DOM Experiment Analysis Notebook

Cat Luria

July 15, 2015

Contents

1 Getting started

First, load all the necessary packages.

```
## Loading required package: tcltk
## Loading required package: vegan
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.3-0
## BiodiversityR 2.5-2: use function 'BiodiversityRGUI()' to launch the BiodiversityR Graphical User In
##
## gdata: read.xls support for 'XLS' (Excel 97-2004) files ENABLED.
##
## gdata: read.xls support for 'XLSX' (Excel 2007+) files ENABLED.
##
## Attaching package: 'gdata'
##
## The following object is masked from 'package:data.table':
##
##     last
##
## The following object is masked from 'package:stats':
##
##     nobs
##
## The following object is masked from 'package:utils':
##
##     object.size
```

2 Alpha Diversity

```
cdata <- ddply(diversitytable, c("Experiment", "Day", "Treatment"), summarise,
               N = length(richness),
               mean = mean(richness),
               sd   = sd(richness),
               se   = sd / sqrt(N)
)

cdata <- as.data.table(cdata)
#cdata$Experiment <- as.factor(cdata$Experiment)
cdata1 <- cdata[which(cdata$Experiment=="1")]
cdata4 <- cdata[which(cdata$Experiment=="4")]


p1 <- ggplot(cdata1, aes(x=Day, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
```

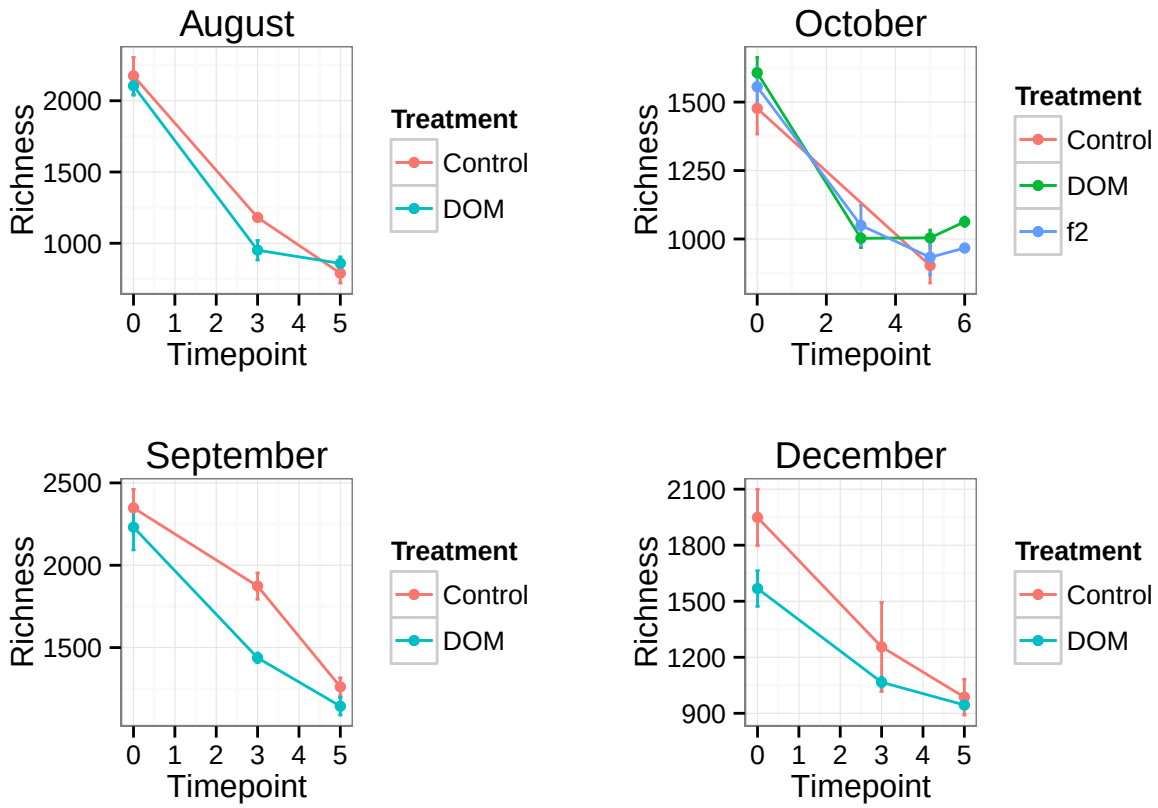

Figure 1: Changes in richness under different experimental treatments.

```
    geom_point() +
    scale_x_continuous(name="Day", breaks=c(0,2,4,6,8,10)) +
    scale_y_continuous(name="Richness") +
    ggtitle("August") +
    theme_bw()

p4 <- ggplot(cdata4, aes(x=Day, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
    geom_point() +
    scale_x_continuous(name="Day", breaks=c(0,2,4,6,8,10)) +
    scale_y_continuous(name="Richness") +
    ggtitle("December") +
    theme_bw()

multiplot(p1, p4, cols=2)
```

```
cdata <- ddply(diversitytable, c("Experiment", "Day", "Treatment"), summarise,
               N = length(richness),
               mean = mean(richness),
               sd   = sd(richness),
               se   = sd / sqrt(N)
)
```

