Regional_nMDS_SIMPER_PERMANOVA

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```r
library(knitr)
hook_output = knit_hooks$get('output')
knit_hooks$set(output = function(x, options) {
  # this hook is used only when the linewidth option is not NULL
  if (!is.null(n <- options$linewidth)) {
    x = knitr:::split_lines(x)
    # any lines wider than n should be wrapped
    if (any(nchar(x) > n)) x = strwrap(x, width = n)
    x = paste(x, collapse = '\n')
  }
  hook_output(x, options)
})
```

nMDS Plots

```r
# Import data
density <- data.frame(read.csv("invert_matrix_transects.csv"))
density[is.na(density)] <- 0

# Create groups using 'newnames' to split data

grp.MPA <- density %>%
  group_by_all() %>%
  mutate(newnames=paste0(Region, Designation, Year))

# Split data into smaller frames

split.MPA <- split(grp.MPA, grp.MPA$newnames)
for (I in 1:length(split.MPA)) {assign(unique(split.MPA[[I]]$newnames),
                                       split.MPA[[I]])}

# Determine average densities of organisms for each Region, Designation,
# and Year

take.avg <- lapply(split.MPA, function(x){

  avgs <- colMeans(x[,9:165])
  avgs <- as.data.frame(avgs)
  t.avgs <- as.data.frame(transpose(avgs))
  colnames(t.avgs) <- rownames(avgs)
  lab <- data.frame(Year=x[1,2], Region=x[1,3], MPA_Group=x[1,4],
```

```r
                       Designation=x[1,7])
  region.unit <- cbind(lab,t.avgs)

})

# Bind rows of new data frames together from the split

only.avgs <- bind_rows(take.avg)

# create data frame of labels (Year, Region, MPA, Designation)

reg.labels <- only.avgs[,1:4]

# create data frame of species matrix

reg.data <- only.avgs[,5:160]

# Generate MDS

set.seed(13)
reg.mds <- metaMDS(reg.data, distance = "bray", k = 2, trymax = 999,
                   autotransform = FALSE)
```

```
## Run 0 stress 0.1275924
## Run 1 stress 0.1275924
## ... New best solution
## ... Procrustes: rmse 2.385989e-06  max resid 7.659247e-06
## ... Similar to previous best
## Run 2 stress 0.1275924
## ... New best solution
## ... Procrustes: rmse 1.844029e-06  max resid 7.034715e-06
## ... Similar to previous best
## Run 3 stress 0.1278416
## ... Procrustes: rmse 0.02874042  max resid 0.1086056
## Run 4 stress 0.2271201
## Run 5 stress 0.1275924
## ... Procrustes: rmse 1.010998e-05  max resid 4.161017e-05
## ... Similar to previous best
## Run 6 stress 0.1275924
## ... Procrustes: rmse 2.369381e-06  max resid 8.113587e-06
## ... Similar to previous best
## Run 7 stress 0.1278416
## ... Procrustes: rmse 0.02874176  max resid 0.1086152
## Run 8 stress 0.1278416
## ... Procrustes: rmse 0.02874434  max resid 0.1086339
## Run 9 stress 0.1275925
## ... Procrustes: rmse 1.607837e-05  max resid 5.72162e-05
## ... Similar to previous best
## Run 10 stress 0.1278416
## ... Procrustes: rmse 0.02874215  max resid 0.1086188
## Run 11 stress 0.1275924
## ... Procrustes: rmse 4.953412e-06  max resid 1.400328e-05
## ... Similar to previous best
## Run 12 stress 0.1275924
```

```
## ... Procrustes: rmse 1.944918e-06  max resid 6.397273e-06
## ... Similar to previous best
## Run 13 stress 0.1275924
## ... Procrustes: rmse 4.155899e-06  max resid 1.615037e-05
## ... Similar to previous best
## Run 14 stress 0.1275924
## ... New best solution
## ... Procrustes: rmse 1.795318e-06  max resid 6.614027e-06
## ... Similar to previous best
## Run 15 stress 0.1275924
## ... Procrustes: rmse 1.895525e-06  max resid 8.2185e-06
## ... Similar to previous best
## Run 16 stress 0.1946847
## Run 17 stress 0.1275924
## ... Procrustes: rmse 6.338998e-07  max resid 1.78383e-06
## ... Similar to previous best
## Run 18 stress 0.1959829
## Run 19 stress 0.1861935
## Run 20 stress 0.2014039
## *** Solution reached
```

```r
# Divide MDS into data and species MDS scores across 2 axes

data.scores <- as.data.frame(scores(reg.mds))
data.scores$site <- rownames(data.scores)
data.scores <- cbind(data.scores,reg.labels)
head(data.scores)
```
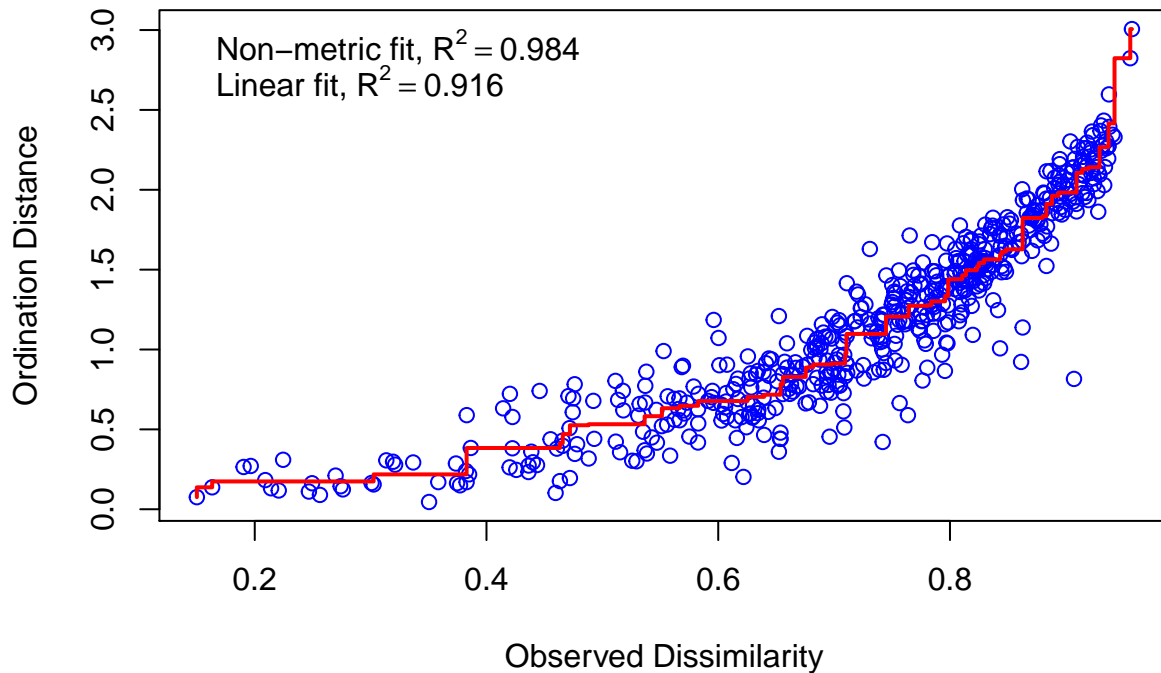
```
##         NMDS1        NMDS2 site Year  Region      MPA_Group Designation
## 1 -0.7680514 -0.51461743    1 2007 Central     Point Lobos         MPA
## 2 -0.9460948 -0.35464770    2 2008 Central    Point Buchon         MPA
## 3 -0.6779332 -1.32543000    3 2009 Central     Point Lobos         MPA
## 4 -1.1065013 -0.03252102    4 2012 Central    Point Buchon         MPA
## 5 -0.2274672 -0.28054402    5 2016 Central Piedras Blancas         MPA
## 6  0.8785583 -0.30256414    6 2019 Central    Point Buchon         MPA
```

