Matabarcoding and Community Ecology

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GitHub Repository (https://github.com/alyssagreen02/BIOL432-Metabarcoding.git)

Question 1

Load Data

```
FS=read.csv("FloristicSurvey.csv")
```

Load Packages

```
library(ggplot2)
library(ape)
library(ggtree)
```

```
## ggtree v3.6.2 For help: https://yulab-smu.top/treedata-book/
##
## If you use the ggtree package suite in published research, please cite
## the appropriate paper(s):
##
## Guangchuang Yu, David Smith, Huachen Zhu, Yi Guan, Tommy Tsan-Yuk Lam.
## ggtree: an R package for visualization and annotation of phylogenetic
## trees with their covariates and other associated data. Methods in
## Ecology and Evolution. 2017, 8(1):28-36. doi:10.1111/2041-210X.12628
##
## Guangchuang Yu, Tommy Tsan-Yuk Lam, Huachen Zhu, Yi Guan. Two methods
## for mapping and visualizing associated data on phylogeny using ggtree.
## Molecular Biology and Evolution. 2018, 35(12):3041-3043.
## doi:10.1093/molbev/msy194
##
## Guangchuang Yu.  Data Integration, Manipulation and Visualization of
## Phylogenetic Trees (1st edition). Chapman and Hall/CRC. 2022,
## doi:10.1201/9781003279242
```

```
##
## Attaching package: 'ggtree'
```

```
## The following object is masked from 'package:ape':
##
##     rotate
```

```
library(vegan)
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.6-4
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following object is masked from 'package:ape':
##
##     where
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

Binary Distance Matrix

```
BinDat=FS
for (row in 1:nrow(FS)) {
  for (col in 1:ncol(FS)) {
    if(BinDat[row,col]>0){
      BinDat[row,col]=1
    }

  }

}

BinDat
```

```
##    Quadrate Population Location Rosettes Bolting Budding Bud_Flw Flower Flw_Sil
## 1         1          1        1        0       0       0       0      0       0
## 2         1          1        1        0       0       0       0      0       0
## 3         1          1        1        0       0       0       0      0       0
## 4         1          1        1        1       1       1       0      0       0
## 5         1          1        1        1       1       1       0      0       0
## 6         1          1        1        0       1       1       0      0       0
## 7         1          1        1        1       1       1       0      0       0
## 8         1          1        1        0       1       1       0      0       0
## 9         1          1        1        0       1       1       0      0       0
## 10        1          1        1        0       0       0       0      0       0
## 11        1          1        1        0       0       0       0      0       0
## 12        1          1        1        0       0       0       0      0       0
## 13        1          1        1        1       1       1       0      0       0
## 14        1          1        1        1       1       1       0      0       0
## 15        1          1        1        1       1       1       0      0       0
## 16        1          1        1        0       0       0       0      0       0
## 17        1          1        1        0       0       0       0      0       0
## 18        1          1        1        0       0       0       0      0       0
## 19        1          1        1        0       0       0       0      0       0
## 20        1          1        1        0       0       0       0      0       0
## 21        1          1        1        0       0       0       0      0       0
## 22        1          1        1        1       1       1       0      0       0
## 23        1          1        1        1       1       1       0      0       0
## 24        1          1        1        0       1       1       0      0       0
## 25        1          1        1        0       0       0       0      0       0
## 26        1          1        1        0       0       0       0      0       0
## 27        1          1        1        0       0       0       0      0       0
## 28        1          1        1        1       1       1       0      0       0
## 29        1          1        1        1       1       1       0      0       0
## 30        1          1        1        1       1       1       0      0       0
##    Sillique Claytonia_virginiana Anemone_hepatica Grass_tuft
## 1         0                    1                1          1
## 2         0                    1                1          1
## 3         0                    1                1          1
## 4         0                    1                0          1
## 5         0                    1                0          1
## 6         0                    1                0          0
## 7         0                    0                0          0
## 8         0                    0                0          0
## 9         0                    0                0          0
## 10        0                    0                0          0
## 11        0                    0                0          0
## 12        0                    0                0          0
## 13        0                    0                0          1
## 14        0                    0                0          1
## 15        0                    0                0          0
## 16        0                    0                0          0
## 17        0                    0                0          0
## 18        0                    0                0          0
## 19        0                    0                0          0
## 20        0                    0                0          0
```

