Dormancy and dispersal structure bacterial communities across ecosystem boundaries

Nathan I. Wisnoski, Mario E. Muscarella, Megan L. Larsen, and Jay T. Lennon

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Initial Setup

First, we'll load the packages we'll need for the analysis, as well as some other functions.

```
# Import Required Packages
library("png")
library("grid")
library("tidyverse")
library("vegan")
library("xtable")
library("viridis")
library("cowplot")
library("adespatial")
library("ggrepel")
library("gganimate")
library("maps")
library("rgdal")
library("iNEXT")
library("officer")
library("flextable") #must have gdtools installed also
library("broom")
library("ggpmisc")
library("pander")
library("lubridate")

source("bin/mothur_tools.R")
se <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}
```

Next, we'll set the aesthetics of the figures we will produce.

```
my.cols <- RColorBrewer::brewer.pal(n = 4, name = "Greys")[3:4]

# Set theme for figures in the paper
theme_set(theme_classic() +
  theme(axis.title = element_text(size = 16),
        axis.title.x = element_text(margin = margin(t = 15, b = 15)),
        axis.title.y = element_text(margin = margin(l = 15, r = 15)),
        axis.text = element_text(size = 14),
        axis.text.x = element_text(margin = margin(t = 5)),
        axis.text.y = element_text(margin = margin(r = 5)),
        #axis.line.x = element_line(size = 1),
        #axis.line.y = element_line(size = 1),
        axis.line.x = element_blank(),
        axis.line.y = element_blank(),
        axis.ticks.x = element_line(size = 1),
```

```
        axis.ticks.y = element_line(size = 1),
        axis.ticks.length = unit(.1, "in"),
        panel.border = element_rect(color = "black", fill = NA, size = 1.5),
        legend.title = element_blank(),
        legend.text = element_text(size = 14),
        strip.text = element_text(size = 14),
        strip.background = element_blank()
        ))
```

Import Data

Here, we read in the processed sequence files from mothur (shared and taxonomy) and a design of the sampling. We also load in the environmental data. We then remove the mock community from the dataset and ensure the the design and OTU table are aligned by row.

```
# Define Inputs
# Design = general design file for experiment
# shared = OTU table from mothur with sequence similarity clustering
# Taxonomy = Taxonomic information for each OTU
design <- "data/UL.design.txt"
shared <- "data/ul_resgrad.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.opti_m
taxon  <- "data/ul_resgrad.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.pick.opti_m

# Import Design
design <- read.delim(design, header=T, row.names=1)

# Import Shared Files
OTUs <- read.otu(shared = shared, cutoff = "0.03")    # 97% Similarity

# Import Taxonomy
OTU.tax <- read.tax(taxonomy = taxon, format = "rdp")

# Load environmental data
env.dat <- read.csv("data/ResGrad_EnvDat.csv", header = TRUE)
env.dat <- env.dat[-c(16,17,18),]

# Subset to just the reservoir gradient sites
OTUs <- OTUs[str_which(rownames(OTUs), "RG"),]
OTUs <- OTUs[-which(rownames(OTUs) == "RGMockComm"),]

# make sure OTU table matches up with design order
design <- design[-c(34:39),]
OTUs <- OTUs[match(rownames(design), rownames(OTUs)),]
design$distance <- max(na.omit(design$distance)) - design$distance
env.dat$distance <- max(na.omit(env.dat$dist.dam)) - env.dat$dist.dam
```

Clean and transform OTU table

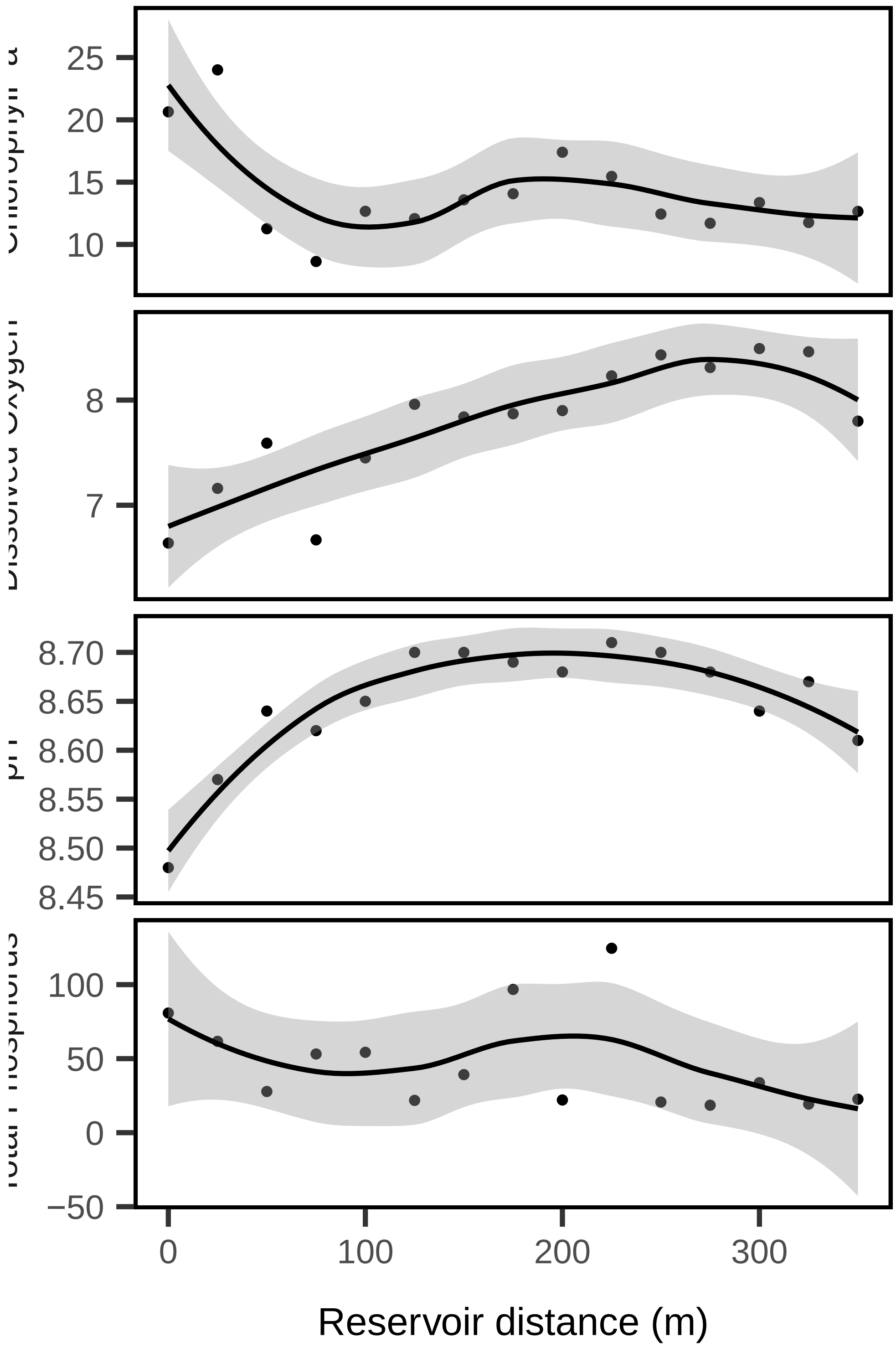
Here, we remove OTUs with low incidence across sites, we remove any samples with low coverage, and we standardize the OTU table by log-transforming the abundances and relativizing by site.

```r
# Remove OTUs with less than two occurences across all sites
#OTUs <- OTUs[, which(colSums(OTUs) >= 2)]

# Sequencing Coverage
coverage <- rowSums(OTUs)

# Remove Low Coverage Samples (This code removes two sites: Site 5DNA, Site 6cDNA)
lows <- which(coverage < 10000)
OTUs <- OTUs[-which(coverage < 10000), ]
design <- design[-which(coverage < 10000), ]
otus.for.inext <-  t(OTUs)
# Remove OTUs with < 2 occurences across all sites
OTUs <- OTUs[, which(colSums(OTUs) >= 2)]
coverage <- rowSums(OTUs)
set.seed(47405)
OTUs <- rrarefy(OTUs, min(coverage))

# Make Relative Abundance Matrices
OTUsREL <- decostand(OTUs, method = "total")

# Log Transform Relative Abundances
OTUsREL.log <- decostand(OTUs, method = "log")
```

Reservoir environmental gradients

Just to see if there are any strong underlying resource or nutrient gradients in the reservoir, we'll plot them along the distance of the reservoir.

```r
facet.labs <- c(`chla` = "Chlorophyll-a",
                `color` = "Color",
                `DO` = "Dissolved Oxygen",
                `pH` = "pH",
                `TP` = "Total Phosphorus")

env.dat %>% select(distance, DO, pH, TP, chla) %>%
  gather(variable, value, -distance) %>%
  ggplot(aes(x = distance, y = value)) +
  geom_point() +
  geom_smooth(method = "loess", color = "black") +
  facet_grid(variable ~., scales = "free", switch = "y",
             labeller = as_labeller(facet.labs)) +
  theme(strip.background = element_blank(),
        strip.text = element_text(size = 14),
        strip.placement = "outside") +
  labs(x = "Reservoir distance (m)",
       y = "") +
  scale_y_continuous()
```



So, there are some weak gradients, but nothing too prevailing.

Analyze Diversity

Now, we will analyze the bacterial diversity in the reservoir and nearby soils to figure out how well they support different mechanisms of community assembly.

How does α-diversity vary along the reservoir?

