

# Functional diversity and composition of Bornean stream frogs

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August 2020

## Introduction

The impact of human disturbances on Bornean stream frogs was assessed by comparing the functional diversity, the functional trait variability and the functional trait composition between primary forests (PF), logged forests (LF) and oil palm plantations (OP). I started to measure the functional diversity of those habitats with three measures: functional richness, functional evenness and functional divergence.

## A. Functional diversity: Functional richness

The FD measure of functional richness described by Petchey and Gaston (2002, 2006) and used by Riemann et al. (2017) will be used in this study. Four steps are required for this calculation which are 1) create a trait dataset, 2) convert the trait dataset into a distance matrix, 3) create a dendrogram based on the distances, 4) Measure the total branch length of the dendrogram (Petchey and Gaston, 2002).

### 1) Trait dataset

Load useful R packages, load the filtered frog dataset from (Konopik et al., 2015) and create subsets removing NAs for the snout-vent length (SVL) and the gape width (GW) traits

```
library(vegan)
library(dplyr)
library(hillR)
library(openxlsx)
library(PMCMR)
library(car)
library(ggplot2)
library(tidyr)
Data<-readWorkbook("OJ_functional_frog_data.xlsx", sheet=1, colNames = TRUE,
startRow = 10)
Data2<-subset(Data,Data$svl!="NA")
Data3<-subset(Data,Data$gw!="NA")
```

### Snout-vent length (SVL)

```
Mean_male_svl<-
Data2%>%filter(sex=="male")%>%group_by(Taxon_name)%>%summarise(Mean.male.svl=
mean(svl), sd.male.svl=sd(svl))
Mean_female_svl<-
Data2%>%filter(sex=="female")%>%group_by(Taxon_name)%>%summarise(Mean.female.
```

```
svl=mean(svl), sd.female.svl=sd(svl))
svl<-merge(Mean_male_svl,Mean_female_svl, by="Taxon_name", all=TRUE)
```

I tested if there was a sexual dimorphism in size across species to determine if it is necessary to separate svl between male and females.

```
t.test(svl$Mean.male.svl,svl$Mean.female.svl)

##
##  Welch Two Sample t-test
##
## data:  svl$Mean.male.svl and svl$Mean.female.svl
## t = -2.3648, df = 57.689, p-value = 0.02142
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -25.848910  -2.147986
## sample estimates:
## mean of x mean of y
##  42.72486  56.72331
```

The difference is significant, which means that there is a sexual dimorphism between males and females. Therefore, the SVL column will be split into males and females to create the trait dataset.

### Gape width (GW)

```
Mean_male_gw<-
Data3%>%filter(sex=="male")%>%group_by(Taxon_name)%>%summarise(Mean.male.gw=m
ean(gw), sd.male.gw=sd(gw))
Mean_female_gw<-
Data3%>%filter(sex=="female")%>%group_by(Taxon_name)%>%summarise(Mean.female.
gw=mean(gw), sd.female.gw=sd(gw))
gw<-merge(Mean_male_gw,Mean_female_gw, by="Taxon_name", all=TRUE)
```

I tested if there was a sexual dimorphism in gape width across species to determine if it is necessary to separate gw between male and females.

```
t.test(gw$Mean.male.gw,gw$Mean.female.gw)

##
##  Welch Two Sample t-test
##
## data:  gw$Mean.male.gw and gw$Mean.female.gw
## t = -1.5251, df = 61.6, p-value = 0.1323
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -8.215978  1.105258
## sample estimates:
## mean of x mean of y
##  14.99875  18.55411
```

The difference is not significant, which means that there is no sexual dimorphism between males and females. Therefore, the gw column will be merged in one column to create the trait dataset.

```
Mean_gw<-Data3%>%group_by(Taxon_name)%>% summarise(mean.gw=mean(gw),
sd.gw=sd(gw))
```

### Gut content (GC)

The gut content of the frogs was available from Konopik et al. (2015). I measured the class richness of the gut content and inserted it in the trait dataset since gut content is a direct information of resource use patterns (Petchey and Gaston, 2006).

```
gut_data<-readWorkbook("OJ_functional_frog_data.xlsx", sheet=2, colNames =
TRUE, startRow = 10)
gut_content <- with(gut_data,
table(gut_data$Taxon_name,gut_data$taxon_class))
gut_content <- data.frame(unclass(gut_content))

qD <- matrix(NA,nrow=nrow(gut_content), ncol=3)
for (j in 1:3) {
  qD[,j] <- hill_taxa(comm=gut_content, q=j-1)
}
```

### Traits

```
Traits<-readWorkbook("OJ_functional_frog_data.xlsx", sheet=3, colNames =
TRUE,rowNames = TRUE)
Traits$BH <-as.factor(Traits$BH)
Traits$CP <-as.factor(Traits$CP)
Traits$RM <-as.factor(Traits$RM)
Traits$BC <-as.factor(Traits$BC)
Traits$RHL <-factor(Traits$RHL, ordered=TRUE,levels=c("1","2","3","4"))
Traits$HW <-factor(Traits$HW, ordered=TRUE,levels=c("1","2","3","4"))
Traits$FW <-factor(Traits$FW, ordered=TRUE,levels=c("1","2","3","4"))
Traits$TD <-factor(Traits$TD, ordered=TRUE,levels=c("1","2","3","4"))
```

head(Traits)

##	BH	CP	Mean.male.SVL	Mean.female.SVL	GW	RHL
## Ansonia leptopus	3	1	36.97755	53.46667	11.046154	3
## Ansonia longidigita	3	1	42.26667	NA	12.983333	4
## Ansonia spinulifer	3	1	35.57170	41.90000	10.585965	4
## Chaperina fusca	3	1	19.13077	22.64286	6.287234	4
## Fejervarya limnocharis	1	1	41.56842	51.41818	14.751282	3
## Hylarana erythrea	1	2	41.10000	61.85000	17.839286	4
##	HW	FW	TD	RM	BC	GC
## Ansonia leptopus	1	3	2	2	2	4
## Ansonia longidigita	1	2	2	2	2	1
## Ansonia spinulifer	1	1	2	2	4	8
## Chaperina fusca	1	1	2	1	4	7

```
## Fejervarya limnocharis 1 1 1 4 2 5
## Hylarana erythrea 1 2 2 1 1 7
```

## 2) Distance Matrix

The function `daisy` helped to convert the trait dataset into a distance matrix based on Gower's distance that allows data of mixed types (Swenson 2014). This function also standardised the trait values to enable equal comparisons.

```
library(cluster)

distance_matrix<-daisy(Traits,metric = "gower")
distance_matrix<-as.matrix(distance_matrix)
```

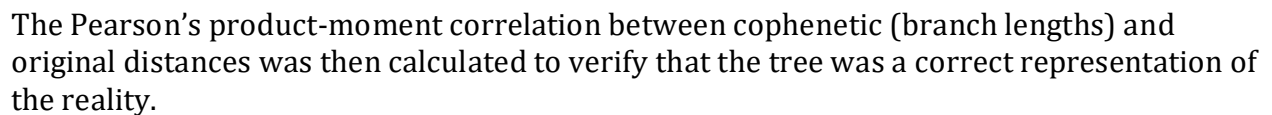
## 3) Dendrogram

The average group clustering method (UPGMA) was performed to create a hierarchical clustering.

```
library(phangorn)

frogs_UPGMA<-upgma(distance_matrix,method="average")
frogs_UPGMA$tip.label<- c("Ansonia leptopus", "Ansonia longidigita", "Ansonia
spinulifer",
                          "Chaperina fusca",
                          "Fejervarya limnocharis",
                          "Hylarana erythrea",
                          "Hylarana megalonesa",
                          "Hylarana nicobariensis",
                          "Hylarana picturata",
                          "Hylarana signata",
                          "Ingerana baluensis",
                          "Ingerophrynus divergens",
                          "Kalophrynus pleurostigma",
                          "Leptobrachella mjobergi",
                          "Leptobrachella parva",
                          "Leptobrachium abboti",
                          "Leptolalax dringi",
                          "Leptolalax gracilis",
                          "Limnonectes finchi",
                          "Limnonectes ibanorum",
                          "Limnonectes kuhlii",
                          "Limnonectes leporinus",
                          "Limnonectes paramacrodon",
                          "Megophrys nasuta",
                          "Meristogenys orphnocnemis",
                          "Meristogenys sp.",
                          "Microhyla perparva",
                          "Microhyla petrigena",
                          "Nyctixalus pictus",
                          "Odorrana hosii",
```

```
plot.phylo(as.phylo(frogs_UPGMA), type="p", edge.col=1, edge.width=1,
show.node.label=TRUE, no.margin=TRUE)
```



