TOS STANDARD OPERATING PROCEDURE: SLS – WETLAND SOIL SAMPLING

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/08/2017	ECO-04372	Initial release
B	01/08/2018	ECO-05308	• Equipment list tables - minor updates for clarity, removed several rows to ensure only additional supplies included • SOP A - added text for when to use long, rectangular 4 mm sample bags vs standard shape 4 mm bags • BOP B, Section B.3 - removed instruction for measuring below-ground sphagnum litter, to be consistent with high-latitude soil sampling updates in the Soil Protocol • SOP B, Section B.4 - added guidance for when to drain water from sample containers • SOP C, Section C.2 - added instruction to record sampleTopDepth and sampleBottomDepth at the time of incubated core deployment
C	02/01/2018	ECO-05390	Removed Maximo numbers from equipment list tables, replaced with supplier names and part numbers
D	03/05/2019	ECO-05980	• Added requirement for DSNY to use wetland method for N-transformation measurement due to highly variable water table fluctuation • Equipment list tables - added shoulder-length gloves, hori hori with plastic handle, folding ruler, high-walled larval tray • Added 4 new figures to demonstrate equipment and methods and improve clarity of instructions • Section 4 and SOP B – inserted guidance on cutting monoliths in thick organic horizon soils • SOP C: added emphasis to make sure bag is well sealed before burying
E	01/27/2020	ECO-06290	• Updated references to Soil protocol sections • SOP B: Revised to cover off-year (microbes) bouts only; added instruction to document unsampled subplots using **samplingImpractical**; guidance to use gridded plot maps to navigate to X,Y locations from nearest plot marker; new field **horizonDetails** to communicate horizon uncertainty

			• SOP C: Revised to cover coordinated bouts, including extra instruction to flag two sub-locations for tinitial and tfinal samples and how to sample M horizons below thick Os; added guidance to bag and incubate O and M t-final samples separately if horizons will be difficult to separate later; revised guidance for how to capture destroyed or lost samples using fate and condition fields • Moved equipment list tables to the end of the document • Minor text edits and clarifications throughout
F	02/08/2024	ECO-07063	• Migrated to SOP template rev E • Updated NEON logo • B1 & B2: Updated instructions for finding and assessing sampling locations to match Soil protocol, including revised Figure 3 that reflects current subplot naming (e.g., 21_400 instead of 21) • B.3: Additional guidance for when to measure soil temperature and litter depth, even with standing water. New Figure 5 to help illustrate. • C.2: Now required, not optional, to add sample metadata in or on the incubation bag; double-bag if needed to protect from sharp roots; OK to widen the borehole if needed to fit and bury the bag • C.3: For single horizon, place entire incubation bag into clean, labeled/barcoded double bag instead of emptying sample into new bag • Minor text updates throughout to clarify procedures and make instructions match the Soil protocol

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 DESCRIPTION

1.1 Overview

This Standard Operating Procedure (SOP) is an extension of TOS Protocol and Procedure: SLS - Soil Biogeochemical and Microbial Sampling (RD[04]). It is designed to enable soil sampling in wetland or wetland-like conditions, where the land is covered by shallow standing water or the water table is at or near the surface. Here, we define shallow standing water as ≤ 50 cm. If water depths exceed this level, soil sampling will not occur. When this SOP is used, the instructions herein are followed for field sampling during off-year soil bouts (SOP B) and field sampling for coordinated bouts including nitrogen transformations (SOP C). There are several steps where technicians are explicitly instructed to use RD[04] while completing the Wetland SOPs, and that document must be on-hand at all times to ensure high-quality sampling.

Plots with wetland or wetland-like conditions are not the focus of the NEON Terrestrial Observation System (TOS), but they are present in certain domains and sites where high water tables are a common seasonal phenomenon (e.g., flooding in low-lying areas following spring snowmelt, permafrost inhibiting drainage in high-latitude sites). Additionally, TOS Protocol and Procedure: Plot Establishment (RD[06]), allows for the establishment of TOS plots as long as > 50% of the plot area is not covered by standing water > 30 cm in depth. As such, areas with standing water or high water tables do occur in space and time across NEON and it is desirable to include them in soil sampling.

We anticipate that only the domains and sites listed in **Table 1** will utilize this SOP. Within those sites, plots requiring wetland sampling techniques will often be classified by the National Land Cover Database (NLCD) as emergent herbaceous wetland, woody wetland, sedge herbaceous, or dwarf shrub. However, this will not always be the case, and field personnel should be prepared to use this SOP to sample plots or subplots in the sites listed below that meet wetland criteria as described in this document regardless of NLCD classification. Field personnel working in NEON domains not listed in Table 1, but who identify plots where conditions may warrant use of this SOP, should notify Science through ServiceNow.

Table 1. List of sites that will use this SOP to sample in wetlands or wetland-like conditions.

Domain	Sites
D01	HARV
D03	DSNY*, OSBS
D05	TREE, UNDE, STEI
D08	DELA, LENO
D09	WOOD, NOGP, DCFS
D18/19	HEAL, TOOL, BARR, BONA

* *The water table at DSNY can rise quickly and unpredictably, thus DSNY will use the modified method detailed below for conducting N-transformation measurements for all plots and bouts*.

The presence of shallow standing water or a high water table yield unique soil sampling challenges. First, collecting soil cores in a standardized fashion is more difficult than in well-drained uplands, and the process may require specialized soil coring equipment and techniques. Second, measuring nitrogen (N) transformations with *in-situ* incubations is complicated by the possibility for leaching losses from incubating covered soil cores. To address such challenges, this SOP provides additional and alternate equipment lists and modified instructions needed to complete the field sampling components of RD[04] in wetland or wetland-like plots. This includes off-year and coordinated bouts. Unlike field sampling components, no special modifications are needed for laboratory processing of wetland or wetland-like soils. Therefore, all lab-based SOPs detailed in RD[04] should be used.

1.2 Purpose

This document outlines modified procedures for soil sampling during off-year and coordinated bouts (including N transformations) in plots covered with shallow standing water or that have a water table at or near the soil surface. It should not be considered a robust wetland monitoring effort, as that is well beyond the scope of the NEON TOS. Instead, this SOP is designed to produce data that can be delivered within the framework of standard NEON soil data products. While not all wetland-relevant variables will be measured, the data produced may still be useful in revealing changes in select wetland soil properties over time and across the bioclimatic gradients present in the Observatory.

1.3 Scope

This document provides a change-controlled version of an Observatory procedure. 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.

