## E8: Part1

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**Filename: E8\_1.Rmd' This markdown file does the following tasks:** 0. Clean and merge 1. Q1: How does invader biomass vary across resource availability in the reference plots?

```
knitr::opts_chunk$set(cache=TRUE)
library(plyr)
library(reshape2)
library(ggplot2) #plotting
library(ggthemes) #ggplot accessories
library(GGally) #????
library(gridExtra) #for grid.arrange fxn
library(lme4) #for mixed-effects models
## Loading required package: Matrix
library(lmerTest) #for lmer p-values
##
## Attaching package: 'lmerTest'
## The following object is masked from 'package:lme4':
##
##
       lmer
##
## The following object is masked from 'package:stats':
##
##
       step
library(ggbiplot) #for pretty biplots
## Loading required package: scales
## Loading required package: grid
library(vegan) #for adonis fxn to do perMANOVAs
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.3-1
source('CODE/mytheme.R')
source('CODE/fxn_FitPlot.R')
figuresPath<-file.path(getwd()[1], "FIGURES_TABLES") #where to put the saved plots
```

```
fig.height<-2.5 #inches
fig.width<- 2.5 #inches
fig.res<-300

synthdataPath<-file.path(getwd()[1], "DATA", "DATA_SYNTHESIZED") #where to put the clean dataframes

soilData<-read.table("DATA/e8_plothalfSoilData.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)
vegData<-read.table("DATA/e8_plothalfVegData.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)
plotLoc<-read.table("DATA/e8_plotLoc.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)
plotTrees<-read.table("DATA/e8_plotTrees.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)

soilData.dict<-read.table("DATA/e8_plothalfSoilData_dictionary.txt", header=TRUE, sep="\t", stringsAsFactors
plotLoc.dict<-read.table("DATA/e8_plotLoc_dictionary.txt", header=TRUE, sep="\t", stringsAsFactors
plotLoc.dict<-read.table("DATA/e8_plotLoc_dictionary.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)

soilData.dict<-read.table("DATA/e8_plotLoc_dictionary.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)

soilData.dict<-read.table("DATA/e8_plotLoc_dictionary.txt", header=TRUE, sep="\t", stringsAsFactors = FALSE)
```

### 0. Clean and merge

Add identifiers, remove extra columns, merge raw datasets Deal with soil N data in years where 0-5cm and 5-15cm layers were measured separately.

```
#############
#A. Clean and merge
##############
# SOIL DATA
soilData$plothalfid1<-paste(soilData$inv,soilData$plotid, sep="_") #add new identifiers
removeCols<-c('plothalfid','site','rep') #prune columns</pre>
soilData.pruned<-soilData[,!colnames(soilData) %in% removeCols]</pre>
#reshape so that each depth x meas has its own column
soilData_melted <- melt(soilData.pruned,</pre>
                         id.vars=c("plotid","plothalfid1",
                                   "inv", "depth", "year"),
                         variable.name = "measCat")
soilData melted$measCat2<-paste(soilData melted$measCat, soilData melted$depth, sep = ' ')</pre>
soilData_cast <- dcast(soilData_melted, plotid + plothalfid1 + inv + year ~ measCat2, value.var="value"</pre>
soilData c<-soilData cast
# VEG DATA
vegData$plothalfid1<-paste(vegData$inv,vegData$plotid, sep="_") #add new identifiers</pre>
vegData.pruned<-vegData[,!colnames(vegData) %in% removeCols] #prune columns
vegData.pruned$total<-vegData.pruned$mv+vegData.pruned$nat #make a total understory biomass variable
vegData.dict[c(2,4),c('v7','v8','v9')]
##
                                 v9
            ν7
                       ν8
            mv
                      nat
## 4 g/.1875m2 g/.1875m2 g/.1875m2
```

```
paste('Dry biomass values are currently in units of g/.1875m2')
## [1] "Dry biomass values are currently in units of g/.1875m2"
ConvertBiom<-function(currVal){newVal <- currVal / 0.1875}</pre>
vegData.pruned$mv g.m2<-ConvertBiom(vegData.pruned$mv)</pre>
vegData.pruned$nat_g.m2<-ConvertBiom(vegData.pruned$nat)</pre>
vegData.pruned$litter g.m2<-ConvertBiom(vegData.pruned$litter)</pre>
vegData.pruned$total_g.m2<-ConvertBiom(vegData.pruned$total)</pre>
colsOldUnits<-c('mv','nat','litter','total')</pre>
vegData_c<-vegData.pruned[,!colnames(vegData.pruned) %in% colsOldUnits] #prune columns</pre>
#add veqData
soilVegData<-merge(soilData_c, vegData_c) #merge soilData_cast and vegData
plotTrees$basalArea.m2<-(plotTrees$dbh * plotTrees$dbh) * 0.00007854 #calculate basal area/m2 from each
#summarize the total basal area/m2 per plot and that which is made up by either AM- or ECM-associated t
plotTrees.summ<-ddply(plotTrees, ~plotid, summarize,</pre>
                      nTrees=length(plotid),
                      BA_total=sum(basalArea.m2, na.rm=T),
                       BA_AM=sum(basalArea.m2[myc=='A'], na.rm=T),
                       BA ECM=sum(basalArea.m2[myc=='E'], na.rm=T),
                       PercBA_AM=(BA_AM/BA_total)*100,
                      PercBA_ECM=(BA_ECM/BA_total)*100)
#update the number of trees (there was a cell that was counted even for plots where there were no trees
plotTrees.summ[plotTrees.summ$plotid %in% c(12,15),'nTrees']<-0</pre>
plotTrees.summ[plotTrees.summ*plotid %in% c(12,15),c('PercBA_AM','PercBA_ECM')]<-NA
tmp<-plotTrees.summ[,c('plotid','nTrees','BA_total','PercBA_AM')]</pre>
trees_c<-tmp
#add tree data
data<-merge(soilVegData, trees_c)</pre>
#get rid of the plots without trees
data<-data[data$nTrees != 0,]</pre>
#str(data)
#############
#B. Deal with soil N data in years where 0-5cm and 5-15cm layers were measured separately
#############
# Plot histogram of values by depth
#subset by year
data.1213 <- subset (data, year %in% c(2012, 2013))
#reshape
data.1213_melted <- melt(data.1213,id.vars=c("plotid","plothalfid1","inv","year"))</pre>
```

data.1213\_melted\_soilVars<-data.1213\_melted[grepl("\_B",data.1213\_melted\$variable) | grepl("\_T",data.121

