Global ocean viral communities

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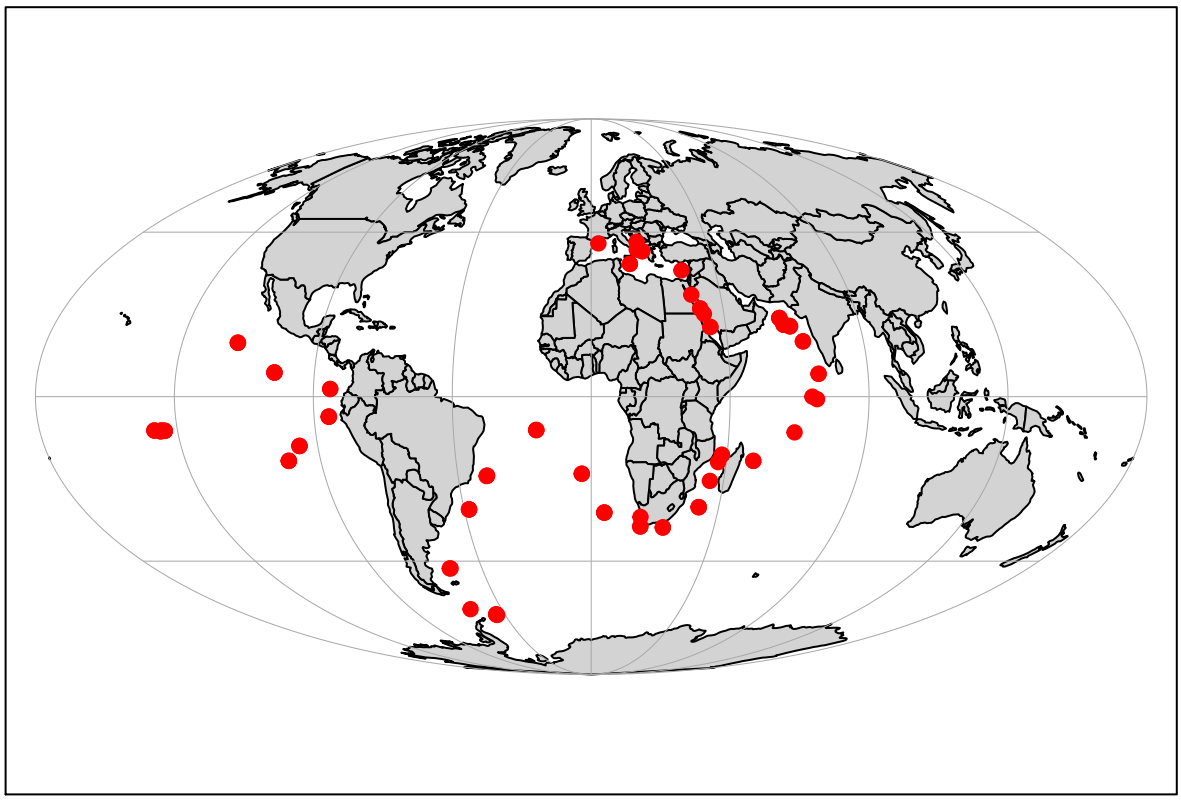
Initialize

```
rm(list=ls())

library('ggplot2')
library('vegan')
library("corrplot")
library("dplyr")
library("reshape2")
library("patchwork")
library("ggpmisc")
library("oce")

setwd("~/GitHub/QB2021_Fishman/2.Worksheets/13.TeamProject/")

df <- read.table("data/env-geo-div-class.txt", sep = "\t", header = TRUE,row.names = 1)
vcs.o <-read.csv("data/vcs.csv", row.names = 1)
vcs <- as.data.frame(t(vcs.o))

# remove outlier
vcs <- subset(vcs, ! rownames(vcs) %in% c("MSP114"))
vcs.0 <- as.data.frame(t(vcs))
df <- subset(df, ! rownames(df) %in% c("MSP114"))

# numerical features
num_cols <- unlist(lapply(df, is.numeric))

# numerical df
df.num <- df[, num_cols]

# imputed data (will show this is done below)
env.data <- read.csv("data/metadata-imputed.csv", row.names = 1)
```

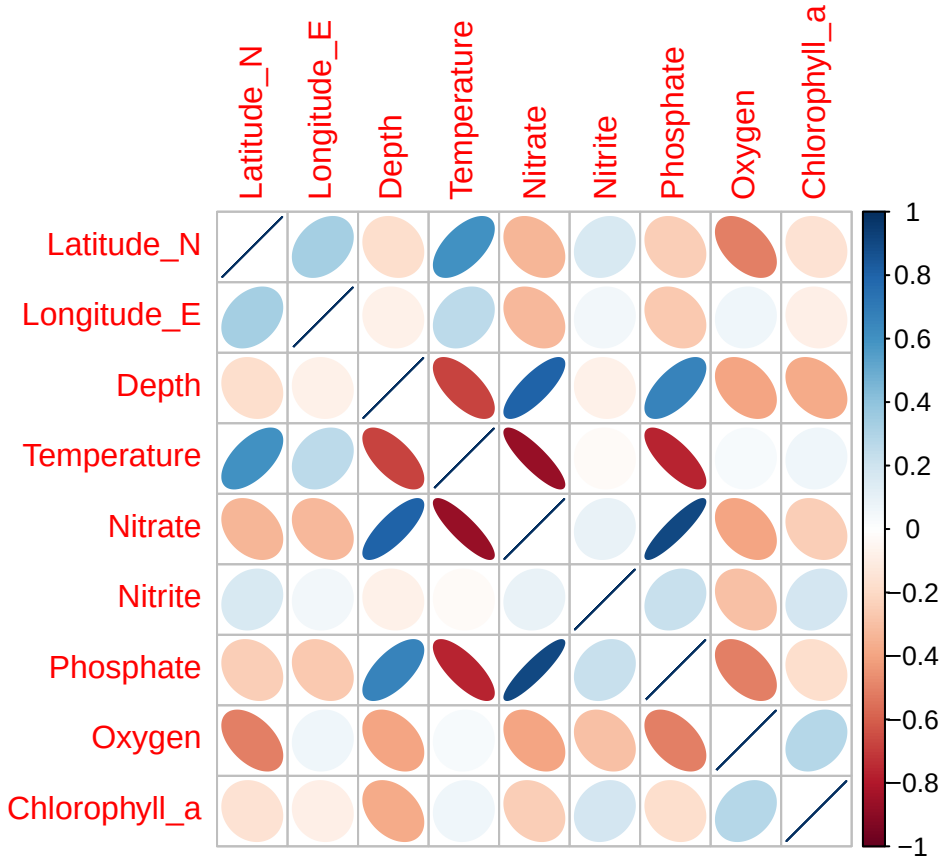
Plot of samples

```
data(coastlineWorld)
par(mar=c(1.5, 1.5, 0.5, 0.5))
mapPlot(coastlineWorld, col="lightgrey")
mapPoints(df.num$Longitude_E, df.num$Latitude_N, pch=19, col="red")
```



Environmental info

```
cor1 <- cor(df.num[,1:9], use="complete.obs")
corrplot(cor1, method = "ellipse")
```



Alpha diversity metrics

```
# richness
S.obs <- function( x = "" ){
  rowSums( x > 0 ) * 1
}
# evenness
Evar <- function(x){
  x <- as.vector(x[x > 0])
  1 - (2/pi)*atan(var(log(x)))
}
# ShannonH
ShanH <- function(x = ""){
  H = 0
  for (n_i in x){
    if(n_i > 0) {
      p = n_i / sum(x)
      H = H - p*log(p)
    }
  }
  return(H)
}
# calculating
vcs.S <- c()
vcs.E <- c()
```

```
vcs.H <- c()
for (i in 1:90) {
  vcs.S[i] = S.obs(vcs[i,])
  vcs.E[i] = Evar(vcs[i,])
  vcs.H[i] = ShanH(vcs[i,])
}
vcs.div <- cbind(vcs.S, vcs.E, vcs.H)
```

Stacked barplots with different grouping strategies

```
#convert to relative abundance table
vcs.rel <- vcs/rowSums(vcs)
# top20 VCs
top <- names(head(sort(colSums(vcs),decreasing = T),20))
top <- as.vector(top)
# recreate a table with the rest VCs classified into "Others"
vcs.rel2 <- vcs.rel
colnames(vcs.rel2)[!colnames(vcs.rel2)%in%top] <- "Others"
others <- rowSums(cbind(vcs.rel2[colnames(vcs.rel2)=="Others"]))
vcs.top <- cbind(vcs.rel2[colnames(vcs.rel2)!="Others"],others)
vcs.top <- vcs.top[,order(colSums(vcs.top))] # ranking
#load group data
env.class.geo.div <- df
vcs.top2 <- as.data.frame(cbind(vcs.top,env.class.geo.div$Layer,env.class.geo.div$Region))
vcs.top2$sample <- rownames(vcs.top2)
vcs.top20 <- melt(vcs.top2,ID="names")
```

```
## Using env.class.geo.div$Layer, env.class.geo.div$Region, sample as id variables
```

```
colnames(vcs.top20)[names(vcs.top20)=="variable"]<-"Taxa"
vcs.top20$`env.class.geo.div$Layer` <- factor(vcs.top20$`env.class.geo.div$Layer`, levels = c("SUR","DCM
# group by layer(zonation)
vcs.top.layer <- aggregate(vcs.top2[,1:21], by = list(Layer = vcs.top2$`env.class.geo.div$Layer`),FUN =
vcs.top.layer$sample <- rownames(vcs.top.layer)
vcs.group.layer <- melt(vcs.top.layer, ID="names")
```

```
## Using Layer, sample as id variables
```

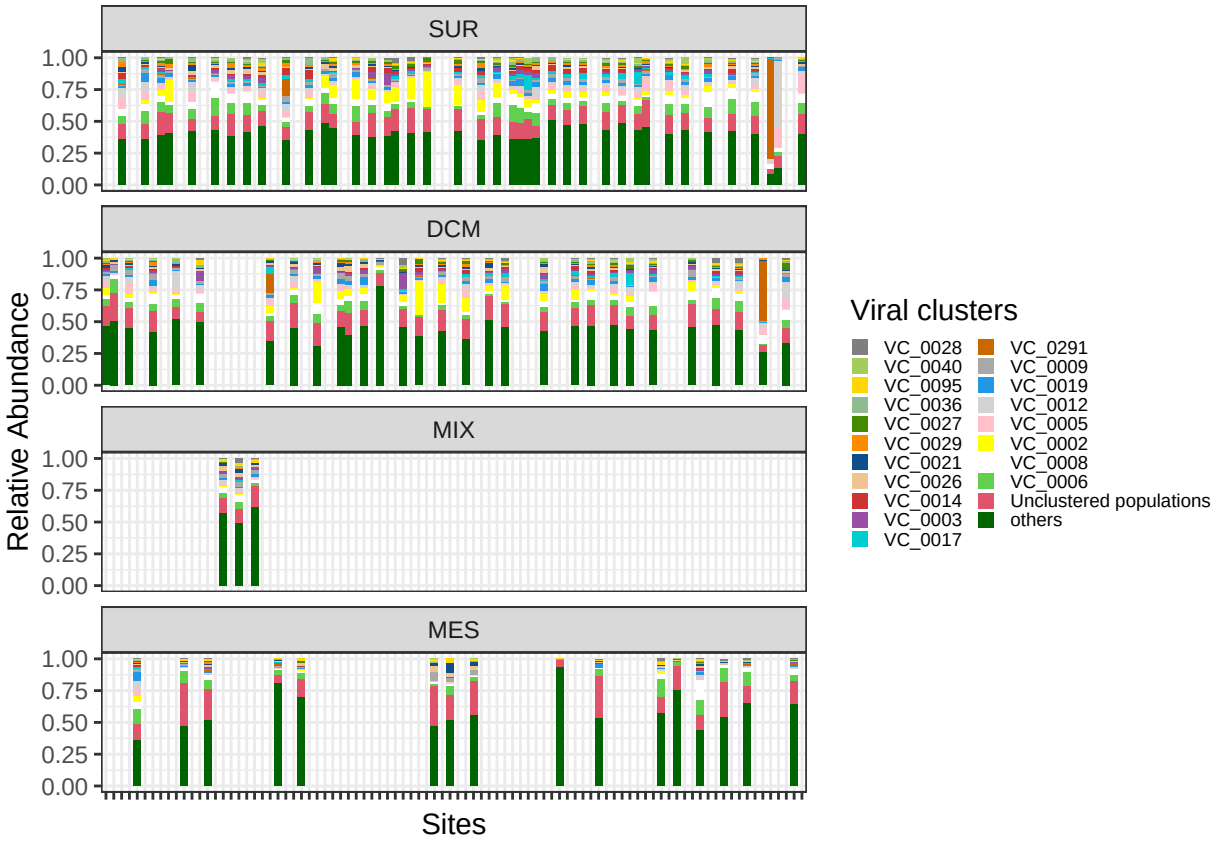
```
colnames(vcs.group.layer)[names(vcs.group.layer)=="variable"]<-"Taxa"
vcs.group.layer$Layer <- factor(vcs.group.layer$Layer, levels = c("SUR","DCM","MIX","MES")) #set order
# group by region
vcs.top.region <- aggregate(vcs.top2[,1:21], by = list(Layer = vcs.top2$`env.class.geo.div$Region`),FUN
vcs.top.region$sample <- rownames(vcs.top.region)
vcs.group.region <- melt(vcs.top.region, ID="names")
```

```
## Using Layer, sample as id variables
```

