Microbial community assembly in a multi-layer dendritic metacommunity

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Initial setup

First, we load the data. This includes the site-by-species matrix (generated in Mothur, v. 1.41.1), the RDP taxonomy, the environmental data, and the phylogenetic tree (generated with FastTreeMP).

Next, we will clean up the data. I'll remove any sample that didn't get 10000 reads. Then also cut those samples from the environment and design tables.

```
# Sequencing Coverage
coverage <- rowSums(OTUs)

# Remove Low Coverage Samples
cutoff <- 10000
lows <- which(coverage < cutoff)
OTUs <- OTUs[-which(coverage < cutoff), ]
design <- design.total[-which(coverage < cutoff), ]
env <- env.total[-which(coverage < cutoff), ]

# Remove OTUs with less than 5 occurances across all sites
OTUs <- OTUs[, which(colSums(OTUs) > 2)]

OTUs <- OTUs[-which(env$sample == "W1_20_W"),]
design <- design[-which(env$sample == "W1_20_W"),]
env <- env[-which(env$sample == "W1_20_W"),]
```

Here, I'll read in the dendritic distances and add a tiny bit of jitter to the spatial distances so nearby sites aren't identical. Then, I'll calculate the earth distance in meters.

```
den.dists <- make.dendritic.dists("data/hja_dendritic-dists.csv")
design$upstreamdist <- as.matrix(den.dists)[1,]

# Read in Distances
# Geo distance Matrix
xy <- cbind(jitter(env$longitude, amount = .0001),
            jitter(env$latitude, amount = .0001))
geo.dists <- SoDA::geoXY(xy[,1], xy[,2])
dist.mat <- fossil::earth.dist(xy) * 1000
```

Next, we will see if any of the environmental variables need to be transformed. I'll then rescale the environmental variables.

```
# Remove orthogonal vectors and make numbers below detection close to zero
env.subs <- env %>% select(habitat, elevation,
```

```
                          temperature, conductivity,
                          ph, TN, TP, DOC) %>%
  mutate(TN = if_else(TN < 0, 0.001, TN),
         TP = if_else(TP < 0, 0.001, TP))

#hist(log(env.subs$TP), breaks = 30)
#hist(log(env.subs$TN), breaks = 30)

env.subs <- env.subs %>% mutate(TN = log(TN), TP = log(TP))

# rescale variables
env.subs <- env.subs %>% mutate_if(is_double, scale_vec)
```

Now, I'll perform some transformations on the abundance data. I'll work with the Hellinger-transformed data for the rest of the analysis.

```
# Rarefy communities
set.seed(47405)
OTUs <- rrarefy(OTUs, min(rowSums(OTUs)))
OTUs <- OTUs[,-which(colSums(OTUs) == 0)]

# Transformations and Standardizations
OTUsREL <- decostand(OTUs, method = "total")
OTUs.PA <- decostand(OTUs, method = "pa")
OTUsREL.log <- decostand(OTUs, method = "log")
OTUsREL.hel <- decostand(OTUs, method = "hellinger")
```

I removed the sites with low coverage, and I removed the OTUs with low abundance across the whole dataset.

Here, we will read in the phylogenetic tree. I pruned the phylogenetic tree to match only the taxa remaining in the dataset. Then, I rooted the tree using the midpoint method.

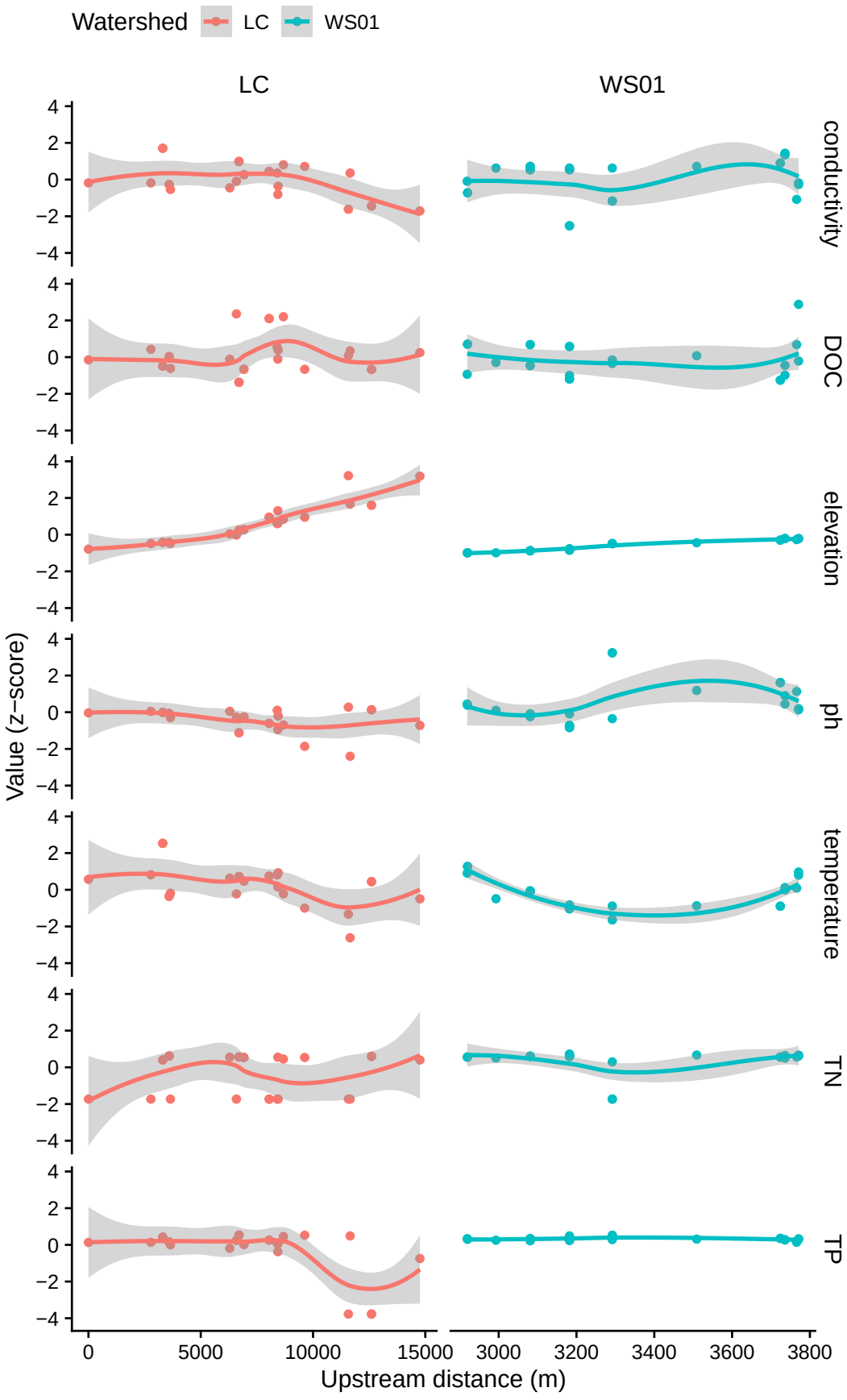
```
# hja.tree <- read.tree("data/hja_streams.tree")
# matched.phylo <- match.phylo.comm(hja.tree, OTUs)
# hja.tree <- matched.phylo$phy
# is.rooted(hja.tree)
# hja.tree.rooted <- midpoint.root(hja.tree)
# is.rooted(hja.tree.rooted)
# saveRDS(object = hja.tree.rooted, file = "temp/hja_tree_rooted.nwk")
hja.tree.rooted <- readRDS(file = "temp/hja_tree_rooted.nwk")
```

Environmental analysis

Here, I'll just plot the environmental variables from downstream to upstream across the watershed.

```
env.subs %>% mutate(upstreamdist = design$upstreamdist, watershed = design$watershed) %>%
  gather(-upstreamdist, -watershed, -habitat, key = variable, value = measurement) %>%
  ggplot(aes(x = upstreamdist, y = measurement, color = watershed)) +
  facet_grid(variable ~ watershed, scales = "free_x") +
  geom_point() +
  geom_smooth() +
  theme(legend.position = "top") +
  scale_x_continuous(labels = scales::wrap_format(10)) +
  labs(x = "Upstream distance (m)",
       y = "Value (z-score)",
```

```
        color = "Watershed")
```


