the cooler with dry ice (mRNA changes very quickly).
17. Enter the metadata in field datasheet.
18. Thoroughly rinse sampling equipment (corer, thermometer, bulb planter, etc).
19. Remove gloves and stow in a dedicated trash bag.
20. Repeat all steps for additional sampling locations.

B.4 Collect soil core for bulk density

1. If required in the job ticket, take a soil core for bulk density
2. Measure the litter layer in the sampling location specified by the job ticket and record depth in datasheet.
3. Remove the litter layer.
4. Collect an organic horizon brownie, note the average depth, and place it in a resealable plastic bag.
5. Label the bag as in step #15 (Collect soil core) with the addition of “bulkDensity” on the bag and place the organic soil inside. Put the sample in the cooler with the ice packs.
6. Use the slide hammer corer (this tool is required for bulk density measurements) to take a core of the mineral soil (to a total borehole—organic AND mineral horizon—depth of 30 cm) and place in a bag labeled as above, with the addition of “bulkDensity”.
7. Write the total length of the core in the datasheet (or PDA) and field notebook, bag this core, and label the bag as above with the addition of “bulkDensity” on the bag. Place in the cooler with the ice packs.

B.5 Sample transport

1. Keep soils for biogeochemistry analysis in the cooler with the ice packs and transfer to 4 C refrigerator upon return to domain lab.
2. Keep soils for microbial analysis in the cooler with the dry ice and transfer to ultralow freezer upon return to domain lab.

SOP C Laboratory Processing and Analysis

Directions are written for field-moist, air-dried, and oven-dried processing of soils. These different processing approaches pertain to different analyses, described below. In all cases, conduct work on a clean lab bench space that has been wiped with ethanol prior to processing samples. Samples for microbial analysis do not require further processing in the domain facility. They should be shipped as soon as possible within 24 hours to the contracted laboratory(ies) (see SOP E), but shipment may be delayed up to 3 days for microbial samples.

C.1 Part 1: Analyzing subsamples for moisture content

Analysis of the moisture present in the soil is important for understanding the field conditions experienced by soil microbial communities, and for some soil nutrient calculations. Conduct the following steps to generate soil moisture data for each horizon of each soil sample. Record the necessary metadata and values in Lab Datasheet: Measuring Soil pH and Moisture (in RD [05]). Soil moisture analysis should be done within 24 h of field collection. In cases where domain staff are working at remote sites, keep samples on fresh ice packs in coolers and process immediately upon return to the domain facility lab.

1. Wear powderless gloves. Use a new glove for each soil sample (Suggestion: use one hand to handle the sample so that you only have to replace one glove. If you use two hands, replace both gloves.).
2. Label foil weighing boat with sampleID and weigh foil boat to nearest 0.01 g and record value in the datasheet.
3. Place 5 g of a field moist organic horizon sample (not sieved) or 10 g of a field moist mineral horizon sample (not sieved) into the weighed foil weighing boat. Record weight to nearest 0.01 g.
4. Repeat Steps 1-3 for all horizon samples.
5. Place all samples into drying oven (recommended on a cafeteria tray) at 105°C for 48 h. Record time in oven on datasheet.
6. At conclusion of drying period, immediately weigh dried sample + weighing boat to nearest 0.01 g and record values in the datasheet. Also record the date and time out of oven.
7. Dispose of soils according to permit requirements and keep all foil weighing boats that are clean and undamaged for reuse.