1.4 Applies To

The procedure described in this document is used in the following protocols:

Doc #	Title
NEON.DOC.014048	TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Sampling

1.5 Acknowledgments

Lisa Windham-Myers of the U.S. Geological Survey and Patrick Inglett of the University of Florida provided helpful input in developing this standard operating procedure. The 2011 National Wetland Condition Assessment (USEPA, 2016), along with the paired NWCA Field Operations Manual (USEPA 2011), were also very helpful resources.

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.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.014048	TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Sampling
RD[05]	NEON.DOC.001577	Datasheets for TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling
RD[06]	NEON.DOC.001025	TOS Protocol and Procedure: PLT – Plot Establishment
RD[07]	NEON.DOC.001717	TOS Standard Operating Procedure: TruPulse Rangefinder Use and Calibration

2.3 Acronyms

Acronym	Definition
NLCD	National Land Cover Database
O	Organic
M	Mineral

2.4 Definitions

Clean technique: Procedures to minimize the introduction of chemical or biological contaminants into a sample. Contamination can result from dust particles, non-purified water, sweat, hair, and other environmental sources.

Fulcrum: Software platform used to create NEON electronic data entry applications.

Mineral (M) horizon: A soil layer where accumulated minerals are the main component. Can vary widely in color based on organic matter content and presence of certain minerals. Often feels gritty.

Monolith: A soil block that is cut with a knife instead of extracted with a coring device. Often more suitable for sampling thick organic soils.

Organic (O) horizon: A soil layer made of organic vegetal material in various states of decomposition, where the mineral fraction is only a small percentage of the layer (much less than half by weight). In general, decomposing plant material is poorly recognizable, though this may not always be true in wetland sites where decomposition is slow. Layer tends to be dark in color, friable (easily crumbled), and sometimes greasy. If more than a couple of mineral grains are detected (grit from sand, stickiness from clay) it is most likely a mineral horizon high in organic matter (OM), not an organic soil.

ServiceNow: Software tool used for problem/incident tracking and resolution.

Sterile technique: Procedures to minimize the introduction of microbial/DNA contaminants into a sample, such as human microbiota or DNA from a different source material or habitat.

Wetland: An area where the water table is frequently at or near the surface, or where the land is covered by shallow standing water. Can occur alongside streams, rivers, lakes, and coasts, in depressions and other low-lying areas, and in association with high-mountain springs. Can be saturated at varying intervals, and have biotic communities adapted to live in conditions ranging from permanently wet to fluctuating wet-dry.

3 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.

Sampling in wetlands should be undertaken with care. Saturated soil may make maneuvering difficult, and caution should be used when walking through flooded plots so as not to incur injury or develop a fungal infection from working in or around flooded plots. Rubber boots, waders, or other protective equipment should be used to keep personnel dry. In some sites, flooded conditions may be accompanied by additional dangers from local wildlife (e.g., alligators, snakes). In such cases, follow all Domain and Manager specific instructions and avoid entering flooded plots when dangers are present.

4 PERSONNEL

4.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

Field personnel must receive training in assembly and operation of the coring device being used to sample unconsolidated substrates, as well as the laser rangefinder if it will be used to locate X,Y coordinates. Additionally, completion of all training requirements described in RD[04], especially how to employ sterilization techniques when handling soil samples and cleaning equipment, is needed to successfully complete this SOP.

4.2 Specialized Skills

The ability to use an appropriate coring device or technique to sample flooded soil substrates from a known depth (typically 30 cm) will be key to successful implementation of this SOP. Additionally, knowledge of the basic characteristics of soils at a given site and the ability to differentiate between organic (O) and mineral (M) horizons is required. Proficiency with the method of delineating random X,Y coordinates described in RD[04] will be needed to ensure sampling occurs at correct locations. A willingness to work in standing water and on unstable surfaces (e.g. mud and sediments) using appropriate PPE is also needed. Finally, all field personnel should be well-versed in sterilization techniques for collection and handling of sensitive microbial samples, as described in depth in RD[04].

5 EQUIPMENT

The equipment listed at the end of this document (**Table 2**, **Table 3**) is used for wetland soil sampling. The lists are supplements to the field sampling equipment lists in RD[04], meaning that only additional and/or alternate equipment required specifically for sampling wetland soils is presented.

The two major equipment modifications in this SOP compared to RD[04] are:

- Substitution of a specialized wetland soil coring device instead of a standard soil corer
- Use of 4 mm thick polyethylene sample bags instead of incubation cylinders for conducting N transformation incubations

Regarding the former, a specialized coring device – such as the AMS Multi-Stage Sludge Sampler (Figure 1), need only be purchased if the domain's standard tool does not allow for collection of high-quality soil cores in the local wetland conditions. This is likely to be the case for mineral soils where the substrate is very unconsolidated and significant amounts of material fall out the bottom when attempting to core. For thick, organic wetland soils, coring devices may have trouble cutting through fibrous organic layers without causing significant compaction. In this case, cutting soil monoliths may be a better alternative. When both organic and mineral horizons are present, a combination of the two techniques may work best. Table 2 provides suggested equipment for different wetland types and conditions – if unsure of the most appropriate device or sampling strategy to use in their domain, Field teams should contact Science to discuss.

Figure 1. Components of the Multi-stage Sludge Sampler.

6 CONTINGENCIES AND NOTES

Similar to non-wetland soil bouts, all sampling for a scheduled bout (e.g., 'peak greenness', 'winter-spring transition') should be completed within a two-week period (i.e., 14 calendar days). If a bout is interrupted by inclement weather or other circumstances, attempt to finish the bout within the two-week window. If sampling cannot be completed within this timeframe, the issue should be reported through ServiceNow, as described in RD[04].

Field personnel must use their judgement to determine when conditions are inclement enough to halt soil collection. As work in wetlands will be an inherently wet endeavor, a light drizzle should not necessarily prevent sampling. However, heavy rains and associated rising water tables, lightning, presence of dangerous animals, or any other condition that threatens the safety of personnel or access to the plots should be taken seriously. In such cases, sampling should be halted. If the issue persists, report the problem through ServiceNow.