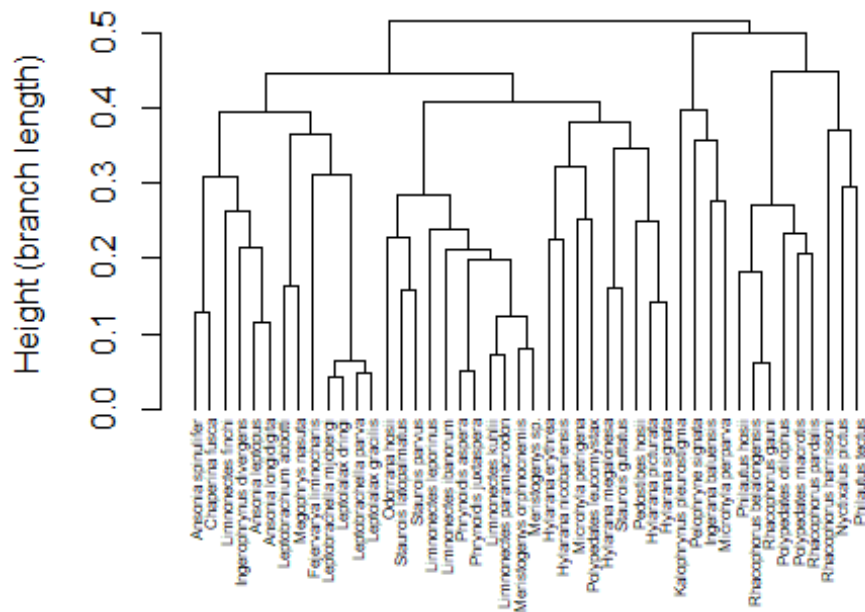
```
library(ape)
library("dendextend")
```

```
frogs_cophenetic<-cophenetic.phylo(frogs_UPGMA)
mantel(distance_matrix, frogs_cophenetic, method="pearson", permutations=999)

##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = distance_matrix, ydis = frogs_cophenetic, method =
## "pearson",      permutations = 999)
##
## Mantel statistic r: 0.6689
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##      90%      95%      97.5%      99%
## 0.0670 0.0845 0.1011 0.1274
## Permutation: free
## Number of permutations: 999
```

The functional dendrogram was then drawn.

```
frogs_hclust<-as.hclust(frogs_UPGMA,hang.dendrogram(0.5))
frogs_dendrogram<-as.dendrogram(frogs_hclust)
frogs_dendrogram<- set(frogs_dendrogram, "labels_cex", 0.53)
plot(frogs_dendrogram,ylab="Height (branch length)")
```



#### 4) Branch length per community

The functional richness (FD) is defined as the total branch length in a trait dendrogram connecting all species, but excluding the unnecessary root segments of the tree (Petchey and Gaston 2006). The community matrix was first created to measure the abundance of each frog species in each community.

```
community<- with(Data, table(Data$stream,Data$Taxon_name))
community<- data.frame(unclass(communitiy),check.names = FALSE)
for (i in ncol(communitiy)){
  number_persp<-colSums(communitiy)
  number_persp<-as.data.frame(number_persp)
}
```

The function treedive, adapted to this study was used to measure the functional richness (Petchey 2002, Riemann 2017).

```
FRic <- numeric(nrow(communitiy))
for (i in 1:nrow(communitiy)) {
  k <- communitiy[i,] > 0
  nit <- sum(k)
  d <- as.dist(frogs_cophenetic[k,k])
  cl_upgma<-upgma(d,method="average")
  cl_hclust<-as.hclust(cl_upgma)
  FRic[i] <- treeheight(cl_hclust)
}
names(FRic) <- rownames(communitiy)
FRic<-as.data.frame(FRic)
FRic
```

```
##          FRic
## 0m        4.4410987
## 120m      3.6316454
## 15m       4.6889508
## 30m       4.8523070
## 5m        0.8893429
## 60m       4.0412744
## Belian    2.9803318
## Elepan    1.8585328
## Gaharu1   2.8064254
## Gaharu2   4.2881231
## Gaharu3   2.8687245
## Injing    4.4234505
## LFE       4.0587512
## Mawang    3.8518765
## Menggaris 3.2488433
## Merbau    2.4456171
## NEW       5.1579634
## Rhino     6.3754759
## Sibut     6.4321558
```

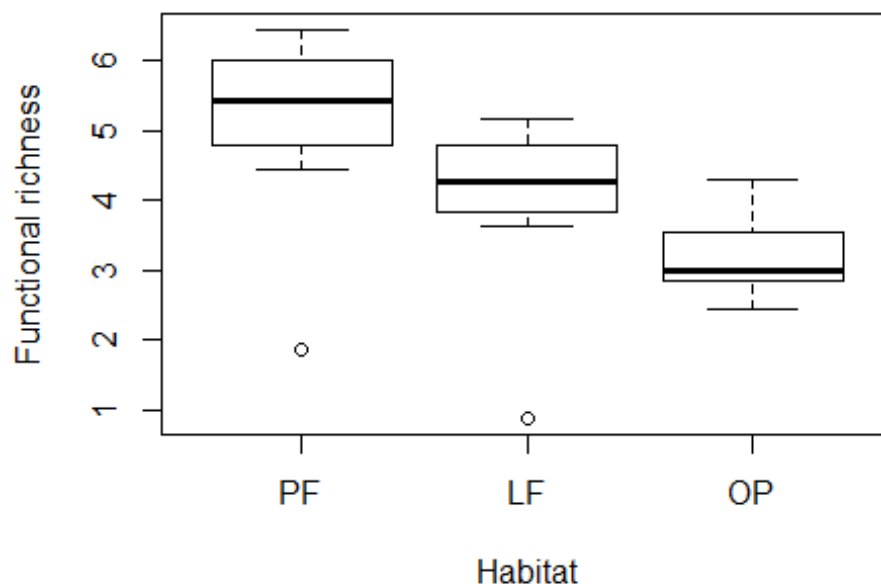
```
## Tembaling 5.6094655
## VJR        5.4199186
## West       5.1408619
```

## Results functional richness

The influence of habitat modifications on frogs functional richness was evaluated. The normality was tested to chose between parametric and non parametric tests. Since the normality was verified, ANOVA and a tukey post-hoc test were done to compare the functional richness between the habitats.

