Lecture 18 - Multivariate Community Analysis

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Lecture 18: Multivariate Community Analysis

Let's import the data and start to explore it

```
# Set theme
theme_set(theme_minimal() +
          theme(text = element_text(size = 12),
                plot.title = element_text(size = 14, face = "bold")))

# Load data
doubs_env <- read_csv("data/DoubsEnv.csv") %>%
  rename(site = 1) %>%
  mutate(site = as.factor(site))

doubs_spe <- read_csv("data/DoubsSpe.csv") %>%
  rename(site = 1) %>%
  mutate(site = as.factor(site))

# Create river reach groups based on distance from source
doubs_env <- doubs_env %>%
  mutate(reach = case_when(
    das <= 30 ~ "Upper",
    das <= 80 ~ "Middle",
    TRUE ~ "Lower"
  ) %>% factor(levels = c("Upper", "Middle", "Lower")))

doubs_spe <- doubs_spe %>%
  left_join(doubs_env %>% select(site, reach), by = "site")

cat("Species data structure:\n")
```

```
Species data structure:
```

```
str(doubs_spe)
```

```
tibble [29 × 29] (S3: tbl_df/tbl/data.frame)
 $ site : Factor w/ 29 levels "1","2","3","4",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ CHA  : num [1:29] 0 0 0 0 0 0 0 0 0 1 ...
 $ TRU  : num [1:29] 3 5 5 4 2 3 5 0 1 3 ...
 $ VAI  : num [1:29] 0 4 5 5 3 4 4 1 4 4 ...
 $ LOC  : num [1:29] 0 3 5 5 2 5 5 3 4 1 ...
 $ OMB  : num [1:29] 0 0 0 0 0 0 0 0 0 1 ...
 $ BLA  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ HOT  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ TOX  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ VAN  : num [1:29] 0 0 0 0 5 1 1 0 2 0 ...
```

```
 $ CHE  : num [1:29] 0 0 0 1 2 2 1 5 2 1 ...
 $ BAR  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ SPI  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ GOU  : num [1:29] 0 0 0 1 2 1 0 0 1 0 ...
 $ BRO  : num [1:29] 0 0 1 2 4 1 0 0 0 0 ...
 $ PER  : num [1:29] 0 0 0 2 4 1 0 0 0 0 ...
 $ BOU  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ PSO  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ ROT  : num [1:29] 0 0 0 0 2 0 0 0 0 0 ...
 $ CAR  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ TAN  : num [1:29] 0 0 0 1 3 2 0 1 0 0 ...
 $ BCO  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ PCH  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ GRE  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ GAR  : num [1:29] 0 0 0 0 5 1 0 4 0 0 ...
 $ BBO  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ ABL  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ ANG  : num [1:29] 0 0 0 0 0 0 0 0 0 0 ...
 $ reach: Factor w/ 3 levels "Upper","Middle",..: 1 1 1 1 1 2 2 2 3 3 ...
```

```
cat("\nEnvironmental data structure:\n")
```

```

Environmental data structure:
```

```
str(doubs_env)
```

```
tibble [29 × 13] (S3: tbl_df/tbl/data.frame)
 $ site : Factor w/ 29 levels "1","2","3","4",..: 1 2 3 4 5 6 7 8 9 10 ...
 $ das  : num [1:29] 0.3 2.2 10.2 18.5 21.5 ...
 $ alt  : num [1:29] 934 932 914 854 849 846 841 752 617 483 ...
 $ pen  : num [1:29] 48 3 3.7 3.2 2.3 3.2 6.6 1.2 9.9 4.1 ...
 $ deb  : num [1:29] 0.84 1 1.8 2.53 2.64 2.86 4 4.8 10 19.9 ...
 $ pH   : num [1:29] 7.9 8 8.3 8 8.1 7.9 8.1 8 7.7 8.1 ...
 $ dur  : num [1:29] 45 40 52 72 84 60 88 90 82 96 ...
 $ pho  : num [1:29] 0.01 0.02 0.05 0.1 0.38 0.2 0.07 0.3 0.06 0.3 ...
 $ nit  : num [1:29] 0.2 0.2 0.22 0.21 0.52 0.15 0.15 0.82 0.75 1.6 ...
 $ amm  : num [1:29] 0 0.1 0.05 0 0.2 0 0 0.12 0.01 0 ...
 $ oxy  : num [1:29] 12.2 10.3 10.5 11 8 10.2 11.1 7.2 10 11.5 ...
 $ dbo  : num [1:29] 2.7 1.9 3.5 1.3 6.2 5.3 2.2 5.2 4.3 2.7 ...
 $ reach: Factor w/ 3 levels "Upper","Middle",..: 1 1 1 1 1 2 2 2 3 3 ...
```

