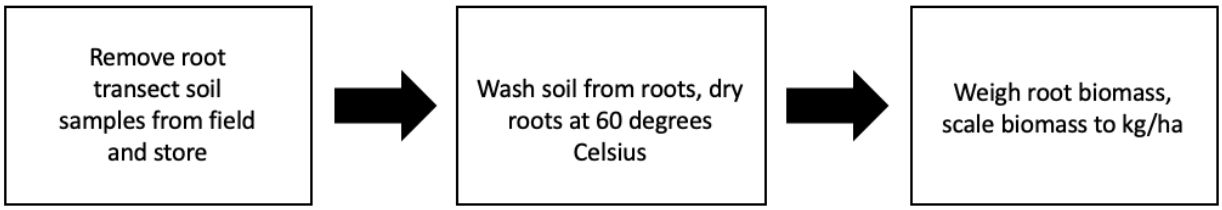
SOP: **Belowground Biomass: Root Transect Sampling**

Overview:

This standard operating procedure (SOP) describes a protocol for in-field sampling and lab sample processing to quantify belowground root biomass using the root transect method. Collecting below-ground root biomass with this method requires no preparation before sampling, but does require a moderate amount of labor to extract samples from the field due to many replicated auger sampling points. Belowground Biomass: In-growth Root Cores SOP describes an alternative method for sampling root biomass with more preparation requirements, but a more streamlined and less complicated field-sampling approach. This Root Transect Sampling method was originally reported by Frasier et al. (2016). Key instruments include soil auger, gallon-size plastic bags, 5-gallon buckets, 2.0 mm sieve, sieve holder, hose with nozzle, tweezers, and metal tins. After field removal, root transect samples are always stored at 4 °C until sample processing.



Safety:

All standard safety protocols and online safety training via UIUC Division of Research Safety (DRS) are required.

Instrumentation:

Field Sampling

- Soil sampling (0.02 m diameter) auger, 35 cm depth
- Gallon size plastic, resealable bags
- Measuring stick as large or larger than space between crop rows

Sample Processing

- At least two 5-gallon buckets
- 2.0 mm sieve
- Hose with nozzle attachment, water source
- Two narrow boards – something to rest sieve over bucket

- Tweezers
- Medium size metal tins (oven safe)

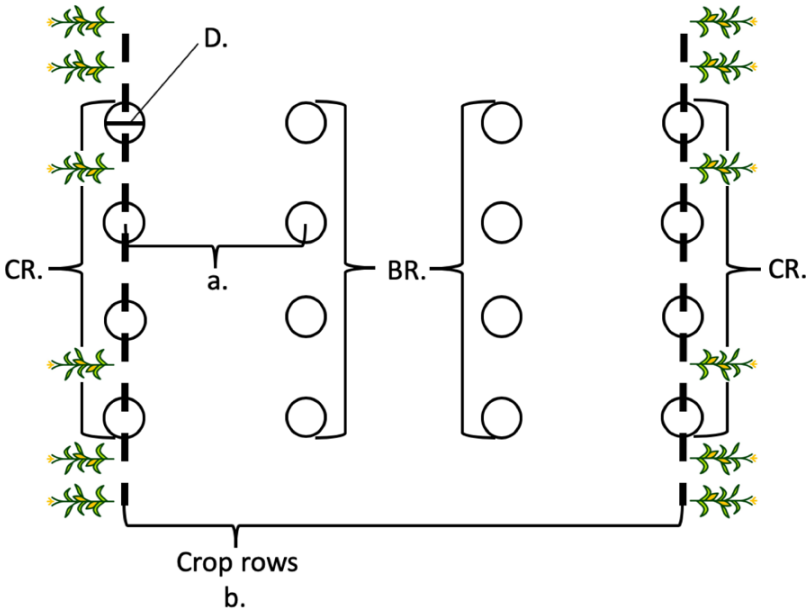
Detailed Procedure:

Note: Considering variability of crops and row spacing, sampling procedure and calculation template should be adjusted to suit specific sampling requirements. Measure row spacing before sampling to determine sampling distance and adjust procedure and calculations accordingly. Depth of auger sampling is set in this procedure as 35 cm, which is appropriate for both soybean and corn. Depending on the research question, some of these measurements (including depth, spacing, and number of transect rows taken) may be adjusted.

