TOS STANDARD OPERATING PROCEDURE: WETLAND SOIL SAMPLING

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1 DESCRIPTION

1.1 Overview

The Standard Operating Procedure (SOP) described in this document is an extension of TOS Protocol and Procedure: 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 frequently at or just below the surface. Here, we define shallow standing water as $\leq$ 50 cm. If water depths exceed this level, soil sampling should not occur.

While plots with wetland or wetland-like conditions are not the focus of the NEON Terrestrial Observation System (TOS), they are present in certain domains and sites where high water tables are a common regional phenomenon. As such, it is desirable to include them in soil sampling.

It is anticipated 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 meeting the wetland criteria described above, 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 NEON's issue tracking software.

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, DEJU

Presence of shallow standing water or a high water table yields unique soil sampling challenges. First, collecting soil cores in a standardized fashion is more difficult than in well-drained uplands, and the process is facilitated with 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 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 soil core collection as well as deployment of soil incubations for N transformation measurements. 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 core collection and deployment of soil incubations for N transformation measurements in plots covered with shallow standing water or that have a water table at or just below 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 are comparable to 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.

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.

1.3 Applies To

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

Doc #	Title
NEON.DOC.014048	Soil Biogeochemical and Microbial Sampling

1.4 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	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.050005	Field Operations Job Instruction Training Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.014048	TOS Protocol and Procedure: 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.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

Wetland: An area where the water table is frequently at or near the surface, or 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.

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 (generally much less than half by weight). Often darker in color. Should not feel gritty or grainy – if it does, it is most likely a mineral horizon high in organic matter, such as an A-horizon, not an organic horizon.

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.

2.5 Safety

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

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

Sampling in wetlands should be undertaken with care. Saturated soil may be difficult to maneuver in, 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 in order to keep personnel dry. In some sites, flooded conditions may be accompanied by additional dangers from local wildlife (e.g., alligators, snakes). In such case, follow all Domain and Manager specific instructions and avoid entering flooded plots when dangers are present.

3 PERSONNEL AND EQUIPMENT

3.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low temperature freezers, etc.

The lists that follow should be considered supplements to the field sampling equipment lists in RD[04], meaning that only additional and/or alternate equipment needed specifically for sampling wetland plots is presented. Refer to Tables 5-7 in RD[04] for full supply lists needed for soil sampling based on bout type.

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, as well as polyethylene sample bags instead of incubation cylinders for conducting N transformation incubations. Regarding the former, a specialized coring device 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. For mineral soils, this will depend on the degree of substrate consolidation and whether or not the coring device allows for sample retrieval without significant amounts of material falling out the corer. For organic soils, this will depend on whether the coring device can cut through fibrous organic material without causing significant compaction. Table 2 provides suggested equipment for different conditions – if unsure of the most appropriate device to use for sampling wetland soils in their domain, Field Operations should contact NEON Science.