C.2 Part 2: Sieving and subsampling

1. Process samples within 24 h of field collection. 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 powderless 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**, skip to Step 8 (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, meeting the standard classification of "soil"). If the sample is too clayey to pass through the sieve, submit a jira ticket to receive further instruction.
6. Discard particles > 2mm according to permit requirements.
7. Record on the Lab Datasheet: Post-field Soil Processing: the metadata (site, eventDate, measuredBy (technician name), plotID, subplotID, coreCoordinateX and coreCoordinateY, sampleID, and horizon) and the sieveDate and sieveTime.
8. If the current year's job ticket specifies that a subsample of soil is being shipped **field moist** to one or more contracted laboratories, take the specified subsample from the sieve (mineral horizon) or field bag (organic horizon) and place in a quart-sized resealable plastic bag labeled with metadata above (step 7). Skip to SOP E for directions about shipping.
9. Subsample organic horizon sample (not sieved) and mineral horizon sample (sieved) in order to **oven-dry** for nutrient/isotopic analysis: fill a 18 ml scintillation vial with each unique sample. Transfer sample information to scintillation vial (Suggestion: write sample information on lab tape and wrap tape completely around middle of scintillation vial.). Do not cap vials.
10. 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 E).
11. Air-drying samples for pH analysis and archiving: place all remaining sample (your organic horizon samples will be in the field resealable plastic bags, and the mineral soil samples will be coming out of the sieve) into individual paper lunch bags labeled with the metadata above.
12. 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 and track the change in weight according to the appropriate fields in the datasheet.

13. Air-drying soil can take several days depending on the initial moisture content. Do not continue with processing until soils are completely dry. Soils are considered completely dry when the change in weight is less than 5% over a 48h period.
14. At the conclusion of air-drying samples, a subsample will be analyzed in the domain facility for pH, and ~200 g of the remaining material should be placed into a labeled quart-sized resealable plastic bag, sealed, and stored in a dry cabinet prior to shipment to an archiving facility. For shipment instructions, see SOP E.

C.3 Part 3: Analyzing subsamples for pH

Soil pH is measured by domain staff on air-dried subsamples. 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. Wear gloves throughout this procedure. If you do not touch the soil samples directly, you do not need to change gloves between samples.
2. 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.
3. Check pH of $CaCl_2$ solution; it should be between 5.0 and 6.5.
4. Adjust one drop at a time with concentrated 6N $Ca(OH)_2$ or 10N HCl if needed (rarely is).
5. Weigh out a subsample of air-dried organic or mineral (fraction ≤ 2 mm) soil and place into 50 – 100 mL cup. Use 10 g for mineral soil and 5 g for organic soil.
6. Add 20 mL of $CaCl_2$ solution. DO NOT STIR. (For measuring pH using water, add 10 mL deionized water).
7. Allow soil to absorb $CaCl_2$ solution.
8. Thoroughly stir for 10 seconds with a glass rod or plastic stir stick.
9. Further stir suspension (for 10 seconds) every 5 minutes for the next 30 minutes.
10. Allow suspension (i.e., the flocculated soil) to settle undisturbed for 30 – 60 minutes. (This will vary by soil type. Look for soil materials to be completely saturated by the solution—see next step.)
11. Look for supernatant (liquid without precipitate) above the flocculated soil. IF not present, then add another aliquot (20 mL) of $CaCl_2$ solution and repeat stirring and settling (steps 6 through 8).
12. Calibrate the pH meter electrode with pH buffers 4, 7, and 10 according to the manual for the probe.
13. 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.
15. Allow reading to stabilize (usually about 1 minute) and record pH value in on datasheet.

16. Clean electrode: rinse thoroughly 2 to 3 times with deionized water and gently dry with a fresh lab tissue.
17. Repeat steps 5 through 16 for each sample.
18. Select 5 soil samples from the group just measured for duplicate (i.e., "Dup") pH measurement. (Choose soil samples that have ample leftover material.)
19. Repeat steps 5 through 16 for the selected five.
20. If the original and duplicate subsamples differ by ≥0.5 in their pH reading, take a third pH reading. Record all original and duplicate values as separate entries in the data ingest.
21. Steps 1-19 describe the procedure for analysis of pH in 0.01 M $CaCl_2$. Repeat Steps 5-20, analyzing subsamples in 20 mL deionized water instead of $CaCl_2$.

C.4 Part 4: Processing bulk density samples

If the job ticket specified soil sample collection included collection for bulk density analysis, you will have a set of soil cores that must be processed as follows. Use the Lab Datasheet: Measuring Bulk Density (in RD[05]).