I. Field Sampling

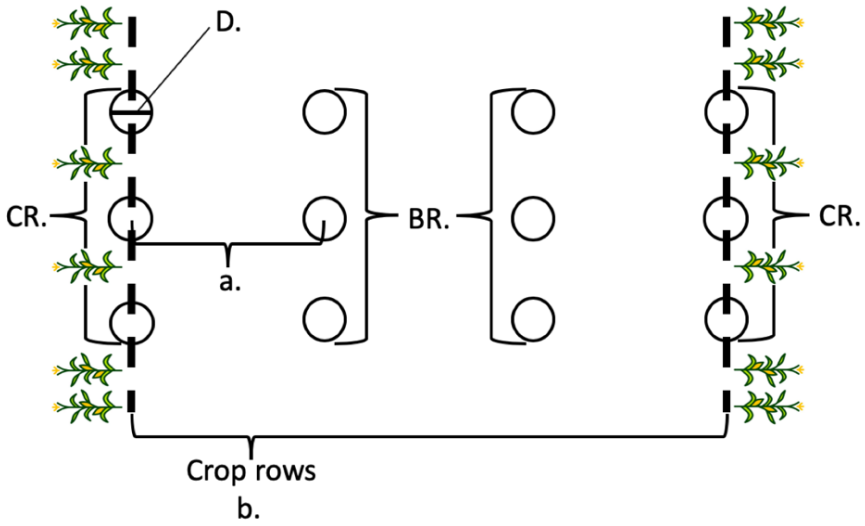
1. Determine space between crop rows. Using a measuring stick, determine sampling points for 4 equidistant sampling points between two crop rows. *I.e., if crop rows are spaced 76.2 cm apart, take samples at: 0 cm (in-row), 25.4 cm (between-row), 50.8 cm (between-row), and 76.2 cm (in-row).* Refer to figure below for sampling diagram.
2. Using the auger, sample soils to 35” depth (for corn or soybean crop) at each equidistant sampling point.
   Composite all samples taken from <u>in-row</u> sampling points in one plastic resealable bag labeled with corresponding ID. Composite all other samples taken from <u>between-row</u> sampling points in separate plastic resealable bag with corresponding ID.
3. Store all samples in their air-tight, resealable bags in cold storage at 4 °C until processing.

a. Equidistant sampling points
b. Distance between rows
D. Auger diameter
BR. Between row sampling points (8)
CR. Crop row sampling points (8)



Above: 4x4 sampling diagram. 8 points will be composited to make each (CR, BR) sample.

a. Equidistant sampling points
b. Distance between rows
D. Auger diameter
BR. Between row sampling points (6)
CR. Crop row sampling points (6)



Above: 3x4 sampling diagram. 6 points will be composited to make each (CR, BR) sample.

II. Sample Processing

1. Weigh metal tins and record weight of each to nearest mg (0.001 g). Label with corresponding sample ID and set aside.
2. Place contents of one bag of soil / roots sample into 5-gallon bucket. Fill bucket with water until all soil / roots are submerged. Keep track of corresponding sample ID, label bucket or place empty labeled sample bag under bucket during soak.
3. Let soak in cool or room temperature overnight (~8 hours) or until the soil has slaked such that the majority of material falls away from roots. It is helpful to gently mix by hand at least once during soak to further break down soil aggregates.

4. Place boards (or whatever is accessible to hold sieve) on top of an empty bucket, open side up. Or, use sieve in a sink with a screen over the drain to avoid soil build up in the drain and allows for roots that may pass through the sieve to be caught before they are lost.

5. Place 2.0 mm (or similar, no larger than 2.8 mm) sieve on top of boards. Pour slaked soil-roots-water mixture over sieve and gently rinse soil through sieve with water, into bucket. Water and soil that pass through the sieve into bucket are waste.
   Note: Depending on crop, typical root size will differ. If a significant amount of root biomass (>5%) is falling through the sieve into the waste bucket, adjust to a small enough sieve size to mitigate root biomass loss.
   If you notice loss while washing a sample, run contents of waste bucket through a smaller sized sieve to recover root biomass, and evaluate the appropriately sized sieve necessary for your sample set.
6. Tweeze out all roots from sieve and place them into labeled metal tin. Dispose of soil that has been separated from roots.

7. Continue steps 5 & 6 until all of the soil-roots-water mixture has been used.
8. Dry tins with root tissue in oven at 60 degrees Celsius for 24 - 48 hours until constant weight is achieved.
9. Once dried, record weight of tin + roots.