Watershed
LC
WS01
LC
WS01
conductivity
DOC
elevation
ph
temperature
TN
TP
Value (z−score)
4
2
0
−2
−4
0
5000
10000
15000
3000
3200
3400
3600
3800
Upstream distance (m)

alpha Diversity analysis

```
alpha.tbl <- tibble(
  habitat = factor(design$habitat, levels = c("sediment", "water"),
                   labels = c("Sediment", "Planktonic")),
  upstream = design$upstreamdist,
  order = design$order,
  sample = rownames(design),
  N0 = rowSums(OTUsREL.hel > 0),
  N1 = exp(diversity(OTUsREL.hel, index = "shannon")),
  N2 = diversity(OTUsREL, index = "invsimpson")
)
summary(lm(N1 ~ habitat, data = alpha.tbl))
```

```
##
## Call:
## lm(formula = N1 ~ habitat, data = alpha.tbl)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1900.9  -284.2   107.3   340.3   945.7
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)         1915.0      115.0  16.645  < 2e-16 ***
## habitatPlanktonic    541.3      149.5   3.619  0.00072 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 514.5 on 47 degrees of freedom
## Multiple R-squared:  0.218,  Adjusted R-squared:  0.2013
## F-statistic:  13.1 on 1 and 47 DF,  p-value: 0.0007205
```

```
alpha.fig <- alpha.tbl %>%
  ggplot(aes(x = habitat, y = N1, fill = habitat, color = habitat)) +
  geom_boxplot(alpha = 0.8) +
  geom_jitter(alpha = 0.2) +
  labs(x = "", y = expression(paste(alpha, "-diversity (species equivalents)"))) +
  scale_fill_manual(values = (my.colors)) +
  scale_color_manual(values = colorspace::darken(my.colors,.4)) +
  guides(fill = FALSE, color = FALSE) +
  scale_y_continuous(limits = c(0, 3500))

summary(lm(N1 ~ habitat * order, data = alpha.tbl))
```

```
##
## Call:
## lm(formula = N1 ~ habitat * order, data = alpha.tbl)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1971.3  -287.7   131.1   336.8   929.0
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)              1946.32     290.10   6.709 2.75e-08 ***
## habitatPlanktonic         634.13     386.76   1.640    0.108
## order                     -13.93     117.94  -0.118    0.907
## habitatPlanktonic:order   -39.83     156.18  -0.255    0.800
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 524.1 on 45 degrees of freedom
## Multiple R-squared:  0.223,  Adjusted R-squared:  0.1712
## F-statistic: 4.304 on 3 and 45 DF,  p-value: 0.009417
```

Unique taxa per habitat

```
sediment.only <- OTUs[, which(
  colSums(OTUs[which(design$habitat == "water"),]) == 0)]
water.only <- OTUs[, which(
  colSums(OTUs[which(design$habitat == "sediment"),]) == 0)]
water.sed <- OTUs[, which(
  colSums(OTUs[which(design$habitat == "sediment"),]) > 1 &
  colSums(OTUs[which(design$habitat == "water"),]) > 1)]
in.water <- OTUs[, which(
  colSums(OTUs[which(design$habitat == "water"),]) > 1)]
in.water.pa <- decostand(in.water, method = "pa")
in.sediment <- OTUs[, which(
  colSums(OTUs[which(design$habitat == "sediment"),]) > 1)]
in.sediment.pa <- decostand(in.sediment, method = "pa")


# what proportion of taxa are unique at each site
water.richness <- rowSums(in.water.pa[which(design$habitat == "water"),])
# in water samples, but not in any sediments
water.unique <- rowSums(in.water.pa[
  which(design$habitat == "water"), # count the number of taxa in each water samp
  which(colSums(in.water.pa[which(design$habitat == "sediment"),]) == 0)]) # whose abund in sediments i
water.unique.frac <- water.unique / water.richness
se(water.unique.frac)
```

```
## [1] 0.009413338
```

```
sed.richness <- rowSums(in.sediment.pa[which(design$habitat == "sediment"),])
# in sediments, but not in any water samples
sed.unique <- rowSums(in.sediment.pa[
  which(design$habitat == "sediment"), # count number of taxa in sed samples
  which(colSums(in.sediment.pa[which(design$habitat == "water"),]) == 0)]) #whose abund in water is zer
sed.unique.frac <- sed.unique / sed.richness
se(sed.unique.frac)
```

```
## [1] 0.002072766
```

```
unique.fracs <- data.frame("unique_frac" = water.unique.frac,
                           "habitat" = "Planktonic") %>%
  rbind.data.frame(., data.frame(
    "unique_frac" = sed.unique.frac,
    "habitat" = "Sediment"))
unique.fracs <- unique.fracs %>% rownames_to_column(var = "sample")
```

```r
unique.fracs$habitat <- factor(unique.fracs$habitat,
                               levels = c("Sediment", "Planktonic"), ordered = F)
unique.fracs <- left_join(unique.fracs, rownames_to_column(design[,c("order","upstreamdist")], var = "sa
summary(lm(unique_frac ~ habitat, data = unique.fracs))
```

```
##
## Call:
## lm(formula = unique_frac ~ habitat, data = unique.fracs)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.126419 -0.013957  0.001765  0.016537  0.085844
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.077675   0.008848   8.779  1.8e-11 ***
## habitatPlanktonic 0.163540   0.011501  14.220  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03957 on 47 degrees of freedom
## Multiple R-squared:  0.8114, Adjusted R-squared:  0.8074
## F-statistic: 202.2 on 1 and 47 DF,  p-value: < 2.2e-16
```

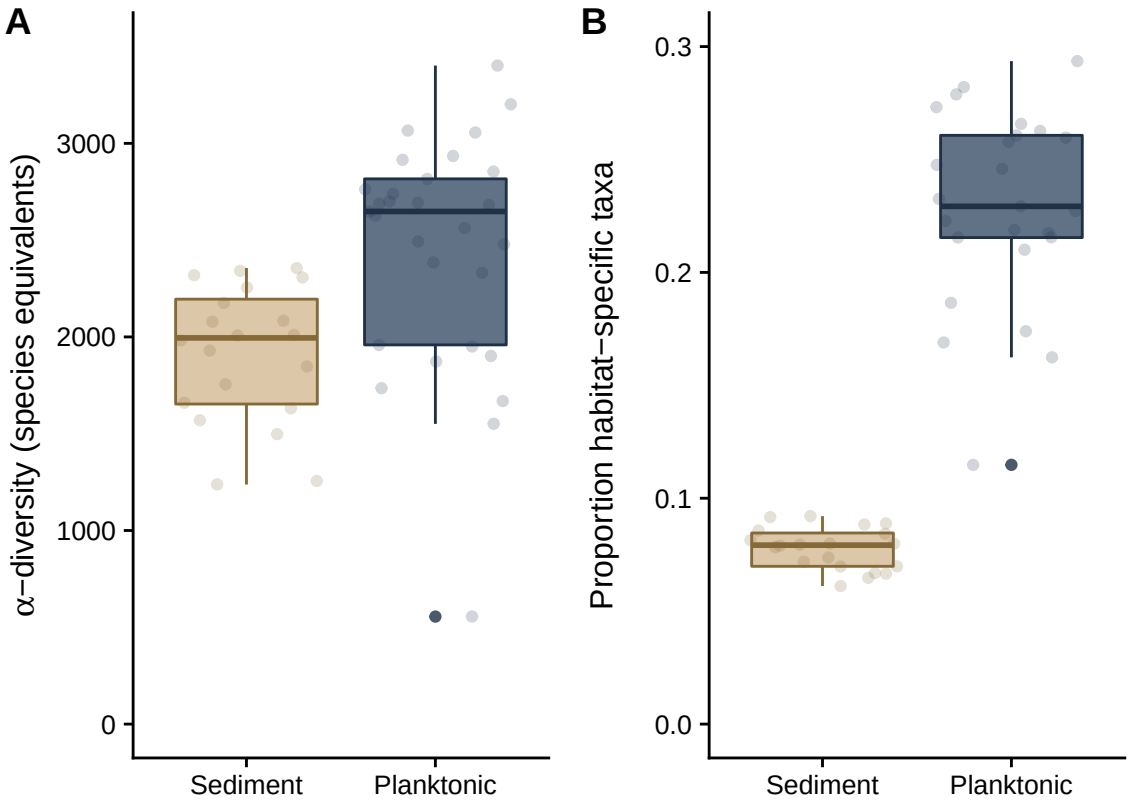
```r
summary(lm(unique_frac ~ habitat, data = unique.fracs)) %>%
  capture.output(file = "tables/unique_compare.txt")
```

```r
unique.fig <- unique.fracs %>%
  ggplot(aes(x = habitat, y = unique_frac, color = habitat, fill = habitat)) +
  geom_boxplot(alpha = 0.8) +
  geom_jitter(alpha = 0.2) +
  labs(x = "", y = "Proportion habitat-specific taxa") +
  scale_fill_manual(values = (my.colors)) +
  scale_color_manual(values = colorspace::darken(my.colors,.4)) +
  guides(fill = FALSE, color = FALSE) +
  scale_y_continuous(limits = c(0, .3))

summary(lm(log10(unique_frac) ~ order*habitat, unique.fracs))
```

```
##
## Call:
## lm(formula = log10(unique_frac) ~ order * habitat, data = unique.fracs)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.25910 -0.02894  0.00473  0.05057  0.18649
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)              -1.13679    0.04463 -25.470  < 2e-16 ***
## order                     0.01070    0.01815   0.590    0.558
## habitatPlanktonic         0.41544    0.05950   6.982 1.08e-08 ***
## order:habitatPlanktonic   0.02967    0.02403   1.235    0.223
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 0.08064 on 45 degrees of freedom
## Multiple R-squared:  0.9061, Adjusted R-squared:  0.8999
## F-statistic: 144.8 on 3 and 45 DF,  p-value: < 2.2e-16
```

```
alpha.to.plot <- unique.fracs %>% left_join(alpha.tbl) %>% select(-N0, -N2) %>%
  gather(unique_frac, N1, key = "metric", value = "value")
alpha.to.plot$metric <- factor(alpha.to.plot$metric)
levels(alpha.to.plot$metric) <- c(
  expression(paste(alpha, "-diversity")),
  expression(paste("Proportion habitat-specific taxa"))
)
alpha.plot <- alpha.to.plot %>%
  ggplot(aes(x = habitat, y = value, fill = habitat, color = habitat)) +
  facet_wrap(~ metric, scales = "free_y", strip.position = "left", labeller = label_parsed, ncol = 2) +
  geom_boxplot(width = .5, alpha = 0.8) +
  geom_jitter(alpha = 0.1) +
  scale_fill_manual(values = (my.colors)) +
  scale_color_manual(values = colorspace::darken(my.colors,0.4)) +
  theme(strip.placement = "outside", legend.position = "none") +
  guides(fill = FALSE) +
  labs(y = "", x = "")


plot_grid(alpha.fig, unique.fig, ncol = 2, align = "hv", labels = c("A", "B"))
```