Today's Data: The Doubs River

About the Doubs River Dataset

Study System:

- Doubs River, France
- 29 sites from upstream to downstream
- Collected by Verneaux (1973)
- Classic community ecology dataset

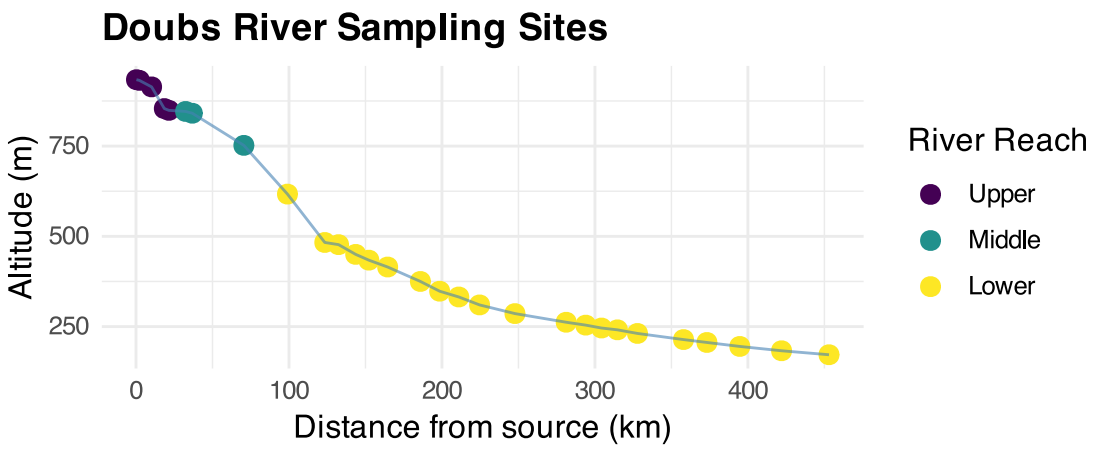
Two Datasets:

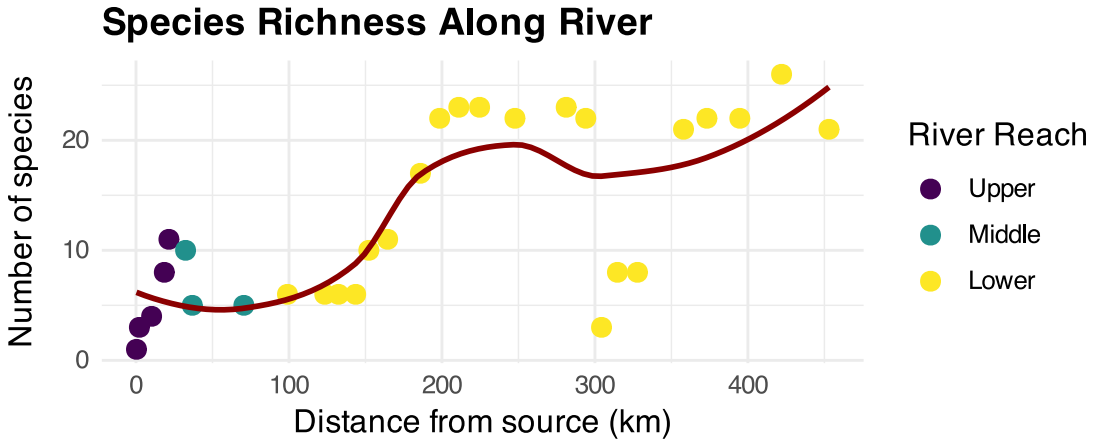
1. **Environmental**: 11 water quality variables
2. **Species**: 27 fish species abundances (0-5 scale)

Research Questions:

- How do fish communities change along the river?
- Which environmental factors drive community composition?
- Are there distinct community types?

```
# Show the sampling design
p1 <- doubs_env %>%
  ggplot(aes(das, alt, color = reach)) +
  geom_point(size = 3) +
  geom_line(color = "steelblue", alpha = 0.6) +
  labs(title = "Doubs River Sampling Sites",
       x = "Distance from source (km)",
       y = "Altitude (m)",
       color = "River Reach") +
  scale_color_viridis_d()

# Show species richness along river
species_richness <- doubs_spe %>%
  select(-site, -reach) %>%
  mutate(richness = rowSums(. > 0)) %>%
  bind_cols(doubs_env %>% select(das, reach))

p2 <- species_richness %>%
  ggplot(aes(das, richness, color = reach)) +
  geom_point(size = 3) +
  geom_smooth(method = "loess", se = FALSE, color = "darkred") +
  labs(title = "Species Richness Along River",
       x = "Distance from source (km)",
       y = "Number of species",
       color = "River Reach") +
  scale_color_viridis_d()

p1 / p2
```





Non-metric Multidimensional Scaling (NMDS)

What is NMDS?

