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## Manuscript title: Grazing Abandonment Negatively Affects Forage Quality in Iberian ##
## Atlantic Grasslands ##
## ##
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#####
## We are indebted to Leps et al. (2011) [1] and Borcard et al. (2011) [2], for their ##
## very useful code ##
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#####
## This code reproduces the analyses presented in the main text, which aimed to test: ##
## 1) whether grazing abandonment promotes the production of forage of lower ##
## nutritive value, by provoking changes in plant community traits. ##
## 2) whether shifts in species abundances (species turnover) and intraspecific ##
## trait variability select for similar traits, reinforcing each other. ##
#####

## 1. Set up working directory and load the required packages
## *****
setwd("Working directory") # Change as required. Unzip Appendix C in the working directory.
source("e6904_trait-flex-v3.R") # load additional functions from Appendix in Leps et al. (2011) [1]
install.packages("FD") # Laliberte et al. (2014) [3]

## 2. Load and prepare data for analysis
## *****
## For the script to work appendix_C must be unzipped in the working directory.
trait<-read.csv("trait.csv",h=T)
height<-read.csv("height.csv",h=T)
comp<-read.csv("composition.csv",h=T)

## Create separate composition data.frames for each site x treatment combination.
alo <- subset(comp,site=="Alotza")
uzk <- subset(comp,site=="Uzkuiti")
alog <- subset(alo,treatment=="G")
aloe <- subset(alo,treatment=="E")
uzkg <- subset(uzk,treatment=="G")
uzke <- subset(uzk,treatment=="E")
alog.comp <- alog[, -c(1:2)]
aloe.comp <- aloe[, -c(1:2)]
uzkg.comp <- uzkg[, -c(1:2)]
uzke.comp <- uzke[, -c(1:2)]

## Create separate plant trait data.frames for each site x treatment combination.
trait.a <- subset(trait,Site=="Alotza")
trait.ag <- subset(trait.a,Treatment=="G")
trait.ae <- subset(trait.a,Treatment=="E")
trait.u <- subset(trait,Site=="Uzkuiti")
trait.ug <- subset(trait.u,Treatment=="G")
trait.ue <- subset(trait.u,Treatment=="E")
height.a <- subset(height,Site=="Alotza")
height.ag <- subset(height.a,Treatment=="G")
height.ae <- subset(height.a,Treatment=="E")
height.u <- subset(height,Site=="Uzkuiti")
height.ug <- subset(height.u,Treatment=="G")
height.ue <- subset(height.u,Treatment=="E")

## 3. Test of the null hypothesis of no effect of grazing abandonment on the composition of
## structural species.
## *****
library(FD)
## dbRDA of the composition data

## Hellinger-transform the composition matrix and compute the Euclidean distance. This results
## on a Hellinger-distance matrix.
comp.h <- decostand(comp[, -c(1,2)], "hellinger")
comp.h.d1 <- dist(comp.h)

## Verify the homogeneity of within-group covariance matrices
# Factor site
comp.h.site.MHV <- betadisper(comp.h.d1, comp$site)
permutest(comp.h.site.MHV) #Permutational test
# Factor treatment
comp.h.treatment.MHV <- betadisper(comp.h.d1, comp$treatment)
permutest(comp.h.treatment.MHV) #Permutational test

## The within-group covariance matrices are homogeneous. We can proceed with dbRDA.
## Test the interaction
interaction.dbrda <- capscale(comp.h~site*treatment+Condition(site*treatment), data=comp, dist="euclidean", add=TRUE)
anova(interaction.dbrda, step=1000, perm.max=1000)
## Interaction is not significant. Proceed to test main effects.
site.treatment.dbrda <- capscale(comp.h~site+treatment, data=comp, dist="euclidean", add=TRUE)
anova(site.treatment.dbrda, step=1000, perm.max=1000)

## This is Appendix Table A2
anova(site.treatment.dbrda, step=1000, perm.max=1000, by="term")
RsquareAdj(site.treatment.dbrda)