```
## 21          0              0              0          0
## 22          0              1              0          0
## 23          0              0              0          0
## 24          0              0              0          1
## 25          0              1              0          0
## 26          0              0              0          0
## 27          0              1              0          0
## 28          0              0              0          0
## 29          0              1              0          0
## 30          0              1              0          0
##      Trillium_grandifolium Erythronium.trout.lily. Acer_saccharum
## 1                        1                       1              1
## 2                        1                       1              1
## 3                        1                       1              1
## 4                        0                       1              0
## 5                        0                       1              1
## 6                        0                       1              0
## 7                        0                       0              1
## 8                        0                       0              1
## 9                        0                       0              1
## 10                       0                       0              1
## 11                       0                       0              1
## 12                       0                       0              1
## 13                       0                       0              1
## 14                       0                       0              1
## 15                       0                       0              1
## 16                       0                       0              1
## 17                       0                       0              1
## 18                       0                       0              1
## 19                       0                       0              0
## 20                       0                       0              0
## 21                       0                       0              0
## 22                       0                       1              0
## 23                       0                       0              0
## 24                       0                       1              0
## 25                       0                       1              1
## 26                       0                       1              1
## 27                       0                       1              1
## 28                       0                       0              1
## 29                       0                       0              0
## 30                       0                       0              1
##      Dicentra_cucularia Bloodroot Gallium_aparine Ulmus_americana Unknown_1
## 1                     0         0               0               0         0
## 2                     1         1               1               1         1
## 3                     1         0               1               0         0
## 4                     0         0               1               1         0
## 5                     0         1               1               1         0
## 6                     0         0               1               1         0
## 7                     0         0               1               0         0
## 8                     0         0               0               0         0
## 9                     0         0               0               0         0
## 10                    0         0               0               0         0
```

```
## 11                    0          0              0              0          0
## 12                    0          0              0              0          0
## 13                    0          0              1              1          0
## 14                    0          0              1              1          0
## 15                    0          0              1              1          0
## 16                    0          0              1              0          0
## 17                    0          0              1              1          0
## 18                    0          0              0              0          0
## 19                    0          0              0              0          0
## 20                    0          0              0              0          0
## 21                    0          0              0              0          0
## 22                    0          0              1              0          0
## 23                    0          0              1              0          0
## 24                    0          0              0              0          0
## 25                    0          0              1              1          0
## 26                    0          0              1              1          1
## 27                    0          0              1              1          0
## 28                    0          0              0              0          0
## 29                    0          0              0              0          0
## 30                    0          0              1              0          1
##      Unknown_2 Unknown_3.rannunculaceae. Unknown_4 Dryopteris_marginalis
## 1            0                         0         0                     0
## 2            0                         0         0                     0
## 3            0                         0         0                     0
## 4            1                         0         0                     0
## 5            1                         0         0                     0
## 6            1                         0         0                     0
## 7            0                         0         1                     0
## 8            0                         0         0                     1
## 9            0                         0         0                     0
## 10           0                         0         0                     0
## 11           0                         0         0                     0
## 12           0                         0         0                     0
## 13           0                         0         1                     0
## 14           0                         0         0                     0
## 15           0                         0         1                     0
## 16           0                         0         0                     0
## 17           0                         0         0                     0
## 18           0                         0         0                     0
## 19           0                         0         0                     0
## 20           0                         0         0                     0
## 21           0                         0         0                     0
## 22           0                         0         1                     0
## 23           0                         0         1                     0
## 24           0                         0         1                     0
## 25           0                         0         1                     0
## 26           0                         0         1                     0
## 27           0                         0         1                     0
## 28           0                         0         0                     0
## 29           0                         1         0                     0
## 30           0                         1         0                     0
##      Ostrich_fern Plantago_lanceolata Violet Rhamnus_frangula Raspberry Unknown_5
```