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

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
	R	AMS Multi-Stage Sludge Sampler (part # 403.31) or similar coring device ***~ alternate for standard soil corer ~***	Collect cores from saturated, unconsolidated soils. Keeps material from falling out the bottom of the coring device	Saturated, unconsolidated soils, standard coring device does not function well	1	N
	R	AMS 5/8" x 1-5' SST Extension Pole (part # 409.06-409.10)*	Extend the soil sampler to a height comfortable for technician use and above the water line	All	1	N
	R	AMS 18" Rubber Coated Cross Handle, 5/8" Threaded (part # 406.04)*	Grip and use the sampler	All	1	N
	S	AMS 2" x 12" Plastic Liner (part # 405.10)	Needed for the sludge coring device to function properly. Sometimes difficult to clean and re-use, helpful to have extras.	All	6-10	N
	S	AMS Serrated MS Sludge Core Tip (part # 403.14)	Assist coring in flooded organic soils, peat, or in thick root mats. Increases sample recovery while reducing compaction	Saturated soils with high organic content or thick root mats	1	N
	S	AMS 2" MS Sludge Auger Tip w/ valve (part # 403.29)	Assist coring in flooded organic soils or peat. Increases sample recovery while reducing compaction	Saturated soils with high organic content	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	'Soil extruder' – dowel, piece of tubing plus end cap, etc. Similar diameter to soil corer	Push/extrude soil from coring device or liner	All	1	N
	S	Bottle brush, 2" diameter (or similar size as coring device)	Clean coring device and liners between samples	All	1	N
MX103931	R	Plastic tray	Separate horizons if both O and M present	All	1	N
MX100721	R	Soil knife	Separate horizons if both O and M present	All	1	N
MX100322	R	Laser Rangefinder, 0.3 m accuracy	Locate x-y coordinates if a meter tape cannot be used due to standing water	Flooding along wester and/or southern borders	1	N
MX104359	R	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	Using laser rangefinder	1	N
MX103218	S	Foliage filter	Use with laser rangefinder in dense vegetation	Using laser rangefinder	1	N
MX107505 MX100494	R	Waders	Keep dry when working in standing water	> ankle-deep standing water	1 per technician	N
	R	Rubber Boots	Keep dry when working in standing water	≤ ankle deep standing water	1 per technician	N
	S	Long survey marking flags or stakes, tall enough to protrude above the water line	Mark locations and probe potential x-y coordinates	All	4	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Meter stick	Measure the depth of standing water	Standing water present		
Consumables						
	R	Laboratory tape	Mark target depth on the coring device	Standing water present	1 roll	N
	S	Paper towels	Dry hands and equipment as needed	All	1 roll	N
Resources						
RD[05]	R	Paper Datasheets	Back-up for recording data	All	1	N

R/S=Required/Suggested

*If another AMS sampler is already in use by the Domain, check for compatibility with extension pole and handle, it may not be necessary to purchase additional items

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

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
MX111250	R	Plastic bags, 4 mm **~replacement for incubation cylinders~**	Contain incubated sample for N transformation measurement, prevent N leaching	All	1 per sample	N
	R	Planting spade or dibble bar (as in Forestry Suppliers part # 77621 or 69042)	Bury incubated sample bag in soil with standing water or in unconsolidated substrates	No intact bore hole to bury bag	1	N
	S	Cleaning gloves, long cuff (as in Home Depot model # 24103-012)	Keep hands and arms dry while burying sample bags in standing water	Standing water	1 per technician	N
MX107049	S	4’ Snow stake	Mark x,y location of incubated sample bag	All	1 per sample	N
Consumable Items						
	R	Small resealable plastic bags, 2 x 2” or similar (as in Forestry Suppliers part #79108)	Contain sampleID label inside sample bag for duration of incubation	All	1 per sample	N
	S	Garbage bag	Line the cooler, buried sample bags will be wet and messy	All	2	N
Resources						
RD[05]	R	Paper Datasheets	Back-up for recording data	All	1	N

R/S=Required/Suggested

3.2 Training Requirements

All technicians must complete required safety and procedure-specific training for safety and implementation of this procedure as required in Field Operations Job Instruction Training Plan (AD[03]).

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 surrounding how to employ sterilization techniques when handling soil samples and cleaning equipment, is needed to successfully complete this SOP.

3.3 Specialized Skills

The ability to use an appropriate coring device to sample loose, unconsolidated 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) when provided proper equipment 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].

4 CONTINGENCIES AND NOTES

Similar to non-wetland soil bouts, all sampling for a given season (e.g., ‘peak greenness’, ‘dry-wet transition’) should occur within a two-week (14 calendar day) period. 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, it will be considered a partial bout and a ticket should be issued through NEON’s issue tracking software.

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, and an informational ticket should be issued through NEON’s issue tracking software.

5 STANDARD OPERATING PROCEDURES

SOP A PREPARATION FOR SAMPLING

1. Complete all steps listed in Section A.1 of RD[04], including:
   - Charge mobile devices
   - Pre-label and organize sample bags
   - Print soil coordinate lists and back-up data sheets
   - Load GPS coordinates for target plots
2. Additionally, gather all equipment needed for wetland-specific sampling, consulting both the equipment lists in Tables 1 and 2 as well as those in RD[04]. Ensure durable items (corer plus parts, waders/boots, etc) are clean and in good working condition. Ensure sufficient quantities of consumable items are available.