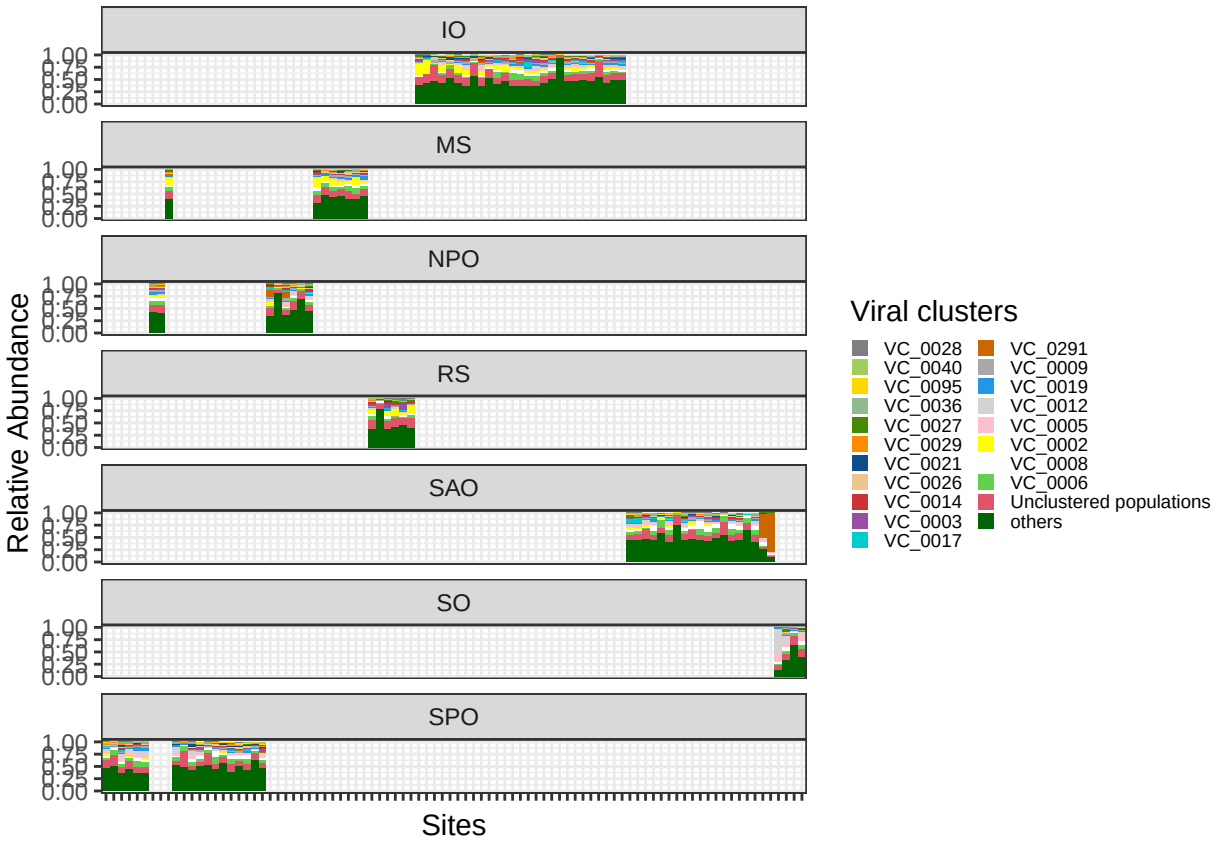
```
colnames(vcs.group.region)[names(vcs.group.region)=="variable"]<-"Taxa"
```

```
# plotting stacked barplot for top 20 viral clusters
colors<-c("grey50","darkolivegreen3","gold","darkseagreen","chartreuse4","darkorange","dodgerblue4","bu
# water depth
ggplot(vcs.top20,aes(x = sample, y = value, fill = Taxa))+
  geom_bar(position = "fill", stat = "identity", width = 1)+
  theme_bw()+
```

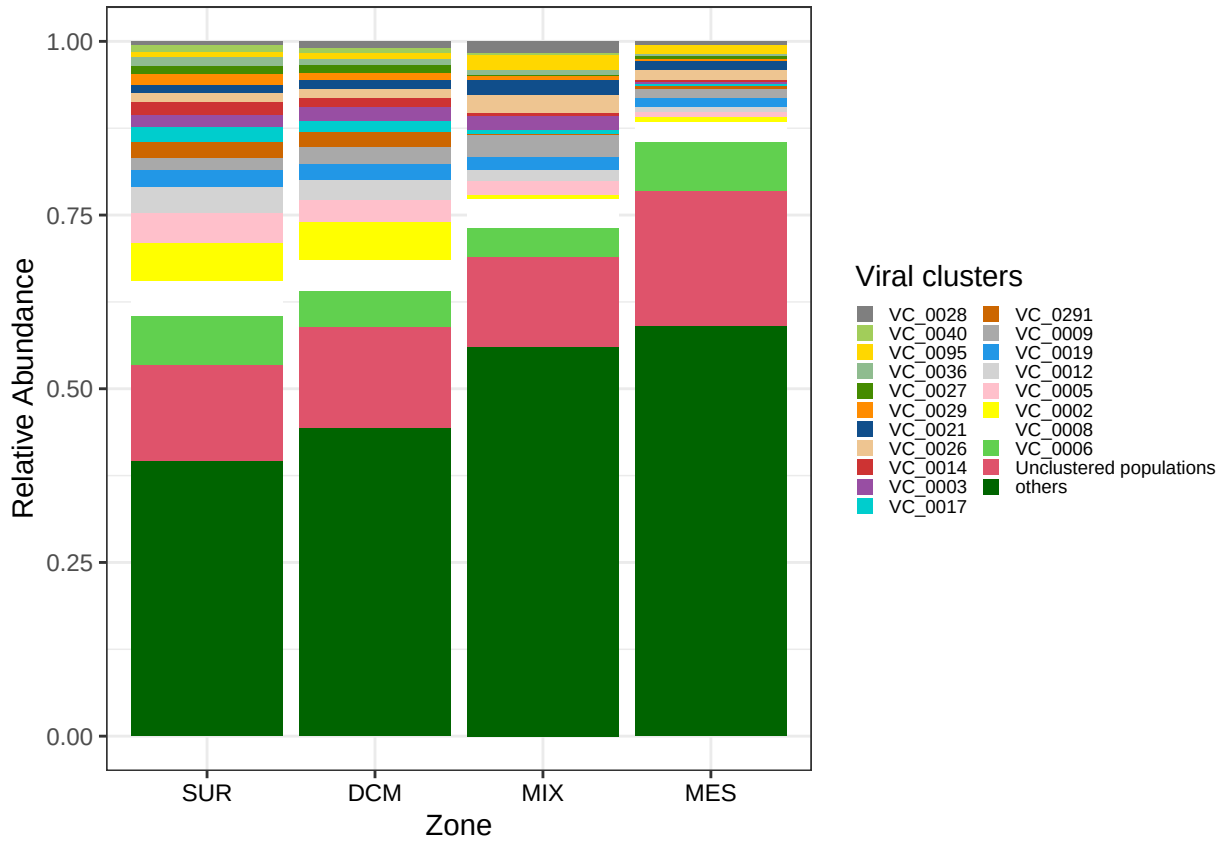
```
  scale_fill_manual(values=colors)+
  facet_wrap(~`env.class.geo.div$Layer`,nrow = 4)+
  labs(x = "Sites",y = "Relative Abundance", fill = "Viral clusters")+
  theme(axis.text.x = element_text(size = 0, color = "transparent"),
        legend.text = element_text(size = 7))+
  guides(fill = guide_legend(keywidth = 0.5, keyheight = 0.5))
```



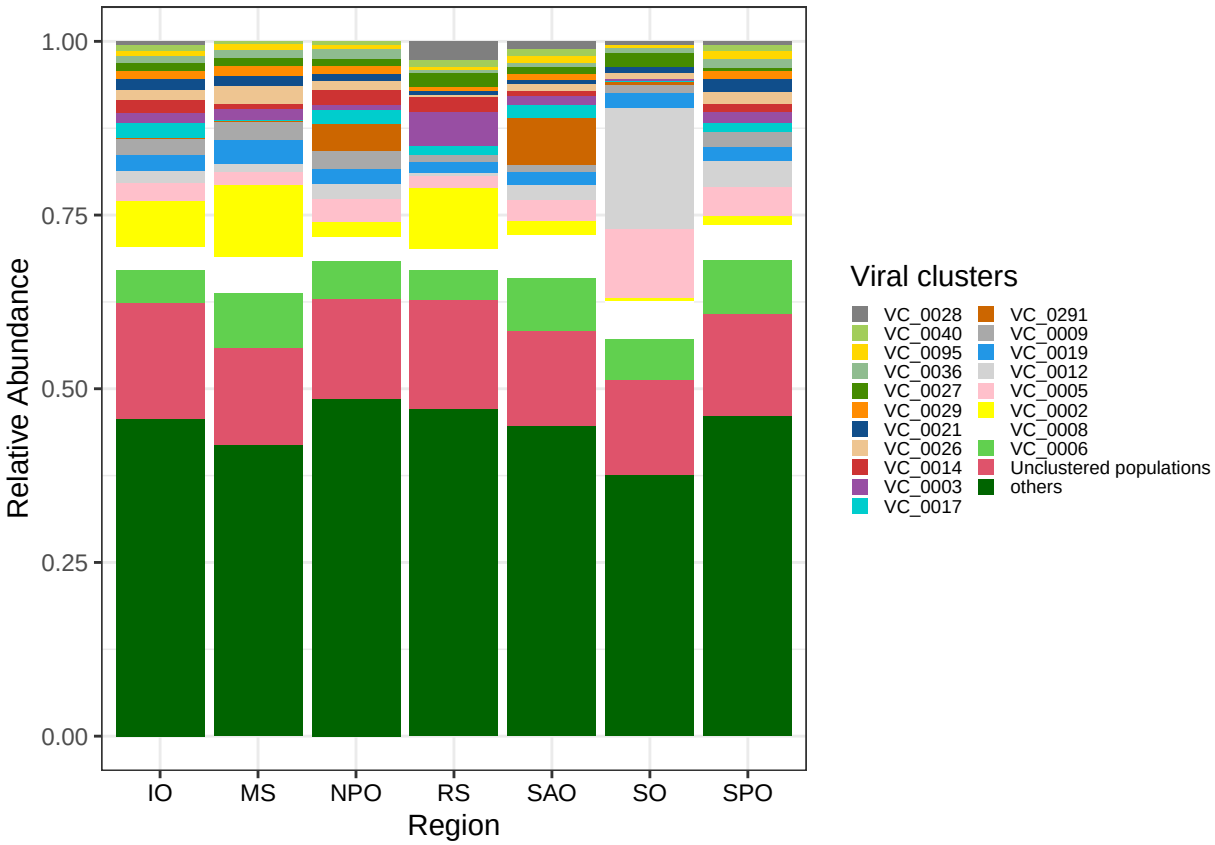

```
# region
ggplot(vcs.top20,aes(x = sample, y = value, fill = Taxa))+
  geom_bar(position = "fill", stat = "identity", width = 1)+
  theme_bw()+
  scale_fill_manual(values=colors)+
  facet_wrap(~`env.class.geo.div$Region`,nrow = 7)+
  labs(x = "Sites",y = "Relative Abundance", fill = "Viral clusters")+
  theme(axis.text.x = element_text(size = 0, color = "transparent"),
        legend.text = element_text(size = 7))+
  guides(fill = guide_legend(keywidth = 0.5, keyheight = 0.5))
```




```
# group by water layer
ggplot(vcs.group.layer,aes(x = Layer, y = value, fill = Taxa))+
  geom_bar(position = "fill", stat = "identity")+
  theme_bw()+
  scale_fill_manual(values=colors)+
  labs(x = "Zone",y = "Relative Abundance", fill = "Viral clusters")+
  theme(axis.text.x = element_text(size = 9, color = "black"),
        legend.text = element_text(size = 7))+
  guides(fill = guide_legend(keywidth = 0.5, keyheight = 0.5))
```




```
# group by region
ggplot(vcs.group.region,aes(x = Layer, y = value, fill = Taxa))+
  geom_bar(position = "fill", stat = "identity")+
  theme_bw()+
  scale_fill_manual(values=colors)+
  labs(x = "Region",y = "Relative Abundance", fill = "Viral clusters")+
  theme(axis.text.x = element_text(size = 9, color = "black"),
        legend.text = element_text(size = 7))+
  guides(fill = guide_legend(keywidth = 0.5, keyheight = 0.5))
```



Seeking relationships with diversity and water depth

```
# linear regression
summary(lm(env.class.geo.div$ShannonH ~ env.class.geo.div$Depth))
```

```
##
## Call:
## lm(formula = env.class.geo.div$ShannonH ~ env.class.geo.div$Depth)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.64947 -0.10602  0.09198  0.31019  0.86107
##
## Coefficients:
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                3.8305984  0.0673619  56.866  < 2e-16 ***
## env.class.geo.div$Depth -0.0008597  0.0002332  -3.687 0.000392 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5566 on 88 degrees of freedom
## Multiple R-squared:  0.1338, Adjusted R-squared:  0.1239
## F-statistic: 13.59 on 1 and 88 DF,  p-value: 0.0003923
```

```
summary(lm(env.class.geo.div$Richness ~ env.class.geo.div$Depth))
```

```
##
## Call:
## lm(formula = env.class.geo.div$Richness ~ env.class.geo.div$Depth)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -160.566  -37.782   -2.865   38.934  186.807
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)              222.21629    8.47596  26.217  < 2e-16 ***
## env.class.geo.div$Depth  -0.13003    0.02934  -4.431 2.69e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 70.04 on 88 degrees of freedom
## Multiple R-squared:  0.1824, Adjusted R-squared:  0.1731
## F-statistic: 19.64 on 1 and 88 DF,  p-value: 2.687e-05
```