```r
species.scores<- as.data.frame(scores(reg.mds, "species"))
species.scores$species <- rownames(species.scores)
head(species.scores)
```

```
##                                 NMDS1       NMDS2
## Acorn.barnacle              1.1080684 -0.11131935
## Aggregated.nipple.sponge   -2.1317519 -0.10495806
## Basket.star                 1.1059118 -1.16115624
## Bat.star                   -0.3149907  0.24852968
## Bat.star.red.sea.star.complex -1.3323847 -0.03665008
## Benthic.siphonophore             NaN         NaN
##                                               species
## Acorn.barnacle                         Acorn.barnacle
## Aggregated.nipple.sponge     Aggregated.nipple.sponge
## Basket.star                               Basket.star
## Bat.star                                     Bat.star
## Bat.star.red.sea.star.complex Bat.star.red.sea.star.complex
## Benthic.siphonophore             Benthic.siphonophore
```

```r
# Plot stress

stressplot(reg.mds)
```


Non−metric fit, $R^2 = 0.984$
Linear fit, $R^2 = 0.916$

(y-axis: Ordination Distance; x-axis: Observed Dissimilarity)

```r
# Plot nMDS in ggplot

ggplot() +
  stat_ellipse(data=data.scores,aes(x=NMDS1,y=NMDS2, color=Region,
                                     linetype=Designation), lwd=2, alpha=0.5) +
  geom_point(data=data.scores,aes(x=NMDS1,y=NMDS2, fill=Region, pch=Designation),
             size=5, alpha=0.3) +
  guides(pch = "none", size = "none") +
  ggtitle("MPA vs. Reference") +
  scale_linetype_manual(values=c(1,2), breaks = c("MPA","Reference")) +
  scale_shape_manual(values=c(21,22)) +
  scale_color_manual(values=c("red","green","blue"),
                     breaks = c("North", "Central", "South")) +
  scale_fill_manual(values=c("red","green","blue"),
                    breaks = c("North", "Central", "South")) +
  theme(axis.text.x = element_blank(),  # remove x-axis text
        axis.text.y = element_blank(), # remove y-axis text
        axis.ticks = element_blank(),  # remove axis ticks
        axis.title.x = element_text(size=18), # remove x-axis labels
        axis.title.y = element_text(size=18), # remove y-axis labels
        panel.background = element_blank(),
        panel.grid.major = element_blank(),  #remove major-grid labels
```
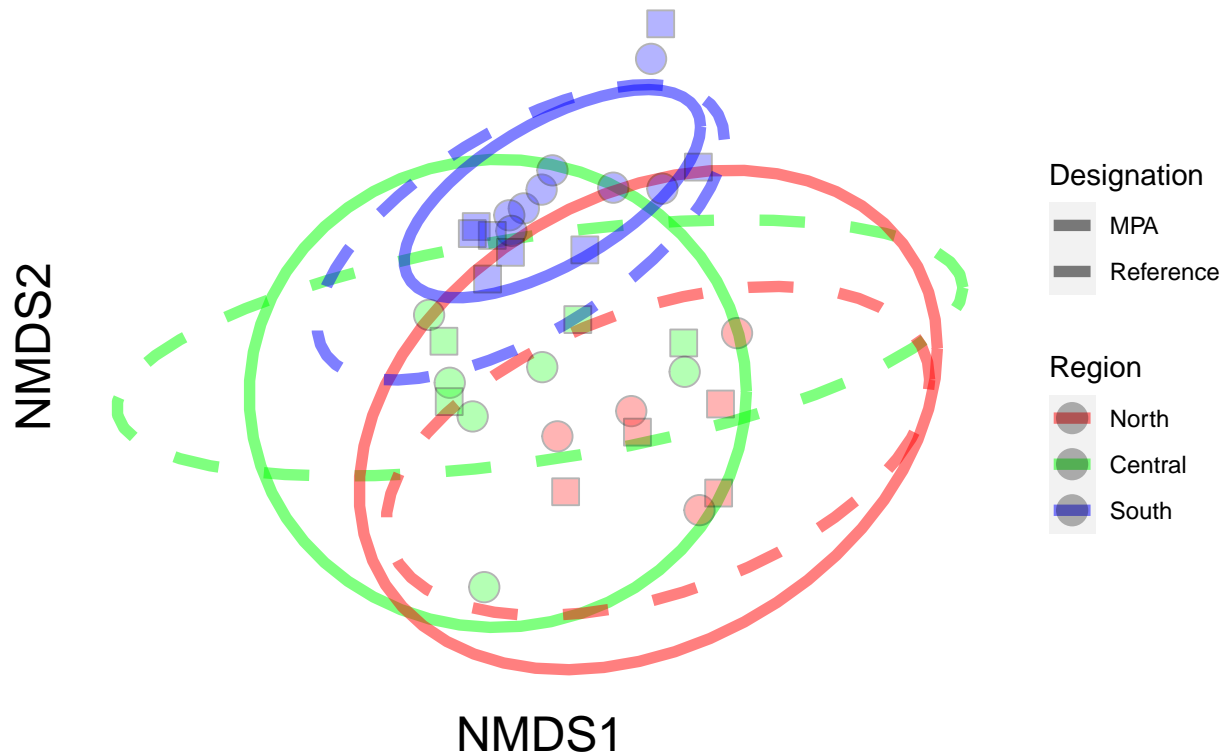
```
        panel.grid.minor = element_blank(),  #remove minor-grid labels
        plot.background = element_blank(),
        plot.title = element_text(size=20))
```

MPA vs. Reference


