

HBP D17 2017/2018 Sorting Subsample Experiment

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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

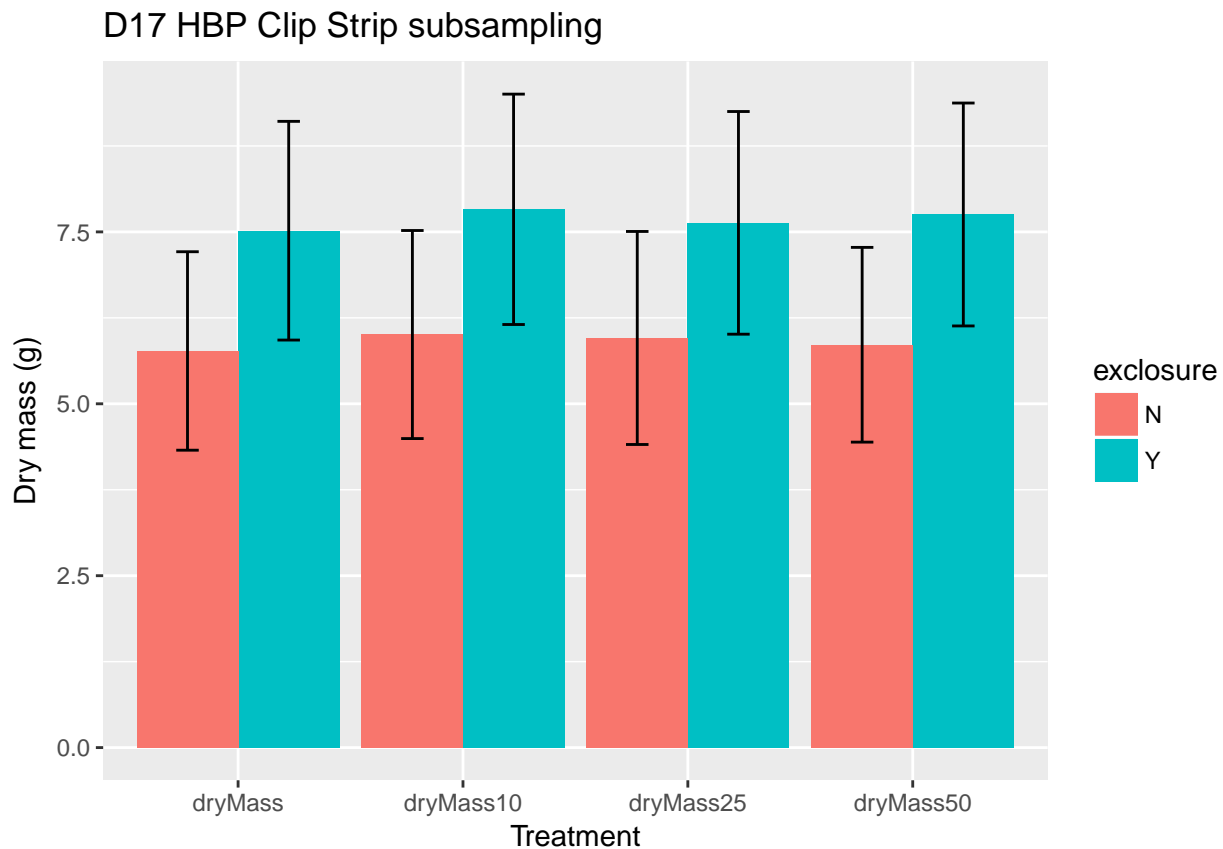
- Select n=10 plots (10 lowest Morton Order), resulting in n=20 clipID to test, due to both **exclosure = Y and N** for each plot. Random spatially-balanced plot locations, and locations of clipIDs within plots, will provide an unbiased estimate of biomass throughout the Tower airshed.
- For each clipID 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 °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** when controlling for **eventID**, 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 + eventID + (1|clipID), longDF, REML = FALSE)
summary(m1ML)
```

```
## Linear mixed model fit by maximum likelihood t-tests use Satterthwaite
## approximations to degrees of freedom [lmerMod]
## Formula: estimatedDryMass ~ treatment + eventID + (1 | clipID)
## Data: longDF
##
##      AIC      BIC    logLik deviance df.resid
##    457.6    478.6   -221.8    443.6      141
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.5590 -0.3427  0.0175  0.2886  3.2982
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## clipID   (Intercept) 24.4391  4.944
```