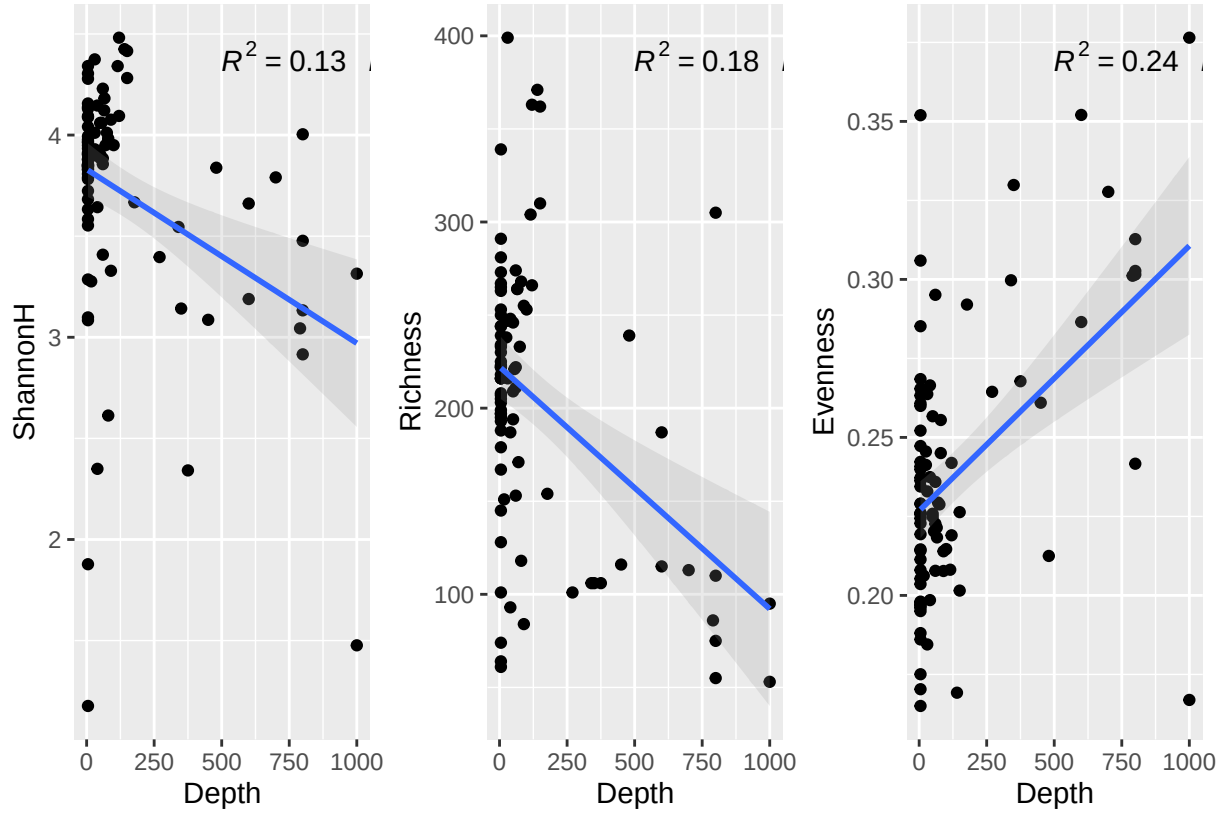
```
summary(lm(env.class.geo.div$Evenness ~ env.class.geo.div$Depth))
```

```
##
## Call:
## lm(formula = env.class.geo.div$Evenness ~ env.class.geo.div$Depth)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.143713 -0.021929 -0.001325  0.016281  0.124779
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)              2.268e-01  4.568e-03  49.639  < 2e-16 ***
## env.class.geo.div$Depth 8.388e-05  1.581e-05   5.304 8.36e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03775 on 88 degrees of freedom
## Multiple R-squared:  0.2422, Adjusted R-squared:  0.2336
## F-statistic: 28.13 on 1 and 88 DF,  p-value: 8.357e-07
```

```
HD <- ggplot(env.class.geo.div, aes(x = Depth, y = ShannonH))+
  geom_point()+
  geom_smooth(method = "lm", alpha = 0.2)+
  stat_poly_eq(formula = y ~ x, aes(label = paste("~~~~~~~~~~~~~~~",..rr.label..,..p.value.label.., sep
SD <- ggplot(env.class.geo.div, aes(x = Depth, y = Richness))+
  geom_point()+
  geom_smooth(method = "lm", alpha = 0.2)+
  stat_poly_eq(formula = y ~ x, aes(label = paste("~~~~~~~~~~~~~~~",..rr.label..,..p.value.label.., sep
ED <- ggplot(env.class.geo.div, aes(x = Depth, y = Evenness))+
  geom_point()+
  geom_smooth(method = "lm", alpha = 0.2)+
  stat_poly_eq(formula = y ~ x, aes(label = paste("~~~~~~~~~~~~~~~",..rr.label..,..p.value.label.., sep
HD + SD + ED
```

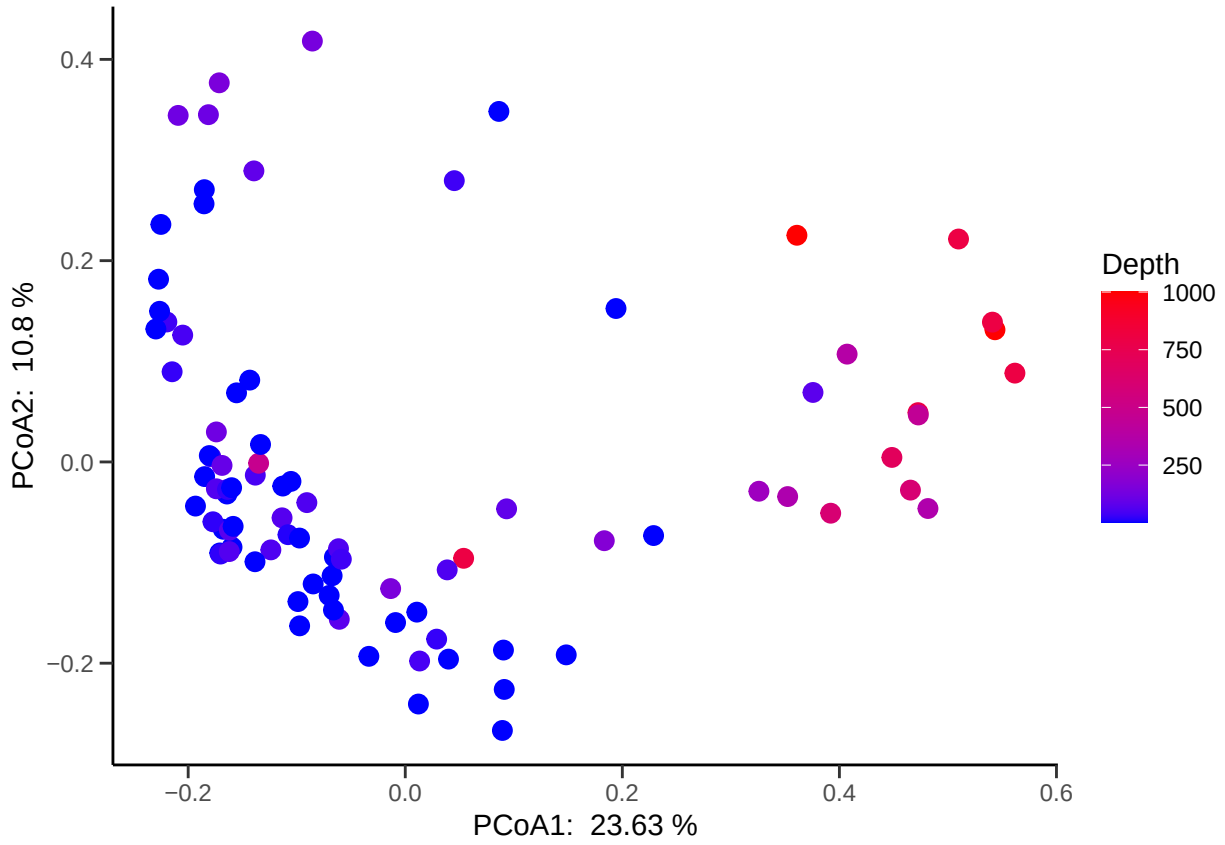
```
## `geom_smooth()` using formula 'y ~ x'
## `geom_smooth()` using formula 'y ~ x'
```

```
## `geom_smooth()` using formula 'y ~ x'
```



PCoA

```
#pcoa with depth gradient
bray.vcs <- vegdist(vcs, method = "bray")
pcoa <- cmdscale(bray.vcs, k =3, eig = TRUE)
pcoa_eig <- (pcoa$eig)[1:2]/sum(pcoa$eig)
site.pcoa <- data.frame(pcoa$points)[1:2]
site.pcoa <- cbind(site.pcoa, df$Depth)
colnames(site.pcoa) <- c("PCoA1","PCoA2","Depth")

ggplot(site.pcoa, aes(PCoA1, PCoA2))+
  geom_point(aes(color = Depth), size = 3)+
  scale_color_gradient(low = "blue", high = "red")+
  labs(x=paste("PCoA1: ", round(100*pcoa_eig[1],2),"%"), y = paste("PCoA2: ", round(100*pcoa_eig[2],2),
   theme(panel.grid.major = element_blank(),
         panel.grid.minor = element_blank(),
         panel.background = element_blank(),
         axis.line = element_line(colour = "black"),
         axis.ticks.length = unit(5,"pt"),)
```



Imputation

```
require(mice)
```

```
## Loading required package: mice
```

```
## Warning: package 'mice' was built under R version 4.0.5
```

```
##
## Attaching package: 'mice'
```

```
## The following object is masked from 'package:stats':
##
##     filter
```

```
## The following objects are masked from 'package:base':
##
##     cbind, rbind
```

```
require(VIM)
```

```
## Loading required package: VIM
```

```
## Warning: package 'VIM' was built under R version 4.0.5
```

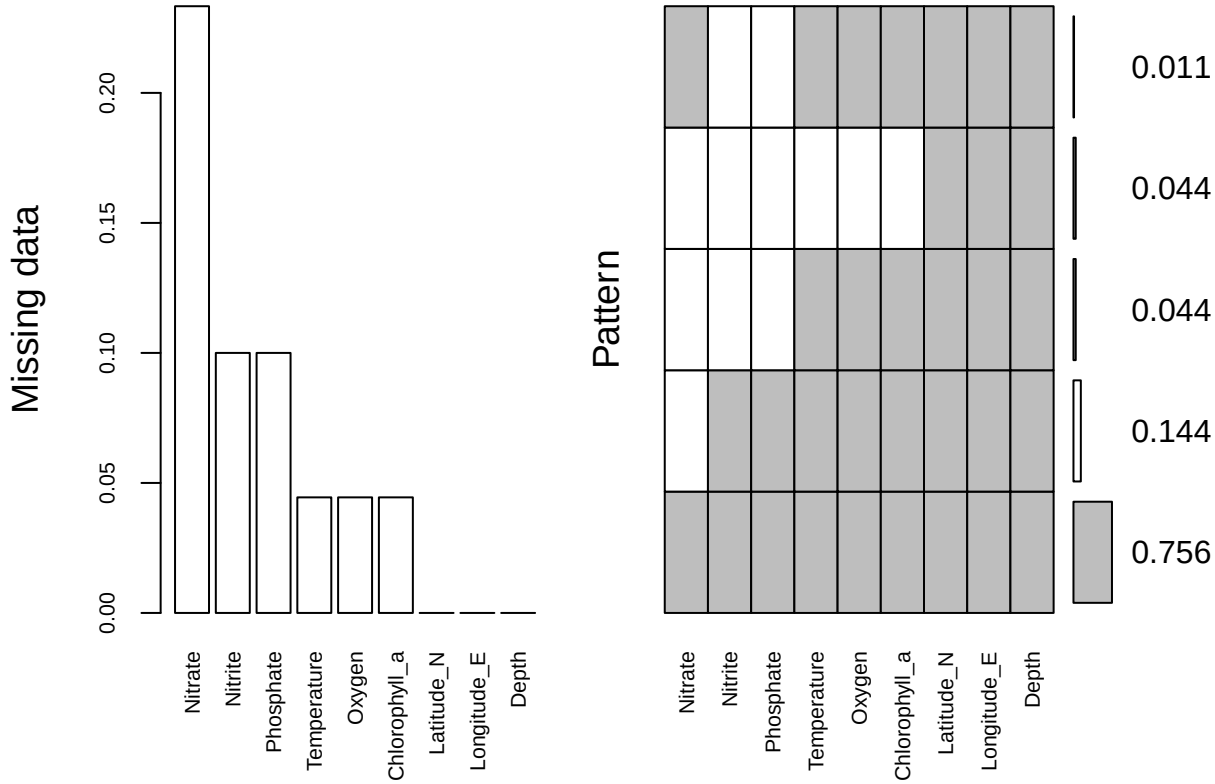
```
## Loading required package: colorspace
```

```
## Warning: package 'colorspace' was built under R version 4.0.5
```

```
## Loading required package: grid
```

```
## VIM is ready to use.

## Suggestions and bug-reports can be submitted at: https://github.com/statistikat/VIM/issues

##
## Attaching package: 'VIM'

## The following object is masked from 'package:datasets':
##
##     sleep
```

```
df.num1 <- subset(df.num, ! rownames(df.num) %in% c("MSP114"))
df.sparse <- subset(df.num1, select=-c(Richness,Evenness,ShannonH))

mice_plot <- aggr(df.sparse, col=c('grey','white'),
                  numbers=TRUE, sortVars=TRUE,
                  labels=names(df.sparse), cex.axis=.7,
                  gap=3, ylab=c("Missing data","Pattern"))
```




```
##
##  Variables sorted by number of missings:
##       Variable      Count
##        Nitrate 0.23333333
##        Nitrite 0.10000000
##      Phosphate 0.10000000
##    Temperature 0.04444444
##         Oxygen 0.04444444
##  Chlorophyll_a 0.04444444
```

```
##     Latitude_N 0.00000000
##    Longitude_E 0.00000000
##          Depth 0.00000000
```

```
df.sparse <- subset(df.sparse, select=-c(Nitrate))
imputed_Data <- mice(df.sparse, m=1, maxit = 100, method = 'pmm')
summary(imputed_Data)
```

```
completeData <- complete(imputed_Data,1)
# write.csv(completeData, file = "data/metadata-imputed.csv")
```