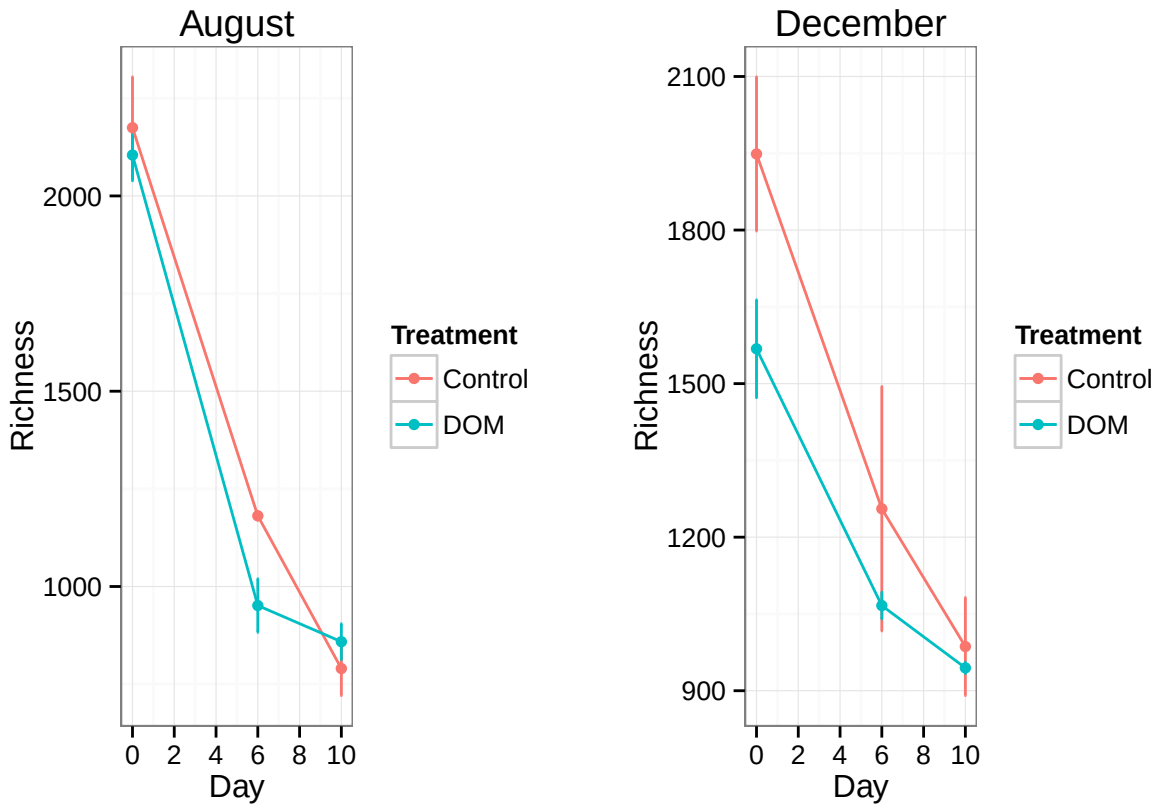

Figure 2:

```
cdata <- as.data.table(cdata)
cdata14 <- cdata[which(cdata$Experiment=="1"|cdata$Experiment=="4")]

exp_labeller <- function(var, value){
  value <- as.character(value)
  if (var=="Experiment") {
    value[value=="1"] <- "August"
    value[value=="4"]   <- "December"
    }
  return(value)
}

p14 <- ggplot(cdata14, aes(x=Day, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
    geom_point() +
    scale_x_continuous(name="Day", breaks=c(0,2,4,6,8,10)) +
    scale_y_continuous(name="Richness") +
    theme_bw()

p14 + facet_grid(.~Experiment, labeller=exp_labeller)
```