```
# Now to use facets to plot several NMDSs at once

base <- ggplot() +
  stat_ellipse(data=data.scores,
               aes(x=NMDS1,y=NMDS2, color=Region, linetype=Designation),
               lwd=2, alpha=0.5) +
  geom_point(data=data.scores,
             aes(x=NMDS1,y=NMDS2, fill=Region, pch=Designation),
             size=5, alpha=0.3) +
  guides(pch = "none", size = "none") +
  ggtitle("MPA vs. Reference") +
  scale_linetype_manual(values=c(1,2),
                        breaks = c("MPA","Reference")) +
  scale_shape_manual(values=c(21,22)) +
  scale_color_manual(values=c("red","green","blue"),
                     breaks = c("North", "Central", "South")) +
  scale_fill_manual(values=c("red","green","blue"),
                    breaks = c("North", "Central", "South")) +
  theme(axis.text.x = element_blank(),  # remove x-axis text
        axis.text.y = element_blank(),  # remove y-axis text
        axis.ticks = element_blank(),   # remove axis ticks
        axis.title.x = element_text(size=18), # remove x-axis labels
```
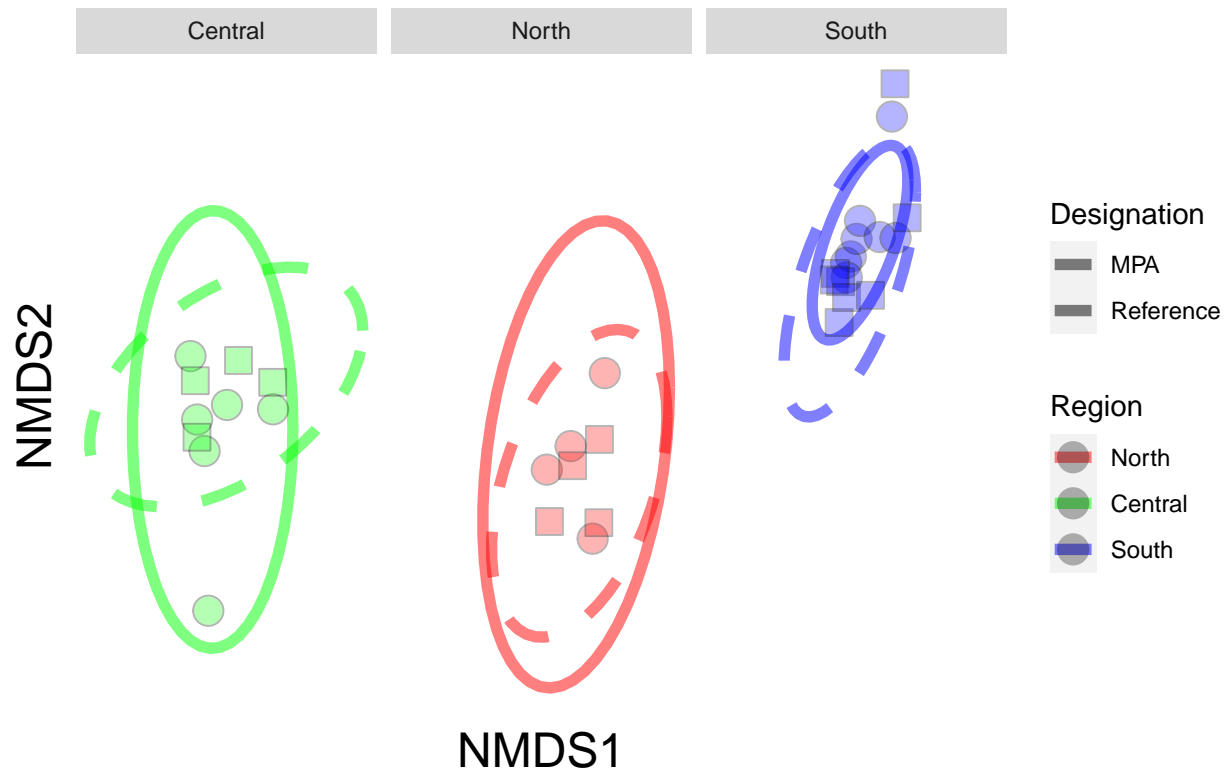
```
        axis.title.y = element_text(size=18), # remove y-axis labels
        panel.background = element_blank(),
        panel.grid.major = element_blank(),  #remove major-grid labels
        panel.grid.minor = element_blank(),  #remove minor-grid labels
        plot.background = element_blank(),
        plot.title = element_text(size=20))

# First by region

base + facet_wrap(~Region)
```



```
# Then by Year

base + facet_wrap(~Year) +
  theme(strip.text.x = element_text(size = 15))
```

```
## Too few points to calculate an ellipse
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## Warning: Removed 34 row(s) containing missing values (geom_path).
```


MPA vs. Reference
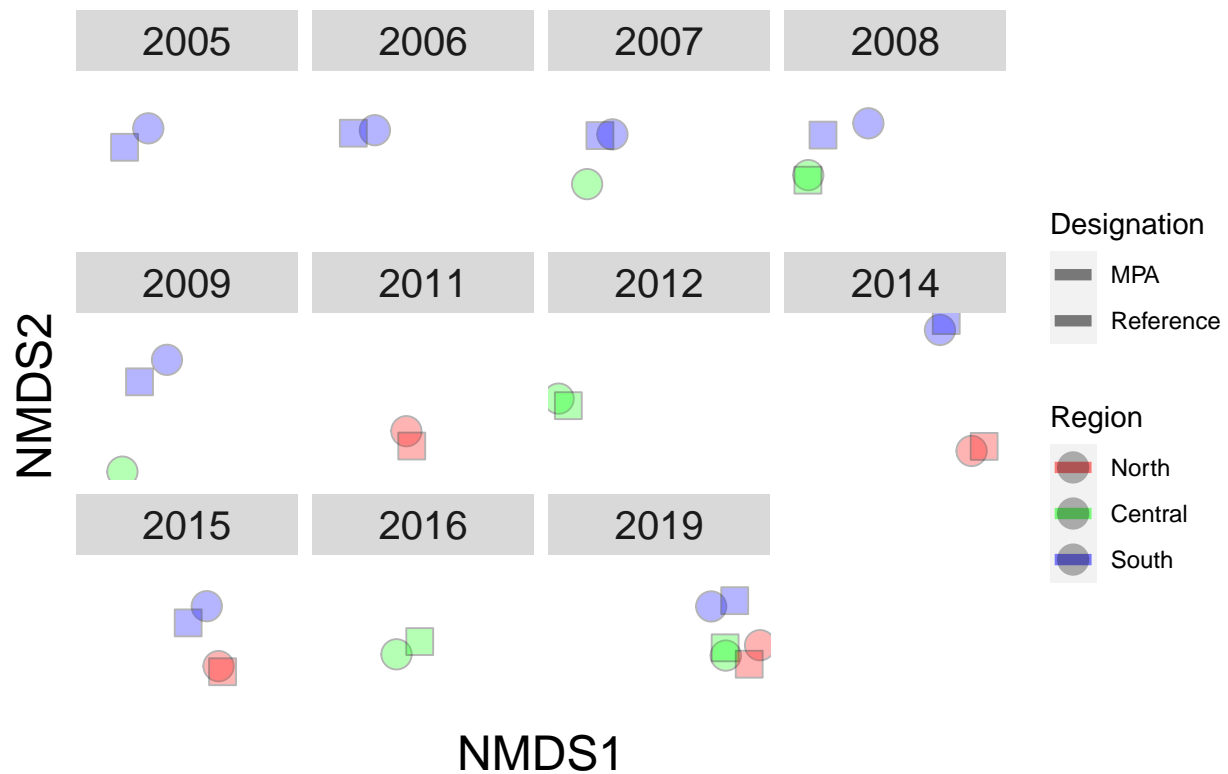
SIMPER analysis

