Vietnam Caves - Bag Experiments

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OVERVIEW

Part of May 2015 mesocosm experiment on Cat Ba Island, Vietnam to investigate mechanisms of methane oxidation in cave ecosystems. General design of experiment was to conduct in situ "bag" experiments where rocks where "live" and "dead" rocks were incubated overnight. The live rock treatment contained ~10 rocks and 150 mL of water in a ~50 gallon polyethylene bag sealed tight. The dead rock treatment contained ~10 rocks and 150 mL of a 10% bleach solution. We measured methane of the air that was used to fill the bags at time zero using a Gasmet. We returned to the cave approximatley 18 hours later and measured methane in the bags at time final. In addition, we had a control, which consisted of measuring methane in a bag containing no rocks, water, or bleach. This generalized experiment was conducted in two caves: Hoa Cuong and Minh Chau.

1) SET WORKING DIRECTORY AND LOAD DATA

```
rm(list=ls())
getwd()
```

```
## [1] "/Users/lennonj/GitHub/radiolyticCH4/code/caves"
```

```
setwd("~/GitHub/radiolyticCH4")
data <- read.table("data/caves/cavebag/bagexp.txt", sep="\t", header=TRUE)
data <- data[-19,] # remove: sample "9e" = cave air measured near bag
```

2) SUBSET DATA AND T-TESTS

Hoa Cuong

```
HC <- data[(data$Cave == "Hoa_Cuong") & (data$Treat == "live"| data$Treat =="dead"),]
HC.dead <- data[(data$Cave == "Hoa_Cuong") & (data$Treat =="dead"),]
HC.means <- tapply(HC$CH4, HC$Treat, mean)
HC.cont <- data[(data$Cave == "Hoa_Cuong") & (data$Treat == "control"),]
HC.means[1] <- HC.cont$CH4
HC.means <- HC.means[c(1,3:4)]
sem <- function(x) sqrt(var(x)/length(x))
HC.sem <- tapply(HC$CH4, HC$Treat, sem)
HC.sem[1] <- 0
HC.sem <- HC.sem[c(1,3:4)]
HC.air <- data[(data$Cave == "Hoa_Cuong") & (data$Treat == "air"),]
HC.t.test <- t.test(HC$CH4 ~ HC$Treat)
HC.t.test
```

1

```
##
##  Welch Two Sample t-test
##
## data:  HC$CH4 by HC$Treat
## t = 6.395, df = 3.522, p-value = 0.004689
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.8629 2.3238
## sample estimates:
## mean in group dead mean in group live
##              1.733               0.140
```

```r
# fold decrease
HC.fold <- mean(HC.means[1],HC.means[2])/HC.means[3]
# percent decrease
HC.per <- (mean(HC.means[1],HC.means[2])-HC.means[3])/mean(HC.means[1],HC.means[2])
# test dead vs. control
HC.t.test.dead <- t.test(HC.dead$CH4, mu = HC.cont$CH4)
```

Minh Chau

```r
MC <- data[(data$Cave == "Minh_Chau") & (data$Treat == "live"| data$Treat =="dead"),]
MC.dead <- data[(data$Cave == "Minh_Chau") & (data$Treat =="dead"),]
MC.means <- tapply(MC$CH4, MC$Treat, mean)
MC.cont <- data[(data$Cave == "Minh_Chau") & (data$Treat == "control"),]
MC.means[1] <- MC.cont$CH4
MC.means <- MC.means[c(1,3:4)]
sem <- function(x) sqrt(var(x)/length(x))
MC.sem <- tapply(MC$CH4, MC$Treat, sem)
MC.sem[1] <- 0
MC.sem <- MC.sem[c(1,3:4)]
MC.air <- data[(data$Cave == "Minh_Chau") & (data$Treat == "air"),]
MC.t.test <- t.test(MC$CH4 ~ MC$Treat)
MC.t.test
```

```
##
##  Welch Two Sample t-test
##
## data:  MC$CH4 by MC$Treat
## t = 10.3, df = 2.126, p-value = 0.00753
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  1.250 2.883
## sample estimates:
## mean in group dead mean in group live
##             2.5100             0.4433
```

```r
# fold decrease
MC.fold <- mean(MC.means[1],MC.means[2])/MC.means[3]
# percent decrease
MC.per <- (mean(MC.means[1],MC.means[2])-MC.means[3])/mean(MC.means[1],MC.means[2])
```

```r
# test dead vs. control
MC.t.test.dead <- t.test(MC.dead$CH4, mu = MC.cont$CH4)
```

Percent reduction in live bags

```r
HC.live <- HC[(HC$Treat == "live"),]
HC.red.1 <- (mean(HC.dead[,5]) - HC.live[1,5])/mean(HC.dead[,5])
HC.red.2 <- (mean(HC.dead[,5]) - HC.live[2,5])/mean(HC.dead[,5])
HC.red.3 <- (mean(HC.dead[,5]) - HC.live[3,5])/mean(HC.dead[,5])

MC.live <- MC[(MC$Treat == "live"),]
MC.red.1 <- (mean(MC.dead[,5]) - MC.live[1,5])/mean(MC.dead[,5])
MC.red.2 <- (mean(MC.dead[,5]) - MC.live[2,5])/mean(MC.dead[,5])
MC.red.3 <- (mean(MC.dead[,5]) - MC.live[3,5])/mean(MC.dead[,5])

re.mean <-mean(c(HC.red.1, HC.red.2, HC.red.3, MC.red.1, MC.red.2, MC.red.3))
re.sem <- sem(c(HC.red.1, HC.red.2, HC.red.3, MC.red.1, MC.red.2, MC.red.3))
```

