Lecture 18 - Class Activity: NMDS and PERMANOVA

Bill Perry

Lecture 18: Non-metric Multidimensional Scaling (NMDS) and PERMANOVA

What is NMDS?

NMDS (Non-metric Multidimensional Scaling) is an ordination technique that: - Visualizes dissimilarity between objects in reduced dimensions - Preserves rank order of distances, not exact distances - Works well with non-linear ecological relationships - Makes few assumptions about data structure

When to Use NMDS

Use NMDS when you have: - **Community data**: Species abundance or presence/absence matrices - **Non-linear relationships**: When PCA assumptions are violated - **Complex ecological gradients**: Multiple environmental factors affecting communities

Key Concepts of NMDS

1. **Dissimilarity matrices** instead of covariance
2. **Stress values** measure goodness of fit (<0.2 is acceptable)
3. **Iterative algorithm** to find optimal configuration
4. **No eigenvalues** - axes have no inherent meaning
5. **Rank-based** - preserves order, not exact distances

> ! Critical First Step
>
> Always check your stress value! Stress < 0.1 is excellent, 0.1-0.2 is good, > 0.2 is poor representation.

Part 1: Data Preparation and Exploration

Load and Prepare Data

We'll use the iris dataset for this analysis, treating it as if the measurements represent abundances of different "species" at different "sites".

```
# Load iris data
iris_df <- read_csv("data/iris.csv") %>%
  clean_names()
```

```
Rows: 150 Columns: 5
── Column specification ────────────────────────────────────────────────────────
Delimiter: ","
chr (1): species
dbl (4): sepal_length, sepal_width, petal_length, petal_width

ℹ Use `spec()` to retrieve the full column specification for this data.
ℹ Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
# View data structure
head(iris_df)
```

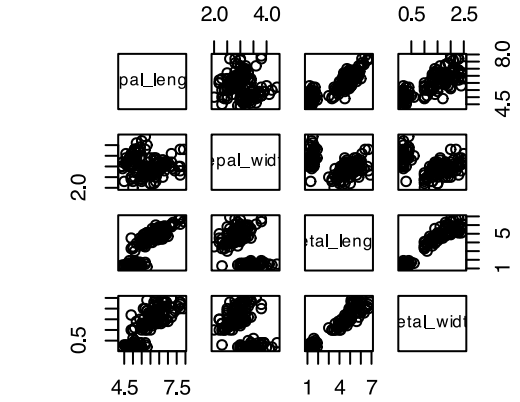
```
# A tibble: 6 × 5
  sepal_length sepal_width petal_length petal_width species
         <dbl>       <dbl>        <dbl>       <dbl> <chr>
1          5.1         3.5          1.4         0.2 setosa
2          4.9         3            1.4         0.2 setosa
3          4.7         3.2          1.3         0.2 setosa
4          4.6         3.1          1.5         0.2 setosa
5          5           3.6          1.4         0.2 setosa
6          5.4         3.9          1.7         0.4 setosa
```

```r
# For NMDS, we need just the numeric columns (our "species" data)
# We'll keep species as our grouping variable
iris_species_df <- iris_df %>%
  dplyr::select(species)

iris_numeric_df <- iris_df %>%
  dplyr::select(-species)

# Check the structure
str(iris_numeric_df)
```

```
tibble [150 × 4] (S3: tbl_df/tbl/data.frame)
 $ sepal_length: num [1:150] 5.1 4.9 4.7 4.6 5 5.4 4.6 5 4.4 4.9 ...
 $ sepal_width : num [1:150] 3.5 3 3.2 3.1 3.6 3.9 3.4 3.4 2.9 3.1 ...
 $ petal_length: num [1:150] 1.4 1.4 1.3 1.5 1.4 1.7 1.4 1.5 1.4 1.5 ...
 $ petal_width : num [1:150] 0.2 0.2 0.2 0.2 0.2 0.4 0.3 0.2 0.2 0.1 ...
```

Visualize the Data

```r
# Create a pairs plot to see relationships
iris_pairs_plot <- iris_df %>%
  dplyr::select(-species) %>%
  pairs(main = "Iris Measurements Relationships")
```



Part 2: Running NMDS

Step 1: Calculate Distance Matrix

```
# Calculate Bray-Curtis distance (common for ecological data)
# For iris data, we'll use Euclidean distance since these are measurements
iris_dist <- dist(iris_numeric_df, method = "euclidean")

# Check the first few distances
iris_dist[1:5]
```

```
[1] 0.5385165 0.5099020 0.6480741 0.1414214 0.6164414
```

