



<i>Title:</i> NEON Procedure and Protocol: Producing TIS Soil Archive Subsamples for Users		<i>Date:</i> 04/12/2022
<i>NEON Doc. #:</i> NEON.DOC.001306	<i>Author:</i> E. Ayres	<i>Revision:</i> D

NEON PROCEDURE AND PROTOCOL: PRODUCING TIS SOIL ARCHIVE SUBSAMPLES FOR USERS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	6/23/2014	ECO-01356	Initial release
B	8/27/2015	ECO-03070	Added instructions for using the finely ground soil for requests of <1g. Other minor updates.
C	09/25/2017	ECO-04951	Changed ethanol concentration from 95% to 70%.
D	04/12/2022	ECO-06798	<ul style="list-style-type: none">Revised logo



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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change controlled version of Observatory protocols for creating soil subsamples for users of the TIS Soil Archive and serve as the version used for external review by subject-matter experts. This document provides the content for training for NEON staff and contractors. Content changes (i.e., changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

1.2 Scope

This document relates to tasks for producing soil subsamples for users of the TIS Soil Archive, as well as directly associated activities and safety practices. This document assumes that a request for samples from the TIS Soil Archive has been received and approved by NEON. This document assumes that users will be sent subsamples from the TIS Soil Archive and that this material will not be returned to the Archive. This document does not describe:

- General safety practices
- Site-specific safety practices
- General maintenance



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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD [01]	NEON.DOC.001307	TIS Soil Pit Sampling Protocol
AD [02]	NEON.DOC.000325	Field and Lab Procedure and Protocol: TIS Soil Archiving

2.2 Reference Documents

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

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.004300	EHS Safety Policy and Program Manual

2.3 External References

External references contain information pertinent to this document, but are not NEON configuration-controlled. Examples include manuals, brochures, technical notes, and external websites.

ER [01]	
ER [02]	
ER [03]	

2.4 Acronyms

Acronym	Explanation
NEON	National Ecological Observatory Network
P&P	Procedure and Protocol
TBD	To be determined
TIS	NEON Terrestrial Instrument System
USDA APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service



3 BACKGROUND AND OBJECTIVES

3.1 Background

Archived soil samples are necessary to serve as a reference of current soil physical and chemical conditions, and for use with future analytical techniques that are yet to be derived or invented (Boone et al. 1999). Previous scientific studies using archived soils have produced a wide range of novel findings, including:

- Assessing changes in lead pollution following the introduction of unleaded gasoline (Friedland et al. 1992);
- Refining estimates of organic matter turnover using ¹⁴C bomb carbon (Trumbore 1993);
- Measuring the accumulation of atmospheric inputs such as sulfur (Lapenis et al. 2004) or pesticides, including DDT, in soils throughout the 20th century (Meijer et al. 2001).

In addition, archived soil samples and associated measurement data can be used to calibrate new techniques/instruments/models to data collected with earlier methods or technologies.

The Terrestrial Instrument System (TIS) Soil Archive consists of air-dried soil collected and stored by soil horizon from a single soil pit at each NEON site. Samples in the TIS Soil Archive will be stored for NEON's 30-year Operational window. The archive is a resource for the scientific community and researchers are expected to request material from the archive for analysis.

3.2 Science Requirements

This protocol fulfills the following Observatory science requirements: NEON.TIS.4.1009

3.3 Data Products

There are currently no data products (DPs) associated with TIS soil archive samples because no measurements are currently planned to be made on these samples by NEON. The archived samples are a resource that NEON shall provide to the community and could provide significant scientific information in the future. There are however, chemical and physical analyzes performed on samples collected in the same location, soil horizon, and time, which are associated with L1 DPs.



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4 PROTOCOL

This document describes how TIS Soil Archive samples are processed to produce a subsample for an Archive User. A description of how soil samples for the TIS Soil Archive are collected in the field is given in AD[01], and how they are processed to get into the archive is described in AD[02].

