NEON FSU Field and Lab Protocol for Ops CPER 2011: Plant Belowground Biomass

PREPARED BY	ORGANIZATION	DATE
Courtney Meier	FSU	3/25/2011

APPROVALS (Name)	ORGANIZATION	APPROVAL DATE
Becky Kao	FSU	09/29/2011

RELEASED BY (Name)	ORGANIZATION	RELEASE DATE
Anne Balsley	CCB ADMINISTRATOR/DCS	10/03/2011

See Configuration Management System for approval history.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
1	3/25/2011	ECO-00148	DRAFT RELEASE
A_DRAFT	09/23/2011	ECO-00280	Update to new document number's/template throughout document.

TABLE OF CONTENTS

1 INTRODUCTION 1
1.1 Purpose 1
1.2 Scope 1
1.3 Acknowledgements 1
2 RELATED DOCUMENTS AND ACRONYMS 2
2.1 Reference documents 2
2.2 Acronyms 2
3 BACKGROUND AND OBJECTIVES 3
3.1 Background 3
3.2 Science requirements 3
3.3 Data products 3
4 PROTOCOL 3
5 QUALITY ASSURANCE AND QUALITY CONTROL 4
6 DECISION TREE 4
7 SAFETY 5
8 PERSONNEL REQUIREMENTS 6
9 TRAINING REQUIREMENTS 6
10 FIELD STANDARD OPERATING PROCEDURE 6
10.1 Sampling frequency and timing 6
10.2 Contingency decisions 7
10.3 Field procedure 7
10.3.1 Equipment and materials 7
10.3.2 Preparation 7
10.3.3 Sample collection in the field: Soil core and crown harvest 8
10.3.4 Sample preservation 10
10.3.5 Sample shipping 10
10.3.6 Data handling 10
10.3.7 Refreshing the field sampling kit 10
10.3.8 Equipment maintenance, cleaning, and storage 11
11 LAB STANDARD OPERATING PROCEDURE 11
11.1 Timing 11
11.2 Lab procedure 11

11.2.1 Equipment and materials 11
11.2.2 Preparation 12
11.2.3 Sample processing in the lab 12
11.2.4 Sample preservation for bioarchive and chemical analysis 14
11.2.5 Sample shipping 15
11.2.6 Data handling 16
11.2.7 Refreshing the laboratory supplies 16
11.2.8 Laboratory maintenance, cleaning, and storage 16
12 DEFINITIONS 16
13 REFERENCES 16
APPENDIX A FIELD DATA SHEETS 17
APPENDIX B LAB DATA SHEETS 18
APPENDIX C CONSIDERATIONS FOR IMPLEMENTATION 19
APPENDIX D PROCEDURE CHECKLIST 20
APPENDIX E FIGURES 21

LIST OF TABLES

Table 1. A summary of field and related lab measurements and the associated NEON Data Products. 3
Table 2. Decision tree associated with the plant belowground biomass harvest, indicating how to respond to unanticipated delays in field or lab work, and consequences of these delays. 4
Table 3. The sampling date range for the belowground biomass harvest at the NEON CPER site. 6
Table 4. Materials and supplies required for the belowground biomass harvest procedure. 7
Table 5. Materials and supplies required for processing plant belowground biomass samples in the laboratory. 11

LIST OF FIGURES

Figure 1. General layout of an Ecosystem Productivity (EP) plot and associated biomass plots at CPER. . 21
Figure 2. Biomass sampling sub-plot located within Ecosystem Productivity plots. 22
Figure 3. Illustration of a perennial grass, and the location of crown material relative to leaves and the soil surface. 23
Figure 4. Soil corer component parts 24
Figure 5. Laboratory sample processing workflow to prepare dried belowground biomass samples for bioarchive and chemical analysis. 25

1 INTRODUCTION

1.1 Purpose

The primary purpose of this document is to provide a change controlled version of Observatory protocols, and is the version 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 this procedure and protocol.

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.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference documents

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
	QA/PA Plan
	DOORS requirements
	ATBD
	NEON.DOC.000243 NEON Glossary of Terms

2.2 Acronyms

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol
EP	Ecosystem Productivity [plot]
CPER	Central Plains Experimental Range

3 BACKGROUND AND OBJECTIVES

3.1 Background

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.

3.2 Science requirements

This protocol fulfills the following Observatory science requirements:
List science requirements from DOORS that are met by this protocol.

3.3 Data products

List Level 0 data products measured by protocol.

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

Measurement	Data Product

4 PROTOCOL

Belowground biomass represents a substantial component of the total plant biomass and plant carbon in terrestrial ecosystems, yet belowground biomass stocks and turnover remain very poorly understood both in space and in time. This is in large part due to the inherent difficulties associated with measuring plant parts that are obscured within soil. Developing a better understanding of how much belowground plant biomass there is, as well as how much of that biomass is produced and decomposed within a given year, is therefore crucial to improving our understanding of how terrestrial ecosystems respond to environmental changes. The soil core samples that NEON collects will enable estimation of the amount of belowground plant biomass at a site, and data from the NEON soil array will provide an insight into how fast that biomass is produced and decomposed. In combination, these two NEON datasets will allow the calculation of belowground plant productivity on a continental scale.

At the NEON CPER site, there are four Ecosystem Productivity (EP) plots, and five biomass sampling plots per EP plot, for a total of 20 plots sampled for belowground biomass within the tower airshed (Figure 1). Biomass sampling plots are referred to throughout this document as “biomass plots.” In Figure 1, permanent markers along the transect are represented by concentric circles spaced every 50 meters.