```
## 1             0              0      0            0       0       0
## 2             0              0      0            0       0       0
## 3             0              0      0            0       0       0
## 4             0              0      0            0       0       0
## 5             0              0      0            0       0       0
## 6             0              0      0            0       0       0
## 7             0              0      0            0       0       0
## 8             0              0      0            0       0       0
## 9             0              0      0            0       0       0
## 10            0              0      0            0       0       0
## 11            0              0      0            0       0       0
## 12            0              0      0            0       0       0
## 13            1              0      0            0       0       0
## 14            0              1      1            1       1       0
## 15            1              0      1            1       0       1
## 16            1              0      0            0       0       1
## 17            1              0      0            0       0       1
## 18            1              0      0            1       1       1
## 19            0              0      0            0       0       0
## 20            0              0      0            0       0       0
## 21            0              0      0            0       0       0
## 22            0              0      0            0       0       1
## 23            0              0      0            1       0       1
## 24            0              0      1            1       1       0
## 25            0              0      0            0       0       0
## 26            0              0      0            0       0       0
## 27            0              0      0            0       1       1
## 28            0              0      0            0       0       0
## 29            1              0      1            0       0       0
## 30            0              0      0            0       1       1
##     Unknown_6 Solidago_canadensis Unknown_7 Dandelion grass viccia_cracca
## 1          0                   0         0         0     0             0
## 2          0                   0         0         0     0             0
## 3          0                   0         0         0     0             0
## 4          0                   0         0         0     0             0
## 5          0                   0         0         0     0             0
## 6          0                   0         0         0     0             0
## 7          0                   0         0         0     0             0
## 8          0                   0         0         0     0             0
## 9          0                   0         0         0     0             0
## 10         0                   0         0         0     0             0
## 11         0                   0         0         0     0             0
## 12         0                   0         0         0     0             0
## 13         0                   0         0         0     0             0
## 14         0                   0         0         0     0             0
## 15         1                   0         0         0     0             0
## 16         0                   1         0         0     0             0
## 17         0                   0         0         0     0             0
## 18         0                   0         1         0     0             0
## 19         0                   0         0         1     1             0
## 20         0                   0         0         1     1             1
## 21         0                   0         0         0     1             0
```

```
## 22          0                  1        0        1    1              0
## 23          0                  1        0        1    1              0
## 24          0                  1        0        1    0              0
## 25          0                  0        0        0    1              0
## 26          0                  0        0        0    1              0
## 27          0                  0        0        0    0              0
## 28          0                  1        0        0    1              0
## 29          0                  1        0        0    0              0
## 30          0                  0        0        0    1              0
##      herb_robert thorny_ash rhamnus_cathartica rhubarb Unknown_8 Unknown_9
## 1             0          0                  0       0         0         0
## 2             0          0                  0       0         0         0
## 3             0          0                  0       0         0         0
## 4             0          0                  0       0         0         0
## 5             0          0                  0       0         0         0
## 6             0          0                  0       0         0         0
## 7             0          0                  0       0         0         0
## 8             0          0                  0       0         0         0
## 9             0          0                  0       0         0         0
## 10            0          0                  0       0         0         0
## 11            0          0                  0       0         0         0
## 12            0          0                  0       0         0         0
## 13            0          0                  0       0         0         0
## 14            0          0                  0       0         0         0
## 15            0          0                  0       0         0         0
## 16            0          0                  0       0         0         0
## 17            0          0                  0       0         0         0
## 18            0          0                  0       0         0         0
## 19            0          0                  0       0         0         0
## 20            0          0                  0       0         0         0
## 21            0          0                  0       0         0         0
## 22            1          0                  0       0         0         0
## 23            1          1                  1       1         0         0
## 24            0          0                  0       0         0         0
## 25            1          0                  0       0         0         0
## 26            1          0                  0       0         0         0
## 27            1          1                  0       0         0         0
## 28            1          0                  0       0         1         0
## 29            0          0                  0       0         0         1
## 30            1          1                  0       0         0         0
##      maianthenum_racemosum
## 1                        0
## 2                        0
## 3                        0
## 4                        0
## 5                        0
## 6                        0
## 7                        0
## 8                        0
## 9                        0
## 10                       0
## 11                       0
```

```
## 12                    0
## 13                    0
## 14                    0
## 15                    0
## 16                    0
## 17                    0
## 18                    0
## 19                    0
## 20                    0
## 21                    0
## 22                    0
## 23                    0
## 24                    0
## 25                    0
## 26                    0
## 27                    0
## 28                    0
## 29                    0
## 30                    1
```

```
BinDat=as.data.frame(BinDat)
```

Make sure all data is numeric

```
#remove quadrate information
FS_nq=FS[,-c(1)]

#Add quadrate as row lable
row.names(FS_nq)=FS$Quadrate

#Convert location into numeric
FS_bin_loc=FS_nq

FS_bin_loc$Location[FS_bin_loc$Location == 'i'] <- 1
FS_bin_loc$Location[FS_bin_loc$Location == 'o'] <- 0