First, we use the method of rarefaction and extrapolation developed by Chao et al. in the iNEXT package.

```
# Observed Richness
S.obs <- rowSums((OTUs > 0) * 1)

# Simpson's Evenness
SimpE <- function(x = ""){
  x <- as.data.frame(x)
  D <- diversity(x, "inv")
  S <- sum((x > 0) * 1)
  E <- (D)/S
  return(E)
}
simpsE <- round(apply(OTUs, 1, SimpE), 3)
shan <- diversity(OTUs, index = "shannon")
exp.shan <- exp(shan)
alpha.div <- cbind(design, S.obs, simpsE, shan, exp.shan)

# define singleton estimator from Chiu and Chao 2016 PeerJ
source("bin/Chao_functions.R")

# # estimate richness
singleton.apply <- function(x){
  singleton.Est(x, "abundance")$corrected.data
}

otus.for.inext <- apply(otus.for.inext, MARGIN = 2, singleton.apply)
# divestim <- estimateD(otus.for.inext, datatype = "abundance",
#           base = "size", conf = 0.95)
# divestim <- iNEXT(otus.for.inext, datatype = "abundance",
#                   size =  min(coverage), nboot = 999)
# divestim$iNextEst
# saveRDS(divestim, file = "intermediate-data/inext-output.rda")
divestim <- read_rds("intermediate-data/inext-output.rda")
divestim
```

```
##          site     m       method order    SC       qD   qD.LCL   qD.UCL
## 1   RGSoil01 37027 interpolated     0 0.955 3889.214 3867.259 3911.169
## 2   RGSoil01 37027 interpolated     1 0.955  752.963  746.212  759.715
## 3   RGSoil01 37027 interpolated     2 0.955  182.356  179.896  184.817
## 4   RGSoil02 37027 interpolated     0 0.945 4392.817 4367.251 4418.382
## 5   RGSoil02 37027 interpolated     1 0.945  666.077  660.260  671.895
## 6   RGSoil02 37027 interpolated     2 0.945  134.658  132.742  136.574
## 7   RGSoil03 37027 interpolated     0 0.950 4191.294 4161.602 4220.987
## 8   RGSoil03 37027 interpolated     1 0.950  773.790  766.732  780.848
## 9   RGSoil03 37027 interpolated     2 0.950  179.353  176.344  182.363
## 10     RGD01 37027     observed     0 0.999  350.000  341.907  358.093
```

```
## 11     RGD01 37027     observed     1 0.999   60.336   59.421   61.251
## 12     RGD01 37027     observed     2 0.999   30.661   30.111   31.211
## 13     RGc01 37027 interpolated     0 0.996  285.774  277.158  294.391
## 14     RGc01 37027 interpolated     1 0.996   21.184   21.034   21.335
## 15     RGc01 37027 interpolated     2 0.996    9.764    9.671    9.858
## 16     RGD02 37027 interpolated     0 0.996  535.631  521.557  549.704
## 17     RGD02 37027 interpolated     1 0.996   70.802   69.799   71.805
## 18     RGD02 37027 interpolated     2 0.996   35.622   35.059   36.185
## 19     RGc02 37027 interpolated     0 0.996  276.963  269.657  284.269
## 20     RGc02 37027 interpolated     1 0.996   31.118   30.905   31.332
## 21     RGc02 37027 interpolated     2 0.996   16.072   15.940   16.204
## 22     RGD03 37027 interpolated     0 0.997  576.480  562.427  590.532
## 23     RGD03 37027 interpolated     1 0.997   67.500   66.450   68.550
## 24     RGD03 37027 interpolated     2 0.997   31.838   31.267   32.409
## 25     RGc03 37027 interpolated     0 0.997  166.654  160.265  173.044
## 26     RGc03 37027 interpolated     1 0.997    7.545    7.497    7.593
## 27     RGc03 37027 interpolated     2 0.997    4.194    4.165    4.223
## 28     RGD04 37027 interpolated     0 0.996  536.871  519.687  554.055
## 29     RGD04 37027 interpolated     1 0.996   71.051   70.051   72.052
## 30     RGD04 37027 interpolated     2 0.996   35.457   34.821   36.092
## 31     RGc04 37027 interpolated     0 0.997  392.580  385.414  399.746
## 32     RGc04 37027 interpolated     1 0.997    2.241    2.218    2.264
## 33     RGc04 37027 interpolated     2 0.997    1.336    1.331    1.341
## 34     RGc05 37027 interpolated     0 0.998  212.420  204.739  220.101
## 35     RGc05 37027 interpolated     1 0.998    4.881    4.840    4.923
## 36     RGc05 37027 interpolated     2 0.998    3.967    3.950    3.984
## 37     RGD06 37027 interpolated     0 0.992  720.373  709.705  731.041
## 38     RGD06 37027 interpolated     1 0.992   61.376   60.858   61.894
## 39     RGD06 37027 interpolated     2 0.992   26.153   25.921   26.386
## 40     RGD07 37027 interpolated     0 0.991 1016.407  994.401 1038.413
## 41     RGD07 37027 interpolated     1 0.991   85.475   83.864   87.085
## 42     RGD07 37027 interpolated     2 0.991   34.786   34.100   35.471
## 43     RGc07 37027 interpolated     0 0.997  171.638  163.075  180.202
## 44     RGc07 37027 interpolated     1 0.997    4.496    4.467    4.524
## 45     RGc07 37027 interpolated     2 0.997    3.192    3.172    3.213
## 46     RGD08 37027 interpolated     0 0.992  835.316  824.174  846.458
## 47     RGD08 37027 interpolated     1 0.992   71.572   70.913   72.230
## 48     RGD08 37027 interpolated     2 0.992   29.885   29.555   30.216
## 49     RGc08 37027 interpolated     0 0.998  165.011  160.172  169.850
## 50     RGc08 37027 interpolated     1 0.998   18.257   18.159   18.355
## 51     RGc08 37027 interpolated     2 0.998   10.562   10.482   10.642
## 52     RGD09 37027 interpolated     0 0.993  962.514  942.906  982.123
## 53     RGD09 37027 interpolated     1 0.993  102.957  101.246  104.668
## 54     RGD09 37027 interpolated     2 0.993   40.437   39.617   41.256
## 55     RGc09 37027 interpolated     0 0.997  264.910  257.723  272.096
## 56     RGc09 37027 interpolated     1 0.997    5.931    5.883    5.979
## 57     RGc09 37027 interpolated     2 0.997    3.899    3.879    3.920
## 58     RGD10 37027 interpolated     0 0.992  979.243  968.583  989.904
## 59     RGD10 37027 interpolated     1 0.992  115.134  114.131  116.138
## 60     RGD10 37027 interpolated     2 0.992   50.536   49.946   51.126
## 61     RGc10 37027 interpolated     0 0.993  728.724  712.946  744.503
## 62     RGc10 37027 interpolated     1 0.993   78.838   77.746   79.930
## 63     RGc10 37027 interpolated     2 0.993   29.012   28.430   29.595
## 64     RGD11 37027 interpolated     0 0.990 1423.107 1411.926 1434.289
```

```
## 65     RGD11 37027 interpolated     1 0.990   161.982  160.650  163.315
## 66     RGD11 37027 interpolated     2 0.990    65.095   64.432   65.759
## 67     RGc11 37027 interpolated     0 0.996   307.585  299.254  315.916
## 68     RGc11 37027 interpolated     1 0.996    36.292   36.060   36.524
## 69     RGc11 37027 interpolated     2 0.996    22.636   22.483   22.788
## 70     RGD12 37027 interpolated     0 0.991 1720.686 1709.731 1731.640
## 71     RGD12 37027 interpolated     1 0.991   252.525  250.280  254.770
## 72     RGD12 37027 interpolated     2 0.991    85.267   84.458   86.077
## 73     RGc12 37027 interpolated     0 0.995   372.791  363.552  382.029
## 74     RGc12 37027 interpolated     1 0.995    24.840   24.682   24.997
## 75     RGc12 37027 interpolated     2 0.995    17.702   17.624   17.780
## 76     RGD13 37027 interpolated     0 0.988   930.870  916.712  945.028
## 77     RGD13 37027 interpolated     1 0.988    56.414   55.942   56.885
## 78     RGD13 37027 interpolated     2 0.988    23.056   22.824   23.287
## 79     RGc13 37027 interpolated     0 0.997   269.903  263.231  276.575
## 80     RGc13 37027 interpolated     1 0.997    15.722   15.619   15.825
## 81     RGc13 37027 interpolated     2 0.997    10.745   10.689   10.800
## 82     RGD14 37027 interpolated     0 0.986 1034.420 1017.730 1051.109
## 83     RGD14 37027 interpolated     1 0.986    73.078   72.401   73.755
## 84     RGD14 37027 interpolated     2 0.986    31.228   30.863   31.592
## 85     RGc14 37027 interpolated     0 0.996   274.400  266.768  282.033
## 86     RGc14 37027 interpolated     1 0.996    24.518   24.418   24.619
## 87     RGc14 37027 interpolated     2 0.996    18.355   18.270   18.441
## 88     RGD15 37027 interpolated     0 0.987 1793.670 1777.615 1809.724
## 89     RGD15 37027 interpolated     1 0.987   203.796  201.493  206.100
## 90     RGD15 37027 interpolated     2 0.987    70.240   69.353   71.127
## 91     RGc15 37027 interpolated     0 0.997   234.673  225.851  243.495
## 92     RGc15 37027 interpolated     1 0.997    25.655   25.508   25.802
## 93     RGc15 37027 interpolated     2 0.997    18.394   18.269   18.519
## 94     RGD16 37027 interpolated     0 0.983 1539.874 1520.207 1559.540
## 95     RGD16 37027 interpolated     1 0.983    39.704   39.088   40.320
## 96     RGD16 37027 interpolated     2 0.983     9.644    9.523    9.765
## 97     RGc16 37027 interpolated     0 0.998   122.606  116.878  128.335
## 98     RGc16 37027 interpolated     1 0.998     2.358    2.345    2.371
## 99     RGc16 37027 interpolated     2 0.998     1.747    1.740    1.755
## 100    RGD17 37027 interpolated     0 0.993 1190.721 1176.273 1205.170
## 101    RGD17 37027 interpolated     1 0.993   126.164  124.455  127.873
## 102    RGD17 37027 interpolated     2 0.993    44.699   44.030   45.368
## 103    RGc17 37027 interpolated     0 0.997   380.131  373.375  386.886
## 104    RGc17 37027 interpolated     1 0.997    12.276   12.171   12.381
## 105    RGc17 37027 interpolated     2 0.997     6.641    6.604    6.679
## 106    RGD18 37027 interpolated     0 0.986 2304.240 2290.738 2317.742
## 107    RGD18 37027 interpolated     1 0.986   296.102  292.933  299.270
## 108    RGD18 37027 interpolated     2 0.986    76.031   75.068   76.993
## 109    RGc18 37027 interpolated     0 0.996   220.000  212.572  227.429
## 110    RGc18 37027 interpolated     1 0.996     4.727    4.704    4.750
## 111    RGc18 37027 interpolated     2 0.996     3.665    3.654    3.676
```

```
divestim.df <- divestim %>%
 mutate(habitat = str_to_title(design[as.character(site),"type"]))
```

Here is the resulting curve, showing the higher diversity in soil samples relative to the lake samples.

```
# divestim.df %>%
#   ggplot(aes(x = x, y = y,
```

```r
#               ymin = y.lwr, ymax = y.upr,
#               color = habitat, fill = habitat, group = site)) +
#   geom_ribbon(data=subset(divestim.df, method == "extrapolated"), alpha = 0.3) +
#   geom_line(data=subset(divestim.df, method == "interpolated"), size = 1, alpha = .8) +
#   geom_line(alpha = 1, linetype = "dashed") +
#   scale_x_continuous(labels = scales::comma, limits = c(0, 90000)) +
#   labs(x = "Sample size", y = "Estimated richness") +
#   theme(legend.position = "none") +
#   #theme(legend.position =  c(.88,.5)) +
#   annotate(label = "Soil", size = 6, geom = "text", x = 85000, y = 5000) +
#   annotate(label = "Water", size = 6, geom = "text", x = 85000, y = 1500) +
#   scale_color_grey(end = .7) +
#   scale_fill_grey(end = .7)
```

Next, we'll extract the estimates for the Hill numbers at different levels of q, which differentially weight common versus rare species.

```r
# hill.estim <- divestim$AsyEst %>% filter(Diversity == "Species richness") %>%
#   left_join(rownames_to_column(alpha.div), by = c("Observed" = "S.obs")) %>%
#   select(Site, rowname, station, molecule, type, distance) %>%
#   left_join(divestim$AsyEst, by = "Site")

hill.water <- divestim.df %>%
  filter(site %in% rownames(OTUs)) %>%
  left_join(rownames_to_column(alpha.div, var = "site")) %>%
  filter(habitat == "Water")
```

```
## Warning: Column `site` joining factor and character vector, coercing into
## character vector
```

```r
hill.water.rich <- subset(hill.water, order == 0)
hill.water.shan <- subset(hill.water, order == 1)
hill.water.simp <- subset(hill.water, order == 2)

hill.water.mod.rich <- lm(qD ~ distance * molecule, data = hill.water.rich)
hill.water.mod.shan <- lm(qD ~ distance * molecule, data = hill.water.shan)
hill.water.mod.simp <- lm(qD ~ distance * molecule, data = hill.water.simp)

# summary(hill.water.mod.rich)
# summary(hill.water.mod.shan)
# summary(hill.water.mod.simp)

# tidy up the model output
hill.water.mods <- as_tibble(rbind.data.frame(
  tidy(hill.water.mod.rich) %>% add_column(Diversity = "Richness"),
  tidy(hill.water.mod.shan) %>% add_column(Diversity = "Shannon"),
  tidy(hill.water.mod.simp) %>% add_column(Diversity = "Simpson")
))
```

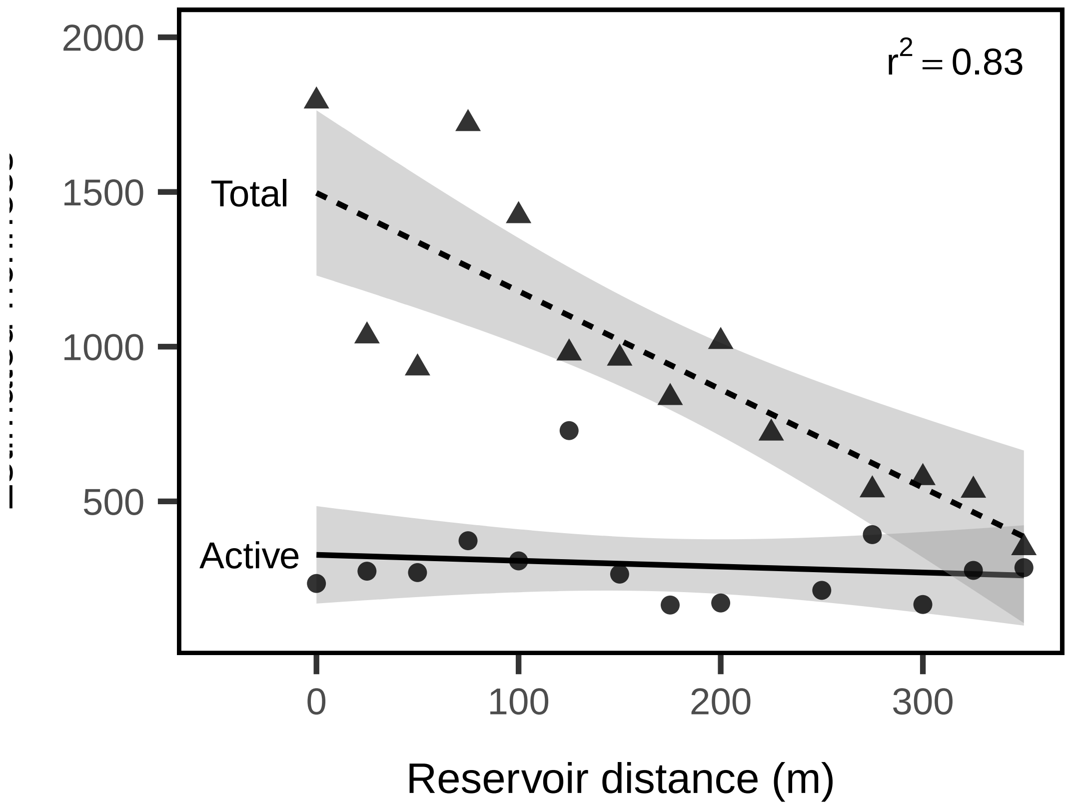
```r
# Summary table of the model results.
hill.water.mods %>%
  group_by(Diversity) %>%
  rename("Term" = term,
         "Estimate" = estimate,
         "Std. Error" = std.error,
```

```
        "Statistic" = statistic,
        "p-value" = p.value) %>%
  select(Diversity, everything()) %>%
  pander(round = 4)
```

