

Assessing source-sink dynamics in invaded habitat using metabarcoding

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Read in data frames

```
# Total samples from collection
collection <- read.csv(file = "collection_data.csv")

# Dataframe including all sequences, without filtering
all_pagio <- read.csv("all_reads.csv")

# Dataframe including all prey sequences following filtering
prey_final <- read.csv("prey_reads.csv")

# Native versus non-native prey status
native_status <- read.csv("native_status.csv")

# Parasites
parasites <- read.csv("parasites.csv")
```

Summaries of results

Collection

```
# By block, transect and site
collection %>%
  group_by(site_status) %>%
  summarise(n_distinct(id)) # 82/86
```

```
## # A tibble: 2 x 2
##   site_status 'n_distinct(id)'
##   <chr>          <int>
## 1 Ginger             82
## 2 Native             86
```

```
collection %>%
  group_by(site_status, transect, block) %>%
  summarise(n = n_distinct(id)) %>%
```

```
ungroup() %>%
summarise(mean(n), plotrix::std.error(n)) # 5.793103
```

```
## # A tibble: 1 x 2
##   'mean(n)' 'plotrix::std.error(n)'
##       <dbl>                <dbl>
## 1      5.79                0.547
```

```
collection %>%
  group_by(transect) %>%
  summarise(n = n_distinct(id)) %>%
  ungroup() %>%
  summarise(mean(n), plotrix::std.error(n)) # 16.8
```

```
## # A tibble: 1 x 2
##   'mean(n)' 'plotrix::std.error(n)'
##       <dbl>                <dbl>
## 1     16.8                2.38
```

Molecular

```
# Reads removed
removed_reads <- sum(all_pagio$count) - sum(preys_final$count) # 1062003
```

```
# From the spiders
all_pagio %>%
  filter(family == "Philodromidae") %>%
  summarize(sum(count) / removed_reads,
            sum(count)) # 679534, 0.6412852
```

```
##   sum(count)/removed_reads sum(count)
## 1              0.6412852    679534
```

```
# From fungi
all_pagio %>%
  filter(kingdom == "Fungi") %>%
  summarize(sum(count),
            sum(count) / removed_reads) # 234630, 0.2214234
```

```
##   sum(count) sum(count)/removed_reads
## 1     234630              0.2214234
```

```
# Hymenopterans - parasitoids
all_pagio %>%
  filter(order == "Hymenoptera") %>%
  summarise(sum(count),
            sum(count) / removed_reads) # 60060, 0.05667941
```

```
##   sum(count) sum(count)/removed_reads
## 1     60060              0.05667941
```

```
# Summary of filtered data set
```

```
# Reads and ASVs per spider
```

```
prey_final %>%  
  group_by(id) %>%  
  summarise(n = sum(count),  
            nasv = n_distinct(asv)) %>%  
  ungroup() %>%  
  summarise(reads_mean = mean(n), reads_stderror = plotrix::std.error(n),  
            asv_mean = mean(nasv),  
            asv_stderror = plotrix::std.error(nasv))
```

```
## # A tibble: 1 x 4  
##   reads_mean reads_stderror asv_mean asv_stderror  
##   <dbl>         <dbl>    <dbl>    <dbl>  
## 1     2288.         288.     2.55     0.126
```

```
# ASVs and specimens by marker
```

```
prey_final %>%  
  group_by(marker) %>%  
  summarise(asv = n_distinct(asv),  
            sample = n_distinct(id))
```

```
## # A tibble: 3 x 3  
##   marker  asv sample  
##   <chr> <int> <int>  
## 1 16s    110   112  
## 2 18s     10    17  
## 3 28s     44    76
```

```
rm(removed_reads)
```

Taxonomy

```
# Total taxonomy breakdown
```

```
prey_final %>%  
  summarise(n_distinct(order), n_distinct(family),  
            n_distinct(genus), n_distinct(species))
```

```
##   n_distinct(order) n_distinct(family) n_distinct(genus) n_distinct(species)  
## 1                9                31                32                23
```

```
# Percent of ASVs with matches
```

```
# Number of ASVs retained with matches
```

```
length(unique(pre_y_final$asv[!is.na(pre_y_final$species)])) /  
length(unique(pre_y_final$asv)) # 0.2317073
```

```
## [1] 0.2317073
```

```
length(unique(pre_final$asv[!is.na(pre_final$genus)])) /
length(unique(pre_final$asv)) # 0.3780488
```

```
## [1] 0.3780488
```

```
length(unique(pre_final$asv[!is.na(pre_final$family)])) /
length(unique(pre_final$asv)) # 0.6158537
```

```
## [1] 0.6158537
```

```
# Percent matches by marker
pre_final %>%
  group_by(marker) %>%
  summarise(mean(percent_match))
```

```
## # A tibble: 3 x 2
##   marker 'mean(percent_match)'
##   <chr>          <dbl>
## 1 16s             91.6
## 2 18s             98.3
## 3 28s             97.7
```