1. Wear powderless gloves, but you DO NOT have to change gloves for each bulk density sample; you can process a whole set of samples with same pair of gloves.
2. Take a pre-processing weight of your collected soil bulk density samples: for each organic and mineral horizon sample, tare the scale using a clean, empty resealable plastic bag of the same size that you used in the field. Weigh each organic and mineral soil sample (plus its bag) and record the weight in the datasheet in 'bulkDensWtPresieve (g)'.
3. **Organic horizon** samples (if collected at your site) do not need to be sieved. Transfer to a paper paper lunch bag and label that bag with sample metadata from resealable plastic bag (field collection).
4. Sieve **mineral horizon** soils through 2mm sieve and place the ≤ 2mm and > 2mm fractions in separate labeled paper paper lunch bags. Clean materials and sieve using 70% ethanol and water between each sample.
5. Place all samples (in their open, labeled paper lunch bags) and 5 empty paper lunch bags into an oven at 105°C for 48 hrs. You only have to dry and weigh one group of 5 paper lunch bags per batch of samples you process.
6. Once soil samples are dry, weigh each bag of sample separately and record the values to the nearest 0.01 g in the datasheet. Discard the material in accordance with permit requirements when weights have been obtained accurately (Note: if samples were collected from a quarantine area and processed in non-quarantine lab, disposal of soils, and water used to wash sieves must follow guidelines in the USDA soils permit).
7. Record the average weight (to the nearest 0.01 g) of the 5 empty paper lunch bags corresponding to the oven-dried batch of samples in the lab datasheet.

SOP D Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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]). Please take care to check your work (i.e., verify that hardcopy and electronic copy match) and to resolve any data issues that occurred in the field or laboratory that may result in data quality issues as you are going through the values at this time. Specific instructions on where to save/store completed data ingest files, and where and how to back-up and store hardcopy datasheets can be found in RD [04].

SOP E 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.

E.1 Part 1: Shipping oven-dried samples for analysis of nutrients and isotopes

1. Take scintillation vial box containing processed samples out of the cabinet for shipment.
2. Wrap the box in bubble wrap and tape securely, then place in a FedEx box for shipment.
3. Include a copy of the USDA soil permit from the contracted laboratory in the box and affix any labels required by the permit, if necessary.
4. Include cover letter explaining shipment, and spreadsheet detailing sample inventory (these forms supplied for each specific contract by NEON Headquarters staff).
5. Address shipping label appropriately and ship ground.

E.2 Part 2: Shipping field moist samples for analysis of nutrients and isotopes

1. Use a "cold-soaked" shipping cooler (or coolers, in order to accommodate samples surrounded by ice packs on four sides).
2. Fill cooler with securely double-freezer-bagged field-moist soils
3. Include a copy of the USDA soil permit from the contracted laboratory in the box and affix any labels required by the permit, if necessary.
4. Include cover letter explaining shipment, and spreadsheet detailing sample inventory (these forms supplied for each specific contract by NEON Headquarters staff).
5. Address shipping label appropriately and send overnight service with "before COB next day" designation. Do NOT ship on Friday or day before holiday.

E.3 Part 3: Shipping air-dried samples for archiving

1. Line a thick-walled box (sized to accommodate sample shipment load) with a trash bag.
2. Pack samples (in freezer resealable plastic bags) securely within the box. Make sure that air is out of all the bags. Fill all open spaces around samples with packing material.
3. Include a copy of the USDA soil permit from the contracted laboratory in the box and affix any labels required by the permit, if necessary.
4. Include cover letter explaining shipment, and spreadsheet detailing sample inventory (these forms supplied for each specific contract by NEON Headquarters staff).
5. Address shipping label appropriately and ship ground.

E.4 Part 4: Shipping ultra-cold samples for microbial analysis

1. Take samples from the ultralow (-80°C) freezer for shipment.
2. Immediately place samples in a sturdy insulated shipping container with a minimum 20 lbs of dry ice.
3. Seal container to preclude spillage (but make sure it is not air-tight).
4. Complete dry ice shipping label.
5. Include a copy of the USDA soil permit from the contracted laboratory in the box and affix any labels required by the permit, if necessary.
6. Include cover letter explaining shipment, and spreadsheet detailing sample inventory (these forms supplied for each specific contract by NEON Headquarters staff).
7. Address shipping label appropriately and send the samples priority overnight mail with tracking number. Do NOT ship on Friday or day before holiday.