Diversity	Term	Estimate	Std. Error	Statistic	p-value
Richness	(Intercept)	1497	100.6	14.88	0
Richness	distance	-3.176	0.4976	-6.381	0
Richness	moleculeRNA	-1170	142.3	-8.222	0
Richness	distance:moleculeRNA	2.985	0.7003	4.263	3e-04
Shannon	(Intercept)	153.7	19.41	7.921	0
Shannon	distance	-0.2941	0.096	-3.062	0.0053
Shannon	moleculeRNA	-123.9	27.46	-4.513	1e-04
Shannon	distance:moleculeRNA	0.2457	0.1352	1.818	0.0815
Simpson	(Intercept)	55.44	6.47	8.57	0
Simpson	distance	-0.0783	0.032	-2.446	0.0221
Simpson	moleculeRNA	-36.78	9.151	-4.019	5e-04
Simpson	distance:moleculeRNA	0.0402	0.045	0.8918	0.3813

```
# hill.estim %>% filter(type == "water") %>%
#   mutate(molecule = ifelse(molecule == "DNA", "Total", "Active")) %>%
#   ggplot(aes(x = distance, y = Estimator,
#              ymin = LCL, ymax = UCL,
#              color = molecule, fill = molecule, shape = molecule)) +
#   geom_point(size =3) +
#   # geom_errorbar(size = .5, aes(ymin = Estimator - s.e., ymax = Estimator + s.e.),
#   #                width = 10, alpha = 0.5) +
#   geom_smooth(method = "lm", aes(linetype = molecule)) +
#   labs(x = "Reservoir distance (m)",
#        y = "") +
#   scale_color_manual(values = my.cols) +
#   scale_fill_manual(values = my.cols) +
#   theme(legend.position = c(.88,.95), strip.placement = "outside",
#         strip.text = element_text(size = 16)) +
#   scale_x_reverse() +
#   facet_grid(Diversity ~ ., scales = "free", switch = "y") +
#   guides(fill = guide_legend(override.aes=list(fill=NA)))
  #facet_grid(Diversity ~ ., scales = "free")

# postitions for labels
xpos = max((na.omit(hill.water$distance)))
yposDNA = predict(hill.water.mod.rich, newdata = data.frame(distance = 0, molecule = "DNA"))
yposRNA = predict(hill.water.mod.rich, newdata = data.frame(distance = 0, molecule = "RNA"))
alpha.fig <- hill.water %>% filter(type == "water", order == 0) %>%
  mutate(molecule = ifelse(molecule == "DNA", "Total", "Active")) %>%
  ggplot(aes(x = distance, y = qD,
             ymin = qD.LCL, ymax = qD.UCL,
             shape = molecule)) +
  # geom_errorbar(size = .5, width = 10, alpha = 0.5) +
  geom_smooth(method = "lm", aes(linetype = molecule), color = "black") +
  geom_point(size =3, alpha = 0.8) +
  labs(x = "Reservoir distance (m)",
```

```
      y = "Estimated richness") +
  scale_y_continuous(breaks = seq(0, 2000, by = 500)) +
  scale_x_continuous(limits = c(-49, 350)) +
  theme(legend.position = "none") +
  guides(fill = guide_legend(override.aes=list(fill=NA))) +
  annotate("text", x = -33, y = yposRNA ,
           label = "Active", size = 5) +
  annotate("text", x = -33, y = yposDNA ,
           label = "Total", size = 5) +
  annotate(geom = "text", x = xpos, y = 2000, hjust = 1, vjust = 1, size = 5,
           label = paste0("r^2== ",round(summary(hill.water.mod.rich)$r.squared, 2)), parse = T) +
  ggsave("figures/alpha_fig.pdf")
alpha.fig
```



So, from the basis of these results, we can make the following conclusions. First, we note that diversity in the total community decays from the stream inlet to the dam of the reservoir. That is, all the lines have a negative slope. However, we do not see this decay in the metabolically active community. Second, we note that the metabolically actively community has much lower diversity than the total community near the soils, but this difference decreases toward the dam. Last, because we quantified diversity across three orders of Hill numbers (q = 0, 1, and 2), we can also say something about the relative importance of rare versus common taxa along the reservoir transect. We see the the significance of the distance-by-molecule interaction term decrease as rare taxa are downweighted in favor of common taxa. This suggests that the differences between the active and total communities along the transect is driven primarily by rare taxa. However, the general trend of higher Simpson diversity across the whole transect suggests that low-activity, but relatively common, taxa are maintained in the reservoir.

Similarity To Terrestrial Habitat Across Gradient (Terrestrial Influence)

Here, we fit a linear model to the similarity of the aquatic community to the soil community.

```
# Similarity to Soil Sample
UL.bray      <- 1-as.matrix(vegdist(OTUsREL.log, method="bray"))
UL.bray.lake <- UL.bray[-c(1:3), 1:3]
bray.mean    <- round(apply(UL.bray.lake, 1, mean), 3)
bray.se      <- round(apply(UL.bray.lake, 1, se), 3)
UL.sim       <- cbind(design[-c(1:3), ], bray.mean, bray.se)

# Calculate Linear Model
model.terr <- lm(bray.mean ~ distance * molecule, data = UL.sim)
predict(model.terr, newdata = data.frame(distance = 0, molecule = c("RNA", "DNA")))
```

```
##          1          2
## 0.03090104 0.16890225
```

```
pander(model.terr)
```

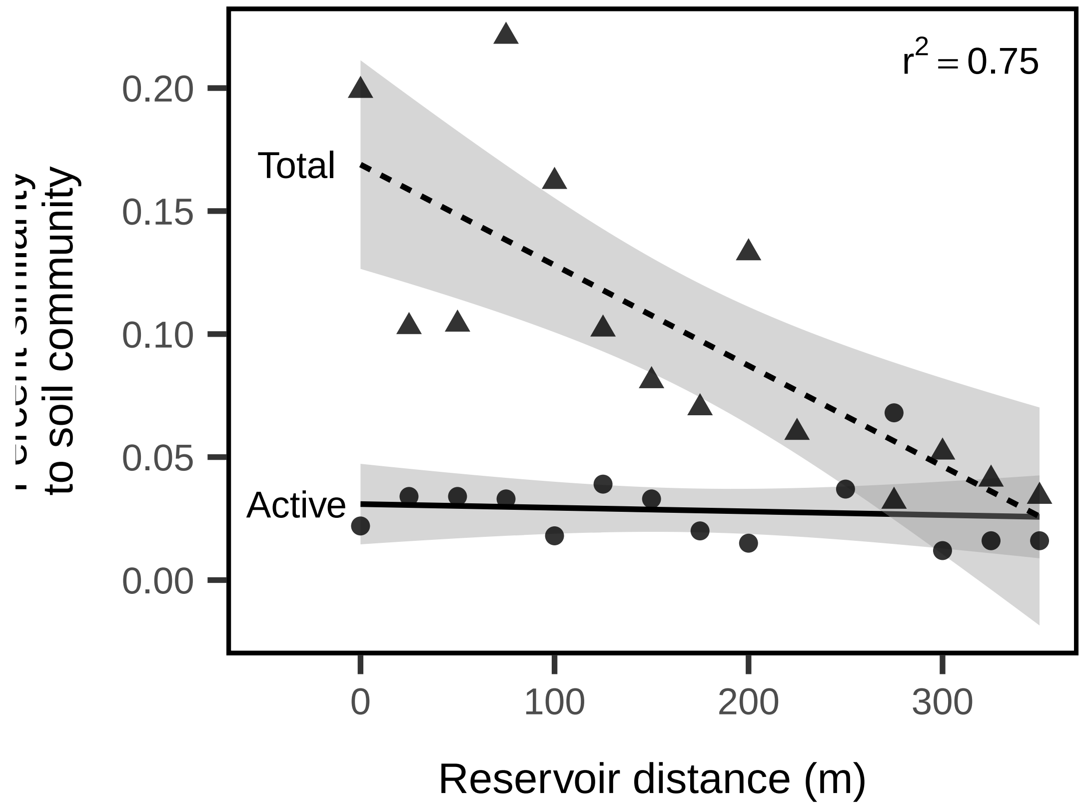
Table 2: Fitting linear model: bray.mean ~ distance * molecule

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.1689	0.01475	11.45	3.279e-11
distance	-0.0004087	7.298e-05	-5.6	9.19e-06
moleculeRNA	-0.138	0.02087	-6.614	7.688e-07
distance:moleculeRNA	0.0003938	0.0001027	3.834	0.0007998

```
# # Calculate Confidance Intervals of Model
# newdata.terr <- data.frame(cbind(UL.sim$molecule, UL.sim$distance))
# conf95.terr <- predict(model.terr, newdata.terr, interval="confidence")
#
# # Dummy Variables Regression Model ("Terrestrial Influence")
# D2 <- (UL.sim$molecule == "RNA")*1
# fit.Fig.3b <- lm(UL.sim$bray.mean ~ UL.sim$distance + D2 + UL.sim$distance*D2)
# D2.R2 <- round(summary(fit.Fig.3b)$r.squared, 2)
# summary(fit.Fig.3b)
#
#
# DNA.int.3b <- fit.Fig.3b$coefficients[1]
# DNA.slp.3b <- fit.Fig.3b$coefficients[2]
# RNA.int.3b <- DNA.int.3b + fit.Fig.3b$coefficients[3]
# RNA.slp.3b <- DNA.slp.3b + fit.Fig.3b$coefficients[4]
```

```
ypred.act <- predict(model.terr, newdata = data.frame(distance = 0, molecule = "RNA"))
ypred.tot <- predict(model.terr, newdata = data.frame(distance = 0, molecule = "DNA"))
similarity.plot <- UL.sim %>%
  mutate(molecule = ifelse(UL.sim$molecule == "DNA", "Total", "Active")) %>%
  ggplot(aes(x = distance, y = bray.mean, shape = molecule)) +
  geom_smooth(method = "lm", aes(linetype = molecule), color = "black", show.legend = T) +
  geom_point(alpha = 0.8, size = 3, show.legend = T) +
  labs(y = str_wrap("Percent similarity to soil community", width = 20),
       x = "Reservoir distance (m)") +
  theme(legend.position = "none") +
```

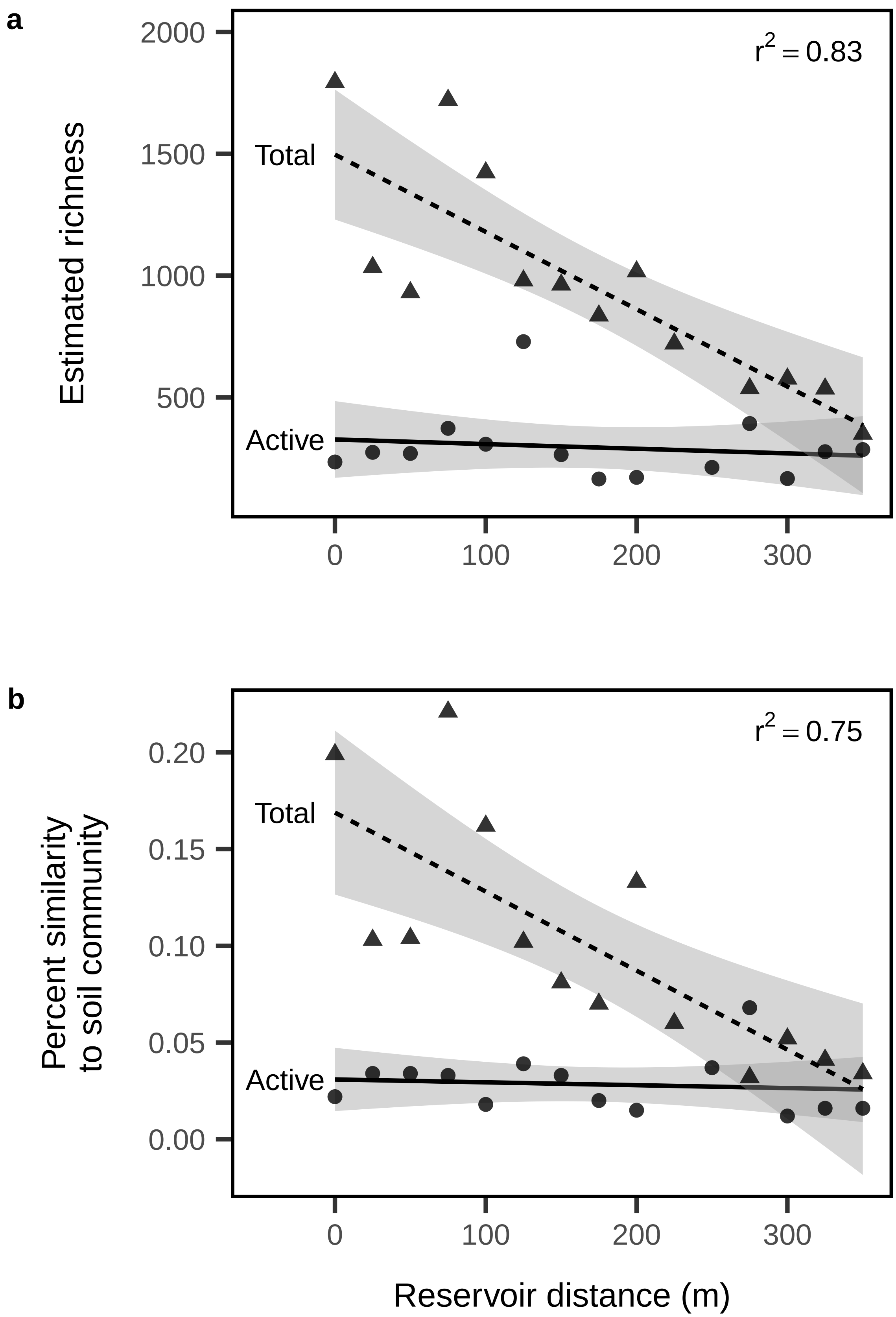
```
  scale_x_continuous(limits = c(-49,350)) +
  annotate(geom = "text", x = 350, y = max(UL.sim$bray.mean), hjust = 1, vjust = 1, size = 5,
           label = paste0("r^2== ",round(summary(model.terr)$r.squared, 2)), parse = T) +
  annotate("text", x = -33, y = ypred.act, label = "Active", size = 5) +
  annotate("text", x = -33, y = ypred.tot, label = "Total", size = 5) +
  ggsave("figures/similarity_fig.pdf")

similarity.plot
```



We find that our model captures most of the variation in community structure ($R^2 = 0.7469401$). We note a significant influence of distance on community similarity and the presence of a significant interaction between distance and whether the comparison is for active or total bacterial communities. This indicates that total communities decay faster with distance to soils than active communities do, which might be explained by the large difference in initial intercept. Active communities are always highly dissimilar to soil communities and remain so across the lake, while total lake communities are initially similar to soils, but this influence dissipates with distance into the reservoir.

