HBP D11 OAES 2023 Sorting Subsample Experiment

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Goal

To determine the efficacy of subsampling various proportions of HBP samples at the D11 OAES site 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

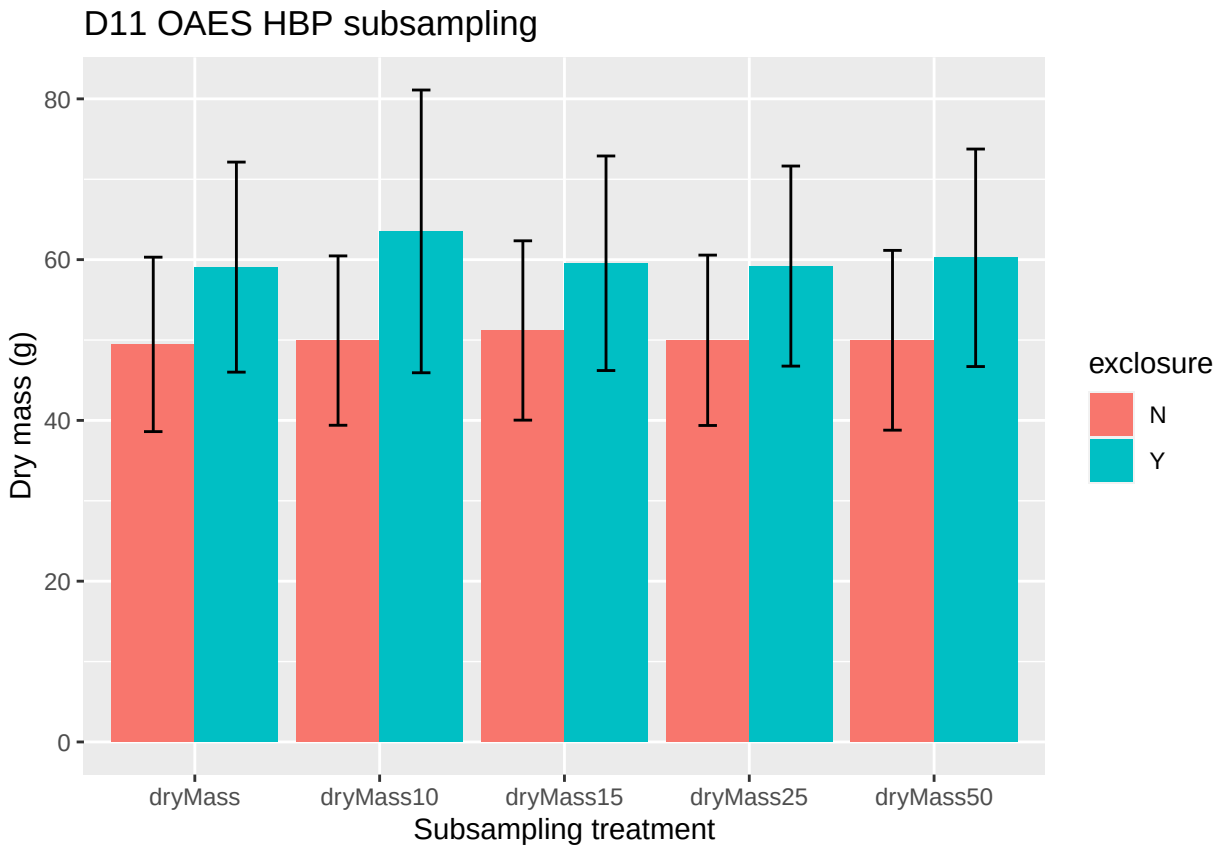
1. Perform clip harvest in the field as normal, and bring clipped biomass back to the laboratory in cold storage as normal.
2. Identify up to n=20 clipIDs (n=10 for `exclosure = Y` and n=10 for `exclosure = N`), originating from each of the 10 plotIDs.
3. Thoroughly mix biomass from each `clipID` to 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 °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.

Data summary

```
## `summarise()` has grouped output by 'treatment'. You can override using the
## `.groups` argument.
```



Results: Mixed-Effects model analysis

1. Models accounting for subsampling effect on **dryMass** and using **clipID** as a random effect (no need to use **exclosure** as a fixed effect since random effect accounts for variation across exclosure treatment).

