NEON FSU Field and Lab Protocol for OPS CPER 2011: Soil Microbes

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Figure 2: Protocols to triangulate the location of a soil core within the Soil Microbe Plot. The Southwest corner is designated by the coordinates (0,0). FSU staff will generate the coordinates for a given core, designated by ⊙, calculate the hypotenuse (Z), and provide the values for X, Y, and Z. If high resolution GSP units are not available, field crew will use three measuring tapes to triangulate to the core location. Measuring tapes for Y and Z can be anchored at (0,0). Measuring tape for X will be anchored at the end point of Y. Two cores will be taken adjacent (< 1 meter apart) to each other at ⊙. The location of the third core is 5 meters along the x-axis from the original core and can be measured with the X-line measuring tape (dashed line). 28

1 INTRODUCTION

1.1 Purpose

[This draft for OPS CPER 2011 <TBR>.]

The primary purpose of this document is to provide change-controlled version of Observatory protocols, which will eventually be used for external review by subject-matter experts. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change-controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to the procedure and protocol for OPS CPER 2011.

1.2 Scope

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

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

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

1.3 Acknowledgements

[If a protocol is based closely on the work of another program or author, note that here <TBR>.]

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

[If you want to reference other procedural documents (e.g. associated Protocol document), drawings, etc. then include filenames in the following sections. <TBR>]

RD[01]	NEON.DOC.000008 NEON Acronym List
RD[02}	EHS Safety Policy and Program Manual
RD]03…]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
RD[06]	NEON.DOC.000243 NEON Glossary of Terms
	QA/PA Plan
	DOORS requirements
	ATBD
	EHS USDA Soil Permit SOP
AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog

2.2 Acronyms

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol

3 BACKGROUND AND OBJECTIVES

3.1 Background

[This draft for OPS CPER 2011 <TBR>.]

Fundamental questions regarding the biogeographical distribution of microorganisms, the roles of soil microbial communities in different ecotypes, and the responses of microbes to global and land-use change remain unanswered. Microbial ecology is a rapidly evolving field, with many unknown microbial functional roles and impacts remaining to be discovered. NEON data and archived samples will inform external research projects within the scientific community that address the ecological roles, constraints, functions and pathogenicity of microbes in the terrestrial environment. NEON will help to expand the temporal and spatial understanding of microbial dynamics, with the long-term aim of capturing the drivers and feedbacks of microbial responses to climate and land use change.

The purpose of the Soil Microbe sampling design is to capture spatially explicit inter- and intra-annual variation in bacterial, archaeal and fungal biodiversity and to relate microbe community composition with function and biogeochemical properties. Microbial community diversity and specific functional gene presence and abundance shall be linked to soil biogeochemical characteristics, plant community composition, and above and belowground ecosystem productivity.

These protocols outline the field and laboratory procedures required to collect, process, and maintain the integrity of the Soil Microbe samples collected during the Field Operations Prototype 2011 at CPER. The following sub-sections contain draft protocols that provide detailed guidance for locating soil microbe plots at a given site, collecting soil cores and associated metadata, laboratory processing of soil cores, and storage and shipment of samples to Analytical Laboratories or Archives.

[This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.

Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page. <TBR>]

3.2 Metadata collection

[This draft for OPS CPER 2011 <TBR>.]

NEON Soil Microbe sample information shall comply with the metadata standards outlined in the Minimum Information about MARKer gene Sequence (MIMARKS). An excel version of the checklist is provided to the community on the Genomic Standards Consortium (GSC) web site at: http://lists.gensc.org/mailman/listinfo/gensc-mimarks-wg.

3.3 Science Requirements

[This draft for OPS CPER 2011 <TBR>.]

This protocol fulfills the following Observatory science requirements:

[List science requirements from DOORS that are met by this protocol <TBR>.]

3.4 Data Products

[This draft for OPS CPER 2011 <TBR>.]

[List Level 0 data products measured by protocol <TBR>].

Table 1 A summary of field and related lab measurements and the associated NEON Data Products

Measurement	Data Product

4 PROTOCOL

[This draft for OPS CPER 2011 <TBR>.]

4.1.1 Plot Location

[This draft for OPS CPER 2011 <TBR>.]

As a first pass, soil microbe samples will be collected at targeted areas that represent, where possible, the dominant soil and vegetation characteristics of a Domain site. The current NEON Soil Microbe design for CPER OPS 2011 uses a sample grid measuring 160 m x 320 m divided into eight 80 m x 80 m cells. This plot will be established within the calculated air shed of the FIU tower location. A GIS technician will work with FSU, FIU and EHS to delineate the Soil Microbe plot location prior to field sampling. The goal with the soil microbe sampling is to coordinate soil microbe communities with tower and soil gas fluxes, plant biodiversity and plant productivity, and soil biogeochemical measurements. The soil microbe sampling is currently focused in the tower airshed but it is expected that sampling will expand to include the entire Domain footprint in operations.

Figure 1

FSU staff is responsible for generating the soil microbe core coordinates. Core coordinates will be randomly generated within each of the eight 80 m x 80 m cells in the Soil Microbe plot and will be available, through the Soil Microbe database, for the field crew to upload to the handheld PDA and/or print to datasheets. Because the field crew may not have high resolution GPS units in the field, core coordinates shall be provided as both UTMs and as (x,y) coordinates that can be triangulated and

navigated to through the use of measuring tapes (see section 10.3.3.1 Locating soil core coordinates within soil microbe plot).

Figure 2

Field crews shall take a waypoint measurement of each core location after it has been collected to account for triangulation errors and variability.

4.1.2 Plot Establishment

[This draft for OPS CPER 2011 <TBR>.]

Currently, FSU staff will establish the Soil Microbe plot delineations prior to field crew sampling. Over the long-term, NEON will contract surveyors using high resolution GPS equipment to delineate plot boundaries.

The Southwest corner of Soil Microbe Plot shall be barcoded and clearly labeled. The requirements of the Domain site owners will determine whether rebar, PVC pipe or flagging will be used to demarcate the plot boundaries.