Beta diversity:

Ordination

```
hja.pcoa <- run.pcoa(comm = OTUsREL.hel, dist.metric = "euclidean", plot = T)
```

```
## PCoA Axis 1 explains 13.1 percent of total variation.
## PCoA Axis 2 explains 8.3 percent of total variation.
```

```
pcoa.ellipse <- ordiellipse(hja.pcoa$pcoa, str_to_title(design$habitat), display = "sites",
            kind = "se", conf = 0.95, label = T)
```

```
pcoa.plot <- cbind.data.frame(vegan::scores(hja.pcoa$pcoa), group = str_to_title(design$habitat))
df_ell <- calc.ellipse(ord = pcoa.plot, ellipse = pcoa.ellipse)


# Run a PERMANOVA
hja.permanova <- adonis(vegdist(OTUsREL, method = "bray") ~ design$habitat + design$order + design$wate
hja.permanova$aov.tab %>% pander::pander()
```

Table 1: Permutation: free

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
design$habitat	1	1.676	1.676	8.907	0.1528	0.001
design$order	1	0.3922	0.3922	2.085	0.03576	0.006
design$watershed	1	0.4342	0.4342	2.308	0.03959	0.005
Residuals	45	8.465	0.1881	NA	0.7719	NA
Total	48	10.97	NA	NA	1	NA

```
capture.output(hja.permanova$aov.tab, file = "./tables/hja_permanova.txt")
```

Now, we'll run an RDA.

```
#hja.rda <- rda(OTUsREL.hel ~ ., env.subs)
#R2.all.vars <- RsquareAdj(hja.rda)$adj.r.squared
#anova(hja.rda, permutations = how(nperm = 999))
#anova(hja.rda, by = "axis", permutations = how(nperm = 999))

hja.dbrda <- dbrda(vegdist(OTUsREL) ~ ., env.subs)

# overall model and 1st two dbRDA axes significant
#anova(hja.dbrda, permutations = how(nperm = 999))
#anova(hja.dbrda, by = "axis", permutations = how(nperm = 999))
```

We see that the RDA is globally significant and the first two canonical axes are also significant. Because the full model was significant, I'll run a model selection procedure.

```
# forward.sel(OTUsREL.hel,
#             model.matrix(~., env.subs)[,-1],
#             adjR2thresh = R2.all.vars,
#             nperm = 999)

# habitat, elevation, and conductivity were selected by Blanchet 2008 method

# reduced model selects habitat, elevation and conductivity. but I'll add back other key env vars that
```

```
hja.rda.reduced <- dbrda(vegdist(OTUsREL) ~ habitat + elevation + conductivity + TP + TN + ph + DOC, env
```

The model selection procedure left us with the model using only habitat, elevation, and conductivity as predictor variables. Now, we have 3 significant RDA axes with the more parsimonious model.

Make figure 2

```
rda.plot <- cbind.data.frame(vegan::scores(hja.rda.reduced)$sites, group = str_to_title(design$habitat)
rda.var1 <- round(eigenvals(hja.rda.reduced)[1] / sum(eigenvals(hja.rda.reduced)) * 100, 1)
rda.var2 <- round(eigenvals(hja.rda.reduced)[2] / sum(eigenvals(hja.rda.reduced)) * 100, 1)
rda.vecs <- as.data.frame(hja.rda.reduced$CCA$biplot)
rda.vecs$predictor <- c("Planktonic\n habitat", "Elevation", "Cond.",
                        "TP", "TN", "pH", "DOC")
rda.vecs$origin <- 0
scale.arrows = 1
hja.rda.fig <-  ggplot(data = rda.plot, aes(x = dbRDA1, y = dbRDA2)) +
  geom_hline(aes(yintercept = 0), linetype = "dashed", alpha = 0.25, size = 0.25) +
  geom_vline(aes(xintercept = 0), linetype = "dashed", alpha = 0.25, size = 0.25) +
  geom_point(aes(fill = group, shape = watershed), size = 2, alpha = .8) +
  # geom_point(data = subset(rda.plot, group == "Sediment"), color = "black", size = 2) +
  # geom_point(data = subset(rda.plot, group == "Water"), color = "black", size = 2) +
  # geom_path(data = df_ell,
  #           aes(x = Dim1, y = Dim2, color = group),
  #           size = .8, alpha = 1, linetype = 2) +
  stat_ellipse(data = rda.plot, alpha = 0.7, aes(color = group, linetype = watershed)) +
  labs(x = paste0("dbRDA1 (", rda.var1, "%)"),
    y = paste0("dbRDA2 (", rda.var2, "%)"),
    color = "Habitat", shape = "Watershed") +
  scale_color_manual(values = my.colors) +
  scale_fill_manual(values = my.colors) +
  scale_shape_manual(values = c(21, 24)) +
  coord_fixed() +
  theme(legend.position = "none") +
  geom_segment(data = rda.vecs, size = .3,
               aes(x = origin, y = origin,
                   xend = scale.arrows*dbRDA1,
                   yend = scale.arrows*dbRDA2),
               alpha = .7, color = "black",
               arrow = arrow(angle = 20,
                             length = unit(.1, "inches"),
                             type = "open")) +
  geom_text_repel(data = rda.vecs, size = 1.8,
                  aes(x = (scale.arrows + .1)*dbRDA1,
                      y = (scale.arrows + .1)*dbRDA2, label = predictor),
                  color = "black",
                  segment.alpha = 0,
                 point.padding = 0,
                 nudge_y = c(-.15, #water
                             0, # elev
                             .035, # conduct
                             0, # TP
                             0, #TN
                             -.05, #ph
```

```
                                .05)) + # DOC
  annotate("text", x = .8, y = 2.2, label = "Benthic", size = 2.5, color = darken(my.colors[1])) +
  annotate("text", x = -.5, y = 2.2, label = "Planktonic", size = 2.5, color = darken(my.colors[2])) +
  ggsave("figures/Fig2.png", width = 8.4, height = 8.4, units = "cm", dpi = 500) +
  ggsave("figures/Fig2.pdf", width = 8.4, height = 8.4, units = "cm")
```

Subset only flow-connected sites

```
flow.connected <- read.csv("data/flow-connected-matrix.csv", row.names = 1)
den.dists.mat <- as.matrix(den.dists)
flow.connected.dists <- as.dist(
  flow.connected[rownames(den.dists.mat), colnames(den.dists.mat)] * den.dists.mat)
```

Null model analysis

```
# Calculate abundance-weighted Raup-Crick dissimilarities
regional.abunds <- t(as.matrix(colSums(OTUs)))
regional.relabunds <- decostand(regional.abunds, method = "total")
occupancy.probs <- t(as.matrix(colSums(decostand(OTUs, method = "pa")) / nrow(OTUs)))
site.abunds <- rowSums(OTUs)
site.rich <- specnumber(OTUs)
a <- regional.relabunds * occupancy.probs

# Create a null community based on Stegen et al. 2015
set.seed(47405)
#rc.nulls <- nullcom.rcabund(OTUs = OTUs, stand = "total", distance = "bray")
#saveRDS(rc.nulls, "temp/rc_nullmodels_bray.rda")
rc.nulls <- readRDS("temp/rc_nullmodels_bray.rda")

obs.bray <- as.matrix(vegdist(OTUsREL, method = "bray"))
site.compares <- expand.grid(site1 = 1:nrow(obs.bray), site2 = 1:nrow(obs.bray))
site.compares <- site.compares[-which(site.compares[,1] == site.compares[,2]),]
RC.bray <- matrix(NA, nrow = nrow(obs.bray), ncol = nrow(obs.bray))

for(row.i in 1:nrow(site.compares)){
  site1 <- site.compares[row.i,1]
  site2 <- site.compares[row.i,2]
  pairwise.null <- rc.nulls[site1,site2,]
  pairwise.bray <- obs.bray[site1,site2]
  num.greater <- sum(pairwise.null > pairwise.bray)
  num.ties <- sum(pairwise.null == pairwise.bray)
  val <- -1*((((1 * num.greater) + (0.5 * num.ties))/999 - 0.5) * 2)
  RC.bray[site1, site2] <- val
}
rownames(RC.bray) <- rownames(design)
colnames(RC.bray) <- rownames(design)
RC.bray.dist <- as.dist(RC.bray)
range(RC.bray.dist)
```

```
## [1] -1  1
```

```
flow_connected_dists_df <- simba::liste(flow.connected.dists, entry = "den_dists")
dists.df <- simba::liste(RC.bray.dist, entry = "RC_bray") %>% add_column(den_dists = flow_connected_dis
dists.df$habitat <- NA
dists.df[str_detect(dists.df$NBX, "_W") & str_detect(dists.df$NBY, "_W"),]$habitat <- str_wrap("Plankto
dists.df[str_detect(dists.df$NBX, "_W") & str_detect(dists.df$NBY, "_S"),]$habitat <- str_wrap("Plankto
dists.df[str_detect(dists.df$NBX, "_S") & str_detect(dists.df$NBY, "_W"),]$habitat <- str_wrap("Plankto
dists.df[str_detect(dists.df$NBX, "_S") & str_detect(dists.df$NBY, "_S"),]$habitat <- str_wrap("Benthic

dists.df$habitat <- factor(dists.df$habitat, levels = c("Planktonic", "Benthic", "Planktonic-Benthic"))
```