Prey diversity and abundance

Summarizing composition

```
# All ASVs
length(unique(pre_final$asv)) # 164
```

```
## [1] 164
```

```
# Shared ASVs
length(intersect(pre_final$asv[pre_final$site_status == "ginger"],
  pre_final$asv[pre_final$site_status == "native"])) # 29
```

```
## [1] 29
```

```
# Shared taxonomy
length(intersect(pre_final$order[pre_final$site_status == "ginger"],
  pre_final$order[pre_final$site_status == "native"])) # 6 orders shared
```

```
## [1] 6
```

```
length(unique(pre_final$order)) # 9
```

```
## [1] 9
```

```
length(intersect(pre_final$family[pre_final$site_status == "ginger"],
  pre_final$family[pre_final$site_status == "native"])) # 11 families shared
```

```
## [1] 11
```

```
length(unique(pre_final$family)) # 31
```

```
## [1] 31
```

```
length(intersect(pre_final$genus[pre_final$site_status == "ginger"],
  pre_final$genus[pre_final$site_status == "native"])) # 10 genera shared
```

```
## [1] 10
```

```
length(unique(pre_final$genus)) # 32
```

```
## [1] 32
```

```
length(intersect(pre_final$species[pre_final$site_status == "ginger"],
  pre_final$species[pre_final$site_status == "native"])) # 5 species shared
```

```
## [1] 5
```

```
length(unique(pre_final$species)) # 23
```

```
## [1] 23
```

Hill numbers

Create community matrices

```
# Create community matrices

# By ASVs
asv_comm <-
  prey_final %>%
  acast(id ~ asv,
    value.var = "count", # Count as values
    fun.aggregate = sum) %>% # Sum counts for site
  as.matrix()

# Transform
asv_hellinger <- decostand(asv_comm, method = "hellinger")

# Incidence
asv_incidence <- asv_hellinger
asv_incidence[asv_incidence > 0] <- 1
```

```

# By taxonomy - family
family_comm <-
  prey_final %>%
  filter(!is.na(family)) %>%
  acast(id ~ family,
        value.var = "count",
        fun.aggregate = sum,
        fill = 0) %>%
  as.matrix()

family_hellinger <- decostand(family_comm, method = "hellinger")

family_incidence <- family_hellinger
family_incidence[family_incidence > 0] <- 1

# By taxonomy - order
order_comm <-
  prey_final %>%
  acast(id ~ order,
        value.var = "count",
        fun.aggregate = sum,
        fill = 0) %>%
  as.matrix()

order_hellinger <- decostand(order_comm, method = "hellinger")

order_incidence <- order_hellinger
order_incidence[order_incidence > 0] <- 1

```

Calculate Hill numbers and perform Welch t-test

```

# ASVs

asv_hill <-
  renyi(asv_hellinger,
        scales = c(0, 1, 2),
        hill = T) %>%
  cbind(collection[collection$id %in% rownames(asv_hellinger),])

# Welch t-test - ASV
asv_q0 <- t.test(asv_hill$`0`[asv_hill$site_status == "Ginger"],
                 asv_hill$`0`[asv_hill$site_status == "Native"])

asv_q1 <- t.test(asv_hill$`1`[asv_hill$site_status == "Ginger"],
                 asv_hill$`1`[asv_hill$site_status == "Native"])

asv_q2 <- t.test(asv_hill$`2`[asv_hill$site_status == "Ginger"],
                 asv_hill$`2`[asv_hill$site_status == "Native"])

hill_asv_sum <-