Create combined figure

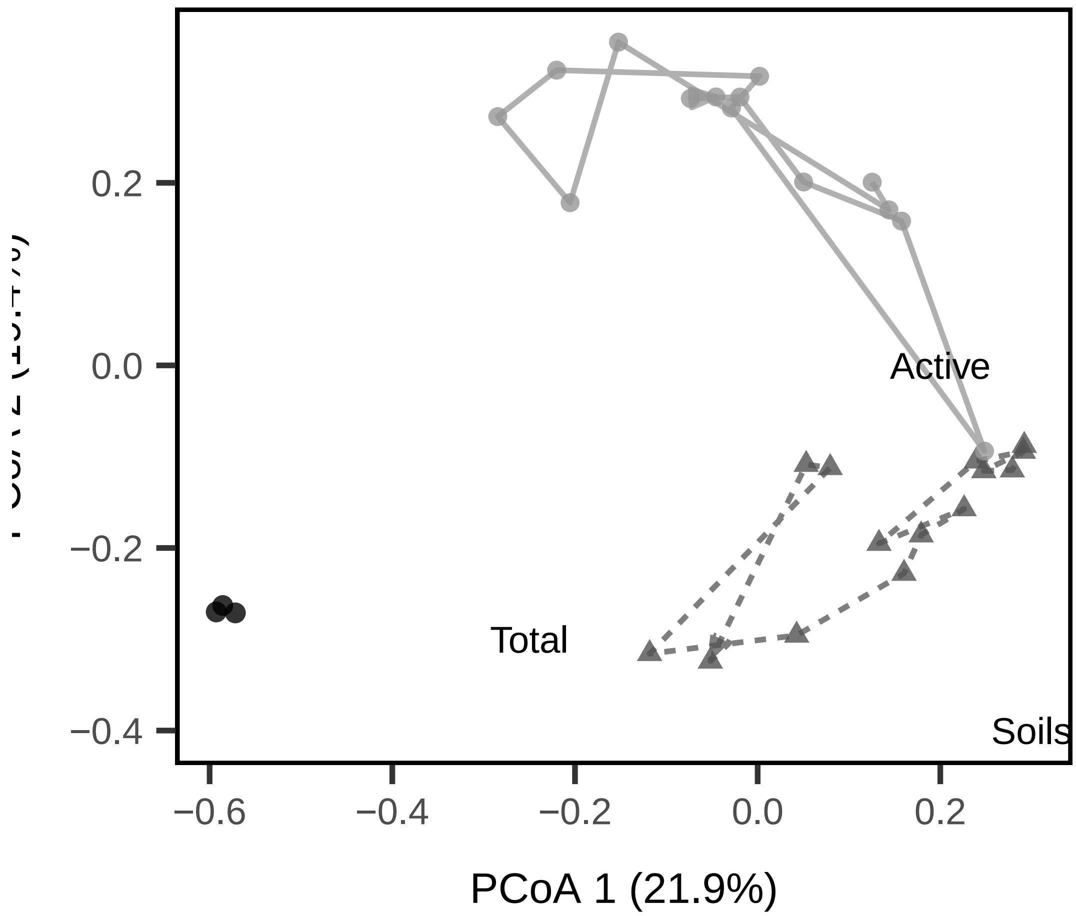
```
plot_grid(alpha.fig + labs(x = ""), similarity.plot,
          align = "hv",
          labels = "auto", ncol = 1) +
  ggsave("figures/alpha_similarity_paneled.pdf")
```


a
b
Estimated richness
Percent similarity to soil community
Reservoir distance (m)
Total
Active
r2 = 0.83
r2 = 0.75
2000
1500
1000
500
0.20
0.15
0.10
0.05
0.00
0
100
200
300

How does community structure change along the gradient?

First, we'll just get an overview of how the communities look along the aquatic transect.

```
ul.pcoa <- cmdscale(vegdist(OTUsREL.log, method="bray"), 2, eig = T, add = T)
explainvars <- round(eigenvals(ul.pcoa)[c(1,2)]/sum(eigenvals(ul.pcoa)),3) *100
water.pcvals <- data.frame(scores(ul.pcoa)) %>%
  rownames_to_column("name") %>%
  left_join(rownames_to_column(design, "name")) %>%
  arrange(desc(distance)) %>% filter(type == "water")
soil.pcvals <- data.frame(scores(ul.pcoa)) %>%
  rownames_to_column("name") %>%
  left_join(rownames_to_column(design, "name")) %>%
  arrange(desc(distance)) %>% filter(type == "soil")
pc_dists <- tibble(
  DNA_dim1 = subset(water.pcvals, molecule == "DNA")$Dim1,
  DNA_dim2 = subset(water.pcvals, molecule == "DNA")$Dim2,
  RNA_dim1 = subset(water.pcvals, molecule == "RNA")$Dim1,
  RNA_dim2 = subset(water.pcvals, molecule == "RNA")$Dim2)

pcoa.fig <- data.frame(scores(ul.pcoa)) %>%
  rownames_to_column("name") %>%
  left_join(rownames_to_column(design, "name")) %>%
  arrange(desc(distance)) %>% filter(type == "water") %>%
  mutate(molecule = ifelse(molecule == "DNA", "Total", "Active")) %>%
  ggplot(aes(x = Dim1, y = Dim2)) +
  geom_path(size = 1, alpha = 0.75, arrow = arrow(angle = 20,
                        length = unit(0.35, "cm"),
                        type = "closed"), aes(color = molecule, linetype = molecule)) +
  geom_point(size = 3, alpha = 0.8, aes(color = molecule, shape = molecule)) +
  geom_point(data = select(soil.pcvals, Dim1, Dim2), col = "black", alpha = .8, size = 3) +
  scale_color_manual("Community Subset", values = my.cols) +
  geom_segment(data = pc_dists,
              aes(x = DNA_dim1, y = DNA_dim2,
                  xend = RNA_dim1, yend = RNA_dim2),
              alpha = 0) +
  coord_fixed(ratio = 1) +
  labs(x = paste0("PCoA 1 (", explainvars[1],"%)"),
       y = paste0("PCoA 2 (", explainvars[2],"%)")) +
  theme(legend.position = "none") +
  annotate(geom = "text", x = .2, y = 0, label = "Active", size = 5) +
  annotate(geom = "text", x = -.25, y = -.3, label = "Total", size = 5) +
  annotate(geom = "text", x = .3, y = -.4, label = "Soils", size = 5) +
  ggsave("figures/pcoa.pdf")
pcoa.fig
```



So, it appears that there is convergence in community structure along the path from stream inlet to the dam. This could reflect a loss of soil-derived taxa in the aquatic samples. To test this, we'll look at β-diversity along the gradient with respect to the soil samples. If we see a decay in similarity to soils, this suggests soil taxa are having a comparatively lower influence with distance from the inlet.

Identifying the Soil Bacteria

Now, we wish to determine whether soil-derived taxa are driving this pattern, and then ask who these influential soil bacteria are.

To classify soil bacteria, we take an incidence-based approach and classify OTUs as:
- present in the soil and present, but never active, in the reservoir
- present in the soil and active in the reservoir

```
# separate lake and soil samples
lake.total <- OTUs[which(design$molecule == "DNA", design$type == "water"),]
soil.total <- OTUs[which(design$molecule == "DNA", design$type == "soil"),]

# which otus are present in both lake and soil samples
lake.and.soil.total <- OTUs[which(design$molecule == "DNA", design$type == "water"),
                            which(colSums(lake.total) > 0 & colSums(soil.total) > 0)]

# isolate just the dna and rna lake communities
w.dna <- OTUs[which(design$molecule == "DNA" & design$type == "water"), ]
```

```r
w.rna <- OTUs[which(design$molecule == "RNA" & design$type == "water"), ]

# pull out the lake rna counts for otus found in lake and soil
lake.and.soil.act <- w.rna[,colnames(lake.and.soil.total)]

# of these lake and soil taxa, which are never active? active?
nvr.act <- which(colSums(lake.and.soil.act) == 0)
yes.act <- which(colSums(lake.and.soil.act) != 0)

# how many otus are active relative to the total number of otus
length(nvr.act) / ncol(lake.and.soil.total)
```

```
## [1] 0.8825537
```

```r
length(yes.act) / ncol(lake.and.soil.total)
```

```
## [1] 0.1174463
```

```r
# of taxa who were never active, what fraction of the total community did they represent?
sum(rowSums(w.dna[,names(nvr.act)]))
```

```
## [1] 23585
```

```r
sum(rowSums(w.dna[,names(yes.act)]))
```

```
## [1] 495479
```

```r
sum(rowSums(w.dna[,names(nvr.act)])) / sum(rowSums(w.dna))
```

```
## [1] 0.04543756
```

```r
# of taxa who became active, what fraction of the active community did they represent?
sum(rowSums(w.rna[,names(nvr.act)]))
```

```
## [1] 0
```

```r
sum(rowSums(w.rna[,names(yes.act)]))
```

```
## [1] 513837
```

```r
sum(rowSums(w.rna[,names(nvr.act)])) / sum(rowSums(w.rna))
```

```
## [1] 0
```

```r
sum(rowSums(w.rna[,names(yes.act)])) / sum(rowSums(w.rna))
```

```
## [1] 0.98993
```

```r
prop.nvr.act <- rowSums(w.dna[,nvr.act]) / rowSums(w.dna)
# cbind.data.frame(design.dna, inactive = prop.nvr.act) %>%
#   ggplot(aes(x = distance, y = inactive)) +
#   geom_point() +
#   geom_line(stat = "smooth", method = "lm", formula = y ~ x, se = F) +
#   labs(x = "Reservoir transect (m)", y = "Rel. abundance of taxa\n that are never active") +
#   scale_x_reverse()
```

We calculate the richness of the soil taxa that are never active in the lake. We calculate richness from the DNA-based samples.

```r
# pull out their dna abundances and calculate richness
terr.lake <- w.dna[ , c(names(nvr.act))]
```

```r
terr.rich <- rowSums((terr.lake > 0) * 1)
terr.REL <- rowSums(terr.lake) / rowSums(w.dna)
design.dna <- design[which(design$molecule == "DNA" & design$type == "water"), ]
terr.rich.log <- log10(terr.rich)
terr.REL.log <- log10(terr.REL)

terr.mod1 <- lm(terr.rich.log ~ design.dna$distance)
summary(terr.mod1)
```

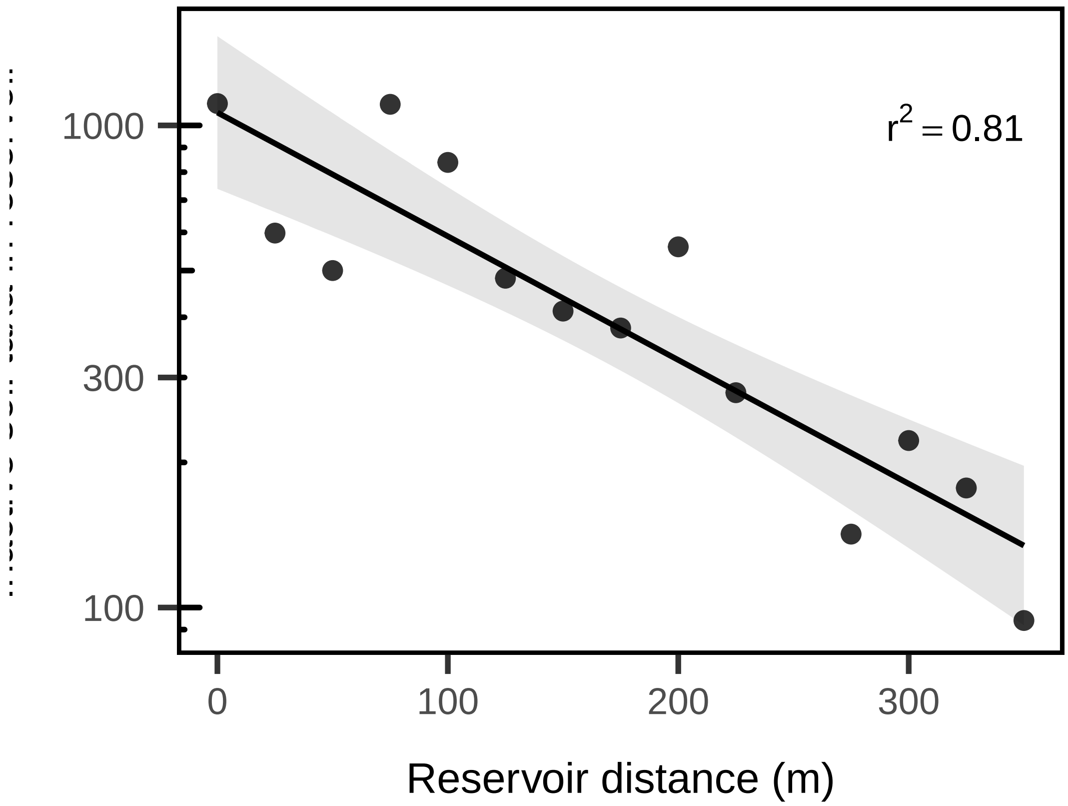
```
##
## Call:
## lm(formula = terr.rich.log ~ design.dna$distance)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.199417 -0.123300 -0.000783  0.080926  0.234711
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)          3.0266909  0.0726577  41.657 2.37e-14 ***
## design.dna$distance -0.0025661  0.0003595  -7.138 1.18e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1478 on 12 degrees of freedom
## Multiple R-squared:  0.8094, Adjusted R-squared:  0.7935
## F-statistic: 50.95 on 1 and 12 DF,  p-value: 1.184e-05
```

```r
T1.R2 <- round(summary(terr.mod1)$r.squared, 2)
T1.int <- terr.mod1$coefficients[1]
T1.slp <- terr.mod1$coefficients[2]
pander(terr.mod1)
```

Table 3: Fitting linear model: terr.rich.log ~ design.dna$distance We find distance is a highly significant predictor of the richness of these soil-derived taxa (on a log-scale).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.027	0.07266	41.66	2.374e-14
design.dna$distance	-0.002566	0.0003595	-7.138	1.184e-05

```r
transient.plot <- tibble(transient_rich = terr.rich, distance = design.dna$distance) %>%
  ggplot(aes(x = distance, y = transient_rich)) +
  geom_smooth(method = "lm", color = "black", fill = "grey") +
  geom_point(size = 3, alpha = .8, color = "black") +
  scale_y_log10() +
  annotation_logticks(sides = "l", size = 1) +
  labs(x = "Reservoir distance (m)",
       y = "Inactive soil taxa in reservoir") +
  annotate("text", x = 350, y = max(terr.rich), hjust = 1, vjust = 1, size = 5,
           label = paste0("r^2== ",T1.R2), parse = T)  +
  ggsave("figures/transients.pdf")
transient.plot
```




```
# plot_grid(alpha.fig,
#           similarity.plot,
#           pcoa.fig + ,
#           transient.plot,
#           align = "hv", axis = "tlbr",
#           labels = "auto", ncol = 2) +
#   ggsave("figures/large_panel.pdf", width = 12, height = 8)
```

What is the fate of soil-derived taxa in the reservoir?

