The geographical ecology of pond bacteria

April 21, 2015

```
# Load Packages
require("sp")          # Classes and methods for handling spatial data
```

```
## Loading required package: sp
```

```
require("geoR")        # Methods for geostatistical analyses
```

```
## Loading required package: geoR
## Loading required package: MASS
## ------------------------------------------------------------
##  Analysis of geostatistical data
##  For an Introduction to geoR go to http://www.leg.ufpr.br/geoR
##  geoR version 1.7-4.1 (built on 2012-06-29) is now loaded
## ------------------------------------------------------------
```

```
require("rgdal")       # Geospatial Data Abstraction Library
```

```
## Loading required package: rgdal
## rgdal: version: 0.9-1, (SVN revision 518)
## Geospatial Data Abstraction Library extensions to R successfully loaded
## Loaded GDAL runtime: GDAL 1.11.1, released 2014/09/24
## Path to GDAL shared files: /usr/local/Cellar/gdal/1.11.1_3/share/gdal
## Loaded PROJ.4 runtime: Rel. 4.8.0, 6 March 2012, [PJ_VERSION: 480]
## Path to PROJ.4 shared files: (autodetected)
```

```
require("raster")      # Methods to create a RasterLayer object
```

```
## Loading required package: raster
##
## Attaching package: 'raster'
##
## The following objects are masked from 'package:MASS':
##
##     area, select
```

```
require("maptools")    # Tools for manipulating and reading geospatial data
```

```
## Loading required package: maptools
## Checking rgeos availability: TRUE
```

```
require("picante")
```

```
## Loading required package: picante
## Loading required package: ape
```

```
##
## Attaching package: 'ape'
##
## The following objects are masked from 'package:raster':
##
##     rotate, zoom
##
## Loading required package: vegan
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.2-1
## Loading required package: nlme
##
## Attaching package: 'nlme'
##
## The following object is masked from 'package:raster':
##
##     getData
```

```
require("ape")
require("seqinr")
```

```
## Loading required package: seqinr
## Loading required package: ade4
##
## Attaching package: 'ade4'
##
## The following object is masked from 'package:vegan':
##
##     cca
##
##
## Attaching package: 'seqinr'
##
## The following object is masked from 'package:nlme':
##
##     gls
##
## The following object is masked from 'package:permute':
##
##     getType
##
## The following objects are masked from 'package:ape':
##
##     as.alignment, consensus
```

```
require("vegan") # biodiversity estimators and related functions
require("fossil")
```

```
## Loading required package: fossil
## Loading required package: maps
## Loading required package: shapefiles
## Loading required package: foreign
```

```
##
## Attaching package: 'shapefiles'
##
## The following objects are masked from 'package:foreign':
##
##     read.dbf, write.dbf
```

```
require("simba")
```

```
## Loading required package: simba
## This is simba 0.3-5
##
## Attaching package: 'simba'
##
## The following object is masked from 'package:picante':
##
##     mpd
##
## The following object is masked from 'package:stats':
##
##     mad
```

C. Load Source Code

In addition to relying on contributed packages, we will also be using a source code file. A source code file has user-defined functions that are required for certain analyses. The benefit of source files is that they contain "vetted" code that can be used across multiple projects. Here, we will be using a source code file that includes a function for reading in the output files from the popular community sequencing software `mothur` (http://www.mothur.org/).

```
load(file = "~/GitHub/Dimensions/Aim3/Mothur/INPond_Initial.RData")
```

Overview

Here, we will explore our primary geographical patterns of interest: the taxa-area relationship (TAR), the phylogenetic diversity-area relationship, and the distance-decay relationship in taxonomic and phylogenetic community similarity.

Study area

We analyzed environmental and bacterial community data from a survey of shallow ponds found east of Bloomington, IN. These ponds were constructed in the 1940s as wildlife refuge ponds, and are scattered throughout Brown County State Park, Yellowood State Forest, and Hoosier National Forest. In the summer of 2013, we visited approximately 50 of these ponds and recorded their geographic locations. We sampled aspects of water chemistry, physical properties, and bacterial community composition.