III. Calculations

Measurement of belowground root biomass is usually expressed in units of g/m^{-2}, and then can be scaled to kg/ha. To input data and calculate, see corresponding Excel calculation template.

1. Calculate the influence-percentage (I%) using diameter of soil sampling auger (D = 0.02 m) and distance between crop rows (b.). CR = in crop row, BR = between crop row.

$$I_{BR}\ (\%) = [(b - (D \times 2))/b] \times 100$$
$$I_{CR}\ (\%) = (D \times 2/b) \times 100$$

2. Using I% (I_{BR}), calculate between-row dry root biomass in g m^{-2}.

*(total mass dry weight BR/number of points BR/(Pi*D^2/4))*IBR/100*
=
$BR\ (g\ m^{-2}) = [(\sum\ dry\ weight\ BR)/(\pi \times D2/4 \times number\ of\ points\ BR)] \times (I_{BR}/100)$

3. Using I% (I_{CR}), calculate in crop-row dry root biomass in g m^{-2}.

*(total mass dry weight CR/number of points CR/(Pi*D^2/4))*ICR/100*
=
$CR\ (g\ m^{-2}) = [(\sum\ dry\ weight\ CR)/(\pi \times D2/4 \times number\ of\ points\ CR)] \times (I_{CR}/100)$

4. Sum crop-row and between-row root dry root biomass to determine the total root biomass (TBR) g m^{-2}.

$$TBR\ (g\ m^{-2}) = BR + CR$$

IV. Example Calculation

1. Calculate I_{BR} (%) and I_{CR} (%) for soybean rows planted 15” (b = 0.381 m) apart. Sampling auger diameter is 0.020 m.

***IBR** (%) = [(b − (D × 2))/b] × 100*
=
*((0.381-(2*0.02))/0.381)*100*
= ***89.501%***

***ICR** (%) = (D × 2/b) × 100*
=
*(0.02*2/0.381)*100*
= ***10.499%***

2. Calculate between-row dry root biomass in g m^{-2}. For this example, there are 6 composited auger samples taken from between-row locations. The sample between-row root biomass dry weight = 0.017 g.

***BR (g m^{-2})** = [(∑ dry weight BR)/(π × D2/4 × number of points BR)] × (I_{BR}/100)*
=
*(0.017/6/(PI()*0.02^2/4))*(89.501/100)*
= ***8.072 g m^{-2}***

3. Calculate <u>in-row</u> dry root biomass in g m^{-2}. For this example, there are 6 composited auger samples taken from in-row locations. The sample in-row root biomass dry weight = 0.023 g.

   CR (g m^{-2}) *= [(∑ dry weight CR)/(π × D2/4 × number of points CR)] × (I_{CR}/100)*
   =
   *(0.023/6/(PI()*0.02^2/4))*(10.499/100)*
   = *1.281 g m^{-2}*

4. Sum crop-row and between-row root dry root biomass to determine the total root biomass (TBR) g m^{-2}.

   TBR (g m^{-2}) *= BR + CR*
   =
   8.072 + 1.281 **= *9.353 g m^{-2}***

5. Multiply g m^{-2} value by 10,000 and then divide by 1000 to scale to kg/ha, effectively multiplying the g m^{-2} value by 10.

   9.353 x 10 **= *93.53 kg/ha.***

(Note: this is a relatively low amount of below-ground biomass)

References:

Frasier, Ileana et al. “Direct field method for root biomass quantification in agroecosystems.” *MethodsX* vol. 3 513-9. 4 Aug. 2016. doi:10.1016/j.mex.2016.08.002

Suggested reading:

Amato, M., Pardo, A. “Root length and biomass losses during sample preparation with different screen mesh sizes.” *Plant Soil* **161,** 299–303 (1994). https://doi.org/10.1007/BF00046401

Frasier, Illeana, et al. “Soil type, land-use and -management as drivers of root-C inputs and soil C storage in the semiarid pampa region, Argentina.” *Soil and Tillage Research*, vol 192, 134-143 (2019) ISSN 0167-1987. https://doi.org/10.1016/j.still.2019.05.010.

Frasier, Ileana et al. “Direct field method for root biomass quantification in agroecosystems.” *MethodsX* vol. 3 513-9. 4 Aug. (2016) doi:10.1016/j.mex.2016.08.002

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