dbRDA

```
dbrda1 <- dbrda(bray.vcs ~ ., as.data.frame(env.data))
dbrda0 <- dbrda(bray.vcs ~ 1, as.data.frame(env.data))

dbrda <- ordiR2step(dbrda0, scope=formula(dbrda1), perm.max=200)
```

```
## Step: R2.adj= 0
## Call: bray.vcs ~ 1
##
##                 R2.adjusted
## <All variables> 0.302408505
## + Depth         0.141441726
## + Temperature   0.119685498
## + Phosphate     0.106243382
## + Oxygen        0.046884733
## + Chlorophyll_a 0.043447490
## + Longitude_E   0.042543083
## + Latitude_N    0.037819787
## + Nitrite       0.003117142
## <none>          0.000000000
##
##         Df    AIC      F Pr(>F)
## + Depth  1 255.49 15.662  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1414417
## Call: bray.vcs ~ Depth
##
##                 R2.adjusted
## <All variables>   0.3024085
## + Longitude_E     0.1858212
## + Temperature     0.1790677
## + Latitude_N      0.1742009
## + Phosphate       0.1714619
## + Oxygen          0.1691391
## + Chlorophyll_a   0.1585865
## + Nitrite         0.1472391
## <none>            0.1414417
##
##               Df    AIC      F Pr(>F)
## + Longitude_E  1 251.68 5.7967  0.002 **
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1858212
## Call: bray.vcs ~ Depth + Longitude_E
##
##                 R2.adjusted
## <All variables>   0.3024085
## + Temperature     0.2246926
## + Phosphate       0.2178631
## + Oxygen          0.2156143
## + Latitude_N      0.2111922
## + Chlorophyll_a   0.1999347
## + Nitrite         0.1922520
## <none>            0.1858212
##
##               Df    AIC      F Pr(>F)
## + Temperature  1 248.24 5.3619  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2246926
## Call: bray.vcs ~ Depth + Longitude_E + Temperature
##
##                 R2.adjusted
## <All variables>   0.3024085
## + Oxygen          0.2616048
## + Latitude_N      0.2501210
## + Chlorophyll_a   0.2437890
## + Phosphate       0.2433198
## + Nitrite         0.2305861
## <none>            0.2246926
##
##          Df   AIC      F Pr(>F)
## + Oxygen  1 244.8 5.2991  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2616048
## Call: bray.vcs ~ Depth + Longitude_E + Temperature + Oxygen
##
##                 R2.adjusted
## <All variables>   0.3024085
## + Latitude_N      0.2783222
## + Chlorophyll_a   0.2782592
## + Phosphate       0.2683694
## + Nitrite         0.2641635
## <none>            0.2616048
##
##              Df    AIC     F Pr(>F)
## + Latitude_N  1 243.67 2.969  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Step: R2.adj= 0.2783222
## Call: bray.vcs ~ Depth + Longitude_E + Temperature + Oxygen + Latitude_N
##
##                 R2.adjusted
## <All variables>   0.3024085
## + Chlorophyll_a   0.2958620
## + Phosphate       0.2847003
## + Nitrite         0.2806636
## <none>            0.2783222
##
##                 Df    AIC      F Pr(>F)
## + Chlorophyll_a  1 242.38 3.0924  0.004 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.295862
## Call: bray.vcs ~ Depth + Longitude_E + Temperature + Oxygen + Latitude_N +      Chlorophyll_a
##
##                 R2.adjusted
## + Phosphate       0.3024665
## <All variables>   0.3024085
## + Nitrite         0.2964497
## <none>            0.2958620
```

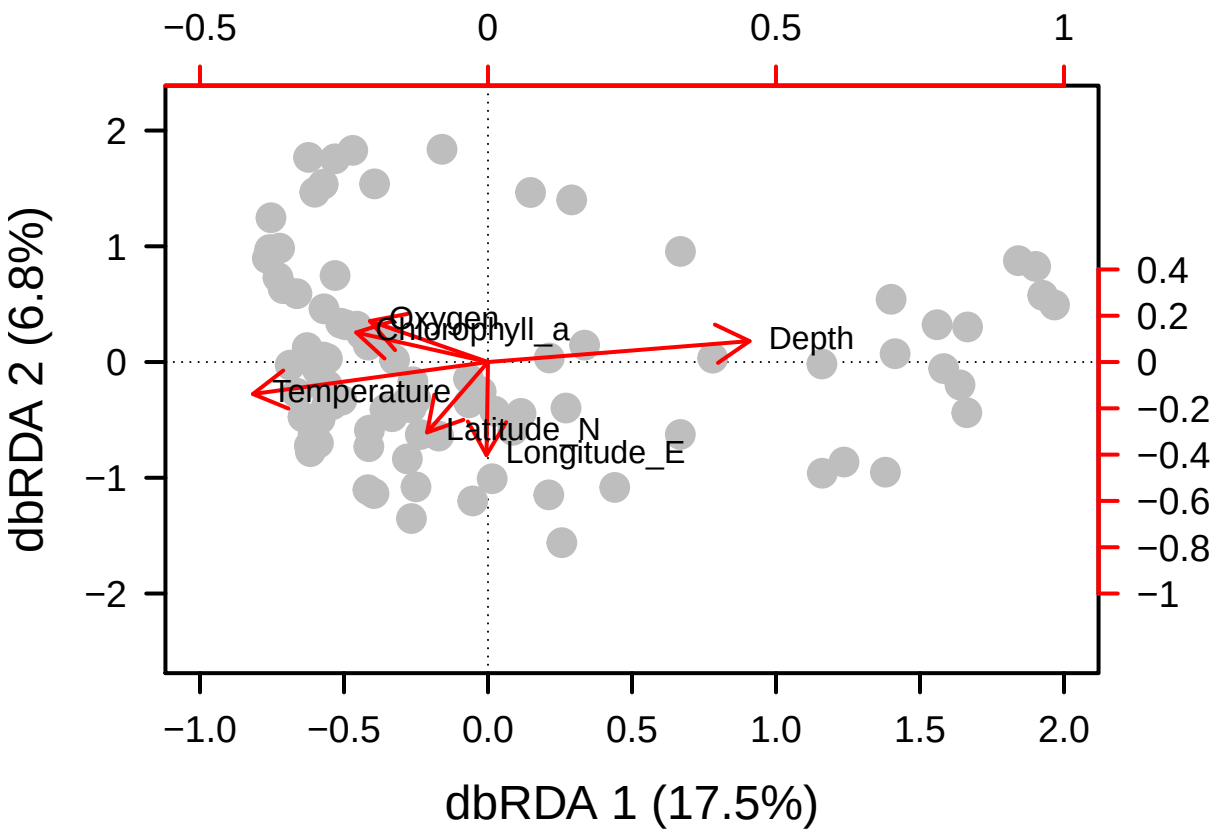
```
permutest(dbrda, permutations=999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = bray.vcs ~ Depth + Longitude_E + Temperature +
## Oxygen + Latitude_N + Chlorophyll_a, data = as.data.frame(env.data))
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     6  6.6874 7.2326  0.001 ***
## Residual 83 12.7905
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
envfit(dbrda, env.data[,c(4,6,7)], perm=999)
```

```
##
## ***VECTORS
##
##               dbRDA1   dbRDA2     r2 Pr(>r)
## Temperature -0.93932 -0.34304 0.5741  0.001 ***
## Phosphate    0.97569  0.21914 0.4749  0.001 ***
## Oxygen      -0.77516  0.63177 0.2075  0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```
dbrda.explainvar1 <- round(dbrda$CCA$eig[1]/sum(c(dbrda$CCA$eig, dbrda$CA$eig)),3) * 100
dbrda.explainvar2 <- round(dbrda$CCA$eig[2]/sum(c(dbrda$CCA$eig, dbrda$CA$eig)),3) * 100


vals <- scores(dbrda, display="wa")
par(mar=c(5,4,2,4) + 0.1)
plot(vals,
     xlim=c(-1, 2), ylim=c(-2.5, 2.2),
     xlab=paste0("dbRDA 1 (", dbrda.explainvar1, "%)"),
     ylab=paste0("dbRDA 2 (", dbrda.explainvar2, "%)"),
     pch=16, cex=2.0, type="n", cex.lab=1.5, cex.axis=1.2, axes=F
)
axis(side=1, labels=T, lwd.ticks=2, cex.axis=1.2, las=1)
axis(side=2, labels=T, lwd.ticks=2, cex.axis=1.2, las=1)
abline(h=0, v=0, lty=3)
box(lwd=2)

points(vals, pch=19, cex=2, bg="grey", col="grey")

vectors <- scores(dbrda, display = "bp")
arrows(0, 0, vectors[,1], vectors[,2], lwd=2, lty=1, length = 0.2, col = "red")
text(vectors[,1], vectors[,2], pos=4, labels=row.names(vectors))
axis(side=3, lwd.ticks = 2, cex.axis=1.2, las=1, col="red", lwd=2.2,
     at = pretty(range(vectors[,1]))*2, labels = pretty(range(vectors[,1])))
axis(side=4, lwd.ticks = 2, cex.axis=1.2, las=1, col="red", lwd=2.2,
     at = pretty(range(vectors[,2]))*2, labels = pretty(range(vectors[,2])))
```



Remove deepest samples

```
vcs.shal <- subset(vcs, df$Layer!="MES")
env.shal <- subset(env.data, df$Layer!="MES")
layers <- subset(df, Layer!="MES", select="Layer")

vcs.shal.rel <- vcs.shal/rowSums(vcs.shal)
vcs.shal.db <- vegdist(vcs.shal.rel, method="bray")
```

Redo ordination without `MES`

```
vcs.shal.pcoa <- cmdscale(vcs.shal.db, eig=T, k=3)

dbrda1.shal <- dbrda(vcs.shal.db ~ ., as.data.frame(env.shal))
dbrda0.shal <- dbrda(vcs.shal.db ~ 1, as.data.frame(env.shal))

dbrda.shal <- ordiR2step(dbrda0.shal, scope=formula(dbrda1.shal), perm.max=200)
```

```
## Step: R2.adj= 0
## Call: vcs.shal.db ~ 1
##
##                 R2.adjusted
## <All variables> 0.233382428
## + Latitude_N    0.068289468
## + Temperature   0.064867149
```

```
## + Longitude_E   0.058183387
## + Depth         0.039783583
## + Oxygen        0.037431878
## + Phosphate     0.035155619
## + Chlorophyll_a 0.020722010
## + Nitrite       0.003067425
## <none>          0.000000000
##
##              Df    AIC      F Pr(>F)
## + Latitude_N  1 169.44 6.3505  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.06828947
## Call: vcs.shal.db ~ Latitude_N
##
##                 R2.adjusted
## <All variables>  0.23338243
## + Temperature    0.12017417
## + Depth          0.10864227
## + Longitude_E    0.10722950
## + Phosphate      0.09186753
## + Oxygen         0.08604975
## + Chlorophyll_a  0.08539670
## + Nitrite        0.07174203
## <none>           0.06828947
##
##               Df    AIC     F Pr(>F)
## + Temperature  1 166.16 5.246  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1201742
## Call: vcs.shal.db ~ Latitude_N + Temperature
##
##                 R2.adjusted
## <All variables>   0.2333824
## + Depth           0.1627800
## + Longitude_E     0.1592136
## + Chlorophyll_a   0.1367697
## + Oxygen          0.1327408
## + Phosphate       0.1275494
## + Nitrite         0.1243791
## <none>            0.1201742
##
##         Df    AIC      F Pr(>F)
## + Depth  1 163.44 4.6132  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.16278
## Call: vcs.shal.db ~ Latitude_N + Temperature + Depth
##
##                 R2.adjusted
```

```
## <All variables>   0.2333824
## + Longitude_E     0.1980321
## + Chlorophyll_a   0.1809458
## + Phosphate       0.1712814
## + Oxygen          0.1697774
## + Nitrite         0.1672336
## <none>            0.1627800
##
##               Df    AIC     F Pr(>F)
## + Longitude_E  1 161.19 4.077  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1980321
## Call: vcs.shal.db ~ Latitude_N + Temperature + Depth + Longitude_E
##
##                 R2.adjusted
## <All variables>   0.2333824
## + Chlorophyll_a   0.2172276
## + Oxygen          0.2070224
## + Phosphate       0.2040181
## + Nitrite         0.2037865
## <none>            0.1980321
##
##                 Df    AIC     F Pr(>F)
## + Chlorophyll_a  1 160.32 2.692  0.006 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2172276
## Call: vcs.shal.db ~ Latitude_N + Temperature + Depth + Longitude_E +      Chlorophyll_a
##
##                 R2.adjusted
## <All variables>   0.2333824
## + Oxygen          0.2257895
## + Phosphate       0.2230164
## + Nitrite         0.2183960
## <none>            0.2172276
##
##          Df    AIC     F Pr(>F)
## + Oxygen  1 160.41 1.752  0.034 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2257895
## Call: vcs.shal.db ~ Latitude_N + Temperature + Depth + Longitude_E +      Chlorophyll_a + Oxygen
##
##                 R2.adjusted
## <All variables>   0.2333824
## + Phosphate       0.2327015
## + Nitrite         0.2261523
## <none>            0.2257895
##
##             Df    AIC      F Pr(>F)
```

```
## + Phosphate  1 160.63 1.6036  0.052 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(dbrda.shal, permutations=999)
```