E.5 Handling Hazardous Material

Technicians must take the necessary precautions for handling dry ice (preservation and shipping of soil samples for microbial community analysis).

E.6 Supplies/Containers

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

E.7 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 Jira ticket.

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

E.8 Conditions

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

E.9 Grouping/Splitting Samples

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

E.10 Return of Materials or Containers

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

E.11 Shipping Inventory

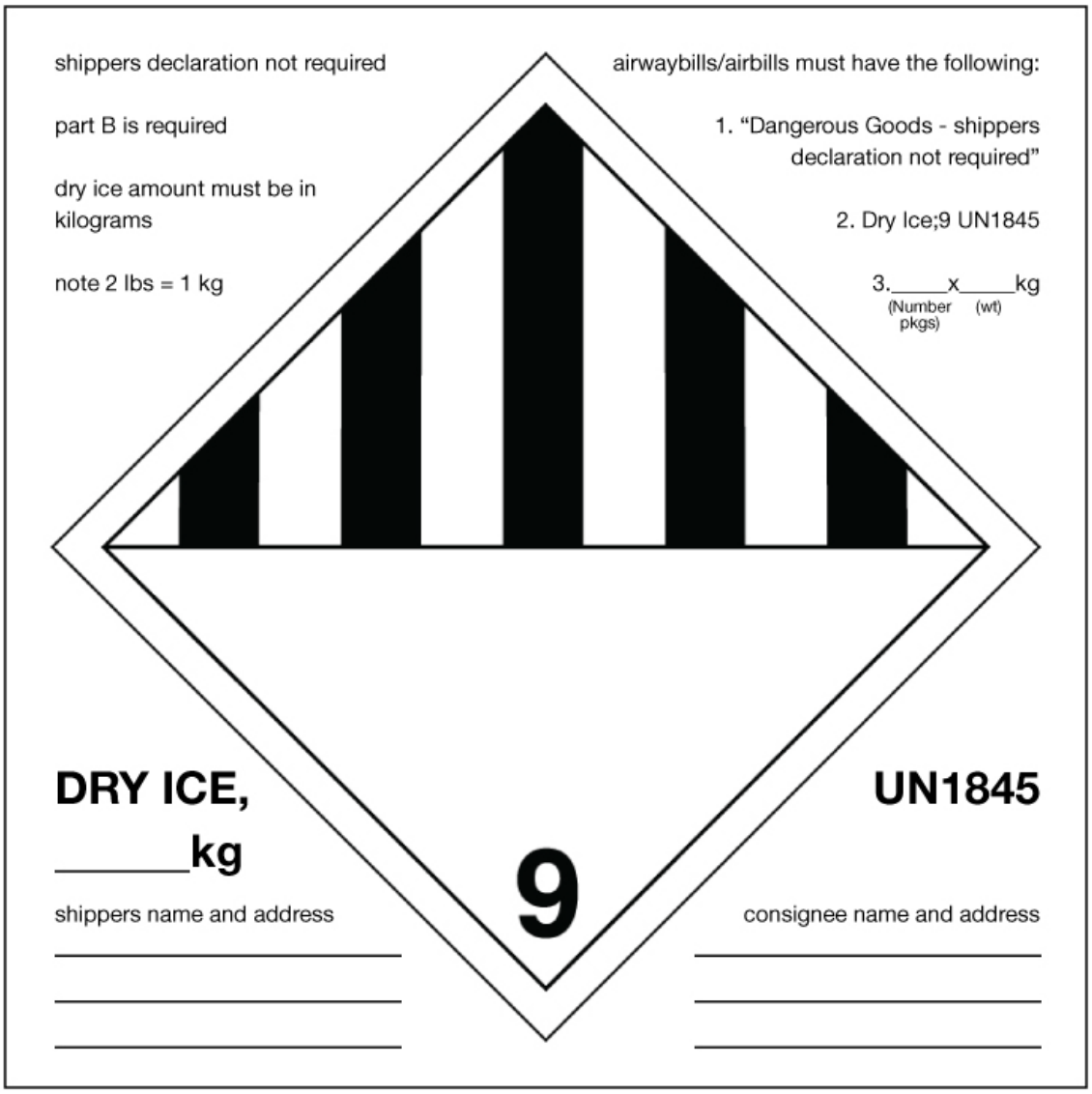
Soil samples shall be shipped according to the USDA Soil Permit requirements: A copy of the receiving laboratory's USDA Soil Permit must be included with foreign soil shipments. A copy of the PPQ form 519 (Compliance agreement) must be included with domestic regulated shipments. The soil permit and appropriate PPQ form must be clearly affixed to the outside of the container.