```r
# Use data frames set up for nMDS to use in SIMPER analysis
# First, compare differences between regions

set.seed(13)
sim <- with(reg.labels, simper(reg.data, Region))
sim
```

```
## cumulative contributions of most influential species:
##
## $Central_North
##  California.sea.cucumber      Slipper.sea.cucumber        Short.red.gorgonian
##                0.1774812                 0.3060658                  0.3772839
##           UI.lobed.sponge  UI.lobed.sponge.tunicate       White.plumed.anemone
##                0.4355861                 0.4839536                  0.5310859
##            Red.sea.urchin       California.hydrocoral       UI.branched.bryozoan
##                0.5774277                 0.6182181                  0.6527903
##         UI.branched.sponge               Red.sea.star
##                0.6852496                 0.7154496
##
## $Central_South
##          White.sea.urchin  UI.lobed.sponge.tunicate
##                0.1726507                 0.2420651
##           UI.lobed.sponge       UI.branched.bryozoan
##                0.3044175                 0.3600304
##             White.sea.pen   California.sea.cucumber
##                0.4129377                 0.4594726
##            Red.sea.urchin                   Bat.star
##                0.5008071                 0.5367493
##                  Sea.whip White.branched.sea.cucumber
##                0.5711367                 0.6037940
##      UI.laced.lobed.bryozoan              Red.sea.star
##                0.6358096                 0.6622935
##           Purple.gorgonian         UI.branched.sponge
##                0.6880643                 0.7127600
##
## $North_South
##      California.sea.cucumber                White.sea.urchin
##                    0.1534472                       0.2741373
##         Slipper.sea.cucumber             Short.red.gorgonian
##                    0.3852217                       0.4451593
##               Red.sea.urchin            White.plumed.anemone
##                    0.4932306                       0.5390345
##         UI.branched.bryozoan White.branched.sea.cucumber
##                    0.5824273                       0.6187451
##                     Bat.star           California.hydrocoral
##                    0.6526233                       0.6841423
##               White.sea.pen
##                    0.7133553
```

```r
# Divide into species and cumulative contributions, then make data frames from these
# vectors

CN_sp <- c("California sea cucumber","Slipper sea cucumber",
```

```
                  "Short red gorgonian", "UI lobed sponge",
                  "UI lobed sponge/tunicate","White plumed anemone",
                  "Red sea urchin","California hydrocoral",
                  "UI branched byrozoan","UI branched sponge","Red sea star")
CN_con <- c(0.1773808,0.3059056,0.3770985,0.4353616,0.4837151,
              0.5308375,0.5771436,0.6178814,0.6524418,0.6848954,0.7150882)
CN_data <- data.frame(CN_sp,CN_con)
CS_sp <- c("White sea urchin","UI lobed sponge tunicate",
              "UI lobed sponge","UI branched bryozoan",
              "White sea pen","California sea cucumber",
              "Red sea urchin","Bat star","Sea whip",
              "White branched sea cucumber","UI laced/lobed bryozoan",
              "Red sea star","Purple gorgonian","UI branched sponge")
CS_con <- c(0.1726507,0.2420651,0.3044175,0.3600304,0.4129377,
              0.4594726,0.5008071,0.5367493,0.5711367,0.6037940,
              0.6358096,0.6622935,0.6880643,0.7127600)
CS_data <- data.frame(CS_sp,CS_con)
NS_sp <- c("California sea cucumber","White sea urchin",
              "Slipper sea cucumber","Short red gorgonian",
              "Red sea urchin","White plumed anemone",
              "UI branched bryozoan","White branched sea cucumber",
              "Bat star","California hydrocoral","White sea pen")
NS_con <- c(0.1533639,0.2740252,0.3850616,0.444977,0.4930209,
              0.5388162,0.5821998,0.6185102,0.6523830,0.6838560,
              0.7130625)
NS_data <- data.frame(NS_sp,NS_con)

# Plot contributions with one graph per region

CN_graph <- ggplot(CN_data, aes(y=CN_con,x=factor(CN_sp,level=CN_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#ff8247',color='gray') +
  ggtitle("SIMPER analysis of species between North and Central CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=15),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
CN_graph
```
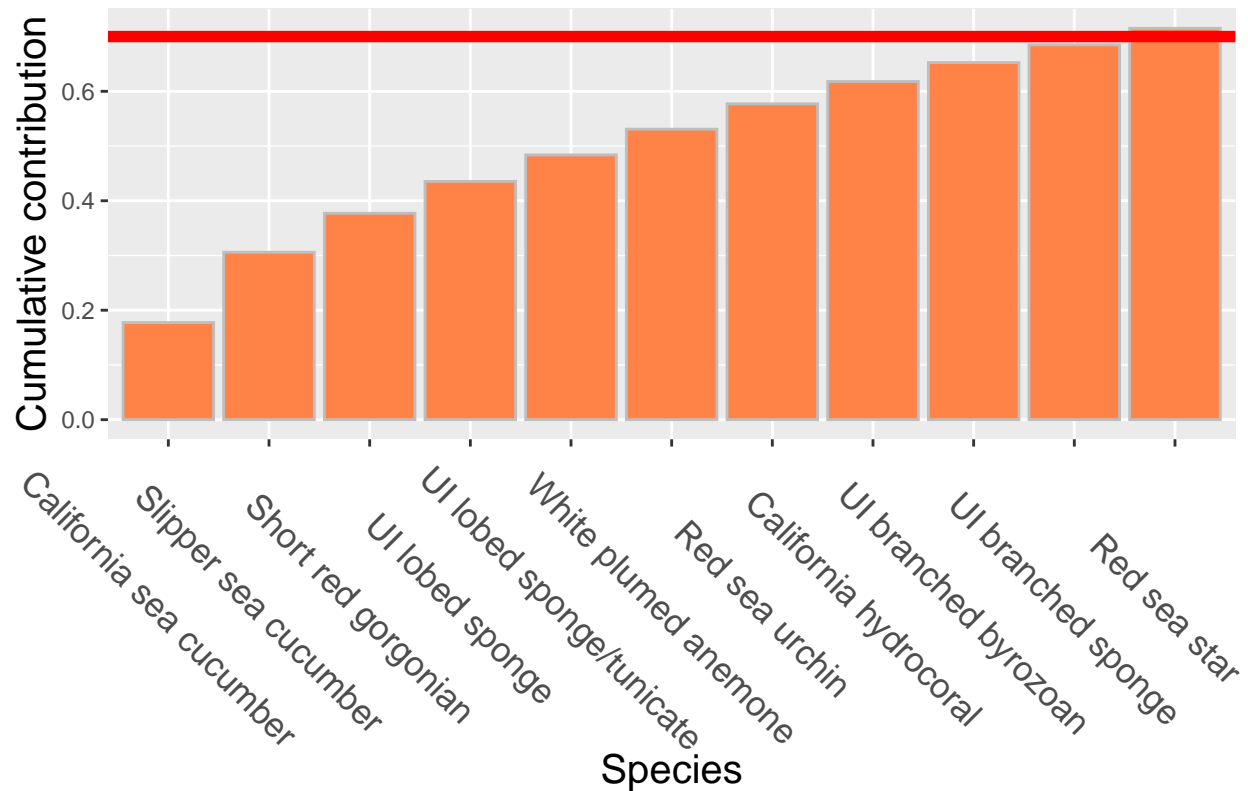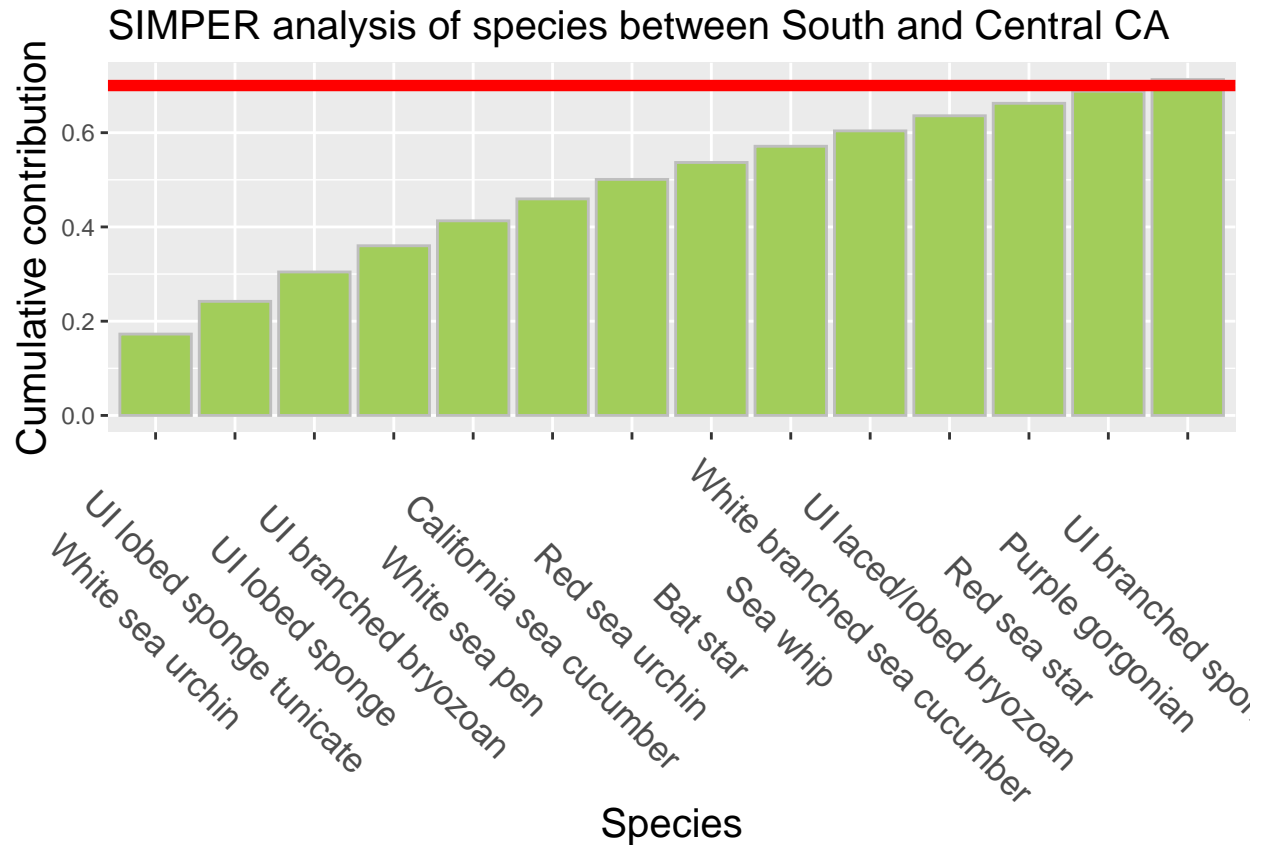
SIMPER analysis of species between North and Central CA