Match phylo

```
matched.phylo <- match.phylo.comm(phy = hja.tree.rooted, comm = OTUs[,which(colSums(OTUs) > 10)])
hja.comm <- matched.phylo$comm
hja.phy <- matched.phylo$phy
```

Make figure 4

```
#mntd.hja <- comdistnt.par(hja.comm, cophenetic(hja.phy), abundance.weighted = T, cores = 32)

#hja.mntd.ses <- ses.comdistnt2(
#  samp = hja.comm,
#  dis = cophenetic(hja.phy),
#  method = "quasiswap",
#  fixedmar = "both",
#  shuffle = "both",
#  strata = NULL,
#  mtype = "count",
#  burnin = 0,
#  thin = 1,
#  abundance.weighted = TRUE,
#  exclude.conspecifics = FALSE,
#  runs = 999,
#  cores = 32)
# saveRDS(mntd.hja, file = "data/mntds.rda")

# # Create null comms
# mntd.null <- array(NA, c(50, 50, 999))
# for(i in 1:999){
#   if(i == 1) pb <- progress_bar$new(total = 999, force = T)
#   pb$update(ratio = i/999)
#   #print(paste("creating null community ", i, " of 999"))
#   temp.mntd <- comdistnt(hja.comm,
#                          cophenetic(tipShuffle(hja.phy)),
#                          abundance.weighted=T)
#   mntd.null[,,i] <- as.matrix(temp.mntd)
#   if(i %% 50 == 0 | i == 999) saveRDS(mntd.null, file = "data/mntds-null-dist.rda")
# }

# read null dists
#mntd.hja <- readRDS(file = "data/mntds.rda")
```

```
#mntds.null <- readRDS(file = "data/mntds-null-dist.rda")

# obs.mntds <- as.matrix(mntd.hja)
#site.compares <- expand.grid(site1 = 1:ncol(obs.mntds), site2 = 1:ncol(obs.mntds))
#bNTI <- matrix(NA, nrow = nrow(obs.mntds), ncol = ncol(obs.mntds))
#for(row.i in 1:nrow(site.compares)){
#  site1 <- site.compares[row.i,1]
#  site2 <- site.compares[row.i,2]
#  pairwise.null <- mntds.null[site1,site2,]
#  pairwise.mntd <- obs.mntds[site1,site2]
#  null.mean <- mean(pairwise.null, na.rm = TRUE)
#  null.sd <- sd(pairwise.null, na.rm = TRUE)
#  val <- (pairwise.mntd - null.mean) / null.sd
#  bNTI[site1, site2] <- val
#}
#colnames(bNTI) <- rownames(hja.comm)
#rownames(bNTI) <- rownames(hja.comm)



hja.mntd.ses <- readRDS("temp/hja.mntd.ses.rda")
bNTI.dist <- as.dist(hja.mntd.ses$comdistnt.obs.z)
sum(bNTI.dist < 2 & bNTI.dist > -2) / length(bNTI.dist) # undom
```

```
## [1] 0.1403061
```

```
sum(bNTI.dist > 2) / length(bNTI.dist) # variable selection
```

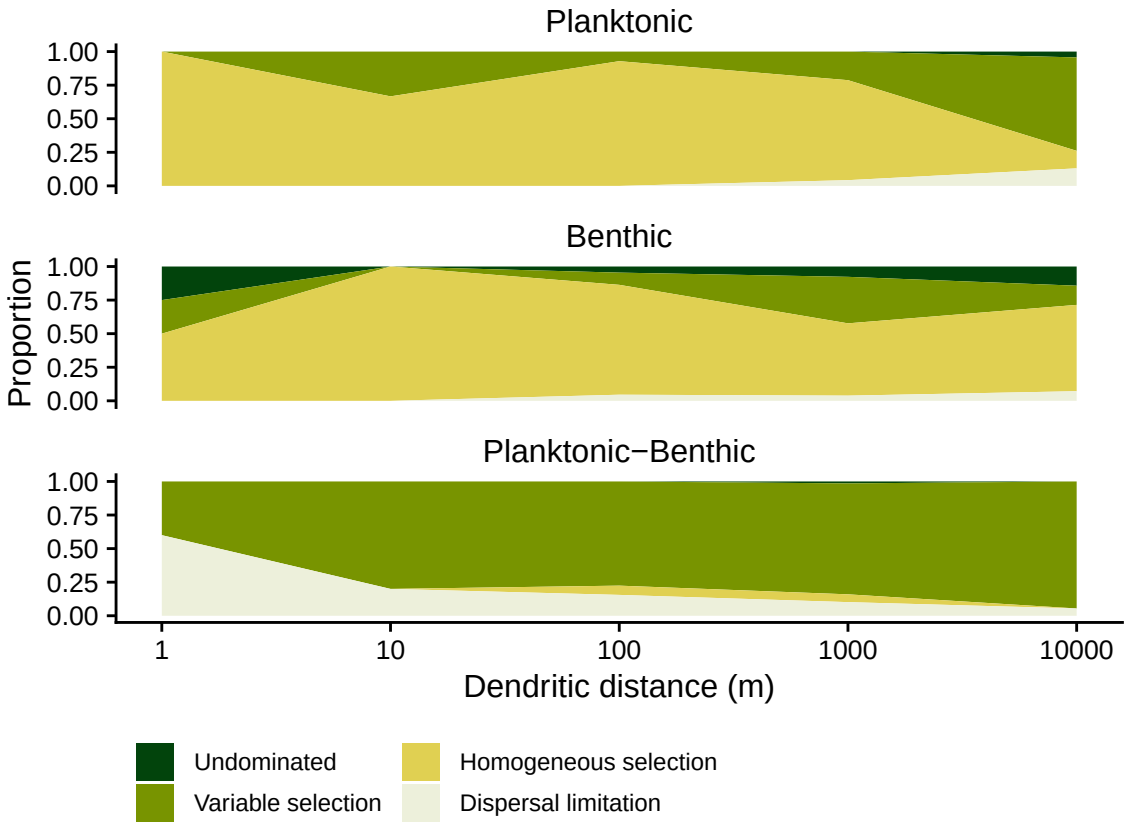
```
## [1] 0.6122449
```

```
sum(bNTI.dist < -2) / length(bNTI.dist) # homogeneous selection
```

```
## [1] 0.247449
```

```
hja.bnti.dist.ls <- simba::liste(bNTI.dist, entry = "bNTI")
hja.rcbray.dist.ls <- simba::liste(RC.bray.dist, entry = "RCbray")
hja.euclid.dist.ls <- simba::liste(dist(SoDA::geoXY(latitude = xy[,2], longitude = xy[,1])), entry = "eu
hja.assembly <- full_join(dists.df, hja.bnti.dist.ls)
hja.assembly <- cbind(hja.assembly, env = simba::liste(dist(env.subs[,-1]))[,3], euclid = hja.euclid.dis


hja.assembly.plot <- hja.assembly %>% filter(den_dists > 0) %>%
  mutate(process = ifelse(bNTI < -2, "Homogeneous selection",
                          ifelse(bNTI > 2, "Variable selection",
                                 ifelse(RC_bray > 0.95, "Dispersal limitation",
                                        ifelse(RC_bray < -0.95, "Mass effects", "Undominated"))))) %>%
  mutate(signif = ifelse(abs(RC_bray) < 0.95 & abs(bNTI) < 2, FALSE, TRUE)) %>%
  gather(RC_bray, bNTI, key = "metric", value = "value")
hja.assembly.plot$metric <- factor(hja.assembly.plot$metric)
levels(hja.assembly.plot$metric) <- c(
  expression(paste(beta,"NTI")),
  expression(beta["RC, Bray-Curtis"])
)

hja.assembly.rounded <- hja.assembly.plot %>%
  mutate(den_dists_log10 = log10(den_dists)) %>%
```

```
  mutate(den_dists_log10_rounded = round(den_dists_log10,0)) %>%
  mutate(den_dists_rounded = 10^den_dists_log10_rounded)


hja.assembly.grouped <- hja.assembly.rounded %>%
  group_by(habitat, den_dists_rounded) %>%
  count(process) %>%
  mutate(proportion = n/sum(n))
hja.assembly.grouped <- hja.assembly.grouped %>%
  full_join(unique(expand.grid(hja.assembly.grouped[,1:3])), fill = 0) %>%
  mutate(proportion = ifelse(is.na(proportion),0, proportion))

write_csv(hja.assembly.grouped, "tables/assembly_counts_by_scale.csv")

hja.assembly.grouped %>%
  arrange(process, den_dists_rounded) %>%
  mutate(process = factor(process, levels = c("Undominated",
                                              "Variable selection",
                                              "Homogeneous selection",
                                              "Mass effects",
                                              "Dispersal limitation"))) %>%
  ggplot(aes(x = den_dists_rounded, y = proportion, fill = process)) +
  geom_area(alpha = 1, position = "stack", color = NA) +
  scale_x_log10() +
  facet_wrap(~ habitat, ncol = 1) +
  scale_fill_manual(values = c("#02440c","#789400","#e0d052", "#edf0db", "#40becd")) +
  labs(x = "Dendritic distance (m)", y = "Proportion") +
  theme(legend.position = "bottom",
        legend.title = element_blank(),
        legend.text = element_text(size = 9)) +
  guides(fill = guide_legend(nrow = 2)) +
  ggsave("figures/Fig4.png", height = 8, width = 4, dpi = 600) +
  ggsave("figures/Fig4.pdf", height = 8, width = 4)
```




```
hja.assembly.grouped %>%
  ungroup() %>%
  group_by(den_dists_rounded) %>%
  count()
```

```
## # A tibble: 5 x 2
## # Groups:   den_dists_rounded [5]
##   den_dists_rounded     n
##               <dbl> <int>
## 1                 1    12
## 2                10    12
## 3               100    12
## 4              1000    12
## 5             10000    12
```

```
hja.assembly.grouped %>%
    count()
```

```
## # A tibble: 15 x 3
## # Groups:   habitat, den_dists_rounded [15]
##    habitat            den_dists_rounded     n
##    <fct>                          <dbl> <int>
##  1 Planktonic                         1     4
##  2 Planktonic                        10     4
##  3 Planktonic                       100     4
##  4 Planktonic                      1000     4
##  5 Planktonic                     10000     4
##  6 Benthic                            1     4
##  7 Benthic                           10     4
```

```
##  8 Benthic                          100     4
##  9 Benthic                         1000     4
## 10 Benthic                        10000     4
## 11 Planktonic-Benthic                 1     4
## 12 Planktonic-Benthic                10     4
## 13 Planktonic-Benthic               100     4
## 14 Planktonic-Benthic              1000     4
## 15 Planktonic-Benthic             10000     4
```

```
hja.assembly.plot %>%
  group_by(habitat) %>%
  count(process)
```

```
## # A tibble: 12 x 3
## # Groups:   habitat [3]
##    habitat            process                 n
##    <fct>              <chr>                 <int>
##  1 Planktonic         Dispersal limitation     10
##  2 Planktonic         Homogeneous selection   142
##  3 Planktonic         Undominated               2
##  4 Planktonic         Variable selection       60
##  5 Benthic            Dispersal limitation      6
##  6 Benthic            Homogeneous selection    88
##  7 Benthic            Undominated              12
##  8 Benthic            Variable selection       28
##  9 Planktonic-Benthic Dispersal limitation     44
## 10 Planktonic-Benthic Homogeneous selection    16
## 11 Planktonic-Benthic Undominated               2
## 12 Planktonic-Benthic Variable selection      286
```

```
hja.assembly.plot %>% count(process)
```

```
##                 process   n
## 1  Dispersal limitation  60
## 2 Homogeneous selection 246
## 3           Undominated  16
## 4    Variable selection 374
```

