FIELD AND LAB PROTOCOL: SOIL PHYSICAL, CHEMICAL, AND MICROBIAL MEASUREMENTS

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A_DRAFT	10/03/2011	ECO-00280	Initial Draft Release
B_DRAFT	01/13/2013	ECO-01140	Updates from 2013. Will be finalized in next rev.

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide change-controlled version of NEON protocols and is the version used for external review by subject-matter experts. This document describes the procedures for collecting soil samples from Terrestrial Observation System plots and initial laboratory processing for determining soil microbial diversity and biogeochemistry. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change-controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices
- site-specific safety practices
- general equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

1.3 Acknowledgements

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 information that shall be applied in the current document. Examples are higher-level requirements documents, standards, rules and regulations.

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

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000906	TOS Science Design Terrestrial Biogeochemistry
RD [04]	NEON.DOC.000908	TOS Science Design for Terrestrial Microbial Ecology
RD [05]	NEON.DOC.000913	TOS Science Design for Spatial Sampling
RD [06]	NEON.DOC.000914	TOS Science Design for Plant Productivity
RD [07]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [08]	NEON.DOC.014051	Field Audit Plan
RD [09]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD[10]	NEON.DOC.001241	NEON Algorithm Theoretical Basis Document for TOS Terrestrial Biogeochemistry: Chemistry of Soils and Plants
RD[11]	NEON.DOC.001242	NEON Algorithm Theoretical Basis Document for TOS Terrestrial Biogeochemistry: Stable Isotopes of Soils and Plants
RD[12]	NEON.DOC.001243	NEON Algorithm Theoretical Basis Document for TOS Microbial Diversity
RD[13]	EHS USDA Soil Permit SOP	

2.3 Acronyms

C	Carbon
^{13}C	Less common stable isotope of carbon
cm	centimeter
m	meter
g	Grams
mg	milligram
h	Hours
^{2}H	Deuterium; the less common stable isotope of hydrogen

^{15}N	Less common stable isotope of nitrogen
^{14}N	Common stable isotope of nitrogen
N	Nitrogen
PDA	Personal Digital Assistant
P	Phosphorus
P&P	Procedure and Protocol
USDA	United States Department of Agriculture

2.4 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 Background

This document describes the required protocol for conducting field sampling of 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. Additionally, 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/m^2). For soil, this calculation requires knowing the dry mass of soil in a known volume (i.e., bulk density, g/cm^3), and the concentration (or amount) of the element per gram of dry soil (e.g., mg/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^-/g dry soil per day. Finally, microbial community structure and function are both determined by high throughput sequencing of nucleic acids extracted from soils.

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 as it ages, 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 for collecting soil samples, 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.

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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 ([RD04]).

4 PROTOCOL

The field protocol used by NEON for collecting soil cores to analyze physical properties, biogeochemical constituents, 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 work order 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 of each plot. In many cases, multiple cores per sampling location must be collected and pooled, both to obtain enough material for analysis and to generate a representative sample at the soil core (i.e., centimeter) scale.

Soil types and horizonation differ throughout the 20 NEON domains. In general, soil shall be sampled by horizon (e.g., separating organic from mineral horizons, where an organic horizon is present) and the horizons shall be analyzed separately. Subclasses of soil horizons will not be analyzed separately, however (e.g., Bt1 and Bt2).

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 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. Other factors that may necessitate relocation of sampling efforts include: obstruction by tree roots, large (i.e., > 4 cm) rocks, or holes (e.g., from small burrowing mammals or previous soil collections). 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 location marked so that subsequent samples are not collected in the same locations.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[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 (RD[06]).