Overview of NMDS:

- **Purpose**: Visualize dissimilarity between objects in 2D/3D space
- **Goal**: Preserve rank order of distances, not exact distances
- **Method**: Iterative repositioning to minimize stress
- **Output**: Ordination plot showing relationships between samples

Key Differences from PCA:

- Uses dissimilarity matrices (not covariance)
- Non-parametric (rank-based)
- Better for non-linear ecological relationships
- No assumption about data distribution

```
# Conceptual diagram of NMDS process
tibble(
  step = 1:4,
  process = c("Calculate\nDissimilarities",
              "Random\nConfiguration",
              "Iterative\nRepositioning",
              "Final\nOrdination"),
  description = c("Create distance matrix\nbetween all samples",
                  "Place points randomly\nin 2D space",
                     "Move points to minimize\nstress (difference between\noriginal and new
```

```
distances)",
                  "Stable configuration\nwith low stress")
) %>%
  ggplot(aes(1, step)) +
  geom_rect(aes(xmin = 0.5, xmax = 1.5, ymin = step - 0.4, ymax = step + 0.4),
            fill = "lightblue", color = "black") +
  geom_text(aes(label = process), fontface = "bold", size = 3) +
  geom_text(aes(label = description, y = step - 0.6), size = 2.5) +
  scale_y_reverse() +
  labs(title = "NMDS Process") +
  theme_void() +
  theme(plot.title = element_text(hjust = 0.5, size = 14, face = "bold"))
```

How NMDS Works

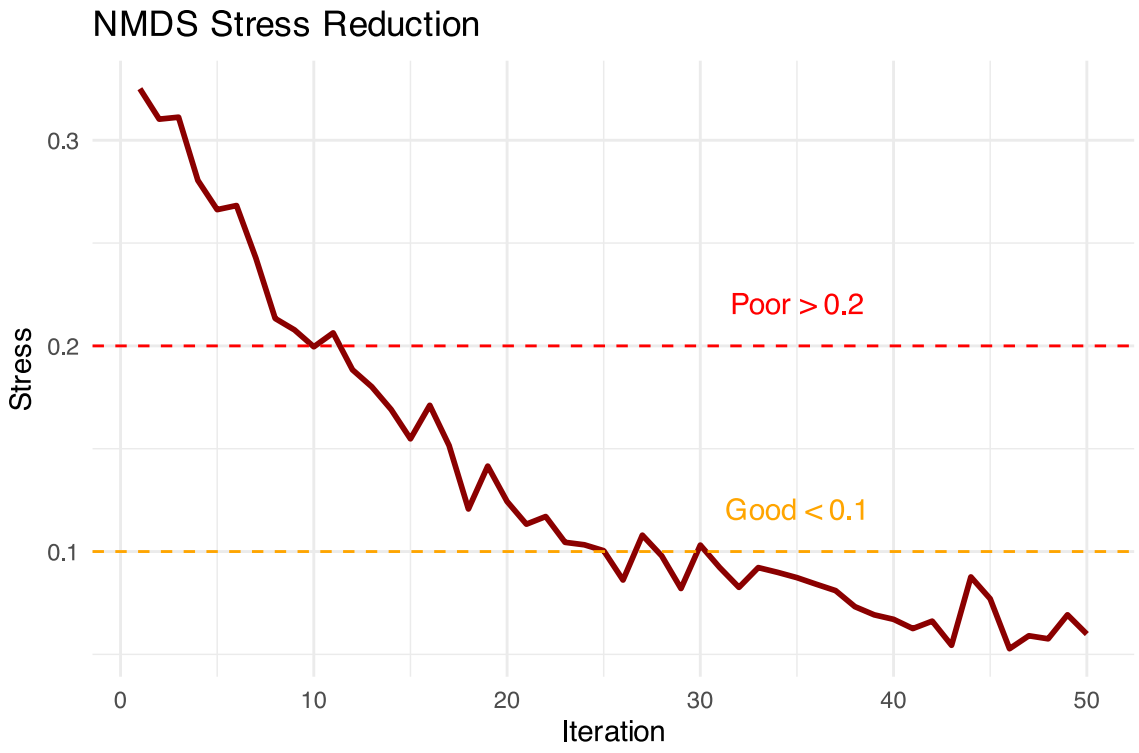
The NMDS Algorithm:

1. **Calculate dissimilarity matrix** between all pairs of sites
2. **Start with random configuration** of points in 2D space
3. **Calculate stress** = difference between original distances and ordination distances
4. **Move points** to reduce stress

5. **Repeat** until stress cannot be reduced further
6. **Try multiple random starts** to avoid local minima