7 STANDARD OPERATING PROCEDURES

SOP A: Preparing to Sample

SOP B: Field Sampling During Off Year Bouts in Wetland Plots

SOP C: Field Sampling for Coordinated Bouts including N Transformations in Wetland Plots

SOP A Preparing to Sample

1. Complete all steps listed in SOP A of RD[04], including:
    - Charge mobile data recorders
    - Pre-label and organize sample containers, including the addition of adhesive barcode labels
    - Print possible X,Y sampling locations from soil coordinate lists
    - Mark target sampling locations on gridded plot maps, using the Soils QC application maintained by Field Science, or by hand
    - Print back-up data sheets
    - Ensure there is a navigation device with target plot locations available
2. Additionally, gather all equipment needed for wetland-specific sampling, consulting both the equipment lists in **Table 2** and **Table 3** as well as those in RD[04]. Use prior knowledge of the target plots as well as reports from other NEON technicians who have recently visited them to assess whether standing water will be present, then ensure appropriate supplies will be available for sampling (waders, shoulder-length gloves, etc).
    a. Durable items (wetland corer plus parts, waders/boots, etc.) must be clean, free of rust, and in good working condition.
    b. Sufficient quantities of consumable items must be available.
3. If the soil team is likely to encounter enough standing water to prevent accurate or efficient use of meter tapes, they should be prepared to use the TruPulse laser rangefinder in **HD** (horizontal distance) mode to locate X,Y sampling locations. In preparation, review RD[07] and complete the following tasks related to using the TruPulse 360R Laser Rangefinder.
    - Check battery and charge (if possible)
    - Clean lenses with lens cloth or lens tissue (if necessary)
    - Check/set correct declination. This is required every time the batteries are changed, when batteries run low, or when moving from one NEON site to the next where declination may be different. See RD[07] for details.
    - Calibrate TruPulse tilt-sensor (only necessary after severe drop-shock; see RD[07] for details).
4. *When preparing for an N-transformations bout*:
    a. Sites where intact soil cores can be taken should plan to use 30 cm long, skinny 4 mm rectangular polyethylene bags for the buried bag method (**Table 3, Figure 2,** right). These will allow intact cores to keep their shape and structure while incubating and are thus preferred.

Figure 2. Different size bags to use for N-transformation incubations. Small bag is for the sample metadata.

b. Sites where the substrate is very unconsolidated and cores fall apart upon removal can use standard square-shaped polyethylene sample bags, as long as they are 4 mm thick (see **Table 3** for part numbers).

c. Regardless of substrate type, ensure that all equipment and supplies needed to conduct potassium chloride (KCl) extractions are ready to go, since samples must be extracted within one day of collection. See RD[04] for detailed supply lists and instructions.

SOP B Field Sampling During Off Year Bouts in Wetland Plots

This SOP is designed to enable soil sampling when plots or subplots are covered with shallow standing water or have a high water table. If plots with wetland or wetland-like NLCD cover classes do not have these conditions at the time of sampling, they need not be sampled using this procedure. Note that SOP B of RD[04] is referenced frequently in the steps outlined below, thus it is mandatory that a hard copy of it be on hand during sampling bouts.

MAXIMUM WATER DEPTH

If standing water depth at a potential X, Y sampling location is > 50 cm, do not attempt to collect soil. Instead, move to the next X, Y coordinate on the coordinate list; if all five potential X, Y coordinates that could be used for the bout have standing water > 50 cm, move to the next designated subplot. If flooding is uniformly > 50 cm across the entire plot, do not collect any samples. For each subplot where soils are not collected due to standing water, record missed sampling using the **samplingImpractical** field, as described in RD[04], and report the issue through ServiceNow. If the flooding is not part of the normal/annual hydrologic cycle but instead results from a disturbance event, ensure that the event is recorded in the Site Management application.

B.1 Identify the plot and sampling location

1. Upon arrival at a plot, use a navigation device to confirm that you are in the correct location. Locate one of the plot markers such as the southwest corner as an additional source of verification. Move along the perimeter as much as possible to minimize foot traffic within the plot.
2. Soil will be collected at three randomly assigned X, Y locations within each plot, one in each randomly assigned subplot. These random sampling locations are identified using the soil X, Y coordinate list, as detailed in RD[04].
3. If not already done, use a laminated, gridded plot map (**Figure 3**) to mark the X,Y coordinates that are the target for sampling. Recall that the X coordinate is the number of meters east, and the Y coordinate is the number of meters north, in relation to the southwest plot corner (point 21).
    a. Keep in mind that rejected coordinates can occur so have back-ups handy.
4. For a particular X, Y coordinate, select the closest plot marker that is available in the field.
5. Next, calculate the distance and direction that must be navigated from each selected plot marker to a coordinate location. This step may be skipped if using pre-printed maps from the Soils QC application as those include distance and direction from nearby plot markers.

a. Using the map, measure the distance you must traverse by counting the number of gridlines from your chosen plot marker to the X, Y coordinate drawn onto the map. Start with the longest distance first. Each gridline corresponds to 1 meter.

b. For each X, Y coordinate, note the distances and directions from the selected plot marker to the X,Y coordinate onto the map. Use these distances and directions to navigate from the selected plot marker to a sample location.

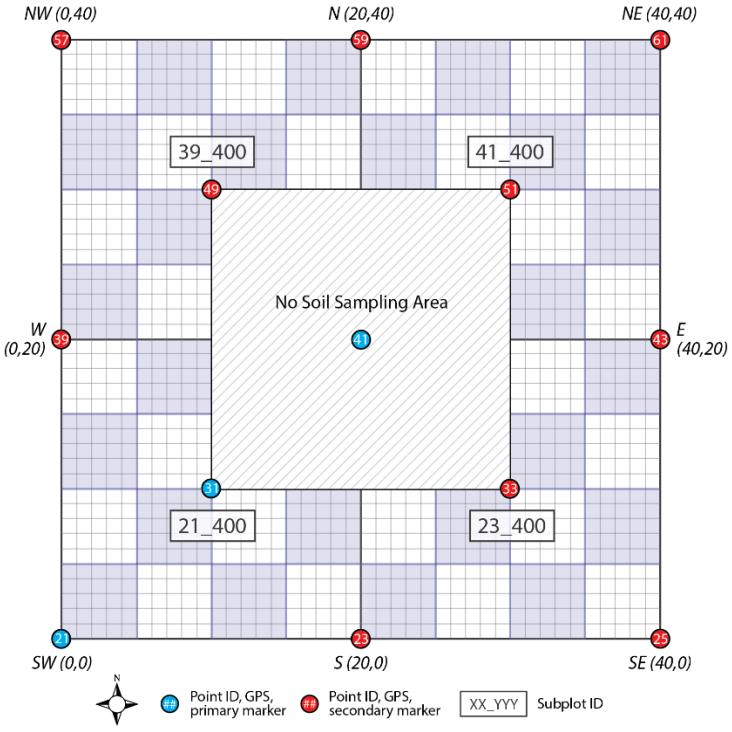

Figure 3. Gridded map of a 40 x 40 m soil plot. Plots are divided into four 20 x 20 m subplots with a soil sampling exclusion zone in the plot center. Subplots are named for the southwest corner pointID (21, 23, 39, 41) and size (400 m^2), and three out of four are sampled per bout. PointIDs and markers for 23, 39, 43, and 59 are not present in small-stature Tower and Distributed plots.