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

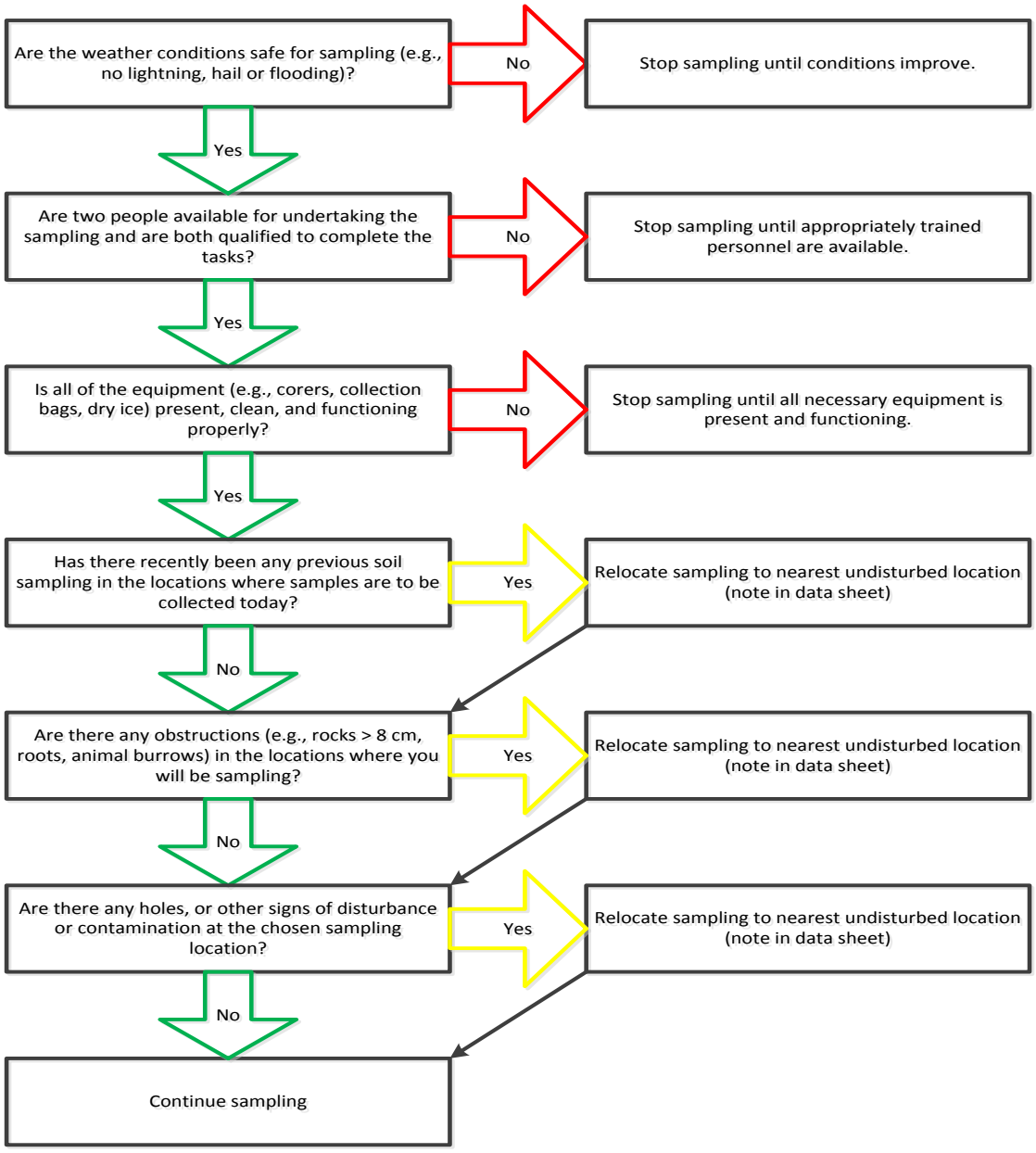

Figure 1. Decision Tree

5.1 Contingent decisions

Soil samples should be collected in undisturbed, uncontaminated locations. If a predetermined sampling location appears to be disturbed when field personnel arrive, they should move to the nearest undisturbed location within the quadrant/plot to sample and note the change in the PDA and field notebook. If samples are being collected during inclement weather, collection should stop if the storm progresses to thunder and lightning, hail, flash flooding, etc. and resume when conditions permit.

6 SAFETY

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). Any Personal Protective Equipment (PPE) required and safety issues specific to the procedure should be detailed here, along with references to any pertinent safety standards.