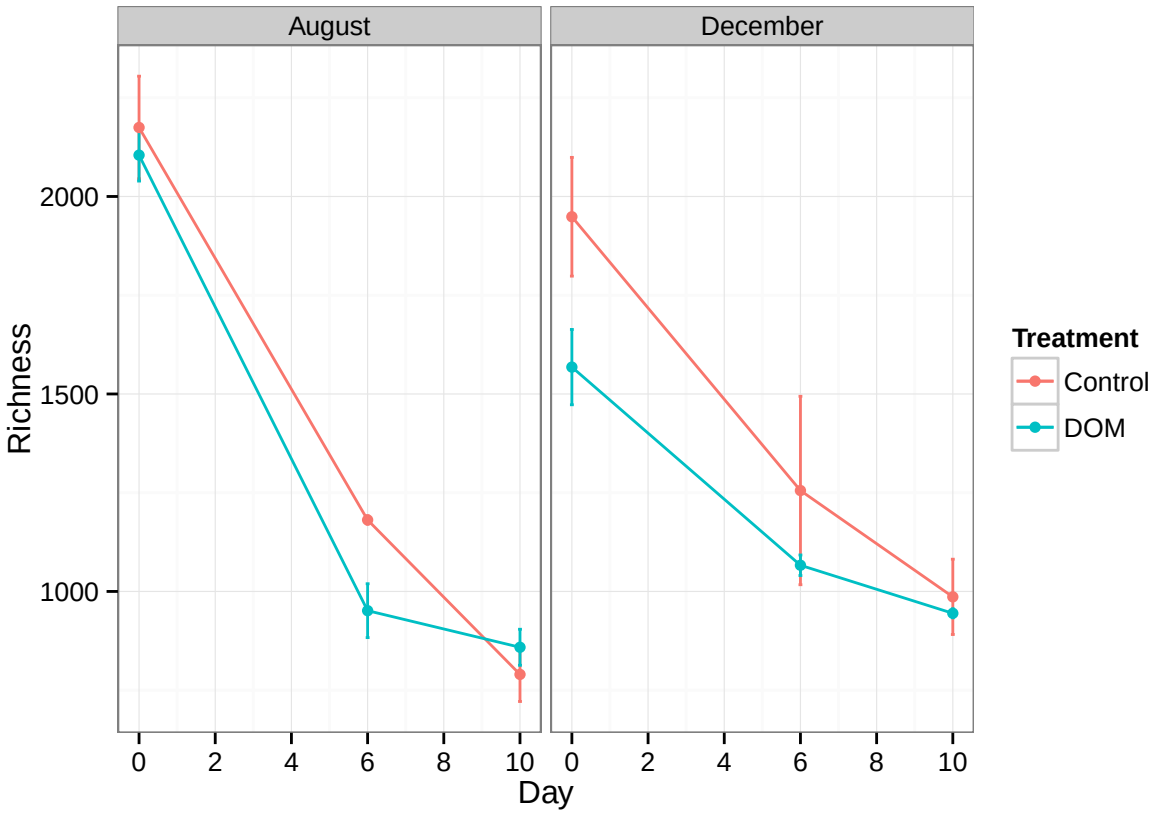
August
December
Richness
2000
1500
1000
0
2
4
6
8
10
Day
Treatment
Control
DOM

Figure 3:

```
ggplot(cdata1, aes(x=Timepoint, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
    geom_point() +
    scale_x_continuous(name="Timepoint") +
    scale_y_continuous(name="Richness") +
    theme_bw()
```

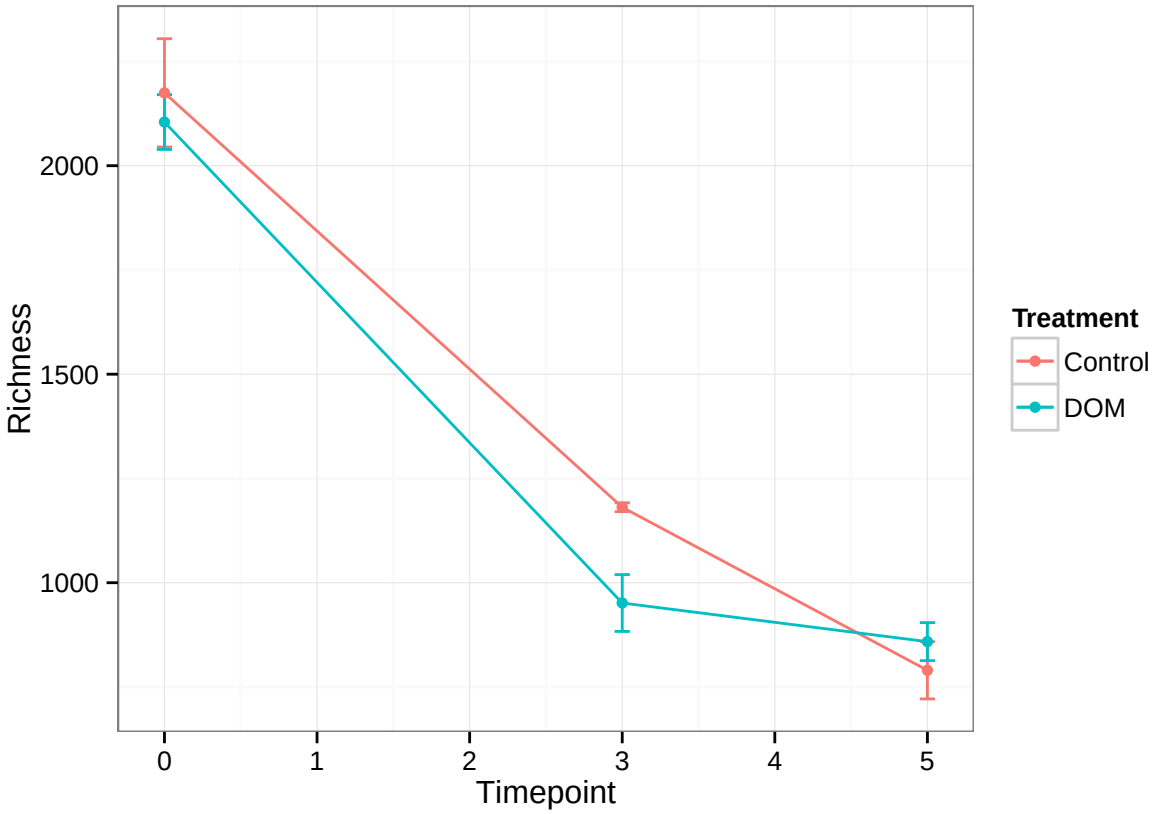

Figure 4:

```
    #theme(legend.position="none")
```

```
ggplot(cdata4, aes(x=Timepoint, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
    geom_point() +
    scale_x_continuous(name="Timepoint") +
    scale_y_continuous(name="Richness") +
    theme_bw()
```

```
    #theme(legend.position="none")
```