6. If there is only shallow or no standing water, use meter tapes to navigate to the target sampling location as follows:

   a. Lay out a meter tape from the selected plot marker in the X (E/W) direction. Use a compass to verify direction.

   b. Pull the tape to the measured distance to the target X coordinate and mark the point.

   c. From that point, navigate in the Y (N/S) direction to the measured distance to the target Y coordinate. Use a compass to verify direction.

   d. Place a marker at the X, Y location.

7. If there is enough standing water in the plot to make meter tapes difficult or inefficient to use, use the laser rangefinder set to HD mode to locate X, Y sampling locations.
    a. Two technicians must work together. One stands at the selected plot marker and operates the laser rangefinder. The second person navigates to the first potential X-location, following the directions of the rangefinder operator and using the reflective tape or reflector stick, so that an accurate horizontal distance measurement can be obtained.
    b. The rangefinder operator must ensure that the angle (azimuth) is as close to 90˚ as possible from True North when measuring the X-coordinate distance.
        i. Anything metal worn by the rangefinder operator will compromise azimuth measurements (glasses with metal arms, watches, rings, etc) and should be removed and kept far enough away from the rangefinder (the larger the amount of metal, the farther away).
    c. Place a marker, such as a pin flag, stake, or other marker of appropriate height given the water level, at the X-location.
    d. The rangefinder operator then moves to stand directly over the marker. Using either a measuring tape or the TruPulse in **HD** mode with a reflective surface, work with the second technician to locate the Y-coordinate location.
    e. Make sure that the angle (azimuth) is as close to 0˚ as possible (True North) and measure the Y-coordinate distance.
    f. Place a clean marker at the potential X, Y location

B.2 Assess sample location

1. Put on a clean pair of Nitrile gloves. At the same plot, gloves can be re-used after rinsing with DI water to remove coarse debris and drying thoroughly. Do NOT reuse gloves between plots.
2. Assess the location for sampling suitability:
    a. Are there obvious disturbances, vegetation, large rocks, insect colonies or other features that would impede sampling within a 0.5 m radius of the X, Y location?
    b. In Tower plots, does the coordinate fall within 2 m of the perimeter of a ground or elevated litter trap?
    c. Locations covered in standing water will also need to be assessed for suitability by probing. Use a sterilized long, clean pin flag or stake to gently probe in the 0.5 m radius of the X, Y location. Determine whether there are unseen impediments to sampling.
3. If any of the above conditions are met, reject the location and record why on the soil coordinate list. Move to the next potential X, Y location. Repeat until a suitable location is found.

- If five X, Y coordinates are rejected, do not sample within that subplot and submit a ServiceNow ticket. Make a record for that subplot and choose "Coordinates not suitable" in the **samplingImpractical** field, with X, Y coordinates and other metadata blank. Do not attempt to sample the fourth subplot not randomly selected for this bout.
- If a disturbance is the dominant condition of a subplot (e.g., more than half the area is covered in debris from logging), do collect a sample but use the **horizonDetails** field along with **remarks** to provide more information on this condition.

4. At a location that appears to be suitable, confirm with additional probing. Start near the exact location of the X, Y coordinate and assess soil depth by probing the soil using a sterilized pigtail stake (or other poking device sufficiently long to accommodate standing water and free of paint and rust). Move outward up to 0.5 m from the X, Y location until a suitable spot is found where the probe suggests protocol target depth can be met.

    a. The target depth for soil sampling is 30 cm, or the entire horizon if thinner. Some sites have characteristically shallow/rocky soils, or restricted sampling thickness due to permafrost. At these sites, a suitable location may not allow coring to 30 cm depth - this is acceptable.

5. Mark the exact target sampling area identified via probing with a flag or stake.

B.3 Collect soil core

1. Once an acceptable location is found, prepare to sample. Ensure that the coring device or hori hori (if planning to collect a monolith) is properly assembled, and that all tools that will be used for sampling have been cleaned with deionized water and sterilized, according to the sterilization instructions provided in RD[04].

Note: *If using a coring device that comes with a plastic 'soil core catcher' (**Figure 4**), but local soils are fairly well consolidated, try to extract a core first without using the catcher, installing it only if needed.*

Figure 4. Example soil core catcher that comes with the AMS Multi-Stage Sampler.

2. If there is no standing water (e.g., the location is only saturated), or if standing water is very patchy/ponded and a dry spot with no standing water can be found at that X, Y location (**Figure 6**), measure soil temperature as described in RD[04], Section B.3.

Figure 5. Soil sampling locations with standing water that is very patchy and heterogenous. D05 on the left, D03 DSNY on the right. In these cases, soil temperature can be measured in a dry spot within the chosen X,Y location.

3. If standing water is uniformly present, the temperature probe cannot be inserted to the proper 10 cm depth, thus temperature measurement should be skipped (leave blank).
4. If there is no standing water, measure litter depth as described in RD[04], Section B.3. It is also possible to record litter depth even with standing water under the following circumstances:
    a. Litter depth is thicker than standing water depth. For example, there is 8 cm of surface leaf litter, upon removing it 2 cm of standing water is revealed – record both values.
    b. In certain ecosystems It may be possible to know that litter depth = 0 cm, even when there is standing water (for example, flooded arctic tundra).
5. Other than these situations, since it is not possible to take an accurate measurement of litter depth through standing water, leave the field blank.
6. Remove any litter that may be present in preparation to sample soil.
7. *In Sphagnum-dominated soils*, use clippers to remove any overlying live sphagnum moss and lichens until the beginning of the O horizon is reached. See RD[04], Appendix D for more detail on how to identify the beginning of the O horizon in these soil types.
8. If there is no standing water, enter 0 for standing water depth in the SLS: Field application and skip to STEP 10.
9. If there *is* standing water, follow these steps to measure and account for it:

a. Insert a ruler, meter stick, or pin flag/stake until it touches the surface of the soil.

    i. For very unconsolidated substrates, the exact soil-water interface may be difficult to determine. Examine the bottom of the measuring tool to make sure there is residue only on the very bottom (if present). Go slow and make a reasonable estimate.

b. Read the standing water depth on the ruler or meter stick (**Figure 6**), or measure the flooded depth of the pin flag/stake using a meter tape.

Figure 6. Measuring standing water depth at a flooded sample location.

c. Record this depth in the SLS: Field application. Then, add depth to 30 cm to get the total target sampling depth for the coring device.