```

```

data.frame(cbind(
  rbind(asv_q0$estimate,
        asv_q1$estimate,
        asv_q2$estimate),
  rbind(asv_q0$p.value,
        asv_q1$p.value,
        asv_q2$p.value)))

colnames(hill_asv_sum) <- c("ginger", "native", "p")
hill_asv_sum$level <- "ASV"
hill_asv_sum$q <- c("q = 0", "q = 1", "q = 2")

# Family

family_hill <- renyi(family_hellinger, scales = c(0, 1, 2), hill = T) %>%
  cbind(collection[collection$id %in% rownames(family_hellinger),])

# Welch t-test - family
fam_q0 <- t.test(family_hill$`0`[family_hill$site_status == "Ginger"],
                 family_hill$`0`[family_hill$site_status == "Native"])

fam_q1 <- t.test(family_hill$`1`[family_hill$site_status == "Ginger"],
                 family_hill$`1`[family_hill$site_status == "Native"])

fam_q2 <- t.test(family_hill$`2`[family_hill$site_status == "Ginger"],
                 family_hill$`2`[family_hill$site_status == "Native"])

hill_fam_sum <- data.frame(cbind(rbind(fam_q0$estimate,
                                       fam_q1$estimate,
                                       fam_q2$estimate),
  rbind(fam_q0$p.value,
        fam_q1$p.value,
        fam_q2$p.value)))

colnames(hill_fam_sum) <- c("ginger", "native", "p")
hill_fam_sum$level <- "Family"
hill_fam_sum$q <- c("q = 0", "q = 1", "q = 2")

# Order

order_hill <- renyi(order_hellinger, scales = c(0, 1, 2), hill = T) %>%
  cbind(collection[collection$id %in% rownames(order_hellinger),])

# Welch t-test - order
order_q0 <- t.test(order_hill$`0`[order_hill$site_status == "Ginger"],
                   order_hill$`0`[order_hill$site_status == "Native"])

order_q1 <- t.test(order_hill$`1`[order_hill$site_status == "Ginger"],
                   order_hill$`1`[order_hill$site_status == "Native"])

order_q2 <- t.test(order_hill$`2`[order_hill$site_status == "Ginger"],
                   order_hill$`2`[order_hill$site_status == "Native"])

```

```

hill_order_sum <- data.frame(cbind(rbind(order_q0$estimate,
  order_q1$estimate,
  order_q2$estimate),
rbind(order_q0$p.value,
  order_q1$p.value,
  order_q2$p.value)))

colnames(hill_order_sum) <- c("ginger", "native", "p")
hill_order_sum$q <- c("q = 0", "q = 1", "q = 2")
hill_order_sum$level <- "Order"

# Combined table
hill_summary <- rbind(hill_asv_sum, hill_fam_sum, hill_order_sum)
print(hill_summary)

```

```

##      ginger  native      p level      q
## 1 2.884615 2.103448 1.447550e-03 ASV q = 0
## 2 2.522093 1.865904 1.122441e-03 ASV q = 1
## 3 2.308503 1.733942 1.041656e-03 ASV q = 2
## 4 1.805970 1.255814 1.570216e-05 Family q = 0
## 5 1.662843 1.188902 1.143474e-05 Family q = 1
## 6 1.586544 1.152156 9.418654e-06 Family q = 2
## 7 1.846154 1.413793 3.962147e-04 Order q = 0
## 8 1.666830 1.325602 9.489919e-04 Order q = 1
## 9 1.578481 1.279930 1.463135e-03 Order q = 2

```

Differences in read abundances

```

reads <-
  prey_final %>%
  group_by(site_status, id) %>%
  summarise(n = sum(count)) %>%
  ungroup()

reads %>%
  group_by(site_status) %>%
  summarise(mean(n), plotrix::std.error(n))

## # A tibble: 2 x 3
##   site_status 'mean(n)' 'plotrix::std.error(n)'
##   <chr>      <dbl>      <dbl>
## 1 ginger      3469.      449.
## 2 native      701.      133.

t.test(reads$n[reads$site_status == "native"],
  reads$n[reads$site_status == "ginger"])

```

```

##
## Welch Two Sample t-test

```



```
##
## data: reads$n[reads$site_status == "native"] and reads$n[reads$site_status == "ginger"]
## t = -5.9106, df = 90.205, p-value = 5.98e-08
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -3698.444 -1837.695
## sample estimates:
## mean of x mean of y
## 700.8793 3468.9487
```