So, we observe that most soil-derived taxa appear to decay once they enter the reservoir. Do any soil-derived taxa persist in the active bacterial community of the reservoir and do they rise to high relative abundances?

```
# identify otus in soil samples and lake samples
in.soil <- OTUs[, which(colSums(OTUs[c(1:3),]) > 0 )]
#in.lake <- OTUs[, which(colSums(OTUs[-c(1:3),]) > 0)]

# isolate just the rna water samples and convert to presence-absence
in.lake.rna <- OTUs[which(design$molecule == "RNA" & design$type == "water"), ]
in.lake.rna.pa <- (in.lake.rna > 0) * 1

# define the 'core' taxa as otus present in 50% of samples
in.lake.core <- w.dna[, which((colSums(in.lake.rna.pa) / nrow(in.lake.rna.pa)) >= 0.75)]

# of the core, how many are also in the soil samples?
in.lake.core.from.soils <- in.lake.core[, intersect(colnames(in.lake.core), colnames(in.soil))]
```

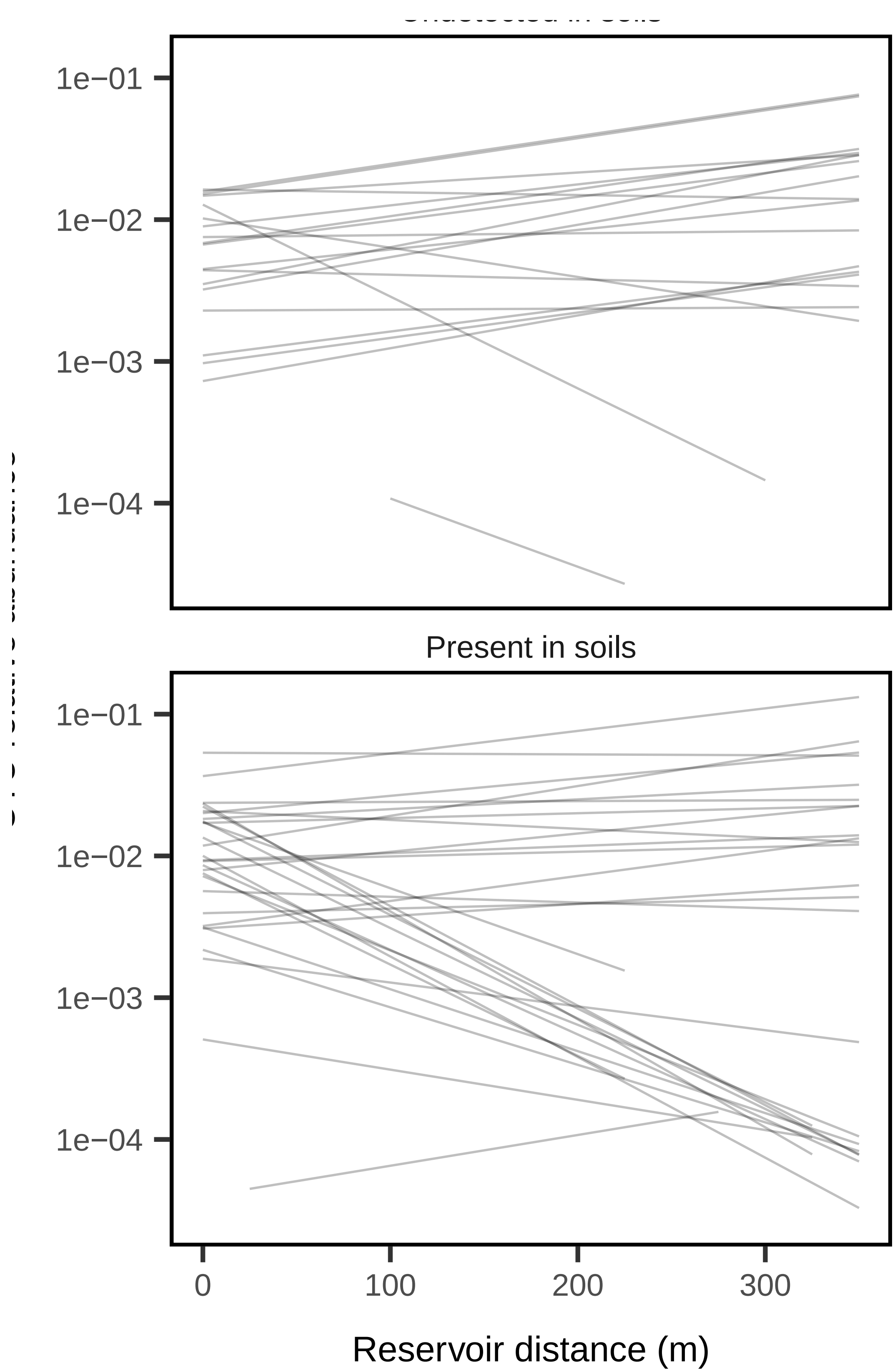
```r
# of the core which are not in the soil samples
in.lake.core.not.soils <- in.lake.core[, setdiff(colnames(in.lake.core), colnames(in.soil))]

# Find the relative abundance of the core taxa and prepare data frame to plot
in.lake.core.from.soils.REL <- in.lake.core.from.soils / rowSums(w.dna)

in.soil.to.plot <- as.data.frame(in.lake.core.from.soils.REL) %>%
  rownames_to_column("sample_ID") %>%
  gather(otu_id, rel_abundance, -sample_ID) %>%
  left_join(rownames_to_column(design.dna, "sample_ID")) %>%
  add_column(found = "soils")

in.lake.core.not.soils.REL <- in.lake.core.not.soils / rowSums(w.dna)

in.lake.to.plot <- as.data.frame(in.lake.core.not.soils.REL) %>%
  rownames_to_column("sample_ID") %>%
  gather(otu_id, rel_abundance, -sample_ID) %>%
  left_join(rownames_to_column(design.dna, "sample_ID")) %>%
  add_column(found = "lake")
```

Now, lets plot the abundances of the OTUs across the reservoir and split them up into whether they were recovered in soils or not.

```r
bind_rows(in.soil.to.plot, in.lake.to.plot) %>%
  ggplot(aes(x = distance, y = rel_abundance, group = otu_id)) +
  labs(x = "Reservoir distance (m)",
       y = "OTU relative abundance") +
  geom_line(alpha = 0.25, stat = "smooth", method = "lm", se = F, show.legend = F) +
  scale_y_log10() +
  facet_wrap(~ found, ncol = 1,
             labeller = as_labeller(c(
               `lake` = "Undetected in soils",
               `soils` = "Present in soils")))
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
```

```
## Warning: Removed 46 rows containing non-finite values (stat_smooth).
```



From this figure, we note a few important points. First, we observe that core reservoir taxa that are not detected in the soil samples tend to increase in relative abundance along the reservoir transect. We also note

that for the taxa that are present in the soil samples, some tend to increase drastically, while others tend to increase, along the transect. This suggests that there may be two classes of soil-derived OTUs that contribute to reservoir bacterial diversity:
- taxa where the reservoir is a sink (i.e., maintained via mass effects from the soils) - aquatic taxa seeded by populations stored in the soils

```
# model distance effect on rel abundance to get slope and pval
soil.core.mods <- apply(in.lake.core.from.soils.REL, MARGIN = 2,
    FUN = function(x) summary(lm(x ~ design.dna$distance))$coefficients[2,c(1,4)])
rownames(soil.core.mods) <- c("slope", "pval")

# classify otus as significantly increasing or decreasing along reservoir
soil.core.decreasing <- as.data.frame(t(soil.core.mods)) %>%
  rownames_to_column("OTU") %>%
  filter(slope < 0) %>%   # rel abund decreases toward dam
  left_join(OTU.tax)
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```
soil.core.increasing <- as.data.frame(t(soil.core.mods)) %>%
  rownames_to_column("OTU") %>%
  filter(slope > 0) %>%   # rel abund increases toward dam
  left_join(OTU.tax)
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```
nonsoil.core.mods <- apply(in.lake.core.not.soils.REL, MARGIN = 2,
    FUN = function(x) summary(lm(x ~ design.dna$distance))$coefficients[2,c(1,4)])
rownames(nonsoil.core.mods) <- c("slope", "pval")
nonsoil.core.decreasing <- as.data.frame(t(nonsoil.core.mods)) %>%
  rownames_to_column("OTU") %>%
  filter(slope < 0) %>%   # rel abund decreases toward dam
  left_join(OTU.tax)
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```
nonsoil.core.increasing <- as.data.frame(t(nonsoil.core.mods)) %>%
  rownames_to_column("OTU") %>%
  filter(slope > 0) %>%   # rel abund increases toward dam
  left_join(OTU.tax)
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

Now we will visualize the significant taxa

```
pander(nonsoil.core.decreasing, caption = "Core taxa not found in soils that get rarer along the transe
```

Table 4: Core taxa not found in soils that get rarer along the transect. (continued below)

OTU	slope	pval	Domain	Phylum
Otu00007	-8.015e-06	0.2431	Bacteria	Proteobacteria
Otu00020	-1.704e-05	0.4607	Bacteria	Proteobacteria
Otu00024	-2.897e-06	0.3675	Bacteria	Bacteroidetes

OTU	slope	pval	Domain	Phylum
Otu00057	-3.017e-05	0.009476	Bacteria	Proteobacteria
Otu00138	-3.401e-05	0.016	Bacteria	Firmicutes
Otu00169	-1.048e-05	0.3397	Bacteria	Bacteria_unclassified
Otu01010	-3.563e-08	0.635	Bacteria	Actinobacteria

Table 5: Table continues below

Class	Order
Betaproteobacteria	Burkholderiales
Betaproteobacteria	Burkholderiales
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Gammaproteobacteria	Methylococcales
Bacilli	Bacillales
Bacteria_unclassified	Bacteria_unclassified
Actinobacteria	Actinomycetales

Family	Genus
Burkholderiaceae	Polynucleobacter
Alcaligenaceae	Alcaligenaceae_unclassified
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Methylococcaceae	Methylococcaceae_unclassified
Bacillaceae_1	Bacillus
Bacteria_unclassified	Bacteria_unclassified
Dermabacteraceae	Brachybacterium

```
pander(nonsoil.core.increasing, caption = "Core taxa not found in soils that get more common along the
```

Table 7: Core taxa not found in soils that get more common along the transect. (continued below)

OTU	slope	pval	Domain	Phylum
Otu00004	0.0001345	1.671e-05	Bacteria	Actinobacteria
Otu00008	3.306e-05	0.02659	Bacteria	Actinobacteria
Otu00015	0.0001372	0.0003621	Bacteria	Actinobacteria
Otu00016	5.151e-05	0.002113	Bacteria	Actinobacteria
Otu00025	4.63e-05	0.006728	Bacteria	Actinobacteria
Otu00038	4.561e-05	0.0001738	Bacteria	Actinobacteria
Otu00040	3.744e-05	2.589e-05	Bacteria	Proteobacteria
Otu00071	4.8e-05	0.0004517	Bacteria	Planctomycetes
Otu00079	8.122e-06	0.001732	Bacteria	Bacteroidetes
Otu00080	1.601e-05	0.1586	Bacteria	Bacteroidetes
Otu00118	6.59e-06	0.03765	Bacteria	Actinobacteria
Otu00156	8.854e-06	0.002739	Bacteria	Bacteria_unclassified

Table 8: Table continues below

Class	Order
Actinobacteria	Actinomycetales
Actinobacteria	Actinomycetales
Actinobacteria	Actinobacteria_unclassified
Actinobacteria	Actinomycetales
Actinobacteria	Actinomycetales
Actinobacteria	Actinomycetales
Alphaproteobacteria	Rhodospirillales
Planctomycetia	Planctomycetales
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Flavobacteriia	Flavobacteriales
Actinobacteria	Actinobacteria_unclassified
Bacteria_unclassified	Bacteria_unclassified

Family	Genus
Actinomycetales_unclassified	Actinomycetales_unclassified
Actinomycetales_unclassified	Actinomycetales_unclassified
Actinobacteria_unclassified	Actinobacteria_unclassified
Microbacteriaceae	Microbacteriaceae_unclassified
Microbacteriaceae	Microbacteriaceae_unclassified
Actinomycetales_unclassified	Actinomycetales_unclassified
Acetobacteraceae	Roseomonas
Planctomycetaceae	Planctomycetaceae_unclassified
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Flavobacteriaceae	Flavobacterium
Actinobacteria_unclassified	Actinobacteria_unclassified
Bacteria_unclassified	Bacteria_unclassified