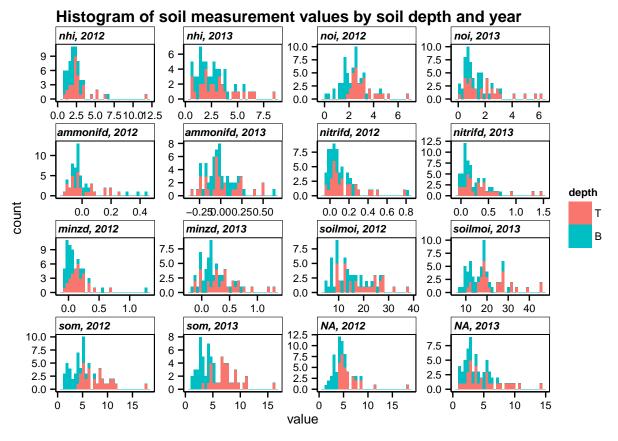
data.1213\_melted\$value<-as.numeric(data.1213\_melted\$value)</pre>

#subset by B and T

```
#add back the separate measCat and depth columns
tmp<-ldply(strsplit(as.character(data.1213_melted_soilVars$variable),"_", fixed=T))
colnames(tmp)<-c('measCat','depth')
data.1213_melted_soilVars<-cbind(data.1213_melted_soilVars, tmp)

#correct the dataframe structure
data.1213_melted_soilVars$measCat<-factor(data.1213_melted_soilVars$measCat, levels=measCat_order)
data.1213_melted_soilVars$depth<-factor(data.1213_melted_soilVars$depth, levels=depth_order)
data.1213_melted_soilVars$inv<-factor(data.1213_melted_soilVars$inv, levels=inv_order)

#plot
PHist.depth<-ggplot(data.1213_melted_soilVars, aes(x=value, fill=depth)) +
    geom_bar() + mytheme + facet_wrap(measCat~year, scales='free') +
    ggtitle("Histogram of soil measurement values by soil depth and year")
pHist.depth</pre>
```



```
#save plot
newfilename<-'pHist_depth.png'
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*4, height = fig.height*4, re
pHist.depth
dev.off()</pre>
```

## pdf ## 2

```
#############
#C. DATASET A : Aggregate values, put aggregated values into full dataset and replace empty _F rows
##############
#aggregate by depth
summ.data.1213_melted_soilVars<-ddply(data.1213_melted_soilVars, ~plotid+plothalfid1+inv+year+measCat,
                     new.value=mean(value, na.rm=T),
                     n=length(plothalfid1),
                     note='aggregated by depth')
#make a dataset without the dis-aggregated data
data_melted <- melt(data,id.vars=c("plotid", "plothalfid1", "inv", "year")) #melt down the full dataset, i
data_melted$value<-as.numeric(data_melted$value) #make values numeric again
uniqWo.B<-unique(data_melted$variable)[!grep1("_B",unique(data_melted$variable))] #isolate only the var
uniqWo.BT<-uniqWo.B[!grepl("_T",uniqWo.B)] #ditto
data_m_woBT<-data_melted[data_melted$variable %in% uniqWo.BT,] #subset data by 'correct' variables
selectvars<-unique(data_m_woBT$variable)[grepl("_F",unique(data_m_woBT$variable))] #select measCat vari
data_m_woBT_e<-data_m_woBT[!(data_m_woBT$year %in% c(2012,2013) & data_m_woBT$variable %in% selectvars)
data_m_woBT_e$note<-NA
\textit{\#make a dataset with the correctly-aggregated data and comparable column names to data\_m\_woBT
summ.data.1213_melted_soilVars<-rename(summ.data.1213_melted_soilVars, replace=c("new.value" = "value")</pre>
summ.data.1213_melted_soilVars$variable<-paste(summ.data.1213_melted_soilVars$measCat, '_F',sep="")#mea
summ.data.1213_melted_soilVars<-summ.data.1213_melted_soilVars[,c('plottid','plothalfid1','inv','year','
#merge
data_a<-rbind(data_m_woBT_e, summ.data.1213_melted_soilVars)</pre>
#ddply(data_a, ~inv+variable, summarize, n=length(plotid)) #check the dataframe structure
#add a column that categorizes the variable types
data_a$varType<-NA
data_a[grepl("_F", data_a$variable),'varType']<-'measCat'</pre>
data_a[data_a$variable %in% c('mv_g.m2','nat_g.m2','litter_g.m2','total_g.m2'),'varType']<-'understoryB
data_a[data_a$variable %in% c('percpar','soiltemp','soilBasin'),'varType']<-'environParam'
data_a[data_a$variable %in% c('nTrees','BA_total','PercBA_AM'),'varType']<-'overstoryParam'</pre>
#add a column that identifies whether the variable type is measured on the plot half or the plot level
plotlevel.vars<-c('soilBasin','nTrees','BA_total','PercBA_AM')</pre>
plothalflevel.vars<-unique(data_a$variable)[!unique(data_a$variable) %in% plotlevel.vars]
data a$measLevel<-NA
data_a[data_a$variable %in% plotlevel.vars,'measLevel']<-'plot'</pre>
data_a[data_a$variable %in% plothalflevel.vars, 'measLevel'] <- 'plothalf'
\#Ammend\ variable\ names\ with\ "\_N"\ or\ "\_I"\ depending\ on\ the\ plothalf
data_a$variable1<-paste(data_a$variable, data_a$inv, sep="_")</pre>
data_a[data_a$measLevel=='plot','variable1'] <-as.character(data_a[data_a$measLevel=='plot','variable'])
#save and export
newfilename<-'data a.txt'
write.table(data_a, file=paste(synthdataPath,newfilename, sep='/'), sep='\t')
```

```
#############
#D. DATASET D : Keep 2012 and 2013 values dis-aggregated and remove 2011 _F values, remove biomass valu
#############
#start with data.1213 melted
data_d<-data.1213_melted[!grepl("_F", data.1213_melted$variable),] #remove variables that were only mea
data_d<-data_d[data_d$variable != 'soiltemp',] #remove variables that were only measured in 2011
#add a column that categorizes the depth types
data_d$depth<-NA
data_d[grepl("_B", data_d$variable),'depth']<-'B'</pre>
data_d[grepl("_T", data_d$variable),'depth']<-'T'</pre>
#add a column that categorizes the variable types
data_d$varType<-NA
data_d[grepl("_B", data_d$variable), 'varType'] <- 'measCat'</pre>
data_d[grepl("_T", data_d$variable), 'varType'] <- 'measCat'</pre>
data_d[data_d$variable %in% c('mv_g.m2', 'nat_g.m2', 'litter_g.m2', 'total_g.m2'), 'varType']<-'understoryB
data_d[data_d$variable %in% c('percpar'), 'varType']<-'environParam'</pre>
data_d[data_d$variable %in% c('nTrees','BA_total','PercBA_AM'),'varType']<-'overstoryParam'</pre>
#add a column that identifies whether the variable type is measured on the plot half or the plot level
plotlevel.vars<-c('nTrees','BA_total','PercBA_AM')</pre>
plothalflevel.vars<-unique(data_d$variable)[!unique(data_d$variable) %in% plotlevel.vars]
data_d\\measLevel < -NA
data_d[data_d$variable %in% plotlevel.vars, 'measLevel'] <- 'plot'</pre>
data_d[data_d$variable %in% plothalflevel.vars, 'measLevel'] <- 'plothalf'
#Ammend variable names with "_{\rm N}" or "_{\rm I}" depending on the plothalf
data_d$variable1<-paste(data_d$variable, data_d$inv, sep="_")
data_d[data_d$measLevel=='plot','variable1']<-as.character(data_d[data_d$measLevel=='plot','variable'])
#save and export
newfilename<-'data_d.txt'
write.table(data_d, file=paste(synthdataPath,newfilename, sep='/'), sep='\t')
#######
# DATA CHOICE : decide which dataset to use
data.choice<-data a
data.choice<-subset(data.choice, year != 2011)</pre>
#######
```