These markers are associated with high-resolution GPS coordinates. The red concentric circle shows the EP plot origin. Grey rectangles represent the biomass plots from which soil cores are sampled. Green lines are spaced every 10 m, and mark locations where Leaf Area Index is measured (see additional documents for LAI protocols). A total of 2 soil cores are taken from each biomass sampling plot (Figure 2), for a total of 40 cores that need to be processed in a given year.

Placeholder for Figure 1: General layout of an Ecosystem Productivity (EP) plot and associated biomass plots at the NEON D10 CPER site.

Placeholder for Figure 2: Biomass sampling sub-plot located within Ecosystem Productivity plots.

The following protocol outlines a multi-day method for collecting and processing belowground biomass in order to obtain weight data per unit area, as well as samples suitable for chemical analysis at an external facility.

The draft belowground biomass sampling design for the D10 2011 Field Operations prototype is based on sampling designs and coring techniques developed over 20 years at the SGS-LTER grassland sites, which were also based at the CPER site. It is NEON's desirement to estimate belowground biomass and productivity to within 10% of the mean, and to adequately capture the range of variability of these measurements within the tower airshed. However, at present it is unclear whether the current design will accomplish this goal.

5 QUALITY ASSURANCE AND QUALITY CONTROL

The NEON QA/QC plan for these measurements is under development and TBD.

6 DECISION TREE

Table 2. Decision tree associated with the plant belowground biomass harvest, indicating how to respond to unanticipated delays in field or lab work, and consequences of these delays.

Delay	Action	Adverse outcome	Outcome for Data Product
Hours	If 1) Delay prevents harvesting all 4 cores from a sub-plot: a) Ensure existing core samples are placed in labeled bags; and b) resume harvest of remaining cores from sub-plot asap.	None	None
	If 2) Delay occurs between sub-plot harvests: resume harvest of cores from next sub-plot asap.	None	None
Day	If 1) Delay prevents harvesting all 4 cores from a sub-plot: a) Ensure existing core samples are placed in labeled bags; b) oven-dry cores as per protocol; and c) resume harvesting remaining cores from sub-plot asap.	None	None

Delay	Action	Adverse outcome	Outcome for Data Product
	If 2) Delay occurs between sub-plot harvests: resume harvest of cores from next sub-plot asap.	None	None
2-7 days	If 1) Delay prevents harvesting all 4 cores from a sub-plot: a) Ensure existing core samples are placed in labeled bags; b) oven-dry cores as per protocol; and c) resume harvesting remaining cores from sub-plot asap.	None	None
	If 2) Delay occurs between sub-plot harvests: resume harvest of cores from next sub-plot asap.	None	None
8-13 days	If 1) Delay prevents harvesting all 4 cores from a sub-plot: a) Ensure existing core samples are placed in labeled bags; b) oven-dry cores as per protocol; and c) resume harvesting remaining cores from sub-plot asap.	Belowground biomass sampled per core may change over this length of time.	Increased error in belowground biomass and NPP estimates.
	If 2) Delay occurs between sub-plot harvests: resume harvest of cores from next sub-plot asap.	Belowground biomass sampled per core may change over this length of time.	Increased error in belowground biomass and NPP estimates.
2 or more weeks	If 1) Delay prevents harvesting all 4 cores from a sub-plot: a) Ensure existing core samples are placed in labeled bags; b) oven-dry cores as per protocol; and c) resume harvesting remaining cores from sub-plot asap.	Belowground biomass sampled per core may change over this length of time.	Increased error in belowground biomass and NPP estimates.
	If 2) Delay occurs between sub-plot harvests: resume harvest of cores from next sub-plot asap.	Belowground biomass sampled per core may change over this length of time.	Increased error in belowground biomass and NPP estimates.

7 SAFETY

Personnel working at a NEON site should be familiar with and practice safe field work 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.

For the field procedures, safety training is required to properly use the soil corer.

For the laboratory procedures, safety training is required to operate drying ovens, the root washer, and the Mixing Mill.

8 PERSONNEL REQUIREMENTS

For the field work, a minimum of 2 field technicians is required for harvesting soil cores. This is because the equipment and the cores themselves weigh a considerable amount. Based on the Domain Manager's judgement, it may be necessary to employ more field technicians to complete the soil coring if the equipment + sample weight becomes too onerous for 2 technicians.

Required skills:

- Demonstrated ability to identify crown material associated with perennial grasses.

For the laboratory work, at least one laboratory technician is required to dry, wash, weigh, grind, and sub-sample belowground biomass samples for shipment to external analytical facilities. Two laboratory technicians are preferred due to increased workflow efficiencies achieved with two people.

9 TRAINING REQUIREMENTS

The NEON training plan associated with these activities is under development and TBD.

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling frequency and timing

Belowground biomass fluctuates substantially throughout the year at CPER in a manner that is not readily predictable. As such, there is no predictable belowground biomass peak that constrains the sampling schedule. Instead, soil core sampling is carried out once per year, and should be timed for the spring or early summer when soils are relatively soft and easily cored – i.e. late May to early June. To provide the best temporal link between the belowground biomass harvest and aboveground biomass, coring for belowground biomass shall take place no more than 5 days following the Spring aboveground biomass sampling bout.

We have estimated that for each plot requiring belowground biomass sampling, it will take 1.5 hours of field work (temporary plot delineation, initial removal of litter, clipping of crown biomass, and soil coring). An additional < 30 min are required at the end of the same day to place biomass samples and soil cores into the drying ovens. As such, field technicians should be able to obtain soil core samples from 5 plots per 8 hour day of field work. Given that there are 20 plots that require belowground biomass sampling, it follows that 2 technicians should be able to process all plots in 4 × 8-hour work days (not including travel time).

Table 3. The sampling date range for the belowground biomass harvest at the NEON CPER site.

Domain, Site	Date	Frequency
D10, CPER	5/23/11 – 6/6/11	1X per year

10.2 Contingency decisions

Please see the Decision Tree in Section 6.