```
CS_graph <- ggplot(CS_data, aes(y=CS_con,x=factor(CS_sp,level=CS_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#a2cd5a',color='gray') +
  ggtitle("SIMPER analysis of species between South and Central CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=15),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
CS_graph
```

SIMPER analysis of species between South and Central CA

```
NS_graph <- ggplot(NS_data, aes(y=NS_con,x=factor(NS_sp,level=NS_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#6495ed',color='gray') +
  ggtitle("SIMPER analysis of species between North and South CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=20),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
NS_graph
```

SIMPER analysis of species between North and
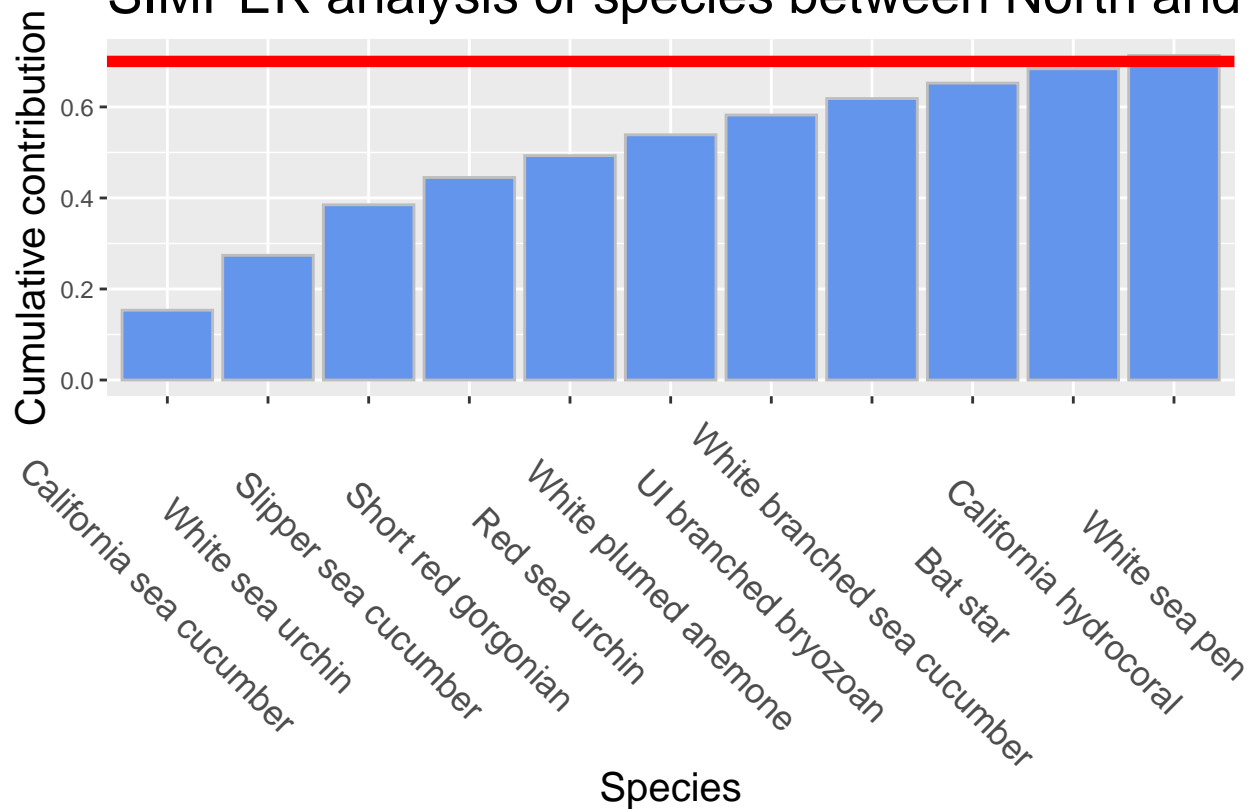

```r
# Now, use SIMPER to analyze differences in MPA vs. Reference designation

set.seed(13)
sim.des <- with(reg.labels, simper(reg.data, Designation))
sim.des

## cumulative contributions of most influential species:
##
## $MPA_Reference
##          White.sea.urchin    California.sea.cucumber
##                 0.1469531                  0.2417720
##      Slipper.sea.cucumber             Red.sea.urchin
##                 0.3017131                  0.3526541
##      UI.branched.bryozoan            UI.lobed.sponge
##                 0.4012280                  0.4459862
##   UI.lobed.sponge.tunicate             White.sea.pen
##                 0.4868980                  0.5273387
##       Short.red.gorgonian White.branched.sea.cucumber
##                 0.5666367                  0.6021512
##                  Bat.star        White.plumed.anemone
##                 0.6327757                  0.6615762
##        UI.branched.sponge        California.hydrocoral
##                 0.6896547                  0.7167302