```
pander(soil.core.decreasing, caption = "Core taxa found in soils that get rarer along the transect.")
```

Table 10: Core taxa found in soils that get rarer along the transect. (continued below)

OTU	slope	pval	Domain	Phylum
Otu00009	-5.159e-05	0.02755	Bacteria	Proteobacteria
Otu00010	-4.34e-05	0.5521	Bacteria	Proteobacteria
Otu00011	-1.949e-05	0.6012	Bacteria	Proteobacteria
Otu00018	-4.676e-05	0.02114	Bacteria	Proteobacteria
Otu00022	-2.524e-05	0.1182	Bacteria	Verrucomicrobia
Otu00028	-3.068e-05	0.02359	Bacteria	Proteobacteria
Otu00030	-2.244e-06	0.2763	Bacteria	Actinobacteria
Otu00039	-8.596e-06	0.1787	Bacteria	Proteobacteria
Otu00045	-8.037e-06	0.5276	Bacteria	Proteobacteria
Otu00059	-6.541e-05	0.02553	Bacteria	Actinobacteria
Otu00065	-5.579e-05	0.02116	Bacteria	Bacteroidetes
Otu00072	-1.895e-05	0.09149	Bacteria	Proteobacteria
Otu00077	-5.886e-05	0.01187	Bacteria	Bacteroidetes
Otu00086	-1.265e-05	0.03621	Bacteria	Proteobacteria
Otu00094	-2.23e-05	0.03169	Bacteria	Proteobacteria

OTU	slope	pval	Domain	Phylum
Otu00095	-3.578e-05	0.03614	Bacteria	Proteobacteria
Otu00170	-2.494e-05	0.02878	Bacteria	Bacteroidetes
Otu00545	-1.236e-06	0.02985	Bacteria	Actinobacteria

Table 11: Table continues below

Class	Order
Gammaproteobacteria	Pseudomonadales
Proteobacteria_unclassified	Proteobacteria_unclassified
Betaproteobacteria	Betaproteobacteria_unclassified
Gammaproteobacteria	Pseudomonadales
Opitutae	Opitutae_unclassified
Gammaproteobacteria	Pseudomonadales
Actinobacteria	Actinomycetales
Betaproteobacteria	Burkholderiales
Betaproteobacteria	Burkholderiales
Actinobacteria	Actinomycetales
Sphingobacteriia	Sphingobacteriales
Alphaproteobacteria	Sphingomonadales
Flavobacteriia	Flavobacteriales
Alphaproteobacteria	Rhizobiales
Betaproteobacteria	Burkholderiales
Betaproteobacteria	Burkholderiales
Sphingobacteriia	Sphingobacteriales
Actinobacteria	Solirubrobacterales

Family	Genus
Pseudomonadaceae	Pseudomonas
Proteobacteria_unclassified	Proteobacteria_unclassified
Betaproteobacteria_unclassified	Betaproteobacteria_unclassified
Pseudomonadaceae	Pseudomonas
Opitutae_unclassified	Opitutae_unclassified
Pseudomonadaceae	Pseudomonas
Micrococcaceae	Micrococcus
Comamonadaceae	Comamonas
Oxalobacteraceae	Oxalobacteraceae_unclassified
Micrococcaceae	Arthrobacter
Sphingobacteriaceae	Pedobacter
Sphingomonadaceae	Sphingomonas
Flavobacteriaceae	Flavobacterium
Bradyrhizobiaceae	Bradyrhizobium
Oxalobacteraceae	Duganella
Comamonadaceae	Comamonadaceae_unclassified
Sphingobacteriaceae	Sphingobacteriaceae_unclassified
Solirubrobacteraceae	Solirubrobacter

```
pander(soil.core.increasing, caption = "Core taxa found in soils that get more common along the transect
```

Table 13: Core taxa found in soils that get more common along the transect. (continued below)

OTU	slope	pval	Domain	Phylum
Otu00001	1.436e-05	0.07999	Bacteria	Proteobacteria
Otu00002	0.0002115	0.002237	Bacteria	Actinobacteria
Otu00003	9.899e-05	0.006441	Bacteria	Verrucomicrobia
Otu00005	3.61e-05	0.01737	Bacteria	Bacteroidetes
Otu00006	6.575e-06	0.1618	Bacteria	Bacteroidetes
Otu00012	7.541e-06	0.09905	Bacteria	Proteobacteria
Otu00014	8.464e-05	0.0007891	Bacteria	Actinobacteria
Otu00023	3.267e-07	0.8	Bacteria	Proteobacteria
Otu00029	3.32e-05	0.004456	Bacteria	Actinobacteria
Otu00032	3.56e-06	0.8341	Bacteria	Bacteroidetes
Otu00033	9.129e-06	0.7085	Bacteria	Proteobacteria

Table 14: Table continues below

Class	Order
Betaproteobacteria	Burkholderiales
Actinobacteria	Actinomycetales
Spartobacteria	Spartobacteria_unclassified
Sphingobacteriia	Sphingobacteriales
Sphingobacteriia	Sphingobacteriales
Betaproteobacteria	Burkholderiales
Actinobacteria	Actinomycetales
Gammaproteobacteria	Pseudomonadales
Actinobacteria	Actinomycetales
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Alphaproteobacteria	Rhizobiales

Family	Genus
Comamonadaceae	Comamonadaceae_unclassified
Actinomycetales_unclassified	Actinomycetales_unclassified
Spartobacteria_unclassified	Spartobacteria_unclassified
Chitinophagaceae	Sediminibacterium
Saprospiraceae	Saprospiraceae_unclassified
Comamonadaceae	Comamonadaceae_unclassified
Actinomycetales_unclassified	Actinomycetales_unclassified
Moraxellaceae	Acinetobacter
Actinomycetales_unclassified	Actinomycetales_unclassified
Bacteroidetes_unclassified	Bacteroidetes_unclassified
Rhizobiales_unclassified	Rhizobiales_unclassified

```
# p1 <- as.data.frame(OTUsREL[,nonsoil.core.increasing$OTU]) %>%
#   rownames_to_column("sampleID") %>%
#   left_join(rownames_to_column(design, "sampleID")) %>%
#   gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
#   filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
```

```r
#   mutate(taxon = paste(Phylum, Class, Order, Family, Genus)) %>%
#   ggplot(aes(x = distance, y = rel_abund, group = OTU)) +
#   #geom_point(alpha = 0.5) +
#   geom_line(stat = "smooth", alpha = 0.5, size = 1,
#             color = "black", method = "loess", span = 1, se = FALSE) +
#   scale_x_reverse() +
#   scale_y_log10(labels = scales::scientific) +
#   theme(legend.position = "none") +
#   guides(color = guide_legend(ncol = 1)) +
#   labs(x = "",
#        y = "Relative Abundance",
#        title = "Absent from soil and significantly increasing")
#
# p2 <- as.data.frame(OTUsREL[,soil.core.increasing$OTU]) %>%
#   rownames_to_column("sampleID") %>%
#   left_join(rownames_to_column(design, "sampleID")) %>%
#   gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
#   filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
#   mutate(taxon = paste(Class, Order)) %>%
#   ggplot(aes(x = distance, y = rel_abund, group = OTU)) +
#   #geom_point(alpha = 0.5) +
#   geom_line(stat = "smooth", alpha = 0.5, size = 1,
#             color = "black", method = "loess", span = 1, se = FALSE) +
#   scale_x_reverse() +
#   scale_y_log10(labels = scales::scientific) +
#   theme(legend.position = "none") +
#   guides(color = guide_legend(ncol = 1)) +
#   labs(x = "",
#        y = "Relative Abundance",
#        title = "Present in soil and significantly increasing")
#
# p3 <- as.data.frame(OTUsREL[,soil.core.decreasing$OTU]) %>%
#   rownames_to_column("sampleID") %>%
#   left_join(rownames_to_column(design, "sampleID")) %>%
#   gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
#   filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
#   mutate(taxon = paste(Class, Order)) %>%
#   ggplot(aes(x = distance, y = rel_abund, group = OTU)) +
#   #geom_point(alpha = 0.5) +
#   geom_line(stat = "smooth", alpha = 0.5, size = 1,
#             color = "black", method = "loess", span = 1, se = FALSE) +
#   scale_x_reverse() +
#   scale_y_log10(labels = scales::scientific) +
#   theme(legend.position = "none") +
#   guides(color = guide_legend(ncol = 1)) +
#   labs(x = "Reservoir Transect (m)",
#        y = "Relative Abundance",
#        title = "Present in soil and significantly decreasing")
#
# cowplot::plot_grid(p1, p2, p3, align = "hv", labels = "AUTO", ncol = 1)

df1 <- as.data.frame(OTUsREL[,nonsoil.core.increasing$OTU]) %>%
  rownames_to_column("sampleID") %>%
```

```r
  left_join(rownames_to_column(design, "sampleID")) %>%
  gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
  filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
  mutate(soils = "Absent from soils", change = "Increasing")
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```r
n1 <- length(unique(df1$OTU))

df2 <- as.data.frame(OTUsREL[,soil.core.increasing$OTU]) %>%
  rownames_to_column("sampleID") %>%
  left_join(rownames_to_column(design, "sampleID")) %>%
  gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
  filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
  mutate(soils = "Present in soils", change = "Increasing")
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```r
n2 <- length(unique(df2$OTU))

df3 <- as.data.frame(OTUsREL[,soil.core.decreasing$OTU]) %>%
  rownames_to_column("sampleID") %>%
  left_join(rownames_to_column(design, "sampleID")) %>%
  gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
  filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
  mutate(soils = "Present in soils", change = "Decreasing")
```

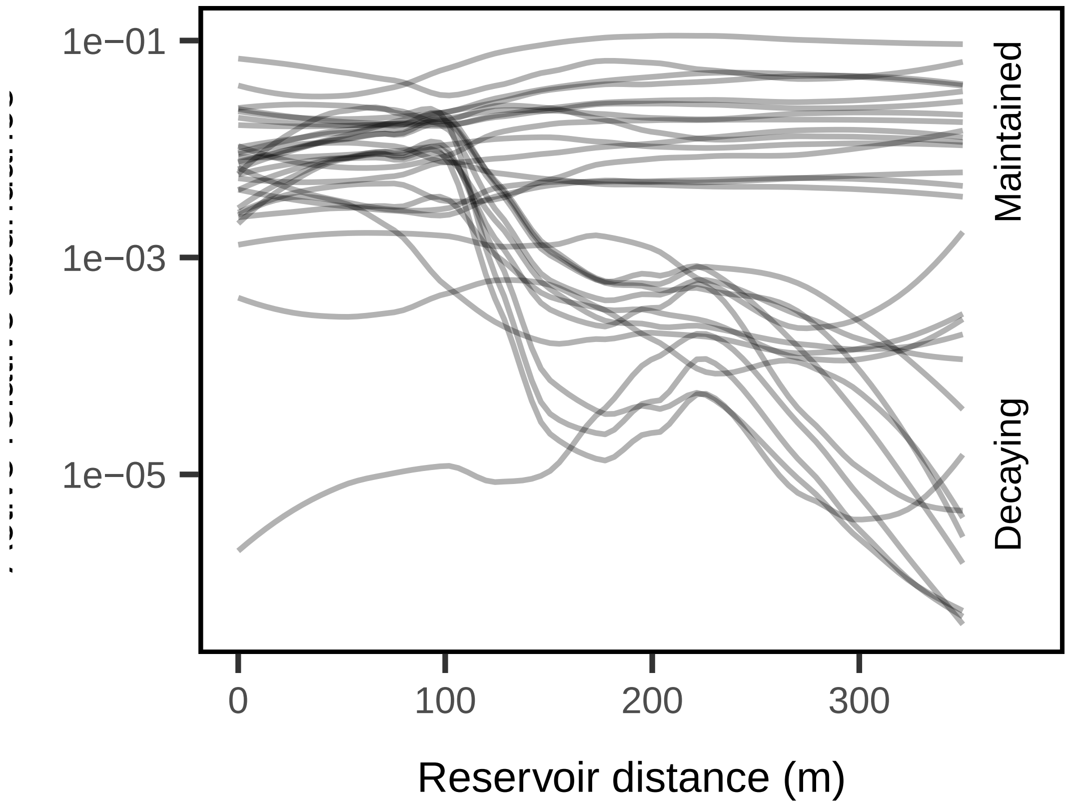
```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

```r
n3 <- length(unique(df3$OTU))

df4 <- as.data.frame(OTUsREL[,nonsoil.core.decreasing$OTU]) %>%
  rownames_to_column("sampleID") %>%
  left_join(rownames_to_column(design, "sampleID")) %>%
  gather(OTU, rel_abund, -station, -molecule, -type, -distance, -sampleID) %>%
  filter(molecule == "DNA") %>% left_join(OTU.tax) %>%
  mutate(soils = "Absent from soils", change = "Decreasing")
```

```
## Warning: Column `OTU` joining character vector and factor, coercing into
## character vector
```