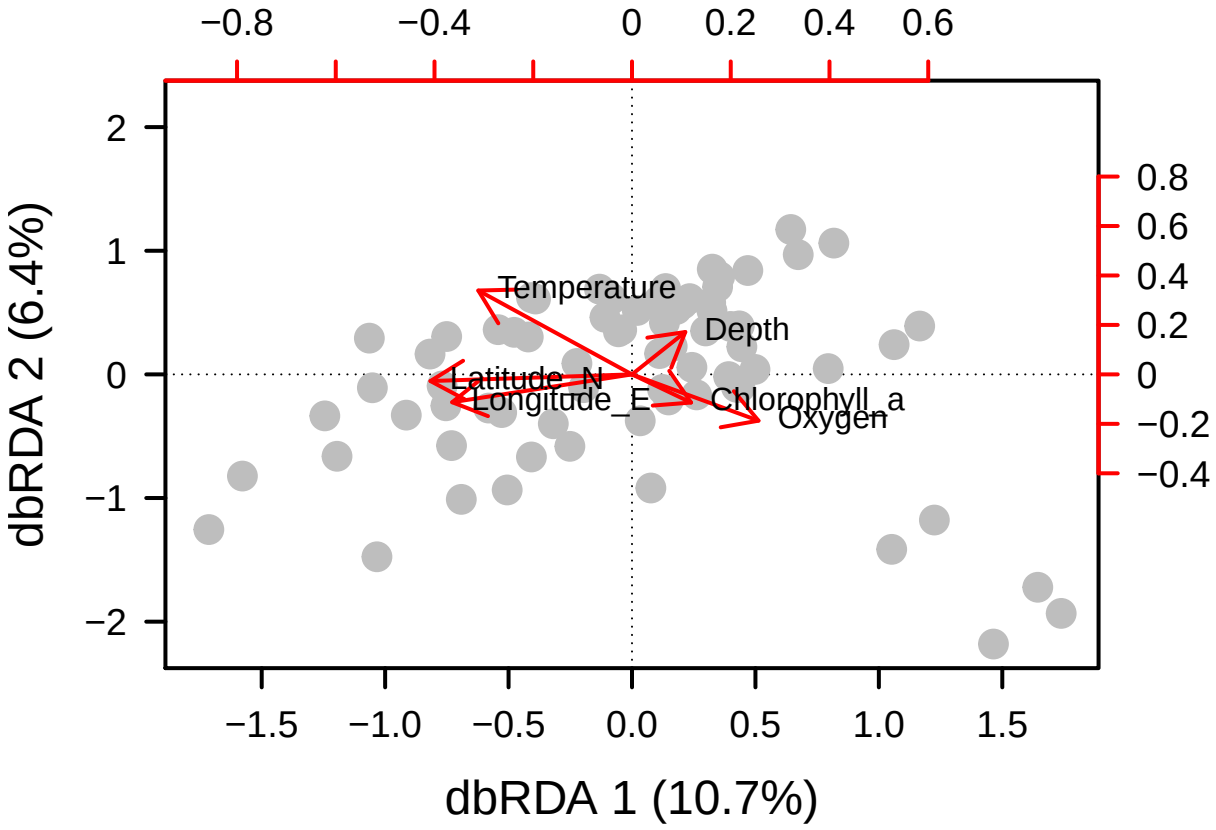
```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = vcs.shal.db ~ Latitude_N + Temperature + Depth +
## Longitude_E + Chlorophyll_a + Oxygen, data = as.data.frame(env.shal))
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     6  2.9858 4.5483  0.001 ***
## Residual 67  7.3307
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
envfit(dbrda.shal, env.shal, perm=999)
```

```
##
## ***VECTORS
##
##                 dbRDA1   dbRDA2     r2 Pr(>r)
## Latitude_N    -0.99892 -0.04636 0.5087  0.001 ***
## Longitude_E   -0.96237 -0.27174 0.4372  0.001 ***
## Depth          0.54085  0.84112 0.1198  0.013 *
## Temperature   -0.69041  0.72342 0.6443  0.001 ***
## Nitrite        0.58277 -0.81263 0.0168  0.550
## Phosphate      0.78188 -0.62342 0.3113  0.001 ***
## Oxygen         0.81434 -0.58039 0.3082  0.001 ***
## Chlorophyll_a  0.73604 -0.67694 0.0831  0.050 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```
dbrda.explainvar1.shal <- round(dbrda.shal$CCA$eig[1]/sum(c(dbrda.shal$CCA$eig, dbrda.shal$CA$eig)),3) *
dbrda.explainvar2.shal <- round(dbrda.shal$CCA$eig[2]/sum(c(dbrda.shal$CCA$eig, dbrda.shal$CA$eig)),3) *


vals.shal <- scores(dbrda.shal, display="wa")
par(mar=c(5,4,2,4) + 0.1)
plot(vals.shal,
     xlim=c(-1.75, 1.75), ylim=c(-2.2, 2.2),
     xlab=paste0("dbRDA 1 (", dbrda.explainvar1.shal, "%)"),
     ylab=paste0("dbRDA 2 (", dbrda.explainvar2.shal, "%)"),
     pch=16, cex=2.0, type="n", cex.lab=1.5, cex.axis=1.2, axes=F
)
axis(side=1, labels=T, lwd.ticks=2, cex.axis=1.2, las=1)
axis(side=2, labels=T, lwd.ticks=2, cex.axis=1.2, las=1)
abline(h=0, v=0, lty=3)
box(lwd=2)
```

```
points(vals.shal, pch=19, cex=2, bg="grey", col="grey")

vectors.shal <- scores(dbrda.shal, display = "bp")
arrows(0, 0, vectors.shal[,1], vectors.shal[,2], lwd=2, lty=1, length = 0.2, col = "red")
text(vectors.shal[,1], vectors.shal[,2], pos=4, labels=row.names(vectors.shal))
axis(side=3, lwd.ticks = 2, cex.axis=1.2, las=1, col="red", lwd=2.2,
     at = pretty(range(vectors.shal[,1]))*2, labels = pretty(range(vectors.shal[,1])))
axis(side=4, lwd.ticks = 2, cex.axis=1.2, las=1, col="red", lwd=2.2,
     at = pretty(range(vectors.shal[,2]))*2, labels = pretty(range(vectors.shal[,2])))
```



Distance decay

```
library(geosphere)
```

```
## Warning: package 'geosphere' was built under R version 4.0.5
```

```
library(ggplot2)
library(ggpmisc)

# geographical distance
vcs.geo <- cbind(df.num$Longitude_E, df.num$Latitude_N)
dist.vcs <- as.dist(distm(vcs.geo, fun = distHaversine))
dist_vcs<-as.data.frame(as.vector(dist.vcs))/1000 #transformed to kilometer
colnames(dist_vcs) <- "dist"
# depth distance
```

```r
dep.m <- matrix(nrow = 90, ncol = 90)
for (i in 1:90){
  for (j in 1:90){
    dep.m[i,j] = abs(df$Depth[i]-df$Depth[j])
  }
}
depth_dis <- as.data.frame(as.vector(as.dist(dep.m)))
colnames(depth_dis) <- "depth"

# bray-curtis dissimilarity
bray.vcs <- vegdist(vcs, method = "bray")
bray_vcs <- as.data.frame(as.vector(bray.vcs))
colnames(bray_vcs)<- "bray"
data.dist <- data.frame(dist_vcs,depth_dis,bray_vcs)
data.dist$bray <- (1-data.dist$bray)*100 #convert to similarity percentage
summary(lm(data.dist$bray ~ data.dist$dist))
```

```
##
## Call:
## lm(formula = data.dist$bray ~ data.dist$dist)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -36.79 -14.53   2.93  14.10  50.27
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)     4.030e+01  5.423e-01  74.301   <2e-16 ***
## data.dist$dist -4.549e-04  5.147e-05  -8.839   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 17.14 on 4003 degrees of freedom
## Multiple R-squared:  0.01914,    Adjusted R-squared:  0.0189
## F-statistic: 78.12 on 1 and 4003 DF,  p-value: < 2.2e-16
```

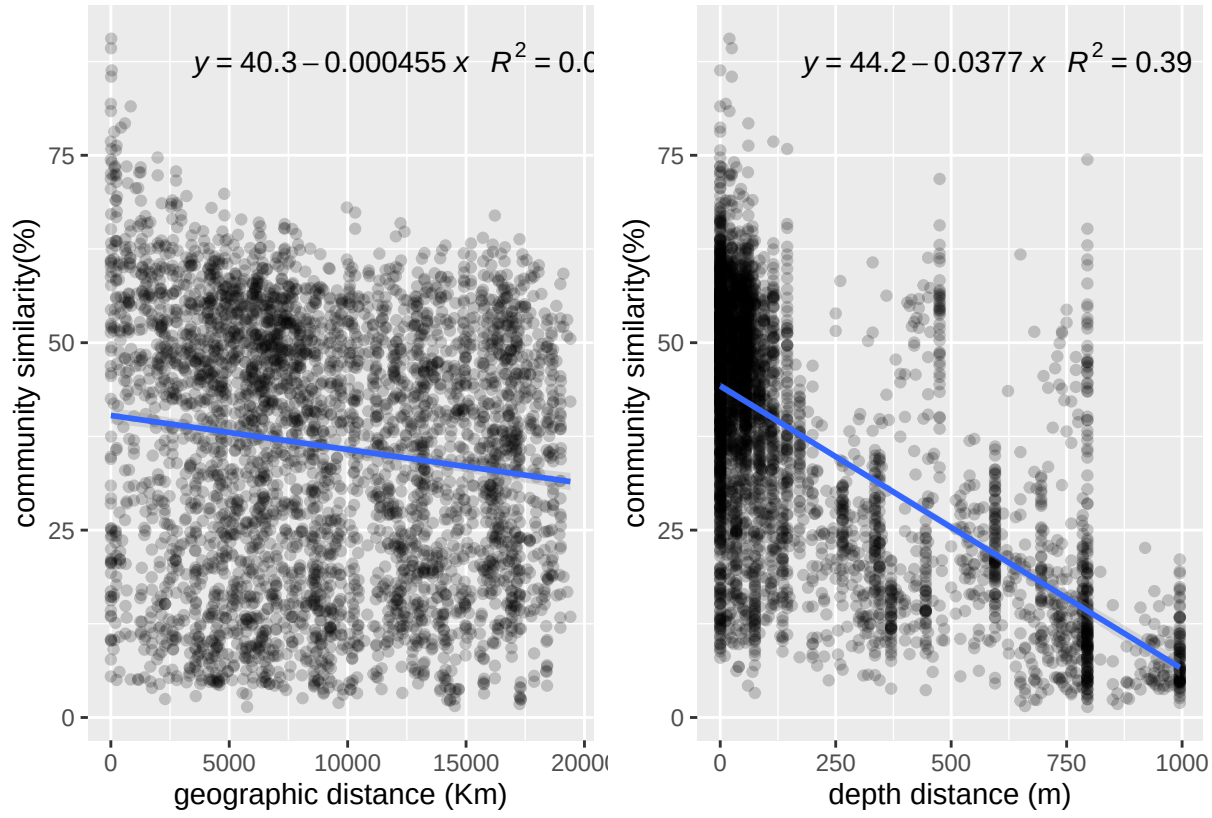
```r
summary(lm(data.dist$bray ~ data.dist$depth))
```

```
##
## Call:
## lm(formula = data.dist$bray ~ data.dist$depth)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -38.135  -8.475   0.432   8.991  60.204
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)     44.2382697  0.2667014  165.87   <2e-16 ***
## data.dist$depth -0.0377462  0.0007453  -50.64   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 13.51 on 4003 degrees of freedom
```

```
## Multiple R-squared:  0.3905, Adjusted R-squared:  0.3904
## F-statistic:  2565 on 1 and 4003 DF,  p-value: < 2.2e-16
```

```
#plot
DDR.geo <- ggplot(data.dist, aes(x = dist, y = bray))+
  geom_point(alpha = 0.2)+
  geom_smooth(method = "lm", alpha = 0.3)+
  labs(x = "geographic distance (Km)", y = "community similarity(%)")+
  stat_poly_eq(formula = y ~ x, aes(label = paste("~~~~~~~~",..eq.label.., ..rr.label..,..p.value.label
DDR.depth <- ggplot(data.dist, aes(x = depth, y = bray))+
  geom_point(alpha = 0.2)+
  geom_smooth(method = "lm", alpha = 0.3)+
  labs(x = "depth distance (m)", y = "community similarity(%)")+
  stat_poly_eq(formula = y ~ x, aes(label = paste("~~~~~~~~",..eq.label.., ..rr.label..,..p.value.label
DDR.geo + DDR.depth
```

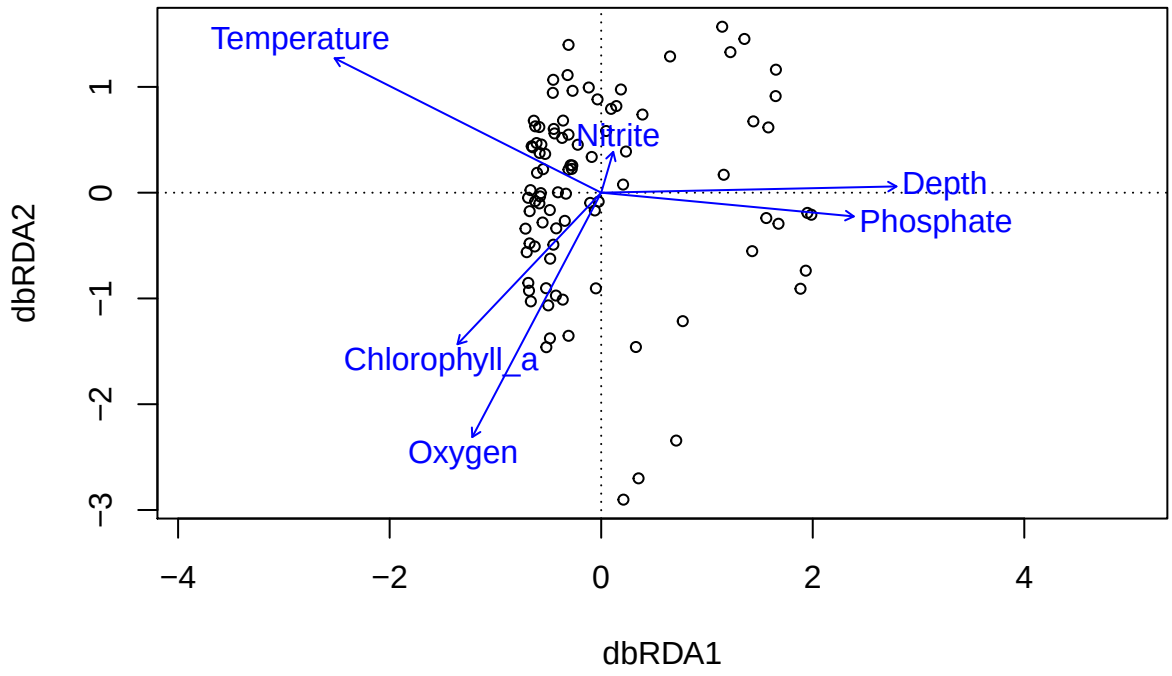
```
## `geom_smooth()` using formula 'y ~ x'
## `geom_smooth()` using formula 'y ~ x'
```