# 1. Q1: Do soil N pools and fluxes shift in response to the presence of Microstegium?

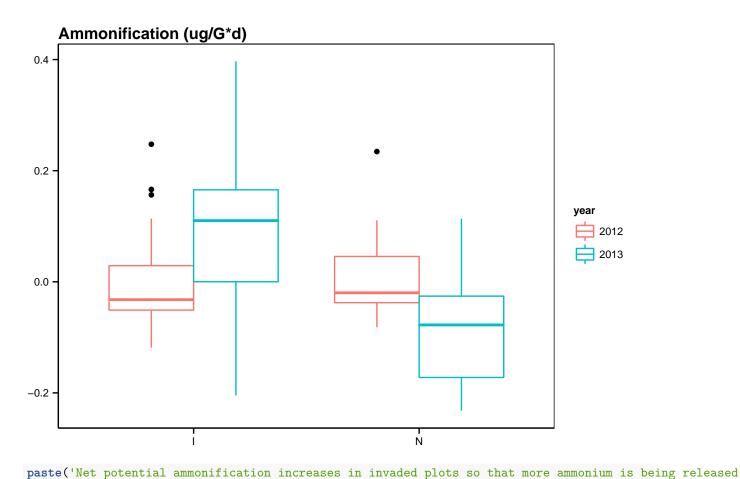
A. Do an ordination of soil N pools and fluxes. B. Test the role of invasion status with permMANOVA C. Investigate individual relationships using mixed effects models with year+site as a random effect. D. Identify N variables that increase/decrease across sites.

```
#############
#A. Do an ordination of soil N pools and fluxes.
##############
#1) Name the plot variable to ordinate
vars<-c('nhi_F','noi_F','ammonifd_F','nitrifd_F','minzd_F') #make vector of terms
varNames<-c('NH4+','NO3-','Ammonif.','Nitrif.','Mineraliz.')</pre>
#2) Subset and reshape
data.vars<-data.choice[data.choice$variable %in% vars, c('plotid','plothalfid1','inv','year','variable'
data.vars$variable<-factor(data.vars$variable, levels=vars)</pre>
data.vars.wide<-dcast(data.vars, plothalfid1 + inv+ year ~ variable, value.var='value')
data.vars.wide$plotInvYear<-paste(data.vars.wide$plothalfid1, data.vars.wide$year, sep="_") #use this t
data.vars.wide$plotYear<-paste(data.vars.wide$plotid, data.vars.wide$year, sep="_") #use this to block
row.names(data.vars.wide)<-data.vars.wide$plotInvYear</pre>
NonValCols<-c('plothalfid1','plotInvYear', 'plotYear','inv','year')
ValCols<-colnames(data.vars.wide)[!(colnames(data.vars.wide) %in% NonValCols)]
#3) Remove rows with missing data and look at correlation matrix
data.vars.wide.rm<-data.vars.wide[!rowSums(is.na(data.vars.wide))>0,]
cor(data.vars.wide.rm[,ValCols])
##
                    nhi F
                                minzd F
## nhi_F
               1.00000000 0.51023042 -0.1704322 0.23675304 0.06605252
               0.51023042 1.00000000 -0.0651940 0.04729761 -0.04983418
## noi_F
## ammonifd_F -0.17043223 -0.06519400 1.0000000 -0.31729409 0.31270435
## nitrifd_F 0.23675304 0.04729761 -0.3172941 1.00000000 0.79373804
## minzd_F
               0.06605252 -0.04983418  0.3127044  0.79373804  1.00000000
#4) Ordinate and save plot
df.ord<-data.vars.wide.rm[,ValCols]</pre>
colnames(df.ord)<-varNames</pre>
df.ord.scaled <-scale(df.ord)
PCA.res<-pre>prcomp(df.ord.scaled)
pPCA<-ggbiplot(PCA.res, groups=data.vars.wide.rm$inv, ellipse=T) + mytheme
newfilename<-'pPCA_invPlot.png'</pre>
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*2, height = fig.height*2, re
pPCA
dev.off()
## pdf
##
#5) Make a dataframe of the pc scores
data.PCA<-data.frame(plotInvYear=rownames(PCA.res$x), PCA.res$x)</pre>
data.vars.PCA<-merge(data.vars.wide.rm, data.PCA[,c(1:3)])</pre>
##############
#B. Test the role of invasion status with permMANOVA
##############
sum(rownames(data.vars.wide.rm) != rownames(df.ord.scaled)) # if this is 0, then rownames match
```

```
## [1] 0
```

```
permMANOVA<-adonis(df.ord.scaled ~ inv, data = data.vars.wide.rm, method='eu')
aov.tab.permmanova<-data.frame(permMANOVA$aov.tab)</pre>
aov.tab.permmanova
                                                        R2 Pr..F.
##
             Df SumsOfSqs MeanSqs F.Model
## inv
                   9.419705 9.419705 1.911189 0.02990382 0.104
                                            NA 0.97009618
## Residuals 62 305.580295 4.928714
             63 315.000000
                                            NA 1.0000000