Dietary composition

Constructing community matrices, by site

```
# Data for sites
sites_data <-
  prey_final %>%
  group_by(site) %>%
  filter(row_number() == 1) %>%
  ungroup() %>%
  select(site, site_status)

# By ASV - all
site_community_asv <-
  prey_final %>%
  acast(site ~ asv, fill = 0,
        value.var = "count", # Count as values
        fun.aggregate = sum)

site_community_asv <- decostand(site_community_asv, method = "hellinger")

# By order
site_community_order <-
  prey_final %>%
  acast(site ~ order, fill = 0,
        value.var = "count", # Count as values
        fun.aggregate = sum)

site_community_order <- decostand(site_community_order, method = "hellinger")
```

Calculate beta diversity using community matrices

```
beta_transform_asv <- beta(site_community_asv, func = "jaccard")
beta_inc_asv <- beta(site_community_asv,
                    func = "jaccard", abund = F)

beta_transform_order <- beta(site_community_order, func = "jaccard")
beta_inc_order <- beta(site_community_order,
                     func = "jaccard", abund = F)
```

Perform NMDS

```
set.seed(200)

# ASV NMDS
site_asv_nmds1 <- metaMDS(beta_transform_asv$Btotal, trymax = 1000)
site_asv_nmds2 <- metaMDS(beta_inc_asv$Btotal, trymax = 1000)

# Order NMDS
site_order_nmds1 <- metaMDS(beta_transform_order$Btotal, trymax = 1000)
site_order_nmds2 <- metaMDS(beta_inc_order$Btotal, trymax = 1000)

# PERMANOVA
adonis2(beta_transform_asv$Btotal ~ site_status,
        data = sites_data, method = "jaccard")

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = beta_transform_asv$Btotal ~ site_status, data = sites_data, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## site_status  1   0.5205 0.04077 1.1477  0.152
## Residual    27  12.2456 0.95923
## Total       28  12.7662 1.00000

adonis2(beta_inc_asv$Btotal ~ site_status,
        data = sites_data, method = "jaccard")

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = beta_inc_asv$Btotal ~ site_status, data = sites_data, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## site_status  1   0.567 0.04574 1.2943  0.042 *
## Residual    27  11.829 0.95426
## Total       28  12.396 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# PERMANOVA
adonis2(beta_inc_order$Btotal ~ site_status,
        data = sites_data, method = "jaccard")

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
```

```
##
## adonis2(formula = beta_inc_order$Btotal ~ site_status, data = sites_data, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## site_status  1   0.3327 0.1036 3.1205  0.036 *
## Residual    27   2.8783 0.8964
## Total       28   3.2109 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(beta_transform_order$Btotal ~ site_status,
        data = sites_data, method = "jaccard")
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = beta_transform_order$Btotal ~ site_status, data = sites_data, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## site_status  1   0.2601 0.04704 1.3328  0.251
## Residual    27   5.2691 0.95296
## Total       28   5.5292 1.00000
```

Order-level diversity

```
prey_final %>%
  group_by(site_status, order) %>%
  # Number of spiders in each site eating each order
  summarise(n = n_distinct(id)) %>%
  ungroup() %>%
  # Add column for total number of spiders in each habitat
  mutate(total = ifelse(site_status == "Ginger",
                        78, 58),
         # Calculate proportion of spiders eating each order
         prop = round(n / total, 3))
```

```
## # A tibble: 15 x 5
##   site_status order      n total  prop
##   <chr>      <chr>   <int> <dbl> <dbl>
## 1 ginger    Araneae         1    58 0.017
## 2 ginger    Coleoptera      15    58 0.259
## 3 ginger    Diptera        29    58 0.5
## 4 ginger    Entomobryomorpha 27    58 0.466
## 5 ginger    Hemiptera       34    58 0.586
## 6 ginger    Lepidoptera     33    58 0.569
## 7 ginger    Neuroptera       1    58 0.017
## 8 ginger    Thysanoptera     4    58 0.069
## 9 native    Coleoptera       2    58 0.034
## 10 native   Diptera        13    58 0.224
## 11 native   Entomobryomorpha 1    58 0.017
## 12 native   Hemiptera      42    58 0.724
```