7 PERSONNEL REQUIREMENTS

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. Other locations, such as Domain 10, an organic horizon may not exist, but other features (e.g., a plow layer, 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.

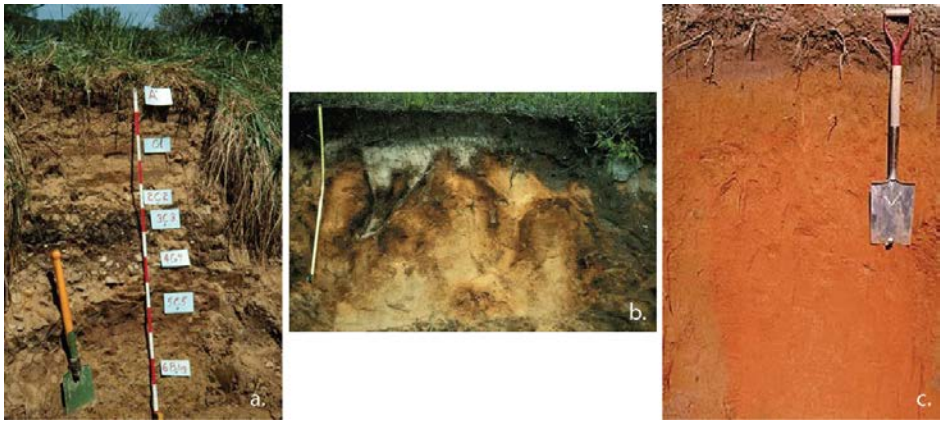

Figure 2.Soil profiles from (a) Maryland, (b) Michigan, and (c) Florida.
(Source: Dr. Ray Weil, University of Maryland (a and b) and the University of Florida (c), http://soil.gsfc.nasa.gov).

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (RD[04]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (RD[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, and safe working practices for field sampling. Refer to NEON Training Plan document [RD07].

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

The timing, frequency, and extent of soil sampling constitute "the science design", and vary by NEON 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 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 minimally, we may 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, we expect that nutrient and microbial dynamics will change more frequently than soil physical properties; hence, these collections follow different schedules.

We estimate that sampling 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).

Table 1. The approximate sampling dates for soil core sampling at all NEON sites

Domain	Estimated Date	Frequency[1]
1	Jan 1 – April 1	Monthly
3	Jan 1 – Dec 31	Monthly
10	Jan 1 – Mar 1	Monthly

[1] Sampling occurs monthly when ground is not frozen, 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.

9.1.1 Criteria for Determining Sampling Dates

9.1.2 Sampling Frequency

Sample monthly except when ground is frozen.

9.1.3 Sampling Timing Parameters

Sampling bouts should be approximately 25-35 days apart.

9.2 Equipment and Materials

Table 2. Soil Sampling Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Backpack (or another suitable container for transporting equipment)			
	Meter tape (at least 2, suggested: 4 for delineating sides of plot to determine x, y locations)			
	Survey flags to mark soil core sites			
	Two slide hammer corers			
	Alternate coring device for situations where rocks and roots are prevalent, which may bend slide hammer corer (e.g., bulb planters). These devices CANNOT be used for bulk density cores, where a known volume of soil is required.			
	2 flexible trays (suggested; can use any surface that can be used to divide soil subsamples and cleaned between sampling locations)			
	Trowel			
	Ruler			
	Hori hori knife			
	Organic horizon cutter template (10 cm x 10 cm inside diameter wooden square frame)			
	One box of powderless gloves			
	Coolers (one with ice packs for soil properties/nutrient stocks/isotopes, one with dry ice for microbial samples)			
	Ziploc freezer bags (2 boxes each, gallon and quart sizes)			
	Disposable paper tissues or cloth for cleaning soil sampling equipment			
	Permanent markers			
	Pencils			
	Field data sheets			

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	PDA			
	DI water for cleaning equipment between samples			
	Trash bag			

9.3 Preparation

Before going into the field:

1) Check the soil sampling kit to make sure that all supplies are packed, clean, and functioning properly (e.g. available dry ice, PDA units are charged, etc.).
2) Fill out site information on field datasheet (Appendix A). Make sure to use proper formats, as detailed in datasheets and described by domain managers. For example, Date (YYYYMMDD) and Time fields must be recorded based on 24 h with no colon (e.g. 1830).
3) "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 for nutrient and isotopic analysis.