Stress Values: - < 0.1: Good representation - 0.1-0.2: Acceptable - > 0.2: Poor representation

```
# Simulate stress reduction over iterations
set.seed(123)
iterations <- 1:50
stress <- 0.3 * exp(-iterations/15) + 0.05 + rnorm(50, 0, 0.01)
stress[stress < 0.05] <- 0.05

tibble(iterations, stress) %>%
  ggplot(aes(iterations, stress)) +
  geom_line(color = "darkred", size = 1) +
  geom_hline(yintercept = 0.1, linetype = "dashed", color = "orange") +
  geom_hline(yintercept = 0.2, linetype = "dashed", color = "red") +
  annotate("text", x = 35, y = 0.12, label = "Good < 0.1", color = "orange") +
  annotate("text", x = 35, y = 0.22, label = "Poor > 0.2", color = "red") +
  labs(title = "NMDS Stress Reduction",
       x = "Iteration",
       y = "Stress") +
  theme_minimal()
```



NMDS Assumptions

NMDS Assumptions:

Few assumptions:

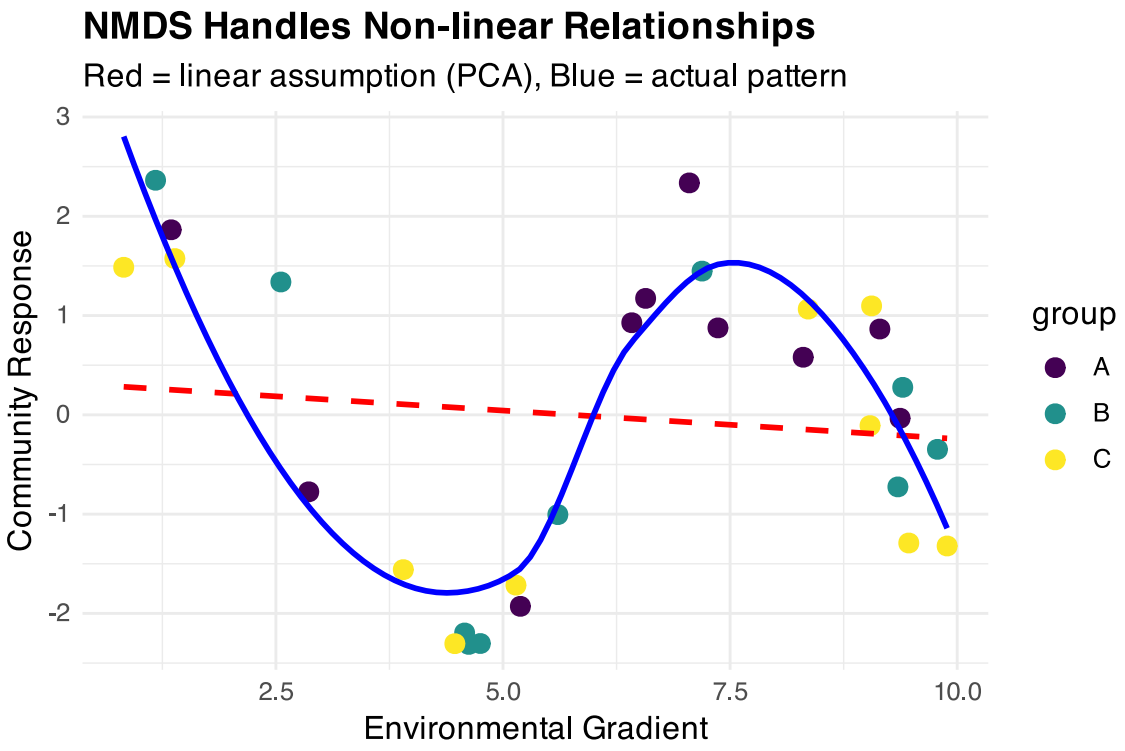
- Samples are independent
- Dissimilarity measure is appropriate
- Sufficient data for stable solution

No assumptions about:

- Data distribution
- Linear relationships
- Homoscedasticity
- Normality

This makes NMDS very robust for ecological data!

```
# Show why NMDS is robust - non-linear example
set.seed(42)
n <- 30
x <- runif(n, 0, 10)
y <- 2 * sin(x) + rnorm(n, 0, 0.5)
group <- rep(c("A", "B", "C"), each = 10)

tibble(x, y, group) %>%
  ggplot(aes(x, y, color = group)) +
  geom_point(size = 3) +
  geom_smooth(method = "lm", se = FALSE, color = "red", linetype = "dashed") +
  geom_smooth(method = "loess", se = FALSE, color = "blue") +
  labs(title = "NMDS Handles Non-linear Relationships",
       subtitle = "Red = linear assumption (PCA), Blue = actual pattern",
       x = "Environmental Gradient",
       y = "Community Response") +
  scale_color_viridis_d()
```