Figure 1. Spatially explicit data on environmental and geographic features.

```
# Load Environmental and Geographical Data
env <- read.table("~/GitHub/Dimensions/Aim3/DATA/EnvData/20130801_PondDataMod.csv", sep = ",", header =
lats <- as.numeric(env[, 3]) # latitudes (north and south)
lons <- as.numeric(env[, 4]) # longitudes (east and west)
```

Environmental data

We measured 19 environmental and geographic variables. These included elevation (m), geographical coordinates (lat-long; data: WGS84), temperature (C), Diameter(m), Depth(m), redox potential (ORP), specific conductivity or SpC (uS/cm), dissolved Oxygen (mg/L), total dissolved solids (g/L), salinity (p.s.u.=ppm), color - measured at absorbance = 660; an estimate of carbon in the water sample, chlorophyll a (ug/ml), dissolved organic carbon (mg/L), dissolved organic nitrogen (mg/L), and total phosphorus (ug/L).

Microbial community data

In addition to measuring a suite of geographic and environmental variables, we characterized the diversity of bacteria in the ponds using molecular-based approaches. Specifically, we amplified the 16S rRNA gene (i.e., "DNA") and 16S rRNA transcripts (i.e., "RNA") of bacteria using barcoded primers on the Illumina MiSeq platform. We then used a `mothur` pipeline to quality-trim our data set and assign sequences to operational taxonomic units (OTU).

For each pond, we used the observed taxonomic richness (S), total number of gene reads (N), and number of gene reads per OTU (Ni) to estimate Shannon's diversity index (H), and Simpson's evenness (D/S). We should estimate a handful of diversity and evenness metrics, as well conduct richness estimation for each site (Chao1, ACE, rarefaction, jacknife). These will provide basic diversity-related variables to explore with respect to geograpy and environmental conditions.

```
# Select DNA Data: Use the `grep()` Command and Rename with `gsub()`
# The active portion, based on cDNA
active.comm <- Pond97[grep("*-cDNA", rownames(Pond97)), ]
rownames(active.comm) <- gsub("\\-cDNA", "", rownames(active.comm))
rownames(active.comm) <- gsub("\\_", "", rownames(active.comm))

# The community without respect to active or not, 16S rRNA gene sequences
all.comm <- Pond97[grep("*-DNA", rownames(Pond97)), ]
rownames(all.comm) <- gsub("\\-DNA", "", rownames(all.comm))
rownames(all.comm) <- gsub("\\_", "", rownames(all.comm))

# Remove Sites Not in the Environmental Data Set
active.comm <- active.comm[rownames(active.comm)  %in% env$Sample_ID, ]
all.comm <- all.comm[rownames(all.comm)  %in% env$Sample_ID, ]

# Remove Zero-Occurrence Taxa
active.comm <- active.comm[ , colSums(active.comm) > 0]
all.comm <- all.comm[ , colSums(all.comm) > 0]

# Import Taxonomy Data Using `read.tax()` from Source Code
#tax <- read.tax(taxonomy = "./Mothur/INPonds.trim.contigs.good.unique.good.filter.unique.precluster.pi
```

```
#### A function to generate observed richness
S.obs <- function(x = ""){ rowSums(x > 0) * 1}
```

```
#### For each site:
# N equals numbers of reads
env$active.N <- as.vector(rowSums(active.comm))
env$all.N <- as.vector(rowSums(all.comm))

# S equals the number of non-zero abundances
```

```
env$active.S <- S.obs(active.comm)
env$all.S <- S.obs(all.comm)

# Diversity is Shannon's
env$active.H <- as.vector(diversity(active.comm, index = "shannon"))
env$all.H <- as.vector(diversity(all.comm, index = "shannon"))

# Evenness is Simpsons; divide Simpson's Diversity by S
env$active.De <- as.vector(diversity(active.comm, index = "invsimpson")/env$active.S)
env$all.De <- as.vector(diversity(all.comm, index = "invsimpson")/env$all.S)
```

Primary geographic patterns

We examined three taxa-level geographic patterns: Distanct-decay (DD), Taxa-area relationship (TAR), and the specific spatial abundance distribution (SSAD). While the DD and TAR have been more or less frequently studied in microbial ecology and microbial biogeography, the SSAD has been mainly, if not entirely examined in studies of macroscopic plants and animals.