Step 2: Run NMDS

```
# Run NMDS with 2 dimensions
set.seed(123)  # For reproducibility
iris_nmds_model <- metaMDS(iris_numeric_df,
                           distance = "euclidean",
                           k = 2,  # Number of dimensions
                           trymax = 100)  # Maximum iterations
```

```
Run 0 stress 0.02525035
Run 1 stress 0.04045544
Run 2 stress 0.03566855
Run 3 stress 0.0268287
Run 4 stress 0.03695952
Run 5 stress 0.03104439
Run 6 stress 0.02682881
Run 7 stress 0.03962709
Run 8 stress 0.03210416
Run 9 stress 0.04325555
Run 10 stress 0.02525031
... New best solution
... Procrustes: rmse 1.741237e-05  max resid 7.129696e-05
... Similar to previous best
```

```
Run 11 stress 0.02525038
... Procrustes: rmse 1.217807e-05  max resid 8.876481e-05
... Similar to previous best
Run 12 stress 0.04000578
Run 13 stress 0.03552087
Run 14 stress 0.0309981
Run 15 stress 0.04078306
Run 16 stress 0.04008785
Run 17 stress 0.03197035
Run 18 stress 0.02525033
... Procrustes: rmse 5.481604e-06  max resid 2.712782e-05
... Similar to previous best
Run 19 stress 0.02682875
Run 20 stress 0.03270101
*** Best solution repeated 3 times
```

```
# Check the results
iris_nmds_model
```

```


Call:
metaMDS(comm = iris_numeric_df, distance = "euclidean", k = 2,      trymax = 100)

global Multidimensional Scaling using monoMDS

Data:     iris_numeric_df
Distance: euclidean

Dimensions: 2
Stress:     0.02525031
Stress type 1, weak ties
Best solution was repeated 3 times in 20 tries
The best solution was from try 10 (random start)
Scaling: centring, PC rotation
Species: expanded scores based on 'iris_numeric_df'
```

Interpretation: - Stress value: 0.025 - This is excellent representation

Step 3: Extract NMDS Scores

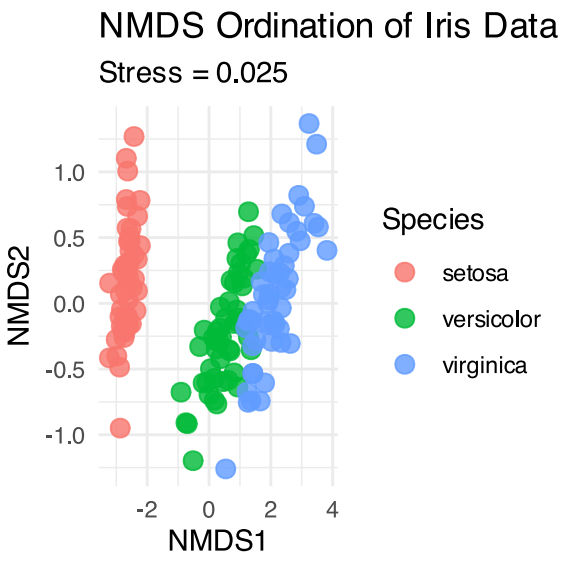
```
# Extract NMDS scores for plotting
nmds_scores_df <- as.data.frame(iris_nmds_model$points) %>%
  rename(nmds1 = MDS1, nmds2 = MDS2) %>%
  bind_cols(iris_species_df)

# View the scores
head(nmds_scores_df)
```

```
      nmds1      nmds2 species
1 -2.687311  0.2758924  setosa
2 -2.717262 -0.1705943  setosa
3 -2.882071 -0.1017541  setosa
4 -2.746720 -0.2603709  setosa
```

```
5 -2.730486  0.2902002  setosa
6 -2.311408  0.6625723  setosa
```

Step 4: Create NMDS Plot

```
# Basic NMDS plot
nmds_basic_plot <- ggplot(nmds_scores_df, aes(x = nmds1, y = nmds2, color = species)) +
  geom_point(size = 3, alpha = 0.8) +
  labs(title = "NMDS Ordination of Iris Data",
       subtitle = paste("Stress =", round(iris_nmds_model$stress, 3)),
       x = "NMDS1",
       y = "NMDS2",
       color = "Species") +
  theme_minimal()

nmds_basic_plot
```