Archive users often request more material than they actually need to guard against spillage or other accidents. As a result some researchers have suggested sending the entire archive sample to the user so that they can take the exact quantity required and return the remaining material to the archive after their analyses are complete (Boone et al. 1999). However, sending out the entire sample (or a large fraction of it) risks the loss or contamination of a large amount of the archived material (e.g., lost or damaged in transit). In addition, it is difficult to ensure the sample storage requirements are met once the sample has left the archive. Because of these concerns, when a user requests material from the TIS Soil Archive, the archive samples will be subsampled and only the subsample will be sent to the user. Any unused material that may be returned to the TIS Soil Archive by the user will NOT be re-combined with the archived sample due to concerns that it may have become contaminated or otherwise altered after it was removed from the archive. Briefly, the process for creating a soil subsample for a TIS Soil Archive user will involve mixing the sample to ensure it is homogeneous, weighing out the quantity required by the user, and returning the remaining soil to its storage container. The subsample will then be shipped to the user.

Soil from several NEON sites, including those from Hawaii, Puerto Rico, and several sites in the southeast US where fire ants are present, must be quarantined according to USDA APHIS regulations. Furthermore, soils samples must be transported following USDA packing standards. To simplify this protocol and minimize the risk of accidental release of quarantined soil, NEON shall treat soil from all sites as quarantined and all parts of this protocol shall comply with NEON’s USDA APHIS soil permit (see permit for details). This includes sterilizing or destroying, using an approved method, anything that came into contact with the soil. Refer to NEON’s USDA APHIS permit for instructions to sterilize soil and material that came into contact with soil prior to disposal.

4.1 Protocol Assumptions

For the purpose of this document, we assume that the TIS Soil Archive will be housed at NEON headquarters. We assume that a request for material from the soil archive was submitted to NEON, was approved, and that NEON has received approval from USDA APHIS to send the material requested to the user.

Soils from several NEON sites are quarantined by USDA APHIS. As a result, we assume all soil samples for the TIS Soil Archive shall be treated as quarantined soil to simplify the processing procedures and minimize the risk of accidental release.



5 QUALITY ASSURANCE AND QUALITY CONTROL

The weight of soil in a TISoil Archive sample shall be compared to the weight of material sent to the user and the weight returned to the archive to ensure that all material has been accounted for.

6 DECISION TREE

Table 1. Decision tree associated with producing soil subsamples for TIS Soil Archive users indicating how to respond to a delay in processing the samples.

Delay	Action	Adverse outcome	Outcome for TIS Soil Archive
>2 hours	Return all sample material to its storage container and return the container to the archive.	Slightly elevated risk of sample spillage due to repeated transfer.	None (assuming no material is lost)



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7 SAFETY

Personnel working at a NEON site should be familiar with and practice safe field and lab work as outlined in the EHS Safety Policy and Program Manual. Additional safety issues associated with this field procedure are outlined below. All employees have the responsibility and right to stop their work in unsafe conditions.

Laboratory safety training is required prior to operating laboratory equipment.



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8 PERSONNEL REQUIREMENTS

Demonstrated ability to follow standard operating procedures in a laboratory setting. Experience using standard laboratory equipment (e.g., balances, drying ovens). Experience working with environmental samples (e.g., soil, plant, or water samples) is desirable. Experience working with quarantined samples is desirable.



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9 TRAINING REQUIREMENTS

The training plan for this procedure is to read this document, read NEON’s USDA APHIS soil permit, discuss the procedure with FIU personnel, and contact FIU personnel when issues or concerns arise. It is essential that any personnel undertaking this procedure are familiar with the current version of NEON’s USDA APHIS soil permit, as this permit dictates how to transport soil samples, and how quarantined soil, and material that has come into contact with quarantined soil, can be safely disposed of or sterilized. Any personnel undertaking this procedure for the first time shall be supervised by personnel that are familiar with the procedure. Note that the USDA APHIS soil permit can be updated at any time, therefore, close communication between NEON Permitting and personnel performing this protocol is required.



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10 FIELD STANDARD OPERATING PROCEDURE

The procedure for collecting samples for the TISoil Archive in the field is described in AD[01].



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11 LAB STANDARD OPERATING PROCEDURE

The procedure shall always fully comply with all restrictions outlined in NEON’s USDA APHIS soil permit. If a discrepancy is identified between the procedure outlined in this document (or other NEON documents) and the USDA APHIS soil permit, the permit has precedence. If this occurs, contact the FIU Manager.

The laboratory procedure has the following goals: 1) thoroughly mix the sample to ensure that the sample is homogeneous; 2) weigh the quantity of material requested into the container that will be sent to the user; 3) return the remaining material to the TIS Soil Archive container and return it to the archive; 4) record and enter all data and metadata associated with each sample; and 5) ship the subsample to the user.