1.) Distance Decay, taxonomic and phylogenetic

Tobler's first law of geography states that "Everything is related to everything else, but near things are more related than distant things" (Tobler 1970). This law is a formulation of the concept of spatial autocorrelation. In short, spatial autocorrelation is the degree to which spatial variables are either clustered in space (positive autocorrelation) or over-dispersed (negative autocorrelation).

The distance-decay relationship is a primary biogeographic pattern of spatial autocorrelation, and captures the rate of decreasing similarity with increasing distance. This pattern addresses whether communities close to one another are more similar than communities that are farther away. The distance-decay pattern can also be used to address whether near environments have greater similarity than far ones. We looked at decay in both taxonomic level compositional similarity via bray-curtis (should also do for Sorensens) and phylogenetic distance via unifrac distance.

RESULTS: Distance-Decay

```
plot.new()
#par(mfrow=c(2, 2))

# Geographic Distances (Kilometers) Among Ponds
long.lat <- as.matrix(cbind(env$long, env$lat))
coord.dist <- earth.dist(long.lat, dist = TRUE)

# Taxonomic Distances Among Ponds (Bray-Curits)
active.bray.curtis.dist <- 1 - vegdist(active.comm)
all.bray.curtis.dist <- 1 - vegdist(all.comm)

# Transform All Distances Into List Format:
active.bray.curtis.dist.ls <- liste(active.bray.curtis.dist, entry = "bray.curtis")
all.bray.curtis.dist.ls <- liste(all.bray.curtis.dist, entry = "bray.curtis")
coord.dist.ls <- liste(coord.dist, entry = "geo.dist")
```

```r
# Create a Data Frame from the Lists of Distances
df <- data.frame(coord.dist.ls, active.bray.curtis.dist.ls[, 3],
          all.bray.curtis.dist.ls[, 3])

names(df)[4:5] <- c("active.bray.curtis", "all.bray.curtis")
attach(df)

# Now, let's plot the DD relationships:

# Set Initial Plot Parameters
par(mfrow=c(1, 2))#, mar = c(1, 5, 2, 1) + 0.1, oma = c(2, 0, 0, 0))

# Make Plot for Taxonomic DD
plot(coord.dist, active.bray.curtis, xlab = "", xaxt = "n", las = 1, ylim = c(0.1, 0.9),
     ylab="Bray-Curtis Similarity",
     main = "Distance Decay, Active taxa", col = "SteelBlue")

# Regression for Taxonomic DD
DD.reg.bc <- lm(active.bray.curtis ~ geo.dist)
summary(DD.reg.bc)
```