#Convert factor to numeric
str(FS_bin_loc$Location)
```

```
##  chr [1:30] "0" "0" "0" "1" "1" "1" "1" "1" "1" "0" "0" "0" "1" "1" "1" "0" ...
```

```
FS_bin_loc$Location=as.numeric(FS_bin_loc$Location)
str(FS_bin_loc$Location)
```

```
##  num [1:30] 0 0 0 1 1 1 1 1 1 0 ...
```

Calculate Distances

```
#Calculate Bray-Curtis dissimilarity
dist=vegdist(FS_bin_loc, method="bray", binary = F)

#Calculate neighbour joining
tree=nj(dist)
```

Plot Tree Figure out how to colour based on location

```
ggtree(tree, layout = "rectangular") %<+% FS +
geom_tiplab(aes(colour=Location)) + theme(legend.position = "right")
```
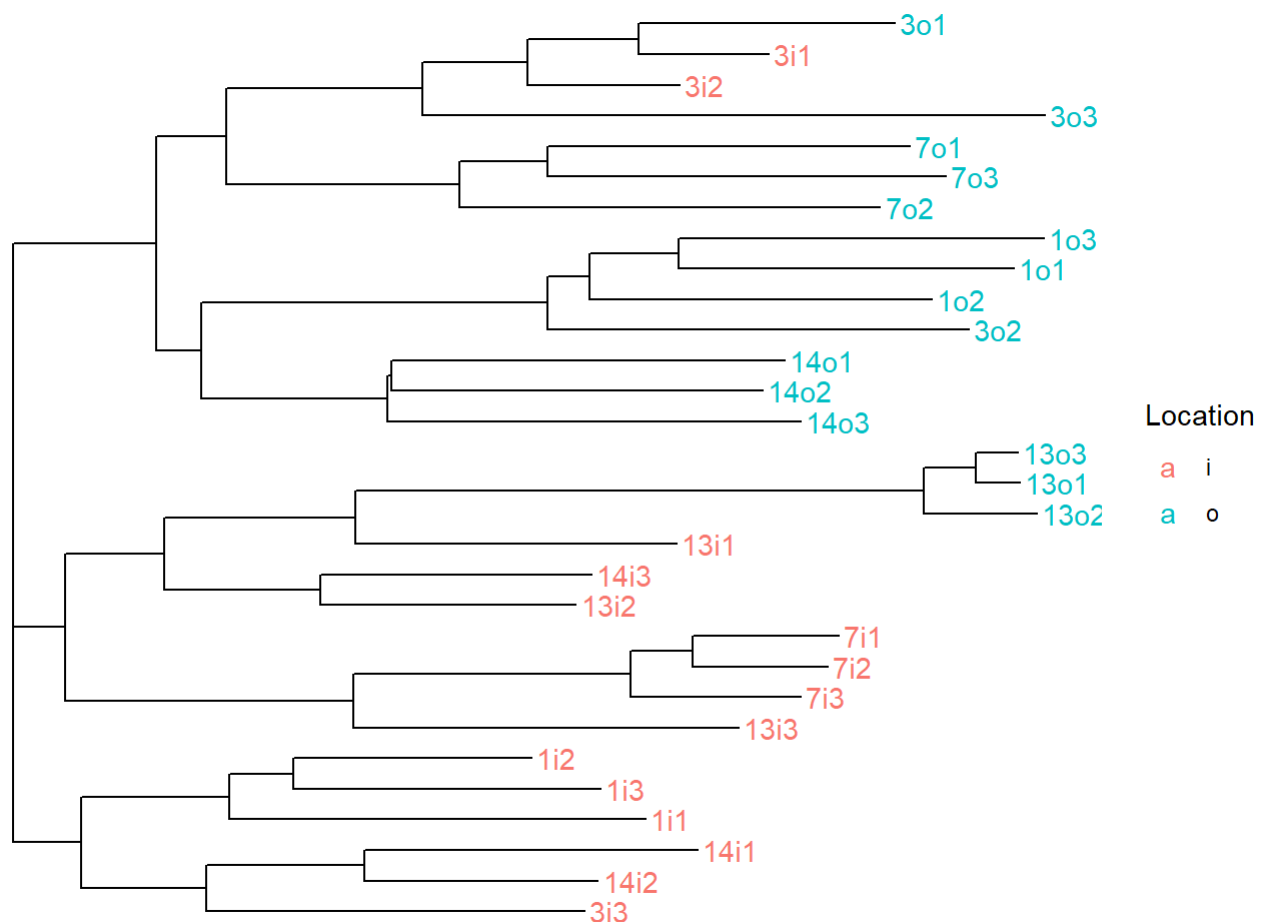

Figure 1: Tree of different quadrates. i indicates that the quadrate patch is inside garlic mustard patches, and o indicates that the quadrate patch is outside the garlic mustard patches. This figure demonstrates that garlic mustard has a large effect on the plan community. We can see this because the quadrates coloured for i and o are in almost completely separate clades, with the exception of 13o3, 13o1, 13o2, 3i1, and 3i2.

Based on the results demonstrated in Figure 1, we can conclude that garlic mustard has a large effect on the plant community. This is because the quadrates coloured for i and o are in almost completely separate clades, with the exception of 13o3, 13o1, 13o2, 3i1, and 3i2. Due, to the small number of outlying quadrates, it is likely that they are located in their positions due to some other property, such as bolding or budding, which when not accounted for, would cause the outliers to move into there respective clades. This would further indicate that garlic mustard has a large effect on the plant community.

Question 2

NMDS