Under no circumstances should wax or wax strippers (NH₄, ammonia) be used on the floor or walls of the soil archive room of for the life of the soil archive. This requirement must be communicated to building managers, FCC, cleaning staff, etc. Use of these materials can contaminate the entire archive.

11.1 Timing

- NEON aims to send approved sample requests to users within 2 months of the request. Since the request first has to be approved by NEON, and then USDA APHIS has to provide approval to NEON to ship material to the user, we assume only 1 month will be available to produce the subsamples for the user. Therefore, processing the samples should begin shortly after a request is fully approved and should be completed within 1 month of being fully approved.
- Additional time may be required if special sample containers are needed (e.g., if they must be made of a certain material).
- Metadata relating to any step in the processing of samples for the TIS Soil Archive shall be entered within 1 business day of completion of that step to minimize mistakes in the metadata (e.g., forgetting how a particular sample was processed).

11.2 Lab Procedure

11.2.1 Equipment and Materials

Table 2. Materials and supplies required for the procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Laboratory gloves (e.g., latex or nitrile)	2/sample	No
Printable labels	1/sample	No
Calibrated mass balance (≤0.1 g accuracy; ≥2 kg capacity)	1	No
Set of calibration standards for mass balance	1	No
Sealable containers for subsamples	1/sample	No
Metal spatulas or spoons	4	No

Data sheets	1 set	No
Pencil	1/technician	No
Ethanol (70%)	0.5 liters	Yes
Paper towels	~4	No
Lint-free/low-lint tissues or wipes	~4	No
Leak-proof shipping container	As needed	No

11.2.2 Preparation

Ensure that you are familiar with the current version of NEON’s USDA APHIS soil permit. Ensure that all storage, processing, and cleaning locations comply with NEON’s USDA APHIS permit at all times (see permit for details).

Ensure that the processing area is clean and that there is sufficient space prior to beginning sample processing. This includes making sure that the floor has been swept so that if a sample is spilt, it can be recovered with minimal contamination (see Appendix C).

11.2.3 Sample Processing in the Lab

Always wear gloves when handling soil for the TISoil Archive. Gloves can be removed and destroyed using an appropriate method as defined in NEON’s USDA APHIS soil permit, whereas it is much harder to appropriately dispose of quarantined soil on your hands.

To minimize contamination, all materials that come into contact with the soil samples (e.g., trays for drying, spoon/spatulas, and storage containers) shall be clean and un-corroded. Materials made of glass and corrosion resistant metal, not plastic, shall be used to process and store samples whenever possible. Glass and metal are recommended since they are unlikely to contaminate the soil samples or compromise any future analyses that may be performed on the samples. To avoid cross-contamination among soil samples, all materials shall be cleaned prior to processing the next sample. If soap is used for cleaning it shall be phosphate-free (e.g., Alconox). Quaternary ammonia must NOT be used for sterilizing soil or equipment used for the TISoil Archive to minimize the risk of contaminating the archive samples. Immediately prior to use, all materials shall be wiped with 70% ethanol and placed in a clean location prior to use.

The TISoil Archive contains at least 4 jars of material for each soil horizon at a NEON site. These jars have the same label, except the last number which is 1, 2, 3, or 4 (or higher if more jars were required). Requests for soil shall always be fulfilled with material from the first (“1”) jar until that jar is exhausted, at which point the second (“2”) jar shall be used until that jar is exhausted, and so on.

1. If the request is for ≥ 1 g soil per sample the subsample shall be generated from the regular archived sample. If the request is for < 1 g soil per sample the subsample shall be generated from the finely ground archived samples, since it is difficult to produce a representative homogenous



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subsample of <1 g unless the soil has been finely ground. See AD[02] for a description of how the finely ground samples are produced.

2. Prepare printed label for each subsample. The subsamples sent to TISoil Archive users shall match the label of the corresponding soil archive sample label, but with the request number appended to the end. For examples, if the archive sample is:

2_Blandy_Pit1_14-23_Archive_Ap2_1

then the subsample for the user shall be labeled:

2_Blandy_Pit1_14-23_Archive_Ap2_1_XXXXX

where XXXXX is a 5-digit number matching the request number.

- a. Use the same approach for labeling subsamples from the finely ground sample. For example, if the archive sample is:

3_Ordway_Pit1_28-125_TISoil Archive_Bw2_1_Powder1

then the subsample for the user shall be labeled:

3_Ordway_Pit1_28-125_TISoil Archive_Bw2_1_Powder1_XXXXX

where XXXXX is a 5-digit number matching the request number.