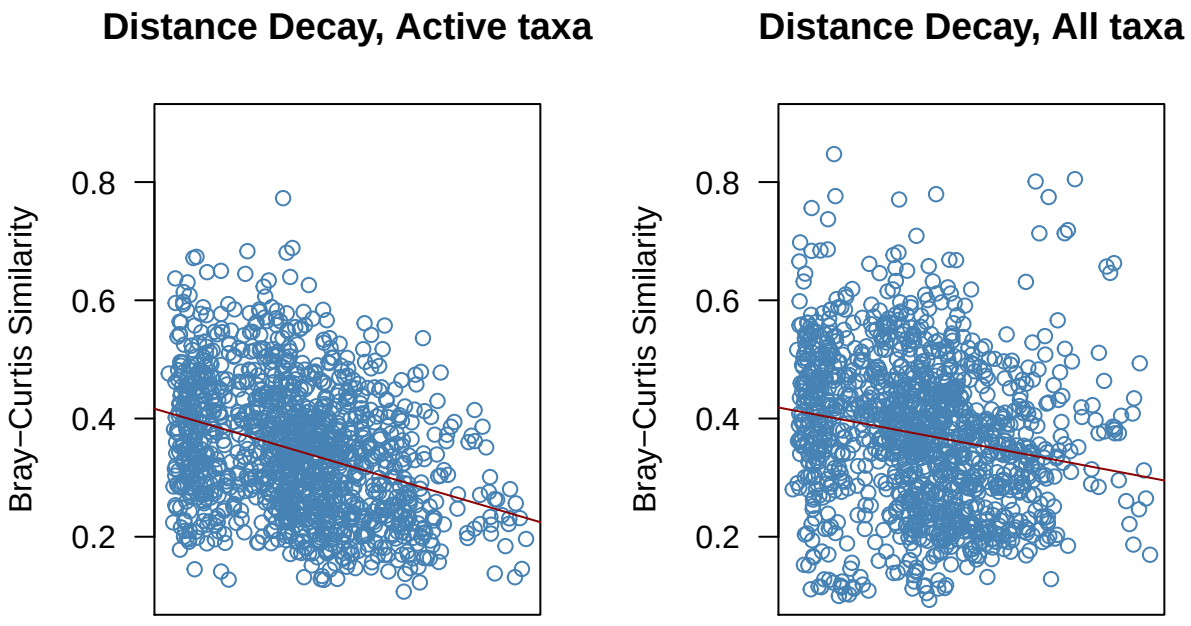
```
##
## Call:
## lm(formula = active.bray.curtis ~ geo.dist)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.25218 -0.08125 -0.00485  0.06762  0.42022
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.4094763  0.0058157   70.41   <2e-16 ***
## geo.dist    -0.0066754  0.0005241  -12.74   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1043 on 1273 degrees of freedom
## Multiple R-squared:  0.113,  Adjusted R-squared:  0.1123
## F-statistic: 162.2 on 1 and 1273 DF,  p-value: < 2.2e-16
```

```r
abline(DD.reg.bc , col = "red4")

# Make Plot for Taxonomic DD
plot(coord.dist, all.bray.curtis, xlab = "", xaxt = "n", las = 1, ylim = c(0.1, 0.9),
     ylab="Bray-Curtis Similarity",
     main = "Distance Decay, All taxa", col = "SteelBlue")

# Regression for Taxonomic DD
DD.reg.bc <- lm(all.bray.curtis ~ geo.dist)
summary(DD.reg.bc)
```

```
##
```

```
## Call:
## lm(formula = all.bray.curtis ~ geo.dist)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.29942 -0.09183  0.00064  0.07955  0.48117
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.4140290  0.0070007  59.141  < 2e-16 ***
## geo.dist    -0.0042916  0.0006309  -6.802 1.58e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1255 on 1273 degrees of freedom
## Multiple R-squared:  0.03507,    Adjusted R-squared:  0.03432
## F-statistic: 46.27 on 1 and 1273 DF,  p-value: 1.579e-11
```

```
abline(DD.reg.bc , col = "red4")

# Add X-Axis Label to Plot
mtext("Geographic Distance, km", side = 1, adj = 0.55,
      line = 0.5, outer = TRUE)
```



```
diffslope(geo.dist, active.bray.curtis, geo.dist, all.bray.curtis)
```

2.) Species- or taxa- area relationship (SAR)