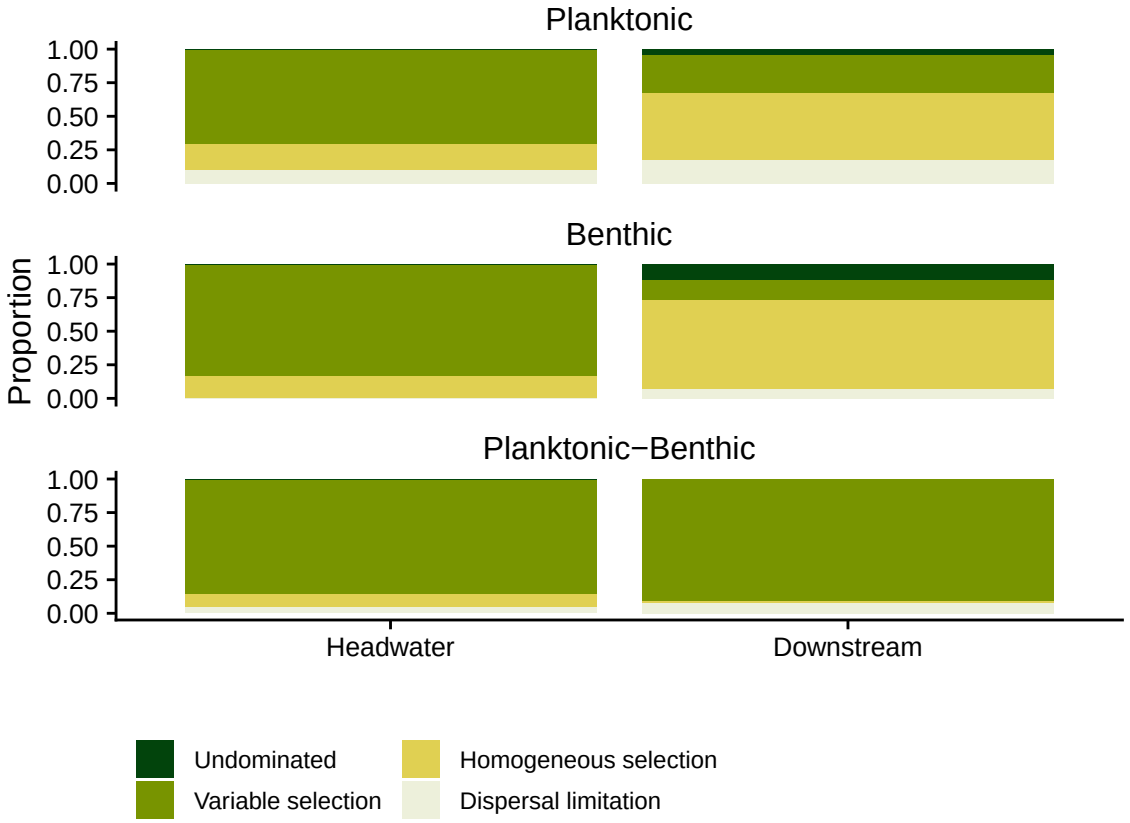
Make figure 5

```
headwaters.dist <- as.dist(as.matrix(bNTI.dist)[which(design$order == 1), which(design$order == 1)])
mainstem.dist <- as.dist(as.matrix(bNTI.dist)[which(design$order != 1), which(design$order != 1)])

headwaters.dist.sed.tax <- as.dist(as.matrix(RC.bray.dist)[
  which(design$order == 1 & design$habitat != "water"),
  which(design$order == 1 & design$habitat != "water")])
mainstem.dist.sed.tax <- as.dist(as.matrix(RC.bray.dist)[
  which(design$order != 1 & design$habitat != "water"),
  which(design$order != 1 & design$habitat != "water")])
headwaters.dist.water.tax <- as.dist(as.matrix(RC.bray.dist)[
  which(design$order == 1 & design$habitat == "water"),
  which(design$order == 1 & design$habitat == "water")])
mainstem.dist.water.tax <- as.dist(as.matrix(RC.bray.dist)[
  which(design$order != 1 & design$habitat == "water"),
```

```
  which(design$order != 1 & design$habitat == "water")])

headwaters.dist.sed.phy <- as.dist(as.matrix(bNTI.dist)[
  which(design$order == 1 & design$habitat != "water"),
  which(design$order == 1 & design$habitat != "water")])
mainstem.dist.sed.phy <- as.dist(as.matrix(bNTI.dist)[
  which(design$order != 1 & design$habitat != "water"),
  which(design$order != 1 & design$habitat != "water")])
headwaters.dist.water.phy <- as.dist(as.matrix(bNTI.dist)[
  which(design$order == 1 & design$habitat == "water"),
  which(design$order == 1 & design$habitat == "water")])
mainstem.dist.water.phy <- as.dist(as.matrix(bNTI.dist)[
  which(design$order != 1 & design$habitat == "water"),
  which(design$order != 1 & design$habitat == "water")])

# now cross-habitat comparisons, not square matrix
headwaters.dist.sed.water.phy <- as.matrix(bNTI.dist)[
  which(design$order == 1 & design$habitat != "water"),
  which(design$order == 1 & design$habitat == "water")]
downstream.dist.sed.water.phy <- as.matrix(bNTI.dist)[
  which(design$order != 1 & design$habitat != "water"),
  which(design$order != 1 & design$habitat == "water")]

headwaters.dist.sed.water.tax <- as.matrix(RC.bray.dist)[
  which(design$order == 1 & design$habitat != "water"),
  which(design$order == 1 & design$habitat == "water")]
downstream.dist.sed.water.tax <- as.matrix(RC.bray.dist)[
  which(design$order != 1 & design$habitat != "water"),
  which(design$order != 1 & design$habitat == "water")]

# construct dfs for headwater downstream comparison
hwsp <- simba::liste(headwaters.dist.sed.phy, entry = "bNTI")
hwwp <- simba::liste(headwaters.dist.water.phy, entry = "bNTI")
dssp <- simba::liste(mainstem.dist.sed.phy, entry = "bNTI")
dswp <- simba::liste(mainstem.dist.water.phy, entry = "bNTI")
hwswp <- simba::liste(headwaters.dist.sed.water.phy, entry = "bNTI")
dsswp <- simba::liste(downstream.dist.sed.water.phy, entry = "bNTI")

hwswt <- simba::liste(headwaters.dist.sed.water.tax, entry = "RC_hel")
dsswt <- simba::liste(downstream.dist.sed.water.tax, entry = "RC_hel")
hwst <- simba::liste(headwaters.dist.sed.tax, entry = "RC_hel")
hwwt <- simba::liste(headwaters.dist.water.tax, entry = "RC_hel")
dsst <- simba::liste(mainstem.dist.sed.tax, entry = "RC_hel")
dswt <- simba::liste(mainstem.dist.water.tax, entry = "RC_hel")

hwsp$position <- "Headwater"
hwwp$position <- "Headwater"
hwswp$position <- "Headwater"
hwst$position <- "Headwater"
hwwt$position <- "Headwater"
hwswt$position <- "Headwater"

dssp$position <- "Downstream"
```

```
dswp$position <- "Downstream"
dsswp$position <- "Downstream"
dsst$position <- "Downstream"
dswt$position <- "Downstream"
dsswt$position <- "Downstream"

hwsp$habitat <- "Benthic"
hwwp$habitat <- "Planktonic"
hwswp$habitat <- "Planktonic-Benthic"
hwst$habitat <- "Benthic"
hwwt$habitat <- "Planktonic"
hwswt$habitat <- "Planktonic-Benthic"

dssp$habitat <- "Benthic"
dswp$habitat <- "Planktonic"
dsswp$habitat <- "Planktonic-Benthic"
dsst$habitat <- "Benthic"
dswt$habitat <- "Planktonic"
dsswt$habitat <- "Planktonic-Benthic"

assembly.by.position <- bind_rows(
  full_join(hwsp, hwst),
  full_join(hwwp, hwwt),
  full_join(hwswp, hwswt),
  full_join(dssp, dsst),
  full_join(dswp, dswt),
  full_join(dsswp, dsswt)
)

assembly.by.position.process <- assembly.by.position %>%
  mutate(process = ifelse(bNTI < -2, "Homogeneous selection",
                          ifelse(bNTI > 2, "Variable selection",
                                 ifelse(RC_hel > 0.95, "Dispersal limitation",
                                        ifelse(RC_hel < -0.95, "Mass effects", "Undominated"))))) %>%
  mutate(signif = ifelse(abs(RC_hel) < 0.95 & abs(bNTI) < 2, FALSE, TRUE))


assembly.position.grouped <- assembly.by.position.process %>%
  group_by(habitat, position) %>%
  count(process) %>%
  mutate(proportion = n/sum(n))

assembly.position.grouped <- assembly.position.grouped %>%
  full_join(unique(expand.grid(assembly.position.grouped[,1:3])), fill = 0) %>%
  mutate(proportion = ifelse(is.na(proportion),0, proportion))

assembly.position.grouped$habitat <- factor(assembly.position.grouped$habitat, levels = c("Planktonic",

assembly.position.grouped %>%
  mutate(position = factor(position, levels = c("Headwater", "Downstream"))) %>%
  mutate(process = factor(process, levels = c("Undominated",
                                              "Variable selection",
                                              "Homogeneous selection",
```

```
                                          "Mass effects",
                                          "Dispersal limitation"))) %>%
  ggplot(aes(x = position, y = proportion, fill = process)) +
  geom_bar(alpha = 1, position = "stack", stat = "identity") +
  facet_wrap(~ habitat, ncol = 1) +
  scale_fill_manual(values = c("#02440c","#789400","#e0d052", "#edf0db", "#40becd")) +
  labs(x = "", y = "Proportion") +
  theme(legend.position = "bottom",
        legend.title = element_blank(),
        legend.text = element_text(size = 9)) +
  guides(fill = guide_legend(nrow = 2)) +
  ggsave("figures/Fig5.png", height = 8, width = 4, dpi = 600) +
  ggsave("figures/Fig5.pdf", height = 8, width = 4)
```




```
assembly.position.grouped %>% count()
```