    - *Example*: 23 cm standing water means target depth for the corer of 23 + 30 = 53 cm.

d. Mark this depth on the extension pole or coring device handle using laboratory tape. Make sure the tape wraps all the way around and overlaps itself or it is likely to fall off.

e. For thick, fibrous O horizons where a submerged monolith will be cut, one of the samplers should hold the hori hori (**Figure 7**), then measure the target depth from the tip of the knife to the appropriate length on their arm. Mark this location with tape, a rubber band, or similar while wearing shoulder-length gloves.

10. Insert the coring device or hori hori into the soil until it reaches 30 cm depth or bedrock. If there is standing water, insert the coring device or gloved arm until the tape mark or rubber band is reached, ensuring it is held perpendicular to the water line.

Figure 7. Technician preparing to collect an O horizon monolith in Alaska, using shoulder-length gloves and a hori hori.

a. For sites where there are thick root mats (example: cattails in Domain 09, **Figure 6**), push aside and/or cut through roots before beginning to core.

11. Slowly remove the core or monolith, watching to ensure that little material falls out the bottom. *With a gloved hand, 'cap' the end of the coring device as it is pulled up to avoid loss of material, or scoop fallen material from the footprint of the monolith*. If a substantial amount of material is lost during removal (>= 50%), do not keep the sample and attempt to take another core from that same X,Y location. If unsure, keep the core but assess how much soil material was recovered (section B.4) and then determine whether it's an acceptable sample.
12. If another coring attempt is needed and the device has a core catcher, install it now, then try taking another core. The core catcher should help to retain the soil material.
    a. If three attempts fail to extract a core without substantial mass loss out the bottom, move to the next suitable X,Y location on the coordinate list. Repeat for up to five X,Y coordinates per subplot.
13. Once a viable soil core or monolith is collected, attempt to find a dry spot outside the plot, or at least in the 'microbes/bgc' destructive sampling zone, to process the subsamples. Try to follow the same path you used to enter in order to minimize disturbance.

B.4 Process soil sample

1. The person who will handle the sample should either clean and re-sterilize their nitrile gloves, or put on a new pair and sterilize them.
2. Empty contents of coring device, or place monolith, onto a sterilized sample tray, using a sterilized extruder if needed.

a. If only a small amount of soil is recovered from the coring device (<= 50%, visual estimate based on core size), suggesting significant mass loss upon removal, discard sample as it is not representative of the target depth and collect another core as described above

3. Remove and discard any loose organic matter from the top of the core or monolith. This includes debris such as twigs, leaves, seeds, live moss, other pieces of intact plant matter, insects, and animal detritus.

    a. For peatland and permafrost sites, refer to RD[04], Appendix D, for more information on how to determine where the soil starts

4. Determine whether both organic (O) and mineral (M) horizons are present. This may be more difficult for wetland soils than in uplands as the boundary between O and M horizons is likely to be poorly defined (**Figure 8**). Use texture and color, refer to RD[04] for more details.

Figure 8. Example of a typical wetland soil core demonstrating a poorly defined boundary between O and M horizons (Photo credit: US Environmental Protection Agency). Note that gloves should be worn by NEON technicians when handling soil samples.

a. If both O and M horizons are present and there is an obvious boundary between them, separate the horizons using a clean, sterilized hori hori. <u>Retain only the O horizon for off-year ('microbes') bouts.</u>

b. If there is no O horizon, or the O horizon is ≤ 1cm, process the entire core as an M horizon sample.

c. If the core appears highly organic but with no clear boundary between O and M layers, process the entire core as one sample and determine which horizon to assign as follows:

TIPS & TRICKS

    i. Take a small mass of soil in your gloved hand and assess the texture. If it feels grainy or gritty, it is likely an M horizon with high organic matter content. If smooth, friable, and a bit greasy, it is probably an O horizon.

ii. Assign whichever horizon is most probable but use the **horizonDetails** field to communicate uncertainty in the horizon designation. If possible, take a photo and seek Science input upon returning from the field.

5. Refer to Sections **B.4** and **B.5** of RD[04] for detailed instructions on how to bag, homogenize, subsample, and store O and M horizon samples, respectively. Remember that the type of subsamples needed depends on site type (core vs gradient) and sample timing (transition vs peak green) - see tables and figures in Appendix A of RD[04].

6. Samples may be extremely wet and include a good deal of water. The goal is to drain off as much of this water as possible without discarding too much of the soil material.

    a. Allow samples to settle for 2 minutes in their 1-gallon bag to enable sand and silt size particles to separate from the water. Then, pour off excess liquid if it is reasonably clear. A useful trick is to insert a sterile scoopula or spoon into the liquid – if the item is visible, the liquid should be poured off, but if not, the water is full of clays and should be kept as part of the homogenized sample.

    b. When subsampling for microbial analysis and archive, transfer only the more solid materials into the cryovials and whirl-paks. Do this by using a sterile scoopula or spoon to target the solids settled at the bottom of the 1-gallon homogenized soil bag. *Do not fill cryovial containers more than 2/3 with very wet samples or they will crack upon freezing.*

7. If it is apparent that not enough material will be available to create all subsamples, or that not enough homogenized soil will be left over after doing so (30 g for O horizon, 60 g for M horizons, for off-year bouts), extract a second core. Return to the X, Y location (ideally following the same path to minimize disturbance) and sample within a 0.5 m radius as described above. Combine material from both cores/monoliths.

8. Follow instructions for recording data in Sections **B.6** of RD[04]. Additionally:

    a. For **samplingProtocolVersion**, make sure to choose this SOP (NEON.DOC.004130vF).

    b. Enter **standingWaterDepth** in centimeters, to the nearest centimeter.

        i. Enter ‘0’ if there was no standing water, but choose ‘water table encountered’ for **horizonDetails** if you hit the water table while sampling.

9. Move to the next designated subplot and repeat all sampling procedures. Once all three subplots per plot have been sampled, remove flags or stakes marking X, Y locations. Travel to the next plot and complete all procedures detailed above.

10. Follow instructions for field clean-up and sample preservation and transport as described in Sections **B.7** and **B.8** of RD[04].

11. Complete all laboratory processing steps as outlined in the SOPs contained in RD[04]

SOP C Feld Sampling for Coordinated Bouts including N Transformations in Wetland Plots

This SOP enables N transformation sampling when plots or subplots are covered with shallow standing water, or have a water table frequently at or near the soil surface (e.g., within 30 cm). **Plots that meet either of these criteria MUST use this SOP, regardless of whether the substrate is consolidated or not (e.g., whether a wetland coring device is used to collect soil).** A good test for presence of a high water table is to extract a 30 cm deep soil core, then check the bore hole. If it begins to fill with water, even if only in the bottom, this SOP must be used **for all samples in that plot** for a given bout (see Box 1).