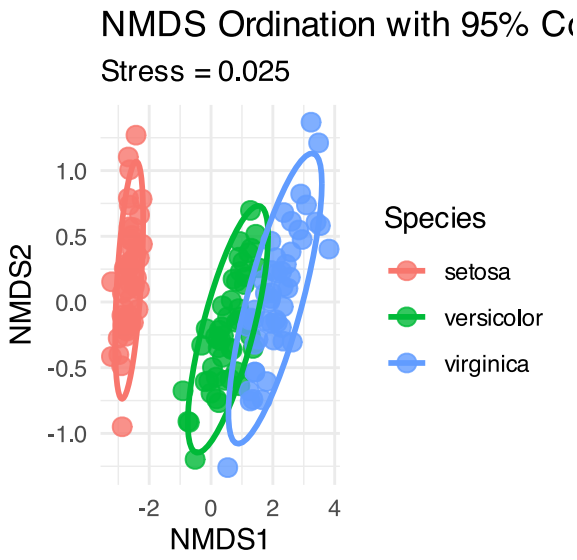


Step 5: Add Confidence Ellipses

```
# NMDS plot with ellipses
nmds_ellipse_plot <- ggplot(nmds_scores_df, aes(x = nmds1, y = nmds2, color = species)) +
  geom_point(size = 3, alpha = 0.8) +
  stat_ellipse(level = 0.95, size = 1) +
  labs(title = "NMDS Ordination with 95% Confidence Ellipses",
       subtitle = paste("Stress =", round(iris_nmds_model$stress, 3)),
       x = "NMDS1",
       y = "NMDS2",
       color = "Species") +
  theme_minimal()
```

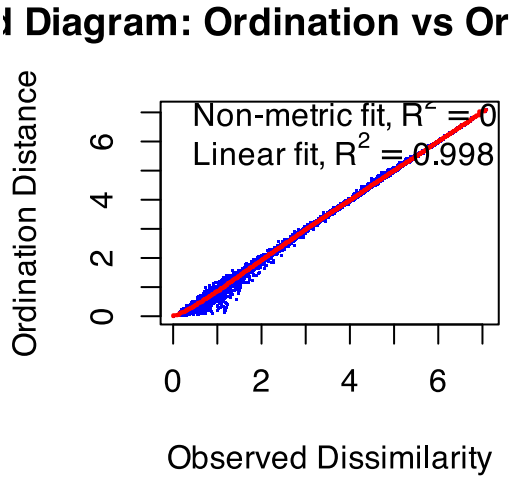
```
Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
ℹ Please use `linewidth` instead.
```

```
nmds_ellipse_plot
```



Step 6: Stress Plot (Shepard Diagram)

```
# Create stress plot to evaluate fit
stressplot(iris_nmds_model, main = "Shepard Diagram: Ordination vs Original Distances")
```



Part 3: PERMANOVA Analysis

What is PERMANOVA?

PERMANOVA (Permutational Multivariate Analysis of Variance) tests whether groups have different multivariate centroids using permutation tests.

Step 1: Run PERMANOVA

```
# Run PERMANOVA to test if species differ in multivariate space
set.seed(456)
iris_permanova_model <- adonis2(iris_numeric_df ~ species,
                                data = iris_df,
                                method = "euclidean",
                                permutations = 999)
```

```r
# View results
iris_permanova_model
```

```
Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = iris_numeric_df ~ species, data = iris_df, permutations = 999, method =
"euclidean")
          Df SumOfSqs      R2      F Pr(>F)
Model      2   592.07 0.86894 487.33  0.001 ***
Residual 147    89.30 0.13106
Total    149   681.37 1.00000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Interpretation: - F-statistic: 487.33 - R^2 (variance explained): 0.869 - p-value: 0.001

Step 2: Check Homogeneity of Dispersions

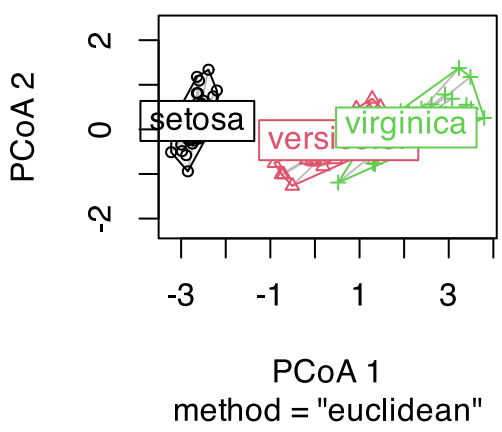
Before interpreting PERMANOVA, we need to check if groups have similar multivariate spread.