Richness dropped off dramtically in Control, f/2+, and DOM+ carboys over the course of all four 10-day incubation experiments. Richness was higher in Control carboys than DOM+ carboys at the midpoint of the

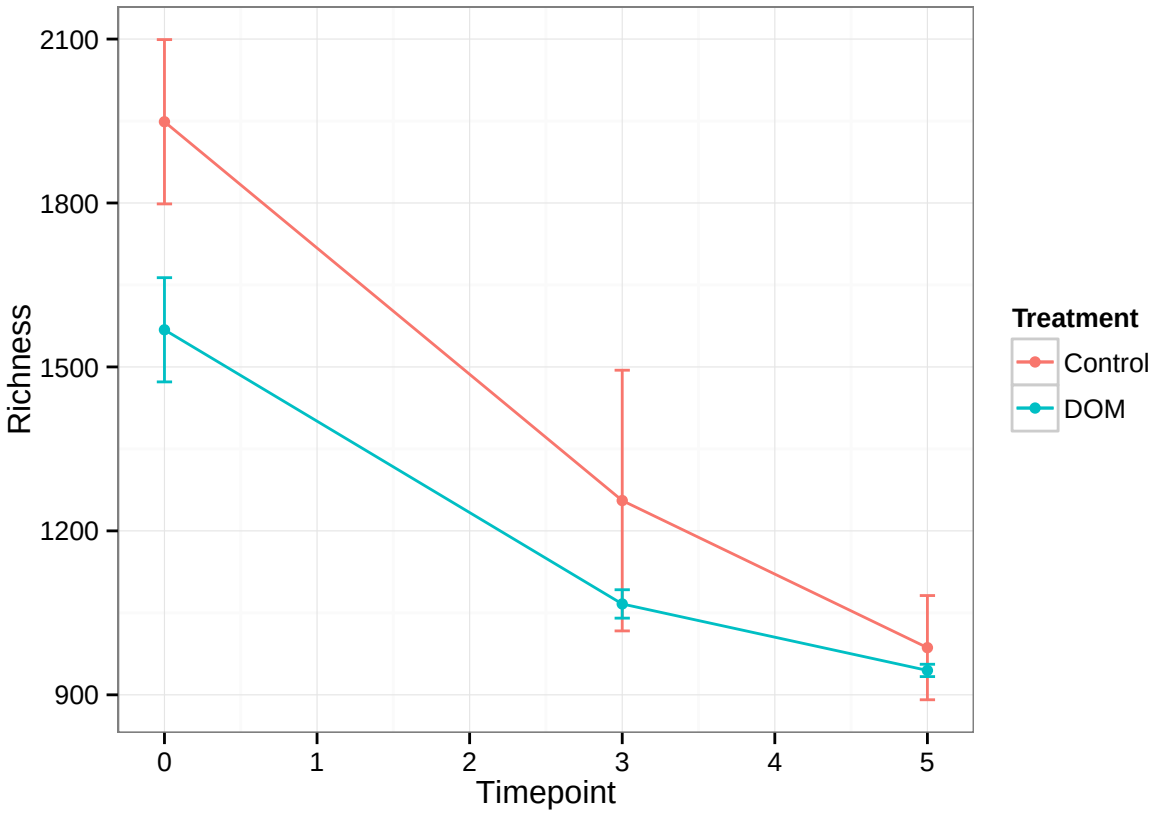
2100
1800
1500
1200
900
Richness
0
1
2
3
4
5
Timepoint
Treatment
Control
DOM

Figure 5:

August and September experiments. Richness began to rebound in the f/2+ and DOM+ carboys toward the end of the October experiment, perhaps suggesting acclimation. The December experiment results are similar to those from August and September, except that the DOM+ carboys had greater richness than the Control carboys at time 0, suggesting some sort of contamination.

3 Taxa plots

Taxa plots from each of the four experiments suggest that container effects are very strong: similar changes occur in both the control and treatment carboys. There does appear to be a difference in timing. By day 6, community composition in the DOM+ carboys has already shifted dramatically and closely resembles composition at day 10. Changes appear to occur more slowly in the control carboys with composition at day 6 representing a more intermediate state. This proposed lag effect can be seen in the August, September, and December experiments. The data from the October experiment are quite different, as discussed below.