For every Soil Microbe Plot, the x-axis shall be aligned W-E and the y-axis shall be aligned N-S. The Southwest corner shall be designated with the coordinates (0,0) and the eight 80 m x 80 m cells shall be labeled A-H in a counter-clockwise pattern (Figure 1).

An overview of Soil Microbe activities occurring over five days for each sampling period is briefly outlined here:

4.1.3 Field & laboratory preparation

[This draft for OPS CPER 2011 <TBR>.]

Preparation involves assembling and inspecting equipment (1 hour), uploading and/or printing datasheets (1 hour), and generating and organizing labels (2-4 hours) and shall occur at least two days prior to field sampling.

4.1.4 Soil core sampling

[This draft for OPS CPER 2011 <TBR>.]

Soil microbe field sampling requires two field technicians. Based on current estimates, two experienced field technicians are able to complete soil sampling in 5-8 hours not including travel to and from the field site. Field sampling shall be completed in one continuous block of time and may require field technicians to work overtime.

4.1.5 Processing soil cores – Stage I

[This draft for OPS CPER 2011 <TBR>.]

Soil microbe laboratory processing requires two technicians. Based on current estimates, two experienced laboratory technicians are able to complete Stage I of soil core processing in 9-14 hours, depending on the soil type and moisture content. Stage I shall be completed in one continuous block of time and may require laboratory technicians to work overtime.

4.1.6 Processing soil cores – Stage II

[This draft for OPS CPER 2011 <TBR>.]

Stage II requires 4-10 hours for final measurements of volumetric water content, laboratory cleanup, equipment storage, and photo and data upload and QC.

4.1.7 Processing soil cores – Stage III

[This draft for OPS CPER 2011 <TBR>.]

Stage III requires 2-4 hours to transfer air-dried soils to archive bags, finalize laboratory cleanup, and prepare frozen and air-dried soils for shipment to Analytical Labs or Archive Facilities.

[Summarize the science rationale (e.g. experiment design), include key citations. Briefly summarize the procedure included in this document and variations in how NEON is implementing this protocol in different locations throughout Observatory (e.g. above ground tree biomass for temperate vs tropical zones). If the protocol is based on existing published procedures, reference those here (e.g. "These methods are based on the amazing work of John Updike (1964). No one has come up with anything better since then.").

A protocol is a formal summary description of a procedure and its related rational. A protocol includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result; can also be called a method.

Read more:
http://wiki.answers.com/Q/What_is_the_difference_between_a_method_and_a_procedure#ixzz1FInck5Na

Identify assumptions or known-unknowns of the chosen protocol. Identify and summarize quantitative aspects of the procedure (timing, # plots, # samples, location of sensors). Provide a simple timeline diagram or table if pre-field activities occur the day prior to the field day, or if the field procedure is a multi-day task. <TBR>]

5 QUALITY ASSURANCE AND QUALITY CONTROL

[Summarize QA/QC plan and reference QA/QC document <TBR>].

6 DECISION TREE

[This draft for OPS CPER 2011 <TBR>.]

Soil core sampling is sensitive to weather conditions and there are circumstances under which soil cores can and cannot be collected. The most important consideration is precipitation, which will be consistent throughout soil core collection. Soil cores may be collected in the rain as long as all cores are collected while it is raining. If it begins to rain or stops raining after soil core collection has begun, all previously collected cores must be discarded and a new sampling campaign must be rescheduled. Due to the temporal component of the soil microbe analyses, soil cores from one site will not be collected greater than 10 h apart.

Soil core processing is time sensitive. Soil cores will not be collected if laboratory processing of cores cannot begin within 24 h of collection.

Delay	Action	Adverse Outcome	Outcome for Data Product
Hours - days	If sampling can be completed by dark, then begin sampling. If not, reschedule ASAP within 14 days.	None	None
1-14 days	Stop sampling and reschedule for a consistent weather day within 14 days	Samples collected during inconsistent weather will be disposed of. New samples will be collected.	None
1-14 days	Do not begin sampling until soil can be processed within 24 hours of sampling date	Data are unreliable	Samples cannot be included in the analysis

[Summarize for the field technician or manager any implementation decisions regarding the protocol <TBR>.]

7 SAFETY

[This draft for OPS CPER 2011 <TBR>.]

Training is required to safely operate the drying oven, dry ice and ultralow freezers.

Personnel working at a NEON site should be familiar with and practice safe fieldwork as outlined in the EHS Safety Policy and Program Manual. 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.

[Any safety issues specific to the procedure should be detailed here, along with references to any pertinent safety standards <TBR>].

8 PERSONNEL REQUIREMENTS

8.1 Field Personnel

[This draft for OPS CPER 2011 <TBR>.]

A minimum of two field technicians is required for soil core sampling in the field.

8.2 Laboratory Personnel

[This draft for OPS CPER 2011 <TBR>.]

A minimum of two laboratory technicians is required to process soil cores within the designated time limit.

[Include special skills or subject matter expertise required (e.g. able to identify regionally specific plants by visual inspection, through use of dissecting microscope and through use of dichotomous key) <TBR>.]

9 TRAINING REQUIREMENTS

[This draft for OPS CPER 2011 <TBR>.]

[Reference NEON Training Plan document relevant to this method <TBR>.]

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling Frequency and Timing

[This draft for OPS CPER 2011 <TBR>.]

Each site within a domain shall be sampled four times during an annual cycle. An exception may be made for the sites in D18 and D19. Unless otherwise noted, sampling intervals will occur no less than 120 days and no greater than 160 days apart. Sampling intervals shall include the beginning and end of a growing season, and may include points representative of annual temperature or precipitation extremes. One sampling period will coincide with peak above and/or belowground productivity. A sampling regime could include the following periods, modified to reflect important temporal characteristics at each site: mid-winter (e.g., plant dormancy), spring (e.g., begin plant physiological activity), mid-summer (e.g., peak plant productivity), fall (e.g., plant senescence).

Table 2 The approximate sample dates for soil microbe sampling at all NEON sites

Domain	Date	Frequency
10	TBD	4 times per year

[Based on sampling frequency information, list estimated dates that correspond with timing rules or estimated dates when timing rules are fulfilled for each NEON site where this procedure is implemented. Include range of scientifically acceptable sampling timing. If the procedure involves multiple sampling

events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table <TBR>.]

