HBP D11 2022 Sorting Subsample Experiment

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2022-10-05

Goal

To determine the efficacy of subsampling various proportions of HBP samples in D11 to reduce long sort times associated with removing OSD from current-year clipped biomass. **Subsampling is only evaluated in the context of clip harvests that do not require sorting to functional group** (i.e., non-peak biomass clips), because the subsample to total mass ratios will not apply to individual herbGroups.

Experimental Setup and Analyses

- Select n=10 plots (all plots for OAES), resulting in a maximum of n=20 `clipID` to test, due to both `exclosure = Y and N` for each plot. Random spatially-balanced plot locations, and locations of clipIDs within plots, will provide an unbiased estimate of biomass throughout the Tower airshed.
- For each `clipID` harvested in the field, test subsampling efficiency at various levels of sorting by creating subsamples (current-year + OSD) with the following percentages of the total `freshMass`:
  - 10%
  - 15%
  - 25%
  - 50%

  The sum of all the subsamples = 100%; that is, the fresh mass of the entire clip strip.
- When subsampling is employed, calculate dryMass as follows: `dM = fM * (ssDM/ssFM)`, where:
  - dM = dryMass of current-year biomass in the clipID (no OSD)
  - fM = total freshMass in the clipID (current-year + OSD)
  - $ssDM$ = subsampleDryMass of current-year biomass in the subsample (no OSD)
  - $ssFM$ = fresh mass of all biomass in the subsample (current-year + OSD)
- Compare `dryMass` results calculated via subsampling with `dryMass` obtained with no subsampling, and use mixed effects models to analyze results.

Procedure

1. Perform clip harvest in the field as normal, and bring clipped biomass back to the laboratory in cold storage as normal.
2. Identify up to n=20 clipIDs (n=10 for `exclosure = Y` and n=10 for `exclosure = N`), originating from each of the 10 plotIDs.
3. Thoroughly mix biomass from each `clipID` to homogenize as thoroughly as possible.
   a. For large amounts of biomass, and when there is more than one bag of biomass for a given `clipID`, use a large bag, box, tray or equivalent vessel to mix the biomass.
4. For each clipID, weigh and record to 0.01 g:

a. **freshMass** = total fresh mass in the clipID (current-year + OSD).
b. It is important that **freshMass** for the entire **clipID** and subsample fresh masses (below) are collected for a given **clipID** as close to each other in time as possible. That is, avoid weighing **freshMass** for a given **clipID** hours apart from the subsample fresh masses as water loss will affect the experimental results.

5. Based on the **freshMass**, calculate the desired subsample fresh masses for testing. For example, assuming **freshMass** = 100 g, the target subsample fresh masses are:
   a. 10% subsample –> 10 g
   b. 15% subsample –> 15 g
   c. 25% subsample –> 25 g
   d. 50% subsample –> 50 g
6. Label a coin envelope for each subsample above with the information below.
   a. **subsampleTest**: 10%, 15%, 25% or 50%
   b. **clipID**
   c. **collectDate**
   d. **exclosure**: Y/N
7. Weigh each fresh subsample created above (current-year + OSD), and record the information below.
   a. **subsampleTest**: as above
   b. **clipID**
   c. **collectDate**
   d. **exclosure**: Y/N
   e. **subsampleFreshMass**: To the nearest 0.01 g; for subsamples < 0.5 g total mass, weigh to the nearest 0.0001 g
8. Sort current-year biomass from OSD for each subsample, and place sorted, current-year biomass into the corresponding labeled coin envelope.
   a. Sorted OSD may be discarded at this point.
9. Dry subsamples until dry; minimum of 48 h @ 65 $^{\circ}$C, track drying progress as normal.
10. Remove dry samples from the oven one at a time, and immediately weigh and record:
    a. **subsampleDryMass**: To the nearest 0.01 g; for masses < 0.5 g, weigh to the nearest 0.0001 g.