3.1 August

At the beginning of the August experiment, Pelagibacteria (alphaproteobacteria), slow-growing oligotrophs, were dominant, but were replaced by gammaproteobacteria (Collwellia) over the course of the experiment.

A very brief search for Collwellia yielded some interesting results:

1. Collwellia were associated with the BP oil spill and supposedly digested alkanes and aromatics 1 2
2. Bidle and Azam conducted experiments in which they allowed diatom detritus to be colonized by natural bacterial assemblages. In one experiment, the pos-incubation assemblage was characterized by y *Collwellia demingiae*. here
3. The group contains some known psychrophiles [here] (http://www.ncbi.nlm.nih.gov/pubmed/16043709)

Flavobacteria also increased over the course of the experiment but did not comprise a large fraction of the community, even at day 10.

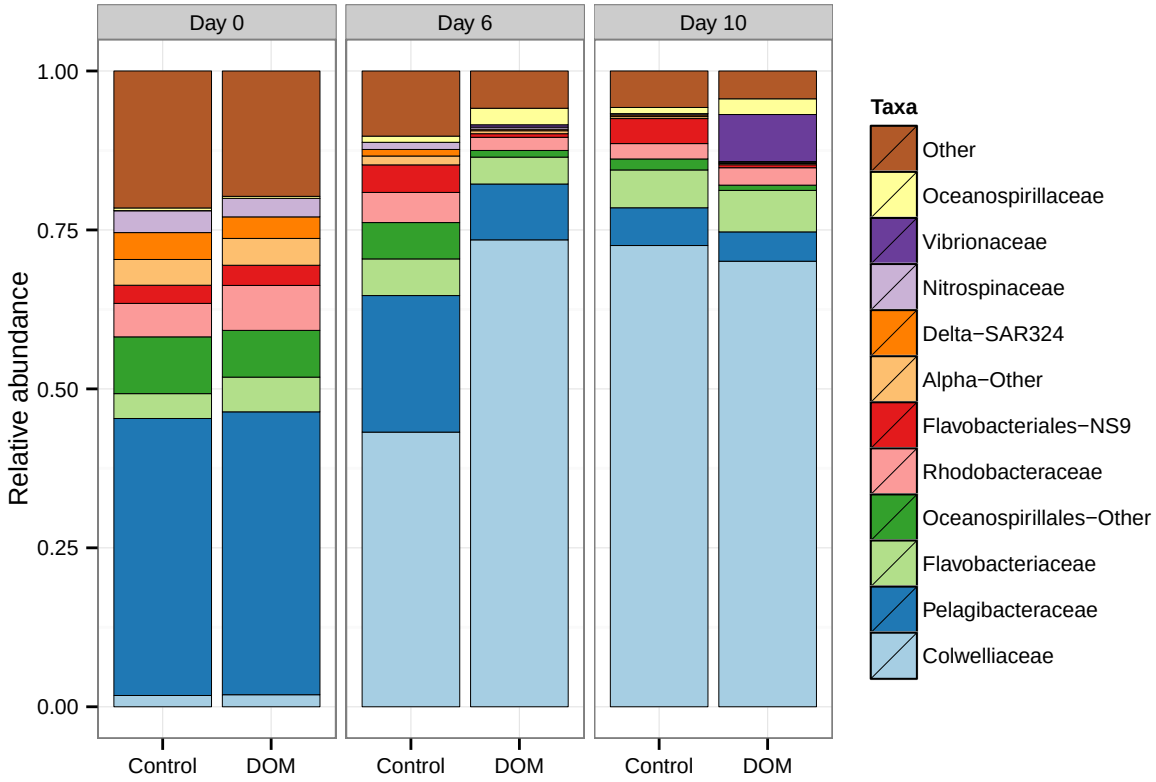

Figure 6: Relative abundance of most common taxa (family level) during DOM addition experiments.

3.2 September

Similar trends (declining Pelagibacteria and increasing Collwellia) occurred during the September experiment. Polaribacter (Flavobacteria blocks) was prevalent in both Control and DOM+ carboys on Day 10. Carbon from phytoplankton die-off (as suggested by disappearing chloroplast sequences) could account for some of the changes that occurred in the Control carboys.