## This is Figure 1 in main text
windows("Figure 1", width=8, height=6)
plot(site.treatment.dbrda, display=c("sp", "cn"), type="n", cex.lab=1.5, xlim=c(-1.5, 1.5))
cn.sc <- scores(site.treatment.dbrda, display="cn", scaling=2)
text(cn.sc, labels=c("Alotza", "Uzkuiti", "E", "G"), col="black", cex=1.5)
sp.sc <- scores(site.treatment.dbrda, display="sp", scaling=2)
text(sp.sc, labels=c("Agr_cap", "Fes_mic", "Gal_sax", "Luz_cam", "Tri_rep"), col="darkgray", cex=1)
arrows(0, 0, x1=sp.sc[, 1], y1=sp.sc[, 2], lty=1, col="darkgray", length=0)

## 4. Test of the null hypothesis of no effect of grazer exclusion in the intraspecific variability
## of measured traits.
## *****
## Two-way ANOVAs for individual species and traits.

## Model of Festuca microphylla/Height.
## The same procedure was used with other species/traits combinations.
Fesmic.h <- subset(height, Species=="Festuca_microphylla")
Fm.h.lm <- aov(Height~Site*Treatment, data= Fesmic.h)

## This is Appendix Table A3
anova(Fm.h.lm)

## Tukey post hoc to determin which of the factor level combiantion differ between them.
## Letters in Table 2 in the main text are obtained from this analysis.
TukeyHSD(Fm.h.lm)

## 5. Test correlations between fixed and specific CWMs of response (H and SLA) and effect (LNC
## and CN) traits.
## *****
## Spearman correlations between fixed and specific CWMs of measured traits
## Fixed CWMs
cor.test(cwm.nonspec$h, cwm.nonspec$CN, method="spearman")
## Specific CWMs
cor.test(cwm.spec$h, cwm.spec$CN, method="spearman")
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## All other trait combinations were similarly analysed.

## 6. Relative contribution of changes in species composition and intraspecific variability to
## community functional response to grazer exclusion.
## *****
## Procedure proposed by Leps et al. (2011) [1]

## Compute fixed or nonspecific trait values for individual species and traits.
nonspec.h <- aggregate(height~Height,by=list(height$Species),mean)
nonspec.SLA <- aggregate(trait$SLA,by=list(trait$Species),mean)
nonspec.LNC <- aggregate(trait$LNC,by=list(trait$Species),mean)
nonspec.CN <- aggregate(trait$CN,by=list(trait$Species),mean)

## Create a data.frame with all fixed trait values.
nonspec.trait<- data.frame(h=nonspec.h[,2],SLA=nonspec.SLA[,2],LNC=nonspec.LNC[,2],CN=nonspec.CN[,2])
rownames(nonspec.trait) <- c("Agrostis_capillaris","Festuca_microphylla","Galium_saxatile","Luzula_campestris","Trifolium_repens")

## Compute fixed community weighted means (CWM) for each site and treatment combination.
cwm.nonspec.ag<-dbFD(nonspec.trait,alog.comp)$CWM
cwm.nonspec.ae<-dbFD(nonspec.trait,aloe.comp)$CWM
cwm.nonspec.ug<-dbFD(nonspec.trait,uzkg.comp)$CWM
cwm.nonspec.ue<-dbFD(nonspec.trait,uzke.comp)$CWM
cwm.nonspec<-data.frame(Site=rep(c("alo","uzk"),each=30),Treatment=rep(c("G","E","G","E"),each=15),
                        rbind(cwm.nonspec.ag,cwm.nonspec.ae,cwm.nonspec.ug,cwm.nonspec.ue))