E.12 Laboratory Contact Information and Shipping/Receipt Days

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

E.13 Additional Directions/Notes

- Shipment of permit-requiring samples is restricted only to analytical labs or archive facilities that hold a USDA soils permit.
- Check USDA list of soil quarantine. If your site/domain fall within any of these quarantined areas, soil shipments must include NEON's USDA quarantine soil permit. Quarantined areas can be found at <7 CFR Part 301 Domestic Quarantine Notices of the Plant Protection Act (7 U.S.C. 7756)>. Quarantine areas can change, so be sure sites/domains are up to date with shipping requirements.
- Microbe samples must be shipped on dry ice. In order to comply with US Department of Transportation and International Air Transport Association regulations, the following information is required on the dry ice label (below) on the package: weight in kg of dry ice, domain office address (shippers name and address) and contract laboratory address.



8 REFERENCES

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

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 5. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001577	Datasheets for TOS Protocol and Procedure: Soil Physical, Chemical, and Microbial Measurements

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

APPENDIX B 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 3 (for a composite sample) organic horizon sample with “brownie cutter”

STEP 5 – Put organic samples into 1 bag, homogenize, and fill a 50 ml vial. Label bag and vial: store vial on dry ice, bagged sample on ice packs.

STEP 6 - Collect 3 (for a composite sample) mineral horizon samples with slide hammer corer (use bulb planter or trowel if needed), place in bag and homogenize, fill 50 ml vial.

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

STEP 8 - Collect bulk density organic horizon sample (use brownie cutter), place in bag, label, and store on ice packs.

STEP 9 - Collect bulk density mineral horizon sample (only use slide hammer corer), place in bag, label and store on ice packs.

STEP 10 - Backfill boreholes in accordance with permit.

STEP 11 – Thoroughly clean equipment using deionized water.

APPENDIX C REMINDERS

COLLECTING QUALITY SOIL SAMPLES FOR BIOGEOCHEMICAL ANALYSIS

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 Data Sheet?
- ☑ Did you record metadata on Data Sheet (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 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., slide hammer corer, tray, brownie cutter, etc…)

Sample Handling: Be sure to…

- ☑ Label sample bags.
- ☑ Store microbial samples in cooler with dry ice.
- ☑ Store chemi/phys samples in cooler with ice packs.

Processing: At end of day…

- ☑ Transfer microbial samples to ultralow freezer in lab.
- ☑ Transfer chemical/physical samples 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 in ultralow freezer (-80° C).
- ☑ Ship samples as soon as possible (within 3 days) to external lab via FedEx, priority overnight.
- ☑ Inform external lab and NEON HQ about Friday shipments/Saturday deliveries.

Bulk Density Samples: Remember to…

- ☑ Sieve, dry, and weigh the ENTIRE soil sample.

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 data sheets into computer?

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in Table 6 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 6. Approximate sampling dates for soil core sampling at NEON sites Note: monthly samples are used for microbial analyses only; biogeochemical analyses will be conducted on the soil cores taken during the growing season *during years 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

APPENDIX E SITE-SPECIFIC INFORMATION

None given. This appendix will be updated with site-specific information once soil characterization work has been completed.