```
## Residual          0.2683  0.518
## Number of obs: 148, groups: clipID, 37
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)      1.9847      1.2029   37.2800   1.650   0.1074
## treatmentdryMass10  0.2741      0.1204  111.0000   2.276   0.0248 *
## treatmentdryMass25  0.1526      0.1204  111.0000   1.267   0.2077
## treatmentdryMass50  0.1612      0.1204  111.0000   1.339   0.1834
## eventIDhbp.2018.SJER.02  8.5734      1.6330   37.0000   5.250  6.5e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trtM10 trtM25 trtM50
## trtmntdrM10 -0.050
## trtmntdrM25 -0.050  0.500
## trtmntdrM50 -0.050  0.500  0.500
## eID.2018.SJ -0.734  0.000  0.000  0.000
```

```
anova(m1ML)
```

```
## Analysis of Variance Table of type III with Satterthwaite
## approximation for degrees of freedom
##              Sum Sq Mean Sq NumDF DenDF F.value    Pr(>F)
## treatment  1.4056  0.4685     3    111  1.7465    0.1617
## eventID    7.3943  7.3943     1     37 27.5618 6.502e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2. Model providing for an interaction with **eventID** and **treatment**, to account for the possibility that subsampling is less/more effective for bouts with greater versus less growth.

```
m2ML <- lmer(estimatedDryMass ~ treatment*eventID + (1|clipID), longDF, REML = FALSE)
summary(m2ML)
```

```
## Linear mixed model fit by maximum likelihood t-tests use Satterthwaite
## approximations to degrees of freedom [lmerMod]
## Formula: estimatedDryMass ~ treatment * eventID + (1 | clipID)
## Data: longDF
##
##      AIC      BIC    logLik deviance df.resid
##    456.6    486.5   -218.3    436.6     138
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.4959 -0.3229  0.0511  0.2733  3.0618
##
## Random effects:
## Groups Name Variance Std.Dev.
## clipID (Intercept) 24.4432  4.9440
## Residual          0.2518  0.5018
## Number of obs: 148, groups: clipID, 37
##
## Fixed effects:
##
##              Estimate Std. Error
## (Intercept)      2.064118   1.205258
## treatmentdryMass10 -0.007459   0.172110
## treatmentdryMass25  0.176645   0.172110
## treatmentdryMass50  0.100871   0.172110
## eventIDhbp.2018.SJER.02 8.426382   1.639328
## treatmentdryMass10:eventIDhbp.2018.SJER.02 0.520831   0.234095
## treatmentdryMass25:eventIDhbp.2018.SJER.02 -0.044446   0.234095
## treatmentdryMass50:eventIDhbp.2018.SJER.02 0.111662   0.234095
##
##              df t value Pr(>|t|)
## (Intercept)  37.570000   1.713  0.0950 .
## treatmentdryMass10 111.000000  -0.043  0.9655
## treatmentdryMass25 111.000000   1.026  0.3070
## treatmentdryMass50 111.000000   0.586  0.5590
## eventIDhbp.2018.SJER.02 37.570000   5.140 8.8e-06 ***
## treatmentdryMass10:eventIDhbp.2018.SJER.02 111.000000   2.225  0.0281 *
## treatmentdryMass25:eventIDhbp.2018.SJER.02 111.000000  -0.190  0.8498
## treatmentdryMass50:eventIDhbp.2018.SJER.02 111.000000   0.477  0.6343
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) trtM10 trtM25 trtM50 eID.20 tM10:I tM25:I
## trtmntdrM10 -0.071
## trtmntdrM25 -0.071  0.500
## trtmntdrM50 -0.071  0.500  0.500
## eID.2018.SJ -0.735  0.052  0.052  0.052
## tM10:ID.201  0.052 -0.735 -0.368 -0.368 -0.071
## tM25:ID.201  0.052 -0.368 -0.735 -0.368 -0.071  0.500
## tM50:ID.201  0.052 -0.368 -0.368 -0.735 -0.071  0.500  0.500
```

```
anova(m2ML)
```

```
## Analysis of Variance Table of type III with Satterthwaite
## approximation for degrees of freedom
##           Sum Sq Mean Sq NumDF DenDF F.value    Pr(>F)
## treatment      1.2072  0.4024     3   111  1.5981    0.19392
## eventID         6.9397  6.9397     1    37 27.5618 6.502e-06 ***
## treatment:eventID 1.8310  0.6103     3   111  2.4241    0.06954 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(m1ML, m2ML)
```

```
## Data: longDF
## Models:
## object: estimatedDryMass ~ treatment + eventID + (1 | clipID)
## ..1: estimatedDryMass ~ treatment * eventID + (1 | clipID)
##           Df      AIC      BIC logLik deviance Chisq Chi Df Pr(>Chisq)
## object    7  457.61  478.59 -221.81   443.61
## ..1      10  456.57  486.54 -218.28   436.57 7.0439     3    0.07051 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

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