3. A preliminary trip to visit target plots and assess standing water depths prior to sampling will streamline field collection and help ensure that appropriate supplies are available. If this is not possible, field personnel should base their preparations on prior knowledge of the target plots as well as reports from other NEON technicians who have recently worked in them.
4. If it is possible that the soil team will encounter standing water along the borders of the plot, which will prevent accurate stretching and anchoring of a meter tape, they should be prepared to use the TruPulse laser rangefinder in **HD** (horizontal distance) mode to locate x,y coordinates. In preparation, review RD[06] 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, and when batteries run low. See RD[06] for details.
   - Calibrate TruPulse tilt-sensor (only necessary after severe drop-shock; see RD[06] for details).

SOP B FIELD SAMPLING FOR SOIL MICROBES AND BIOGEOCHEMICAL STOCKS

SOP B is designed to enable soil sampling when plots or subplots are covered with shallow standing water or have mucky, unconsolidated substrates due to a persistently 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 depths at potential sampling locations exceed 50 cm, do not attempt to collect soil. Attempt to sample at the next x,y coordinate on the coordinate list; if that fails, try another designated subplot. If flooding is uniformly > 50 cm across the entire plot, do not collect any samples and issue an informational ticket to Science through NEON's issue tracking software.

B.1 Identify the plot

1. Navigate to the southwest corner of the plot.
2. Lay out meter tapes on the western and southern borders of the plot as directed in RD[04].
3. If there is standing water along these borders and meter tapes cannot be used, prepare to use the laser rangefinder in **HD** mode to locate x,y coordinates.

B.2 Assess sample location

1. Locate the first potential x,y sampling location on the coordinate list. If there is standing water, use the TruPulse laser rangefinder in **HD** (horizontal distance) mode as follows:
    a. Two technicians must work together. One stands at the southwest corner of the plot (0,0) and operates the laser rangefinder in **HD** mode. The other navigates to the first potential x-location, following the directions of the rangefinder operator and using the reflective tape so that an accurate horizontal distance measurement can be obtained.
        1) 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.
    b. Place a pin flag, stake, or other marker of appropriate height at this first potential x-location.
    c. The rangefinder operator then moves to stand directly over the pin flag or marker. Using either a measuring tape or the TruPulse in **HD** mode with a reflective surface, they will work with the second technician to locate the y-coordinate location.
        1) Make sure that the angle (azimuth) is as close to 0˚ as possible (True North) when measuring the y-coordinate distance.

d. Place a clean pin flag, stake, or other marker at the potential x,y location

2. Assess the x,y location for sampling suitability, defined as a location where one or two cores is likely to produce sufficient soil material for all samples and subsamples.

    a. Look for obvious visual impediments – are there disturbances, vegetation, large rocks, or other features **within a 0.5 m radius** of the x,y location that will prohibit sampling?

    b. Locations covered in standing water will also need to be assessed for suitability by probing. Use a 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.

    c. If the location is deemed suitable, prepare to sample.

    d. If the location is deemed unsuitable, reject it. Record why on the coordinate list and move to the next potential x,y location. Repeat until a suitable location is found, attempting up to 3 x,y coordinates per subplot. Do not spend more than 1 hour trying to find a suitable location in a single subplot.

B.3 Collect soil core

1. Once an acceptable x,y location is found, prepare to sample it. Ensure that the coring device is properly assembled and has been cleaned with deionized water and sterilized, according to the sterilization instructions provided in RD[04].

TIPS & TRICKS

Note: *If using a coring device that comes with a plastic 'soil core catcher' (**Figure 1**), but local soils are fairly well consolidated, try to extract a core first without using the catcher, installing it only if needed.*

Figure 1 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):

    a. Measure soil temperature and litter depth, as described in RD[04], SOP B.