9.4 Sample Collection in the Field

Prior to sample collection, plots and sub-plots where soil samples will be collected should be identified and marked.

1) Navigate to the southwest corner of the first plot where sampling is to occur.
2) Lay out meter tapes on the west and south sides of the plot (suggested: lay them out on all sides to easily locate sampling locations in the NE and SE quadrants of the plot).
3) Find the first x, y sampling location using the tapes as guides.
4) Put on a clean pair of powderless gloves (you only need 1 pair per random sampling location to take the three cores that constitute a sample, but you MUST put on a new pair at each location; do not reuse gloves).
5) Collect three soil cores and record associated data. (If you experience difficulty obtaining a core due to rocks, roots, etc, see Figure 1. Decision Tree). The cores will be pooled to make one sample, and should be taken within 1 m of one another (see Figure 3 below).
    a) Measure the depth of the litter layer (cm) above each core location and record the value. Be careful not to compact the litter layer where you are taking your measurement.
    b) Remove the litter layer.
    c) If an organic horizon is present
        i) Cut out an organic horizon "brownie" using the cutter tool. Measure the depth of each side and record the average value.
        ii) On the cafeteria tray, cut the brownie into halves; place one half into a bag for microbial analysis, and the other half into a second bag for physical/elemental/isotopic analysis.

Repeat steps 2-5 at two other locations within 1 m of the first location (see Note). Put the brownie halves into the same two bags; you will have 3 brownie halves per bag.

iii) Label each bag with the sample location information, date, time, organic horizon, and your initials. Place the microbial sample in the cooler with dry ice, and the physical/elemental/isotopic sample into a cooler with ice packs.

d) Use the slide hammer corer to take three mineral soil cores:

i) If significant obstructions such as rocks are present, another approved device may be used to get the soil out of the ground.

ii) 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.

iii) 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.

e) Place each of three mineral soil cores into the same Ziploc bag.

f) Break up the cores and homogenize (mix) in the bag.

g) Remove half of the soil and place in a second Ziploc bag. NOTE: Samples for microbial analysis are split off and preserved differently than those for nutrients/isotopes/soil properties (see Figure 3).

h) Label the bags with the sample location, date, depth/horizon, and your name.

i) Put one bag in the cooler with the ice packs, and the other in the cooler with the dry ice.

j) Enter the metadata (plot ID, date/time of collection, soil temp, x, y coordinates) in field sheet.

k) Remove gloves and stow in a dedicated trash bag.

6) If required in the work order, take a soil core for bulk density

a) Remove the litter layer in a fourth spot of the sampling location.

b) Collect an organic horizon brownie, note the average depth, and place it in a Ziploc bag.

c) Label the bag as above, with the addition of “bulk density” on the bag and place the organic soil inside. Put the sample in the cooler with the ice packs.

7) Use the slide hammer corer to take a core of the mineral soil (to a depth of 30 cm). Write the total length of the core in the PDA and field notebook, bag this core, and label the bag as above with the addition of “bulk density” on the bag. Place in the cooler with the ice packs.