### Influence of habitat on functional richness

```
Habitat<-
c(rep("LF",6),"OP","PF",rep("OP",3),"PF","LF",rep("OP",3),"LF",rep("PF",5))
FRic<-cbind(Habitat,FRic)
FRic$Habitat <- factor(FRic$Habitat , levels=c("PF", "LF", "OP"))
boxplot(FRic$FRic~FRic$Habitat,xlab="Habitat", ylab="Functional richness")
```



```
shapiro.test(FRic$FRic)

##
##  Shapiro-Wilk normality test
##
## data:  FRic$FRic
## W = 0.98268, p-value = 0.9525
```



```

Fit<-aov(FRic$FRic~FRic$Habitat)
summary(Fit)

##              Df Sum Sq Mean Sq F value Pr(>F)
## FRic$Habitat  2  11.77   5.885    3.76 0.0421 *
## Residuals    19  29.74   1.565
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

tukey<- TukeyHSD(x=Fit, 'FRic$Habitat', conf.level=0.95)
tukey

##    Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = FRic$FRic ~ FRic$Habitat)
##
## $`FRic$Habitat`
##              diff              lwr              upr              p adj
## LF-PF -1.0669563 -2.711941    0.5780286 0.2507645
## OP-PF -1.8242742 -3.523207   -0.1253411 0.0341144
## OP-LF -0.7573179 -2.402303    0.8876670 0.4849577

```

### functional richness vs taxonomic richness

Functional diversity being generally highly correlated to taxonomic richness (Petchey 2002), their correlation was tested.  $q_0$  refers to Hill numbers and corresponds to species richness.

```

qD_taxo <- as.data.frame(specnumber(community))
qD_taxo<-qD_taxo%>% rename(q0 = `specnumber(community)`)
FRic<-cbind(FRic,qD_taxo)

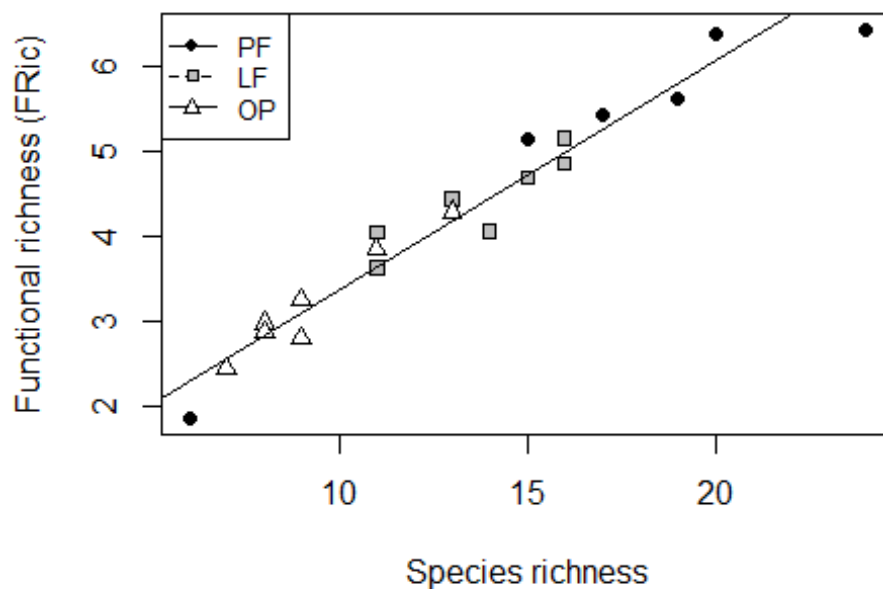
Mod2<-lm(FRic$FRic~FRic$q0)
summary(Mod2)

##
## Call:
## lm(formula = FRic$FRic ~ FRic$q0)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.71422 -0.17510  0.07377  0.20167  0.42230
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.67219    0.17531   3.834  0.00104 **
## FRic$q0      0.26976    0.01295  20.834 4.94e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```
##
## Residual standard error: 0.3024 on 20 degrees of freedom
## Multiple R-squared: 0.956, Adjusted R-squared: 0.9538
## F-statistic: 434.1 on 1 and 20 DF, p-value: 4.943e-15

plot(FRic$FRic[FRic$Habitat=="PF"]~FRic$q0[FRic$Habitat=="PF"],xlab="Species
richness", ylab="Functional richness (FRic)",pch=21,bg="black")
points(FRic$FRic[FRic$Habitat=="LF"]~FRic$q0[FRic$Habitat=="LF"],xlab="Specie
s richness", ylab="Functional richness (FRic)",pch=22,bg="grey")
points(FRic$FRic[FRic$Habitat=="OP"]~FRic$q0[FRic$Habitat=="OP"],xlab="Specie
s richness", ylab="Functional richness (FRic)",pch=24,bg="white")
abline(Mod2)
legend("topleft", legend=c("PF", "LF", "OP"),pch =c(21,22,24),
pt.bg=c("black", "grey","white"), lty=1:2, cex=0.8)
```



### Null models functional richness

Because functional richness was highly correlated with species richness, species richness was kept constant by null models to allow comparison between communities and investigate variations of functional richness beyond species richness (Viller et al., 2008; Swenson, 2014; Riemann et al., 2017).

```
library(picante)
```

```
#The dendrogram tips were shuffled to randomize the functional traits while
the functional richness did not change.
#The function suffle the tips but change the order of the tips. Therefore,
```

*they needed to be ordered alphabetically.*

```
rand.FRic.fun<-function(community, x){
  tmp.phylo<-tipShuffle(x)
  tmp.phylo_hclust<-as.hclust(tmp.phylo)
  r<- cophenetic(tmp.phylo_hclust)
  ordering <- sort(attr(r, "Labels"))
  m2 <- as.matrix(r)[ordering, ordering]

  div2 <- numeric(nrow(community))
  for (i in 1:nrow(community)) {
    k <- community[i,] > 0
    nit <- sum(k)
    d <- as.dist(m2[k,k])
    tree<-upgma(d,method="average")
    cl_hclust<-as.hclust(tree)
    div2[i] <- treeheight(cl_hclust)
  }
  names(div2) <- rownames(community)
  div2<-as.data.frame(div2)
}

null.output<-replicate(999,rand.FRic.fun(community,frogs_UPGMA))
null.output<-as.data.frame(null.output)
```

### Deviation from the null model: Standardised effect size (SES)

The standardized effect size (SES), comparing the expected functional richness (FRic\_exp) obtained from the null models and the observed functional richness (FRic), was measured for each habitat to assess deviation from the null model.

```
library(genefilter)

FRic_ex<-matrix(NA,nrow = nrow(null.output))
FRic_ex_sd<-matrix(NA,nrow = nrow(null.output))
for(i in 1:nrow(null.output)){
  FRic_ex[i,<-rowMeans(null.output[i,])
  FRic_ex_sd[i,<-as.numeric(rowSds(null.output[i,]))
}
FRic<-cbind(FRic,FRic_ex,FRic_ex_sd)
FRic
```

##	Habitat	FRic	q0	FRic_ex	FRic_ex_sd
## 0m	LF	4.4410987	13	4.5681975	0.2952085
## 120m	LF	3.6316454	11	4.0104505	0.2946688
## 15m	LF	4.6889508	15	5.0994495	0.3243851
## 30m	LF	4.8523070	16	5.3527498	0.3241351

## 5m	LF	0.8893429	2	0.8986067	0.1681726
## 60m	LF	4.0412744	11	4.0009378	0.2856085
## Belian	OP	2.9803318	8	3.1105708	0.2462672
## Elepan	PF	1.8585328	6	2.4541391	0.2341416
## Gaharu1	OP	2.8064254	9	3.4135456	0.2726535
## Gaharu2	OP	4.2881231	13	4.5770754	0.2984289
## Gaharu3	OP	2.8687245	8	3.1051702	0.2539152
## Injing	PF	4.4234505	13	4.5677630	0.3039243
## LFE	LF	4.0587512	14	4.8477048	0.3246868
## Mawang	OP	3.8518765	11	4.0120669	0.2853960
## Menggaris	OP	3.2488433	9	3.4159949	0.2740884
## Merbau	OP	2.4456171	7	2.7876682	0.2438270
## NEW	LF	5.1579634	16	5.3597681	0.3306816
## Rhino	PF	6.3754759	20	6.3499169	0.3425952
## Sibut	PF	6.4321558	24	7.2734395	0.3355039
## Tembaling	PF	5.6094655	19	6.1196857	0.3338345
## VJR	PF	5.4199186	17	5.6116555	0.3276679
## West	PF	5.1408619	15	5.1062328	0.3264782