## Compute specific trait values for individual species and traits.
spec.h.ag <- aggregate(height.ag$Height,by=list(height.ag$Species),mean)
spec.h.ae <- aggregate(height.ae$Height,by=list(height.ae$Species),mean)
spec.h.ug <- aggregate(height.ug$Height,by=list(height.ug$Species),mean)
spec.h.ue <- aggregate(height.ue$Height,by=list(height.ue$Species),mean)
spec.SLA.ag <- aggregate(trait.ag$SLA,by=list(trait.ag$Species),mean)
spec.SLA.ae <- aggregate(trait.ae$SLA,by=list(trait.ae$Species),mean)
spec.SLA.ug <- aggregate(trait.ug$SLA,by=list(trait.ug$Species),mean)
spec.SLA.ue <- aggregate(trait.ue$SLA,by=list(trait.ue$Species),mean)
spec.LNC.ag <- aggregate(trait.ag$LNC,by=list(trait.ag$Species),mean)
spec.LNC.ae <- aggregate(trait.ae$LNC,by=list(trait.ae$Species),mean)
spec.LNC.ug <- aggregate(trait.ug$LNC,by=list(trait.ug$Species),mean)
spec.LNC.ue <- aggregate(trait.ue$LNC,by=list(trait.ue$Species),mean)
spec.CN.ag <- aggregate(trait.ag$CN,by=list(trait.ag$Species),mean)
spec.CN.ae <- aggregate(trait.ae$CN,by=list(trait.ae$Species),mean)
spec.CN.ug <- aggregate(trait.ug$CN,by=list(trait.ug$Species),mean)
spec.CN.ue <- aggregate(trait.ue$CN,by=list(trait.ue$Species),mean)

## Create data.frames with specific trait values.
spec.trait.ag <- data.frame(h=spec.h.ag[,2],SLA=spec.SLA.ag[,2],LNC=spec.LNC.ag[,2],CN=spec.CN.ag[,2])
rownames(spec.trait.ag) <- c("Agrostis_capillaris","Festuca_microphylla","Galium_saxatile","Luzula_campestris","Trifolium_repens")
spec.trait.ae <- data.frame(h=spec.h.ae[,2],SLA=spec.SLA.ae[,2],LNC=spec.LNC.ae[,2],CN=spec.CN.ae[,2])
rownames(spec.trait.ae) <- c("Agrostis_capillaris","Festuca_microphylla","Galium_saxatile","Luzula_campestris","Trifolium_repens")
spec.trait.ug <- data.frame(h=spec.h.ug[,2],SLA=spec.SLA.ug[,2],LNC=spec.LNC.ug[,2],CN=spec.CN.ug[,2])
rownames(spec.trait.ug) <- c("Agrostis_capillaris","Festuca_microphylla","Galium_saxatile","Luzula_campestris","Trifolium_repens")
spec.trait.ue <- data.frame(h=spec.h.ue[,2],SLA=spec.SLA.ue[,2],LNC=spec.LNC.ue[,2],CN=spec.CN.ue[,2])
rownames(spec.trait.ue) <- c("Agrostis_capillaris","Festuca_microphylla","Galium_saxatile","Luzula_campestris","Trifolium_repens")

## Compute specific CWM for each site and treatment combination.
cwm.spec.ag<-dbFD(spec.trait.ag, alog.comp)$CWM
cwm.spec.ae<-dbFD(spec.trait.ae,aloe.comp)$CWM
cwm.spec.ug<-dbFD(spec.trait.ug,uzkg.comp)$CWM
cwm.spec.ue<-dbFD(spec.trait.ue,uzke.comp)$CWM
cwm.spec<-data.frame(Site=rep(c("alo","uzk"),each=30),Treatment=rep(c("G","E","G","E"),each=15),
                    rbind(cwm.spec.ag,cwm.spec.ae,cwm.spec.ug,cwm.spec.ue))

## Create definitive data.frames to apply Leps' function
#h
cwm.h <- data.frame(cwm.spec[,1:2],spec.h=cwm.spec[,3],nonspec.h=cwm.nonspec[,3])
#SLA
cwm.SLA <- data.frame(cwm.spec[,1:2],spec.SLA=cwm.spec[,4],nonspec.SLA=cwm.nonspec[,4])
#LNC
cwm.LNC <- data.frame(cwm.spec[,1:2],spec.LNC=cwm.spec[,5],nonspec.LNC=cwm.nonspec[,5])
#CN
cwm.CN <- data.frame(cwm.spec[,1:2],spec.CN=cwm.spec[,6],nonspec.CN=cwm.nonspec[,6])