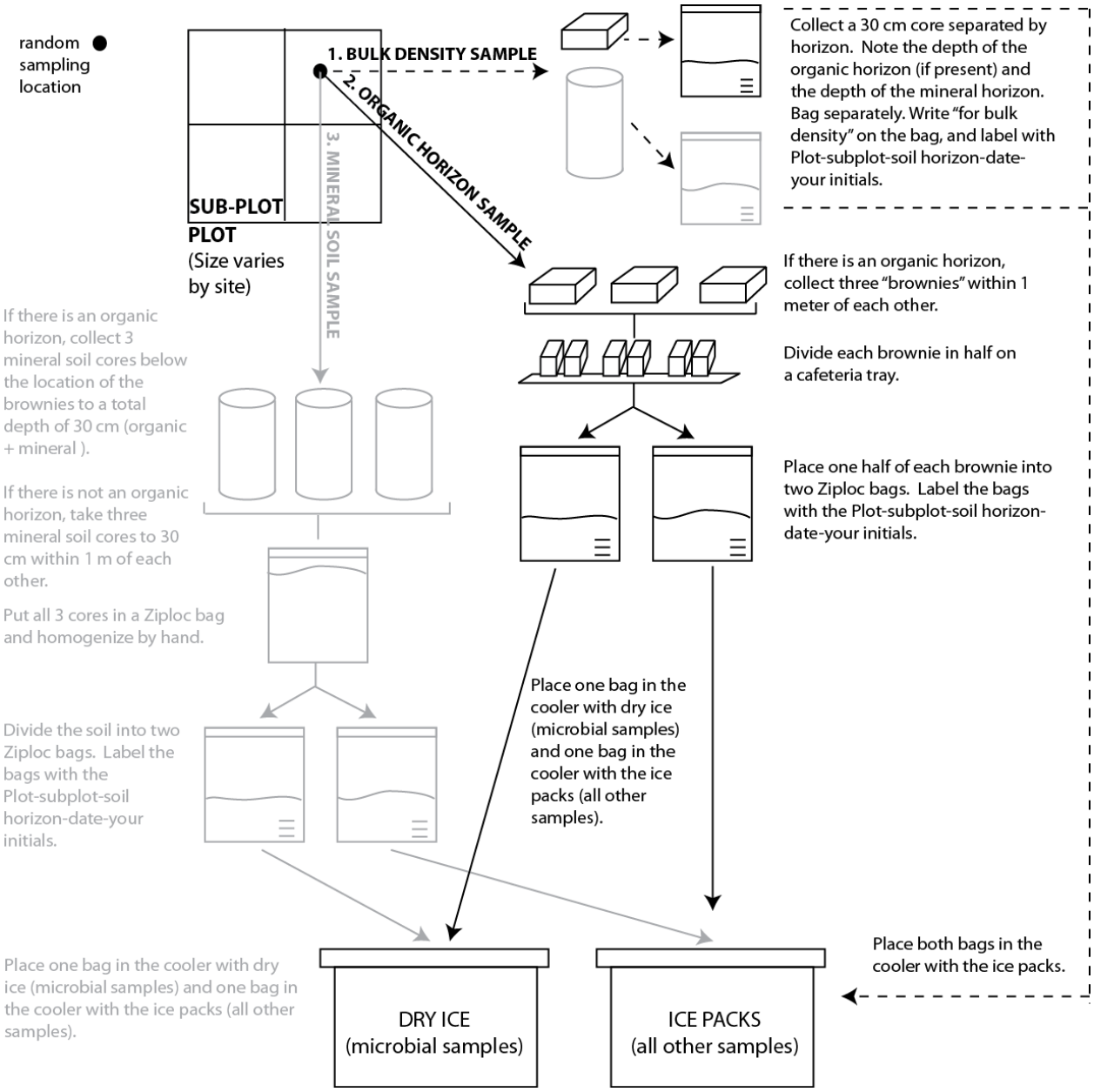

Figure 3. Workflow for the soil sample collection

9.5 Sample Preservation

1) Keep soils for biogeochemistry analysis in the cooler with the ice packs and transfer to 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.

9.6 Data Handling

1)
   a)
   b)
2)

9.7 Refreshing the Sampling Kit

Restock the sampling kit (backpack) with new Ziploc bags, permanent markers, nitrile gloves, flags, etc. Refer to 'Equipment List', Section 10.3.1

9.8 Equipment Maintenance, Cleaning and Storage

1) Recharge the batteries for the GPS, and PDA.
2) Make sure that the soil corers are clean and functioning properly.
3) Clean the knife, trowel, sieve, and organic horizon cookie cutter.