The normality of the distribution was verified for each habitat type and the deviation from null models was tested.

```
# PF
FRic_PF<-FRic %>% filter(Habitat=="PF")
shapiro.test(FRic_PF$FRic)

##
## Shapiro-Wilk normality test
##
## data:  FRic_PF$FRic
## W = 0.83678, p-value = 0.0927

shapiro.test(FRic_PF$FRic_ex)

##
## Shapiro-Wilk normality test
##
## data:  FRic_PF$FRic_ex
## W = 0.94236, p-value = 0.6601

t.test(FRic_PF$FRic, FRic_PF$FRic_ex, paired=TRUE)

##
## Paired t-test
##
## data:  FRic_PF$FRic and FRic_PF$FRic_ex
## t = -2.5025, df = 6, p-value = 0.04637
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.628075095 -0.007059679
## sample estimates:
```

```

## mean of the differences
##          -0.3175674

#LF
FRic_LF<-FRic %>% filter(Habitat=="LF") #significant => normality not
respected => NP => Wilcoxon signed rank test
shapiro.test(FRic_LF$FRic)

##
##  Shapiro-Wilk normality test
##
## data:  FRic_LF$FRic
## W = 0.76788, p-value = 0.01285

shapiro.test(FRic_LF$FRic_ex)

##
##  Shapiro-Wilk normality test
##
## data:  FRic_LF$FRic_ex
## W = 0.7419, p-value = 0.006643

wilcox.test(FRic_LF$FRic, FRic_LF$FRic_ex, paired=TRUE)

##
##  Wilcoxon signed rank test
##
## data:  FRic_LF$FRic and FRic_LF$FRic_ex
## V = 2, p-value = 0.02344
## alternative hypothesis: true location shift is not equal to 0

#OP
FRic_OP<-FRic %>% filter(Habitat=="OP")
shapiro.test(FRic_OP$FRic)

##
##  Shapiro-Wilk normality test
##
## data:  FRic_OP$FRic
## W = 0.92525, p-value = 0.5113

shapiro.test(FRic_OP$FRic_ex)

##
##  Shapiro-Wilk normality test
##
## data:  FRic_OP$FRic_ex
## W = 0.91216, p-value = 0.411

t.test(FRic_OP$FRic, FRic_OP$FRic_ex, paired=TRUE)

##
##  Paired t-test

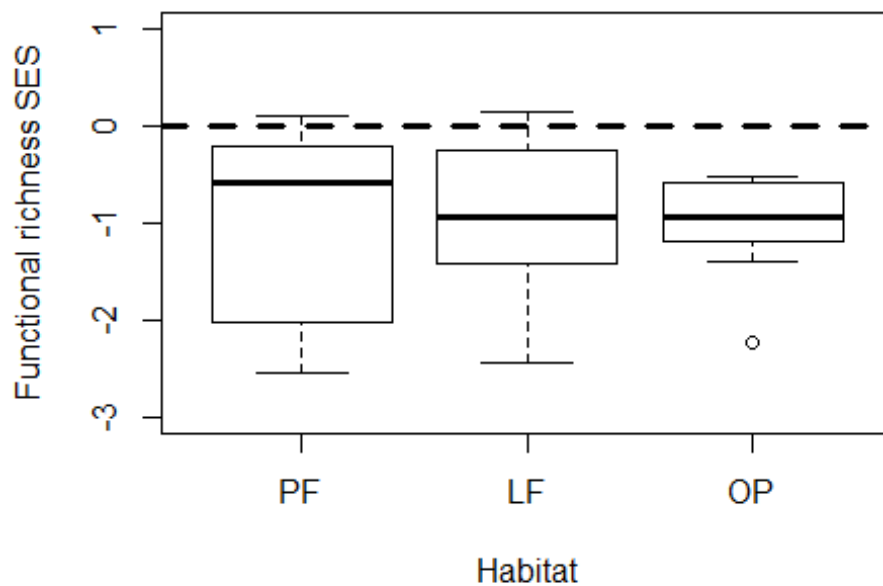
```

```
##
## data: FRic_OP$FRic and FRic_OP$FRic_ex
## t = -4.4411, df = 6, p-value = 0.004371
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.4281025 -0.1239405
## sample estimates:
## mean of the differences
## -0.2760215
```

### Test whether these SES values vary with habitat type

The standardised effect size was compared across the habitats to test whether functional richness SES is influenced by habitat type. Since the normality was respected, an ANOVA was carried out.

```
FRic$FRic_SES<-(FRic$FRic-FRic$FRic_ex)/FRic$FRic_ex_sd
FRic$Habitat <- factor(FRic$Habitat , levels=c("PF", "LF", "OP"))
boxplot(FRic$FRic_SES~FRic$Habitat, xlab="Habitat", ylim=c(-3,1),
ylab="Functional richness SES")
abline(h=0,lty="dashed",lwd=3)
```



```
shapiro.test(FRic$FRic_SES)
```

```
##
## Shapiro-Wilk normality test
##
```

```
## data: FRic$FRic_SES
## W = 0.91957, p-value = 0.07449

mod_SES<-aov(FRic$FRic_SES~FRic$Habitat)
summary(mod_SES)

##              Df Sum Sq Mean Sq F value Pr(>F)
## FRic$Habitat  2   0.07  0.0351   0.044  0.957
## Residuals    19  15.09  0.7944
```

## B. Functional diversity: Functional evenness and functional divergence

The functional evenness and the functional divergence (Rao's quadratic entropy) of Bornean frogs were calculated with the indices developed by Vileger et al. (2008) and Botta-Dukat (2005) and improved by Laliberte and Legendre (2010).

```
library(FD)
library(picante)

dbFD<-dbFD(distance_matrix, community,w.abun =
TRUE,calc.FRic=FALSE,stand.FRic=TRUE, scale.RaoQ = TRUE,calc.FGR =
FALSE,calc.CWM = FALSE,calc.FDiv = FALSE,messages = TRUE)

## FEve: Could not be calculated for communities with <3 functionally
singular species.

FEve<-dbFD$FEve
RaoQ<-dbFD$RaoQ
FD<-cbind(FRic, FEve, RaoQ)
```

## Null models for functional evenness and functional divergence

To obtain functional evenness and functional divergence values independent of species richness and remove its effect on those indices, null models with 999 randomisations were used (De Coster et al., 2015).

```
#Observed FD measures
obs.FEve<-FD$FEve
obs.RaoQ<-FD$RaoQ

#Randomize species and obtain null distribution for n random communities
(maintaining fragment species richness)
nsamp=999
niter=999
nsites=dim(community)[1]
nspecies=dim(community)[2]

FEve.rand<-matrix(data=NA,nrow=nsites,ncol=nsamp)
row.names(FEve.rand) = row.names(FD)
RaoQ.rand<-matrix(data=NA,nrow=nsites,ncol=nsamp)
row.names(RaoQ.rand) = row.names(FD)
```

```

i=1
while (i < nsamp+1) {
  print(i) #a counter to know how far the simulation is #
  random.community=matrix(data=0,nrow=nsites, ncol=nspecies,dimnames =
list(rownames(community),colnames(community)) )
  random.community=randomizeMatrix(community, null.model = "richness",
iterations = niter)

  for (j in 1:nsites) {
    random.community[j,]= random.community[j,]
  }
  if (all(apply(random.community, 2, sum) != 0)) { #condition needed to run
dbFD

FD.community.1sample=dbFD(distance_matrix,a=random.community,w.abun=TRUE,
calc.CWM=FALSE,calc.FRic = FALSE,stand.FRic=FALSE,scale.RaoQ = TRUE,calc.FDiv
= FALSE)
  FD.community.1sample=as.data.frame(FD.community.1sample)
  FEve.rand[,i]=FD.community.1sample$FEve
  RaoQ.rand[,i]=FD.community.1sample$RaoQ
  i=i+1
}
}

FEve.rand<-as.matrix(FEve.rand)
RaoQ.rand<-as.matrix(RaoQ.rand)

write.csv(FEve.rand, file = "FEve.rand_null_model_n999.csv", row.names=TRUE)
write.csv(RaoQ.rand, file = "RaoQ.rand_null_model_n999.csv", row.names=TRUE)