10.2 Contingent decisions

[This draft for OPS CPER 2011 <TBR>.]

Soil core sampling is sensitive to weather conditions and there are circumstances under which soil cores can and cannot be collected. The most important consideration is precipitation, which will be consistent throughout soil core collection. Soil cores may be collected in the rain as long as all cores are collected while it is raining. If it begins to rain or stops raining after soil core collection has begun, all previously collected cores must be discarded and a new sampling campaign must be rescheduled. Due to the temporal component of the soil microbe analyses, soil cores from one site will not be collected greater than 10 h apart.

Soil core processing is time sensitive. Soil cores will not be collected if laboratory processing of cores cannot begin within 24 h of collection.

[Summarize what-if decisions (how to accommodate site-specific or changing conditions) <TBR>.]

10.3 Field Procedure

[Write the procedure as if a PDA were not available. This way, when the asteroid hits the earth and disrupts all electrical equipment, the field personnel can reference the "old school" procedure and collect data. These non-PDA procedures also help CI to define PDA and data ingest requirements, and provide sufficient information for an external reviewer to assess the procedure without the need of a PDA <TBR>.]

10.3.1 Equipment and Materials

[This draft for OPS CPER 2011 <TBR>.]

A preliminary list of required field and laboratory equipment for microbe sampling for CPER OPS 2011 is provided here. This list will be updated. Further details regarding quantity needed and suggested manufacturer are provided in a separate document.

Table 3 Field materials and supplies required for the Microbe Sampling Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
field notebook (all-weather paper)		
pens		
pencils		
permanent markers, black, blue, red		
external frame field pack	2	
digital camera	1	

PVC camera frame at 1 m height	1	
AA batteries (reusable)	8	
AA 8 position battery charger (with car adaptor)		
dry ice	15 lbs	Yes
fiberglass measuring tape (30 m) english/metric	2	
PVC stakes		
metal stakes	24	
roll of flagging tape	1	
antimicrobial alcohol hand wipes		
cooler bags	4	
fiberglass measuring tape (100 m) english/metric	2	
soil temperature probe	2	
16 oz rubber mallet	4	
9 Qt cooler	1	
Hori-Hori soil knife	2	
Turf-Tec Pocket Tubular ½ inch diameter soil corer	1	
3.5", 10 schedule, aluminum pipe (1 ft)	25	
Kaput covers, 3˝ Polyethylene Liner Caps	50	
GPS	2	
Watch	2	
Quart size Ziplock bags	50	
Sand for filling holes		
Bucket or equivalent for carrying sand		

[Include all standard and unique equipment and capabilities required to execute the procedures in this document, including:

- *A detailed list of materials (e.g. equipment, sampling gear, sample containers, chemical preservatives) used in the field.*
- *Do not include materials used for separate but related activity; that will be included in the Procedure for that activity (i.e. lab vs. field materials).*
- *Describe the chemicals being used or as a preservative when samples are immediately returned to the field – exact chemical constituents and strength, and bottle size. Leave a place-marker in the draft if unknown.*
- *Illustrations of materials (e.g. sampling gear) – all in jpeg format, 3 inches wide*
- *Can be in bullet point or table format, but be consistent throughout the document <TBR>.]*

10.3.2 Field & Laboratory Preparation

[This draft for OPS CPER 2011 <TBR>.]

All necessary field equipment shall be assembled at least two days prior to field sampling to ensure batteries are charged, databases are uploaded to the PDA and/or printed on water resistant paper, soil core sleeves are sterilized and labeled, and all consumables are available.

Dry ice is needed for field sampling and should be ordered in advance.

Sand is needed for filling in soil core holes and should be ordered in advance.

Laboratory Equipment and bench space shall be available for use within 24h of soil core collection. This will include ensuring that distilled water, bleach solution, and solution for pH meter calibration are available, and ensuring that the drying oven, refrigerators, and freezers are at the correct temperatures and have space for samples.

The Soil Microbe field crew is responsible for transferring the appropriate files to the handheld PDA and/or printing the appropriate water resistant datasheets before field sampling begins. The file formats are <TBD> but will include instructions and mapping tools to find the Soil Microbe Plot and the designated Soil Microbe Core locations and a database for entering GPS waypoints, metadata, and comments during sample collection.

Field or Laboratory technicians are responsible for generating and organizing sample labels, which should take no longer than 4 hours. All sample labels will be created before the field campaign begins in order to expedite the laboratory processing, which is time sensitive. Consumables that only require temporary labels (e.g., soil core sleeves, aluminum pans, weigh boats, pH sampling cups, drying tins) will be marked with laboratory tape and sharpie makers. Storage containers for sample archive (e.g., Ziploc bags, paper bags, cryovials) will require permanent barcoded labels. The format of the barcode labels is TBD.

The following labeled containers are required for each soil core and will be created before the field campaign begins. All thirteen containers pertaining to one sample will be kept together to minimize the risk of cross-contamination across samples and to ensure all appropriate subsamples are collected.

1. marker flags or flagging tape (label type TBD) – Field

Figure 3 marker flags “image pending”

2. soil core sleeves (temporary label) – Field

Figure 4 soil core sleeve “image pending”

3. aluminum pan for sieving weighing (temporary label)

Figure 5 aluminum pan “image pending”

4. large plastic weigh boat (temporary label)

Figure 6 large plastic weigh boat “image pending”

5. plastic cup for pH (temporary label)

Figure 7 plastic cup for pH “image pending”

6. aluminum tin for volumetric water content (temporary label)

Figure 8 aluminum tin for volumetric water content "image pending"

7. quart-sized Ziploc bags for RNA archive (-80 °C storage, permanent label) – Field
8. quart-sized Ziploc bags or 3-5 ml cryovial for 5 g soil for DNA extract (-80 °C storage, permanent label)

Figure 9 3-5 ml cryovial "image pending"

For operations, a system will be designed to weigh multiple 5 g aliquots of soil for archive and analysis. This will change procedures and equipment requirements and could increase labwork time estimates by hours.