```
set.seed(13)
NMDSdat=metaMDS(dist, k=2, trymax = 100)
```

```
## Run 0 stress 0.177062
## Run 1 stress 0.1878424
## Run 2 stress 0.177062
## ... Procrustes: rmse 3.111086e-05  max resid 0.0001234516
## ... Similar to previous best
## Run 3 stress 0.1822645
## Run 4 stress 0.1812268
## Run 5 stress 0.1744617
## ... New best solution
## ... Procrustes: rmse 0.06201532  max resid 0.2113024
## Run 6 stress 0.1822645
## Run 7 stress 0.1822645
## Run 8 stress 0.1744617
## ... Procrustes: rmse 4.452285e-05  max resid 0.0001904364
## ... Similar to previous best
## Run 9 stress 0.177062
## Run 10 stress 0.1744396
## ... New best solution
## ... Procrustes: rmse 0.002791267  max resid 0.01220943
## Run 11 stress 0.1822645
## Run 12 stress 0.177062
## Run 13 stress 0.1812268
## Run 14 stress 0.2795768
## Run 15 stress 0.1844849
## Run 16 stress 0.181227
## Run 17 stress 0.1822646
## Run 18 stress 0.1812265
## Run 19 stress 0.1744617
## ... Procrustes: rmse 0.002795924  max resid 0.0122497
## Run 20 stress 0.1882835
## Run 21 stress 0.1744396
## ... New best solution
## ... Procrustes: rmse 2.122644e-06  max resid 5.307102e-06
## ... Similar to previous best
## *** Best solution repeated 1 times
```

Create data for plottin

```
row.names(FS)=FS$Quadrate
row.names(FS)
```

```
##  [1] "7o3"   "7o1"   "7o2"   "7i3"   "7i2"   "7i1"   "3i1"   "3i2"   "3i3"   "3o1"
## [11] "3o2"   "3o3"   "1i3"   "1i2"   "1i1"   "1o1"   "1o2"   "1o3"   "13o1"  "13o2"
## [21] "13o3"  "13i2"  "13i1"  "13i3"  "14o2"  "14o1"  "14o3"  "14i2"  "14i1"  "14i3"
```

```
PDat=data.frame(NMDS1=NMDSdat$points[,1],
                NMDS2=NMDSdat$points[,2],
                Quadrate=row.names(FS))
```

Add labels

```
PDat=merge(PDat, FS, by="Quadrate", all.x=T, all.y =F)
```

Plot

```
PDat$Population=as.factor(PDat$Population)
qplot(x=NMDS1, NMDS2, alpha=I(0.6), data = PDat, shape=Location, colour=Population)+theme_bw()
```