```

Standardised effect sizes (SES) were calculated for functional evenness and functional divergence to quantify the deviation of observed values from expected null models. SES was calculated by subtracting the mean of the expected functional evenness and divergence values from each observed value and dividing it by the standard deviation of the expected values.

```

obs.FEve<-FD$FEve
obs.RaoQ<-FD$RaoQ

nsamp=999
niter=999
nsites=dim(community)[1]
nspecies=dim(community)[2]

FEve.rand = as.matrix(read.csv("FEve.rand_null_model_n999.csv",header=T,
row.names=1))
RaoQ.rand = as.matrix(read.csv("RaoQ.rand_null_model_n999.csv",header=T,
row.names=1))

```



```

mean.FEve.rand=apply(FEve.rand,1,mean)
sd.FEve.rand=apply(FEve.rand,1,sd)

mean.RaoQ.rand=apply(RaoQ.rand,1,mean)
sd.RaoQ.rand=apply(RaoQ.rand,1,sd)

SESFve<-rep(NA,nsites)
names(SESFve)=rownames(community)
SESRaoQ<-rep(NA,nsites)
names(SESRaoQ)=rownames(community)
for (j in 1:nsites) {
  SESFve[j]=(obs.FEve[j] - mean.FEve.rand[j])/sd.FEve.rand[j]
  SESRaoQ[j]=(obs.RaoQ[j] - mean.RaoQ.rand[j])/sd.RaoQ.rand[j]
}
SESFd=cbind(FD,SESFve,SESRaoQ)

```

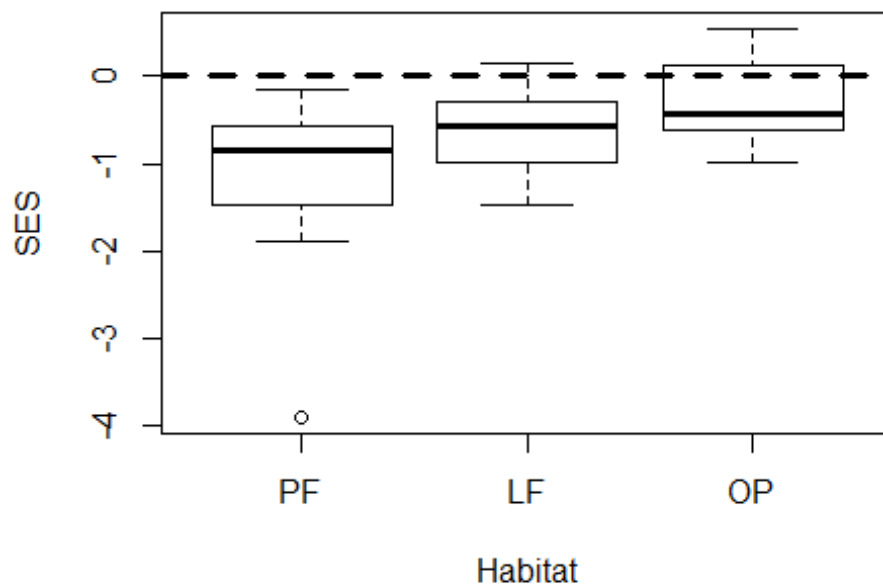
### Test whether these SES values vary with habitat type

As the functional evenness and functional divergence followed non-parametric distributions, the comparison across habitats was evaluated with a Kruskal-Wallis analysis of variance test. ##### FEve

```

SESFd$Habitat <- factor(SESFd$Habitat , levels=c("PF", "LF", "OP"))
boxplot(SESFd$SESFve~SESFd$Habitat, xlab="Habitat",ylab="SES")
abline(h=0,lty="dashed",lwd=3)

```



```
shapiro.test(SESFD$SESFEve)

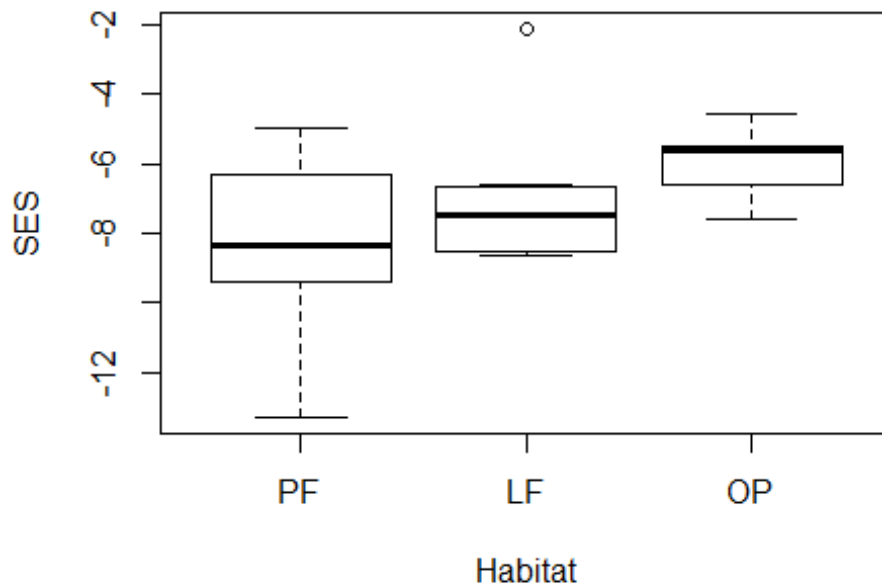
##
##  Shapiro-Wilk normality test
##
## data:  SESFD$SESFEve
## W = 0.84002, p-value = 0.002873

mod_SES_FEve<-kruskal.test(SESFD$SESFEve~SESFD$Habitat)
mod_SES_FEve

##
##  Kruskal-Wallis rank sum test
##
## data:  SESFD$SESFEve by SESFD$Habitat
## Kruskal-Wallis chi-squared = 3.5918, df = 2, p-value = 0.166
```