9. quart-sized Ziploc for litter layer (-80 °C storage, permanent label)
10. quart-sized Ziploc for roots (-80 °C storage, permanent label)
11. quart-sized Ziploc for soil chemical assays (-80 °C storage, permanent label)
12. gallon-sized Ziploc bag for archive (-80 °C storage, permanent label)

Figure 10 quart-sized Ziploc bag "image pending"

13. quart-sized paper bag for archive (air-dried storage, permanent label)

Figure 11 quart-sized paper bag "image pending"

[Describe all activities that must occur prior to arrival in the field, for example equipment calibration or preparation. A detailed list of tasks, using the numbering format shown below. Be consistent.

- *Break each step down into actionable steps*
- *Described and list the tasks in chronological order*
- *If pre-field tasks occur over multiple days, break Section 7.1 down further (e.g.:*
  - *One week prior to sample collection, do this*
  - *Two days prior to sample collection, do this*
- *Be explicit and use language geared toward 3rd yr undergraduate student. Assume the user has no previous knowledge of the activity.*
- *Include a description of what sampling gear, equipment, etc. they need to verify are in working order and properly packaged for the field day.*
- *Define safe practices (e.g. use gloves to mix chemicals in the fume hood), where necessary at each step. Do not assume this information is written elsewhere – include all relevant safety procedures in every document. Each document should be written as a stand-alone document.*
- *Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3" wide.*

<TBR>]

10.3.3 Sample Collection in the Field

10.3.3.1 Locating soil core coordinates within soil microbe plot

[This draft for OPS CPER 2011 <TBR>.]

For every Soil Microbe Plot, the Southwest corner shall be designated with the coordinates (0,0) and the eight cells shall be labeled A-H in a counter-clockwise pattern (Figure 1).

Field crews will first navigate to the Southwest corner of the Soil Microbe Plot and scan the barcode to ensure they are in the correct location. Soil core collection will begin in subplot A. If a high resolution GPS is not available or if its accuracy is not at the 1 meter scale, field crews will need to use measuring tapes to locate soil core locations. Field crews will use three measuring tapes units to triangulate to the core location (Figure 2). Measuring tapes for (Y) and (Z) can be anchored at (0,0).

Once three soil cores have been collected within A, the field crew will move to cell B and use (0,80) as an anchoring point to measure (Y) and (Z). The field crew will scan the barcode at (0,80) to ensure they are in cell B. The following anchoring points will be used in each cell and the field crew will scan the barcode at each point before sampling in that cell:

A (0,0); B (0,80); C (0,160); D (0,240), E (80,240); F (80,160); G (80,80); H (80,0)

10.3.3.2 Soil core metadata collection

[This draft for OPS CPER 2011 <TBR>.]

Twenty-four soil cores shall be collected at each sampling period.

1. A digital photograph of each soil core location will be taken before sampling for microsite characterization. Field technicians will use a digital camera affixed to a modified tripod with a 0.5 m^2 quadrat base to take two photos of each microsite. Each photo will include a scale for distance and color and the flag or label that corresponds to the soil core.

Figure 12 step 1 “image pending”

2. Prior to collecting each soil core the following metadata shall be recorded in the PDA and/or the appropriate datasheets:

    a. Time of day (h:m)
    b. Air temperature (^{o}C)
    c. Soil temperature (^{o}C)
        - Insert the soil temperature probe to 10 cm depth so the face of the probe is flush with the soil where the core will be collected and allow 30 seconds to equilibrate. Record the soil temperature.

Figure 13 step 2c “image pending”

    d. Litter depth (cm)

- The pin of the soil temperature probe can be used to measure litter depth. Press the pin through the litter until it touches the soil surface and use your index finger and thumb to indicate the top of the litter on the pin. Remove the pin and align it against a measuring tape to determine the litter depth. Record the measurement in centimeters.

Figure 14 step 2d "image pending"

e. Snow depth (cm, when appropriate)
   - If snow cover is sparse, the pin of the soil temperature probe can be used to measure snow depth as above. If snow cover is high, push a sturdy measuring tape though the snow until it touches the soil surface and record the measurement in centimeters.

Figure 15 step 2e "image pending"

f. Additional comments (e.g., raining, area flooded, animal carcass, other ongoing experiments in the area, etc., if needed)

3. The following labeled containers and markers are required for each soil core and will be generated by technicians before fieldwork begins (see 10.3.4):

   a. 24 marker flags or flagging tape (label type TBD)
   b. 24 soil core sleeve (labeled with tape and sharpie for temporary use)
   c. 24 quart-sized Ziploc bags for RNA archive (barcode labeled for permanent storage)

Three soil cores will be collected in each of the eight 80 m x 80 m cells. The final sampling scheme is TBD. Currently, two soil cores will be collected adjacent to each other at the randomly designated location provided by FSU staff. Adjacent cores will be no greater than 1 meter apart. Field crew will use the measuring tape for X (Figure 2) to extend 5 meters along the x-axis of the cell to collect the third core.

4. If the PDA is functioning, all samples will be date-time stamped to correspond to automated instrument measurements.

5. A soil core is collected after a photograph of the core location has been taken and soil temperature, litter depth, and snow depth (when applicable) measurements have been made.

10.3.3.3 Soil core collection

[This draft for OPS CPER 2011 <TBR>.]

1. Wear nitrile gloves; never touch soils with bare hands.

2. Soil for RNA archive is collected first. Use the Turf-Tec Pocket Tubular ½ inch diameter soil corer.

3. Clear a small patch of litter from the area to be cored with the Turf-Tec soil corer. Place the Turf-Tec soil corer directly on the soil surface and push to 5 cm depth. There should be no litter in the core.

Figure 16 step 3 "image pending"

4. Place the corer into a barcode-labeled quart-sized Ziploc bag and knock out the soil.

5. Double bag in an unmarked Ziploc taking care to ensure that the label is visible through the second bag and immediately place on dry ice in the small cooler.

Figure 17 step 5 "image pending"

6. Rinse soil particles from corer with water, sterilize corer with ethanol wipes and air dry before reusing. Store ethanol wipes in a gallon sized Ziploc bag for disposal.
7. Soil for the remaining analyses and archive are collected with 7.5 cm diameter and 30 cm depth soil core sleeves. Each soil core sleeve will be marked at 10 cm (blue line) and at 15 cm (red line) depth.