## Total
                                  NA
                                                                NA
newfilename<-'permMANOVA_invPlot.txt'
write.table(aov.tab.permmanova, file=paste(figuresPath,newfilename, sep='/'), sep='\t')
##############
#C. Investigate individual relationships using mixed effects models with year+site as a random effect.
#############
#1) Make a fxn to run the models with invasion status predicting different y variables
InvEffect<-function(Yvars, YvarNames, df){</pre>
  anova.list<-list()</pre>
  figure.list<-list()
  i<-0
  for(i in 1:length(Yvars)){
    curr.Yvar<-Yvars[i]</pre>
    y<-df[,curr.Yvar]
    #regression model
    mod<-lmer(y~inv + (1|plotYear), data=df)</pre>
    anova.list[[i]]<-anova(mod)</pre>
    #plot
    df$year<-factor(df$year)</pre>
    p<-ggplot(df, aes_string(x='inv', y=curr.Yvar, color='year')) + geom_boxplot() +
      mytheme + xlab(NULL) + ylab(NULL) + ggtitle(YvarNames[i])
    figure.list[[i]]<-p
  names(anova.list)<-Yvars</pre>
  names(figure.list)<-Yvars</pre>
  #anova summary
  anovaSummary<-ldply(lapply(anova.list, data.frame))</pre>
  anovaSummary[,-1]<-round(anovaSummary[,-1], digits=4)</pre>
  anovaSummary<-rename(anovaSummary, c(.id='yVar', Pr..F.='pValue'))</pre>
 results<-list(anovaSummary=anovaSummary, figures=figure.list)</pre>
 return(results)
}
```

```
#2) Run models
result<-InvEffect(Yvars=c('noi_F', 'nitrifd_F', 'ammonifd_F'),</pre>
                  YvarNames=c('Nitrate (ug/G)','Nitrification (ug/G*d)','Ammonification (ug/G*d)'),
                  df=data.vars.PCA)
#3) Save anova summary table
data.frame(result[['anovaSummary']])
           yVar Sum.Sq Mean.Sq NumDF
                                       DenDF F.value pValue
          noi F 0.3584 0.3584
## 1
                                   1 61.0000 0.3832 0.5382
## 2 nitrifd_F 0.0049 0.0049
                                   1 61.0000 0.1424 0.7072
## 3 ammonifd_F 0.1215 0.1215
                                   1 62.0001 8.0397 0.0062
newfilename<-'anovaSummary_inv.txt'
write.table(data.frame(result[['anovaSummary']]), file=paste(figuresPath,newfilename, sep='/'), sep='\t
#4) Plot
#grid.arrange(do.call(arrangeGrob, result[['figures']])) #all panels
ylabel<-textGrob("Plot variable", rot=90)</pre>
xlabel<-textGrob("Invasion status")</pre>
newfilename<-'pScat inv.png'</pre>
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*1.5, height = fig.height*3,
grid.arrange(
  ylabel, # topleft
  do.call(arrangeGrob, result[['figures']]),
  textGrob(" "), #bottom left
  xlabel, #bottom right
  widths = unit.c(unit(2.5, "lines"), unit(1, "npc") - unit(2.5, "lines")),
  heights = unit.c(unit(1, "npc") - unit(2.5, "lines"), unit(2.5, "lines")),
  nrow=2, ncol=2
dev.off()
## pdf
##
#D. Identify N variables that increase/decrease across sites.
#############
result[['figures']]$ammonifd_F
```



## [1] "Net potential ammonification increases in invaded plots so that more ammonium is being released

## [1] "Although net ammonification increases in invaded plots, ammonium pools do not differ. This ma

# 2. Q2: Do reference plot conditions and/or Microstegium biomass predict impact magnitudes?

paste('Although net ammonification increases in invaded plots, ammonium pools do not differ.