# Use the provided information to create a data frame

Des_sp <- c("White sea urchin","California sea cucumber",
```

```r
            "Slipper sea cucumber","Red sea urchin",
            "UI branched bryozoan","UI lobed sponge",
            "UI lobed sponge/tunicate","White sea pen",
            "Short red gorgonian","White branched sea cucumber",
            "Bat star","White plumed anemone","UI branched sponge",
            "California hydrocoral")
Des_con <- c(0.1469395,0.2417168,0.3016337,0.3525581,0.4011254,
             0.4458663,0.4867733,0.5272089,0.5664956,0.6020061,
             0.6326269,0.6614230,0.6894982,0.7165490)
Des_data <- data.frame(Des_sp,Des_con)

# And plot it

Des_graph <- ggplot(Des_data, aes(y=Des_con,x=factor(Des_sp,level=Des_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#bf3eff',color='gray') +
  ggtitle("SIMPER analysis of species between MPAs and Reference sites") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=20),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
Des_graph
```
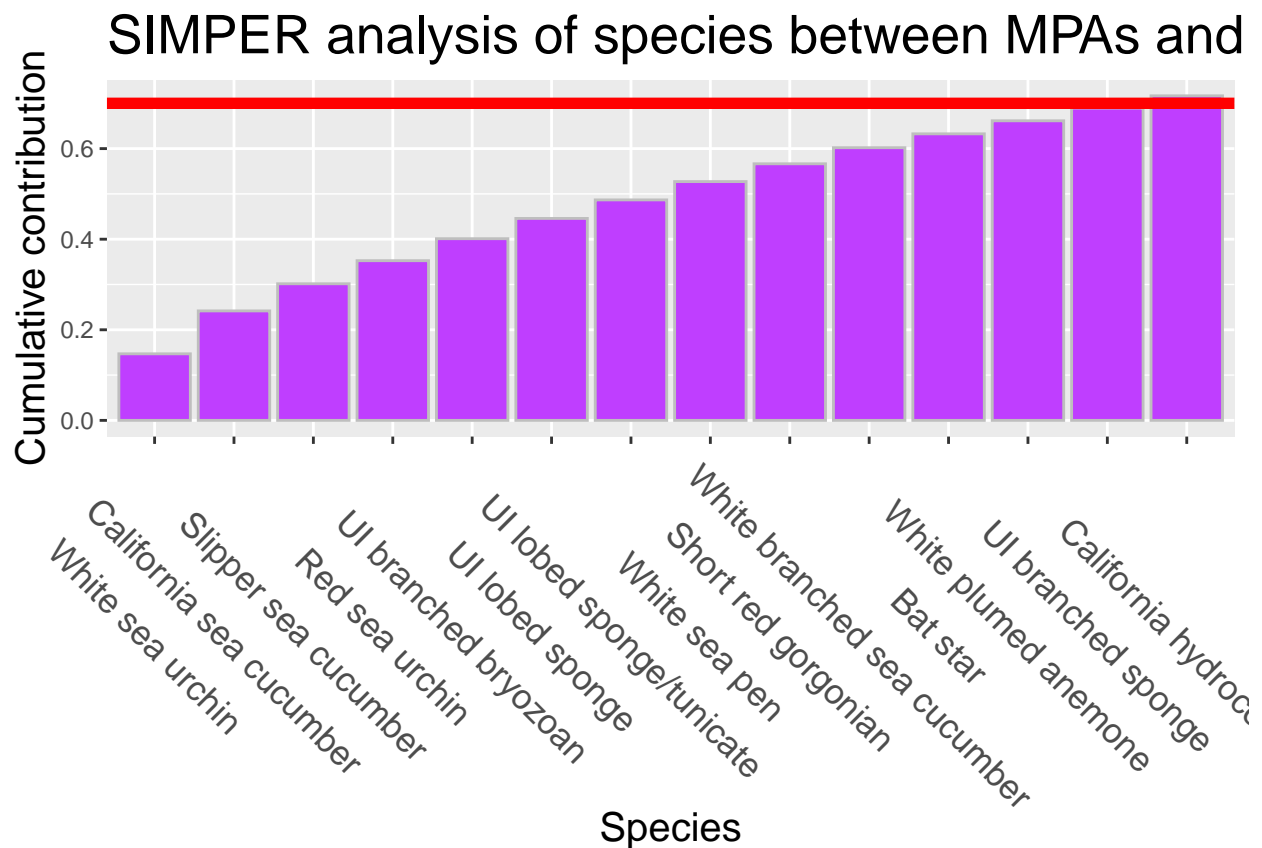


```r
# Now, to subset by region & SIMPER by designation separately
```

```
North.avg <- subset(only.avgs, Region == "North")
Central.avg <- subset(only.avgs, Region == "Central")
South.avg <- subset(only.avgs, Region == "South")

# North

north.labels <- North.avg[,1:4]
north.data <- North.avg[,5:160]
set.seed(13)
north.sim <- with(north.labels, simper(north.data, Designation))
north.sim
```
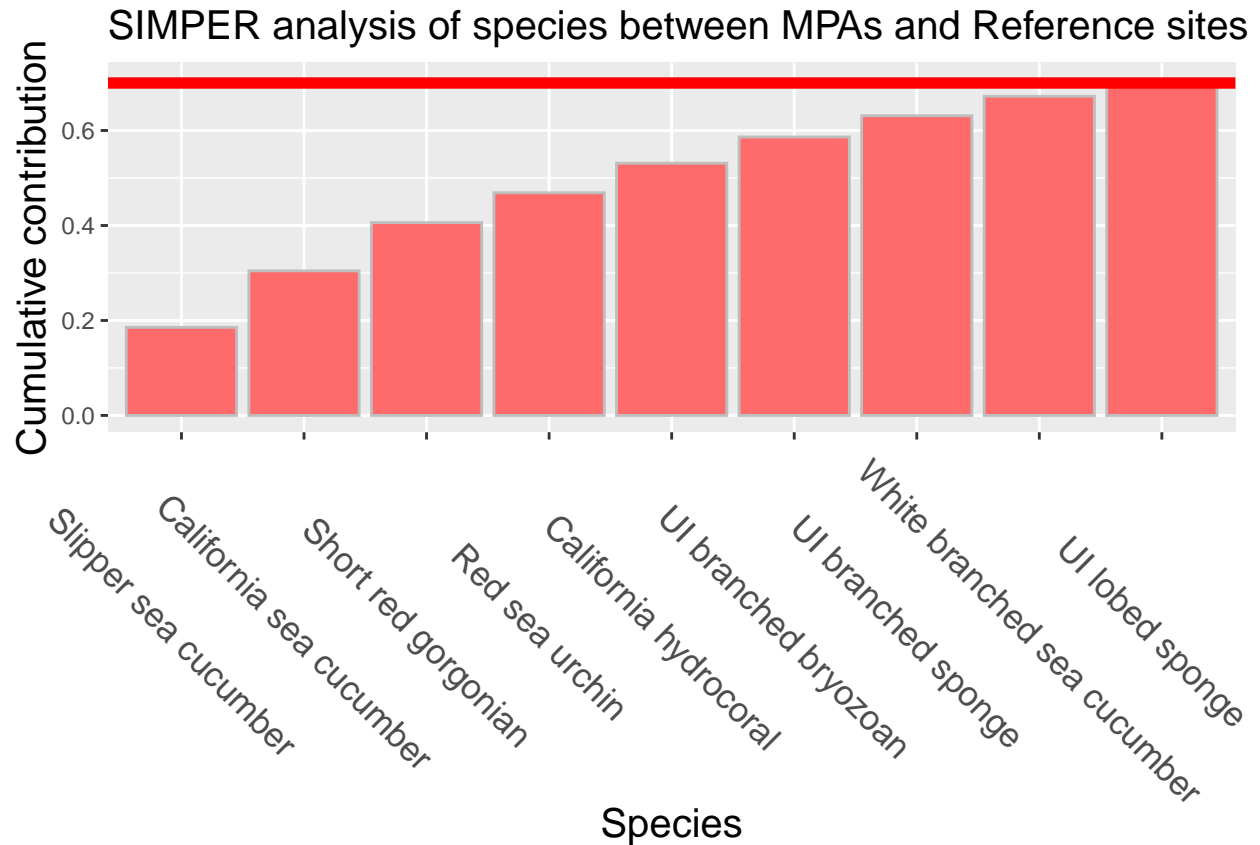
```
## cumulative contributions of most influential species:
##
## $MPA_Reference
##        Slipper.sea.cucumber     California.sea.cucumber
##                   0.1857117                   0.3047077
##        Short.red.gorgonian             Red.sea.urchin
##                   0.4064165                   0.4693606
##        California.hydrocoral       UI.branched.bryozoan
##                   0.5317557                   0.5870058
##        UI.branched.sponge White.branched.sea.cucumber
##                   0.6316958                   0.6728573
##           UI.lobed.sponge
##                   0.7081504
```