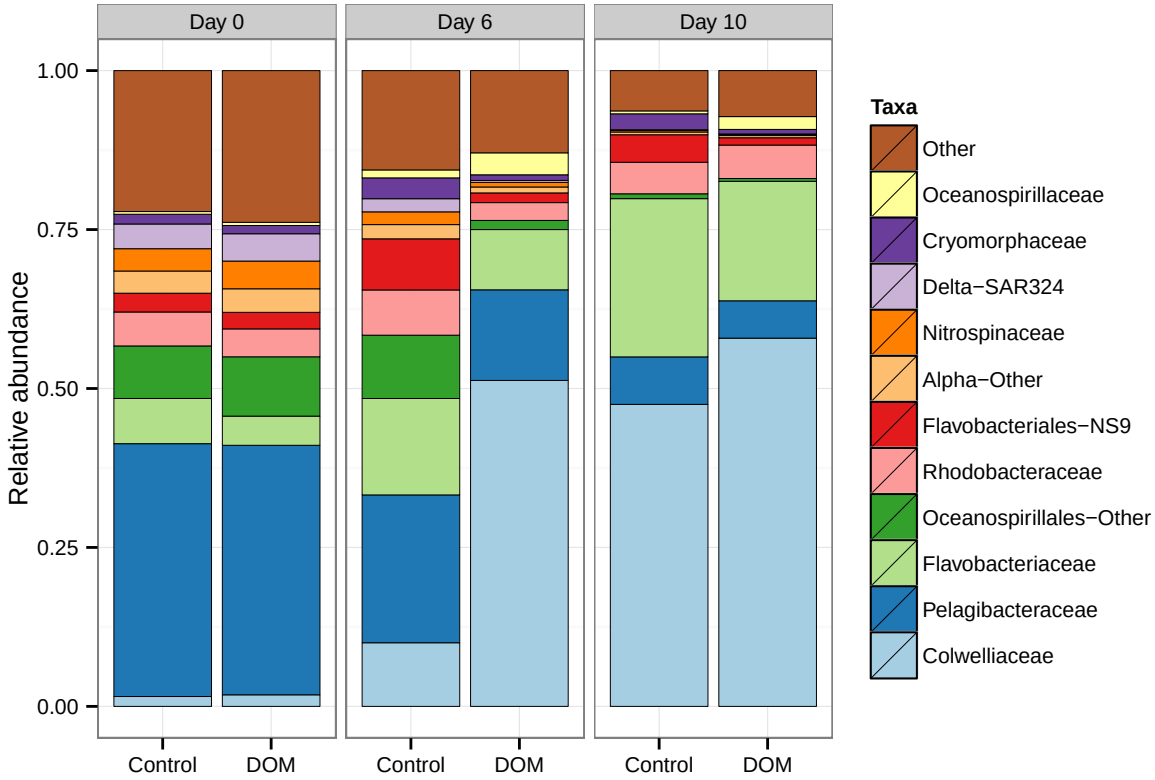

Figure 7: Relative abundance of most common taxa (family level) during DOM addition experiments.

3.3 October

The results from the October experiment, which included an f/2+ treatment, are strange. At the beginning of the experiment, Rickettsiales comprises large fractions of all of the communities. The grey blocks are made up of Haptophyceae (chloroplasts) and Oceanospirillales. At day 6, we have no data from the control carboys. The f/2+ and DOM+ carboys are both dominated by flavobacteria. These flavobacter OTUs are classified as Polaribacter. The DOM+ carboys also have a significant block of Alteromonadales. On day 10, the control and f/2+ carboys are very similar, while the DOM+ carboys are dominated to a greater extent by Polaribacter. A few additional samples were collected on day 11, which I find to be suspect. My inclination for this experiment would be to only consider data from day 0 and day 10. Again, carbon from phytoplankton die-off (as suggested by disappearing chloroplast sequences) could account for some of the changes that occurred in the Control carboys.

3.4 December

In the December experiment, the day 0 communities had a number of chloroplasts (grey blocks). Both Control and DOM+ carboys acquired Polaribacter (Flavobacteria) over the course of the experiment. Collwellia appeared in the DOM+ carboys but not the Control carboys.