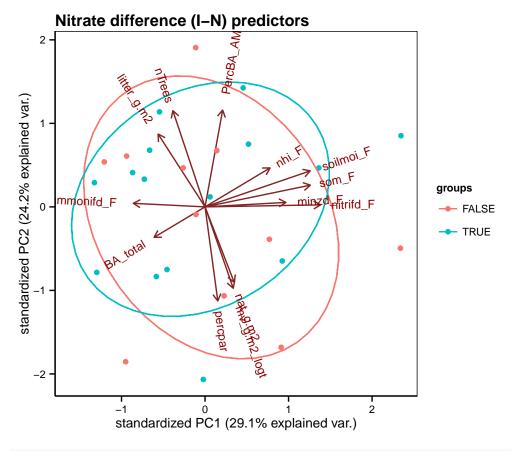
A. Calculate impact magnitude for each target variable (nitrate, nitrification, other variables detected in Q1 ordination) B. Test the role of reference plot conditions and Microstegium biomass on impacts individually using mixed effects models with year as a random effect

```
#calculate diff
data.vars.wideIN$Diff<-data.vars.wideIN$I - data.vars.wideIN$N</pre>
#subset select diff variables and reshape
diffVars<-c('noi_F','nitrifd_F','ammonifd_F')</pre>
data.diffVars<-data.vars.wideIN[data.vars.wideIN$variable %in% diffVars, c('plotid','year','variable','
data.diffVars.wide<-dcast(data.diffVars, plotid + year ~ variable, value.var='Diff')</pre>
colnames(data.diffVars.wide)[-c(1:2)] <-paste('Diff', colnames(data.diffVars.wide)[-c(1:2)], sep=" ")
##############
#B. Test the role of reference plot conditions and Microstegium biomass on impacts individually using m
#############
#1. Identify reference plot condition variables
refVars<-c('nhi_F', 'noi_F', 'ammonifd_F', 'nitrifd_F', 'minzd_F', 'soilmoi_F', 'som_F',
        'nat_g.m2','litter_g.m2','percpar','nTrees','BA_total','PercBA_AM')
data.refVars<-data.choice[data.choice$variable %in% refVars, c('plotid', 'plothalfid1', 'inv', 'year', 'var
data.refVars$variable<-factor(data.refVars$variable, levels=refVars)</pre>
data.refVars<-subset(data.refVars, inv == 'N')</pre>
data.refVars.wide<-dcast(data.refVars, plotid + year ~ variable, value.var='value')</pre>
#2. Identify microstegium biomass
mvVar<-c('mv_g.m2')</pre>
data.mvVar<-data.choice[data.choice$variable %in% mvVar, c('plotid', 'plothalfid1', 'inv', 'year', 'variabl
data.mvVar<-subset(data.mvVar, inv == 'I')</pre>
data.mvVar.wide<-dcast(data.mvVar, plotid + year ~ variable, value.var='value')
pHist.mv<-ggplot(data.mvVar.wide, aes(x=log(mv_g.m2)))+geom_histogram() #log-transform mv biomass to im
data.mvVar.wide$mv_g.m2_logt<-log(data.mvVar.wide$mv_g.m2)</pre>
#3. Merge the reference plot condition columns and the microstegium biomass columns
data.q2.indp<-merge(data.refVars.wide, data.mvVar.wide)</pre>
#4. Merge the impact variables and the independant variables
data.q2<-merge(data.diffVars.wide, data.q2.indp)</pre>
data.q2$plotYear<-paste(data.q2$plotid,data.q2$year, sep="_")</pre>
#5. Make a fxn to run the models with reference conditions (excluding linked response variable) and Mic
#testing
#diffVarShort='nitrifd_F'; refVars=refVars; mvVar='mv_g.m2_logt'; df=data.q2
ExplainDiffVar<-function(diffVarShort, refVars, mvVar, df){</pre>
  #identify variables
  select.diffVar<-paste('Diff',diffVarShort, sep='_')</pre>
  y<-df[,select.diffVar]
  select.refVars<-refVars[!refVars %in% diffVarShort]</pre>
  xVars<-c(select.refVars, 'mv_g.m2_logt')</pre>
  #individual model for each reference condition
  anova.list<-list()</pre>
  pBivar.list<-list()</pre>
```

```
for(i in 1:length(xVars)){
    #identify current dataframe
    curr.Xvar<-xVars[i]
    x<-df[,curr.Xvar]
    df.i<-data.frame(x, y, year=df$year)</pre>
    df.i.rm<-df.i[!rowSums(is.na(df.i)),]</pre>
    df.i.rm$year<-factor(df.i.rm$year)</pre>
    #regression model
    mod<-lmer(y~x + (1|year), data=df.i.rm)</pre>
    anova.list[[i]]<-anova(mod)</pre>
    #bivariate plot
    p<-ggplot(df.i.rm, aes(x=x, y=y, color=year)) + geom_point() + mytheme +
      xlab(curr.Xvar) + ylab(select.diffVar)
    pBivar.list[[i]]<-p
  #save anovaSummary
  names(anova.list)<-xVars</pre>
  anovaSummary<-ldply(lapply(anova.list, data.frame))</pre>
  anovaSummary[,-1]<-round(anovaSummary[,-1], digits=4)</pre>
  anovaSummary<-rename(anovaSummary, c(.id='yVar', Pr..F.='pValue'))</pre>
  anovaSummary
  #save plots
  names(pBivar.list)<-xVars</pre>
  #full model multivariate
  select.df<-data.frame(df[,c('plotYear','year')],Diff=df[,select.diffVar],df[,c(select.refVars,mvVar)]</pre>
  select.df.rm<-select.df[!rowSums(is.na(select.df))>0,]
  select.df.rm$DiffColor<-factor(select.df.rm$Diff>0)
  select.df.rm.scaled <-scale(select.df.rm[,!colnames(select.df.rm) %in% c('DiffColor','plotYear', 'year
  row.names(select.df.rm.scaled)<-select.df.rm$plotYear</pre>
  #make ordination
  PCA.res<-pre>prcomp(select.df.rm.scaled)
  pPCA<-ggbiplot(PCA.res, group=select.df.rm$DiffColor, ellipse = T) + mytheme + ggtitle(diffVarShort)
  #test permanova
  data.PCA<-data.frame(plotYear=rownames(PCA.res$x), PCA.res$x)</pre>
  data.vars.PCA<-merge(select.df.rm, data.PCA[,c(1:3)])</pre>
  permMANOVA<-adonis(select.df.rm.scaled ~ Diff, data = data.vars.PCA, method='eu')</pre>
  aov.tab.permmanova<-data.frame(permMANOVA$aov.tab)</pre>
  results <-list(anovaSummary=anovaSummary, permAOV=aov.tab.permmanova, pPCA=pPCA, pBivar.list=pBivar.li
  return(results)
}
#A. Predict nitrate diff
result <- ExplainDiff Var (diff Var Short = 'noi_F', ref Vars = ref Vars, mv Var = 'mv_g.m2_logt', df = data.q2)
result[['anovaSummary']] #save
```

```
##
              yVar Sum.Sq Mean.Sq NumDF DenDF F.value pValue
                                           30
## 1
             nhi_F 0.0812 0.0812
                                      1
                                               0.1395 0.7114
                          0.0839
## 2
        ammonifd F 0.0839
                                               0.1441 0.7069
## 3
        nitrifd_F 0.0811
                          0.0811
                                           30
                                               0.1394 0.7115
                                      1
## 4
           minzd_F 0.2143
                          0.2143
                                      1
                                               0.3711 0.5470
## 5
                                           30 0.2018 0.6565
         soilmoi F 0.1172 0.1172
                                      1
             som_F 0.0074 0.0074
                                           30 0.0126 0.9114
## 6
                                      1
## 7
          nat_g.m2 0.0703
                           0.0703
                                      1
                                           30 0.1207 0.7307
## 8
       litter_g.m2 0.0474
                           0.0474
                                      1
                                           30 0.0812 0.7776
## 9
           percpar 0.0071
                           0.0071
                                      1
                                           24 0.0105 0.9190
## 10
           nTrees 0.8314
                           0.8314
                                           30 1.4929 0.2313
                                      1
## 11
          BA_total 0.1882
                                           30 0.3254 0.5726
                           0.1882
                                      1
## 12
         PercBA_AM 0.0637
                           0.0637
                                           30 0.1093 0.7432
                                      1
                                           30 0.0291 0.8658
## 13 mv_g.m2_logt 0.0170 0.0170
```