```r
# Test homogeneity of multivariate dispersions
iris_dist_full <- dist(iris_numeric_df)
dispersion_model <- betadisper(iris_dist_full, iris_df$species)

# Test for differences in dispersion
dispersion_test <- anova(dispersion_model)
dispersion_test
```

```
Analysis of Variance Table

Response: Distances
           Df  Sum Sq Mean Sq F value  Pr(>F)
Groups      2  2.9092 1.45458  10.748 4.4e-05 ***
Residuals 147 19.8941 0.13533
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Step 3: Visualize Dispersions

```r
# Plot dispersions
plot(dispersion_model, main = "Multivariate Dispersion by Species")
```



Step 4: Pairwise PERMANOVA

```r
# Function for pairwise PERMANOVA comparisons
pairwise_permanova <- function(data_matrix, groups, distance_method = "euclidean") {
  # Get unique group combinations
  group_levels <- unique(groups)
  comparisons <- combn(group_levels, 2)

  # Initialize results
  results_df <- data.frame(
    group1 = character(),
    group2 = character(),
    f_statistic = numeric(),
    r_squared = numeric(),
    p_value = numeric()
  )

  # Run pairwise comparisons
  for(i in 1:ncol(comparisons)) {
    # Subset data
    group1 <- comparisons[1, i]
    group2 <- comparisons[2, i]
    subset_indices <- which(groups %in% c(group1, group2))

    subset_data <- data_matrix[subset_indices, ]
    subset_groups <- groups[subset_indices]

    # Run PERMANOVA
    temp_result <- adonis2(subset_data ~ subset_groups,
                           method = distance_method,
                           permutations = 999)

    # Store results
    results_df <- rbind(results_df, data.frame(
      group1 = group1,
      group2 = group2,
      f_statistic = temp_result$F[1],
      r_squared = temp_result$R2[1],
      p_value = temp_result$"Pr(>F)"[1]
```

```
    ))
  }

  # Adjust p-values for multiple comparisons
  results_df$p_adjusted <- p.adjust(results_df$p_value, method = "bonferroni")

  return(results_df)
}

# Run pairwise comparisons
pairwise_results_df <- pairwise_permanova(iris_numeric_df, iris_df$species)
pairwise_results_df
```

```
      group1     group2 f_statistic r_squared p_value p_adjusted
1     setosa versicolor    551.0039 0.8489994   0.001      0.003
2     setosa  virginica    943.7992 0.9059320   0.001      0.003
3 versicolor  virginica     86.7697 0.4696100   0.001      0.003
```

Part 4: ANOSIM Analysis

What is ANOSIM?

ANOSIM (Analysis of Similarities) tests whether there is a significant difference between groups using rank dissimilarities.

Step 1: Run ANOSIM

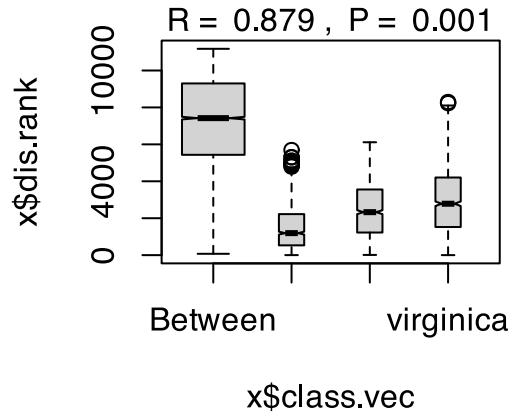
```
# Run ANOSIM
set.seed(789)
iris_anosim_model <- anosim(iris_dist_full, iris_df$species, permutations = 999)

# View results
iris_anosim_model
```

```


Call:
anosim(x = iris_dist_full, grouping = iris_df$species, permutations = 999)
Dissimilarity: euclidean

ANOSIM statistic R: 0.8794
      Significance: 0.001

Permutation: free
Number of permutations: 999
```

Interpretation: - R statistic: 0.879 - p-value: 0.001 - R close to 1 indicates strong separation between groups

Step 2: Plot ANOSIM Results

```
# Plot ANOSIM results
plot(iris_anosim_model, main = "ANOSIM Results: Distribution of Permuted R Statistics")
```



Part 5: Environmental Fitting (Optional)

If we had environmental variables, we could fit them to the ordination.