## 13 native	Lepidoptera	21	58	0.362
## 14 native	Psocoptera	1	58	0.017
## 15 native	Thysanoptera	2	58	0.034

NMDS for Hemiptera and Lepidoptera

```
# NMDS is doing the same thing because ASVs are distinct
set.seed(200)

# Hemiptera
hemi <-
  prey_final %>%
  filter(order == "Hemiptera" & marker == "16s") %>%
  acast(site ~ asv, fill = 0, value.var = "count",
        fun.aggregate = sum) %>%
  as.matrix()

hemi_phylo_16s <-
  BAT::beta(decostand(hemi, method = "hellinger"),
            tree = read.tree("16s_hemiptera_tree.newick"),
            abund = F)

hemi_nmms <- metaMDS(hemi_phylo_16s$Btotal,
                    trymax = 1000, k = 2)

# Lepidoptera
lep <-
  prey_final %>%
  filter(order == "Lepidoptera" & marker == "16s") %>%
  #filter(!site %in% problem) %>%
  acast(site ~ asv, fill = 0, value.var = "count",
        fun.aggregate = sum) %>%
  as.matrix()

lep_phylo_16s <-
  BAT::beta(decostand(lep, method = "hellinger"),
            tree = read.tree("16s_lepidoptera_tree.newick"),
            abund = F)

lep_nmms <- metaMDS(lep_phylo_16s$Btotal,
                    trymax = 1000, k = 2)
```

Native versus non-native prey

```
# Count identified
length(unique(native_status$asv[native_status$status != "unknown"])) # 53
```

```
## [1] 53
```

```
length(unique(preying_final$asv)) # 164
```

```
## [1] 164
```

```
# Determining prey composition for each individual
composition <-
  preying_final %>%
  mutate(status = case_when(
    asv %in% native_status$asv[native_status$status == "Native"] ~ "Native",
    asv %in% native_status$asv[native_status$status == "Non-native"] ~ "Non-native",
    TRUE ~ "unknown"
  )
) %>%
group_by(id) %>%
mutate(preying_comp = case_when(
  any(status == "Non-native") & any(status == "Native") ~ "Both",
  any(status == "Non-native") & !any(status == "Native") ~ "Non-native",
  !any(status == "Non-native") & any(status == "Native") ~ "Native",
  !any(status == "Non-native") & !any(status == "Native") ~ "Unknown"
)) %>%
filter(row_number() == 1) %>%
ungroup()
```

Parasites

```
length(unique(parasites$id))
```

```
## [1] 61
```

```
# First, identify number of parasite ASVs and relative reads
```

```
parasite_summary <-
  parasites %>%
  acast(id ~ asv, fill = 0, value.var = "count") %>%
  as.matrix() %>%
  decostand(method = "hellinger") %>%
  melt() %>%
  filter(value != 0) %>%
  rename(id = Var1,
         asv = Var2,
         rel_read = value) %>%
  left_join(parasites, by = c("id", "asv")) %>%
  group_by(id, order, site, site_status) %>%
  summarise(n_asv = n_distinct(asv),
            n_read = sum(rel_read))
```

```
# Add in spiders that had no parasitism
```

```
parasite_summary <-
  preying_final %>%
```

```

filter(!id %in% parasite_summary$id) %>%
group_by(id) %>%
filter(row_number() == 1) %>%
mutate(n_asv = 0,
       n_read = 0,
       order = NA) %>%
select(id, site, site_status, order, n_asv, n_read) %>%
rbind(parasite_summary) %>%
ungroup()

t.test(parasite_summary$n_asv[parasite_summary$site_status == "native"],
       parasite_summary$n_asv[parasite_summary$site_status == "ginger"]) # 0.0002957

```

```

##
## Welch Two Sample t-test
##
## data: parasite_summary$n_asv[parasite_summary$site_status == "native"] and parasite_summary$n_asv[p
## t = -3.7048, df = 152.01, p-value = 0.0002957
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.6621241 -0.2015435
## sample estimates:
## mean of x mean of y
## 0.3593750 0.7912088

```

```

t.test(parasite_summary$n_read[parasite_summary$site_status == "native"],
       parasite_summary$n_read[parasite_summary$site_status == "ginger"]) # 0.00223

```

```

##
## Welch Two Sample t-test
##
## data: parasite_summary$n_read[parasite_summary$site_status == "native"] and parasite_summary$n_read
## t = -3.1139, df = 143.25, p-value = 0.00223
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.423945 -0.094709
## sample estimates:
## mean of x mean of y
## 0.307696 0.567023