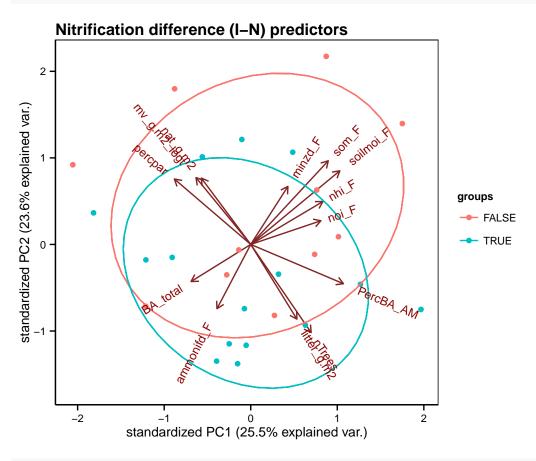
```
#save anova table
newfilename<-'anovaTable_noiDiff.txt'
write.table(result[['anovaSummary']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
#save permMANOVA table
newfilename<-'permMANOVA_noiDiff.txt'
write.table(result[['permAOV']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
result[['pPCA']] + ggtitle('Nitrate difference (I-N) predictors')</pre>
```



paste('Neither reference conditions nor Microstegium biomass explain variation in nitrate')

## [1] "Neither reference conditions nor Microstegium biomass explain variation in nitrate"

```
#B. Predict nitrification diff
result<-ExplainDiffVar(diffVarShort='nitrifd_F', refVars=refVars, mvVar='mv_g.m2_logt', df=data.q2)
#save anova table
newfilename<-'anovaTable_nitrifDiff.txt'
write.table(result[['anovaSummary']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
#save permMANOVA table
newfilename<-'permMANOVA_nitrifDiff.txt'
write.table(result[['permAOV']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
result[['pPCA']] + ggtitle('Nitrification difference (I-N) predictors')</pre>
```



paste('Reference mineralization rate, soil moisture, and soil organic matter explain variation in nitri

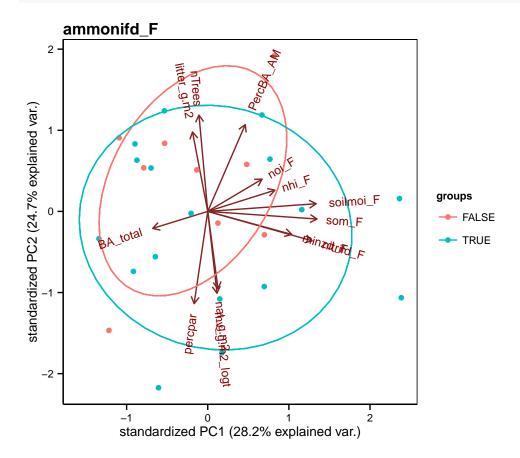
## [1] "Reference mineralization rate, soil moisture, and soil organic matter explain variation in nitr

```
#plot signif vars (x) vs nitrif diff (y)
#save plot
ylabel<-textGrob("Nitrification difference (I-N)", rot=90)
xlabel<-textGrob("Reference condition")
pMinz<-result[['pBivar.list']]$minzd_F + geom_smooth(method='lm') + guides(color=FALSE) +
    xlab(NULL) + ylab(NULL) + ggtitle('Mineralization (ug/G*d)')
pSoilmoi<-result[['pBivar.list']]$soilmoi_F + geom_smooth(method='lm') + guides(color=FALSE) +
    xlab(NULL) + ylab(NULL) + ggtitle('Soil moisture (%)')
pSom<-result[['pBivar.list']]$som_F + geom_smooth(method='lm') + guides(color=FALSE) +
    xlab(NULL) + ylab(NULL) + ggtitle('Soil organic matter (%)')
newfilename<-'pScat_noiDiff.png'</pre>
```

```
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*1, height = fig.height*3, re
grid.arrange(
  ylabel, # topleft
  arrangeGrob(pMinz, pSoilmoi, pSom),
  textGrob(" "), #bottom left
  xlabel, #bottom right
  widths = unit.c(unit(2.5, "lines"), unit(1, "npc") - unit(2.5, "lines")),
  heights = unit.c(unit(1, "npc") - unit(2.5, "lines")),
  nrow=2, ncol=2
)
dev.off()
```