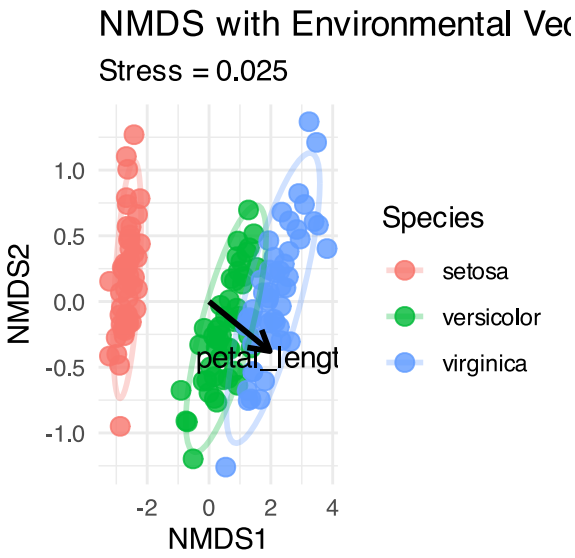
```
# For demonstration, let's use petal_length as an "environmental" variable
env_data_df <- data.frame(petal_length = iris_df$petal_length)

# Fit environmental vector
env_fit_model <- envfit(iris_nmds_model, env_data_df, permutations = 999)
env_fit_model
```

```
***VECTORS

               NMDS1    NMDS2     r2 Pr(>r)
petal_length  0.98205 -0.18862 0.9979  0.001 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999
```

Visualize Environmental Vectors

```
# Extract vector coordinates
env_coords_df <- as.data.frame(env_fit_model$vectors$arrows * 2)  # Scale for visibility
env_coords_df$variable <- rownames(env_coords_df)

# NMDS plot with environmental vector
nmds_env_plot <- ggplot(nmds_scores_df, aes(x = nmds1, y = nmds2, color = species)) +
  geom_point(size = 3, alpha = 0.8) +
  stat_ellipse(level = 0.95, size = 1, alpha = 0.3) +
  geom_segment(data = env_coords_df,
               aes(x = 0, y = 0, xend = NMDS1, yend = NMDS2),
               arrow = arrow(length = unit(0.3, "cm")),
               color = "black", size = 1) +
  geom_text(data = env_coords_df,
            aes(x = NMDS1 * 1.1, y = NMDS2 * 1.1, label = variable),
```

```
            color = "black", size = 4) +
  labs(title = "NMDS with Environmental Vector",
       subtitle = paste("Stress =", round(iris_nmds_model$stress, 3)),
       x = "NMDS1",
       y = "NMDS2",
       color = "Species") +
  theme_minimal()

nmds_env_plot
```



Summary Checklist for NMDS and PERMANOVA

Analysis Checklist

1. **Prepare your data** - ensure numeric matrix format
2. **Choose appropriate distance measure**
   - Bray-Curtis for abundance data
   - Euclidean for measurement data
3. **Run NMDS** with sufficient iterations
4. **Check stress value** - must be < 0.2
5. **Create ordination plots** with groups identified
6. **Test homogeneity of dispersions** before PERMANOVA
7. **Run PERMANOVA** to test group differences
8. **Consider ANOSIM** as complementary test
9. **Fit environmental variables** if available

Key Points to Remember

- **NMDS preserves rank order** of distances, not exact values
- **Stress < 0.2** is acceptable, < 0.1 is excellent
- **PERMANOVA tests centroids**, ANOSIM tests overlap
- **Check dispersion homogeneity** - violated assumption affects interpretation
- **Multiple comparisons** require p-value adjustment
- **Axes have no inherent meaning** in NMDS (unlike PCA)
- **Use appropriate distance measures** for your data type

! Key Takeaways from NMDS/PERMANOVA Analysis

1. **NMDS is flexible** - works with any distance measure and makes few assumptions
2. **Stress indicates fit quality** - always report and check this value
3. **PERMANOVA is powerful** but assumes homogeneous dispersions
4. **ANOSIM is complementary** - provides different perspective on group separation
5. **Visualization is crucial** - always plot your ordination results
6. **Environmental fitting** helps interpret ecological patterns
7. **Permutation tests** avoid distributional assumptions

Remember: NMDS is iterative and may find different solutions - always set a seed for reproducibility!