10.3 Field procedure

There are 5 biomass plots per EP plot, and four EP plots within the tower airshed. A total of 2 soil cores are harvested from each biomass plot, for a total of 40 soil cores that will need to be processed in a given year for belowground biomass. Prior to harvesting the soil core, crown biomass from perennial grasses is also harvested (see Figure 3); in the short grass steppe ecosystem, crown biomass can be substantial, though crowns grow very slowly and contribute only nominally to productivity in a given year.

Placeholder for Figure 3: Illustration of a perennial grass, and the location of crown material relative to leaves and the soil surface.

10.3.1 Equipment and materials

The equipment listed here is sufficient for a team of 2 people to collect soil cores from biomass plots located within Ecosystem Productivity plots.

Table 4. Materials and supplies required for the belowground biomass harvest procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Soil corer assembly	1 (see Figure 4 for a diagram of required component parts)	NA
Hi-lift jack with bumper-lift	1	NA
Juno SB GPS unit	1	NA
Hand shears	1	NA
Hori-hori knife	1	NA
Paper bags, heavy-duty 25# kraft	40	NA
Paper bags, 8# kraft	40	NA
Pre-printed labels	80	NA
Pre-marked string and stake sets to visually mark plot boundaries and buffers	5	NA
Sharpies	2	NA
Mechanical pencils	2	NA
Rite-in-the-Rain “Field core QC” checklist	1	NA

Placeholder for Figure 4: Soil corer component parts.

10.3.2 Preparation

At least 10 days prior to field work, check to make sure that all consumables required for this field work are available for use (i.e. flagging tape, 25# and 8# kraft paper bags, pre-printable labels, and water resistant paper). Re-order these items as necessary prior to field work.

At least 2 days prior to field work, all necessary field equipment for vegetation structure measurements and aboveground biomass clip harvests shall be assembled to ensure that:

- Batteries are charged in the GPS unit(s)
- Waypoints, layers, and background images are loaded onto the GPS units
- Datasheets and/or QC checklists are printed on water resistant paper and organized into a field binder
- Labels for paper bags used for the biomass harvest are pre-printed, with appropriate space available for data that must be recorded manually in the field
- Pre-marked string and stake sets are fabricated, marked, and labeled
- Soil core assembly is clean and in good working condition
- A 40 cm length is clearly marked on the soil core tube (measured from the bottom of the bit, not the bottom of the soil core tube; use electrical tape to wrap the core tube and mark the correct length)
- Blades on hand shears are sharp

Finally, sufficient space within drying ovens shall be available the same day as biomass harvests occur in the field.

Note that CPER rules require that holes resulting from soil coring shall be backfilled with sand immediately following coring. This prevents injury to livestock, and provides an obvious on-the-ground cue as to where previous soil cores have been harvested. Sand can either be purchased, or can be obtained several days prior to sampling from Owl Creek.

10.3.3 Sample collection in the field: Soil core and crown harvest

1. Use pre-loaded GPS waypoints and the "Field core QC" checklist to locate the EP plot and biomass plot from which soil cores will be sampled. See Figure 1 for a general layout of EP and biomass plots at the site. In Figure 1, biomass plots are marked as small grey rectangles.
2. Within the biomass plot, locate the area adjacent to the correct harvest strip that is to be cored – i.e. the harvest strip just clipped for the Spring aboveground biomass harvest (see Figure 2).
    a. The plot corner that is closest to the origin of the plot center-line is hereafter referred to as the bottom-left corner of the plot (in Figure 2, the bottom-left corner is marked with a circle).
3. Find the permanent markers for the biomass plot corners, and use two sets of the pre-marked strings and stakes to temporarily mark the bottom-left to the bottom-right corners of the plot, and from the top-left to the top-right corners of the plot. Place the remaining two pre-marked string and stake sets along the buffer lines that are parallel to the left and right boundaries of the plot – i.e. the buffer lines marked in red in Figure 2.
    a. Markings on the strings should indicate where plot boundaries, buffer boundaries, and harvest strips are located.
    b. Do not walk in the current year's harvest strip. To avoid trampling the other harvest strips in the plot, walk in the buffer area of the plot during sampling rather than in the harvest area (if possible).

c. Use the "Notes" section of the "Field biomass QC" checklist to record relevant observations – e.g. missing plot markers, vandalization of plots, etc.

4. For each of two soil cores, label one 25# heavy-duty kraft paper bag to hold the core, and another small paper bag to hold any crown material from perennial grasses. Labels may be pre-printed and should include the following information:
    a. Date
    b. Site
    c. EP plot #
    d. Biomass plot #
    e. Core # within biomass plot (this will be either "1" or "2")
    f. Biomass code (either "CROWN" or "ROOT", depending on tissue type).
5. Two 66.5 mm i.d. (3-inch o.d.) × 40 cm length soil cores are taken from the buffer area of the plot that is adjacent to the current year's harvest strip.
    a. See for soil core locations relative to the current year's harvest strip.
    b. If rocks are encountered that prevent coring to 40 cm depth, note this on the "Field core QC" checklist, and choose a new location for the core – e.g. cores may be taken from within the spring harvest strip if necessary.
6. Use hand shears to clip aboveground plant leaves and stems from the area that is to be cored. Clip forbs and sub-shrubs at the soil surface. For perennial grasses, clip just above the crown (approx. 1 cm – 2 cm above the soil surface), and ***DO NOT*** clip crown biomass at this point.
    a. See Figure 3 for an illustration of where crown biomass becomes leaf biomass. Leaves emerge from the top of the crown.
7. Remove all litter from the soil surface by hand.
8. Assemble the soil corer: Attach the corer bit by threading tightly to the bottom of the soil core tube, and use the pin to attach the drive-head assembly and slide hammer to the top of the soil core tube.
    a. See Figure 4 for an illustration of soil corer parts and the assembled soil corer.
9. At the desired coring location, score the ground with the soil corer bit. Then use a hori-hori knife to loosen and remove the soil around any perennial grass crown material growing within the scored area. Remove soil from around the crown until the transition from crown to root material is visible.
    a. See Figure 3 for an illustration of where crown biomass becomes root biomass.
10. Clip crown material from the roots with the hand shears and place crown material into the appropriately labeled small paper bag.
11. Position the soil core bit back over the scored area, and make sure the soil core assembly is perpendicular to the ground. Use the slide hammer to pound the soil corer to 40 cm depth.