3. Ensure the balance is level and was calibrated within the last 12 months.
4. Put on gloves. Weigh and record the weight in grams of the TISoil Archive sample including the jar lid and compare the weight to the last recorded weight for that sample. If the weight differs by >1 g do not proceed with that sample and contact the FIU Manager, otherwise proceed to the next step.
5. Tip the sealed jar containing the sample onto its side and rotate the sample 4 times around its long axis to homogenize the material in the jar. If it appears that the sample is still not homogenous this step can be repeated until the sample appears homogeneous.
6. Label the container that will be sent to the user and record its weight in grams, including container lid.
7. Remove the container lid and tare the balance. Then weigh the requested weight of soil into the container and record the weight in grams.
8. Return all remaining soil to the TISoil Archive jar. Weigh the jar (including lid) and record the weight in grams.
9. Ensure that all soil is accounted for using the following equation:



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$$x = A - B - D$$

Where

A is the initial weight of the TIS Soil Archive jar (*Step 4*)

B is the final weight of the TIS Soil Archive jar (*Step 8*)

D is the weight of soil added to the container (*Step 7*)

x is the weight of soil that is unaccounted for

If *x* is ≤ 0.15 g and ≥ -0.15 g we assume all soil was accounted for, with the discrepancy due to loss of trace amounts during processing and uncertainty introduced by the balance. If *x* is outside this range check all the weights to determine the mistake. If the discrepancy cannot be resolved contact the FIU Manager.

- Once all the subsamples for a TIS Soil Archive user have been prepared they shall be sent to the user via a method that complies with the current USDA APHIS permit held by NEON. At present this specifies that each sample shall be placed in a leak-proof container, which will be placed within a second leak-proof container for shipping.

11.2.4 Sample Preservation

Samples shall be stored in the TIS Soil Archive when not in use. Samples shall always be returned to their allocated location in the archive (e.g., shelf 4, cabinet 2). This is important to ensure that samples can be found quickly and easily whenever requested.

Samples for a user shall be stored in the TIS Soil Archive until they are ready to be sent to the user. Samples shall never be stored on the floor to minimize the risk of loss due to flooding. To minimize degradation of soil samples due to light exposure lights shall be switched off when the archive is not in use.

11.2.5 Sample Shipping

The shipping procedure shall comply with NEON's USDA APHIS Permit to Receive Soil.

11.2.6 Data Handling

It is preferable that all data and metadata recorded during this procedure be entered directly into electronic form (rather than a data sheet, which must then be transcribed to electronic form), as this will eliminate one source of errors.

If physical datasheets are used, all data and metadata shall be entered in electronic form to the appropriate database (TBD) within 1 day. The original data sheet shall be archived within the NEON soil archive.



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11.2.6.1 Metadata

Metadata for this protocol shall include the date that the user’s subsample was produced, the name of the technician, as well as any relevant notes.

11.2.7 Refreshing the Laboratory Supplies

Order new supplies as user requests are received.

11.2.8 Laboratory Maintenance, Cleaning, and Storage

Prior to beginning this procedure and at the end of every day when samples are processed, all work surfaces shall be cleaned with 70% ethanol and the floor shall be swept clean

All materials shall be placed in labeled locations (i.e., draws, cupboards, racks) when not in use.

All waste that came into contact with soil shall be disposed of in compliance with NEON’s USDA APHIS permit.



12 REFERENCES

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13 APPENDIX A FIELD DATA SHEETS

See AD[01].



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14 APPENDIX B LAB DATA SHEETS

The following data sheet serves as a backup for times when electronic data collection devices are not available. An ongoing digital datasheet will be kept; data should either be directly entered into this datasheet or transcribed as soon as possible.

Archive Sample ID	Subsample ID	Initial Archive sample weight (g)	Labeled container+lid weight (g)	Soil weight (g)	Final Archive sample weight (g)	Unaccounted soil (g)	Subsampling date	Technician name	Shipping date	Notes



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15 APPENDIX C CONSIDERATIONS FOR IMPLEMENTATION

If an entire sample is spilt, try to salvage parts of the sample that are least likely to have been contaminated. For example, if a subsample jar is dropped and smashes and the soil is in a pile on the floor, collect the soil from the top of the pile that does not appear to have come into direct contact with the floor. Record details of the incident in the metadata and note the quantity of sample that was salvaged. This subsample should only be used after all other material from the same sample has been used.