### RaoQ

```
SESFD$Habitat <- factor(SESFD$Habitat , levels=c("PF", "LF", "OP"))
boxplot(SESFD$SES RaoQ~SESFD$Habitat, xlab="Habitat",ylab="SES")
abline(h=0,lty="dashed",lwd=3)
```



```
shapiro.test(SESFD$SES RaoQ)

##
##  Shapiro-Wilk normality test
##
```

```
## data: SESFD$SES RaoQ
## W = 0.95388, p-value = 0.3762

mod_SES_RaoQ<-kruskal.test(SESFD$SES RaoQ~SESFD$Habitat)
mod_SES_RaoQ

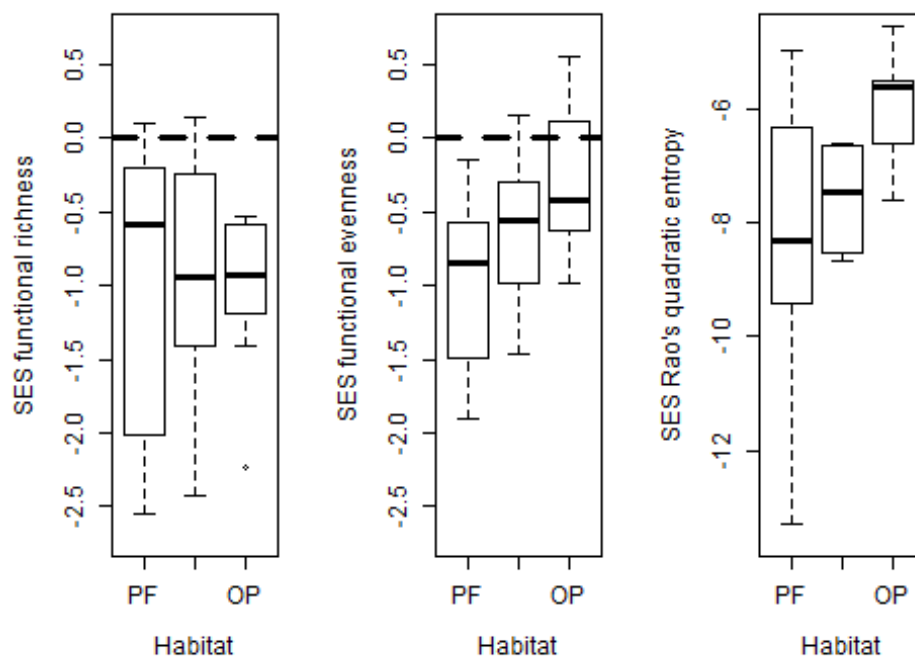
##
## Kruskal-Wallis rank sum test
##
## data: SESFD$SES RaoQ by SESFD$Habitat
## Kruskal-Wallis chi-squared = 4.0401, df = 2, p-value = 0.1326

par(mfrow=c(1,3))
FD$Habitat <- factor(FD$Habitat , levels=c("PF", "LF", "OP"))
SESFD$Habitat <- factor(SESFD$Habitat , levels=c("PF", "LF", "OP"))

boxplot(FD$Fric_SES~SESFD$Habitat, xlab="Habitat",ylab="SES functional
richness", ylim=c(-2.7,0.7),cex.axis=1.1,cex.lab=1.1)
abline(h=0,lty="dashed",lwd=3)

boxplot(SESFD$SESFEve~SESFD$Habitat, xlab="Habitat",ylab="SES functional
evenness",ylim=c(-2.7,0.7),cex.axis=1.1,cex.lab=1.1)
abline(h=0,lty="dashed",lwd=3)

boxplot(SESFD$SES RaoQ~SESFD$Habitat, xlab="Habitat",ylab="SES Rao's quadratic
entropy", ylim=c(-13.5,-4.7),cex.axis=1.1,cex.lab=1.1)
abline(h=0,lty="dashed",lwd=3)
```



## C. Functional trait variability: Community weighted mean

To investigate whether some functional traits vary across the different habitats, the community weighted mean of each trait was compared between all the habitat types. The community weighted mean is defined as the mean value of each trait weighted by the relative abundance of each species (Lavorel et al., 2008). Traits with parametric distributions were evaluated with a one-way ANOVA followed by a Tukey post-hoc test. Traits with non-parametric distributions were evaluated with a Kruskal-Wallis analysis of variance test followed by a Nemenyi post-hoc test.

```
Traits2<-readWorkbook("OJ_functional_frog_data.xlsx", sheet=3, colNames =
TRUE,rowNames = TRUE)
community<-as.matrix(community)
CWM1<-functcomp(Traits2,community)
CWM<-cbind(Habitat,CWM1)
CWM$Habitat <- factor(CWM$Habitat , levels=c("PF", "LF", "OP"))

#cp
shapiro.test(CWM$CP)
boxplot(CWM$CP~CWM$Habitat,xlab="Habitat",ylab="CWM_CP")
Mod_cwm_cp<-kruskal.test(CWM$CP~CWM$Habitat)
Mod_cwm_cp

#BH
shapiro.test(CWM$BH)
boxplot(CWM$BH~CWM$Habitat,xlab="Habitat",ylab="CWM_BH")
Mod_cwm_BH<-aov(CWM$BH~CWM$Habitat)
summary(Mod_cwm_BH)

#Mean male SVL
shapiro.test(CWM$Mean.male.SVL)
boxplot(CWM$Mean.male.SVL~CWM$Habitat,xlab="Habitat",ylab="CWM_Mean.male.SVL"
)
Mod_cwm_Mean.male.SVL<-kruskal.test(CWM$Mean.male.SVL~CWM$Habitat)
Mod_cwm_Mean.male.SVL

#Mean female SVL
shapiro.test(CWM$Mean.female.SVL)
boxplot(CWM$Mean.female.SVL~CWM$Habitat,xlab="Habitat",ylab="CWM_Mean.female.
SVL")
Mod_cwm_Mean.female.SVL<-kruskal.test(CWM$Mean.female.SVL~CWM$Habitat)
Mod_cwm_Mean.female.SVL

#GW
shapiro.test(CWM$GW)
boxplot(CWM$GW~CWM$Habitat,xlab="Habitat",ylab="CWM_GW")
Mod_cwm_GW<-kruskal.test(CWM$GW~CWM$Habitat)
Mod_cwm_GW
```

```

#RHL
shapiro.test(CWM$RHL)
boxplot(CWM$RHL~CWM$Habitat,xlab="Habitat",ylab="CWM_RHL")
Mod_cwm_RHL<-aov(CWM$RHL~CWM$Habitat)
summary(Mod_cwm_RHL)

#HW
shapiro.test(CWM$HW)
boxplot(CWM$HW~CWM$Habitat,xlab="Habitat",ylab="CWM_HW")
Mod_cwm_HW<-aov(CWM$HW~CWM$Habitat)
summary(Mod_cwm_HW)

#FW
shapiro.test(CWM$FW)
boxplot(CWM$FW~CWM$Habitat,xlab="Habitat",ylab="CWM_FW")
Mod_cwm_FW<-aov(CWM$FW~CWM$Habitat)
summary(Mod_cwm_FW)

#TD
shapiro.test(CWM$TD)
boxplot(CWM$TD~CWM$Habitat,xlab="Habitat",ylab="CWM_TD")
Mod_cwm_TD<-aov(CWM$TD~CWM$Habitat)
summary(Mod_cwm_TD)

#RM
shapiro.test(CWM$RM)
boxplot(CWM$RM~CWM$Habitat,xlab="Habitat",ylab="CWM_RM")
Mod_cwm_RM<-kruskal.test(CWM$RM~CWM$Habitat)
Mod_cwm_RM

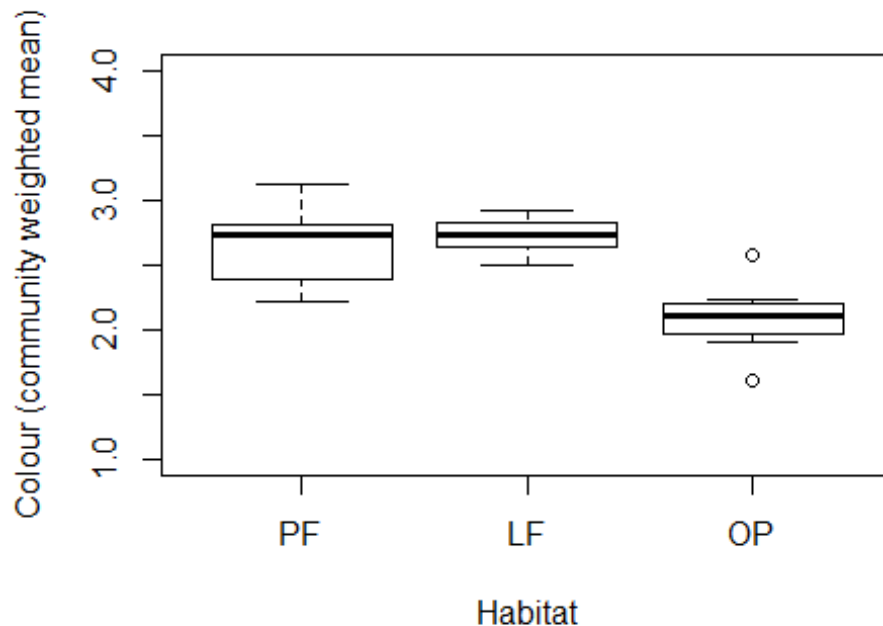
#BC
shapiro.test(CWM$BC)
boxplot(CWM$BC~CWM$Habitat,xlab="Habitat",ylab="Colour (community weighted
mean)", ylim=c(1,4))
Mod_cwm_BC<-aov(CWM$BC~CWM$Habitat)
summary(Mod_cwm_BC)
Tukey_BC<-TukeyHSD(x=Mod_cwm_BC, 'CWM$Habitat', conf.level=0.95,)
Tukey_BC

#GC
shapiro.test(CWM$GC)
boxplot(CWM$GC~CWM$Habitat,xlab="Habitat",ylab="CWM_GC")
Mod_cwm_GC<-aov(CWM$GC~CWM$Habitat)
summary(Mod_cwm_GC)
Tukey_GC<-TukeyHSD(x=Mod_cwm_GC, 'CWM$Habitat', conf.level=0.95)
Tukey_GC