a. The 40 cm core length should be pre-marked on the soil core tube with electrical tape. ***This 40 cm length is measured from the bottom of the soil core bit.***

b. ***Once the soil corer is in the ground, do not turn the unit counter-clockwise, as this will unscrew the bit from the core tube underground, resulting in loss of the expensive bit.***

12. Once the soil corer has been pounded to 40 cm depth, follow these steps:
    a. Push the corer back and forth several times to loosen it within the soil profile.
    b. Remove the adapter pin and drive head assembly, then re-insert the adapter pin.
    c. Use the hi-lift jack and bumper-lift chain (hooked onto the adapter pin of the soil corer) to remove the soil corer assembly from the ground.
    d. Remove the core from inside the bit and soil core tube.
    e. Place the core into a labeled heavy-duty 25# kraft paper bag.
    f. Back-fill the hole with sand (either obtained previously from Owl Cr. or purchased).
13. If rocks are encountered that prevent coring to 40 cm depth, remove the corer from the ground as instructed immediately above, and select a new location for coring.
    a. If it is not possible to core in the buffer area of the plot that is adjacent to the current year's harvest strip, select an alternate area within the Spring harvest section of the current year's strip (see Figure 2).
    b. Note that soil cores should be sampled ≥ 50 cm from each other.
14. Fill in the required information on the "Field core QC" checklist to ensure that all biomass plots are sampled for belowground biomass (roots and crowns).
15. If time permits, proceed to additional biomass plots and continue soil core sampling.

10.3.4 Sample preservation

Not applicable to this procedure.

10.3.5 Sample shipping

Not applicable to this procedure.

10.3.6 Data handling

Following field work, all information from field QC checklists should be entered and saved as soon as possible to the appropriate MS Access database or Excel spreadsheet.

10.3.7 Refreshing the field sampling kit

Make sure that the following consumable supplies are available in sufficient quantity for the next time belowground biomass is harvested:

- Paper bags, 25# and 8# kraft
- Pre-printable labels
- Water resistant paper

10.3.8 Equipment maintenance, cleaning, and storage

Maintain equipment for the next sampling day/bout by doing the following:

- Clean the soil core assembly, and check that all parts are in good working condition
- Clean and check blades are sharp on hand shears
- Use the ZTS battery tester to check and recharge batteries (if necessary) for the GPS unit(s)

After completing cleaning and maintenance tasks, return all equipment to the appropriate storage location in the Support Facility laboratory.

11 LAB STANDARD OPERATING PROCEDURE

The following goals are associated with the belowground biomass laboratory procedure: 1) dry crown and soil core samples; 2) wash soil cores to remove soil from root biomass; 3) dry root biomass; 4) weigh and record data for dried crown and root samples; and 5) grind biomass samples, and create sub-samples suitable for shipment to an external facility for chemical analysis.

11.1 Timing

- Harvested soil cores and crown material must be placed within a drying oven the same day as harvest, in order to halt decomposition.
- Following drying, crowns may be weighed any time within the next 30 days.
- Following drying, soil cores may be washed and processed any time within the next 90 days.
- Grinding and sub-sampling of biomass for bioarchive and shipment to external facilities for chemical analysis may take place as time permits.

11.2 Lab procedure

11.2.1 Equipment and materials

Table 5. Materials and supplies required for processing plant belowground biomass samples in the laboratory.

Item Description	Quantity per sampling event	Hazardous Chemical
Drying ovens	2	NA
Mass balance (0.01 g accuracy)	1	NA
Large weigh boats	80	NA
Retsch Mixing Mill	1	NA
20 mL scintillation vials (Wheaton or equivalent)	700	NA
Pre-printable labels	700	NA
Paper bags, 8# kraft	40	NA
Datasheets and QC checklists	1 set	NA
Sharpies	1 per person	NA
Mechanical pencils	1 per person	NA

11.2.2 Preparation

At least 10 days in advance, check inventories of all laboratory consumables (i.e. weigh boats, scint vials, pre-printable labels) to ensure that sufficient time is available to re-order items as needed prior to laboratory sample processing.

At least 2 days prior to the anticipated use date, the root washer in the "wet" lab should be checked to ensure that it is clean and in full working condition.

On the same day that soil core collection occurs in the field, make sure that space within drying ovens is available for crown and soil core samples.

11.2.3 Sample processing in the lab

11.2.3.1 Initial sample drying, and weighing of crowns

On the same day that soil cores and crown material are harvested from the field, return all biomass samples to the Support Facility lab for drying. Immediately drying samples halts decomposition and loss of biomass from root samples, and allows the field crew to focus on harvesting all cores from the field in as short an amount of time as possible.