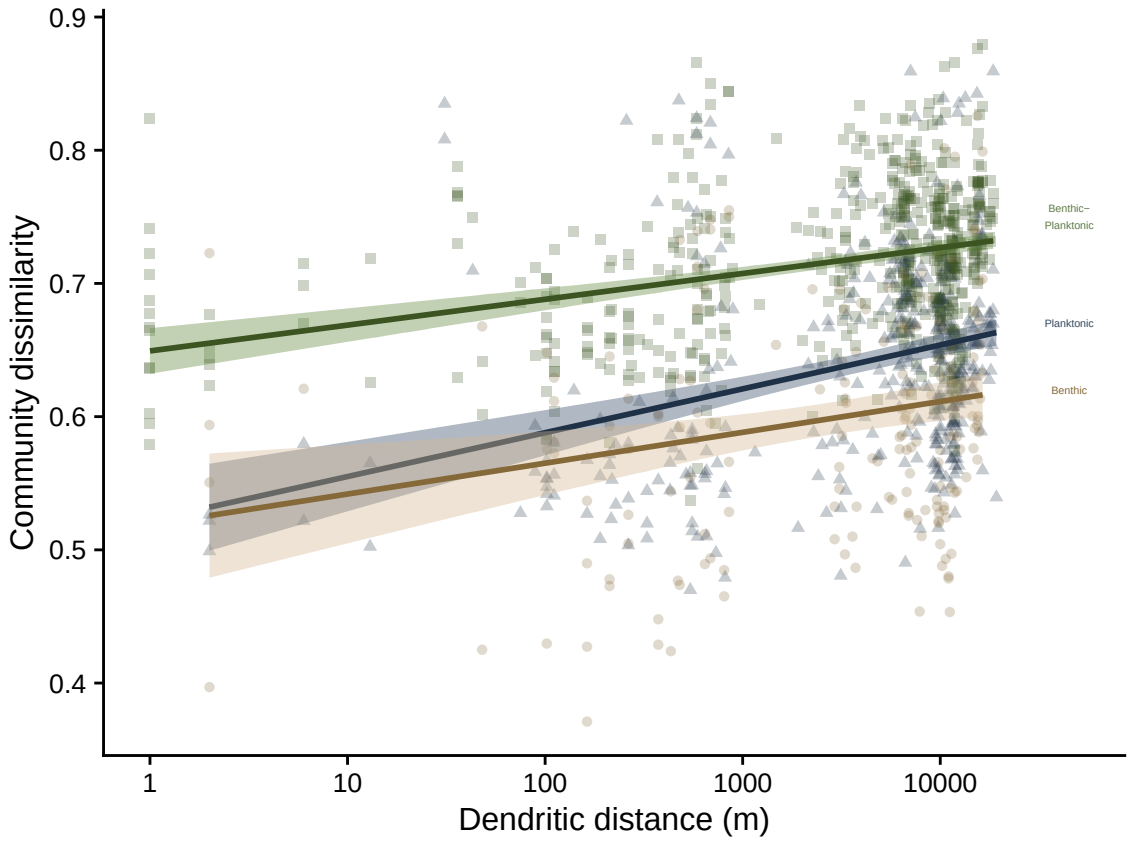
```
## # A tibble: 6 x 3
## # Groups:   habitat, position [6]
##   habitat            position       n
##   <fct>              <chr>      <int>
## 1 Planktonic         Downstream     4
## 2 Planktonic         Headwater      4
## 3 Benthic            Downstream     4
## 4 Benthic            Headwater      4
## 5 Planktonic-Benthic Downstream     4
## 6 Planktonic-Benthic Headwater      4
```

```
assembly.by.position.process %>%
  group_by(position, habitat) %>%
  count(process)
```

```
## # A tibble: 20 x 4
## # Groups:   position, habitat [6]
##    position   habitat            process                  n
##    <chr>      <chr>              <chr>                <int>
##  1 Downstream Benthic            Dispersal limitation     9
##  2 Downstream Benthic            Homogeneous selection    79
##  3 Downstream Benthic            Undominated              15
##  4 Downstream Benthic            Variable selection       17
##  5 Downstream Planktonic         Dispersal limitation     49
##  6 Downstream Planktonic         Homogeneous selection   137
##  7 Downstream Planktonic         Undominated              12
##  8 Downstream Planktonic         Variable selection       78
##  9 Downstream Planktonic-Benthic Dispersal limitation     29
## 10 Downstream Planktonic-Benthic Homogeneous selection     8
## 11 Downstream Planktonic-Benthic Undominated               1
## 12 Downstream Planktonic-Benthic Variable selection      346
## 13 Headwater  Benthic            Homogeneous selection     1
## 14 Headwater  Benthic            Variable selection        5
## 15 Headwater  Planktonic         Dispersal limitation      1
## 16 Headwater  Planktonic         Homogeneous selection     2
## 17 Headwater  Planktonic         Variable selection        7
## 18 Headwater  Planktonic-Benthic Dispersal limitation      1
## 19 Headwater  Planktonic-Benthic Homogeneous selection     2
## 20 Headwater  Planktonic-Benthic Variable selection       17
```