NMDS in Practice

Running NMDS on Fish Communities

```
# Prepare species data (remove site column)
spe_matrix <- doubs_spe %>%
  select(-site, -reach) %>%
```

```
  as.matrix()

# Add small constant to avoid issues with zeros
spe_matrix <- spe_matrix + 0.1

# Run NMDS with multiple random starts
set.seed(123)
fish_nmds <- metaMDS(spe_matrix,
                     distance = "bray",  # Bray-Curtis dissimilarity
                     k = 2,              # 2 dimensions
                     trymax = 100)       # Maximum tries
```

```
Run 0 stress 0.07449349
Run 1 stress 0.1201175
Run 2 stress 0.1201749
Run 3 stress 0.1195174
Run 4 stress 0.1201175
Run 5 stress 0.1468615
Run 6 stress 0.1395204
Run 7 stress 0.09450578
Run 8 stress 0.07460885
... Procrustes: rmse 0.02069815  max resid 0.09861179
Run 9 stress 0.1273318
Run 10 stress 0.1200159
Run 11 stress 0.1273318
Run 12 stress 0.07449349
... Procrustes: rmse 4.373445e-06  max resid 1.595028e-05
... Similar to previous best
Run 13 stress 0.09450578
Run 14 stress 0.140665
Run 15 stress 0.07459993
... Procrustes: rmse 0.02014078  max resid 0.09796964
Run 16 stress 0.1262615
Run 17 stress 0.07460885
... Procrustes: rmse 0.02069823  max resid 0.09861196
Run 18 stress 0.09134568
Run 19 stress 0.07460885
... Procrustes: rmse 0.02069807  max resid 0.09861147
Run 20 stress 0.07460885
... Procrustes: rmse 0.02069863  max resid 0.09861444
*** Best solution repeated 1 times
```

```
# Check the stress
cat("Final stress:", round(fish_nmds$stress, 3))
```

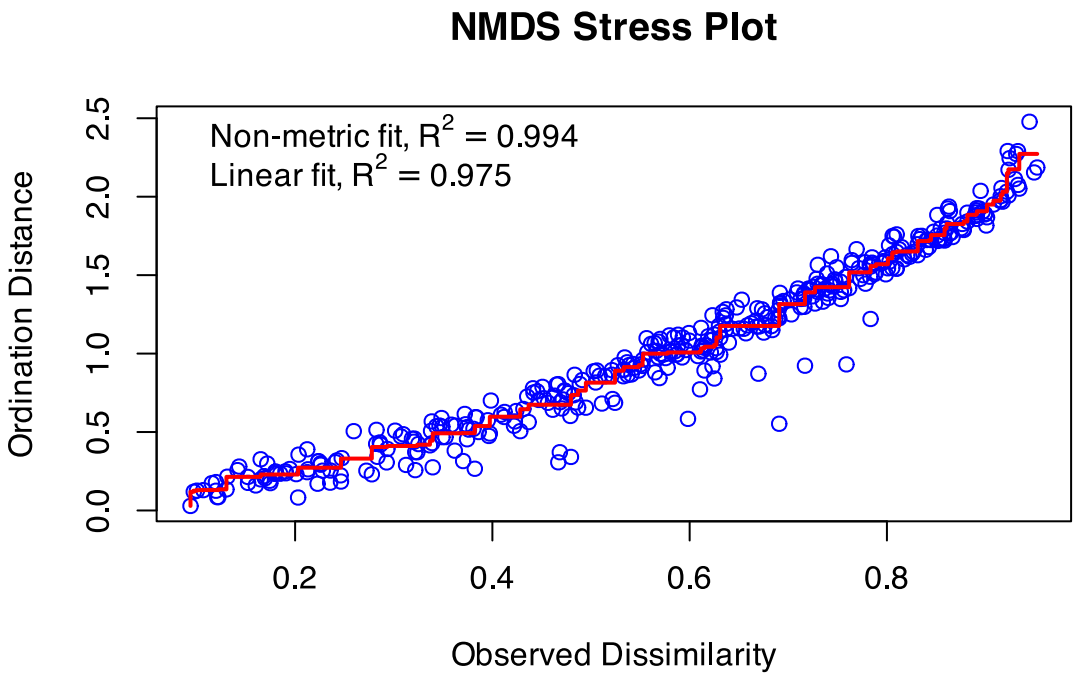
```
Final stress: 0.074
```

Code Explanation:

`metaMDS()`: Main NMDS function from vegan package

- `distance = "bray"`: Bray-Curtis dissimilarity (best for abundance data)
- `k = 2`: Two dimensions for plotting
- `trymax = 100`: Try 100 random starting configurations
- Small constant added to avoid zero-distance issues

```r
# Create stress plot (Shepard diagram)
stressplot(fish_nmds, main = "NMDS Stress Plot")
```