1. Prior to drying, perform the following checks with the "Lab core QC" checklist:
    a. Count all bags again.
    b. Compare this independent count of the bag numbers and IDs to the field count on the "Field core QC" checklist to make sure no bags have been misplaced.
2. As soon as possible after returning from the field, place sample bags into a 55 ˚C drying oven for 72 h – 120 h (3 d – 5 d).
    a. In order to assess how long each bag has been in the drying oven, label each bag with the date and time it was placed in the oven.
    b. Check that soil cores and crown material are truly dry by sequentially weighing the same sub-set of 5 samples on day 3, 4, 5, etc. Weigh 5 sub-samples of crown material, and 5 sub-samples of the soil cores.
        i. Let soil cores come to room temperature prior to weighing.
    c. Use the "Lab core QC" checklist to record sample weights during drying.
    d. Samples are dry when weight is constant from one day to the next.
3. Once soil core and crown samples are dry, follow (a) below for crown material, and (b) below for soil cores.
    a. Weighing crown material from perennial grasses
        i. Weigh dried crown samples as soon as possible following drying with a mass balance to the nearest 0.01 grams.
        ii. Record all weight data, and all metadata from the paper bag, into the "Belowground biomass" datasheet.

iii. Return the weighed sample to its paper bag, and proceed to the next sample.

iv. Use the "Lab core QC" checklist to ensure that no samples have been missed.

v. Once all weights have been recorded, return all paper bags containing crown material to temporary storage. Samples in temporary storage can then be prepared for tissue chemistry analysis at an external facility as time permits.

b. Dried soil cores and root material

i. Use the "Lab core QC" checklist to make sure all cores are accounted for.

ii. Place dried soil cores into temporary storage for up to 90 days to await further processing as time permits (see section immediately below). Group the 2 cores from the same biomass plot together in order to speed processing at a later date.

11.2.3.2 Preparing and weighing roots from soil core samples

1. There are 2 cores from each biomass plot. As part of the NEON Quality Control procedure, process pairs of cores from the same biomass plot at the same time.
2. Separate root material from soil using the Delta-T root washer located in the Support Facility "wet" lab. The root washer has 4 buckets, allowing simultaneous processing of 4 soil cores.
    a. Place the labeled paper bags that were used to store the soil cores on the root washer so that it is clear which samples are in a given root washer bucket.
    b. Operate the root washer according to the manufacturer's instructions, bearing in mind that filters may require frequent cleaning to remove sediment.
    c. Wash the root samples until they are free of soil and organic matter debris, then manually squeeze out as much water as possible from the clean root sample.
    d. Use the "Lab core QC" checklist to ensure that all soil cores have been washed, and that no samples have been missed.
3. Place washed root material from each core into its own new, small 8# kraft paper bag for drying. Label small paper bags with the following, which may be pre-printed on a self-adhesive label:
    a. Date sampled in the field
    b. Site
    c. EP plot #
    d. Biomass plot #
    e. Core # within biomass plot (either "1" or "2")
    f. Plant tissue type ("ROOT" in this case).
    g. Date drying begins
4. Place root material in a drying oven at 55 ˚C for 72 h – 120 h (3 d – 5 d).
    a. Check that root samples are truly dry by weighing the same sub-set of 5 samples on day 3, 4, 5, etc.

b. Use the "Lab core QC" checklist to record sample weights during drying.

c. Samples are dry when weight is constant from one day to the next.

5. Weigh dried root samples from each core with a mass balance to the nearest 0.01 grams.
    a. Record weight data and all metadata from the paper bag into the "Belowground biomass" datasheet.
    b. Return dried root material to the appropriate labeled paper bag.
    c. Use the "Lab core QC" checklist to ensure that no samples have been missed.
6. Once all weights have been recorded, return bags of dried root samples to temporary storage. Samples in temporary storage can then be prepared as time permits for tissue chemistry analysis at an external facility.

11.2.4 Sample preservation for bioarchive and chemical analysis

The following procedure is similar to that described for aboveground biomass, but a Mixing Mill (MM) is used at all times to grind crown and root biomass rather than a Wiley Mill. The high mineral content of belowground biomass causes the Wiley Mill blades to dull very quickly, and the MM does not suffer from this problem. Crown and root samples are first ground to 1.5 mm with the MM, and this first grind is used for fiber analysis (i.e. lignin, cellulose, hemicellulose). Biomass samples are then re-ground to 0.2 mm with the MM, and samples from this second grind are used for all other chemical analyses. In order to speed throughput, two people may simultaneously perform two tasks: 1) grind biomass samples; and 2) sub-sample previously ground biomass samples with the splitter. See Figure 5 for a flow diagram of the laboratory processing associated with each belowground biomass sample.

Placeholder for Figure 5: Laboratory sample processing workflow to prepare dried belowground biomass samples for bioarchive and chemical analysis.

1. Locate bags of dried biomass in temporary storage that are to be prepared for bioarchive and chemical analyses.
2. For each bag of dried biomass to be ground, prepare eight 20 mL scintillation vials for collection of ground sub-samples. Sub-samples will be used for the following analyses:
    a. Carbon and nitrogen (1 vial); code = CN
    b. "Majors" elemental analysis (1 vial); code = MAJ
    c. Fiber analysis – i.e. cellulose, hemicellulose, and lignin (1 vial); code = FIBER
    d. Ash content (1 vial); code = ASH
    e. Isotope analyses (3 vials); code = ISO
    f. Bioarchive (1 bioarchive container); code = BIO
3. Label each vial with a pre-printed label containing the following information:
    a. Date
    b. Site
    c. EP plot #

d. Biomass plot #

e. Plant material code (CSAG, CSPG, BOGR/BODA, WSPG, OSD, ROOT, CROWN, etc.)

f. Analysis code (listed above)

4. To obtain a relatively homogeneous sample suitable for fiber analysis, pass the dried unprocessed belowground biomass samples from each bag through the Mixing Mill one bag at a time, using a 1.5 mm sieve screen.

   a. Collect ground biomass from each bag in a large beaker or equivalent, and mix thoroughly.

   b. For each core, if more than one bag was collected in the field for a given type of plant material (e.g. 2 bags of crown material from a given core were collected), combine all ground material from the same plant material type and mix thoroughly into one batch.