Make figure 3

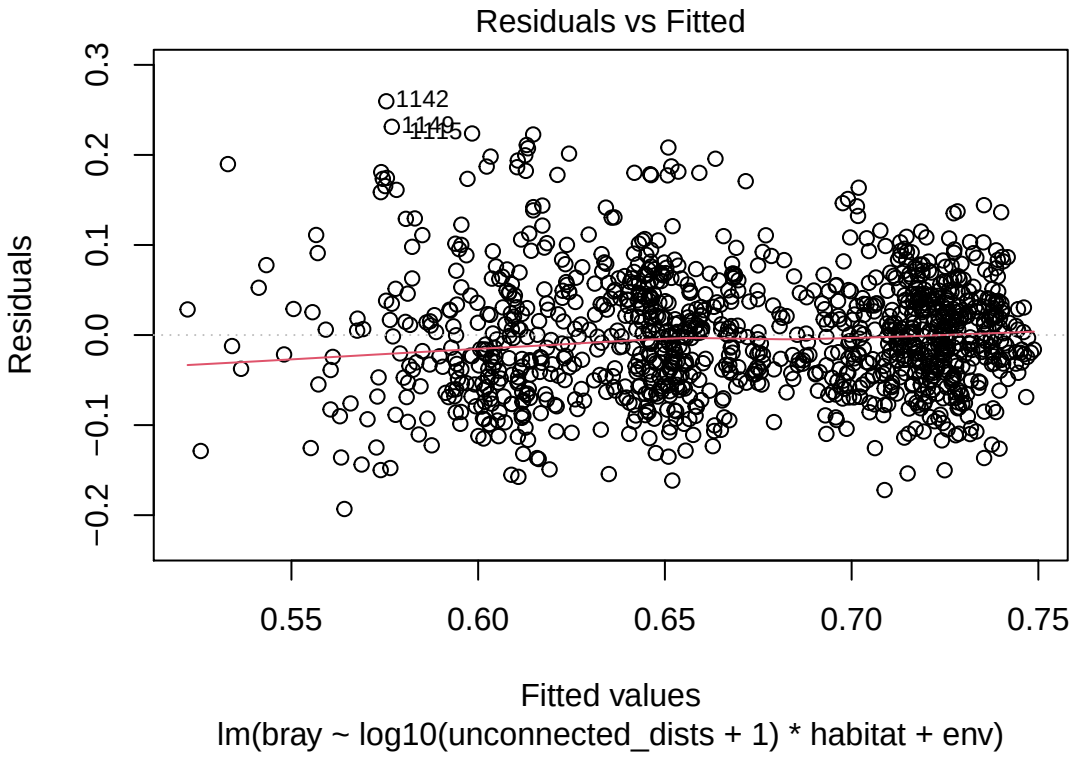
```
hja.assembly$hel <- vegdist(OTUsREL.hel, method = "euc")
hja.assembly$bray <- vegdist(OTUsREL, method = "bray")
my.colors <- c("#3a4f6a", "#d5ba94")
hja.assembly %>% left_join(simba::liste(den.dists, entry = "unconnected_dists")) %>%
  ggplot(aes(x = unconnected_dists +1, y = bray, color = habitat, fill = habitat, shape = habitat)) +
  geom_point(alpha = 0.25) +
  geom_smooth(method = "lm") +
  scale_x_log10(lim = c(1, 50000)) +
  scale_color_manual(values=colorspace::darken(c(my.colors, "#688c45"),0.4)) +
  scale_fill_manual(values=c(my.colors, "#688c45", my.colors,   "#688c45")) +
  scale_shape_manual(values=c(17, 16, 15)) +
  labs(x = "Dendritic distance (m)",
       y = "Community dissimilarity") +
  theme(legend.position = "none") +
  annotate("text", x = 45000, y = 0.75, label = "Benthic-\nPlanktonic", size = 1.5, color = darken("#688
  annotate("text", x = 45000, y = 0.67, label = "Planktonic", size = 1.5, color = darken("#3a4f6a")) +
  annotate("text", x = 45000, y = 0.62, label = "Benthic", size = 1.5, color = darken("#d5ba94",.4)) +
  ggsave("figures/Fig3.pdf", width = 8, height = 8*3/4, units = "cm") +
  ggsave("figures/Fig3.png", width = 8, height = 8*3/4, units = "cm", dpi = 600)
```

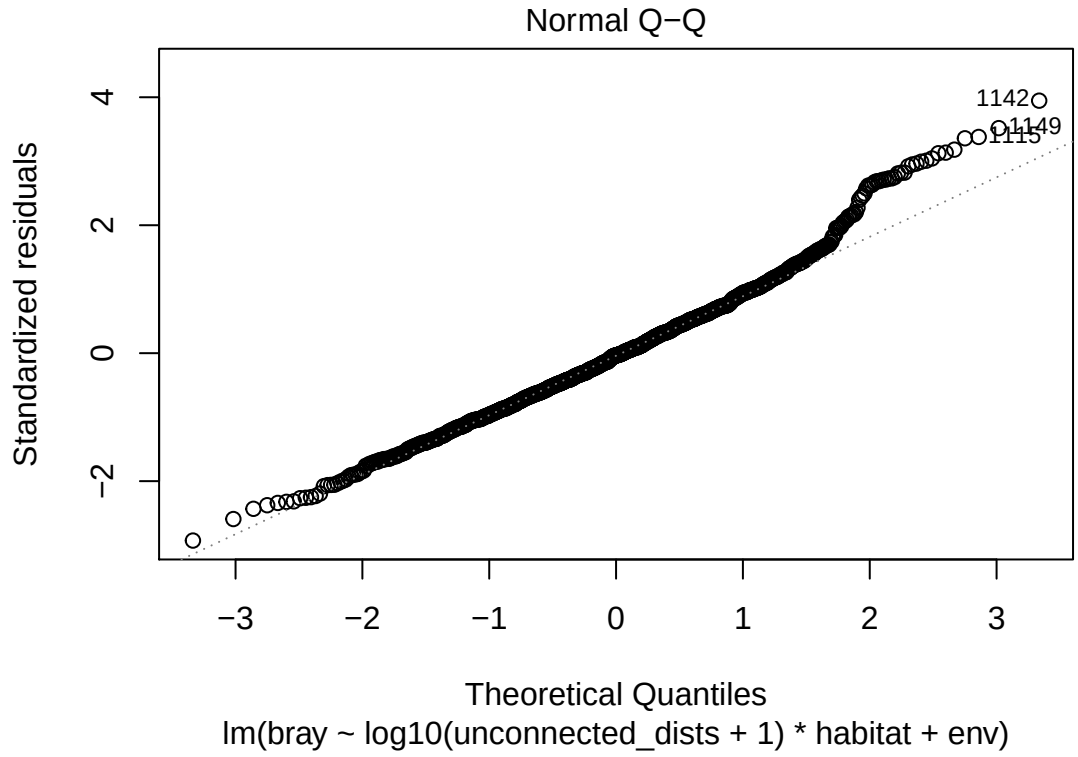


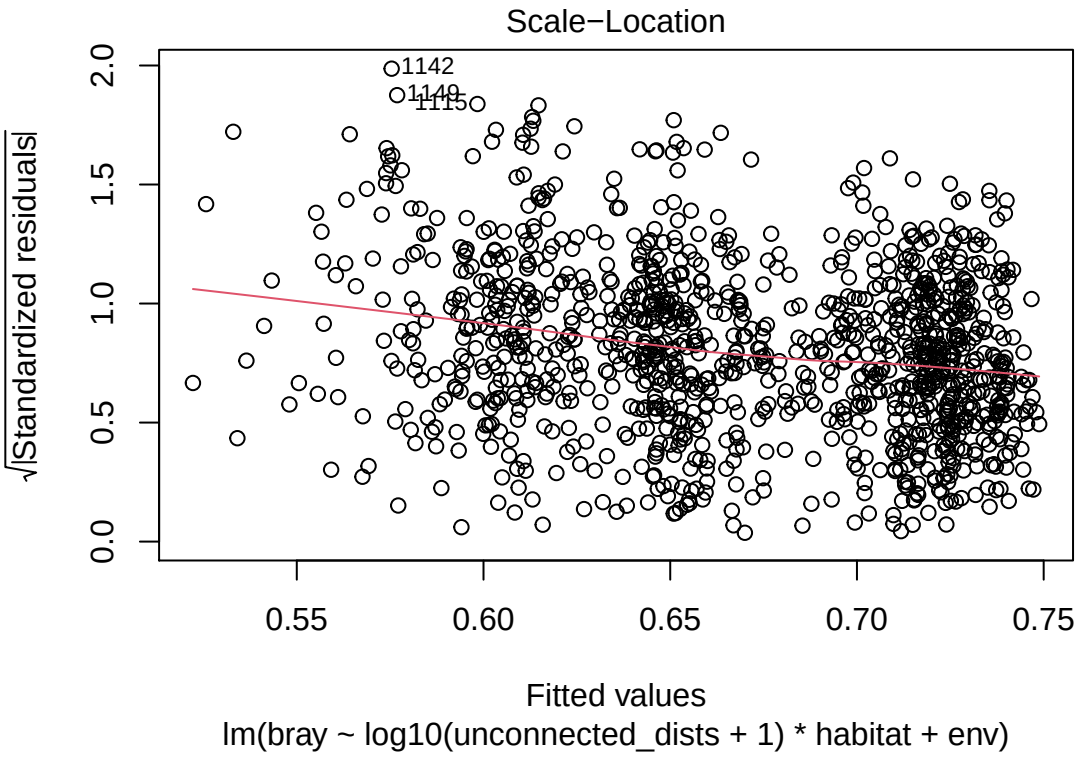

```
ddr_formod <- hja.assembly %>% left_join(simba::liste(den.dists, entry = "unconnected_dists")) %>% muta

ddr_envdist_mod <- lm(bray ~ log10(unconnected_dists+1) * habitat + env, data = ddr_formod)
AIC(ddr_envdist_mod)
```