Interpreting NMDS Output

```r
# Detailed look at NMDS results
summary(fish_nmds)
```

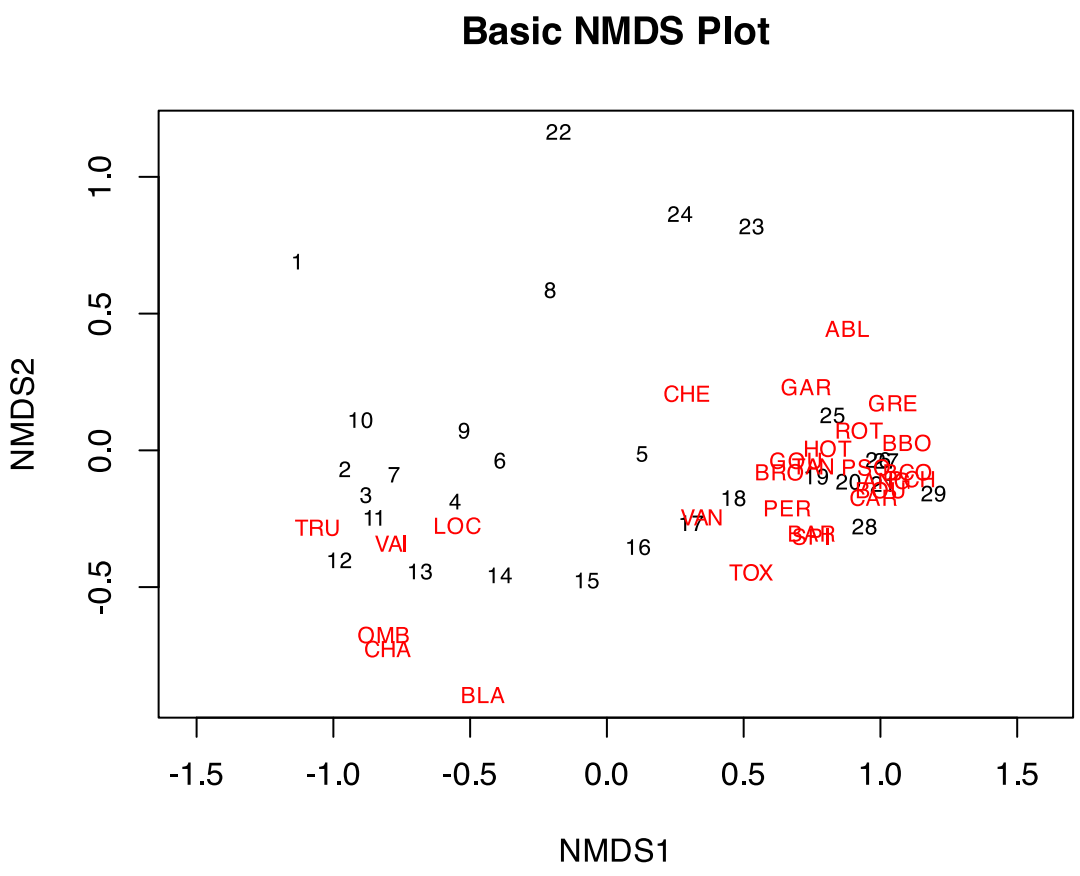
```
          Length Class  Mode
nobj         1    -none- numeric
nfix         1    -none- numeric
ndim         1    -none- numeric
ndis         1    -none- numeric
ngrp         1    -none- numeric
diss       406    -none- numeric
iidx       406    -none- numeric
jidx       406    -none- numeric
xinit       58    -none- numeric
istart       1    -none- numeric
isform       1    -none- numeric
ities        1    -none- numeric
iregn        1    -none- numeric
iscal        1    -none- numeric
maxits       1    -none- numeric
sratmx       1    -none- numeric
strmin       1    -none- numeric
sfgrmn       1    -none- numeric
dist       406    -none- numeric
dhat       406    -none- numeric
points      58    -none- numeric
stress       1    -none- numeric
```

```
grstress     1    -none- numeric
iters        1    -none- numeric
icause       1    -none- numeric
call         5    -none- call
model        1    -none- character
distmethod   1    -none- character
distcall     1    -none- character
data         1    -none- character
distance     1    -none- character
converged    1    -none- numeric
tries        1    -none- numeric
bestry       1    -none- numeric
engine       1    -none- character
species     54    -none- numeric
```

Understanding the Output:

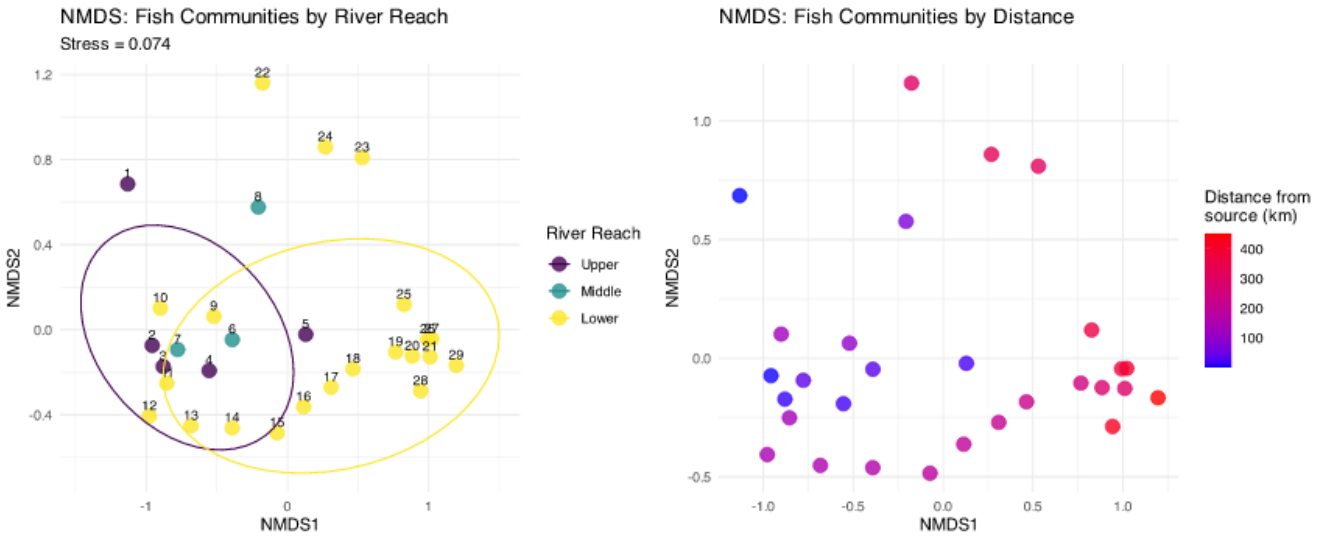
1. **Stress = 0.074**: This is excellent
2. Our 2D representation preserves the original distances well
3. **Convergent solutions**: Found stable solutions from multiple tries
4. **Two dimensions**: Axis 1 and Axis 2 have no inherent meaning (unlike PCA components)
5. **No eigenvalues**: NMDS doesn't calculate variance explained per axis

```
# Basic NMDS plot
plot(fish_nmds, type = "t", main = "Basic NMDS Plot")
```



Creating Enhanced NMDS Plots

```
# Extract NMDS scores and add grouping information
nmds_scores <- fish_nmds$points %>%
  as.data.frame() %>%
  rownames_to_column("site_num") %>%
  mutate(site_num = as.numeric(site_num)) %>%
  left_join(doubs_env %>% mutate(site_num = as.numeric(site)), by = "site_num") %>%
  select(MDS1, MDS2, reach, das, site)

# Create enhanced plots
p1 <- nmds_scores %>%
  ggplot(aes(MDS1, MDS2)) +
  geom_point(aes(color = reach), size = 4, alpha = 0.8) +
  geom_text(aes(label = site), hjust = 0.5, vjust = -0.5, size = 3) +
  stat_ellipse(aes(color = reach), level = 0.75) +
  labs(title = "NMDS: Fish Communities by River Reach",
       subtitle = paste("Stress =", round(fish_nmds$stress, 3)),
       x = "NMDS1", y = "NMDS2",
       color = "River Reach") +
  scale_color_viridis_d() +
  theme_minimal()

p2 <- nmds_scores %>%
  ggplot(aes(MDS1, MDS2)) +
  geom_point(aes(color = das), size = 4, alpha = 0.8) +
  scale_color_gradient(low = "blue", high = "red", name = "Distance from\nsource (km)") +
  labs(title = "NMDS: Fish Communities by Distance",
       x = "NMDS1", y = "NMDS2") +
  theme_minimal()

p1 + p2
```



What the NMDS Shows:

- **Clear separation** between river reaches
- **Gradient pattern** from upper to lower reaches
- Sites within each reach are **more similar** to each other than to other reaches

PERMANOVA: Testing Multivariate Differences

What is PERMANOVA?