Figure 18 step 7 "image pending"

8. Remove the core sleeve caps and place the soil core sleeve on top of the litter of the area to be cored. The RNA soil core hole should fall within the larger core.

Figure 19 step 8 "image pending"

9. Use a rubber mallet to pound the soil core sleeve into the soil to obtain a minimum sample depth of 10 cm.

Figure 20 step 9 "image pending"

10. If a core depth of 10 cm can be reached, try for a sample depth of 15 cm and record the final depth reached.
11. If a core depth of 10 cm cannot be reached, chose a location immediately adjacent to the original, try again and record the final depth reached.
12. Use a sterile hand-held soil knife to carefully remove the soil core sleeve from the soil, twist the sleeve in the soil to loosen, if necessary. Rinse the soil knife with water, sterilize with ethanol wipes and air dry before reusing. Store ethanol wipes in a gallon sized Ziploc bag for disposal.

Figure 21 step 12 "image pending"

13. Do not touch the soil in the sleeve with bare hands; wear gloves if necessary to prevent sample loss.

Figure 22 step 13 "image pending"

14. Cap the soil core sleeves at both ends using the covers provided. Blue cap corresponds to the end of the sleeve with a blue line. Red cap corresponds to the end of the sleeve with a red line.

Figure 23 step 14 "image pending"

15. Maintain the soil core sleeves in an upright position to prevent horizons from mixing.

16. Transfer the soil core sleeve in an upright position into an insulated external frame backpack or equivalent.

Figure 24 step 16 "image pending"

17. Take a waypoint of the core location.

Figure 25 step 17 "image pending"

18. CPER requires the hole be filled in with sand. Once the hole has been filled, secure a labeled flag in the sample location.

Figure 26 step 18 "image pending"

19. Collect metadata following the protocols in section 10.3.5.2 for the adjacent core, change or sterilize nitrile gloves with ethanol wipes before sampling. The adjacent core should be no greater than 1 meter from the original core but can be taken N, S, W or E of the original core.

20. Repeat 1-18 for the adjacent core.

21. Use the X line measuring tape (Figure 2) to measure 5 m on the x-axis to collect the third core of the cell.

Figure 27 step 21 "image pending"

22. Collect metadata following the protocols in section 10.3.5.2 and change or sterilize nitrile gloves with ethanol wipes before sampling. Repeat 1-18.

23. When three cores have been collected from a cell, move to the next cell and locate the core coordinates following the protocols in section 10.3.3.1**.**

[A detailed list of tasks:

- *Break each step down*
- *Describe and list the tasks in chronological order*
- *List decision criteria used to implement procedure based on plot/sampling location conditions (i.e. sampling plot composition, stream width, etc.).*
- *If field tasks occur over multiple days or are complex, break this section down further (as shown in previous Section)*
- *Be explicit and use language geared toward 3rd yr undergraduate student*
- *Include detailed instructions on assembly of sampling gear, if gear is assembled in the field. If gear is assembled/disassembled in the Domain Office (e.g. not in the field), include that detail in the Pre- or Post-Field Task sections*
- *Include safety issues and practices*
- *Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3" wide. <TBR>]*

10.3.4 Sample Preservation

10.3.4.1 Soil core transport and storage prior to processing

[This draft for OPS CPER 2011 <TBR>.]

1. The soils stored on dry ice in the field must be transferred to ultralow storage (-80 °C) before the dry ice sublimes. Fifteen pounds of dry ice should be sufficient for one soil collection campaign.
2. The soil cores sleeves must remain upright to prevent horizons from mixing.
3. Insulated external frame backpacks or the equivalent will be provided so that two field crew technicians can carry a total of 24 soil cores in an upright position at ambient temperatures from the field and during transport to the laboratory.
4. Soil cores sleeves shall be refrigerated at 4 °C within eight hours of collection.
5. Soil cores may remain at 4 °C for up to 24 h before processing.
6. Soil cores shall not be collected if they cannot be processed within 24 hours.

[Detail the task in order from start to finish

- *Include how long (time/days/yrs/indefinitely) sample can be preserved in this mode.*

- *Only include activities that relate to the Field Task.*
- *If a sample is processed at the Domain Lab, that work is a written in the next Section or as a separate document if complex. <TBR>]*

10.3.5 Sample Shipping (may not be applicable for all Field SOPs)

[Provide detail on shipping specifics (e.g. wrap the sample containers in a plastic bag, seal the top. Place containers upright in cooler labeled "Elephant Samples", include four reusable ice packs. Seal the container. In addition to the shipping label, the following hazmat labels are required: Check with EHS for label requirements <TBR>]

10.3.6 Data Handling (may not applicable for all Field SOPs)

[Briefly explain data upload steps (e.g. enter data into excel file name "XXXXX", plug in PDA, etc., etc.)<TBR>.]

10.3.7 Refreshing the Field Sampling Kit

- *[Provide detail on how to restock the sampling kit with non-perishable items. Best practice is to restock the sampling kit after the sample event, with a check at the start of each sample event that the kit is appropriately stocked*
- *Reference the materials list, above*
- *Be explicit and ensure information does not overlap or refute early info (ex. If preservatives used in the field have to remain cold, then the 'refreshment' of the preservative is a detailed in Section 7.2 and not here. <TBR>]*

10.3.8 Equipment Maintenance, Cleaning, and Storage

- *[Include maintenance of sampling gear as they relate to this Procedure, such as battery recharge.*
- *Do not include vehicle maintenance or maintenance of gear commonly used in the field such as a mosquito net or boots. Maintenance of common field items will be a different document.*
- *Include relevant safety issues and practices*
- *Be explicit*
- *Illustrations or photographs of tasks that would benefit from an illustration, jpeg 3" wide.<TBR>]*

11 LAB STANDARD OPERATING PROCEDURE

[Write the lab procedures as if a PDA were not available. Use the same sections as the field protocol, above. Separate the lab procedures into multiple sections and add on to the above title. For example:
11 Lab Standard Operating Procedure - Plant identification and drying

12 Lab Standard Operating Procedure - Plant mounting <TBR>]

11.1 Timing

[This draft for OPS CPER 2011 <TBR>.]