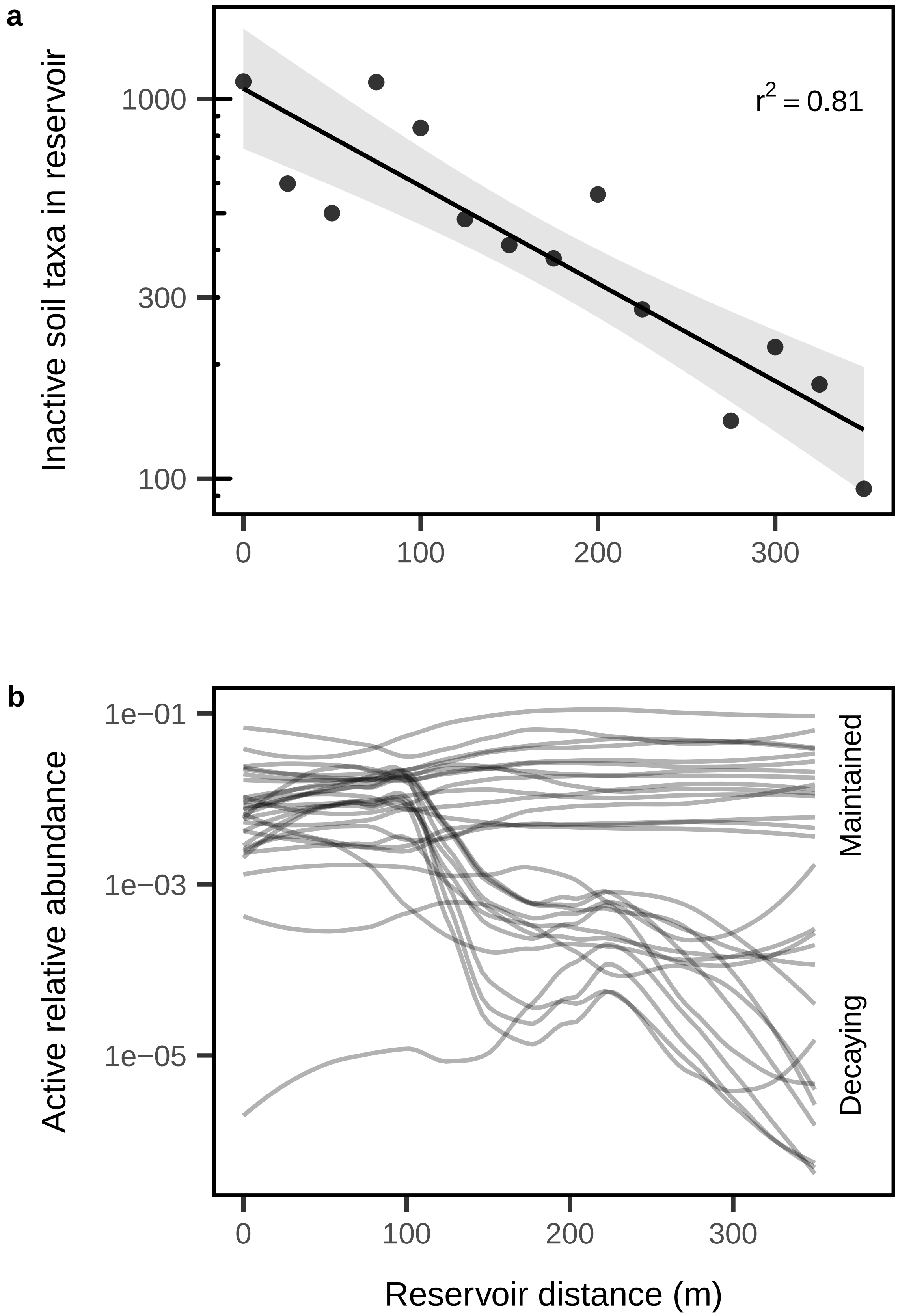
```r
n4 <- length(unique(df4$OTU))


df.plot <- as_tibble(rbind.data.frame(df1, df2, df3, df4)) %>% filter(type == "water")

taxon_fate.plot <- df.plot %>% mutate(rel_abund = ifelse(rel_abund == 0, 1e-6, rel_abund)) %>%
  filter(soils == "Present in soils") %>%
  #mutate(change = ifelse(change == "Increasing",
  #                       paste0("Increasing (n = ", n2,")"),
  #                       paste0("Decreasing (n = ", n3,")"))) %>%
  ggplot(aes(x = distance, y = rel_abund, group = OTU)) +
  #geom_jitter(alpha = 0.15) +
```

```r
  geom_line(stat = "smooth", alpha = 0.3, size = 1,
            method = "loess", span = .7, se = FALSE) +
  scale_y_log10(labels = scales::scientific) +
  scale_x_continuous(limits = c(0,380)) +
  #theme(legend.position = "none") +
  #guides(color = guide_legend(ncol = 1)) +
  labs(x = "Reservoir distance (m)",
       y = "Active relative abundance") +
  annotate("text", x = 365, y = 1e-1, size = 5, hjust = 1, vjust = 1, angle = 90,
           label = "Maintained") +
  annotate("text", x = 365, y = 1e-5, size = 5, hjust = 0.5, vjust = 1, angle = 90,
           label = "Decaying") +
  ggsave("figures/taxa_origins.pdf")
taxon_fate.plot
```




```r
# how much do the different core components contribute to total abundances
in.lake.core.soil.REL <- rowSums(in.lake.core.from.soils) / rowSums(w.dna)
in.lake.core.water.REL <- rowSums(in.lake.core.not.soils) / rowSums(w.dna)
```

```r
plot_grid(transient.plot + labs(x = ""),
          taxon_fate.plot,
          align = "hv", axis = "rltb",
          labels = "auto",
          ncol = 1) +
  ggsave("figures/fate_panel.pdf")
```


a
Inactive soil taxa in reservoir
1000
300
100
$r^2 = 0.81$
0
100
200
300
b
Active relative abundance
1e−01
1e−03
1e−05
Maintained
Decaying
0
100
200
300
Reservoir distance (m)

```r
# soil.mods <- t(soil.core.mods) %>% as.data.frame()
# soil.mods$habitat <- "Present in soils"
# soil.mods <- soil.mods %>% rownames_to_column(var = "OTU")
# nonsoil.mods <- t(nonsoil.core.mods) %>% as.data.frame()
# nonsoil.mods$habitat <- "Absent from soils"
# nonsoil.mods <- nonsoil.mods %>% rownames_to_column(var = "OTU")
# rbind.data.frame(soil.mods, nonsoil.mods) %>%
#   filter(pval < 0.05) %>%
#   ggplot(aes(x = -slope, fill = habitat, color = habitat)) +
#   geom_line(stat = "density", alpha = 0.5, adjust = .8) +
#   geom_density(color = NA, adjust = .8, alpha = 0.2)
```

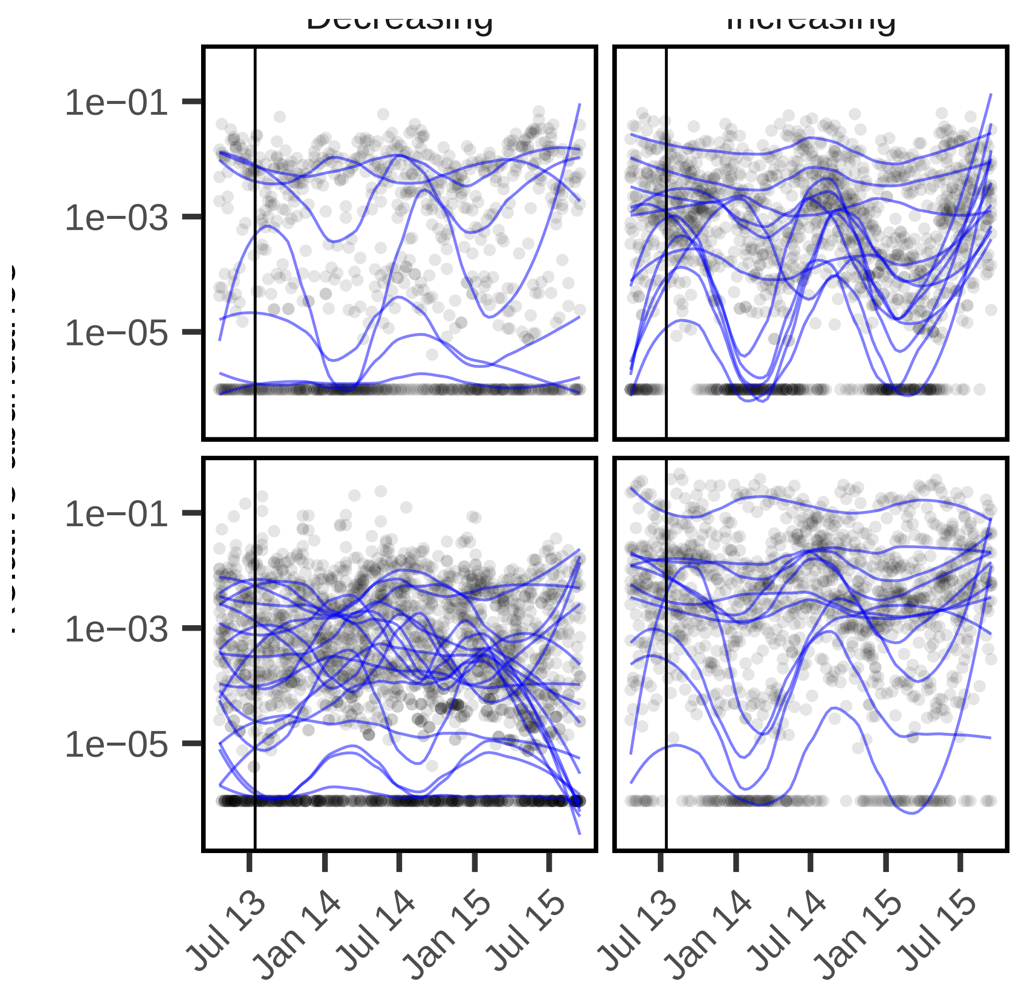
Are the "persistent" reservoir taxa really representative? Look over time...

```r
total.OTUs <- read.otu(shared = shared, cutoff = "0.03")    # 97% Similarity

# Import Taxonomy
total.OTU.tax <- read.tax(taxonomy = taxon, format = "rdp")

# Subset to just the time series sites
UL.ts.OTUs <- total.OTUs[str_which(rownames(total.OTUs), "UL"),]

# make sure OTU table matches up with design order
UL.ts.design <- read_csv("data/UL_timeseries_design.csv")
UL.ts.OTUs <- UL.ts.OTUs[match(UL.ts.design$sample.name, rownames(UL.ts.OTUs)),]
UL.ts.OTUs.RNA <- decostand(UL.ts.OTUs[which(UL.ts.design$sample.type == "RNA"),], method = "total")
UL.ts.OTUs.DNA <- decostand(UL.ts.OTUs[which(UL.ts.design$sample.type == "DNA"),], method = "total")


env.ts.data <- read.table("data/ul-seedbank.env.txt", sep="\t", header=TRUE)
env.ts.data$date <- as.Date(parse_date_time(env.ts.data$date, "m d y"))
env.ts.data$doc[which(env.ts.data$doc == "**")] <- NA
env.ts.data$doc <- as.numeric(env.ts.data$doc)
summary(env.ts.data)
```

```
##    sample.id          date                 temp            spc
##  Min.   :  1.00   Min.   :2013-04-19   Min.   : 2.21   Min.   :0.3300
##  1st Qu.: 31.75   1st Qu.:2013-11-20   1st Qu.: 5.50   1st Qu.:0.4600
##  Median : 62.50   Median :2014-06-23   Median :17.73   Median :0.5320
##  Mean   : 62.50   Mean   :2014-06-24   Mean   :16.18   Mean   :0.5172
##  3rd Qu.: 93.25   3rd Qu.:2015-01-25   3rd Qu.:25.05   3rd Qu.:0.5660
##  Max.   :124.00   Max.   :2015-09-14   Max.   :29.77   Max.   :0.6700
##                                        NA's   :2       NA's   :2
##      oxygen         salinity          secchi             ph
##  Min.   : 1.870   Min.   :0.1500   Min.   :0.200   Min.   : 6.890
##  1st Qu.: 5.237   1st Qu.:0.2200   1st Qu.:1.200   1st Qu.: 7.920
##  Median : 8.355   Median :0.2550   Median :1.600   Median : 8.415
##  Mean   : 8.961   Mean   :0.2487   Mean   :1.668   Mean   : 8.567
##  3rd Qu.:10.178   3rd Qu.:0.2700   3rd Qu.:2.200   3rd Qu.: 9.123
##  Max.   :22.240   Max.   :0.3200   Max.   :3.600   Max.   :10.860
```

```
##  NA's   :2        NA's   :2        NA's   :1       NA's   :2
##       chla               tp                 tn               doc
##  Min.   :  0.92   Min.   :   8.26   Min.   : 0.407   Min.   :  2.00
##  1st Qu.: 12.63   1st Qu.:  26.30   1st Qu.: 0.882   1st Qu.: 32.25
##  Median : 37.67   Median :  34.85   Median : 1.210   Median : 61.50
##  Mean   : 79.25   Mean   :  84.25   Mean   : 1.889   Mean   : 61.57
##  3rd Qu.:121.31   3rd Qu.:  47.95   3rd Qu.: 1.490   3rd Qu.: 90.75
##  Max.   :523.56   Max.   :3200.00   Max.   :42.600   Max.   :121.00
##  NA's   :2        NA's   :2         NA's   :3        NA's   :2
##       orp              air.temp
##  Min.   :-41.800   Min.   :-11.60
##  1st Qu.:  9.325   1st Qu.:  7.00
##  Median : 21.700   Median : 18.50
##  Mean   : 50.507   Mean   : 15.57
##  3rd Qu.:104.975   3rd Qu.: 24.00
##  Max.   :225.200   Max.   : 32.00
##  NA's   :68        NA's   :2
```

```
UL.ts.design <- left_join(UL.ts.design, env.ts.data[,c("sample.id", "date")])
env.ts.data <- env.ts.data[-which(!(env.ts.data$date %in% UL.ts.design$date)),]

OTUs.in.core <- UL.ts.OTUs.RNA[, which(colnames(UL.ts.OTUs) %in% df.plot$OTU)]
cbind.data.frame(UL.ts.design[which(UL.ts.design$sample.type == "RNA"),], OTUs.in.core) %>% as_tibble()
  gather(-sample.name, -sample.type, -sample.id, -date, key = OTU, value = rel_abund) %>%
  mutate(soils = ifelse(OTU %in% unique(c(df2$OTU, df3$OTU)),
                        "Present in soils", "Absent from soils")) %>%
  mutate(change = ifelse(OTU %in% unique(c(df3$OTU, df4$OTU)),
                        "Decreasing", "Increasing")) %>%
  mutate(rel_abund = ifelse(rel_abund == 0, 1e-6, rel_abund)) %>%
  ggplot(aes(x = date, y = rel_abund, group = OTU)) +
  geom_point(alpha = .1) +
  geom_line(stat = "smooth", method = "loess", color = "blue",
            alpha = 0.5, span = .5, se = F) +
  geom_vline(aes(xintercept = as_date("2013-07-15"))) +
  scale_y_log10() +
  scale_x_date(labels = scales::date_format(format = "%b %y")) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  facet_grid(soils ~ change) +
  labs(x = "",
       y = "Relative abundance")
```



Many of them do appear to track the seasons quite well, suggesting there could be a seasonality component to the role of terrestrial inputs into the reservoir.