10 LABORATORY STANDARD OPERATING PROCEDURE

10.1 Sample Processing Timing

Process samples within 24 hr of collection. In cases where technicians are working remotely, keep samples in coolers on cold ice packs and dry ice (for chemical and microbial analysis, respectively) until at the domain lab, then process immediately.

10.2 Equipment and Materials

Table 3. Sample Processing Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Powderless gloves			
	Sieves			
	Ziploc bags (gallon and quart sizes)			
	Paper bags			
	Coin envelopes			
	18 ml plastic scintillation vials			

Table 4. Sample Shipping Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Shipping coolers			
	Dry ice			
	Dry ice packing labels			
	Ice packs			
	Packing tape			
	USDA Soil Permit from contracted facility (see Safety section)			
	Boxes			

10.3 Preparation

1) <TBD IN NEXT REV>
   a)
   b)
2)

10.4 Sample Processing in the Lab

Directions are written for field-moist and air-dried processing of soils; follow the appropriate protocol according to contract laboratory specifications.

Samples for analysis of nutrients, isotopes, and soil properties (stored in cooler with ice packs)..

1) Wear powderless gloves. Use a new glove for each soil sample (using one hand to handle the sample allows you to only have to replace one glove).
2) With gloved hand, stir soil sample to homogenize (mix), breaking up any soil clods completely. At the same time, remove rocks, roots, leaves, and debris.
3) Shake 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").
4) Discard particles > 2mm according to permit requirements.
5) Place sieved sample (≤ 2mm material) in a new Ziploc bag. Label the bag with the information on the field collection bag.
6) Record on the Soil Lab Drying Data Sheet:
    a) Metadata: Sample Date, Sampler Name, Lab Tech Name, Plot ID, Quadrant, (X,Y) Coordinates, Sample #, and Horizon.
    b) Date and time of sieving
7) For samples shipped **field moist**, ship immediately in cold-soaked cooler with ice packs.
8) For samples shipped sieved and **air-dried**:
    a) Leave the bag open with the sides rolled down to air-dry. Shake up soil to expose new surfaces once or twice each day.
    b) Air-drying soil can take several days depending on the initial moisture content. Do not continue with processing until soils are completely dry.
    c) For nutrients and isotopic analysis:
        i) Fill a 18 ml scintillation vial with the dried material.
        ii) Cap and store in the scintillation box (holds 100 vials) in a dry cabinet prior to shipment.
    d) For soil properties place ~200 g of the remaining soil into a labeled quart-sized Ziploc bag, seal, and store in a dry cabinet prior to shipment.
    e) For archived material place ~200 g of the remaining material into a labeled quart-sized bag, seal, and store in a dry cabinet prior to shipment.

Samples for **bulk density**

1) Weigh samples collected for bulk density in ziploc bags, remove sample from bag and re-weigh bag. Record 'pre-sieved weight' in PDA or lab datasheet.
2) Sieve soils through 2mm sieve and place the ≤ 2mm and ≥ 2mm fractions in separate labeled paper lunch bags. Clean materials and sieve using 70% ethanol and water between each sample.
3) Place bulk density cores and 5 empty paper lunch bags into an oven at 105°F (60°C) for 48 hours.
4) Once soil samples are dry, weigh dried material for each fraction and record the values to the nearest 0.01 g. 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).

5) Record the average weight of the 5 empty paper lunch bags corresponding to the oven-dried batch of samples in the PDA and lab datasheet.

Samples for **microbial analysis** (stored in cooler with dry ice)

1) No laboratory processing is required for samples stored on dry ice.

10.5 Sample Preservation

1) Samples for analysis of nutrients, isotopes, and soil properties (air-dried)
   a) Store in a dry, clean cabinet.
2) Samples for analysis of nutrients, isotopes, and soil properties (field-moist)
   a) Store in a refrigerator (4°C)
3) Samples for microbial analysis (stored in cooler with dry ice)
   a) The soils stored on dry ice in the field must be transferred to ultralow storage (-80°C) before the dry ice sublimes.