The species-area relationshp describes the rate at which species are discovred with increasing area. The SAR one of ecology's oldest and most intensively studied patterns. Arrhenius (1921) first described the general form of the *species-area relationship (SAR)* as a power-law: $S = cA^z$ where S is species richnness and A is area. Arrhenius's formula predicts a rate of increase in richness that is approximately linear in log-log space. That is, $log(S) = c + zlog(A)$, where z is the scaling exponent.

```
# A function to generate the species-area relationship by
# Random Accumulating Sites

SAR.rand.accum <- function(com){
  Alist <- c()
  Slist <- c()

  num.ponds <- c(1,2,4,6,8,12,16,24,32,42,51)
  for (i in num.ponds) {
    areas <- c() # hold iterated area values
    Ss <- c() # hold iterated S values

    for(j in 1:50){
      pond.sample <- sample(51, replace = FALSE, size = i)
      area <- 0
      cum.abs <- vector(length = length(com[1, ]))

      for (k in pond.sample) { # Loop through each randomly drawn pond
        area <- area + pond.areas[k] # aggregating area
        cum.abs <- cum.abs + com[k, ]
        if (length(cum.abs) > length(com[1,])) {
          print('cum.abs is too long')
          break
          }
      } # End random pond samples loop

      Ss <- c(Ss, length(cum.abs[cum.abs > 0]))
      areas <- c(areas, area)
    }

    Alist <- rbind(Alist, mean(areas))
    Slist <- rbind(Slist, mean(Ss))
    print(c(mean(areas), mean(Ss)))
  }
  print(length(com[1,]))
  print(mean(Ss))
  return(cbind(log10(Alist), log10(Slist)))
}
```

```
# A function to generate the species-area relationship by
# accumulating area according to distance
```

```
SAR.accum.dist <- function(com){
  Alist <- c()
  Slist <- c()
  num.ponds <- c(1,2,4,6,8,12,16,24,32,42,51)

  for (i in num.ponds) {
    areas <- c() # hold iterated area values
    Ss <- c() # hold iterated S values

    for(j in 1:50){
      pondID <- sample(51, size = 1)
      Area <- as.numeric(pond.areas[pondID]) # aggregating area
      cum.abs <- com[pondID, ]
      used <- c()

      for (k in 2:i) { # Loop through ponds
        sdata <- subset(coord.dist.ls, FALSE == is.element(NBX, used) & FALSE == is.element(NBY, used))
        sdata <- subset(sdata, NBX == pondID | NBY == pondID)
        sdata <- subset(sdata, geo.dist == min(sdata[, 3]))

        if (dim(sdata)[1] > 1) {
          x <- sample(dim(sdata)[1], size=1)
          sdata <- sdata[x,]
          }

        sdata <- t(as.matrix(as.numeric(as.matrix(sdata))))
        used <- c(used, as.integer(pondID))
        Area <- Area + as.numeric(pond.areas[pondID]) # aggregating area
        cum.abs <- cum.abs + com[pondID, ]

        if (sdata[1] - pondID == 0) {
          pondID <- sdata[2]
        } else {
          pondID <- sdata[1]
        }
      }
      Ss <- c(Ss, length(cum.abs[cum.abs > 0]))
      areas <- c(areas, Area)
    }
    # End random pond samples loop
    Alist <- rbind(Alist, mean(areas))
    Slist <- rbind(Slist, mean(Ss))
    print(c(mean(areas), mean(Ss)))
  }
  return(cbind(log10(Alist), log10(Slist)))
}
```