Model: Data from eventID = "HBP.2023.OAES.24" only

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: estDryMass ~ treatment + (1 | clipID)
##    Data: longDF %>% dplyr::filter(eventID == "HBP.2023.OAES.24")
##
##      AIC      BIC   logLik deviance df.resid
##    672.6    690.9   -329.3    658.6       93
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.9356 -0.3435 -0.0927  0.4176  3.8149
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 199.77   14.134
##  Residual              19.19    4.381
## Number of obs: 100, groups:  clipID, 20
##
## Fixed effects:
##                    Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)         32.3385     3.3088 23.0967   9.774 1.13e-09 ***
```

```
## treatmentdryMass10  -0.0150     1.3852 80.0000  -0.011    0.991
## treatmentdryMass15   0.2005     1.3852 80.0000   0.145    0.885
## treatmentdryMass25   1.4835     1.3852 80.0000   1.071    0.287
## treatmentdryMass50   0.4175     1.3852 80.0000   0.301    0.764
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) trtM10 trtM15 trtM25
## trtmntdrM10 -0.209
## trtmntdrM15 -0.209  0.500
## trtmntdrM25 -0.209  0.500  0.500
## trtmntdrM50 -0.209  0.500  0.500  0.500
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## treatment 30.896   7.724     4    80  0.4025 0.8063
```

Model: Data from eventID = "HBP.2023.OAES.36" only

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: estDryMass ~ treatment + (1 | clipID)
##    Data: longDF %>% dplyr::filter(eventID == "HBP.2023.OAES.36")
##
##      AIC      BIC   logLik deviance df.resid
##    840.2    858.0   -413.1    826.2       88
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.8516 -0.2981  0.0556  0.1988  7.0689
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 4707.1   68.61
##  Residual              122.2   11.05
## Number of obs: 95, groups:  clipID, 19
##
## Fixed effects:
##                    Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)         77.5984    15.9427 19.7910   4.867 9.59e-05 ***
## treatmentdryMass10   5.1689     3.5862 76.0000   1.441    0.154
## treatmentdryMass15   2.0226     3.5862 76.0000   0.564    0.574
## treatmentdryMass25  -0.9163     3.5862 76.0000  -0.256    0.799
## treatmentdryMass50   1.2926     3.5862 76.0000   0.360    0.720
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) trtM10 trtM15 trtM25
## trtmntdrM10 -0.112
## trtmntdrM15 -0.112  0.500
## trtmntdrM25 -0.112  0.500  0.500
## trtmntdrM50 -0.112  0.500  0.500  0.500
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
```

```
##           Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## treatment 415.43  103.86     4    76  0.8501  0.498
```

Model: Data from both eventIDs

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: estDryMass ~ treatment * eventID + (1 | clipID)
##    Data: longDF
##
##      AIC      BIC   logLik deviance df.resid
##   1605.2   1644.5   -790.6   1581.2      183
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.7691 -0.2097  0.0132  0.2436  9.3973
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 2395.63  48.945
##  Residual               69.36   8.328
## Number of obs: 195, groups:  clipID, 39
##
## Fixed effects:
##                                            Estimate Std. Error       df t value
## (Intercept)                                 32.3385    11.1018  40.8116   2.913
## treatmentdryMass10                          -0.0150     2.6337 156.0000  -0.006
## treatmentdryMass15                           0.2005     2.6337 156.0000   0.076
## treatmentdryMass25                           1.4835     2.6337 156.0000   0.563
## treatmentdryMass50                           0.4175     2.6337 156.0000   0.159
## eventIDHBP.2023.OAES.36                     45.2599    15.9055  40.8116   2.846
## treatmentdryMass10:eventIDHBP.2023.OAES.36   5.1840     3.7733 156.0000   1.374
## treatmentdryMass15:eventIDHBP.2023.OAES.36   1.8221     3.7733 156.0000   0.483
## treatmentdryMass25:eventIDHBP.2023.OAES.36  -2.3998     3.7733 156.0000  -0.636
## treatmentdryMass50:eventIDHBP.2023.OAES.36   0.8751     3.7733 156.0000   0.232
##                                            Pr(>|t|)
## (Intercept)                                 0.00578 **
## treatmentdryMass10                          0.99546
## treatmentdryMass15                          0.93941
## treatmentdryMass25                          0.57405
## treatmentdryMass50                          0.87425
## eventIDHBP.2023.OAES.36                     0.00690 **
## treatmentdryMass10:eventIDHBP.2023.OAES.36  0.17146
## treatmentdryMass15:eventIDHBP.2023.OAES.36  0.62984
## treatmentdryMass25:eventIDHBP.2023.OAES.36  0.52571
## treatmentdryMass50:eventIDHBP.2023.OAES.36  0.81690
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) trtM10 trtM15 trtM25 trtM50 eIDHBP tM10:I tM15:I tM25:I
## trtmntdrM10 -0.119
## trtmntdrM15 -0.119  0.500
## trtmntdrM25 -0.119  0.500  0.500
## trtmntdrM50 -0.119  0.500  0.500  0.500
```