1. Soil cores sleeves shall be refrigerated at 4 °C within eight hours of collection.
2. Soil cores may remain at 4 °C for up to 24 h before processing.
3. Soil cores shall not be collected if processing cannot begin within 24 hours.

[Provide details on preferred timing of sample processing at the domain labs AND the maximum time between field collection and lab processing If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table. <TBR>]

11.2 Lab Procedure

11.2.1 Establishing Workspace

[This draft for OPS CPER 2011 <TBR>.]

Laboratory Equipment and bench space shall be available for use within 24h of soil core collection. This will include ensuring that distilled water, bleach solution, and solution for pH meter calibration are available, and ensuring that the drying oven, refrigerators, and freezers are at the correct temperatures and have space for samples.

The following space, large equipment and calibrations of equipment are required for soil processing:

- 15 ft of bench space (workspace for sieving, weighing, and measuring pH for two people)

Figure 28 workspace "image pending"

- 7 ft of bench space for air-drying soil samples for 48 h
- Refrigerator (4 °C; must be empty because 24 soil core sleeves fill one entire refrigerator)
- Ultralow freezer (-80 °C)
- Drying oven (105 °C; must be turned on and calibrated to correct temperature 24 h before use)
- pH meter (must be calibrated immediately before use)
- Sink (with mesh or other material to prevent debris from going down the drain)
- Bleach solution bucket (for sterilizing sieves and knives between use and for final sterilization)
- Balance (up to 1 kg, accurate to 0.01 g; must be stabilized before use)

11.2.2 Equipment and Materials

[This draft for OPS CPER 2011 <TBR>.]

A list of required field and laboratory equipment for microbe sampling for CPER OPS 2011 is provided here. Details regarding quantity needed and suggested manufacturer are provided in a separate document.

Table 4 Laboratory materials and supplies required for the Soil Microbe sampling procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Ultralow Freezer (-80 C)		Yes
Refrigerator (4 C)		
Drying oven (105 C)		Yes
Balance (0.01 g accuracy)		
pH meter		
pH buffer (pH 4, pH 7, pH 10)		
Nitex screen (1 roll = 10 yds x 45 inches)		
nominal opening 4 mm sieve, U.S. Std #5		
instant-recall memory timer (digital lab clock)		
lab washer squirt bottle		
graduated cylinder - 25 ml		
graduated cylinder - 100 ml		
glass beakers (50 ml)		
heavy duty aluminium foil		
quart freezer zip-top bags		
gallon freezer zip-top bags		
tin sample canisters		
weigh boats (sm)		
paper bags (lunch bag size)		
8" aluminum pie pan		
weigh boats (lg)		
spatula		
2 " standard tape dispenser		
lab tape		
packing tape		
26 x 19-3/4 x 10-1/2" insulated shipping cooler		
DI (or distilled water)		
lab coat		
5 gallon bucket		
handbroom & dustpan		
Nitrile gloves – L		
Nitrile gloves – M		
Nitrile gloves - S		
Paper towels		
bleach		Yes

11.2.3 Sample Processing in the Lab

11.2.3.1 Processing Soil Cores: Stage I

[This draft for OPS CPER 2011 <TBR>.]

1. Turn the pH meter on at least 1 h prior to use.

2. Wear nitrile gloves and laboratory coats.

3. Process each core separately to avoid cross-contamination. Change gloves each time a new core is handled.

4. Use clean wax paper or aluminum foil to cover 1 ½ feet (0.5 m) of workspace. Change wax paper or aluminum foil each time a new core is processed.

Figure 29 step 3 "image pending"

5. Retrieve soil core sleeve from refrigerator (keeping it upright), remove both covers and extrude the soil core from the soil core sleeve by banging a rubber mallet against the sleeve.

6. Note the number of horizons, which can be determined by different soil color and texture. If more than one horizon is present, label extra Ziploc bags and use _H1, _H2 to distinguish (H1 = horizon closest to soil surface). Record the number of horizons in the database.

Figure 30 step 5 "image pending"

7. Use tape and a sharpie to create a temporary label of the sample and take a digital photograph of the soil core with the label.

Figure 31 step 6 "image pending"

8. If more than one horizon is present, process horizons separately.

9. Use a sterile soil knife to cut the litter layer from the soil core and place the litter layer in the appropriately labeled quart size Ziploc bag. Squeeze the air from the bag and close. Double bag the sample in an unlabeled Ziploc taking care to ensure that the label is visible through the second bag and store at -80 ^{o}C.

Figure 32 step 8 "image pending"

10. Sieved soil will be collected in sterile, disposable aluminum pans or suitable alternatives.

11. Tare the aluminum pan on the balance and then place the pan below a sterile 4 mm mesh sieve.

Figure 33 step 10 "image pending"

12. Pour the contents of the soil core onto the sieve and use a gloved hand to remove roots. Tare the Ziploc bag that will contain the roots. Place the roots in the appropriately labeled quart size Ziploc bag. Squeeze the air from the bag, close and weigh the bag. Double bag the sample in an unlabeled Ziploc taking care to ensure that the label is visible through the second bag and store at -80 ^{o}C.

Figure 34 step 11 "image pending"

13. Remove plant tissue, rocks or other debris and store in a bucket separate from other waste to dispose of separately.

14. Use a gloved hand move the soil through the sieve and remove any large debris that has passed through the sieve.

Figure 35 step 13 "image pending"

15. Return the aluminum pan with sieved soil to the balance, record the bulk weight of the soil sample, remove the pan and zero the balance.

16. Use the database or a calculator to subtract 70 g from the bulk weight of the sample. Multiply the remaining weight by 0.66 to determine the amount for soil for archive at -80 °C. The amount remaining is for air-dried archive.

Example: Bulk weight = 187 g.
Subtract 70 g (for DNA extract, volumetric water, pH, soil chemistry) = 117 g.
Multiply 117 x 0.66 (for -80 °C archive) = 77.2 g.
The difference between 77.2 and 117 g is the amount available for air-dried archive (39.8 g).