RESULTS: Taxa-area relationship

```
plot.new()
par(mfrow=c(1, 1))
```

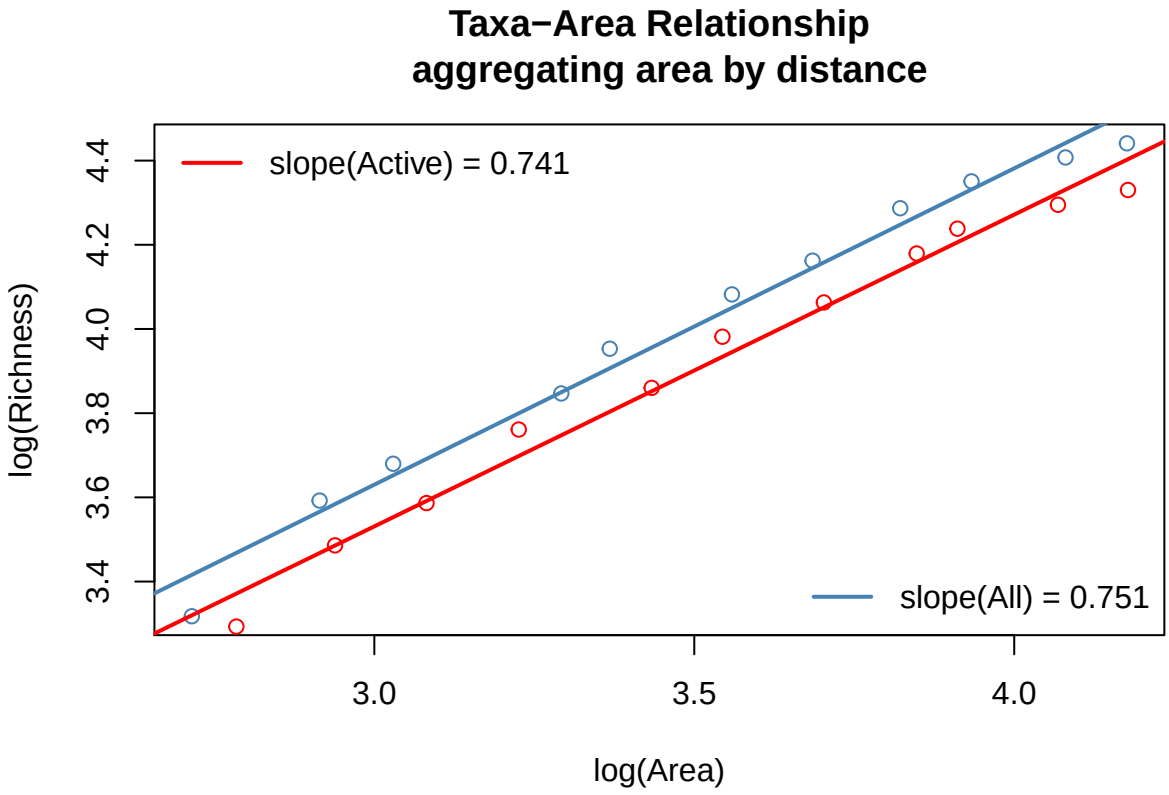
```
pond.areas <- as.vector(pi * (env$Diameter/2)^2) # Find areas of all 51 ponds

sar <- SAR.accum.dist(all.comm)
```

```
## [1]  821.1974 3912.1400
## [1]  518.5513 2077.9200
## [1] 1070.15 4784.00
## [1] 1959.507 7027.360
## [1] 2333.091 8975.840
## [1]  3621.032 12081.140
## [1]  4840.246 14530.260
## [1]  6634.64 19354.54
## [1]  8575.313 22420.520
## [1] 12025.88 25555.48
## [1] 15002.57 27602.76
```

```
sar <- as.data.frame(sar)
plot(sar, xlab = "log(Area)", ylab = "log(Richness)",
  main = "Taxa-Area Relationship
  aggregating area by distance", col = "SteelBlue")

OLS <- lm(sar$V2 ~ sar$V1)
abline(OLS, col = "SteelBlue", lw = 2)
slope <- round(coefficients(OLS)[2], 3)
  legend("bottomright", legend = paste("slope(All) =", slope),
         bty = "n", lw = 2, col = "SteelBlue")

sar <- SAR.accum.dist(active.comm)
```

```
## [1]  868.1421 3061.6000
## [1]  608.6207 1963.8400
## [1] 1206.454 3858.660
## [1] 1681.481 5769.940
## [1] 2712.867 7246.320
## [1] 3499.835 9585.720
## [1]  5041.147 11563.100
## [1]  7040.586 15113.500
## [1]  8151.015 17309.760
## [1] 11704.41 19725.22
## [1] 15057.85 21386.56
```

```
sar <- as.data.frame(sar)
points(sar, xlab = "log(Area)", ylab = "log(Richness)",
  main = "Species-Area Relationship (Active)", col = "red")

OLS <- lm(sar$V2 ~ sar$V1)
abline(OLS, col = "red", lw = 2)
slope <- round(coefficients(OLS)[2], 3)
  legend("topleft", legend = paste("slope(Active) =", slope),
         bty = "n", lw = 2, col = "red")
```




```
par(mfrow=c(1, 1))

sar <- SAR.rand.accum(all.comm)
```