        1) *In Sphagnum-dominated soils*, the majority of the litter layer is likely comprised of decaying Sphagnum, which is difficult to measure. The best way to approximate is to measure the length of brown stem sections 2-4 cm below the growing tip (capitulum) of the Sphagnum and record this as litter depth (Bregazza et al. 2012). If other litter

(leaves, needles) has accumulated on top of the Sphagnum, and its depth can be reasonably measured, record and add to the Sphagnum litter for total litter depth.

b. Remove the litter layer in preparation to sample soil.

1) *In Sphagnum-dominated soils*, also remove any overlying sphagnum moss until the beginning of the O horizon is reached.

3. If there *is* standing water on top of that x,y location, do not measure temperature or litter depth but do determine the approximate depth of the standing water.

a. Insert a clean meter stick (or pin flag/stake) until it touches the surface of the soil.

) 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 any is present). Go slowly and make a reasonable estimate.

b. Read the standing water depth on the meter stick, or measure the flooded depth of the pin flag/stake using a meter tape.

c. Add this depth to 30 cm to get the total target sampling depth for the coring device.

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

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

4. Insert the coring device into the soil until it reaches 30 cm or bedrock. If there is standing water, insert the coring device until the tape mark is reached, ensuring it is held perpendicular to the water line. *Use a twisting motion to help avoid compaction*.

5. Slowly remove the coring device, watching to ensure that little material falls out the bottom. If a substantial amount of material is lost during removal (more than half), do not keep the soil and attempt to take another core from that same x,y location.

a. If the device has a core catcher, you may install it now, then try taking another core. The core catcher should help to retain the soil material.

b. 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 three x,y coordinates, not spending more than one hour attempting to sample a single subplot.

6. Once a viable soil core is collected, walk out of the plot or subplot, or to a dry spot in the 'microbes/bgc' destructive sampling zone. Try to follow the same path you used to enter in order to minimize disturbance.

B.4 Process soil core

1. The person who will handle the sample should put on a clean, sterilized pair of nitrile gloves (1 pair per x,y location, do not re-use gloves between coordinates).
2. Empty contents of corer or core liner onto a sterilized sample tray, using a sterilized extruder if needed.Refer to RD[04] for sterilization instructions.
3. Remove and discard any loose organic matter from the top of the core. This includes debris such as twigs, leaves, moss, other pieces of in-tact plant matter, insects, and animal detritus.
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 2**).

Figure 2. Example of a typical wetland soil core (Photo credit: US Environmental Protection Agency)

   a. If both O and M horizons are present and there is an obvious boundary between them, separate the horizons using a clean, sterilized soil knife. Retain either only the O horizon (for microbe-only bouts) or both the O and M horizons (for bouts that include biogeochemistry). If keeping both, process as separate samples.

   b. If there is no clear distinction between O and M horizons, or the O horizon is < 1 cm, process the core as one sample. Choose an O or M designation dependent upon whether the 30 cm core is composed of mostly mineral or mostly organic material.

      1) If unsure, take a small mass of soil in your gloved hand and assess the texture. If it feels grainy or gritty, it is likely a mineral soil with high organic matter content. If smooth and a bit greasy, it is probably an organic soil.

      2) If still uncertain: make your best guess, record uncertainty in **remarks** upon data entry, take a photo, and issue a ticket to Science.

5. Refer to Sections **B.4.4** and **B.4.7-9 of RD[04]** for detailed instructions on how to bag, homogenize, and subsample the soil sample(s). Remember that the types of subsamples needed depends on the bout type and sample timing - see Tables 16 and 17 in RD[04].

) If it is clear that insufficient material will be available to create all subsamples, or that not enough soil will be left over after doing so, extract a second core. Return to the x,y location (ideally following the same path to minimize disturbance) and sample within a 0.5 cm radius as described above. Combine material from both cores.

6. Follow instructions for how to stow (sub)samples, enter metadata, and clean and sterilize equipment as described in Sections **B.4.10-14** of RD[04].

7. When recording data:

    a. For **samplingProtocolVersion**, make sure to choose this SOP.

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

8. Move to the next designated subplot and repeat. Once three locations per plot have been sampled, move to the next plot and complete all procedures detailed above.