Instead of using incubated covered cores, which are ideal in well-drained uplands, the 'buried bag' method will be used in plots with standing water or a high water table. This is because a high water table can cause nitrogen leaching from the soil sample when using the covered core method, and this compromises the integrity of N transformation estimates. Four millimeter (4 mm) thick polyethylene sample bags allow for gas exchange but are impermeable to liquids. Thus, they prevent leaching losses and allow reaction products to accumulate while exposing the sample to ambient temperatures, making them similar in nature, though with more disturbance, compared to the covered core method (Binkley and Hart 1989).

***Note**: If a high water table is suspected in the plot, locate and flag the coordinates in all three subplots first, then choose to begin sampling at the coordinate that is in the lowest lying area. This is where you are most likely to encounter the high water table.*

Box 1. Guidelines for N transformation field sampling in wetland or wetland-like plots. The goal is to use a consistent method within a given plot for each bout.

Situation	Action
At least one subplot (X,Y location) in the plot is covered with shallow standing water, or has a water table at or near (within 30 cm) the surface	Follow this SOP (buried bag method) to conduct N transformation sampling for the entire plot
SOP C of RD[04] (covered core method) has already been used in one or two subplots when you discover that another target subplot in the plot has a water table at or near (within 30 cm of) the surface	Return to the subplot(s) where covered cores have been installed, remove cores from the ground, and extrude soil into 4 mm polyethylene sample bags. Bury and cover bags as described in this SOP and change **incubationMethod** to buried bag in the SLS: Nitrogen Transformations application or paper datasheet
This SOP (buried bag method) was utilized in a flooded plot during the spring sampling bout, but by summer or fall the plot has dried out	Use SOP C of RD[04] (covered core method) to conduct standard N transformation sampling once the plot has dried out
SOP C of RD[04] (covered core method) was used in a plot during the spring sampling bout, but in summer or fall the plot has become flooded	Follow this SOP (buried bag method) to conduct N transformation sampling in summer or fall if the plot has become flooded

C.1 Collection of initial soil cores

1. Follow the instructions described above in **SOP B** to collect, bag, homogenize, subsample and store soil samples to be used for 'initial' N-transformation and microbial measurements. <u>However, the following points differ during coordinated bouts</u>:
    a. Once the X, Y location has been accepted, mark two sub-locations for sampling – one for the initial core and one for the incubated sample. Sub-locations should be no closer than 0.2 m of each other and ideally within 0.5 m of each other, and both core locations should be within 0.5 m of the actual X, Y location (Figure 19, RD[04]). *Ok to skip if there is deep standing water*.
    b. Note and record bottom depth of the T-initial sample following collection. The bottom depth of the paired T-final incubation sample should match this depth **± 5 cm.**
    c. If both O and M horizons are present and possible to separate, collect and keep material from both. They will be treated as separate samples and each requires a record in the SLS: Field Sampling application.
        i. If using the monolith method, it may be necessary to cut just the O-horizon with the hori hori, then use a coring device to sample the M-horizon exposed beneath. This depends on whether the M is too unconsolidated to be removed as a block.
    d. For coordinated bouts, more sample is needed for laboratory analysis and subsampling. Ensure that the homogenized sample has at least this much mass remaining after all subsampling is complete:
        i. boutType = microbesBiomass (transition seasons): 50 g O, 100 g M
        ii. boutType = microbesBiomassBGC (peak greenness): 75 g O, 160 g M
    e. As described in SOP C of RD[04], N transformation processing and extraction must occur the day of or one day after samples are collected.

C.2 Deployment of incubated soil cores

1. After collecting an initial soil sample, collect a sample from the second sub-location – no closer than 0.2 m and ideally within 0.5 m of the first coring location, and both should be within 0.5 m of the actual X, Y coordinate. Match bottom depth to the T-initial sample, **± 5 cm.**
2. Remove core from the coring device, or extract monolith.
3. Bag it as follows:
    a. If core/monolith is unconsolidated and contains two horizons, place the O and M horizons in separate bags and incubate separately. This is because they will be difficult or impossible to separate at the time of retrieval. Use a tray to assist with this if needed.

b. If core/monolith is well-consolidated and only contains one horizon, or both O and M are present but very in-tact and will be easy to separate later, transfer the entire sample to one 4 mm polyethylene sample bag, disturbing the structure as little as possible. Use an appropriate shape sample bag as described in SOP A.

4. Ensure that each sample bag is labeled with the following pieces of metadata so that it can be identified upon collection.

   - Record **plotID**, **subplotID**, **sampleTiming,** and **horizon** (if known)
   - Also estimate and record **sampleTopDepth** and **sampleBottomDepth**, as this may be easier to assess at the time of deployment versus collection.

   A weatherproof label applied to the bag exterior may fall off/disintegrate, so use an alternate labeling method. One option is to write on a piece of all-weather copy paper using pencil, place that in a small size re-sealable plastic bag (**Figure 2**), rinse exterior with DI or local standing water, then place inside the larger incubation bag. Another option is to label the incubation bag with a weatherproof label, but add a second plastic bag around the first to protect the label.

5. It is possible to write the key metadata on a pin-flag or flagging tape left at the sample location, but do this *in addition to labeling inside the bag*, not instead. If the bag gets dislodged from its original location, it will be important to have sample metadata included in or on the sample bag.

6. Close the sample bag and make sure to remove all air. This may require some 'squishing' of the core or monolith and that is acceptable - it is important to remove all air from the bag.

7. Make sure the bag is completely sealed. If soil particles interfere with closure of the bag, or in areas that contain many sharp roots that may pierce the bag when putting it back into the borehole, add a second, clean bag and make sure that one is properly sealed.