## pdf ## 2

```
#C. Predict ammonification diff
result<-ExplainDiffVar(diffVarShort='ammonifd_F', refVars=refVars, mvVar='mv_g.m2_logt', df=data.q2)
#save anova table
newfilename<-'anovaTable_ammonifDiff.txt'
write.table(result[['anovaSummary']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
#save permMANOVA table
newfilename<-'permMANOVA_ammonifDiff.txt'
write.table(result[['permAOV']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
result[['pPCA']]</pre>
```



```
paste('Microstegium biomass explains variation in ammonification')
```

## [1] "Microstegium biomass explains variation in ammonification"

### 3. Q3: Do reference plot conditions predict Microstegium biomass?

A. Do an ordination of reference conditions. B. Test the relationship between Microstegium biomass and reference conditions using permMANOVA. C. Investigate individual relationships using mixed effects models with year as a random effect.

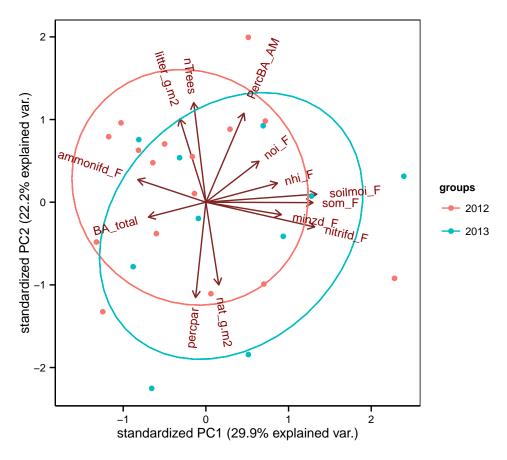
```
#1. Identify dataframe with (x) reference plot condition variables and (y) Microstegium biomass
#refVars
data.q3<-data.q2.indp
data.q3$plotYear<-paste(data.q3$plotid, data.q3$year, sep="_")

#2. Ordinate reference conditions
data.q3.rm<-data.q3[!rowSums(is.na(data.q3))>0,]
tmp<-data.q3.rm[,refVars]
cor(tmp) #look at correlation matrix</pre>
```

```
noi_F ammonifd_F
##
                                              nitrifd F
                  nhi F
                                                            minzd F
## nhi F
              1.00000000 0.228928023 -0.40703592 0.35909440 0.10965729
              0.22892802 \quad 1.000000000 \quad -0.15042511 \quad 0.13248114 \quad 0.03498599
## noi F
## ammonifd_F -0.40703592 -0.150425113 1.00000000 -0.54414358 0.13400374
## nitrifd_F
            0.35909440 0.132481143 -0.54414358 1.00000000 0.75840269
## minzd_F
              0.10965729 \quad 0.034985991 \quad 0.13400374 \quad 0.75840269 \quad 1.000000000
              0.36263779 0.432467789 -0.44254127 0.72619591 0.51254044
## soilmoi_F
## som_F
              ## nat_g.m2
             -0.13698505 -0.032439353 -0.11123402 0.18133659 0.13060454
## litter_g.m2 0.20273076 0.219876083 0.50310183 -0.40893547 -0.09327104
## percpar
              0.01268812 -0.303452291 -0.05176894 0.02578535 -0.00206067
## nTrees
             -0.07118420 0.002642215 0.11352848 -0.09210334 -0.02830722
## BA total
             -0.42345093 -0.271278802 0.23082878 -0.23115841 -0.09471401
## PercBA_AM
              ##
               soilmoi F
                               som_F
                                      nat_g.m2 litter_g.m2
                                                              percpar
## nhi_F
              0.362637788 \quad 0.503814194 \quad -0.13698505 \quad 0.202730757 \quad 0.01268812
              ## noi F
## ammonifd_F -0.442541267 -0.240368561 -0.11123402 0.503101827 -0.05176894
```

```
## nitrifd F
## minzd F
             ## soilmoi F
             1.000000000 0.786122334 0.19823184 -0.138069439 -0.21633329
## som_F
             0.786122334 1.000000000 0.14148840 -0.008757706 -0.04707648
## nat g.m2
             0.198231838 0.141488398 1.00000000 -0.357597640 0.19719854
## litter g.m2 -0.138069439 -0.008757706 -0.35759764 1.000000000 -0.38023773
## percpar
            -0.216333289 -0.047076483 0.19719854 -0.380237733 1.00000000
## nTrees
            -0.005851712 -0.115328018 -0.57268724 0.391036473 -0.63121063
## BA total
            -0.322432174 -0.349825442 0.45247833 0.030849064 -0.21459259
            ## PercBA_AM
##
                  nTrees
                          BA_total PercBA_AM
## nhi_F
            -0.071184204 -0.42345093 0.19137324
## noi F
             0.002642215 -0.27127880 0.25117978
## ammonifd_F
            ## nitrifd_F
            -0.092103335 -0.23115841 0.16487359
## minzd_F
             -0.028307217 -0.09471401 0.05245243
## soilmoi_F
            -0.005851712 -0.32243217 0.31811180
## som F
            -0.115328018 -0.34982544 0.13714394
## nat_g.m2
            ## litter g.m2 0.391036473 0.03084906 0.20679677
## percpar
            -0.631210635 -0.21459259 -0.63141277
## nTrees
            1.000000000 0.09391344 0.50260561
## BA_total
            0.093913441 1.00000000 -0.15152227
## PercBA AM
             0.502605609 -0.15152227 1.00000000
row.names(tmp)<-data.q3.rm$plotYear</pre>
tmp.scaled<-scale(tmp)</pre>
PCA.res<-pre>prcomp(tmp.scaled)
summary(PCA.res)
## Importance of components:
##
                         PC1
                               PC2
                                     PC3
                                           PC4
                                                 PC5
                                                        PC6
                                                               PC7
## Standard deviation
                      1.9712 1.6979 1.2651 1.1627 1.0726 0.86978 0.65346
## Proportion of Variance 0.2989 0.2218 0.1231 0.1040 0.0885 0.05819 0.03285
## Cumulative Proportion 0.2989 0.5206 0.6438 0.7478 0.8363 0.89444 0.92729
##
                                PC9
                                      PC10
                                             PC11
                                                    PC12
                                                            PC13
                          PC8
## Standard deviation
                      0.57207 0.45718 0.42108 0.37217 0.30516 0.006864
## Proportion of Variance 0.02517 0.01608 0.01364 0.01065 0.00716 0.000000
## Cumulative Proportion 0.95246 0.96854 0.98218 0.99283 1.00000 1.000000
```