```
north_sp <- c("Slipper sea cucumber","California sea cucumber",
              "Short red gorgonian","Red sea urchin",
              "California hydrocoral","UI branched bryozoan",
              "UI branched sponge","White branched sea cucumber",
              "UI lobed sponge")
north_con <- c(0.1855598,0.3043917,0.4060153,0.4688773,0.5311454,
               0.5863474,0.6310009,0.6721305,0.7073433)
north_data <- data.frame(north_sp,north_con)
north_graph <- ggplot(north_data,
                      aes(y=north_con,x=factor(north_sp,level=north_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#ff6a6a',color='gray') +
  ggtitle("SIMPER analysis of species between MPAs and Reference sites in Northern CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=15),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
north_graph
```

SIMPER analysis of species between MPAs and Reference sites


```r
# Central

central.labels <- Central.avg[,1:4]
central.data <- Central.avg[,5:160]
set.seed(13)
central.sim <- with(central.labels, simper(central.data, Designation))
central.sim
```

```
## cumulative contributions of most influential species:
##
## $MPA_Reference
## UI.lobed.sponge.tunicate          UI.lobed.sponge    California.sea.cucumber
##              0.1278430                 0.2486069                 0.3276743
##        White.sea.urchin         Short.red.gorgonian         Purple.sea.urchin
##              0.3883098                 0.4344837                 0.4778682
##            Red.sea.star         White.plumed.anemone       California.hydrocoral
##              0.5206175                 0.5596661                 0.5978635
##      UI.branched.sponge                    Bat.star       UI.branched.bryozoan
##              0.6357187                 0.6703310                 0.7013164
```

```r
central_sp <- c("UI lobed sponge/tunicate","UI lobed sponge",
          "California sea cucumber","White sea urchin",
          "Short red gorgonian","Purple sea urchin",
          "Red sea star","White plumed anemone",
          "California hydrocoral","UI branched sponge",
          "Bat star","UI branched bryozoan")
central_con <- c(0.1278430,0.2486069,0.3276743,0.3883098,0.4344837,
```

```
                       0.4778682,0.5206175,0.5596661,0.5978635,0.6357187,
                       0.6703310,0.7013164)
central_data <- data.frame(central_sp,central_con)
central_graph <- ggplot(central_data, aes(y=central_con,x=factor(central_sp,level=central_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#76ee00',color='gray') +
  ggtitle("SIMPER analysis of species between MPAs and Reference sites in Central CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=15),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
central_graph
```


SIMPER analysis of species between MPAs and Reference sites
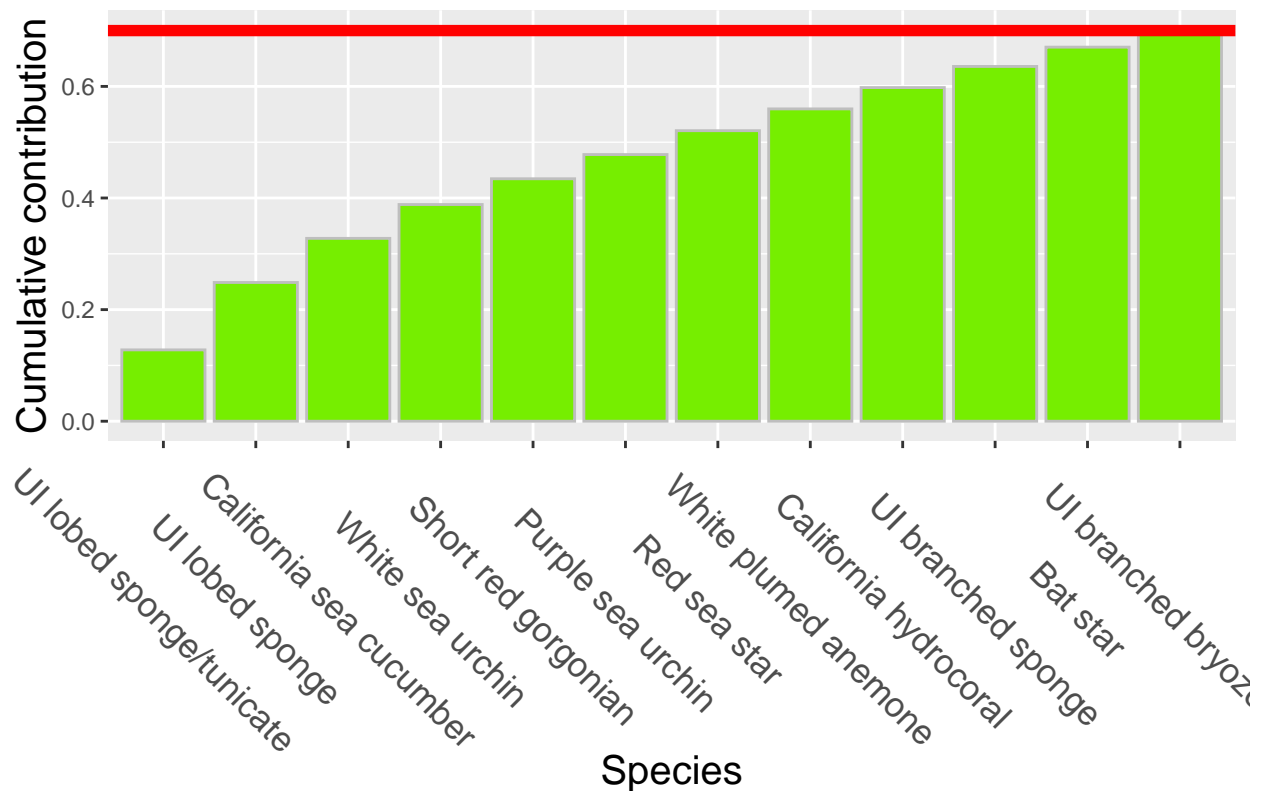
```
# South

south.labels <- South.avg[,1:4]
south.data <- South.avg[,5:160]
set.seed(13)
south.sim <- with(south.labels, simper(south.data, Designation))
south.sim