17. Record the calculated values in the database.

18. To measure volumetric water content, place the aluminum tin and lid on the balance and record the weight of the tin and lid.

19. Remove lid, add 10 g (accuracy to ± 0.2 g) of soil to the aluminum tin, replace lid and record the weight.

Figure 36 step 18 "image pending"

20. Place aluminum tin in the drying oven at 105 °C for 24 h. The lid should be secured loosely to allow circulation in the tin.

21. Use a large plastic weigh boat or aluminum pan (tare the balance after each measurement) to partition the remaining sieved soil as follows:

- 5 g transferred to a quart-sized Ziploc bag or 3-5 ml cryovial and stored immediately at -80 °C (DNA extract)
- 50 g transferred to a quart-sized Ziploc bag, double-bagged, and stored immediately at -80 °C (soil chemistry)
- 5 g transferred to a pH cup
- XX g (the amount calculated for -80 °C archive) transferred to a gallon- sized Ziploc bag, double-bagged, and stored immediately at -80 °C (archive)

22. Transfer the remaining soil to the plastic weigh boat or aluminum pan, record the weight and compare to the calculation for air-dried archive. The values should be similar.

Figure 37 step 21 "image pending"

23. Return the soil to the aluminum pan and set aside on a laboratory bench under mesh screen for 48 h. (see section 11.2.4 for final storage protocol).

24. Add 10 ml distilled water to the 5 g of soil in the pH cup. Shake well until all soil dissolves into solution. Set aside until all pH samples are ready to be measured.

Figure 38 step 23 "image pending"

25. One laboratory technician will be designated to measure pH while the other cleans up the equipment (see section 11.2.8 for detailed protocol).

26. Immediately before use, calibrate the pH meter following the manufacturer's instructions using a minimum of two pH standards.

27. To measure the pH of a sample, gently swirl the slurry before placing the pH probe in the cup. Allow the probe to equilibrate for 1 minute and record the value. Do not disturb the slurry. Allow the probe to equilibrate for 30 seconds more and record the value. The PDA/database will calculate the average pH and error.

Figure 39 step 26 "image pending"

28. Remove the probe, rinse with distilled water and repeat for the next sample.

29. The soil slurry can be discarded into a bucket separate from other waste and disposed of separately.

11.2.3.2 Processing soil cores: Stage II

[This draft for OPS CPER 2011 <TBR>.]

1. Final weight for volumetric water content will be measured after 24 h. Remove all aluminum tins from the drying oven and set aside on the laboratory bench for 10 minutes.

Figure 40 step 1 "image pending"

2. To measure final weight, zero the balance, weigh the aluminum tin with soil contents and lid and record the weight in the database.

3. All soil can be discarded into a bucket separate from other waste and disposed of separately.

4. Remove the temporary labels from the aluminum tins, clean the inside of the tins with ethanol wipes to remove any soil particles, allow tins to dry and then return to storage.

5. Soil for dry storage will be air-dried for up to 48 h on laboratory benches under mesh cloth.

Figure 41 step 5 "image pending"

6. After 24 h, air-dried soil should be shaken in the aluminum pan to separate particles and expose more soil to the air.

7. Soil should then be dried for an additional 24h. Extremely wet soil may require longer drying time.

11.2.3.3 Processing soil cores: Stage III

[This draft for OPS CPER 2011 <TBR>.]

1. When air-dried soil is completely dried it will be transferred to barcoded paper bags for permanent storage at ambient temperature.
2. Aluminum pans may be re-used if they are sterilized as detailed in section 11.2.8.
3. Sample preparation for shipment (see section 11.2.5.1) will occur on Day 3 unless other arrangements have been made.

11.2.3.4 Additional Requirements

[This draft for OPS CPER 2011 <TBR>.]

There are Domain-specific restrictions for soil processing and shipping. CPER, the location of the Field Operations Prototype 2011, does not have soil permit restrictions. NEON shall adhere to the requirements of the USDA Soil Permit held by EHS for soil processing and shipping. The requirements can be found in the NEON USDA Soil Permit SOP here XXXX.

CPER requires that soil microbe core holes be filled in with sand.

11.2.4 Sample Preservation

[Detail the task in order from start to finish

- *Include how long (time/days/yrs/indefinitely) sample can be preserved in this mode.*
- *Only include activities that relate to the Field Task.*
- *If a sample is processed at the Domain Lab, that work is a written in the next Section or as a separate document if complex. <TBR>]*

11.2.5 Sample Shipping

11.2.5.1 Sample shipment to analytical laboratory or archive

[This draft for OPS CPER 2011 <TBR>.]

1. Frozen and air-dried samples shall be shipped according to the USDA Soil Permit requirements.

2. A copy of NEON's USDA Soil Permit will be included with foreign soil shipments and a copy of the PPQ Form 519 (Compliance Agreement) will be included with domestic regulated soil shipments.

Figure 42 step 2 "image pending"

3. The soil permit and appropriate PPQ form will be clearly affixed to the outside of the container.

Figure 43 step 3 "image pending"

4. Frozen samples shall be stored in an ultralow freezer (-80 °C) until they can be shipped to an Analytical Lab or Archive Facility that holds a USDA Soil Permit.

5. Samples stored at -80 °C shall be shipped priority overnight mail with tracking number and a minimum of 20 lbs of dry ice to the Analytical Lab or Archive Facility in a well-sealed, sturdy, leak proof cooler (or Styrofoam container within another box), which will preclude spillage or pest escape in transit and while samples await processing.

Figure 44 step 5 "image pending"

6. Samples shall be clearly labeled and a printout of the spreadsheet detailing each sample will be included in the container. The spreadsheet should be sealed in a Ziploc bag.

7. Air-dried samples will be shipped to Archive Facilities via standard mail or the most economical option in a well-sealed, sturdy box, which will preclude spillage or pest escape in transit and while samples await processing.