```

```

# Wasps
wasps <-
  parasites %>%
  filter(order == "Hymenoptera")

wasps %>%
  summarise(n_distinct(id),
            n_distinct(asv))

```

```

##   n_distinct(id) n_distinct(asv)
## 1              49             29

```

```
wasps %>%
  group_by(site_status) %>%
  summarise(n_distinct(id),
            n_distinct(asv))
```

```
## # A tibble: 2 x 3
##   site_status 'n_distinct(id)' 'n_distinct(asv)'
##   <chr>          <int>          <int>
## 1 ginger             31             20
## 2 native             18             13
```

```
wasps %>%
  filter(percent_match >= 92) %>%
  group_by(family, site_status) %>%
  summarise(n_distinct(id))
```

```
## # A tibble: 14 x 3
## # Groups:   family [11]
##   family      site_status 'n_distinct(id)'
##   <chr>      <chr>          <int>
## 1 Aphelinidae ginger             1
## 2 Aphelinidae native             3
## 3 Bethylinidae ginger             1
## 4 Braconidae  ginger            11
## 5 Braconidae  native             6
## 6 Diapriidae  native             1
## 7 Eucharitidae ginger             3
## 8 Eulophidae  ginger             1
## 9 Halictidae  ginger             1
## 10 Ichneumonidae ginger             3
## 11 Ichneumonidae native             6
## 12 Pteromalidae native             1
## 13 Signiphoridae native             1
## 14 Sphecidae   native             2
```

```
wasps <-
  parasite_summary %>%
  filter(order == "Hymenoptera" | is.na(order))

t.test(wasps$n_asv[wasps$site_status == "native"],
       wasps$n_asv[wasps$site_status == "ginger"]) # 0.01165
```

```
##
## Welch Two Sample t-test
##
## data: wasps$n_asv[wasps$site_status == "native"] and wasps$n_asv[wasps$site_status == "ginger"]
## t = -2.5617, df = 120.96, p-value = 0.01165
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.61419547 -0.07869618
## sample estimates:
## mean of x mean of y
## 0.3492063 0.6956522
```

```
t.test(wasps$n_read[wasps$site_status == "native"],
       wasps$n_read[wasps$site_status == "ginger"]) # 0.03998
```

```
##
## Welch Two Sample t-test
##
## data: wasps$n_read[wasps$site_status == "native"] and wasps$n_read[wasps$site_status == "ginger"]
## t = -2.0749, df = 129.37, p-value = 0.03998
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.378179954 -0.008994213
## sample estimates:
## mean of x mean of y
## 0.3034158 0.4970029
```

```
# Fungi
fungi <-
  parasites %>%
  filter(order != "Hymenoptera")

fungi %>%
  summarise(n_distinct(id),
            n_distinct(asv))
```

```
##   n_distinct(id) n_distinct(asv)
## 1              23              6
```

```
fungi %>%
  group_by(site_status) %>%
  summarise(n_distinct(id),
            n_distinct(asv))
```

```
## # A tibble: 2 x 3
##   site_status 'n_distinct(id)' 'n_distinct(asv)'
##   <chr>          <int>          <int>
## 1 ginger             22             5
## 2 native             1             1
```

```
fungi <-
  parasite_summary %>%
  filter(order != "Hymenoptera" | is.na(order))

t.test(fungi$n_asv[fungi$site_status == "native"],
       fungi$n_asv[fungi$site_status == "ginger"]) # 3.777e-06
```

```
##
## Welch Two Sample t-test
##
## data: fungi$n_asv[fungi$site_status == "native"] and fungi$n_asv[fungi$site_status == "ginger"]
## t = -5.0233, df = 69.464, p-value = 3.777e-06
## alternative hypothesis: true difference in means is not equal to 0
```



```
## 95 percent confidence interval:
## -0.5284656 -0.2280562
## sample estimates:
## mean of x mean of y
## 0.02173913 0.40000000
```

```
t.test(fungi$n_read[fungi$site_status == "native"],
       fungi$n_read[fungi$site_status == "ginger"]) # 6.097e-06
```

```
##
## Welch Two Sample t-test
##
## data: fungi$n_read[fungi$site_status == "native"] and fungi$n_read[fungi$site_status == "ginger"]
## t = -4.9237, df = 65.16, p-value = 6.097e-06
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.3877765 -0.1639845
## sample estimates:
## mean of x mean of y
## 0.01255109 0.28843163
```