## cumulative contributions of most influential species:
##
## $MPA_Reference
##         White.sea.urchin              White.sea.pen
##                0.3150594                  0.3927712
```

```
##              Red.sea.urchin              UI.branched.bryozoan
##                   0.4616202                         0.5259156
## White.branched.sea.cucumber                  Purple.gorgonian
##                   0.5901728                         0.6422199
##       UI.laced.lobed.bryozoan                          Sea.whip
##                   0.6887477                         0.7325898
```

```r
south_sp <- c("White sea urchin","White sea pen","Red sea urchin",
              "UI branched bryozoan","White branched sea cucumber",
              "Purple gorgonian","UI laced lobed bryozoan","Sea whip")
south_con <- c(0.3150594,0.3927712,0.4616202,0.5259156,0.5901728,
               0.6422199,0.6887477,0.7325898)
south_data <- data.frame(south_sp,south_con)
south_graph <- ggplot(south_data,
                      aes(y=south_con,x=factor(south_sp,level=south_sp))) +
  geom_bar(position="dodge", stat="identity", fill='#6495ed',color='gray') +
  ggtitle("SIMPER analysis of species between MPAs and Reference sites in Southern CA") +
  geom_hline(yintercept = 0.7,color='red',size=2) +
  theme(axis.text.x = element_text(angle = 315,size=13),
        plot.title = element_text(size=15),
        axis.title = element_text(size=15)) +
  xlab("Species") +
  ylab("Cumulative contribution")
south_graph
```
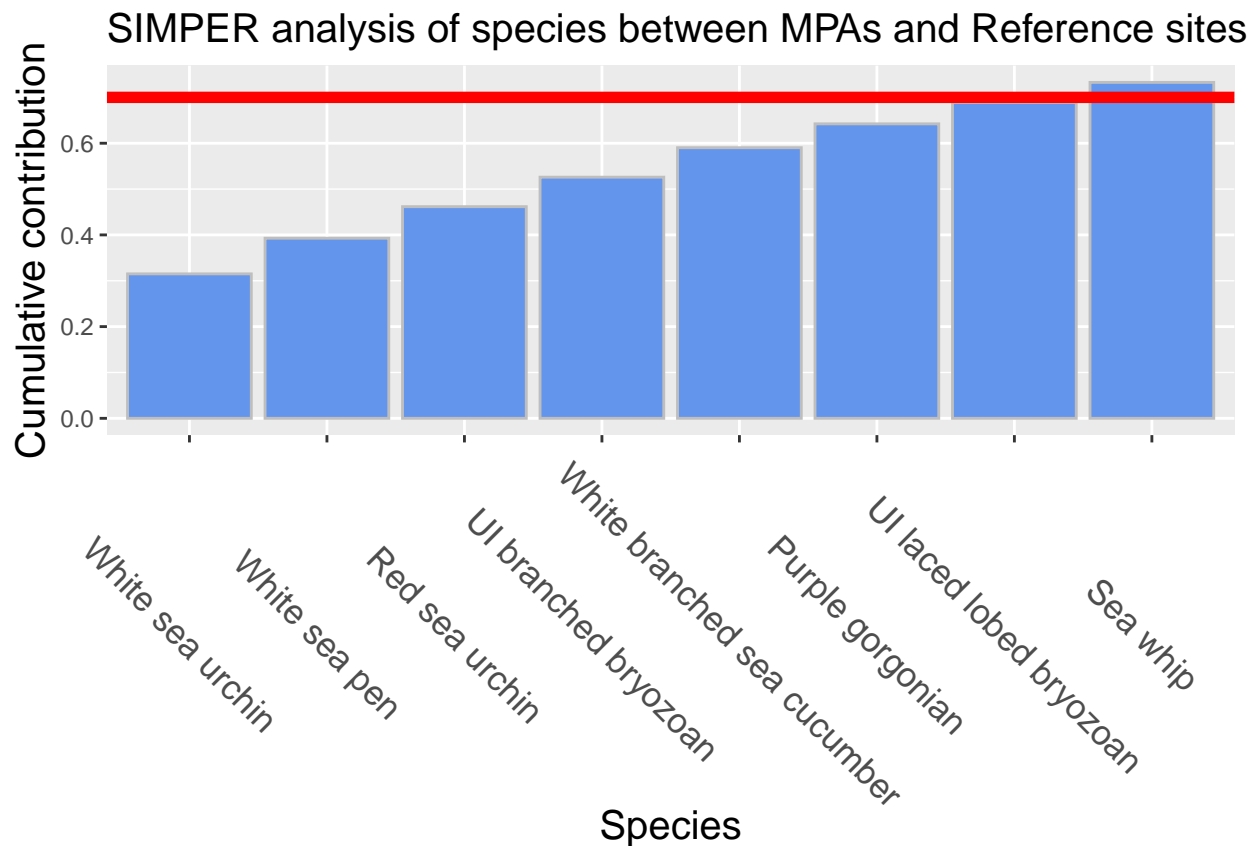

PERMANOVA

```
# PERMANOVA assumptions

# 1) Objects in the data set are exchangable under the
# null hypothesis

# 2) Exchangable objects (sites, samples, observations,
# etc.) are independent

# 3) Exchangable objects have similar multivariate
# dispension(i.e. each group has a similar degree of
# multivariate scatter)

# Basically, so long as the data is theoretically exchangable
# (one site COULD be identical to another) and the observations
# are independent, then the test meets assumptions and can be run.

# Run with Designation*Region

set.seed(13)
reg.div <- adonis2(reg.data ~ Region*Designation,
                   data = reg.labels, permutations = 999,
                   method = "bray")
reg.div
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = reg.data ~ Region * Designation, data = reg.labels, permutations = 999,
method = "bray")
## Df SumOfSqs R2 F Pr(>F)
## Region 2 2.5605 0.28157 6.0456 0.001 ***
## Designation 1 0.2378 0.02615 1.1228 0.310
## Region:Designation 2 0.3658 0.04023 0.8637 0.618
## Residual 28 5.9295 0.65205
## Total 33 9.0936 1.00000
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Run with just Designation to see if there is a significant difference

set.seed(13)
reg.div2 <- adonis2(reg.data ~ Designation,
                    data = reg.labels, permutations = 999,
                    method = "bray")
reg.div2
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = reg.data ~ Designation, data = reg.labels, permutations = 999, method =
"bray")
```

```
## Df SumOfSqs R2 F Pr(>F)
## Designation 1 0.2357 0.02592 0.8515 0.544
## Residual 32 8.8579 0.97408
## Total 33 9.0936 1.00000
```

```
# No change in significance, use the interaction analysis in final results.
```