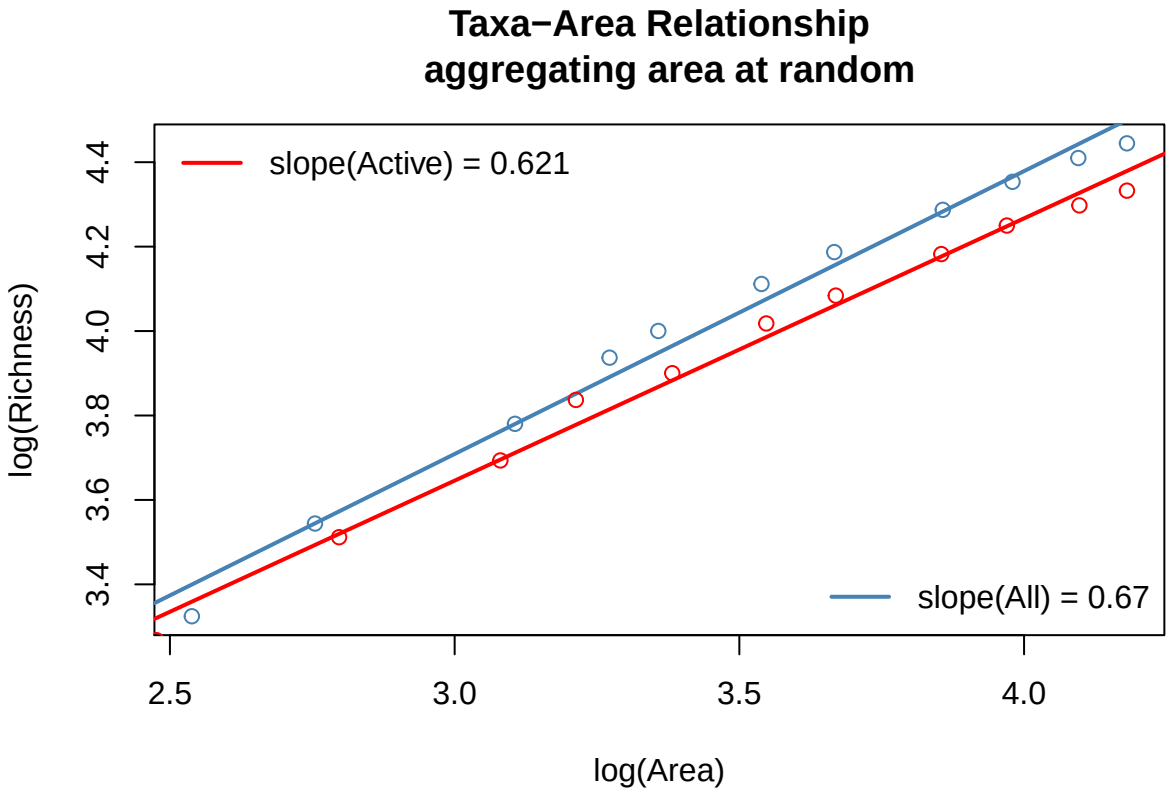
```
## [1]  345.5326 2110.8600
## [1]  568.5687 3500.0400
## [1] 1276.408 6029.040
## [1] 1870.893 8650.040
## [1]  2278.559 10004.480
## [1]  3458.432 12926.800
## [1]  4641.365 15383.960
## [1]  7198.865 19371.960
## [1]  9542.366 22570.820
## [1] 12452.17 25696.80
## [1] 15159.69 27839.00
## [1] 27839
## [1] 27839
```

```
sar <- as.data.frame(sar)
plot(sar, xlab = "log(Area)", ylab = "log(Richness)",
  main = "Taxa-Area Relationship
  aggregating area at random", col = "SteelBlue")

OLS <- lm(sar$V2 ~ sar$V1)
abline(OLS, col = "SteelBlue", lw = 2)
```

```
slope <- round(coefficients(OLS)[2], 3)
  legend("bottomright", legend = paste("slope(All) =", slope),
         bty = "n", lw = 2, col = "SteelBlue")

sar <- SAR.rand.accum(active.comm)
```

```
## [1]  299.3847 1852.6600
## [1]  626.7638 3248.5000
## [1] 1203.219 4937.460
## [1] 1632.675 6868.220
## [1] 2410.811 7946.860
## [1]  3526.118 10424.380
## [1]  4670.37 12133.02
## [1]  7156.388 15216.560
## [1]  9327.312 17779.060
## [1] 12508.08 19846.20
## [1] 15159.69 21510.00
## [1] 21510
## [1] 21510
```

```
sar <- as.data.frame(sar)
points(sar, xlab = "log(Area)", ylab = "log(Richness)",
  main = "Species-Area Relationship (Active)", col = "red")

OLS <- lm(sar$V2 ~ sar$V1)
abline(OLS, col = "red", lw = 2)
slope <- round(coefficients(OLS)[2], 3)
  legend("topleft", legend = paste("slope(Active) =", slope),
         bty = "n", lw = 2, col = "red")
```


Taxa−Area Relationship
aggregating area at random
slope(Active) = 0.621
slope(All) = 0.67
log(Richness)
3.4
3.6
3.8
4.0
4.2
4.4
2.5
3.0
3.5
4.0
log(Area)