9. Follow instructions for sample preservation and transport as described in Section **B.5** of RD[04].

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

SOP C FIELD SAMPLING FOR N TRANSFORMATIONS

This SOP enables N transformation sampling when plots or subplots are covered with shallow standing water, or have a water table 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 specialized coring device is needed 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. It if 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).

This is because a high water table can cause nitrogen leaching from the soil sample when using the method outlined in RD[04], which compromises the integrity of N transformation estimates. Thus, 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. 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).

Box 1. Guidelines for N transformation field sampling in wetland or wetland-like plots

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) of the surface	Follow this SOP (buried bag method) to conduct N transformation sampling
SOP F of RD[04] (covered core method) has already been used in one or two subplots when it is revealed that an additional 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** in the data entry 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 F of RD[04] (covered core method) to conduct standard N transformation sampling once the plot has dried out
SOP F 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 (or the procedures in RD[04] if there is no standing water and soil is well-consolidated) to collect, bag, homogenize, subsample and stow soil samples to be used for 'initial' N-transformation measurements.
    a. If the substrate is very fibrous (such as *Sphagnum* peat organic soil) and there is little to no standing water, it may be helpful to cut around the sampling tube or coring device with a soil knife while gently working the corer into the ground. This will allow for extraction of a sample while avoiding compaction.
2. Ensure that both O and M horizons are collected, if present and possible to separate.
3. As described in SOP F of RD[04], N transformation processing and extraction must occur within 24 hours of sample collection.

C.2 Deployment of incubated soil cores

1. After collecting an initial soil core, collect another one from as close as possible to the first coring location, making sure to stay within 0.5 cm of the x,y coordinate.
2. Prepare a label for the second core by writing the **sampleID** on a piece of all-weather copy paper using pencil, then placing that piece of paper into a small size resealable plastic bag.
) Recall that **sampleID** is composed of plotID-horizon-coreCoordinateX-coreCoordinateY-collectDate. However, since **collectDate** of the incubated core is not known at the time of deployment, this part should be blank (example: ONAQ_001-M-8.5-21-).
3. Remove core from the coring device or liner and transfer it to a 4 mm polyethylene sample bag, disturbing the core structure as little as possible. Place the label bag inside the sample bag.

4. Close the sample bag and **make sure to remove all air**. This may require some 'squishing' of the core and that is acceptable - <u>it is more important to remove all air from the bag</u>.
5. 'Bury' the bag
    a. If the bore hole is visible (little or no standing water), place the bag back inside of it.
        1) Cover with a few centimeters of leaf litter, loose soil, and other detritus so that the bag will not be in direct sun.
        2) Site-host permitting, mark the location with a pin flag or stake. If not, mark the location with a GPS waypoint, labeling it with the sampleID (no collectDate). This will assist in recovering the sample at the end of the incubation period.
    b. If the bore hole is not visible or it collapses (standing water on the plot), use a planting spade or dibble bar to bury the bag.

1) Insert planting spade or dibble bar at ~ 45⌈ angle and pry open a slot in the soil/substrate.
2) Using long-cuffed gloves (only if desired/needed to keep hands dry), place the bag into the slot. Try to position it so that the entire bag is buried.
3) Remove the planting spade or dibble bar and allow the soil to collapse around the bag.
4) Pat down soil on top of the bag. This will help make sure it stays in place.
5) Site-host permitting, place a 4 ft stake or other appropriate long marker at the location of the buried sample. If not, mark the location with a GPS waypoint, labeling it with the sampleID (no collectDate). This will assist in recovering the sample at the end of the incubation period.

C.3 Collection of incubated soil cores

1. Navigate to the location of a buried bag.
2. Using long-cuffed gloves (only if desired/needed to keep hands 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, still collect but 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 and do not create a record in the data entry application or paper datasheet. Notify Science with an issue ticket.
3. Dry the bag enough to write on it with a permanent marker and write **collectDate.** This will help in completing the **sampleID** upon return to the domain lab.
4. If there is only one horizon type, or the O horizon is < 1 cm or impossible to separate, no further field processing is needed. If both O and M horizons are clearly present and possible to separate, follow the instruction above in Section B.4 to separate and bag horizons individually.
5. Record all relevant metadata about the sample(s).

   a. For **samplingProtocolVersion**, make sure to choose this SOP.
   b. Enter **standingWaterDepth** in centimeters to the nearest centimeter.
   c. For **incubationMethod**, choose 'buried bag.'
6. Transfer sample(s) to a cooler with ice packs and then to the lab for processing within 24 hours. Complete all laboratory processing steps as outlined in RD[04].