3) PLOT DATA - Hoa Cuong and Mingh Chau

```r
#```{r}
png(filename="~/GitHub/radiolyticCH4/figures/cave.bags.png", width = 1500,
    height = 900, res = 96*2)

plot.new()
layout <- layout(cbind(1, 2), height = c(1, 1))
par(mar = c(6, 0.5, 5, 0.5))
par(oma = c(1, 5, 1, 2))

HC.plot <- plot(HC.means, ylim = c(0, 3), xlim = c(0.5, 3.5),
     main = "Hoa Cuong", pch = 22, bg = "white", lwd = 2, cex = 3, yaxt = "n", xaxt = "n",
     cex.lab = 2, cex.axis = 1.5, las = 1, ylab = "", xlab = "")
     box(lwd = 2)

#Add standard errors
arrows(x0 = 2:3, y0 = HC.means[2:3], y1 = HC.means[2:3] - HC.sem[2:3], angle = 90,
   length = 0.05, lwd = 2)
 arrows(x0 = 2:3, y0 = HC.means[2:3], y1 = HC.means[2:3] + HC.sem[2:3], angle = 90,
   length = 0.05, lwd = 2)

# Add y-label
mtext(expression('CH'[4]*' (ppm)'), side = 2, outer = TRUE, cex = 1.75,
      line = 2, adj = 0.55)

# Add p-value for t-test
mtext(expression(~italic("P")~"= 0.005"), line = -1.25, cex = 1, at = 0.9)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.5, las = 1,
```

```r
        labels = c(0, 1, 2, 3), at = c(0, 1, 2, 3))

#axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
#     at=c(0, 1, 2, 3), labels = F)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    labels = c("control", "dead", "live"), at = c(1, 2, 3))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3), labels = F)

# Add atmosphere line
abline(h = HC.air$CH4, col = "black", lty = 3, lwd = 2)

# Add Ming Chau panel to the plot

MC.plot <- plot(MC.means, ylim = c(0, 3), xlim = c(0.5, 3.5),
       main = "Minh Chau", pch = 22, bg = "white", lwd = 2, cex = 3, yaxt = "n", xaxt = "n",
       cex.lab = 2, cex.axis = 1.5, las = 1, ylab = "", xlab = "")
       box(lwd = 2)

#Add standard errors
arrows(x0 = 2:3, y0 = MC.means[2:3], y1 = MC.means[2:3] - MC.sem[2:3], angle = 90,
   length = 0.05, lwd = 2)
 arrows(x0 = 2:3, y0 = MC.means[2:3], y1 = MC.means[2:3] + MC.sem[2:3], angle = 90,
   length = 0.05, lwd = 2)

# Add p-value for t-test
mtext(expression(~italic("P")~"= 0.008"), line = -1.25, cex = 1, at = 0.9)

# Major Axes
#axis(side = 2, lwd.ticks = 2, cex.axis = 1.5, las = 1,
#     at=c(0, 1, 2, 3), labels = F)

axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at=c(0, 1, 2, 3), labels = F)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    labels = c("control", "dead", "live"), at = c(1, 2, 3))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3), labels = F)

# Add atmosphere line
abline(h = MC.air$CH4, col = "black", lty = 3, lwd = 2)

dev.off() # this writes plot to folder
```

```
## pdf
##   2
```

```r
graphics.off() # shuts down open devices
```

5) RATES OF CH4 OXIDATION

Use concentraiton data, time of incbuation, and rock surface area to estimate CH4 oxidation rates per surface area rock in the bags.

```r
# Load surface area data (cm2)
sa.cm2 <- read.table("~/GitHub/radiolyticCH4/data/caves/cavebag/surface.area.txt",
                sep="\t", header=TRUE)
sa.cm2 <- read.table("~/GitHub/radiolyticCH4/data/caves/cavebag/surface.area.final.txt",
                sep="\t", header=TRUE)
names(sa.cm2)[3]<-paste("sa.cm2")
sa.conv <- 10000 # cm2 to m2 conversion
sa.m2 <- sa.cm2[,3]/sa.conv # create m2 data
sa.data <- cbind(sa.cm2, sa.m2) # bind data

# Experiment durations
HC.time <- 18/24 # hr/hr = d
MC.time <- 18/24 # hr/hr = d

# Conversion factors
gas.mol <- 22.4 # L occupied by 1 mole of gas
bag.vol <- 200 # L; operating volume of bags
ppm.conv <- 10^-6
m.umol.conv <- 10^6
gfw.ch4 <- 16.042

# Rate calculations

# X (M) = (x ppm * 10^-6/ 1 L) * (1 mole / 22.4 L) * (bag vol, L)
HC.diff.ppm <- (data[(data$Cave == "Hoa_Cuong") & data$Treat == "live",]$CH4 - HC.cont$CH4)
HC.diff.umol <- (HC.diff.ppm * ppm.conv) * (1/gas.mol) * bag.vol * m.umol.conv
HC.rate.umol <- HC.diff.umol / sa.data[sa.data$cave == "HC",]$sa.m2 / HC.time
HC.rate.mg <- HC.rate.umol * gfw.ch4 / 1000

MC.diff.ppm <- (data[(data$Cave == "Minh_Chau") & data$Treat == "live",]$CH4 - MC.cont$CH4)
MC.diff.umol <- (MC.diff.ppm * ppm.conv) * (1/gas.mol) * bag.vol * m.umol.conv
MC.rate.umol <- MC.diff.umol / sa.data[sa.data$cave == "MC",]$sa.m2 / MC.time
MC.rate.mg <- MC.rate.umol * gfw.ch4 / 1000

rate.range <- abs(c(max(HC.rate.mg, MC.rate.mg), min(HC.rate.mg, MC.rate.mg)))
```