Variation partitioning (two-factor)

```
# vcs table without site #MSP114
vcs.db <- vegdist(vcs, method = "bray", upper = TRUE, diag = TRUE)
# env with depth
env <- as.matrix(env.data[,3:8])
#perform dbRDA
```

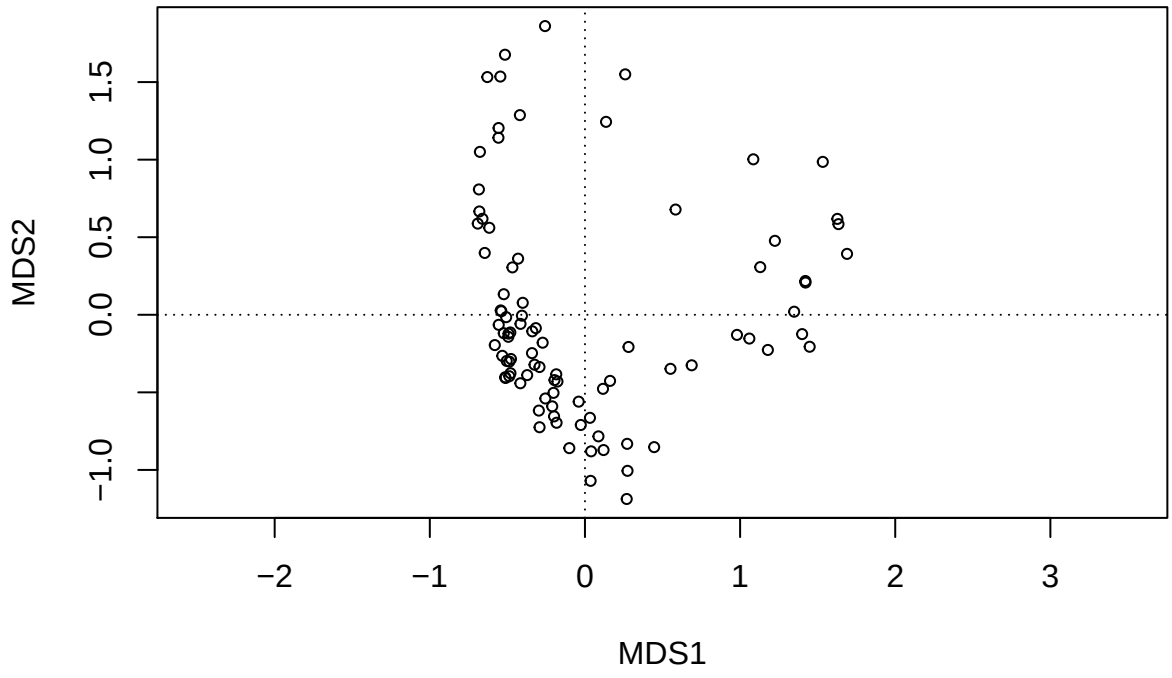
```
vcs.dbrda <- dbrda(vcs.db ~ ., as.data.frame(env))
ordiplot(vcs.dbrda)
```




```
psych::corr.test(env)
```

```
## Call:psych::corr.test(x = env)
## Correlation matrix
##               Depth Temperature Nitrite Phosphate Oxygen Chlorophyll_a
## Depth          1.00       -0.66   -0.06      0.64  -0.37         -0.35
## Temperature   -0.66        1.00   -0.02     -0.71   0.04          0.06
## Nitrite       -0.06       -0.02    1.00      0.23  -0.28          0.20
## Phosphate      0.64       -0.71    0.23      1.00  -0.55         -0.16
## Oxygen        -0.37        0.04   -0.28     -0.55   1.00          0.29
## Chlorophyll_a -0.35        0.06    0.20     -0.16   0.29          1.00
## Sample Size
## [1] 90
## Probability values (Entries above the diagonal are adjusted for multiple tests.)
##               Depth Temperature Nitrite Phosphate Oxygen Chlorophyll_a
## Depth          0.00        0.00    1.00      0.00   0.00          0.01
## Temperature    0.00        0.00    1.00      0.00   1.00          1.00
## Nitrite        0.56        0.83    0.00      0.22   0.06          0.39
## Phosphate      0.00        0.00    0.03      0.00   0.00          0.64
## Oxygen         0.00        0.73    0.01      0.00   0.00          0.05
## Chlorophyll_a  0.00        0.58    0.06      0.13   0.01          0.00
##
##  To see confidence intervals of the correlations, print with the short=FALSE option
```

```r
# Environmental variables are significantly correlated.
vcs.dbrda.mod0 <- dbrda(vcs.db ~ 1, as.data.frame(env))
ordiplot(vcs.dbrda.mod0)
```




```r
vcs.dbrda.mod1 <- dbrda(vcs.db ~ ., as.data.frame(env))
vcs.dbrda <- ordiR2step(vcs.dbrda.mod0, vcs.dbrda.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: vcs.db ~ 1
##
##                  R2.adjusted
## <All variables> 0.237389871
## + Depth         0.141441726
## + Temperature   0.119685498
## + Phosphate     0.106243382
## + Oxygen        0.046884733
## + Chlorophyll_a 0.043447490
## + Nitrite       0.003117142
## <none>          0.000000000
##
##         Df    AIC      F Pr(>F)
## + Depth  1 255.49 15.662  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1414417
```

```
## Call: vcs.db ~ Depth
##
##                 R2.adjusted
## <All variables>   0.2373899
## + Temperature     0.1790677
## + Phosphate       0.1714619
## + Oxygen          0.1691391
## + Chlorophyll_a   0.1585865
## + Nitrite         0.1472391
## <none>            0.1414417
##
##               Df    AIC      F Pr(>F)
## + Temperature  1 252.43 5.0333  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1790677
## Call: vcs.db ~ Depth + Temperature
##
##                 R2.adjusted
## <All variables>   0.2373899
## + Oxygen          0.2098507
## + Chlorophyll_a   0.1998654
## + Phosphate       0.1952861
## + Nitrite         0.1844586
## <none>            0.1790677
##
##          Df    AIC      F Pr(>F)
## + Oxygen  1 249.95 4.3894  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2098507
## Call: vcs.db ~ Depth + Temperature + Oxygen
##
##                 R2.adjusted
## <All variables>   0.2373899
## + Chlorophyll_a   0.2286253
## + Phosphate       0.2196416
## + Nitrite         0.2103001
## <none>            0.2098507
##
##                 Df    AIC      F Pr(>F)
## + Chlorophyll_a  1 248.73 3.0932  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2286253
## Call: vcs.db ~ Depth + Temperature + Oxygen + Chlorophyll_a
##
##                 R2.adjusted
## + Phosphate       0.2381854
## <All variables>   0.2373899
## <none>            0.2286253
```

```
## + Nitrite         0.2281003
```

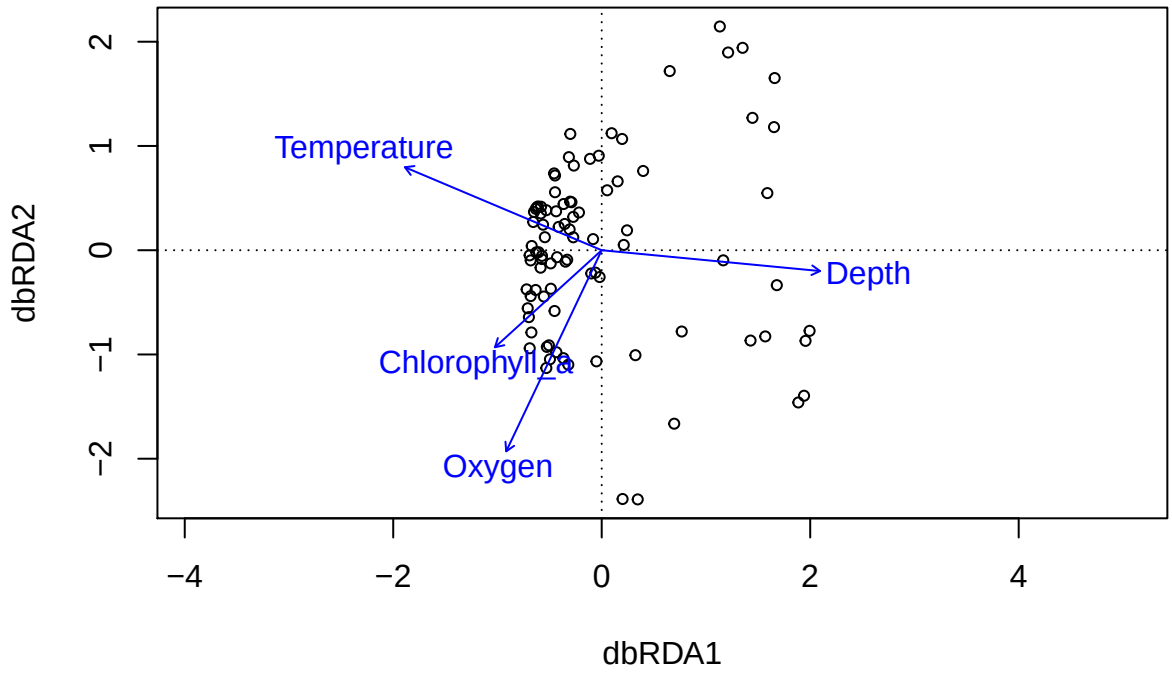
```
vcs.dbrda$call
```

```
## dbrda(formula = vcs.db ~ Depth + Temperature + Oxygen + Chlorophyll_a,
##     data = as.data.frame(env))
```

```
vcs.dbrda$anova
```

```
##                  R2.adj Df    AIC       F Pr(>F)
## + Depth         0.14144  1 255.49 15.6622  0.002 **
## + Temperature   0.17907  1 252.43  5.0333  0.002 **
## + Oxygen        0.20985  1 249.95  4.3894  0.002 **
## + Chlorophyll_a 0.22862  1 248.73  3.0932  0.002 **
## <All variables> 0.23739
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ordiplot(vcs.dbrda)
```




```
env.mod <- model.matrix(~ Depth + Temperature + Oxygen + Chlorophyll_a, as.data.frame(env))[,-1]
```

```
# spatial model with PCNM
rs.vcs <- rowSums(vcs)/sum(vcs)
vcs.dis <-cbind(env.data$Latitude_N, env.data$Longitude_E)
vcs.pcnmw <- pcnm(dist(vcs.dis), w = rs.vcs, dist.ret = T)
vcs.pcnmw$values>0
```

```
##  [1]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
```

```
## [13]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [25]  TRUE  TRUE  TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [37] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
```