```

Only the community weighted mean of the “Basic colouration” trait differed with habitat type. The other traits were not affected.

```
boxplot(CWM$BC~CWM$Habitat,xlab="Habitat",ylab="Colour (community weighted mean)", ylim=c(1,4))
```



```
shapiro.test(CWM$BC)

##
##  Shapiro-Wilk normality test
##
## data:  CWM$BC
## W = 0.95699, p-value = 0.4309

Mod_cwm_BC<-aov(CWM$BC~CWM$Habitat)
summary(Mod_cwm_BC)

##              Df Sum Sq Mean Sq F value    Pr(>F)
## CWM$Habitat   2  1.727   0.8635   12.98 0.000279 ***
## Residuals    19  1.264   0.0665
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Tukey_BC<-TukeyHSD(x=Mod_cwm_BC, 'CWM$Habitat', conf.level=0.95,)
Tukey_BC

##    Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = CWM$BC ~ CWM$Habitat)
##
```

```
## $`CWM$Habitat`
##           diff           lwr           upr           p adj
## LF-PF  0.09497503 -0.2440848  0.4340349  0.7596816
## OP-PF -0.54497757 -0.8951571 -0.1947981  0.0023419
## OP-LF -0.63995260 -0.9790124 -0.3008928  0.0003543
```

## D. Functional composition: Functional integrity

Functional composition was first assessed with the functional integrity index developed by De Coster et al.(2015). The community weighted mean dataset was used to calculate the pairwise distance between sites based on the gower distance. The resulting distance matrix was reduced by a classical multidimensional scaling ordination. Functional integrity was measured by subtracting the mean values of primary forests sites from each logged forest site and each oil palm plantation sites.

### Measure functional integrity

```
dist_cwm<-as.matrix(vegdist(CWM1,method="gower", binary=FALSE))
cmd<-cmdscale(dist_cwm,k=2,eig=TRUE)$points[,1]

cmd.PF<-cmd[names.PF<-
c("Elepan","Injing","Rhino","Sibut","Tembaling","VJR","West")]
cmd.LF<-cmd[names.LF<-c("0m","120m","15m","30m","5m","60m","LFE","NEW")]
cmd.OP<-cmd[names.OP<-
c("Belian","Gaharu1","Gaharu2","Gaharu3","Mawang","Menggaris","Merbau")]

posneg=ifelse (mean(cmd.PF)< mean(cmd.LF),-1,1)
# -1 if primary forest sites have, on average, smaller values than logged
forest sites; 1 otherwise
FI_LF= (cmd.LF-mean(cmd.PF))*posneg + 1
#The obtained values were multiplied by posneg to assure higher values of
primary forests sites and a positive range was created by adding one to all
the values.

posneg2<-ifelse (mean(cmd.PF)< mean(cmd.OP),-1,1)
# -1 if primary forest sites have, on average, smaller values than oil palm
plantation sites; 1 otherwise
FI_OP=(cmd.OP-mean(cmd.PF))*posneg2 + 1
#The obtained values were multiplied by posneg2 to assure higher values of
primary forests sites and a positive range was created by adding one to all
the values.
```

### Test whether functional integrity changed from primary forests to logged forests and from primary forests to oil palm plantations

```
FI_table<-readWorkbook("Functional integrity.xlsx", sheet=1)
t.test(FI_table$PF,FI_table$LF)

##
## Welch Two Sample t-test
##
```

```
## data: FI_table$PF and FI_table$LF
## t = 0.45906, df = 7, p-value = 0.6601
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.1181232 0.1750358
## sample estimates:
## mean of x mean of y
## 1.0000000 0.9715437

t.test(FI_table$PF,FI_table$OP)

##
## Welch Two Sample t-test
##
## data: FI_table$PF and FI_table$OP
## t = 0.77404, df = 6, p-value = 0.4683
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.08734583 0.16817532
## sample estimates:
## mean of x mean of y
## 1.0000000 0.9595853
```

## E. Functional composition: Functional beta-diversity

Functional composition of Bornean frogs was then measured with the tree-based beta-diversity index which can be used to compute functional beta-diversity. Functional beta-diversity represent the trait composition dissimilarity between a pair of communities by measuring the unique (non-shared) fraction of the functional tree between each community pair (Swenson, 2014).

### Functional beta-diversity

```
frogs_rev_dendrogram<-rev(frogs_dendrogram) # change the order of the
branches by reversing the nodes to compute unifrac
frogs_rev_dendrogram<-as.phylo(frogs_rev_dendrogram) # transform the reversed
dendrogram into a tree of class "phylo", required for unifrac

frogs_rev_cophenetic<-cophenetic.phylo(frogs_rev_dendrogram)

#verify that the branch length dissimilarity matrix of the reverse tree
correspond to the original dissimilarity matrix between the traits
mantel(distance_matrix, frogs_rev_cophenetic, method="pearson",
permutations=999)

##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = distance_matrix, ydis = frogs_rev_cophenetic, method =
"pearson", permutations = 999)
```



```
##
## Mantel statistic r: 0.6689
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##      90%      95%    97.5%      99%
## 0.0612 0.0799 0.0981 0.1161
## Permutation: free
## Number of permutations: 999

# verify that the branch length dissilarity matrix of the reversed tree
correspond to the brach length of the original tree
mantel(frogs_cophenetic,frogs_rev_cophenetic,method="pearson",permutations =
999)

##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = frogs_cophenetic, ydis = frogs_rev_cophenetic,      method =
"pearson", permutations = 999)
##
## Mantel statistic r:      1
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##      90%      95%    97.5%      99%
## 0.0654 0.0856 0.1029 0.1194
## Permutation: free
## Number of permutations: 999

#measure the functional beta-diversity
uw_beta.funct<-as.matrix(unifrac(community,frogs_rev_dendrogram))
```