8. 'Bury' the bag.

   a. If the bore hole or monolith footprint is visible (little or no standing water), place the bag back inside of it. If O and M are bagged separately, place M bag on bottom, O bag on top.

      i. Cover with a few centimeters of leaf litter, loose soil, and other detritus so that the bag will not be in direct sun. If needed, it is OK to deepen and/or widen the original borehole to ensure the bag can fit back inside and be thoroughly buried.

      ii. Site-host permitting, place a few pin flags or stakes surrounding the location where the bag is buried.

      iii. If leaving a flag or stake is not permitted but a less intrusive marker type is acceptable, take an ~18" long segment of brightly colored paracord, bury one end along with the bag and allow the other end to stick out of the ground 6-8."

iv. If no marker can be left at all, record the location with a GPS waypoint, labeling it with the plotID, subplot, sampleTiming. This will assist in recovering the sample at the end of the incubation period.

b. If the bore hole or monolith footprint is not visible or it collapses (standing water on the plot), use a planting spade or dibble bar to bury the bag.

i. Insert planting spade or dibble bar at ~ 45° angle and pry open a slot in the soil/substrate.

ii. Wearing shoulder-length gloves (as desired/needed to keep arms dry), place the bag into the slot. Try to position it so that the entire bag is buried. If O and M are bagged separately, place M bag on bottom, O bag on top.

iii. Remove the planting spade or dibble bar and allow the soil to collapse around the bag.

iv. Pat down soil on top of the bag. This will help make sure it stays in place.

v. Site-host permitting, place a few 4 ft stakes or other appropriate long markers surrounding the location where the bag is buried. If not permitted, mark the location with a GPS waypoint, labeling it with the plotID, subplot, sampleTiming. This will assist in recovering the sample at the end of the incubation period.

C.3 Collection of incubated samples

1. After the appropriate incubation period has passed (refer to RD[04] Appendix C for site-specific guidance), navigate to the location of a buried bag.
2. Using shoulder-length gloves (as desired/needed to keep arms dry), remove the buried bag.

a. If there is minor damage to the bag – for example, an animal has torn a small hole in it, or it has leaked and there is extra water inside, still collect but choose the appropriate value for **incubationCondition** (or 'other' if needed) and make a note in remarks.

b. If there is major damage to the bag – for example, an animal has torn the bag completely open and there is a gaping hole, do not save the sample. Create a record in the SLS: Field Sampling application or paper datasheet, but note 'lost' for **sampleFate,** 'sample destroyed, not saved' for **incubationCondition,** and only record minimal sample information**.**

c. If the bag is damaged but severity is difficult to assess, err on the side of collecting the sample, but take a photo, note issue in **incubationCondition** and remarks fields, and contact NEON Science for input.

d. If the bag cannot be found despite best efforts (up to 30 minutes of search), create a record in the SLS: Field Sampling application or paper datasheet, but note 'lost' for

sampleFate, 'sample not found' for **incubationCondition,** and only record minimal sample information**.**

3. If there is only one horizon type in the bag, or the O horizon is ≤ 1 cm or impossible to separate, place the entire sample in its incubation bag (likely to be very dirty) into a new, labeled and barcoded plastic bag. No further field processing is needed.
4. If both O and M horizons are present in a single bag and possible to separate, pull them apart and transfer each to its own clean, labeled and barcoded plastic bag. If pressed for time, this step can be done in the lab, as long as it won't compromise your ability to separate the horizons.
5. Record all relevant metadata about the sample(s).

   a. For **samplingProtocolVersion**, make sure to choose this SOP (NEON.DOC.004130vF).
   b. Enter **standingWaterDepth** in centimeters to the nearest centimeter. Enter '0' if there was no standing water.
   c. For **incubationMethod**, choose 'buried bag.'
6. Transfer sample(s) to a cooler with ice packs and then to the lab for processing the day of or the day following collection. Complete all laboratory processing steps as outlined in RD[04].

8 REFERENCES

Binkley, D. and S. C. Hart. 1989. The components of nitrogen availability assessments in forest soils. Advances in soil science. Springer New York, 1989. 57-112.

U.S. Environmental Protection Agency. 2011. National Wetland Condition Assessment: Field Operations Manual. EPA-843-R-10-001. U.S. Environmental Protection Agency, Washington, DC.

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APPENDIX A QUICK REFERENCES

OFF-YEAR BOUTS IN WETLAND PLOTS

REMINDER: Use sterile techniques as much as possible.

STEP 1 – Navigate to a designated soil sampling plot, wearing waders or rubber boots as needed.

STEP 2 – Use plotID and X,Y coordinate lists to identify a suitable sampling location. If needed due to standing water presence, use the laser rangefinder in **HD** mode to locate X,Y locations.

STEP 3 – Use appropriate, sterilized coring equipment to collect a 30-cm deep soil core or monolith, accounting for standing water depth.

STEP 4 – Separate organic (O) and mineral (M) horizons if both are present and a clear distinction exists. Keep only the top horizons for off-year bouts.

STEP 5 – Bag, homogenize, subsample, and store samples as outlined in TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling. Subsampling depends upon site type and sample timing.

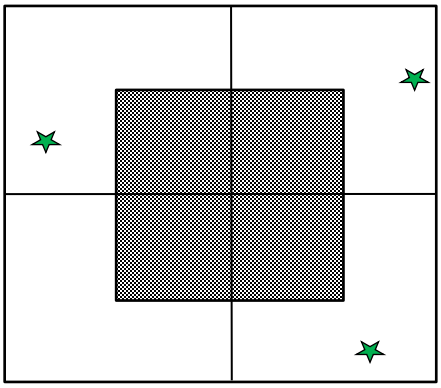

Figure 9. Schematic of a TOS soil sampling plot, with green star indication the three X,Y sampling locations.

COORDINATED BOUTS INCLUDING N TRANSFORMATIONS IN WETLAND PLOTS

STEP 1 – Collect an 'initial' soil core as above, but keep both O and M if present. Create all required microbial subsamples as needed.

STEP 2 – Collect an additional soil core from the same X,Y location and place into a 4 mm polyethylene sample bag. Remove all air and seal. Bag O and M separate if they will be difficult to separate later.

STEP 3 – Bury sample bag in the soil at that X,Y location and mark. Leave in place for the site-specific durations specified in RD[04].

STEP 4 – Return to that X,Y location and recover the sample. Keep chilled.

STEP 5 – Process and extract initial and incubated soil samples within 1 day of collection, as specified in TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling.

APPENDIX B REMINDERS

COLLECTING QUALITY SOIL SAMPLES

Pre-sampling: Be sure to…

- ☑ Charge mobile data recorders.
- ☑ Pre-label and organize sample bags and containers.
- ☑ Print soil coordinate lists, gridded plot maps, and back-up data sheets.
- ☑ Ensure samplers have a navigation device that will enable navigating to the plot.
- ☑ Obtain dry ice and ice packs plus appropriately sized coolers.
- ☑ Assemble all wetland-specific sampling equipment (coring device, wader/rubber boots, shoulder-length gloves, etc).

At soil sample location: Check…

- ☑ Are you at the correct potential X,Y location?
- ☑ Are there visual disturbances or impediments to sampling?
- ☑ Did you probe the area within 0.5 m of X,Y coordinates to find a suitable location?
- ☑ If a location was rejected, did you record why on the coordinate list?