```
## Warning: `qplot()` was deprecated in ggplot2 3.4.0.
```
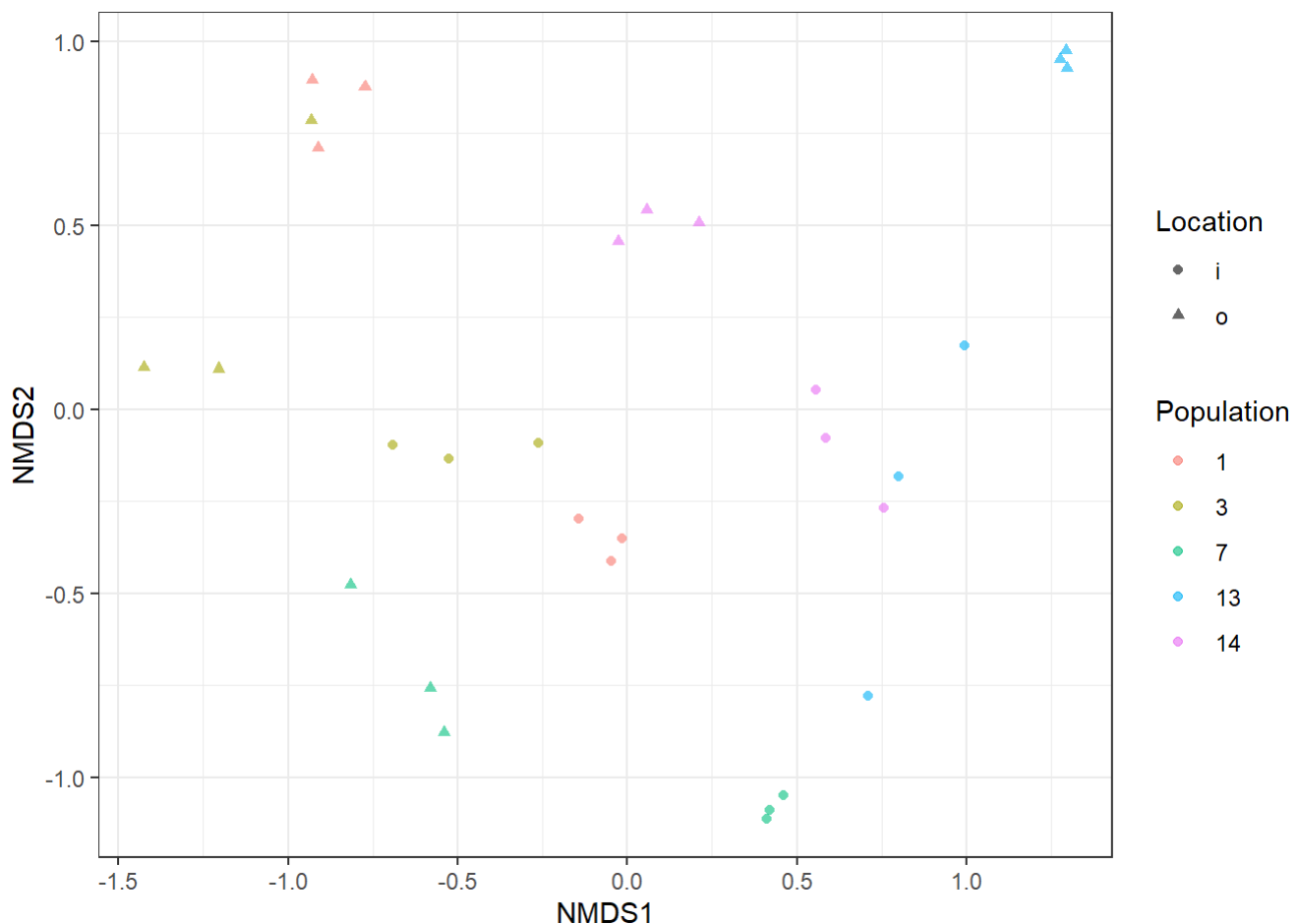

Figure 2: NMDS Plot. i indicates that the quadrate patch is inside garlic mustard patches, and o indicates that the quadrate patch is outside the garlic mustard patches. This figure demonstrates the distinction of samples based on

location and population. From this figure we can see that the locations are well clustered, divided across the 45 degree line. We can also see that the populations are not as well clustered with different colours located sporatically across the plot.

Based on the results demonstrated in Figure 2, we can conclude that the presence/absence of garlic mustard (location : in(i)/out(o)) has a stronger effect on plant communities than sampling population. This is seen because the presence/absence of garlic mustard is well clustered, divided across the 45 degree line, with a few deviants. Whereas, the populations are not clustered as well, with different colours located sporatically across the plot.