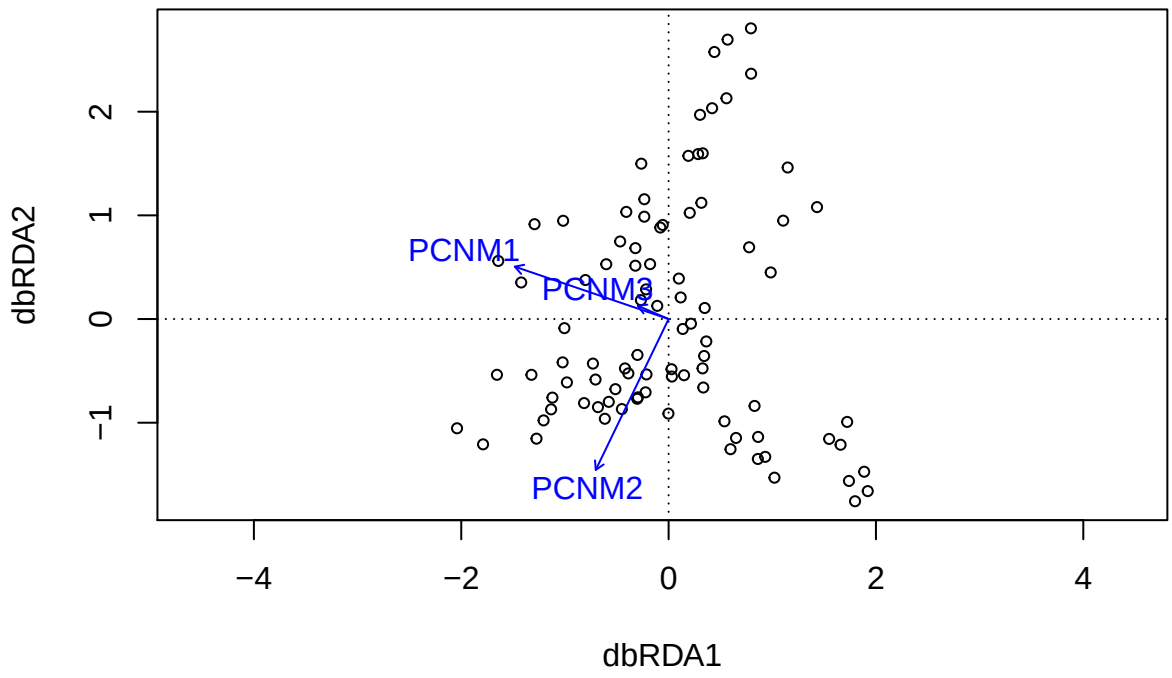
```
#perform model selection for spatial matrix
vcs.space <-as.data.frame(scores(vcs.pcnmw))
vcs.pcnm.mod0 <- dbrda(vcs.db ~ 1, vcs.space)
vcs.pcnm.mod1 <- dbrda(vcs.db ~ ., vcs.space)
step.pcnm <- ordiR2step(vcs.pcnm.mod0, vcs.pcnm.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: vcs.db ~ 1
##
##                   R2.adjusted
## <All variables>  0.0962770053
## + PCNM1          0.0372899800
## + PCNM2          0.0337864325
## + PCNM15         0.0170560648
## + PCNM3          0.0119662318
## + PCNM4          0.0102935626
## + PCNM5          0.0090794575
## + PCNM14         0.0081380356
## + PCNM6          0.0032382404
## + PCNM19         0.0031318508
## + PCNM9          0.0013037913
## + PCNM17         0.0003197189
## <none>           0.0000000000
## + PCNM24        -0.0002517278
## + PCNM7         -0.0002650326
## + PCNM8         -0.0003707471
## + PCNM18        -0.0009237171
## + PCNM11        -0.0020593000
## + PCNM12        -0.0021780594
## + PCNM20        -0.0027678804
## + PCNM16        -0.0034954716
## + PCNM22        -0.0043393010
## + PCNM21        -0.0045196004
## + PCNM10        -0.0062164050
## + PCNM26        -0.0064536481
## + PCNM23        -0.0065543363
## + PCNM25        -0.0066384102
## + PCNM13        -0.0069773378
## + PCNM27        -0.0071189589
##
##         Df    AIC      F Pr(>F)
## + PCNM1  1 265.79 4.4474  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.03728998
## Call: vcs.db ~ PCNM1
##
##                  R2.adjusted
## <All variables>  0.09627701
## + PCNM2          0.07129410
```

```
## + PCNM15         0.05497074
## + PCNM3          0.05061667
## + PCNM4          0.04927912
## + PCNM14         0.04856839
## + PCNM5          0.04715579
## + PCNM19         0.04097574
## + PCNM6          0.04055086
## + PCNM9          0.03913683
## + PCNM17         0.03796937
## + PCNM7          0.03746023
## + PCNM24         0.03733292
## <none>           0.03728998
## + PCNM8          0.03706036
## + PCNM18         0.03635466
## + PCNM11         0.03570246
## + PCNM12         0.03539870
## + PCNM16         0.03489007
## + PCNM20         0.03456254
## + PCNM22         0.03347677
## + PCNM21         0.03316719
## + PCNM10         0.03131661
## + PCNM26         0.03122488
## + PCNM25         0.03099457
## + PCNM23         0.03093995
## + PCNM27         0.03050025
## + PCNM13         0.03032424
##
##         Df    AIC      F Pr(>F)
## + PCNM2  1 263.53 4.2221  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.0712941
## Call: vcs.db ~ PCNM1 + PCNM2
##
##                 R2.adjusted
## <All variables>  0.09627701
## + PCNM3          0.08535091
## + PCNM15         0.08492789
## + PCNM14         0.08326007
## + PCNM5          0.08287832
## + PCNM4          0.08174324
## + PCNM6          0.07589838
## + PCNM19         0.07532932
## + PCNM9          0.07249684
## <none>           0.07129410
## + PCNM8          0.07109266
## + PCNM24         0.07079342
## + PCNM18         0.07076584
## + PCNM7          0.07062606
## + PCNM17         0.07031123
## + PCNM12         0.06992536
## + PCNM16         0.06923239
## + PCNM11         0.06903507
```

```
## + PCNM20         0.06903058
## + PCNM22         0.06808286
## + PCNM21         0.06751725
## + PCNM10         0.06568101
## + PCNM23         0.06567405
## + PCNM26         0.06563057
## + PCNM25         0.06492882
## + PCNM13         0.06488942
## + PCNM27         0.06422087
##
##         Df    AIC      F Pr(>F)
## + PCNM3  1 263.11 2.3371   0.01 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.08535091
## Call: vcs.db ~ PCNM1 + PCNM2 + PCNM3
##
##                 R2.adjusted
## + PCNM15         0.09818299
## + PCNM14         0.09765421
## + PCNM5          0.09722787
## <All variables>  0.09627701
## + PCNM4          0.09589324
## + PCNM6          0.09028626
## + PCNM19         0.08976652
## + PCNM9          0.08690710
## + PCNM8          0.08546337
## <none>           0.08535091
## + PCNM24         0.08490813
## + PCNM7          0.08454180
## + PCNM12         0.08445000
## + PCNM17         0.08424619
## + PCNM18         0.08366646
## + PCNM16         0.08340938
## + PCNM20         0.08324390
## + PCNM11         0.08320767
## + PCNM22         0.08225460
## + PCNM21         0.08146340
## + PCNM23         0.07994569
## + PCNM10         0.07980778
## + PCNM26         0.07948376
## + PCNM25         0.07913680
## + PCNM13         0.07906406
## + PCNM27         0.07843556
```

```
plot(step.pcnm)
```




```
step.pcnm$anova
```

```
##                   R2.adj Df    AIC      F Pr(>F)
## + PCNM1         0.037290  1 265.79 4.4474  0.002 **
## + PCNM2         0.071294  1 263.53 4.2221  0.002 **
## + PCNM3         0.085351  1 263.11 2.3371  0.010 **
## <All variables> 0.096277
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
space.mod <- model.matrix(~ PCNM1 + PCNM2 + PCNM3, vcs.space)[,-1]
# First conduct constrained ordinations
vcs.total.env <- dbrda(vcs.db ~ env.mod)
vcs.total.space <- dbrda(vcs.db ~ space.mod)
# Next construct partial constrained ordinations
vcs.env.cond.space <- dbrda(vcs.db ~ env.mod + Condition(space.mod))
vcs.space.cond.env <- dbrda(vcs.db ~ space.mod + Condition(env.mod))
# Next test for significance of the dbRDA fractions.
permutest(vcs.env.cond.space, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = vcs.db ~ env.mod + Condition(space.mod))
```

```
## Permutation test for all constrained eigenvalues
##          Df Inertia     F Pr(>F)
## Model     4  4.7544 7.822  0.001 ***
## Residual 82 12.4605
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(vcs.space.cond.env, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = vcs.db ~ space.mod + Condition(env.mod))
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     3   1.889 4.1437  0.001 ***
## Residual 82  12.460
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(vcs.total.env, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = vcs.db ~ env.mod)
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     4  5.1284 7.5946  0.001 ***
## Residual 85 14.3495
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(vcs.total.space, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = vcs.db ~ space.mod)
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     3   2.263 3.7684  0.001 ***
## Residual 86  17.215
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
#Using the built-in varpart() function
vcs.varpart <- varpart(vcs.db, env.mod, space.mod, transfo = "hel")
```

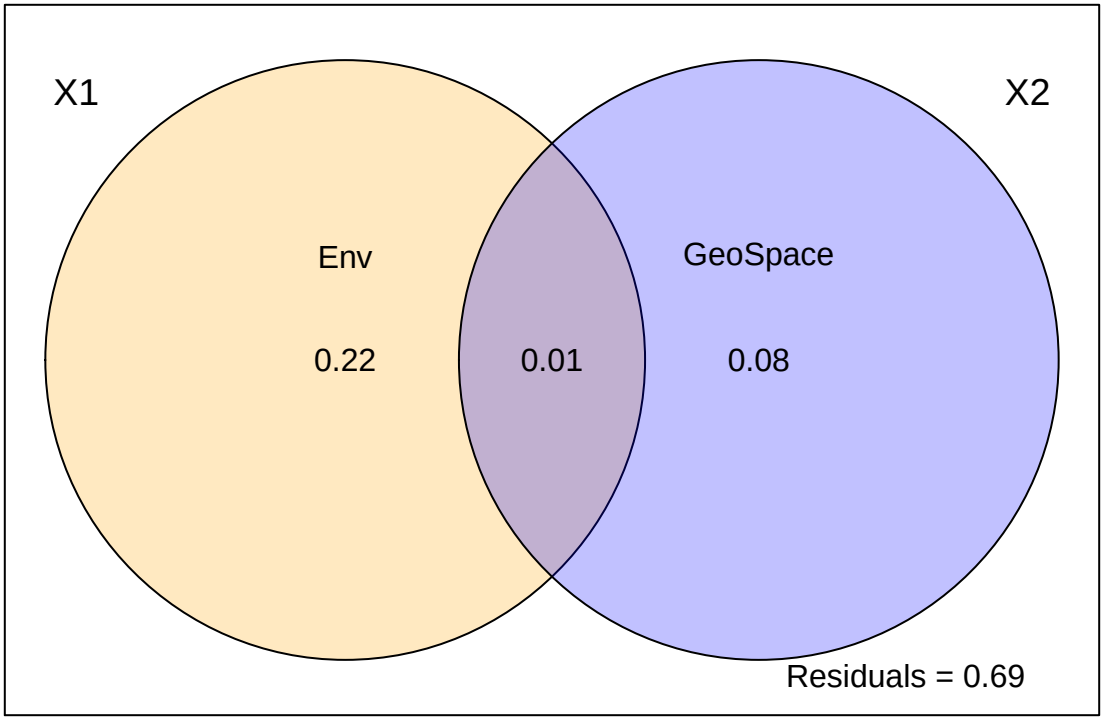
```
## arguments 'transfo' and 'scale' are ignored with distances
```

```
## arguments 'scale' and 'transfo' ignored: valid only in RDA
```

```r
vcs.varpart
```

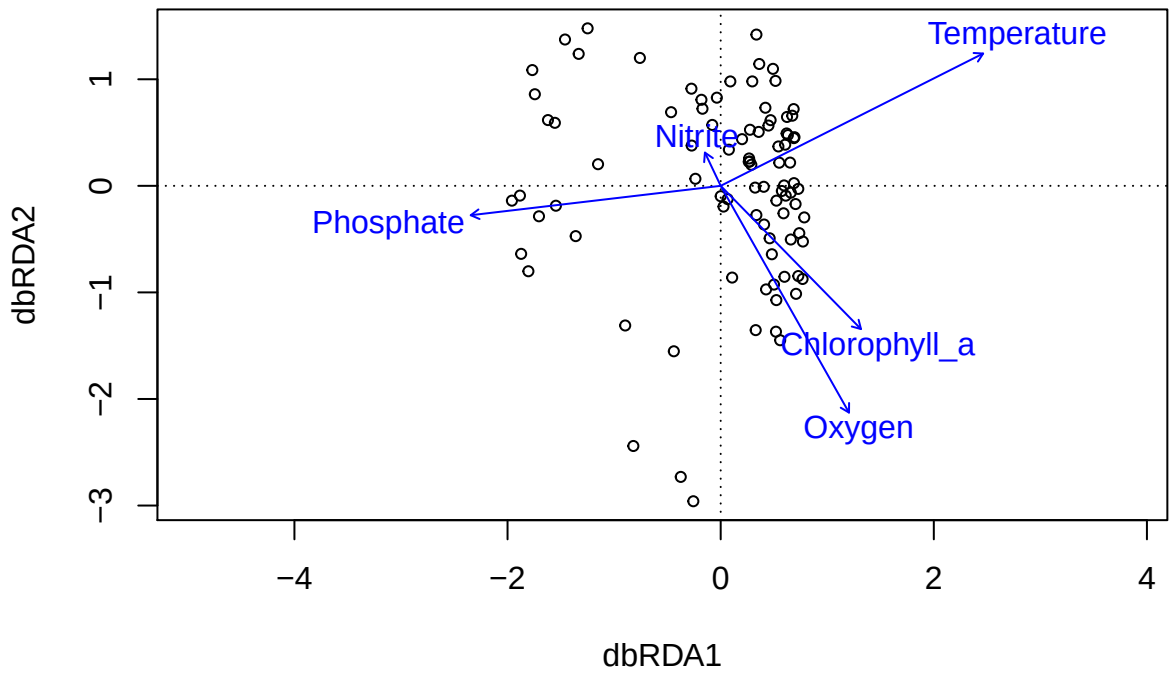
```
##
## Partition of squared Bray distance in dbRDA
##
## Call: varpart(Y = vcs.db, X = env.mod, space.mod, transfo = "hel")
##
## Explanatory tables:
## X1:  env.mod
## X2:  space.mod
##
## No. of explanatory tables: 2
## Total variation (SS): 19.478
## No. of observations: 90
##
## Partition table:
##                       Df R.squared Adj.R.squared Testable
## [a+b] = X1             4   0.26329       0.22863     TRUE
## [b+c] = X2             3   0.11618       0.08535     TRUE
## [a+b+c] = X1+X2        7   0.36027       0.30566     TRUE
## Individual fractions
## [a] = X1|X2            4                 0.22031     TRUE
## [b]                    0                 0.00831    FALSE
## [c] = X2|X1            3                 0.07704     TRUE
## [d] = Residuals                          0.69434    FALSE
## ---
## Use function 'dbrda' to test significance of fractions of interest
```

```r
par(mar = c(2,2,2,2))
plot(vcs.varpart, bg = c("orange", "blue"))
text(1, 0.25, "GeoSpace")
text(0, 0.25, "Env")
```



Variation partitioning (Three-factor)

```
# env without depth
envpure <- as.matrix(env.data[,4:8])
#perform dbRDA
vcs.dbrda.p <- dbrda(vcs.db ~ ., as.data.frame(envpure))
ordiplot(vcs.dbrda.p)
```