5. Use a sample splitter with hi-back pans to split out one aliquot of approximately 2 g sample ground to 1.5 mm size.

   a. This is the sub-sample used for fiber analysis. Place the sub-sample into the 20 mL scint vial labeled with "FIBER".

   b. Clean the splitter between samples using compressed air.

6. Install the 0.2 mm sieve screen into the MM, and pass the remainder of the 1.5 mm ground samples through the mill again. This will create a fine, homogeneous powder suitable for all chemical analyses (except the FIBER analysis).

7. Use either the sample splitter or a scoopula to divide the 0.2 mm ground sample among the seven remaining 20 mL scintillation vials. The splitter will generate considerable amounts of dust if ground material is poured too quickly.

   a. The "CN" and "ISO" vials will require ~0.25 g ground plant material each.

   b. The remaining ground plant material should be distributed evenly between the "MAJ", "ASH", and "BIO" vials.

   c. If there is very little sample to work with, and the "MAJ", "ASH" and "BIO" vials ultimately contain < 0.25 g ground material each, then re-distribute the 0.2 mm ground biomass equally among all 7 of the "CN", "ISO", "MAJ", "ASH", and "BIO" vials.

8. Clean the Mixing Mill with 70% or 95% ethanol, KimWipes, and compressed air in between grinding the contents of each biomass bag (or group of bags of the same plant material type).

9. Use the "Biomass prep QC" checklist to ensure that all bags of dried biomass are processed, and that all sub-samples are accounted for (i.e. 8 scint vials per sample).

11.2.5 Sample shipping

Collate sub-sample vials with the same analysis code, and prepare for shipment to the appropriate external facility for analysis. For example, "CN" vials from all samples should be grouped together, etc. Record the following in the "Biomass prep QC" checklist:

1. The address of the external analytical facility to which samples are shipped.
2. The contact name and telephone number of the sample manager at the external facility.
3. The date of shipment.

The Domain Lab Manager should record in the "Biomass prep QC" checklist the date that data are received from an external analytical facility for a given batch of sub-samples.

11.2.6 Data handling

Enter crown and root biomass data from the "Belowground biomass" datasheet(s) into the appropriate MS Access database or Excel spreadsheet as soon as possible after recording the data.

Following initial grinding and sub-sampling, enter data from the "Biomass prep QC" checklist into the appropriate MS Access database or Excel spreadsheet. Once data are received from an external facility, the Domain Manager should coordinate entry of additional QC data (e.g. which datasets have been received from whom, turnaround time, etc.) and sample analysis data into the appropriate MS Access database or Excel spreadsheet. The NEON Plant Ecologist or Biogeochemist is responsible for performing any further QA/QC analysis on the returned data.

11.2.7 Refreshing the laboratory supplies

Make sure the following consumable supplies are available in sufficient quantity for processing the next round of belowground biomass samples:

- Large weigh boats
- 20 mL scintillation vials (Wheaton or equivalent)
- Pre-printable labels
- Paper bags, 8# kraft

Consult with the Domain Manager to re-order consumable supplies as necessary.

11.2.8 Laboratory maintenance, cleaning, and storage

At the end of every day, the Retsch Mixing Mill and the sample splitter require cleaning with ethanol (either 70% or 95%) and compressed air. These pieces of equipment also require cleaning between different samples or between different batches of similar samples (as described in the procedure above).

12 DEFINITIONS

Define all protocol specific technical terms in alphabetical format.

13 REFERENCES

Use Ecology style.

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.

Field datasheets to be prepared for the D10 2011 Field Ops prototype include:

- None – no data (other than QC data) are collected in the field for this procedure.

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.

Laboratory datasheets to be prepared for the D10 2011 Field Ops prototype include:

- Belowground biomass

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

APPENDIX D Procedure checklist

Field and laboratory QC checklists to be prepared for the D10 2011 Field Ops prototype include:

- Field core QC
- Lab core QC
- Biomass prep QC

APPENDIX E Figures

Figure 1. General layout of an Ecosystem Productivity (EP) plot and associated biomass plots at CPER.