11.2.6 Data Handling

[Briefly explain data upload steps (e.g. enter data into excel file name "XXXXX", plug in PDA, etc., etc.). <TBR>]

11.2.7 Refreshing the Laboratory Supplies

[Provide detail on how to restock the sampling kit with non-perishable items. Best practice is to restock the sampling kit after the sample event, with a check at the start of each sample event that the kit is appropriately stocked

- *Reference the materials list, above*
- *Be explicit and ensure information does not overlap or refute early info (ex. If preservatives used in the field have to remain cold, then the 'refreshment' of the preservative is a detailed in Section 7.2 and not here. <TBR>]*

11.2.8 Laboratory Maintenance, Cleaning, Storage

[This draft for OPS CPER 2011 <TBR>.]

1. All equipment that will be re-used shall be sterilized by scrubbing in soapy water with an abrasive sponge or brush, rinsed in an approximately 5 % bleach (5.25% sodium hypochlorite) solution, and air-dried overnight. The dilute bleach solution should be made up in a large bucket or Tupperware tub with tap water. For disposal, dilute bleach solution can be poured down the drain while the tap water is running.

2. Laboratory bench tops and other used surfaces shall be sprayed with dilute bleach solution or 70% Ethanol and wiped clean.

[Include maintenance of sampling gear as they relate to this Procedure, such as battery recharge.

- *Do not include vehicle maintenance or maintenance of gear commonly used in the field such as a mosquito net or boots. Maintenance of common field items will be a different document.*
- *Include relevant safety issues and practices*
- *Be explicit*
- *Illustrations or photographs of tasks that would benefit from an illustration, jpeg 3" wide. <TBR>]*

12 DEFINITIONS

[Define all protocol specific technical terms in alphabetical format. <TBR>]

13 REFERENCES

[Use Ecology style <TBR>]

APPENDIX A Field Data Sheets

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

[This draft for OPS CPER 2011 <TBR>.]

Column headers

date
technician names
site
core label
plot
core ID
sleeve ID
x coordinate
y coordinate
angle coordinate
time of day (h:m)
photo ID
latitude decimal degrees
longitude decimal degrees
snow depth (cm)
litter depth (cm)
soil temp (C)
core depth (cm)
notes

[Include copies of all data sheets – jpg format (data sheets are useful for CI to define PDA and data ingest requirements) <TBR>]

APPENDIX B Lab Data Sheets

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

[This draft for OPS CPER 2011 <TBR>.]

Column headers

date
technician names
site
core label
plot
core ID
sleeve ID
Number of Horizons
litter (g)
total core weight (g)
tin & wet weight (g)
(24 h later) tin & dry weight (g)
pH weight (ca. 5 g)
adjusted total(g)
DNA (store at -80 C) (g)
a (2/3 adjusted total store at -80 C) (g)
b (remaining air dry storage) (g)
pH-1
pH-2
pH-3
notes

[Include copies of all data sheets – jpg format (data sheets are useful for CI to define PDA and data ingest requirements) <TBR>]

APPENDIX C Considerations for implementation

[Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.

Describe any component of the process that may interfere with the accuracy of the final product.

Discuss how to avoid common errors in sampling or common ways samples can be contaminated.

Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results")... i.e. call out weird issues and folklore explicitly. See: http://en.wikipedia.org/wiki/Phantom_of_Heilbronn <TBR>]

APPENDIX D Procedure Checklist
APPENDIX E Tables
APPENDIX F Figures
[This draft for OPS CPER 2011 <TBR>.]

Figure 1: Current Soil Microbe Plot design measuring 320 meters (X-axis) by 160 meters (Y-axis). The Southwest corner is designated by the coordinates (0,0). Eight cells measuring 80 m x 80 m are labeled counterclockwise A-H.

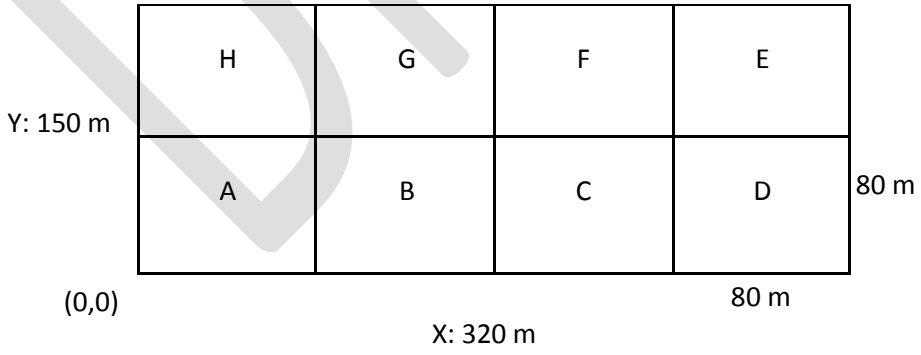
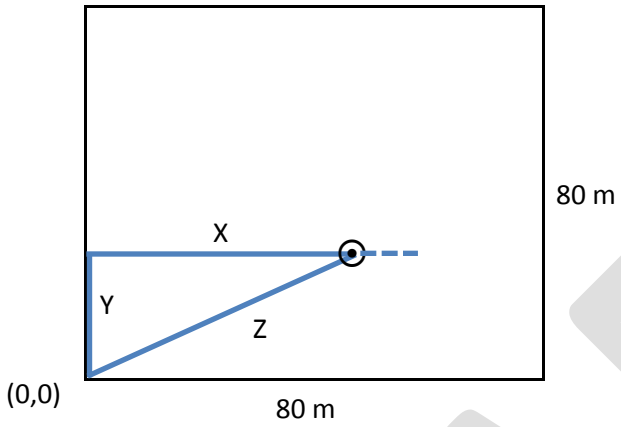


Figure 2: Protocols to triangulate the location of a soil core within the Soil Microbe Plot. The Southwest corner is designated by the coordinates (0,0). FSU staff will generate the coordinates for a given core, designated by ⊙, calculate the hypotenuse (Z), and provide the values for X, Y, and Z. If high resolution

GSP units are not available, field crew will use three measuring tapes to triangulate to the core location. Measuring tapes for Y and Z can be anchored at (0,0). Measuring tape for X will be anchored at the end point of Y. Two cores will be taken adjacent (< 1 meter apart) to each other at ⊙. The location of the third core is 5 meters along the x-axis from the original core and can be measured with the X-line measuring tape (dashed line).



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