ggbiplot(PCA.res, groups=factor(data.q3.rm\$year), ellipse = T) + mytheme



```
df.PCA<-data.frame(plotYear=row.names(PCA.res$x), PCA.res$x)</pre>
data.q3.PCA<-merge(data.q3.rm, df.PCA[,c(1:3)])</pre>
#3. Do reference area and plot characteristics explain variation in Mv biomass?
#a) perMANOVA on mv biomass
perMANOVA <-adonis(tmp.scaled ~ mv g.m2 logt, data = data.q3.PCA, method='eu')
aov.tab.permanova<-data.frame(perMANOVA$aov.tab)</pre>
#save anova table
newfilename<-'permMANOVA_RefxMv.txt'
write.table(aov.tab.permanova, file=paste(figuresPath,newfilename, sep='/'), sep='\t')
#b) mixed effects model that uses 1st two PC scores to predict mu biomass
data.q3.PCA$year<-factor(data.q3.PCA$year)</pre>
mod<-lmer(mv_g.m2_logt~PC1*PC2 + (1|year), data=data.q3.PCA)</pre>
anovaTable_mv.PC<-data.frame(round(anova(mod),digits=4))</pre>
#save anova table
newfilename<-'anovaTable_mvPC.txt'</pre>
write.table(anovaTable_mv.PC, file=paste(figuresPath,newfilename, sep='/'), sep='\t')
pScat_PC2.mv<-ggplot(data.q3.PCA, aes(x=PC2, y=mv_g.m2_logt, color=year)) +
  geom_point() + mytheme + geom_smooth(method='lm') + ylab('Microstegium (log trans. g/m2)')
#save plot
newfilename<-'pScat RefPC2xMv.png'
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*1.5, height = fig.height*1,
pScat PC2.mv
dev.off()
```

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

```
#c) Look at how Mv biomass corresponds with variables associated with PC2
ExplainMvVar<-function(sub.refVars, mvVar, df){</pre>
  anova.list<-list()</pre>
  figure.list<-list()
  i<-0
  for(i in 1:length(sub.refVars)){
    #identify current data frame
    curr.refVar<-sub.refVars[i]</pre>
    x<-df[,curr.refVar]</pre>
    y<-df[,mvVar]
    df.i<-data.frame(x=x, y=y, year=df$year)</pre>
    df.i$year<-factor(df.i$year)</pre>
    #regression model
    mod<-lmer(y~x + (1|year), data=df)</pre>
    anova.list[[i]]<-anova(mod)</pre>
    #plot
    p<-ggplot(df.i, aes(y=y, x=x, color=year)) + geom_point() +</pre>
      mytheme + xlab(NULL) + ylab(NULL) + ggtitle(sub.refVars[i]) + guides(color=FALSE)
    figure.list[[i]]<-p
  names(anova.list)<-sub.refVars</pre>
  anovaSummary<-ldply(lapply(anova.list, data.frame))</pre>
  anovaSummary[,-1]<-round(anovaSummary[,-1], digits=4)</pre>
  anovaSummary<-rename(anovaSummary, c(.id='yVar', Pr..F.='pValue'))</pre>
  names(figure.list)<-sub.refVars</pre>
  results<-list(anovaSummary=anovaSummary, figures=figure.list)
  return(results)
  }
result<-ExplainMvVar(sub.refVars=c('percpar','nTrees','PercBA_AM','nat_g.m2','litter_g.m2'),
                      mvVar='mv_g.m2_logt',
                      df=data.q3.PCA)
#save anova table
newfilename<-'anovaSummary_RefINDxMv.txt'
write.table(result[['anovaSummary']], file=paste(figuresPath,newfilename, sep='/'), sep='\t')
\#grid.arrange(do.call(arrangeGrob, result[['figures']])) \ \#all panels
ylabel<-textGrob("Microstegium biomass (log trans. g/m2)", rot=90)
xlabel<-textGrob("Reference area variable")</pre>
#save plot
newfilename<-'pScat_RefxMv.png'
png(paste(figuresPath,newfilename, sep='/'), units='in', width = fig.width*1.5, height = fig.height*3,
grid.arrange(
```

## pdf ## 2

#### Trends over time

- a. Does Microstegium biomass increase over time?
- b. Do soil N pools and fluxes in reference (and invaded) plots shift over time?
- c. Do impact magnitudes shift over time?
- d. Does the influence of reference conditions on Microstegium biomass shift over time?

### Invasion front study design

a. What environmental factors besides soil N pools and fluxes differ among paired invaded and reference plots?

### %AM

- a. Does Microstegium biomass vary with %AM?
- b. Does %AM vary with multivariate soil N conditions in reference plots?