Sampling: Remember to…

- ☑ Clean and sterilize durable equipment before use at every sample location.
- ☑ Wear clean gloves. Can clean between samples, but change between plots.
- ☑ Measure soil temperature and leaf litter depth at each sample location *if* not under water.
- ☑ Collect a core or monolith to 30 ± 1 cm, accounting for the standing water depth.
- ☑ Separate O and M horizons; process both for coordinated bouts, else only keep top horizon.
- ☑ Homogenize and drain water from samples prior to field subsampling.

Sample Handling: Be sure to…

- ☑ Label sample bags and double check labels against SLS: Field Sampling application.
- ☑ Store microbial samples (-gen, -ga, -comp) in cooler with dry ice, transfer to -80°C freezer in lab.
- ☑ Store all other sample types in cooler with ice packs, transfer to 4°C refrigerator in lab.

Cleanup: Remember to…

- ☑ Thoroughly clean and dry equipment using the proper cleaning method, especially the coring device (see RD[04], SOP D). Ensure all soil residue is removed.
- ☑ Replenish consumables so that sufficient materials are available for next bout

APPENDIX C EQUIPMENT

The following equipment is needed to implement the procedures in this document. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc. Refer to the Field Sampling equipment list tables in RD[04] for full supply lists needed for sampling based on bout type. Equipment lists are organized by task.

Table 2. Equipment list – Additional and alternate supplies for soil sampling in wetland or wetland-like plots, all bout types.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
	N	AMS Multi-Stage Sludge Sampler (2”) or similar coring device designed for wetland soil types ***alternate for standard soil core***	Collect cores from saturated, unconsolidated soils. Device has mechanism to prevent material from falling out the bottom of the coring device	1
	N	AMS 5/8” x 1-5’ SST Extension Pole. *If another AMS sampler is already in use by the Domain, check for compatibility with extension pole and handle*	Extend the AMS sludge sampler to a height comfortable for technician use	1
	N	AMS 18" Rubber Coated Cross Handle, 5/8" Threaded	Grip and use the AMS sludge sampler	1
	N	AMS 2” x 12” Plastic Liner	Needed for the AMS sludge sampler to function properly. Can be difficult to clean, helpful to have extras	6-10
	N	AMS Serrated MS Sludge Core Tip	Use with the AMS sludge sampler to core in flooded organic soils, peaty and/or with thick root mats. Reduces compaction	1
	N	AMS 2” MS Sludge Auger Tip w/ valve	Use with the AMS sludge sampler to core in flooded organic soils or peat. Reduces compaction	1
	N	‘Soil extruder’ – dowel, capped piece of tubing, etc. Similar diameter to soil corer	Push/extrude soil from coring device or liner	1
	N	Bottle brush, 2” diameter (or similar size as coring device)	Clean coring device and liners between samples	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Clippers	Cut away live sphagnum to reach the start of the O horizon, peatland and permafrost sites	
	N	Plastic tray - larval type with high walls works well	Separate horizons if both O and M present	1
	N	Serrated soil knife (Hori Hori), plastic handle	Separate horizons if both O and M present; Cut soil monoliths	1
Forestry Supplier 91567	Y	TruPulse 360R Laser Rangefinder, 0.3 m accuracy	Locate X, Y coordinates if a meter tape is difficult to use due to standing water	1
	N	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	1
Compass Tools, 703512 Forestry Supplier, 90998	Y	Foliage filter	Use with laser rangefinder in dense vegetation	1
	N	Waders	Keep dry when working in standing water	1 per technician
	N	Rubber Boots	Keep dry when working in standing water	1 per technician
	N	Long survey marking flags or stakes, to protrude above the water line	Mark locations and probe potential X, Y coordinates	4
	N	Meter stick	Measure the depth of standing water	
	N	Folding ruler with metric system gradations	Measure the depth of standing water	
Consumable Items				
	N	Laboratory tape	Mark target depth on the coring device	1 roll
	N	Shoulder-length disposable gloves	Keep arms dry when sampling in standing water	1 per technician
	N	Paper towels OR shop towels	Dry hands and equipment as needed	1 roll

Table 3. Equipment list – Additional and alternate supplies for conducting a N transformation incubation in wetland or wetland-like plots.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable Items				
	N	Sharpshooter planting spade or dibble bar	Bury incubated sample bag in soil with standing water or in unconsolidated substrates	1
	N	4’ Snow stake	Mark X, Y location of incubated sample bag in deep standing water	1 per sample
	N	Brightly colored paracord	Less intrusive option to mark X, Y location of incubated sample bag, if no standing water	18” per location
Consumable Items				
	N	Plastic bags, various sizes, must be 4 mm thickness ***replacement for incubation cylinders***	Contain incubated sample for N transformation measurement, prevent N leaching	1 per sample plus several extras
	N	Small resealable plastic bags, 2 x 2” or similar	Contain sample metadata inside sample bag for duration of incubation	1 per sample
	N	Shoulder-length disposable gloves	Keep arms dry when sampling in standing water	1 per technician
	N	Garbage bag	Line the cooler if needed, buried sample bags can be messy	2
	N	Paper towels OR shop towels	Dry hands and equipment as needed	1 roll

APPENDIX D SITE-SPECIFIC INFORMATION

D05 - TREE, UNDE, STEI	
Issue: In cedar and wooded sphagnum swamps, there may be an organic soil layer perched on top of dense tree root mats, with an air gap between this perched soil layer and the start of the 'true' soil surface below.	***Solution:*** If a perched organic soil layer is present at an X,Y location deemed suitable for sampling, collect it, then measure the thickness of the perched layer. If less than 30 cm, continue sampling from the true soil surface below, excluding the height of the air gap from the sample depth measurement. For example, if a 5 cm perched O-horizon is sampled, sample up to an additional 25 cm from below the true soil surface. Combine perched O horizon with any additional O-horizon material collected. Make a note in the **remarks** field that 'soil perched on root mats' was included in the sample. During coordinated bouts, material from above and below an air-gap can be combined into a single bag for N-transformation incubations. This bag should be completely buried in the true soil layer – the hole may be deepened or widened if needed.
D03 - DSNY	
Issue: The water table at DSNY can rise quickly and unpredictably. If covered cores are used because a plot is dry at the time of installation, there is a good chance they will become flooded during the incubation period.	***Solution:*** All N-transformation bouts at DSNY will use the buried bag method instead of covered cores for conducting incubations. This applies to all plots and bouts, regardless of the observed water level.