Ecosystem functions

```
metab <- read.table("data/res.grad.metab.txt", sep="\t", header=TRUE)
colnames(metab) <- c("dist", "BP", "BR")
BGE <- round((metab$BP/(metab$BP + metab$BR)),3)
metab <- cbind(metab, BGE)
metab <- metab[-c(16:18),]
metab$dist <- 350 - metab$dist


# Quadratic regression for BP
dist <- metab$dist
dist2 <- metab$dist^2
BP.fit <- lm(metab$BP ~ dist + dist2)
BP.R2 <- round(summary(BP.fit)$r.squared, 2)

# Simple linear regression for BR
BR.fit <- lm(metab$BR ~ metab$dist)
```

```r
BR.R2 <- round(summary(BR.fit)$r.squared, 2)
BR.int <- BR.fit$coefficients[1]
BR.slp <- BR.fit$coefficients[2]

# Simple linear regression for BGE
BGE.fit <- lm(metab$BGE ~ metab$dist)
BGE.R2 <- round(summary(BGE.fit)$r.squared, 2)
BGE.int <- BGE.fit$coefficients[1]
BGE.slp <- BGE.fit$coefficients[2]

BP.R2
```

```
## [1] 0.36
```

```r
BR.R2
```

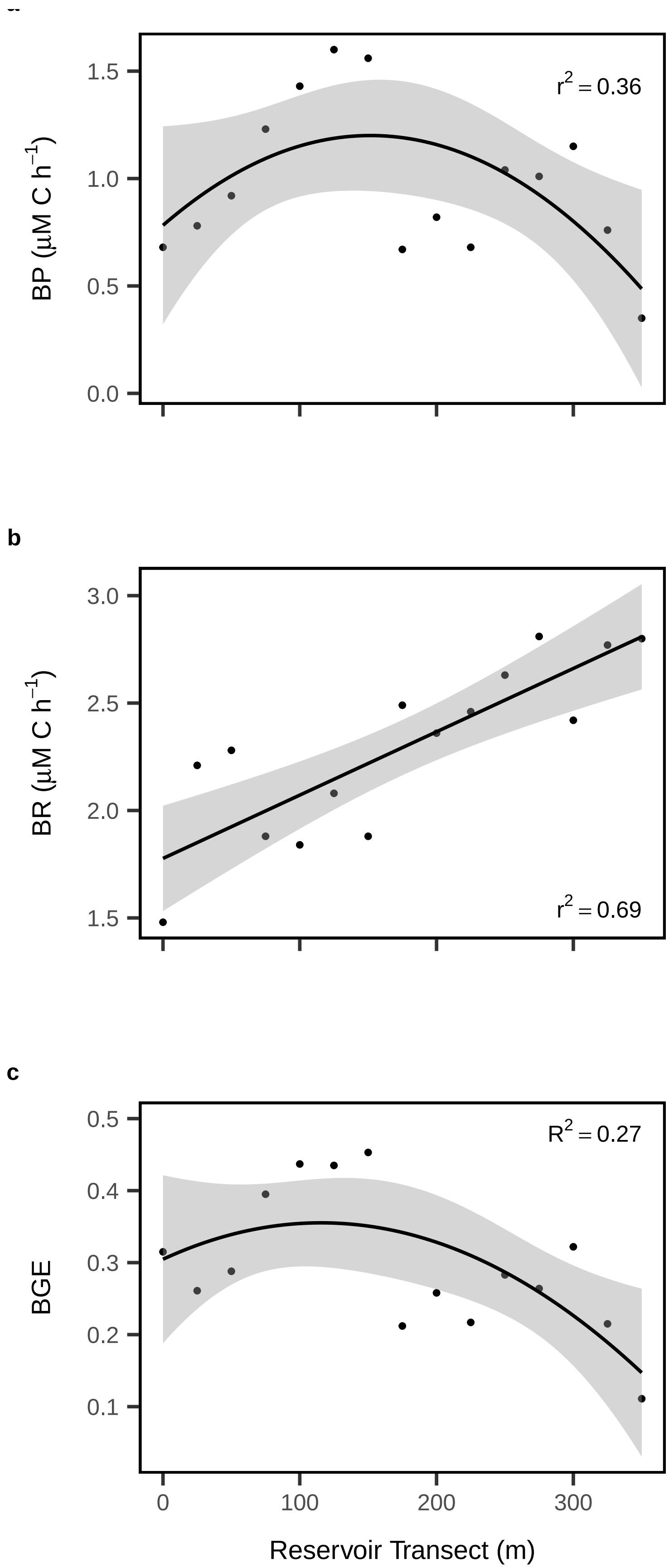
```
## [1] 0.69
```

```r
BGE.R2
```

```
## [1] 0.27
```

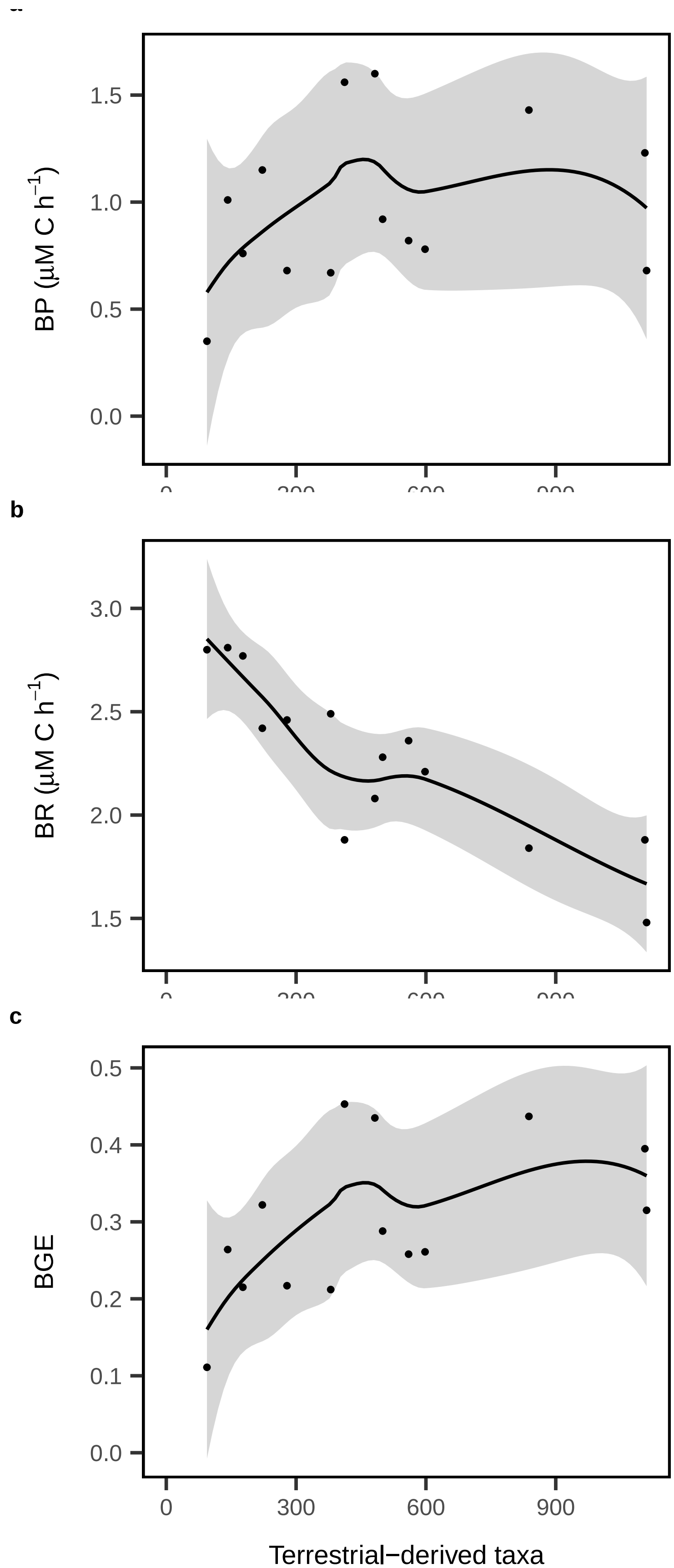
```r
BP.plot <- ggplot(metab, aes(x = dist, y = BP)) +
  geom_point() +
  geom_smooth(method = "lm", formula = y ~ x + I(x^2), color = "black") +
  annotate(geom = "text", x = 350, y = 1.5, size = 5, hjust = 1, vjust = 1,
           label = paste0("r^2== ",BP.R2), parse = T) +
  labs(y = expression(paste('BP (', mu ,'M C h'^-1* ')')),
       x = "Reservoir Transect (m)")
BR.plot <- ggplot(metab, aes(x = dist, y = BR)) +
  geom_point() +
  geom_smooth(method = "lm", formula = y ~ x, color = "black") +
  annotate("text", x = 350, y = 1.5, size = 5, hjust = 1, vjust = 0,
           label = paste0("r^2== ",BR.R2), parse = T ) +
  labs(y = expression(paste('BR (', mu ,'M C h'^-1* ')')),
       x = "Reservoir Transect (m)")
BGE.plot <- ggplot(metab, aes(x = dist, y = BGE)) +
  geom_point() +
  geom_smooth(method = "lm", formula = y ~ x + I(x^2), color = "black") +
  annotate("text", x = 350, y = .5, size = 5, hjust = 1, vjust = 1,
           label = paste0("R^2== ",BGE.R2), parse = T ) +
  labs(y = "BGE",
       x = "Reservoir Transect (m)")
```

```r
plot_grid(BP.plot + theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
                          plot.margin = unit(c(1, 1, -1, 0), "cm")),
          BR.plot + theme(axis.title.x = element_blank(), axis.text.x = element_blank(),
                          plot.margin = unit(c(-1, 1, -1, 0), "cm")),
          BGE.plot + theme(plot.margin = unit(c(-1, 1, 0, 0), "cm")),
          align = "hv", ncol = 1, labels = "auto")
```


b
c
BP (μM C h−1)
r2 = 0.36
1.5
1.0
0.5
0.0
BR (μM C h−1)
r2 = 0.69
3.0
2.5
2.0
1.5
BGE
R2 = 0.27
0.5
0.4
0.3
0.2
0.1
0
100
200
300
Reservoir Transect (m)

Relation of ecosystem functions and community structure

```
metab.joined <- cbind.data.frame(design.dna, metab[-5,])

transient.metabolism <- cbind.data.frame(transients = terr.rich, metab.joined)

p1 <- transient.metabolism %>%
  ggplot(aes(x=transients, y = BP)) +
  geom_smooth(color = "black") +
  geom_point() +
  scale_x_continuous(limits = c(0, NA)) +
  labs(x = "Terrestrial-derived taxa",
       y = expression(paste('BP (', mu ,'M C h'^-1* ')'))) +
  theme(axis.title.x = element_blank(),
                          plot.margin = unit(c(1, 1, 0, 0), "cm"))
p2 <- transient.metabolism %>%
  ggplot(aes(x=transients, y = BR)) +
  geom_smooth(color = "black") +
  geom_point() +
  scale_x_continuous(limits = c(0, NA)) +
  labs(x = "Terrestrial-derived taxa",
       y = expression(paste('BR (', mu ,'M C h'^-1* ')'))) +
  theme(axis.title.x = element_blank(),
                          plot.margin = unit(c(0, 1, 0, 0), "cm"))
p3 <- transient.metabolism %>%
  ggplot(aes(x=transients, y = BGE)) +
  geom_smooth(color = "black") +
  geom_point() +
  scale_x_continuous(limits = c(0, NA)) +
  labs(x = "Terrestrial-derived taxa") +
  theme(plot.margin = unit(c(0, 1, 0, 0), "cm"))

plot_grid(p1, NULL, p2, NULL, p3,
          rel_heights = c(1, -.15, 1, -.15, 1), align = "hv",
          ncol = 1, labels = c("a", "NULL", "b", "NULL", "c")) +
  ggsave("figures/functions.pdf")
```


b
c
BP (μM C h−1)
BR (μM C h−1)
BGE
Terrestrial−derived taxa
0
300
600
900
1.5
1.0
0.5
0.0
3.0
2.5
2.0
0.5
0.4
0.3
0.2
0.1