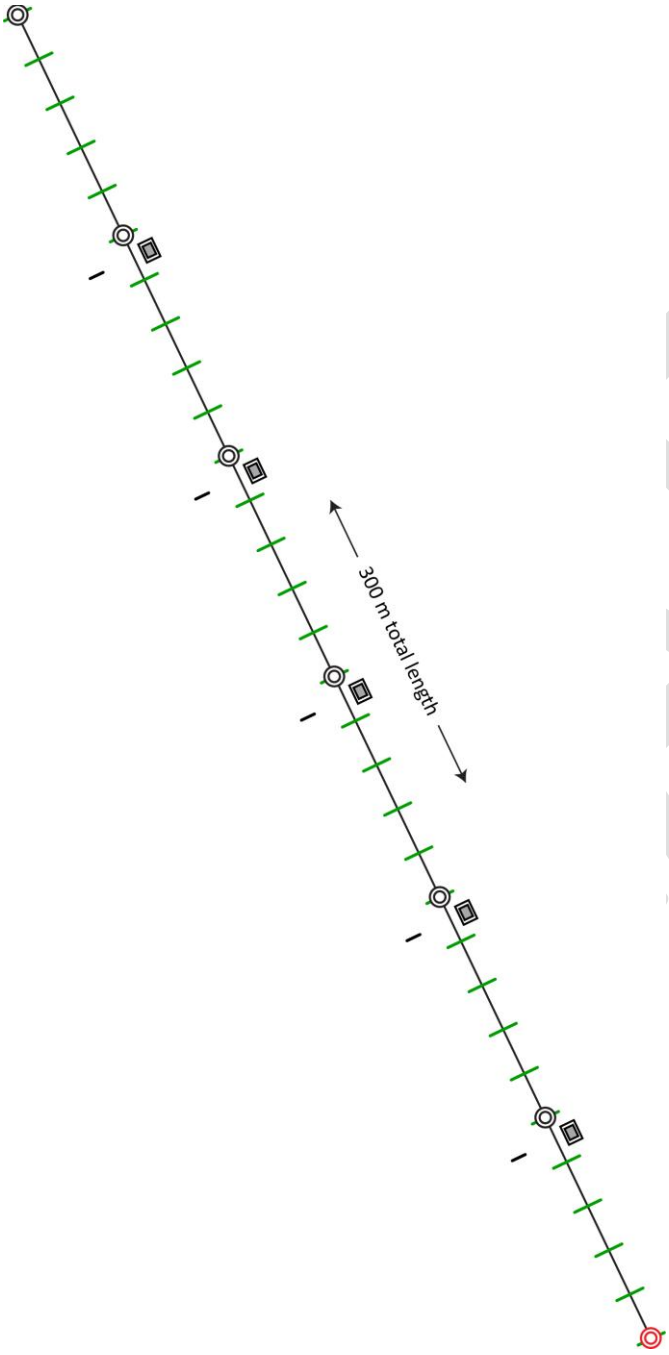

Figure 2. Biomass sampling sub-plot located within Ecosystem Productivity plots.

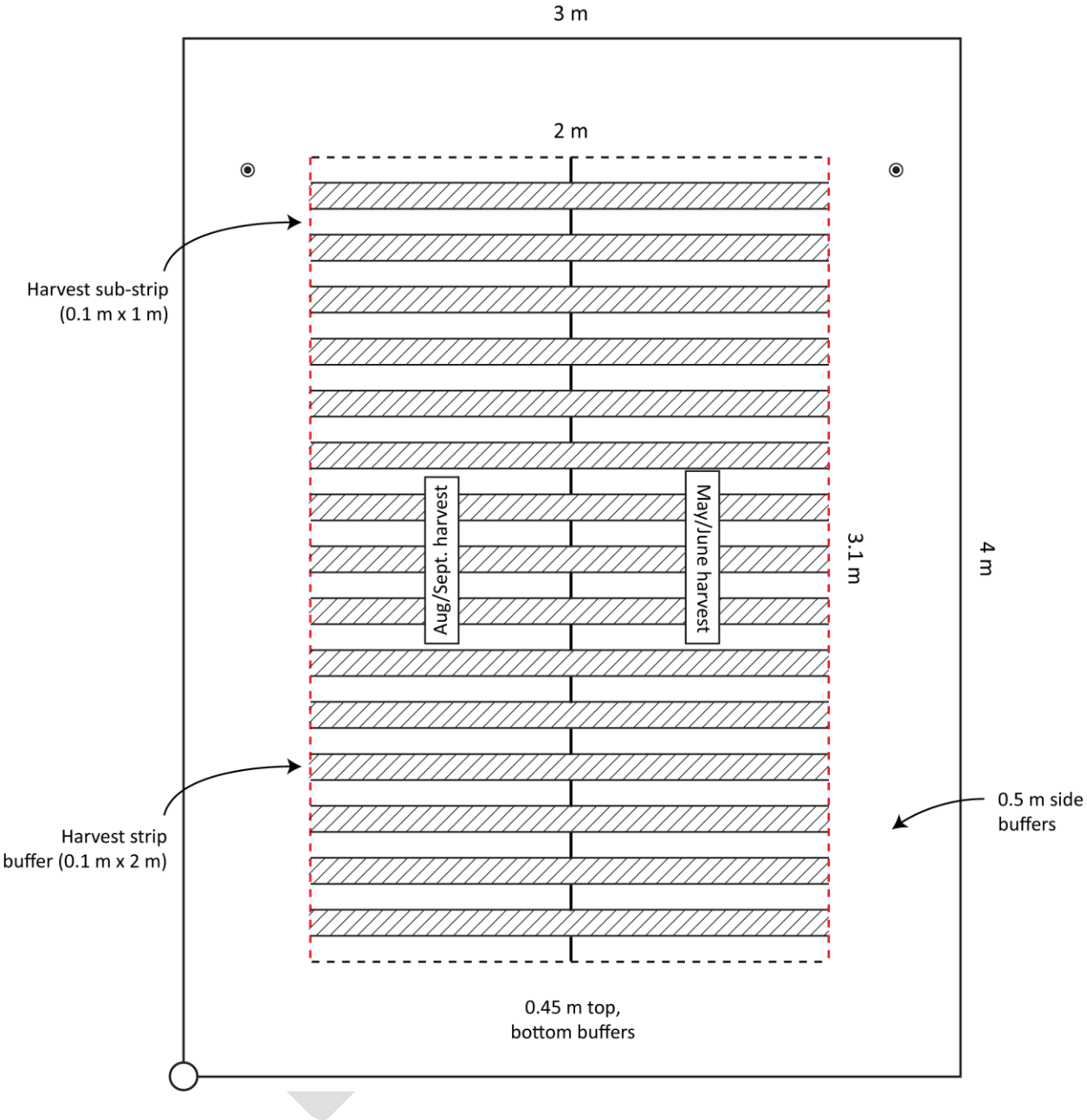

Figure 3. Illustration of a perennial grass, and the location of crown material relative to leaves and the soil surface.