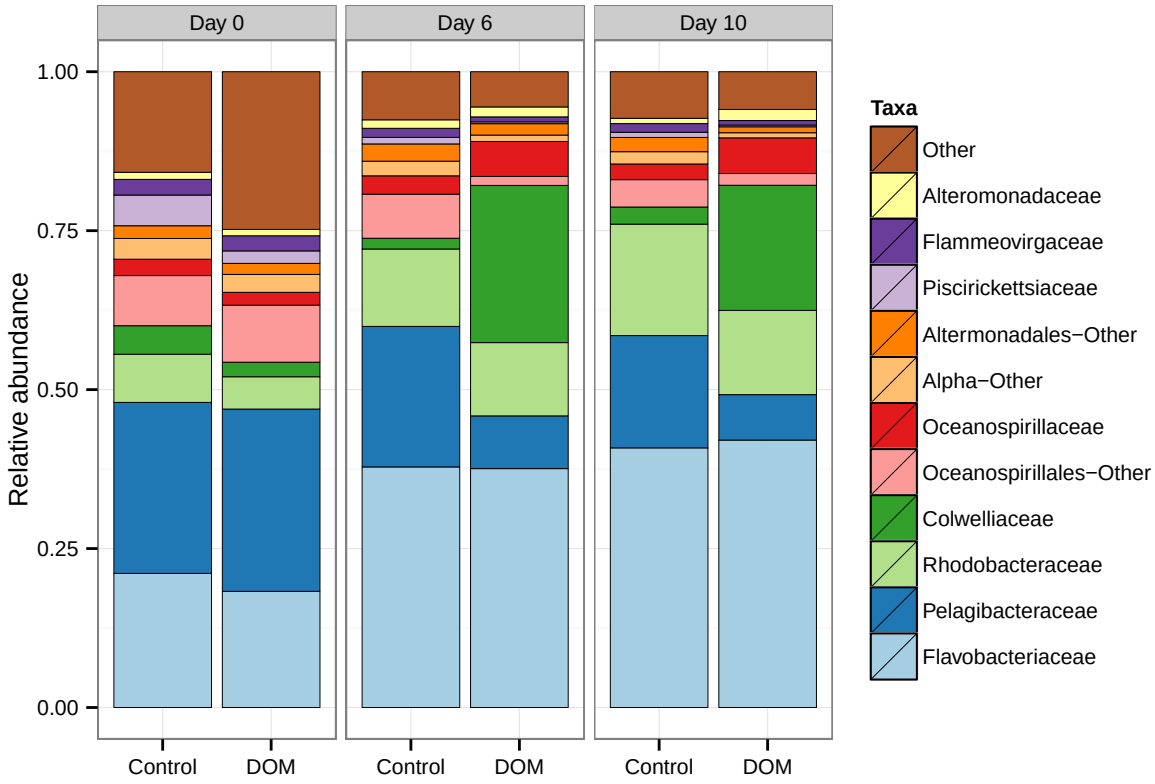

Figure 8: Relative abundance of most common taxa (family level) during DOM addition experiments.

4 NMDS

```
# for large datasets that crash R
# didn't need for this dataset
# compile the c++ code from within R
Rcpp::sourceCpp('bc_sparse.cpp')

dis <- my_bray_curtis(otutable_bac_samples_t)
m <- monoMDS(dis)
plot(m)
```

```
## Run 0 stress 0.095330541
## Run 1 stress 0.095330507
## ... New best solution
## ... procrustes: rmse 2.5991522e-05  max resid 0.0001901818
## *** Solution reached
```

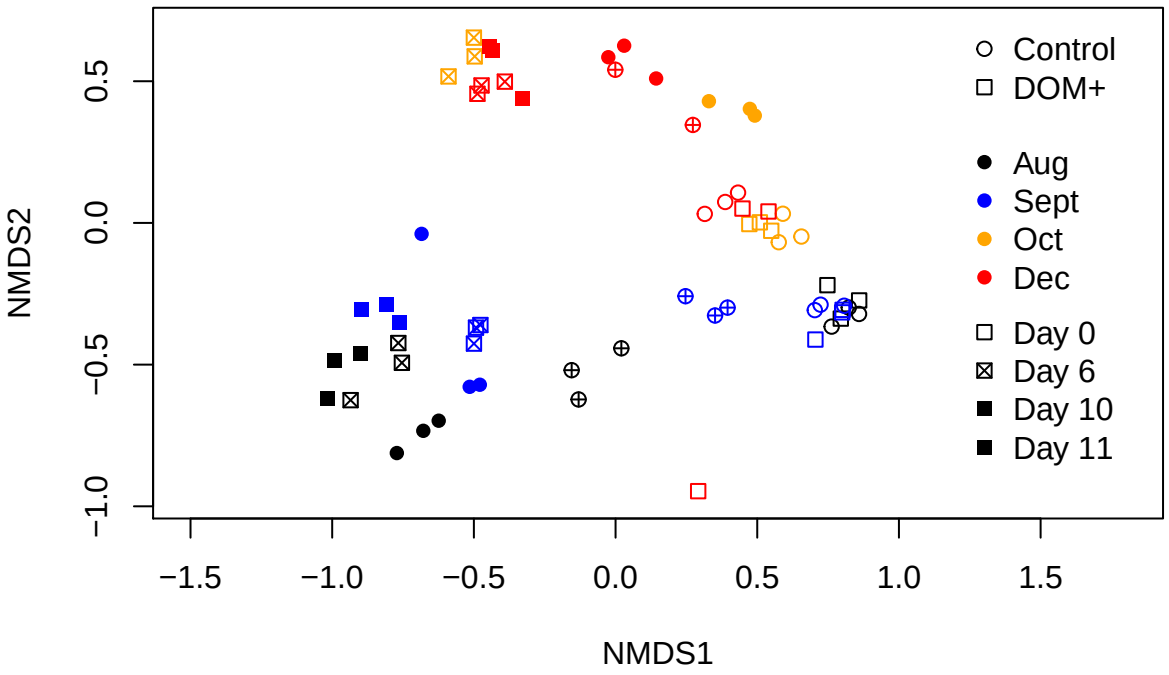

Figure 9: NMDS of samples from four DOM experiments (conducted in August, September, October, and December 2013). Two treatment (Control and DOM+) are shown, one (f/2+ is not). Carboys were samples on days 0, 6, and 10 except in one case when samples were also collected on day 11.

4.1 August

The Control and DOM+ carboys are similar to each other on both Day 0 and Day 10. As seen previously in the taxa plots, DOM+ carboys seems to more rapidly approach final Day 10 community composition. Day 6 DOM+ carboys are clustered with Day 10 DOM+ carboys, while the Day 6 control carboys form their own cluster. Note that the replicate carboys do, in fact, cluster together.

4.2 September

The September NMDS results are similar to the August results. Day 0 and Day 10 carboys all group together regardless of treatment. The Day 6 DOM+ carboys are more similar to the Day 10 cluster, while the Day 6 Control carboys are more similar to the Day 0 cluster.

