TOS STANDARD OPERATING PROCEDURE: WETLAND SOIL SAMPLING

PREPARED BY	ORGANIZATION	DATE
Samantha Weintraub	SCI	12/07/2018

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	03/05/2019
Mike Stewart	PSE	03/05/2019

RELEASED BY	ORGANIZATION	RELEASE DATE
Anne Balsley	CM	03/05/2019

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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

TABLE OF CONTENTS

1 DESCRIPTION 1

1.1 Overview 1

1.2 Purpose 2

1.3 Applies To 2

1.4 Acknowledgments 2

2 RELATED DOCUMENTS AND ACRONYMS 3

2.1 Applicable Documents 3

2.2 Reference Documents 3

2.3 Acronyms 3

2.4 Definitions 3

2.5 Safety 4

3 PERSONNEL AND EQUIPMENT 5

3.1 Equipment 5

3.2 Training Requirements 11

3.3 Specialized Skills 11

4 CONTINGENCIES AND NOTES 11

5 STANDARD OPERATING PROCEDURES 12

SOP A PREPARATION FOR SAMPLING 12

SOP B SOIL CORE COLLECTION IN WETLAND PLOTS 14

SOP C FIELD SAMPLING FOR N TRANSFORMATIONS IN WETLAND PLOTS 21

6 REFERENCES 25

APPENDIX A QUICK REFERENCES 26

APPENDIX B REMINDERS 27

APPENDIX C SITE-SPECIFIC INFORMATION 28

LIST OF TABLES AND FIGURES

Table 1 List of sites that will use this SOP to sample in wetlands or wetland-like conditions. 1
Table 2. Equipment list – Additional and alternate supplies for soil sampling in wetland or wetland-like plots, all bout types..... 6
Table 3. Equipment list – Additional and alternate supplies for conducting an N transformation incubation in wetland or wetland-like plots. 9

Figure 1 Components of the Multi-stage Sludge Sampler..... 5
Figure 2 Different size bags to use for N-transformation incubations. Small bag is for the sample ID. 12
Figure 3 Example soil core catcher that comes with the AMS Multi-Stage Sampler 15
Figure 4 Measuring standing water depth at a flooded sample location..... 16
Figure 5 Technician preparing to collect an O horizon monolith in Alaska, using shoulder-length gloves and a hori hori. 17
Figure 6. 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. 18
Figure 7 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). 26

1 DESCRIPTION

1.1 Overview

This Standard Operating Procedure (SOP) 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 at or just below 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 soil core collection (SOP B) and field sampling for nitrogen transformations (SOP C). However, there are several steps where technicians are explicitly instructed to use RD[04] while completing these SOPs.

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 or summer monsoon rains, 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.

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 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 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

* *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 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 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.

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 context of conducting sampling for 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.001025	TOS Protocol and Procedure: 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

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.

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). In general, decomposing plant material is poorly recognizable, except in high-latitude, high-

altitude, or **wetland** sites where decomposition is very slow. Layer should be darker in color and friable (easily crumbled), and is sometimes greasy. If you feel more than a couple of mineral grains (grit from sand, stickiness from clay) it is most likely a mineral horizon high in organic matter (OM), not an organic soil.

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.

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 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 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 cases, 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 required specifically for sampling wetland soils 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 4mm 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 found in wetlands in Domains 3 and 9 where the substrate is very unconsolidated and significant amounts of material fall out the bottom when attempting to core. For thick organic wetland soils like those found in Domains 5, 18, and 19, 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. 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 Operations should contact Science through NEON's issue tracking platform.

Figure 1 Components of the Multi-stage Sludge Sampler

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

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items							
AMS, Inc.	403.31	S	AMS Multi-Stage Sludge Sampler (2”) or similar coring device ***~ alternate for standard soil corer ~***	Collect cores from saturated, unconsolidated soils. Keeps mineral material from falling out the bottom of the coring device	Saturated, unconsolidated mineral soils, standard coring device does not function well	1	N
AMS, Inc.	409.06-409.10	S	AMS 5/8” x 1-5’ SST Extension Pole *	Extend the soil sampler to a height comfortable for technician use and above the water line	With multi-stage sludge sampler	1	N
AMS, Inc.	406.04	S	AMS 18" Rubber Coated Cross Handle, 5/8" Threaded*	Grip and use the sampler	With multi-stage sludge sampler	1	N
AMS, Inc.	405.10	S	AMS 2” x 12” Plastic Liner	Needed for the sludge coring device to function properly. Can be difficult to clean and re-use, helpful to have extras.	With multi-stage sludge sampler	6-10	N
AMS, Inc.	403.14	S	AMS Serrated MS Sludge Core Tip	Core in flooded organic soils, peaty and/or with thick root mats. Increases sample recovery while reducing compaction	Saturated soils with high organic content or thick root mats	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
AMS, Inc.	403.29	S	AMS 2" MS Sludge Auger Tip w/ valve	Core flooded organic soils or peat. Increases sample recovery while reducing compaction	Saturated soils with high organic content	1	N
		S	'Soil extruder' – dowel, capped piece of tubing, 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
Fisher Bioquip	1523911 1426B	R	Plastic tray - larval type with high walls works well	Separate horizons if both O and M present	All	1	N
MLTools	P8246	S	Serrated soil knife (Hori Hori), plastic handle	Separate horizons if both O and M present; Cut soil monoliths	All; Flooded plots	1	N
		R	Hand clippers	Remove overlying live vegetation to reach the top of the O horizon prior to sampling	Peatland and permafrost soils (Domains 5, 18, 19)	1	N
Forestry Supplier	91567	R	Laser Rangefinder, 0.3 m accuracy	Locate x-y coordinates if a meter tape cannot be used due to standing water	Flooding along western and/or southern borders	1	N
Grainger	5B317	R	White reflector or reflective tape	Reflective target for laser rangefinder, aids in measuring distance to target accurately	Using laser rangefinder	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Compass Tools; Forestry Supplier	703512 90998	S	Foliage filter	Use with laser rangefinder in dense vegetation	Using laser rangefinder	1	N
Cabela's	IK832337 (select size)	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, to protrude above the water line	Mark locations and probe potential X,Y coordinates	All	4	N
		S	Meter stick	Measure the depth of standing water	Standing water present		
Grainger	30PC15	S	Folding ruler	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
Grainger Neogen	8AKM7 043-TA843 698688	S	Shoulder-length disposable gloves	Keep arms dry when sampling in standing water	Standing water present	1 per technician	N
		S	Paper towels	Dry hands and equipment as needed	All	1 roll	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.

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items							
Fisher Grainger Grainger	6255-0613 5CNK5 8YAT5	R	Plastic bags, various sizes, 4 mm thickness **~replacement for incubation cylinders~**	Contain incubated sample for N transformation measurement, prevent N leaching	All	1 per sample	N
Forestry Suppliers	77621 or 69042	R	Sharpshooter planting spade or dibble bar	Bury incubated sample bag in soil with standing water or in unconsolidated substrates	No intact bore hole to bury bag	1	N
Grainger Neogen	8AKM7 043-TA843 698688	S	Shoulder-length disposable gloves	Keep arms dry when sampling in standing water	Standing water present	1 per technician	N
Amazon	Various	S	4’ Snow stake	Mark X,Y location of incubated sample bag	All	1 per sample	N
Consumable Items							
Forestry Supplier	79108	S	Small resealable plastic bags, 2 x 2” or similar	Contain sampleID label inside sample bag for duration of incubation	All	1 per sample	N
		S	Brightly colored paracord	Less intrusive option to mark X,Y location of incubated sample bag	No standing water but high water table	18” per location	N

Supplier	Supplier Number	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
		S	Garbage bag	Line the cooler, buried sample bags will be wet and messy	All	2	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 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 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].

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, the issue should be reported 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. If the issue persists, report the problem through NEON’s issue tracking software.

5 STANDARD OPERATING PROCEDURES

SOP A PREPARATION FOR SAMPLING

1. Complete all steps listed in SOP A of RD[04], including:
    - Charge mobile data recorders
    - Pre-label and organize sample bags, including the addition of adhesive barcode labels
    - Print possible sampling locations from 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 2 and 3 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. *When preparing for an N-transformations bout*:
    a. Sites where prior experience shows that intact soil cores can be taken should plan to use 30 cm long rectangular polyethylene bags (Table 3, Figure 2 right). These will allow intact cores to keep their shape and structure while incubating and are thus preferred.
    b. Sites where prior experience shows that 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).

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

4. A preliminary trip to visit target plots and assess standing water depths prior to sampling may streamline field collection and help ensure that appropriate supplies are available. Such a visit will help determine whether waders, shoulder-length gloves, the TruPulse (see below), or a wetland coring device will be needed for the bout. If such a trip 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.

5. If the soil team is likely to encounter standing water along the borders of the plot, which would prevent accurate stretching and anchoring of meter tapes, they should be prepared to use the TruPulse laser rangefinder in **HD** (horizontal distance) mode to locate X,Y coordinates. 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).

SOP B SOIL CORE COLLECTION IN WETLAND PLOTS

This SOP is designed to enable soil sampling when plots or subplots are covered with shallow standing water or have 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 X,Y sampling locations exceed 50 cm, do not attempt to collect soil. Instead, move to the next X,Y coordinate on the coordinate list; if that location also has standing water > 50 cm, try another designated subplot. If flooding is uniformly > 50 cm across the entire plot, do not collect any samples, record the issue in the Site Management data entry application, and report the issue through NEON's issue tracking platform.

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], Section B.1.
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 Locate and 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.
        2) Anything metal worn by the rangefinder operator will compromise azimuth measurements (glasses with metal arms, watches, rings, etc) and should be removed.
    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, live or dead woody 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 five 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 or hori hori (if planning to collect a monolith) 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 3**), but local soils are fairly well consolidated, try to extract a core first without using the catcher, installing it only if needed.*

Figure 3 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], Section B.3.

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

1) *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 E for more detail on how to identify the beginning of the O horizon in these soil types.

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 ruler, 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 ruler or meter stick (**Figure 4**), or measure the flooded depth of the pin flag/stake using a meter tape.

Figure 4 Measuring standing water depth at a flooded sample location

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 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 5), 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.

Figure 5 Technician preparing to collect an O horizon monolith in Alaska, using shoulder-length gloves and a hori hori.

4. 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. For monoliths, use the hori hori to cut out a block of soil to the target depth.
    a. For sites where there are thick root mats (example: cattails in Domain 09, **Figure 4**), push aside and/or cut through roots before beginning to core.
5. Slowly remove the core or monolith, watching to ensure that little material falls out the bottom. *With a gloved hand, you can '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*. However, if a substantial amount of material is lost during removal, do not keep sample and attempt to take another from that same X,Y location. If it is unclear, keep the core but assess how much soil material was recovered (section B.4) and determine whether it's an acceptable sample.
6. 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, not spending more than one hour attempting to sample a single subplot.

7. Once a viable soil core or monolith is collected, mark the X,Y location with a long flag or stake, then 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 sample

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). Refer to RD[04] for glove sterilization instructions.
2. Empty contents of corer or core liner, or place monolith, onto a sterilized sample tray, using a sterilized extruder if needed. Refer to RD[04] for sterilization instructions.
    a. If only a small amount of soil is recovered from a 30-cm core, 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 plant matter, insects, and animal detritus.
    a. For peatland and permafrost sites, refer to RD[04], Appendix E, 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 6).

Figure 6. 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. Retain either only the O horizon (for

'microbes' bouts) or both the O and M horizons (for coordinated bouts, 'microbesBiomass' or 'microbesBiomassBGC'). If keeping both, process as separate samples.

b. If there is no clear distinction between O and M horizons, or the O horizon is ≤ 1cm, 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 an M horizon with high organic matter content. If smooth, friable, and a bit greasy, it is probably an O horizon.

2) If still uncertain: make your best guess, record the soil characteristics in **remarks** upon data entry, take a photo, and seek Science input via NEON's issue tracking platform.

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 types of subsamples needed depends on bout type and sample timing - see Tables 1, 17 and 18 in RD[04].

a. 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.

1) 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.