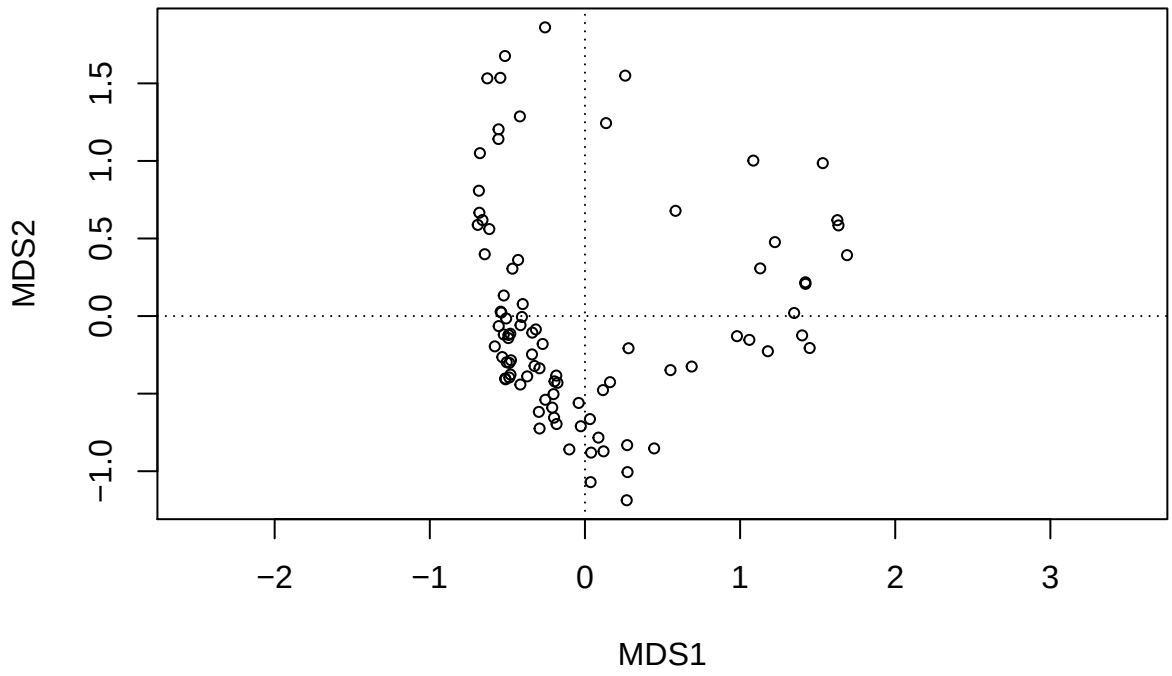



```
print(psych::corr.test(envpure),short = FALSE)
```

```
## Call:psych::corr.test(x = envpure)
## Correlation matrix
##               Temperature Nitrite Phosphate Oxygen Chlorophyll_a
## Temperature          1.00   -0.02     -0.71   0.04          0.06
## Nitrite             -0.02    1.00      0.23  -0.28          0.20
## Phosphate           -0.71    0.23      1.00  -0.55         -0.16
## Oxygen               0.04   -0.28     -0.55   1.00          0.29
## Chlorophyll_a        0.06    0.20     -0.16   0.29          1.00
## Sample Size
## [1] 90
## Probability values (Entries above the diagonal are adjusted for multiple tests.)
##               Temperature Nitrite Phosphate Oxygen Chlorophyll_a
## Temperature          0.00    1.00      0.00   1.00          1.00
## Nitrite              0.83    0.00      0.19   0.05          0.32
## Phosphate            0.00    0.03      0.00   0.00          0.51
## Oxygen               0.73    0.01      0.00   0.00          0.04
## Chlorophyll_a        0.58    0.06      0.13   0.01          0.00
##
##  Confidence intervals based upon normal theory.  To get bootstrapped values, try cor.ci
##              raw.lower raw.r raw.upper raw.p lower.adj upper.adj
## Tmprt-Nitrt     -0.23 -0.02      0.18  0.83     -0.23      0.18
## Tmprt-Phsph     -0.80 -0.71     -0.59  0.00     -0.83     -0.53
## Tmprt-Oxygn     -0.17  0.04      0.24  0.73     -0.20      0.27
## Tmprt-Chlr_     -0.15  0.06      0.26  0.58     -0.19      0.31
```

```
## Nitrt-Phsph      0.02  0.23      0.41  0.03     -0.05      0.47
## Nitrt-Oxygn     -0.46 -0.28     -0.08  0.01     -0.52      0.00
## Nitrt-Chlr_     -0.01  0.20      0.39  0.06     -0.08      0.44
## Phsph-Oxygn     -0.68 -0.55     -0.39  0.00     -0.72     -0.31
## Phsph-Chlr_     -0.36 -0.16      0.05  0.13     -0.41      0.10
## Oxygn-Chlr_      0.09  0.29      0.47  0.01      0.01      0.53
```

```
# Temperature, Phosphate, Nitrite and Oxygen are significantly correlated.
vcs.dbrda.p.mod0 <- dbrda(vcs.db ~ 1, as.data.frame(envpure))
ordiplot(vcs.dbrda.p.mod0)
```




```
vcs.dbrda.p.mod1 <- dbrda(vcs.db ~ ., as.data.frame(envpure))
vcs.dbrda.p <- ordiR2step(vcs.dbrda.p.mod0, vcs.dbrda.p.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: vcs.db ~ 1
##
##                  R2.adjusted
## <All variables> 0.200596042
## + Temperature   0.119685498
## + Phosphate     0.106243382
## + Oxygen        0.046884733
## + Chlorophyll_a 0.043447490
## + Nitrite       0.003117142
## <none>          0.000000000
##
##               Df    AIC    F Pr(>F)
```

```
## + Temperature  1 257.74 13.1  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1196855
## Call: vcs.db ~ Temperature
##
##                 R2.adjusted
## <All variables>   0.2005960
## + Oxygen          0.1653014
## + Chlorophyll_a   0.1590395
## + Phosphate       0.1426166
## + Nitrite         0.1240118
## <none>            0.1196855
##
##          Df    AIC      F Pr(>F)
## + Oxygen  1 253.92 5.8092  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1653014
## Call: vcs.db ~ Temperature + Oxygen
##
##                 R2.adjusted
## <All variables>   0.2005960
## + Chlorophyll_a   0.1919971
## + Phosphate       0.1747048
## + Nitrite         0.1689095
## <none>            0.1653014
##
##                 Df    AIC      F Pr(>F)
## + Chlorophyll_a  1 251.96 3.8744  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.1919971
## Call: vcs.db ~ Temperature + Oxygen + Chlorophyll_a
##
##                 R2.adjusted
## + Phosphate       0.2010239
## <All variables>   0.2005960
## <none>            0.1919971
## + Nitrite         0.1918469
```

```
vcs.dbrda.p$call
```

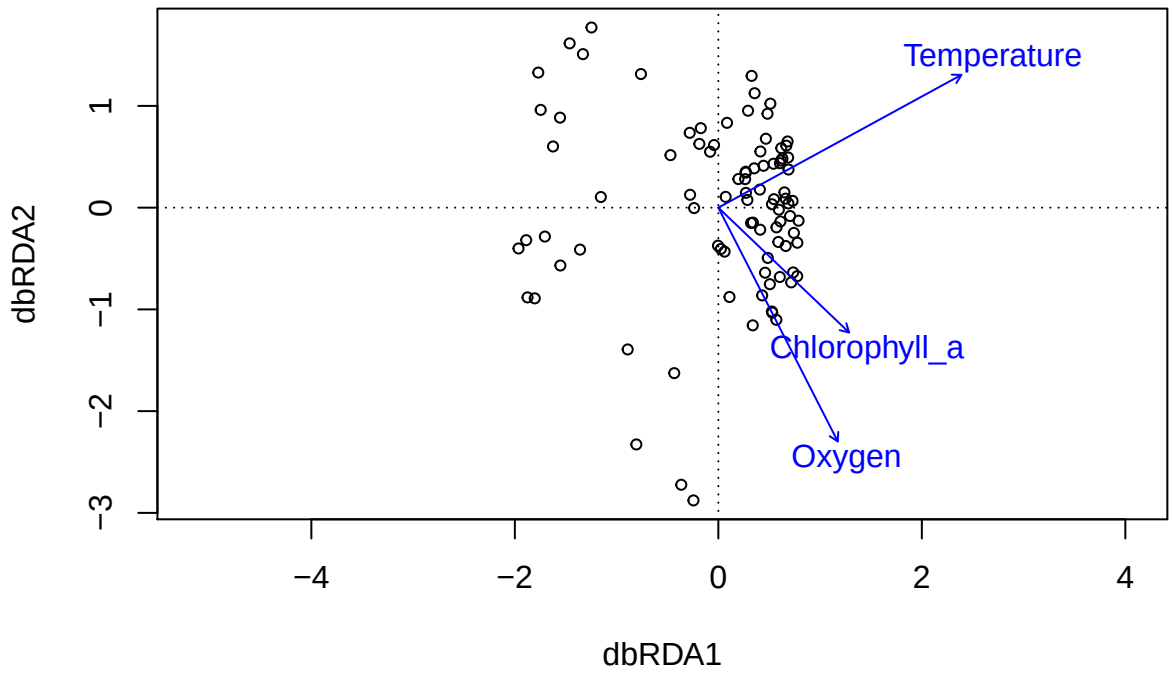
```
## dbrda(formula = vcs.db ~ Temperature + Oxygen + Chlorophyll_a,
##     data = as.data.frame(envpure))
```

```
vcs.dbrda.p$anova
```

```
##                   R2.adj Df    AIC       F Pr(>F)
## + Temperature   0.11968  1 257.74 13.1002  0.002 **
## + Oxygen        0.16530  1 253.92  5.8092  0.002 **
## + Chlorophyll_a 0.19200  1 251.96  3.8744  0.002 **
```

```
## <All variables> 0.20060
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ordiplot(vcs.dbrda.p)
```




```
envpure.mod <- model.matrix(~ Temperature + Oxygen + Chlorophyll_a, as.data.frame(envpure))[,-1]
vcs.varpart.3 <- varpart(vcs.db, envpure.mod, space.mod, env.data[3], transfo = "hel")
```

```
## arguments 'transfo' and 'scale' are ignored with distances
```

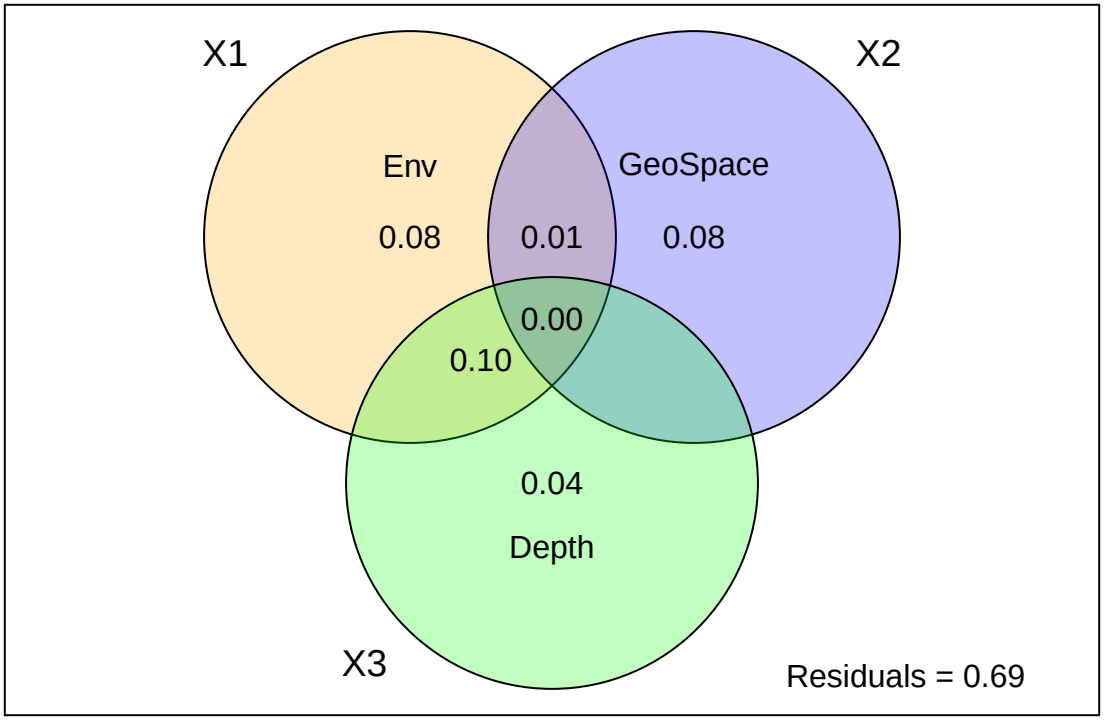
```
## arguments 'scale' and 'transfo' ignored: valid only in RDA
```

```
vcs.varpart.3
```

```
##
## Partition of squared Bray distance in dbRDA
##
## Call: varpart(Y = vcs.db, X = envpure.mod, space.mod, env.data[3],
## transfo = "hel")
##
## Explanatory tables:
## X1:  envpure.mod
## X2:  space.mod
## X3:  env.data[3]
##
## No. of explanatory tables: 3
## Total variation (SS): 19.478
```

```
## No. of observations: 90
##
## Partition table:
##                         Df R.square Adj.R.square Testable
## [a+d+f+g] = X1           3  0.21923      0.19200     TRUE
## [b+d+e+g] = X2           3  0.11618      0.08535     TRUE
## [c+e+f+g] = X3           1  0.15109      0.14144     TRUE
## [a+b+d+e+f+g] = X1+X2    6  0.31697      0.26760     TRUE
## [a+c+d+e+f+g] = X1+X3    4  0.26329      0.22863     TRUE
## [b+c+d+e+f+g] = X2+X3    4  0.25996      0.22513     TRUE
## [a+b+c+d+e+f+g] = All    7  0.36027      0.30566     TRUE
## Individual fractions
## [a] = X1 | X2+X3         3                0.08053     TRUE
## [b] = X2 | X1+X3         3                0.07704     TRUE
## [c] = X3 | X1+X2         1                0.03806     TRUE
## [d]                      0                0.00665    FALSE
## [e]                      0               -0.00144    FALSE
## [f]                      0                0.10171    FALSE
## [g]                      0                0.00310    FALSE
## [h] = Residuals                           0.69434    FALSE
## Controlling 1 table X
## [a+d] = X1 | X3          3                0.08718     TRUE
## [a+f] = X1 | X2          3                0.18225     TRUE
## [b+d] = X2 | X3          3                0.08369     TRUE
## [b+e] = X2 | X1          3                0.07560     TRUE
## [c+e] = X3 | X1          1                0.03663     TRUE
## [c+f] = X3 | X2          1                0.13978     TRUE
## ---
## Use function 'dbrda' to test significance of fractions of interest
```

```
par(mar = c(2,2,2,2))
plot(vcs.varpart.3, bg = c("orange", "blue", "green"))
text(0.5, -1.1, "Depth")
text(1, 0.25, "GeoSpace")
text(0, 0.25, "Env")
```



Values <0 not shown