4.3 October

Recall the the October experiment sampling scheme had a few discrepancies (no Control of Day 6, additional samples collected on Day 11). In this experiment, the f/2+ carboys clustered with the DOM+ carboys midway through the experiment but clustered with the control carboys at the end of the experiment. The DOM+ carboys changed between Day 0 and Day 6 but did not change after that.

4.4 December

In this experiment, both the Control carboys and the DOM+ carboys appeared to reach their final states by Day 6.

4.5 Add ellipses (not working right now)

```
bpdata <- ddply(diversitytable, c("Experiment", "Day", "Treatment"), summarise,
               N = length(Leucine),
               mean = mean(Leucine),
               sd   = sd(Leucine),
               se   = sd / sqrt(N)
)

bpdata <- as.data.table(bpdata)
bpdata14 <- bpdata[which(bpdata$Experiment=="1"|bpdata$Experiment=="4")]

exp_labeller <- function(var, value){
  value <- as.character(value)
  if (var=="Experiment") {
    value[value=="1"] <- "August"
    value[value=="4"]   <- "December"
    }
  return(value)
}

bp14 <- ggplot(bpdata14, aes(x=Day, y=mean, colour=Treatment)) +
    geom_errorbar(aes(ymin=mean-se, ymax=mean+se), width=.1) +
    geom_line() +
    geom_point() +
    scale_x_continuous(name="Day", breaks=c(0,2,4,6,8,10)) +
    scale_y_continuous(name="Bacterial production (pM 3H-leucine/hr)") +
    theme_bw()

bp14 + facet_grid(.~Experiment, labeller=exp_labeller)
```

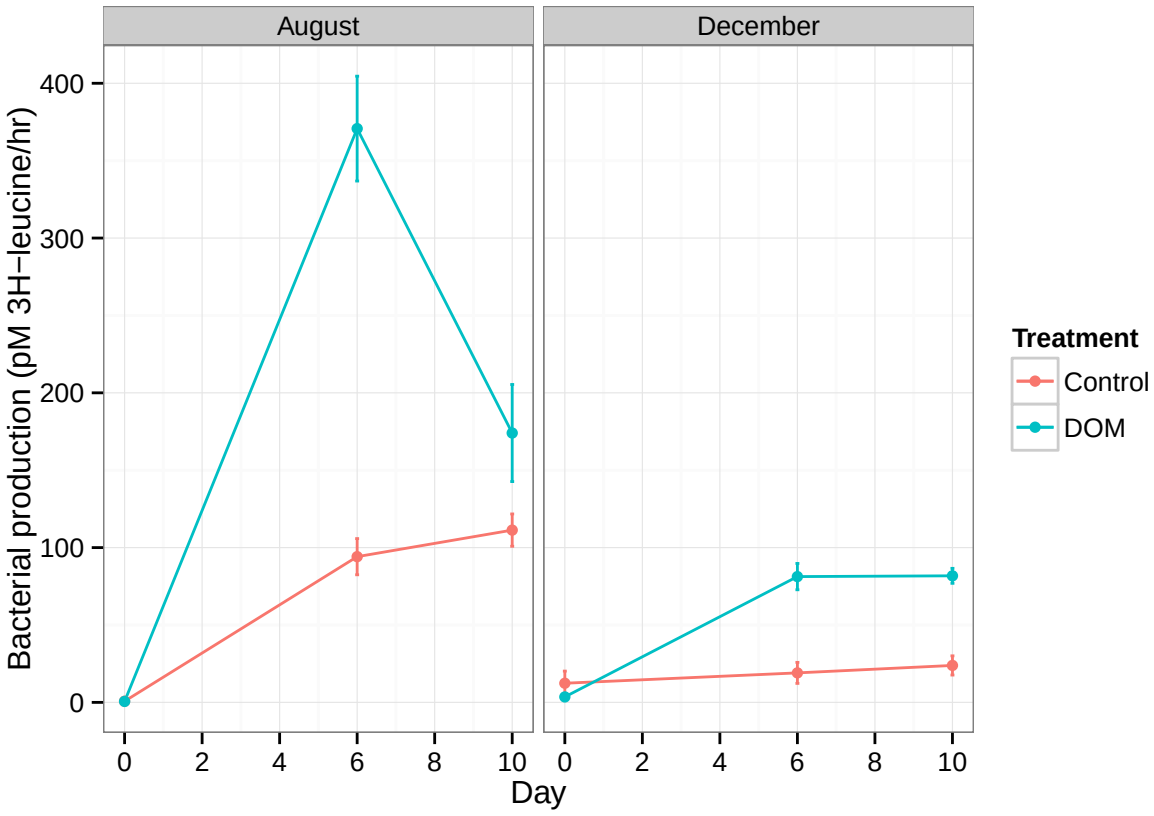
August
December
Bacterial production (pM 3H−leucine/hr)
400
300
200
100
0
0
2
4
6
8
10
Day
Treatment
Control
DOM

Figure 10: