HBP D17 2017/2018 Sorting Subsample Experiment

Courtney Meier

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Goal

To determine the efficacy of subsampling various proportions of HBP samples in D17 in order 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 (10 lowest Morton Order), resulting in 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 procedure by creating subsamples (current-year + OSD) with the following percentages of the total freshMass:
  - 10%
  - 25%
  - 50%
  - 100% (no subsampling)
- 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 paired t-test 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 n=20 clipIDs (n=10 for `exclosure = Y` and n=10 for `exclosure = N`), originating from the 10 plotIDs with the lowest Morton Order numbers.
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)
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. 25% subsample –> 25 g
c. 50% subsample –> 50 g

6. Label a coin envelope for each subsample above with the information below. Label an additional coin envelope for the residual clipped biomass that was not subsampled.

   a. **subsampleTest**: 10%, 25% or 50%; use **subsampleTest** = residual for remaining biomass that was not subsampled.
   b. **clipID**
   c. **collectDate**
   d. **exclosure**: Y/N

7. Weigh each subsample created above (current-year + OSD), and record the information below. For **subsampleTest** = residual, leave **subsampleFreshMass** = NULL.

   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.

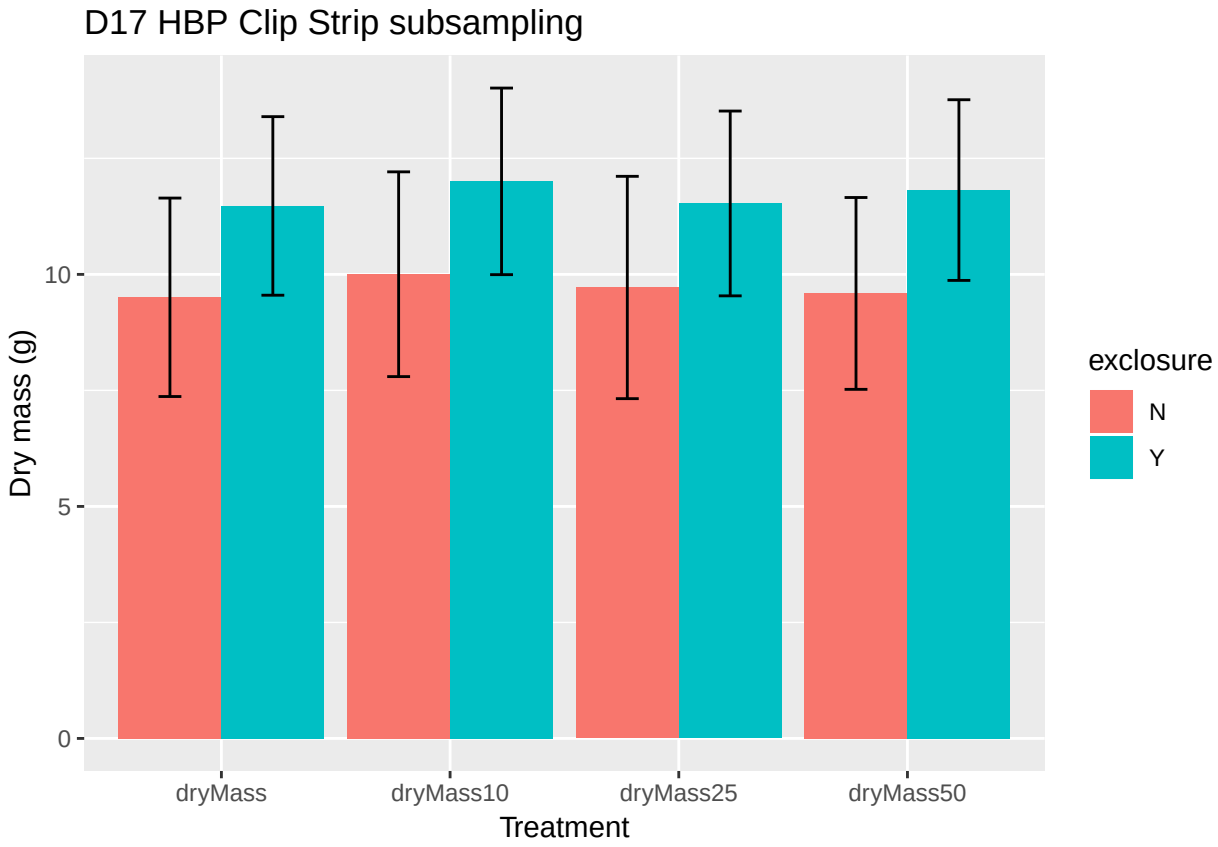
   a. Also sort remaining fresh mass that was not subsampled, and place into the **subsampleTest** = residual envelope.
   b. Sorted OSD may be discarded at this point.

9. Dry subsamples and residual current-year mass 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; record the dry mass for **subsampleTest** = residual in this field as well.

Analyses



Results: Mixed-Effects model analysis

1. Model 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).

```
m1ML <- lmer(estimatedDryMass ~ treatment + (1|clipID), longDF, REML = FALSE)
summary(m1ML)
```

```
## Linear mixed model fit by maximum likelihood . t-tests use
##   Satterthwaite's method [lmerModLmerTest]
## Formula: estimatedDryMass ~ treatment + (1 | clipID)
##    Data: longDF
##
##      AIC      BIC   logLik deviance df.resid
##    287.6    301.9   -137.8    275.6       74
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.72645 -0.44160  0.04646  0.43571  2.38772
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 39.909   6.3173
##  Residual              0.414   0.6434
## Number of obs: 80, groups:  clipID, 20
```

```
##
## Fixed effects:
##                     Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)          10.4905     1.4199 20.3112   7.388 3.54e-07 ***
## treatmentdryMass10    0.5134     0.2035 60.0000   2.523   0.0143 *
## treatmentdryMass25    0.1322     0.2035 60.0000   0.650   0.5184
## treatmentdryMass50    0.2125     0.2035 60.0000   1.045   0.3004
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) trtM10 trtM25
## trtmntdrM10 -0.072
## trtmntdrM25 -0.072  0.500
## trtmntdrM50 -0.072  0.500  0.500
```

```
anova(m1ML)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value  Pr(>F)
## treatment 2.8422 0.94742     3    60  2.2883 0.08759 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2. Null model, and using **clipID** as a random effect.

```
m3ML <- lmer(estimatedDryMass ~ 1 + (1|clipID), longDF, REML = FALSE)
summary(m3ML)
```

```
## Linear mixed model fit by maximum likelihood . t-tests use
##   Satterthwaite's method [lmerModLmerTest]
## Formula: estimatedDryMass ~ 1 + (1 | clipID)
##    Data: longDF
##
##      AIC      BIC   logLik deviance df.resid
##    288.1    295.3   -141.1    282.1       77
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.70708 -0.45281 -0.08922  0.31257  2.70243
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  clipID   (Intercept) 39.8967  6.3164
##  Residual              0.4614  0.6793
## Number of obs: 80, groups:  clipID, 20
##
## Fixed effects:
##             Estimate Std. Error     df t value Pr(>|t|)
## (Intercept)   10.705      1.414 20.000   7.568 2.72e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(m1ML, m3ML)
```

```
## Data: longDF
## Models:
## m3ML: estimatedDryMass ~ 1 + (1 | clipID)
## m1ML: estimatedDryMass ~ treatment + (1 | clipID)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## m3ML  3 288.13 295.28 -141.06   282.13
## m1ML  6 287.63 301.92 -137.81   275.63 6.4998      3    0.08967 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
m1LM <- lm(estimatedDryMass ~ exclosure*treatment, data = longDF)
summary(m1LM)
```

```
##
## Call:
## lm(formula = estimatedDryMass ~ exclosure * treatment, data = longDF)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -9.2766 -4.6119 -0.5442  3.3540 14.6367
##
## Coefficients:
##                               Estimate Std. Error t value Pr(>|t|)
## (Intercept)                    9.50600    2.09005   4.548 2.14e-05 ***
## exclosureY                     1.96900    2.95578   0.666    0.507
## treatmentdryMass10             0.49713    2.95578   0.168    0.867
## treatmentdryMass25             0.21117    2.95578   0.071    0.943
## treatmentdryMass50             0.08341    2.95578   0.028    0.978
## exclosureY:treatmentdryMass10  0.03249    4.18010   0.008    0.994
## exclosureY:treatmentdryMass25 -0.15795    4.18010  -0.038    0.970
## exclosureY:treatmentdryMass50  0.25823    4.18010   0.062    0.951
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.609 on 72 degrees of freedom
## Multiple R-squared:  0.02585,    Adjusted R-squared:  -0.06886
## F-statistic: 0.2729 on 7 and 72 DF,  p-value: 0.9625
```

```
anova(m1LM)
```

```
## Analysis of Variance Table
##
## Response: estimatedDryMass
##                      Df  Sum Sq Mean Sq F value Pr(>F)
## exclosure             1   80.18  80.176  1.8354 0.1797
## treatment             3    2.84   0.947  0.0217 0.9956
## exclosure:treatment   3    0.44   0.147  0.0034 0.9997
## Residuals            72 3145.19  43.683
```

Outcomes

1. Random effect associated with **clipID** explains considerable variability in the data. See summary output for model = m1ML.
2. Summary output for Mixed Effects model indicates that D17 Clip Strips should be subsampled at the 25% level for all bouts with **herbGroup** = ALL.
    - Estimates of dryMass using a 10% subsample are significantly higher than the entire sorted sample, using data from one bout collected late February and just before peak green.
    - Other subsamples are not significantly different than the entire sorted subsample –> go with 25%
3. Effect of `exclosure*treatment`, as evaluated in standard linear model:
    - Effect of `exclosure` not significant (m1LM), and effect of `treatment` also not significant. No detection of `exclosure:treatment` interaction effect.