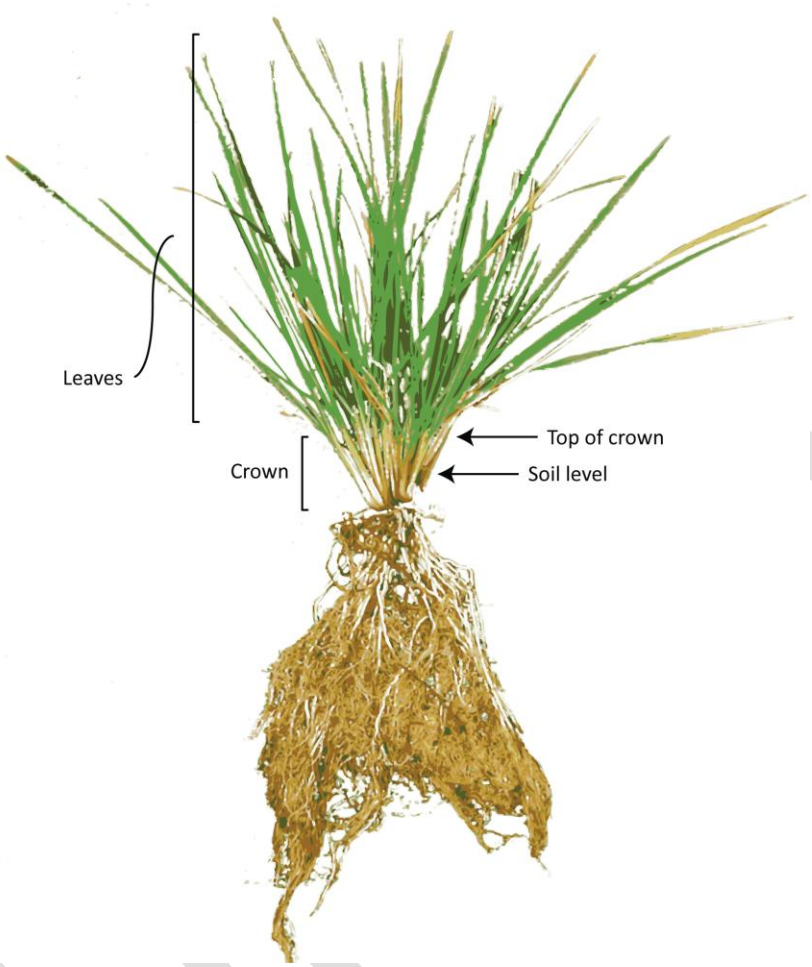

Figure 4. Soil corer component parts.

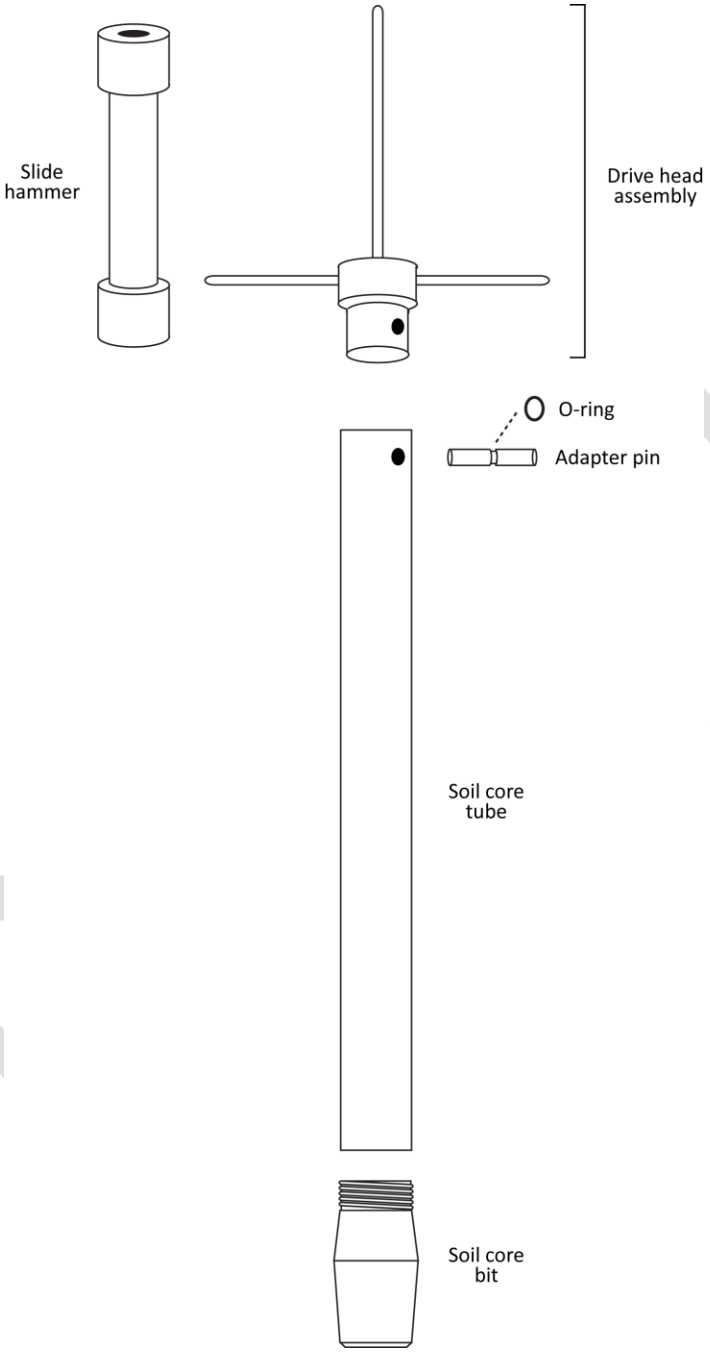

Figure 5. Laboratory sample processing workflow to prepare dried belowground biomass samples for bioarchive and chemical analysis.