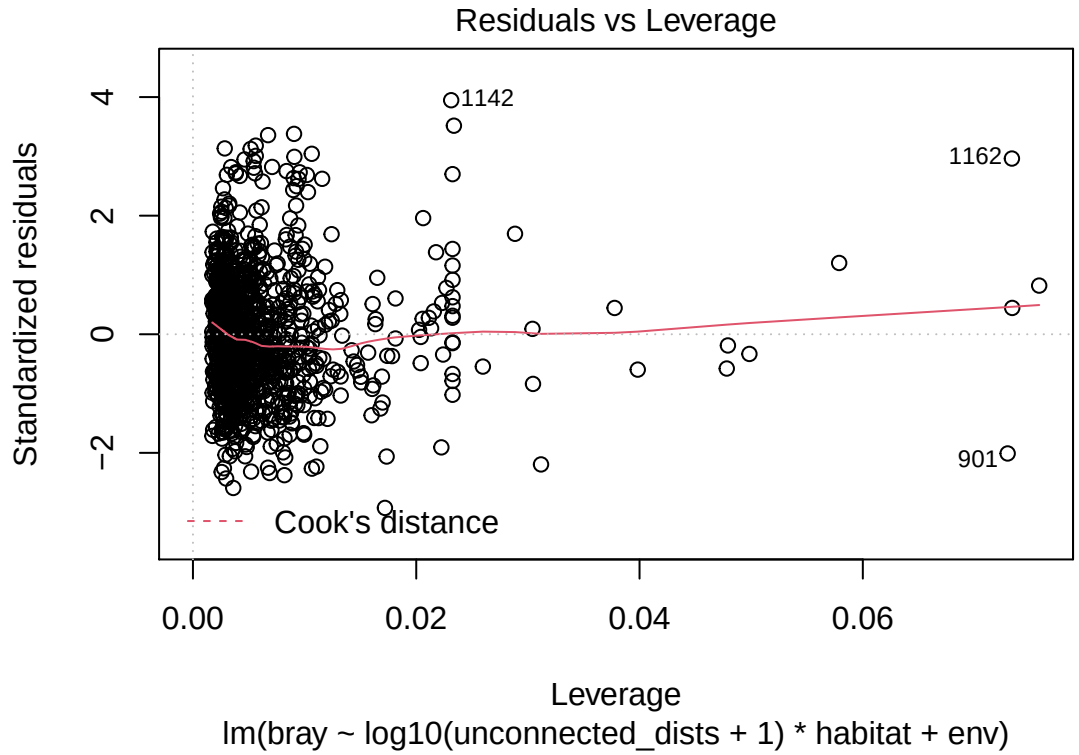
```
## [1] -3027.704
```

```
plot(ddr_envdist_mod)
```


Residuals vs Fitted
Residuals
Fitted values
lm(bray ~ log10(unconnected_dists + 1) * habitat + env)
1142
1149
1115


Normal Q−Q
Standardized residuals
Theoretical Quantiles
lm(bray ~ log10(unconnected_dists + 1) * habitat + env)
1142
1149
1115



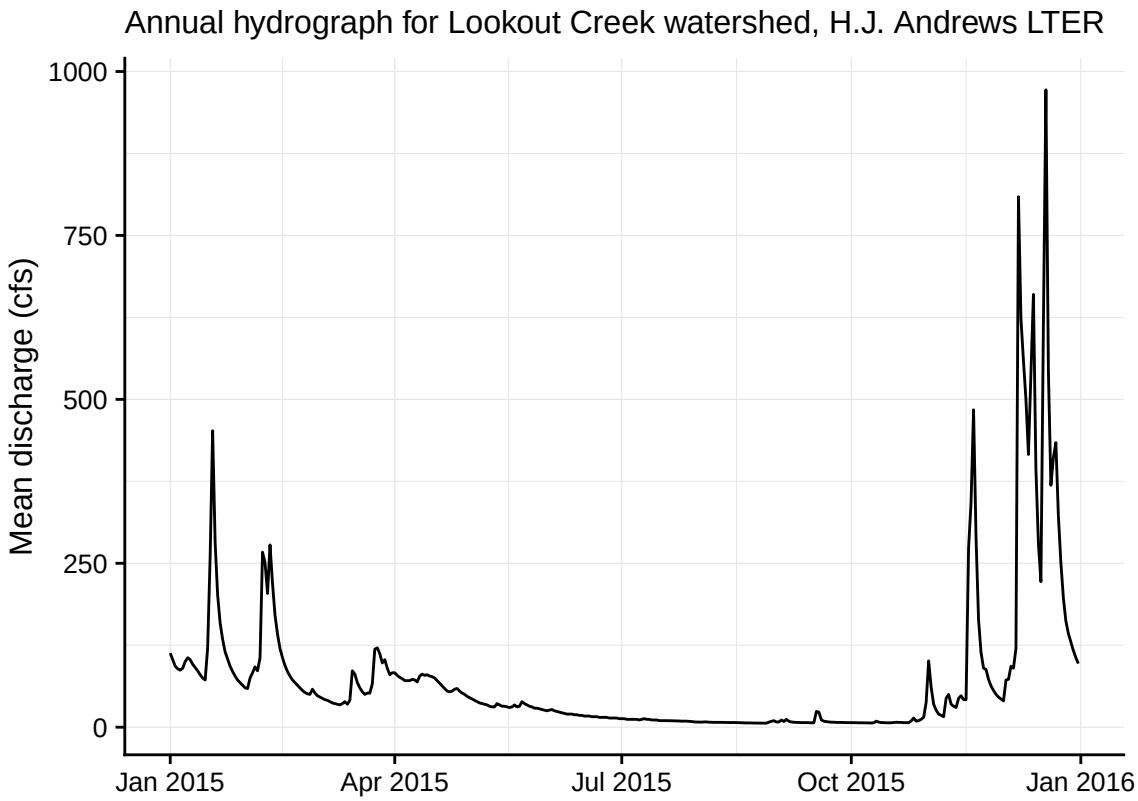



```
summary(ddr_envdist_mod)
```

```
##
## Call:
## lm(formula = bray ~ log10(unconnected_dists + 1) * habitat +
##     env, data = ddr_formod)
```

```
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.193150 -0.044140 -0.002145  0.038978  0.259542
##
## Coefficients:
##                                                          Estimate Std. Error
## (Intercept)                                              0.516084   0.016717
## log10(unconnected_dists + 1)                             0.029122   0.004681
## habitatBenthic                                          -0.010557   0.026598
## habitatPlanktonic-Benthic                                0.125375   0.019924
## env                                                      0.005600   0.001475
## log10(unconnected_dists + 1):habitatBenthic             -0.007878   0.007418
## log10(unconnected_dists + 1):habitatPlanktonic-Benthic -0.013038   0.005488
##                                                         t value Pr(>|t|)
## (Intercept)                                              30.873  < 2e-16 ***
## log10(unconnected_dists + 1)                              6.221 6.86e-10 ***
## habitatBenthic                                           -0.397 0.691505
## habitatPlanktonic-Benthic                                 6.293 4.40e-10 ***
## env                                                       3.796 0.000155 ***
## log10(unconnected_dists + 1):habitatBenthic              -1.062 0.288440
## log10(unconnected_dists + 1):habitatPlanktonic-Benthic  -2.376 0.017680 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.06653 on 1169 degrees of freedom
## Multiple R-squared:  0.3764, Adjusted R-squared:  0.3732
## F-statistic: 117.6 on 6 and 1169 DF,  p-value: < 2.2e-16
```

```
HF00402_v12 <- read_csv("data/HF00402_v12.csv")

HF00402_v12 %>%
  filter(SITECODE == "GSLOOK") %>%
  filter(lubridate::year(DATE) == "2015") %>%
  ggplot(aes(x = DATE, y = MEAN_Q)) +
  geom_line() +
  theme(panel.grid.major = element_line(size = .2, color = "gray90"),
        panel.grid.minor = element_line(size = .1, color = "gray90")) +
  labs(x="", y = "Mean discharge (cfs)", subtitle = "Annual hydrograph for Lookout Creek watershed, H.J
```


Annual hydrograph for Lookout Creek watershed, H.J. Andrews LTER
Mean discharge (cfs)
1000
750
500
250
0
Jan 2015
Apr 2015
Jul 2015
Oct 2015
Jan 2016