## Test the treatment effect in fixed, specific and intraspecific CWM.
## Fixed or nonspecific CWM is only affected by changes in the turnover of structural species.
## Specific CWM is affected by both turnover and intraspecific variability of structural species.
## Intraspecific CWM is computed by subtracting the fixed CWM from specific CWM, and it is only affected
## by the intraspecific variability of structural species.
##height
#nonspec.h
nonspec.h.lm <- lm(nonspec.h~Site*Treatment,data= cwm.h)
#intraspecific.h = spec.h - nonspec.h
intraspecific.h <- cwm.h$spec.h - cwm.h$nonspec.h
intraspecific.h.lm <- lm(intraspecific.h~Site*Treatment,data= cwm.h)
#spec.h
spec.h.lm <- lm(spec.h~Site*Treatment,data=cwm.h)
## The same procedure was followed with other traits.

## This is Appendix Table A4
anova(nonspec.h.lm)
anova(intraspecific.h.lm)
anova(spec.h.lm)

## This is Figure 2 in the main text
## With Height, Same procedure was followed with the other traits.
sitel <- as.factor(c("alo","alo","uzk","uzk"))
treatl <- as.factor(c("G","E","G","E"))
nonspec.h.predict <- predict(nonspec.h.lm,newdata=data.frame(Site=sitel,Treatment=treatl),interval="confidence")
spec.h.predict <- predict(spec.h.lm,newdata=data.frame(Site = sitel, Treatment = treatl),interval="confidence")
SITE <- as.factor(c("Alotza","Alotza","Uzkuiti","Uzkuiti","Alotza","Alotza","Uzkuiti","Uzkuiti"))
TREAT <- as.factor(c("G","E","G","E","G","E","G","E"))
TREAT <- relevel(TREAT,ref = "G")
TYPE <- as.factor(c(rep("Fixed",4),rep("Specific",4)))
h.df <- data.frame(SITE,TREAT,TYPE,rbind(nonspec.h.predict, spec.h.predict)) # Ignore the warning
prepanel.ci <- function(x, y, ly, uy, subscripts, ...)
{
  x <- as.numeric(x)
  ly <- as.numeric(ly[subscripts])
  uy <- as.numeric(uy[subscripts])
  list(ylim = range(y, uy, ly, finite = TRUE))
}
panel.ci <- function(x, y, ly, uy, subscripts, pch = 16, ...)
{
  x <- as.numeric(x)
  y <- as.numeric(y)
  ly <- as.numeric(ly[subscripts])
  uy <- as.numeric(uy[subscripts])
  panel.arrows(x, ly, x, uy, col = "black",
               length = 0.1, unit = "native",
               angle = 90, code = 3)
  panel.xyplot(x, y, pch = pch, ...)
}

xyplot(fit~TREAT|SITE,data=h.df,groups=TYPE,type="b",lty=c(1,2),col="black",pch=c(15,0),xlab="Treatment",ylab="Height (cm)",
      ly = h.df$lywr,uy = h.df$upr,prepanel = prepanel.ci,panel = panel.superpose,panel.groups=panel.ci,
      par.settings = list(strip.background=list(col="lightgrey")))
```

## Apply the Leps' function to disentangle the contributions of turnover and intraspecific variability  
## to community response to grazing.

```
## Height. The same procedure was followed with other traits.
h.flex<-trait.flex.anova(~Site*Treatment, spec.h, nonspec.h, data=cwm.h)

## This is Figure 3 in the main text.
plot(h.flex, plot.covar=F, use.percentage=T,plot.total=T, main="Height")

##*****REFERENCES*****
# [1] Leps, J. et al. Ecography 2011, 34:856-863.
# [2] Borcard, D. et al. 2011. Numerical Ecology with R. Springer.
# [3] Laliberté, E. et al. 2014. FD: measuring functional diversity from multiple traits, and other tools for
#       functional ecology. R package version 1.0-12.
```