10.6 Sample Shipping

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. Shipment of permit-requiring samples are restricted only to analytical labs or archive facilities that hold a USDA soils permit.

1) Samples for analysis of nutrients and isotopes (air-dried)
   a) Take scintillation vial box containing processed samples out of the cabinet for shipment.
   b) Wrap the box in bubble wrap and tape securely, then place in a FedEx box for shipment.
   c) Include a copy of the USDA soil permit (AD[13]) in the box and affix the PPQ to packaging, if necessary.
   d) Address shipping label appropriately and ship ground.
2) Samples for analysis of nutrients and isotopes (field-moist)
   a) Use a "cold-soaked" shipping cooler (or coolers, in order to accommodate samples surrounded by ice packs on four sides) with ice packs.
   b) Fill cooler with securely double-Ziploc-bagged field-moist soils
   c) Include a copy of the USDA soil permit (AD[13]) in the box and affix the PPQ to packaging, if necessary.
   d) Address shipping label appropriately and send overnight service. Do NOT ship on Friday or day before holiday.
3) Samples for soil properties (air-dried)
   a) Line a box with a trash bag.

b) Pack samples (in Ziploc bags) securely within the box.
c) Include a copy of the USDA soil permit (AD[13]) in the box and affix the PPQ to packaging, if necessary.
d) Address shipping label appropriately and ship ground.

4) Samples for archiving (air-dried)
   a) Line a thick-walled box with a trash bag.
   b) Pack samples (in Ziploc bags) securely within the box.
   c) Include a copy of the USDA soil permit (AD[13]) in the box and affix the PPQ to packaging, if necessary.
   d) Address shipping label appropriately and ship ground.
5) Samples for microbial analysis (stored in cooler with dry ice)
   a) Take samples from the -80C freezer for shipment.
   b) Immediately place samples in a sturdy insulated shipping container with a minimum 20 lbs of dry ice.
   c) Seal container to preclude spillage.
   d) Complete dry ice shipping label.
   e) Include a copy of the USDA soil permit (AD[13]) in the box and affix the PPQ to packaging, if necessary.
   f) Address shipping label appropriately and send the samples priority overnight mail with tracking number. Do NOT ship on Friday or day before holiday.

10.7 Data Handling

Dependent on soil biogeochemical and physical measurements to be determined with DT and KL, and CI interface.

Enter data from field datasheet into … spreadsheet [CI]

Enter data from lab datasheet into … spreadsheet [CI]

10.8 Equipment Maintenance, Cleaning and Storage

1) Wipe down any surfaces used to prepare soils (e.g., bench top, lunch tray) with 70% ethanol and rinse with water.

11 REFERENCES

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

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 FIELD DATA SHEET

Field Data Sheets: NEON SOIL CORE SAMPLING page ___ of ___

Plot ID: _____

Quadrant: NE NW SE SW _____

AOP Plot (if appropriate): _____

eventDate (YYYYMMDD): _____

eventTime (24 HR):

Soil Core (microbes)
Core 1 coordinate X:
Core 1 coordinate Y:
Litter layer 1 depth (cm):
Organic horizon 1 depth (cm):
Mineral horizon 1 depth (cm):
Soil Core (bulk density or biogeochemisty-only collect if indicated by NEON biogeochemist)
Core 2 coordinate X:
Core 2 coordinate Y:
Litter layer 2 depth (cm):
Organic horizon 2 depth (cm):
Mineral horizon 2 depth (cm):

Sample Type:

Remarks:

Field Tech Initials: _____

APPENDIX B LAB DATASHEET

Lab Data Sheets: NEON SOIL CORE SAMPLING

Plot ID: _____

Quadrant: NE NW SE SW _____

AOP Plot (if appropriate): _____

eventDate (YYYYMMDD): _____

Horizon (organic or mineral): _____

Total Weight Pre Sieve: _____

Wet weight >2mm (+bag): _____

Wet weight <2mm (+bag): _____

Dry weight >2mm (+bag): _____

Dry weight <2mm (+bag): _____

Average weight of 5 dried bags: _____

Remarks:

Field Tech Initials: _____