## Test whether functional beta-diversity is affected by habitat type

Then, a permutational multivariate analysis of variance (perMANOVA) with 999 permutations was used to test whether the functional beta-diversity (dissimilarity between functional traits) was affected by habitat type.

```
# model estimate
adonis(uw_beta.funct~FD$Habitat)

##
## Call:
## adonis(formula = uw_beta.funct ~ FD$Habitat)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
```

```
##
##              Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## FD$Habitat  2    1.4256 0.71279  5.6076 0.37118  0.001 ***
## Residuals  19    2.4151 0.12711          0.62882
## Total      21    3.8407          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Arbizu (2020) created a function that compute a pairwise perMANOVA. This
# function was used to compare the functional beta-diversity across the three
# different habitats
pairwise.adonis2 <- function(x, data, strata = NULL, nperm=999, ... ) {

  ##describe parent call function
  ststri <- ifelse(is.null(strata), 'Null', strata)
  fostri <- as.character(x)
  #list to store results

  ##copy model formula
  x1 <- x
  ## extract left hand side of formula
  lhs <- x1[[2]]
  ##extract factors on right hand side of formula
  rhs <- x1[[3]]
  ## create model.frame matrix
  x1[[2]] <- NULL
  rhs.frame <- model.frame(x1, data, drop.unused.levels = TRUE)

  ## create unique pairwise combination of factors
  co <- combn(unique(as.character(rhs.frame[,1])),2)

  ## create names vector
  nameress <- c('parent_call')
  for (elem in 1:ncol(co)){
    nameress <- c(nameress, paste(co[1,elem], co[2,elem], sep='_vs_'))
  }
  ##create results list
  res <- vector(mode="list", length=length(nameress))
  names(res) <- nameress

  ##add parent call to res
  res['parent_call'] <- list(paste(fostri[2], fostri[1], fostri[3], ', strata
  =', ststri))

  ##start iteration trough pairwise combination of factors
  for(elem in 1:ncol(co)){

  ##reduce model elements
```

```

    if(inherits(eval(lhs), 'dist')){
      xred <- as.dist(as.matrix(eval(lhs))[rhs.frame[,1] %in%
c(co[1,elem],co[2,elem]),
      rhs.frame[,1] %in% c(co[1,elem],co[2,elem])])
    }else{
      xred <- eval(lhs)[rhs.frame[,1] %in% c(co[1,elem],co[2,elem]),]
    }

    mdat1 <- data[rhs.frame[,1] %in% c(co[1,elem],co[2,elem]),]

## redefine formula
    if(length(rhs) == 1){
      xnew <- as.formula(paste('xred',as.character(rhs),sep='~'))
    }else{
      xnew <- as.formula(paste('xred' ,
      paste(rhs[-1],collapse= as.character(rhs[1])),
      sep='~'))}

##pass new formula to adonis
    if(is.null(strata)){
      ad <- adonis(xnew,data=mdat1, ... )
    }else{
      perm <- how(nperm = nperm)
      setBlocks(perm) <- with(mdat1, mdat1[,ststri])
      ad <- adonis(xnew,data=mdat1,permutations = perm, ... )}

    res[nameres[elem+1]] <- ad[1]
  }
  #names(res) <- names
  class(res) <- c("pwadstrata", "list")
  return(res)
}
pairwise.adonis2(uw_beta.funct~Habitat,FD)

## $parent_call
## [1] "uw_beta.funct ~ Habitat , strata = Null"
##
## $LF_vs_OP
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Habitat    1   0.17137 0.171368  16.846 0.56443 0.002 **
## Residuals 13   0.13224 0.010173    0.43557
## Total     14   0.30361          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##

```

```
## $LF_vs_PF
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model    R2 Pr(>F)
## Habitat     1  0.016319 0.016319  1.4122 0.09799  0.23
## Residuals  13  0.150222 0.011555          0.90201
## Total       14  0.166541          1.00000
##
## $OP_vs_PF
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model    R2 Pr(>F)
## Habitat     1  0.137366 0.137366 16.524 0.5793 0.002 **
## Residuals  12  0.099756 0.008313          0.4207
## Total       13  0.237122          1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## attr(,"class")
## [1] "pwadstrata" "list"
```

## NMDS

A non-metric multidimensional scaling ordination method was used to visualise the dissimilarity of trait composition (measured by functional beta-diversity) between the different habitats.

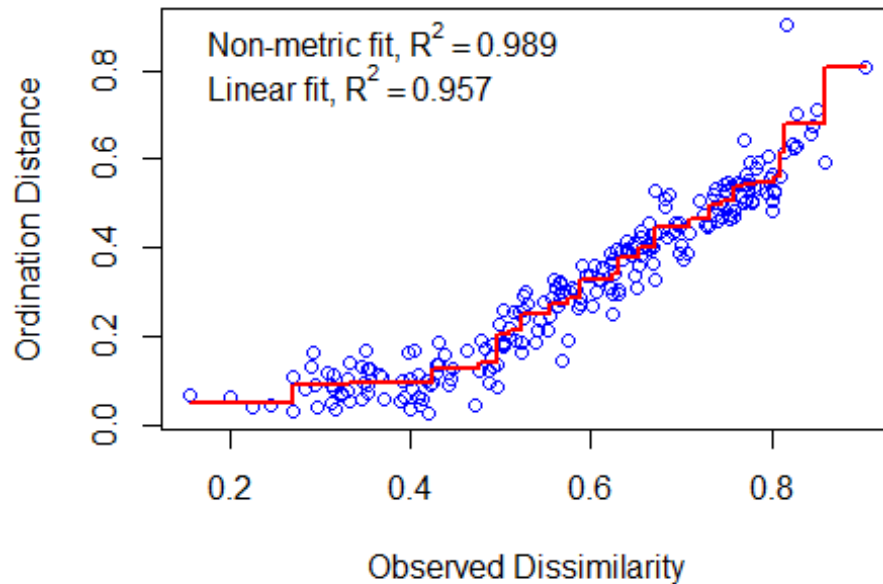
```
frogs_metaMDS<-metaMDS(uw_beta.funct,k=2,try =2, trymax =2,engine ="monoMDS",
trace=TRUE,plot = FALSE,wascores = TRUE)

## Run 0 stress 0.1089512
## Run 1 stress 0.106937
## ... New best solution
## ... Procrustes: rmse 0.08683262  max resid 0.3389088
## Run 2 stress 0.1049079
## ... New best solution
## ... Procrustes: rmse 0.02256529  max resid 0.06585383
## *** No convergence -- monoMDS stopping criteria:
##      2: stress ratio > sratmax

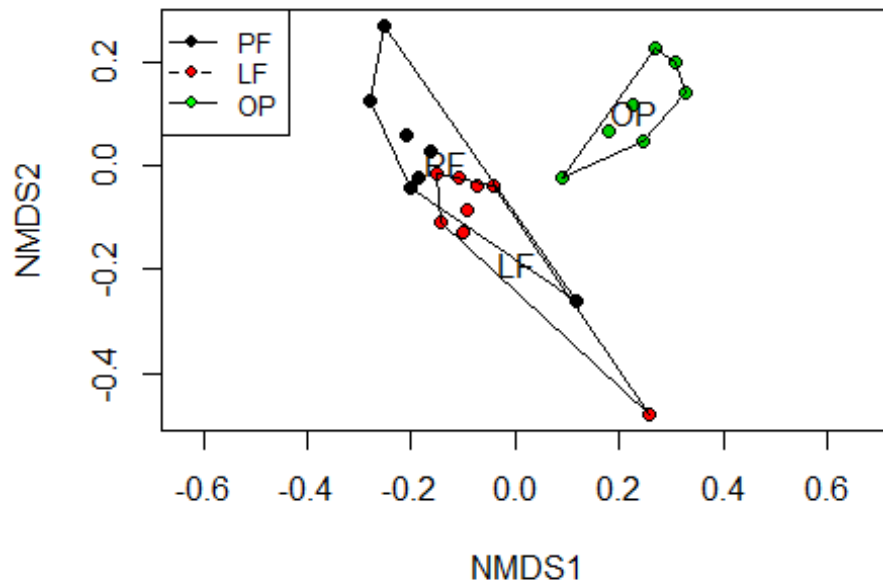
frogs_metaMDS$stress # stress measures the fit between observed
dissimilarities and the ordination distances

## [1] 0.1049079
```

```
stressplot(frogs_metaMDS) # Shepard plot represent the fit between observed  
dissimilarities and the ordination distances
```



```
ordiplot<-ordiplot(frogs_metaMDS,type = "n")  
## species scores not available  
points(ordiplot,what = "sites",bg=CWM$Habitat,pch=21)  
legend("topleft", legend=c("PF", "LF","OP"),pch =21, pt.bg=c("black", "red",  
"green"), lty=1:2, cex=0.8)  
ordihull(ordiplot,FD$Habitat,label=TRUE)
```



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