```
## eIDHBP.2023 -0.698  0.083  0.083  0.083  0.083
## tM10:IDHBP.  0.083 -0.698 -0.349 -0.349 -0.349 -0.119
## tM15:IDHBP.  0.083 -0.349 -0.698 -0.349 -0.349 -0.119  0.500
## tM25:IDHBP.  0.083 -0.349 -0.349 -0.698 -0.349 -0.119  0.500  0.500
## tM50:IDHBP.  0.083 -0.349 -0.349 -0.349 -0.698 -0.119  0.500  0.500  0.500
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                   Sum Sq Mean Sq NumDF DenDF F value   Pr(>F)
## treatment         156.97   39.24     4   156  0.5658 0.687851
## eventID           602.74  602.74     1    39  8.6897 0.005381 **
## treatment:eventID 299.22   74.80     4   156  1.0785 0.369134
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2. Null model, and using **clipID** as a random effect. Theoretically, if all subsampling results in the same dryMass as the full sort, the null model should be no different than the "treatment" model.

```
## Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's
##   method [lmerModLmerTest]
## Formula: estDryMass ~ 1 + (1 | clipID)
##    Data: longDF
##
##      AIC      BIC   logLik deviance df.resid
##   1601.3   1611.2   -797.7   1595.3      192
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.9879 -0.1959 -0.0171  0.1825  9.6311
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 2931.93  54.147
##  Residual               72.22   8.498
## Number of obs: 195, groups:  clipID, 39
##
## Fixed effects:
##             Estimate Std. Error     df t value Pr(>|t|)
## (Intercept)   55.340      8.692 39.000   6.367  1.6e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Data: longDF
## Models:
## m4ME: estDryMass ~ 1 + (1 | clipID)
## m3ME: estDryMass ~ treatment * eventID + (1 | clipID)
##      npar    AIC    BIC  logLik deviance  Chisq Df Pr(>Chisq)
## m4ME    3 1601.3 1611.2 -797.67   1595.3
## m3ME   12 1605.2 1644.5 -790.60   1581.2 14.151  9     0.1171
```

3. Linear model, using treatment*exclosure, and no random effect.

```
##
## Call:
## lm(formula = estDryMass ~ exclosure * treatment + eventID, data = longDF)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -79.356 -20.643  -5.911   8.659 292.004
##
## Coefficients:
##                                Estimate Std. Error t value Pr(>|t|)
## (Intercept)                     27.6124    12.1799   2.267   0.0246 *
## exclosureY                       8.3994    16.3104   0.515   0.6072
## treatmentdryMass10               0.4747    16.5170   0.029   0.9771
## treatmentdryMass15               1.7300    16.5170   0.105   0.9167
## treatmentdryMass25               0.5095    16.5170   0.031   0.9754
## treatmentdryMass50               0.5121    16.5170   0.031   0.9753
## eventIDHBP.2023.OAES.36         46.1195     7.2963   6.321 1.91e-09 ***
## exclosureY:treatmentdryMass10    3.9698    23.0648   0.172   0.8635
## exclosureY:treatmentdryMass15   -1.2515    23.0648  -0.054   0.9568
## exclosureY:treatmentdryMass25   -0.3805    23.0648  -0.016   0.9869
## exclosureY:treatmentdryMass50    0.6469    23.0648   0.028   0.9777
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 50.91 on 184 degrees of freedom
## Multiple R-squared:  0.186,  Adjusted R-squared:  0.1417
## F-statistic: 4.203 on 10 and 184 DF,  p-value: 2.911e-05
```

```
## Analysis of Variance Table
##
## Response: estDryMass
##                       Df Sum Sq Mean Sq F value    Pr(>F)
## exclosure              1   5079    5079  1.9595    0.1632
## treatment              4    147      37  0.0142    0.9996
## eventID                1 103552  103552 39.9548 1.912e-09 ***
## exclosure:treatment    4    157      39  0.0151    0.9995
## Residuals            184 476876    2592
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Summary of subsampling results

Table 1: Difference in estimated dryMass to total dryMass for subsampling (%)

eventID	ss10	ss15	ss25	ss50
HBP.2023.OAES.24	0.0	0.6	4.6	1.3
HBP.2023.OAES.36	6.7	2.6	-1.2	1.7

Conclusions

Summary of D11 OAES results:

1. Random effect associated with **clipID** explains considerable variability in the data. See summary outputs for all mixed-effects models.
2. Summary output for Mixed Effects model indicates that D11 OAES Clip Strips can be subsampled at the 15% level for all bouts with **herbGroup** = ALL.
    - Comparing results from two separate bouts, estimates of dryMass using a 10% subsample are more variable relative to total dryMass from the full sort compared to other subsampling levels, even though the difference is not significant.
    - When eventIDs are analyzed separately (m1ME, m2ME), other subsamples are not significantly different than the entire sorted subsample ($p > 0.29$) –> go with 15% subsample.
    - With both eventIDs analyzed together, there are no differences between the full sort dryMass and estimated dryMass from subsampling ($p = 0.68$).
3. Effect of `exclosure*treatment`, as evaluated in standard linear model (m1LM):
    - Effect of `exclosure` is not significant ($p = 0.16$).
    - Effect of `treatment` not significant ($p = 0.99$).
    - No detection of `exclosure:treatment` interaction effect ($p = 0.99$).
    - Effect of `eventID` highly significant ($p < 0.0001$)