2) 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.*

b. If it becomes apparent that insufficient material will be available to create all subsamples, or that not enough homogenized soil will be left over after doing so (25-75 g for O horizon, 50-150 g for M horizons, depending on boutType), extract a second core. Return to the X,Y location (ideally following the same path to minimize disturbance) and sample within a 0.5m radius as described above. Combine material from both cores/monoliths.

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

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

b. Enter **standingWaterDepth** in centimeters, to the nearest centimeter. Enter 0 if there was no standing water.

7. Remove flags or stakes, move to the next designated subplot, and repeat. Once all three subplots per plot have been sampled, move to the next plot and complete all procedures detailed above.
8. Follow instructions for sample preservation and transport as described in SOP B of RD[04].
9. Complete all laboratory processing steps as outlined in the SOPs contained in RD[04].

SOP C FIELD SAMPLING FOR 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. 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 covered core method, and this compromises the integrity of N transformation estimates. 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. 4 mm 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 by bout combination.

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 for the entire plot
SOP F of RD[04] (covered core method) has already been used in one or two subplots when it is revealed 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 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 to collect, bag, homogenize, subsample and store soil samples to be used for 'initial' N-transformation measurements.
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 sample, collect another one from as close as possible to the first coring location, making sure to stay within 0.5 m of the X,Y coordinate.
2.  Remove core from the coring device or extract monolith and transfer it to a 4 mm polyethylene sample bag, disturbing the core/monolith structure as little as possible. Use an appropriate shape sample bag as described in SOP A.
3. Prepare a label for the incubated sample by either writing the pieces of information below on a piece of all-weather copy paper using pencil, then placing that piece of paper into a small size re-sealable plastic bag, or writing them on a pin-flag or flagging tape that will be left at the sample location.
   - Record **plotID**, **coreCoordinateX**, **coreCoordinateY**, 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.
4. If using, place the label bag inside the sample bag.
5. 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.
6. Make sure the bag is completely sealed. If soil interferes with closure of the bag, add a second, clean bag.
7. '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.
      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, place a few pin flags or stakes surrounding the location where the bag is buried.

3) 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."

4) If no marker can be left at all, record 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 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.

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

2) 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.

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 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 sampleID (no collectDate). 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 E 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 data entry application or paper datasheet, but note 'destroyed' for **sampleFate** 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 remarks, and contact NEON Science for input.
3. If there is only one horizon type, or the O horizon is ≤ 1 cm or impossible to separate, transfer the entire incubated sample into a new, prelabeled 1-gallon bag. 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.

4. Record all relevant metadata about the sample(s).

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

   b. Enter **standingWaterDepth** in centimeters to the nearest centimeter. Enter 0 if there was no standing water.

   c. For **incubationMethod**, choose 'buried bag.'

5. 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 SOP G of 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. 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.
3. 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 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.

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

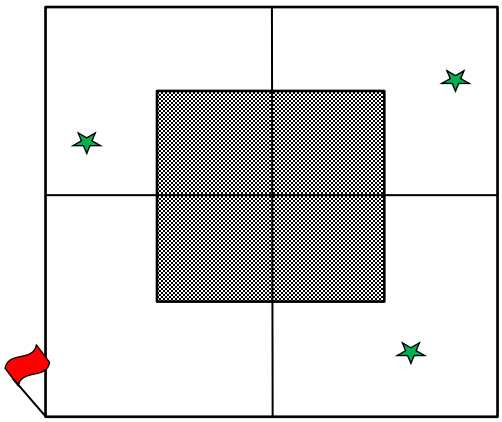

Figure 7 Schematic of a TOS 'bgc' soil sampling plot, with green stars indicating the three X,Y sampling locations and red flag indicating the southwest corner (0,0).

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 the site-specific durations specified in RD[04].

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, SOP G.

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 and back-up data sheets.
- ☑ Load GPS coordinates for target plots.
- ☑ Obtain dry ice and cold soak coolers if needed.
- ☑ 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. Either change or clean gloves between samples.
- ☑ 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 and process separately if both are present.
- ☑ Homogenize and drain water from samples prior to field subsampling.

Sample Handling: Be sure to…

- ☑ Label sample bags and double check labels against datasheets or data entry 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, 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.