```
## summary from lme4 is returned
## some computational error has occurred in lmerTest

## Linear mixed model fit by maximum likelihood ['lmerMod']
## Formula: estimatedDryMass ~ 1 + (1 | clipID)
## Data: longDF
##
##      AIC      BIC    logLik deviance df.resid
##    475.3    484.3   -234.7    469.3     145
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.4399 -0.3120 -0.0440  0.2561  3.4720
##
## Random effects:
## Groups Name Variance Std.Dev.
## clipID (Intercept) 42.6909  6.534
## Residual          0.2809  0.530
## Number of obs: 148, groups: clipID, 37
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    6.766      1.075    6.294
```

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

```
## Data: longDF
## Models:
## ..1: estimatedDryMass ~ 1 + (1 | clipID)
## object: estimatedDryMass ~ treatment + eventID + (1 | clipID)
##      Df    AIC    BIC logLik deviance Chisq Chi Df Pr(>Chisq)
## ..1     3 475.33 484.32 -234.67   469.33
## object  7 457.61 478.59 -221.81   443.61 25.718     4 3.608e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Results: Linear model analysis

4. Linear model, using treatment, eventID, exclosure, and no random effect.

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

```
##
## Call:
## lm(formula = estimatedDryMass ~ eventID + exclosure + treatment,
##     data = longDF)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -8.9469 -2.4268 -0.5746  1.3005 14.4916
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.2622     1.0149   1.244  0.2157
## eventIDhbp.2018.SJER.02  8.5282     0.8273  10.308 <2e-16 ***
## exclosureY        1.5353     0.8249   1.861  0.0648 .
## treatmentdryMass10    0.2741     1.1657   0.235  0.8145
## treatmentdryMass25    0.1526     1.1657   0.131  0.8960
## treatmentdryMass50    0.1612     1.1657   0.138  0.8902
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 5.014 on 142 degrees of freedom
## Multiple R-squared:  0.4387, Adjusted R-squared:  0.419
## F-statistic: 22.2 on 5 and 142 DF, p-value: < 2.2e-16
AIC(m1ML, m1LM)

##      df      AIC
## m1ML   7 457.6142
## m1LM   7 905.0900
```


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

```
m2LM <- lm(estimatedDryMass ~ eventID*exclosure + treatment, data = longDF)
summary(m2LM)
```

```
##
## Call:
## lm(formula = estimatedDryMass ~ eventID * exclosure + treatment,
##     data = longDF)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -9.1804 -2.2441 -0.7553  1.0411 14.6444
##
## Coefficients:
##                                Estimate Std. Error t value Pr(>|t|)
## (Intercept)                   1.5216     1.1014   1.381   0.169
## eventIDhbp.2018.SJER.02       8.0354     1.1544   6.961 1.18e-10
## exclosureY                    0.9840     1.2208   0.806   0.422
## treatmentdryMass10            0.2741     1.1683   0.235   0.815
## treatmentdryMass25            0.1526     1.1683   0.131   0.896
## treatmentdryMass50            0.1612     1.1683   0.138   0.890
## eventIDhbp.2018.SJER.02:exclosureY 1.0181     1.6592   0.614   0.540
##
## (Intercept)
## eventIDhbp.2018.SJER.02          ***
## exclosureY
## treatmentdryMass10
## treatmentdryMass25
## treatmentdryMass50
## eventIDhbp.2018.SJER.02:exclosureY
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 5.025 on 141 degrees of freedom
## Multiple R-squared:  0.4402, Adjusted R-squared:  0.4164
## F-statistic: 18.48 on 6 and 141 DF,  p-value: 8.946e-16
```

```
anova(m1LM, m2LM)
```

```
## Analysis of Variance Table
##
## Model 1: estimatedDryMass ~ eventID + exclosure + treatment
## Model 2: estimatedDryMass ~ eventID * exclosure + treatment
##   Res.Df    RSS Df Sum of Sq    F Pr(>F)
## 1     142 3569.6
## 2     141 3560.1  1     9.5079 0.3766 0.5404
```

Outcomes

- 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 both bouts.
 - Other subsamples are not significantly different than the entire sorted subsample -> go with 25%
 - No overall interaction effect for **eventID:treatment**, but sorting at 10% level leads to higher estimates of **dryMass** when total biomass is higher. Not a huge issue since subsampling at 25% is not affected.
- Effect of **exclosure**, as evaluated in standard linear model:
 - Effect of **exclosure** not significant across both bouts (m1LM), and effect of **exclosure** not bout dependent, as **eventID:exclosure** is not significant (m2LM).
- Random effect associated with **clipID** explains considerable variability in the data. See summary output for model = m1ML.