6 REFERENCES

1. 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.
2. Bragazza, L., A. Buttler, J. Habermacher, L. Brancaleoni, R. Gerdol, H. Fritze, P. Hanajík, R. Laiho and D. Johnson. 2012. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Glob Change Biol, 18: 1163–1172.
3. 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.
4. U.S. Environmental Protection Agency. 2016. National Wetland Condition Assessment. EPA-843-R-15-005. U.S. EPA, Washington, DC.

APPENDIX A QUICK REFERENCES

SOIL SAMPLING 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 plot boundaries are covered in standing water, use the laser rangefinder in **HD** mode to locate x,y locations.

STEP 3 – Use an appropriate, sterilized coring device to collect a 30 cm deep soil core, accounting for standing water depth.

STEP 4 – Separate organic (O) and mineral (M) horizons if both are present and a clear distinction exists.

STEP 5 – Bag, homogenize, subsample, and stow samples as outlined in TOS Protocol and Procedure: Soil Biogeochemical and Microbial Sampling, dependent upon bout type and sample timing.

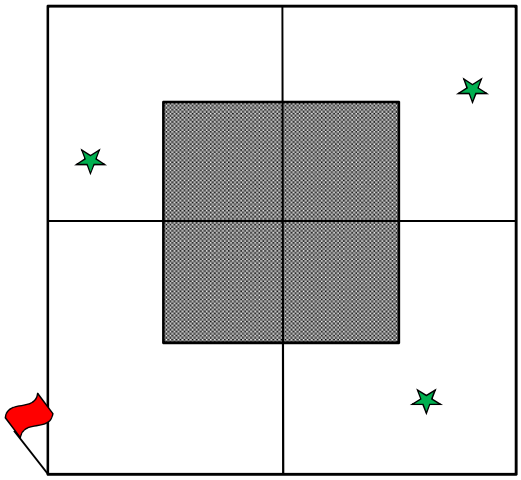

Figure 3 Schematic of a TOS 'bgc' soil sampling plot, with green stars indicating the three x,y sampling locations and red flag indicating the southwest corner (0,0).

N TRANSFORMATIONS IN WETLAND PLOTS

STEP 1 – Collect an 'initial' soil core and distribute required 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.

STEP 3 – Bury sample bag in the soil at that x,y location and mark. Leave in place for two to four weeks.

STEP 4 – Return to that x,y location and recover the core. Keep chilled.

STEP 5 – Process and extract initial and incubated soil samples within 24 hours, 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 devices.
- ☑ Pre-label and organize sample bags.
- ☑ Print soil coordinate lists and back-up data sheets.
- ☑ Load GPS coordinates for target plots and review job ticket.
- ☑ Obtain dry ice and cold soak coolers if needed.
- ☑ Assemble all wetland-specific sampling equipment (coring device, wader/rubber boots, 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?
- ☑ Did you record required metadata (plotID, collectDate, standingWaterDepth, etc)?

Coring: Remember to…

- ☑ Clean and sterilize durable equipment before use at every sample location.
- ☑ Wear clean gloves. Either change or clean gloves between samples.
- ☑ Measure soil temperature and leaf litter depth at each sample location if not under water.
- ☑ Core to 30 ± 1 cm, accounting for the standing water depth.
- ☑ Separate O and M horizons and process separately if both are present.
- ☑ Homogenize samples prior to field subsampling.

Sample Handling: Be sure to…

- ☑ Label sample bags and double check labels against datasheets.
- ☑ Store microbial molecular samples 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, especially coring device. Ensure all soil residue is removed.
- ☑ Replenish consumables so that sufficient materials are available for next bout

APPENDIX C 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.