

HBP D09 2017 Sorting Subsample Experiment

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Goal

To determine the efficacy of subsampling various proportions of HBP samples in D09 in order to reduce long sort times associated with removing OSD from current-year clipped biomass within the context of dense Kentucky Blue Grass mats. 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.

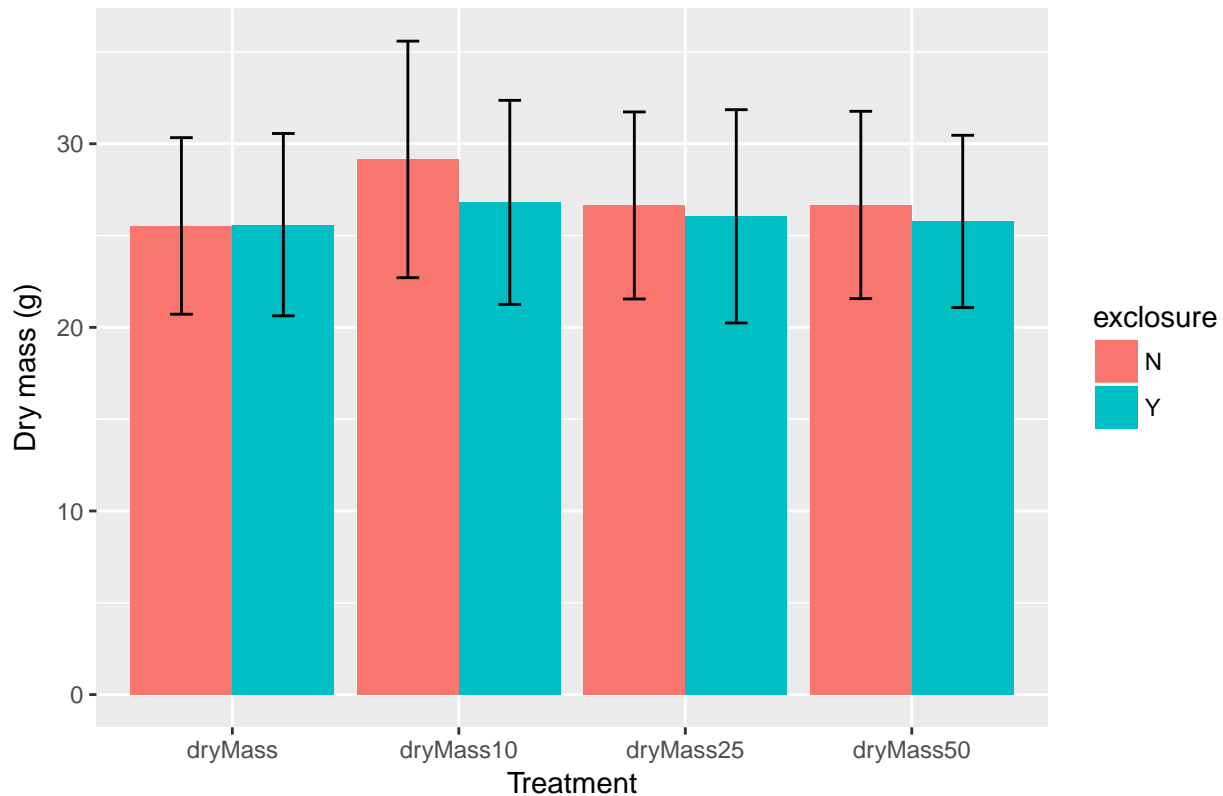
Procedure

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

D09 HBP Clip Strip subsampling



Results: Mixed-Effects model analysis

1. Model accounting for subsampling effect on **dryMass** when controlling for **enclosure**, and using **clipID** as a random effect.

```
m1ML <- lmer(estimatedDryMass ~ treatment + enclosure + (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 + enclosure + (1 | clipID)
## Data: longDF
##
##      AIC      BIC   logLik deviance df.resid
##    527.3    543.9   -256.6    513.3      73
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.00181 -0.50945  0.03055  0.21983  3.00784
##
## Random effects:
## Groups   Name                Variance Std.Dev.
## clipID   (Intercept)         244.70    15.64
## Residual                    11.84     3.44
```

```

## Number of obs: 80, groups: clipID, 20
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)    26.0290     5.0209  20.7200   5.184 4.05e-05 ***
## treatmentdryMass10  2.4196     1.0879  60.0000   2.224  0.0299 *
## treatmentdryMass25  0.7842     1.0879  60.0000   0.721  0.4738
## treatmentdryMass50  0.6597     1.0879  60.0000   0.606  0.5465
## enclosureY      -0.9400     7.0378  20.0000  -0.134  0.8951
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trtM10 trtM25 trtM50
## trtmntdrM10 -0.108
## trtmntdrM25 -0.108  0.500
## trtmntdrM50 -0.108  0.500  0.500
## enclosureY  -0.701  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  63.456  21.1521     3    60  1.78709  0.1593
## enclosure   0.211   0.2111     1    20  0.01784  0.8951

```

2. Model accounting only for effect of **exclosure**, and using **clipID** as a random effect.

```
m2ML <- lmer(estimatedDryMass ~ exclosure + (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 ~ exclosure + (1 | clipID)
## Data: longDF
##
##      AIC      BIC    logLik deviance df.resid
##    526.4    535.9   -259.2    518.4      76
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.9965 -0.3383 -0.0623  0.1392  3.2291
##
## Random effects:
## Groups Name Variance Std.Dev.
## clipID (Intercept) 244.43  15.634
## Residual          12.89   3.591
## Number of obs: 80, groups: clipID, 20
##
## Fixed effects:
##              Estimate Std. Error    df t value Pr(>|t|)
## (Intercept)  26.995      4.976 20.000   5.424 2.61e-05 ***
## exclosureY   -0.940      7.038 20.000  -0.134  0.895
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr)
## exclosureY -0.707
```

```
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)
## exclosure 0.23001 0.23001      1    20 0.017839 0.8951
```

```
AIC(m1ML, m2ML)
```

```
##      df      AIC
## m1ML  7 527.2659
## m2ML  4 526.4011
```

3. Model only accounting for random effect of **clipID**.

```
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
##    524.4    531.6  -259.2    518.4      77
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.9983 -0.3391 -0.0622  0.1375  3.2307
##
## Random effects:
## Groups Name Variance Std.Dev.
## clipID (Intercept) 244.65  15.641
## Residual          12.89   3.591
## Number of obs: 80, groups: clipID, 20
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)    26.52      3.52    7.534
```

```
AIC(m1ML, m2ML, m3ML)
```

```
##      df      AIC
## m1ML  7 527.2659
## m2ML  4 526.4011
## m3ML  3 524.4189
```

Outcomes

- D09 Clip Strips should be subsampled at the 25% level for all bouts with **herbGroup** = ALL.
 - AIC for ML models with and without **treatment** are virtually identical, but the 10% subsample group is significantly higher than the other groups.
 - lmerTest Anova results indicate **treatment** is not significant
 - Clip Strips sorted at 10% are different from all others, but effect is < 5-10% compared to unsorted.
- Effect of **exclosure** is also not significant
 - AIC for ML model with and without **exclosure** are virtually identical.
 - lmerTest Anova results indicate **exclosure** is not significant at given sample size.
- Random effect associated with **clipID** explains considerable variability in the data.
 - Most variability in the data is explained by random variation among clipID.