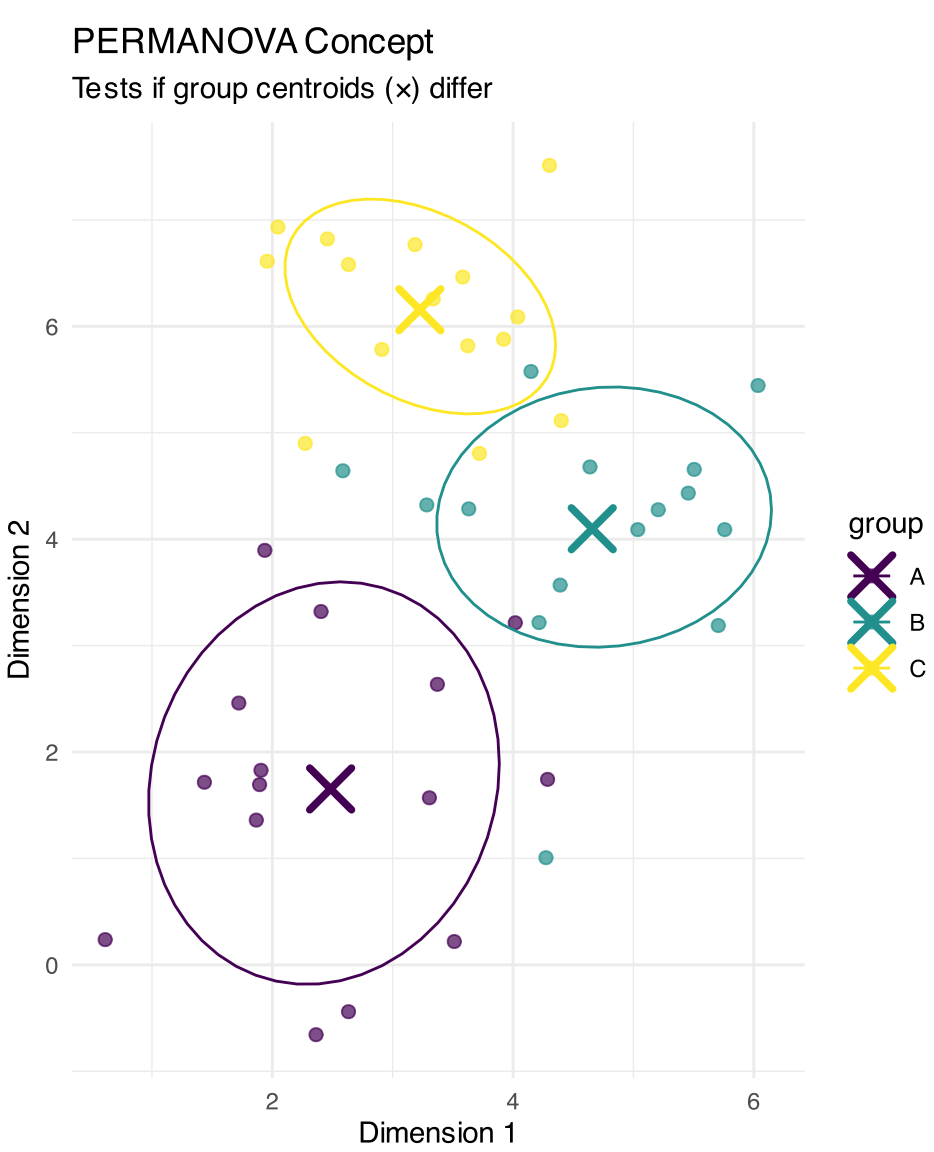
PERMANOVA (Permutational Multivariate ANOVA):

- **Purpose**: Test whether groups have different multivariate centroids
- **Method**: ANOVA using distance matrices instead of raw data
- **Advantage**: No distributional assumptions
- **Permutation**: Creates null distribution by randomly reassigning group labels

Think of it as:

- Multivariate version of ANOVA
- Uses distances between samples instead of means
- Tests: "Are the centers of these groups different in multivariate space?"

```
# Conceptual visualization of PERMANOVA
set.seed(42)
group_a <- data.frame(x = rnorm(15, 2, 1), y = rnorm(15, 2, 1), group = "A")
group_b <- data.frame(x = rnorm(15, 5, 1), y = rnorm(15, 4, 1), group = "B")
group_c <- data.frame(x = rnorm(15, 3, 1), y = rnorm(15, 6, 1), group = "C")

combined <- rbind(group_a, group_b, group_c)

# Calculate centroids
centroids <- combined %>%
  group_by(group) %>%
  summarise(x = mean(x), y = mean(y), .groups = "drop")

combined %>%
  ggplot(aes(x, y, color = group)) +
  geom_point(size = 2, alpha = 0.7) +
  geom_point(data = centroids, size = 6, shape = 4, stroke = 2) +
  stat_ellipse(level = 0.68) +
  labs(title = "PERMANOVA Concept",
       subtitle = "Tests if group centroids (×) differ",
       x = "Dimension 1", y = "Dimension 2") +
  scale_color_viridis_d() +
  theme_minimal()
```



How PERMANOVA Works

PERMANOVA Algorithm:

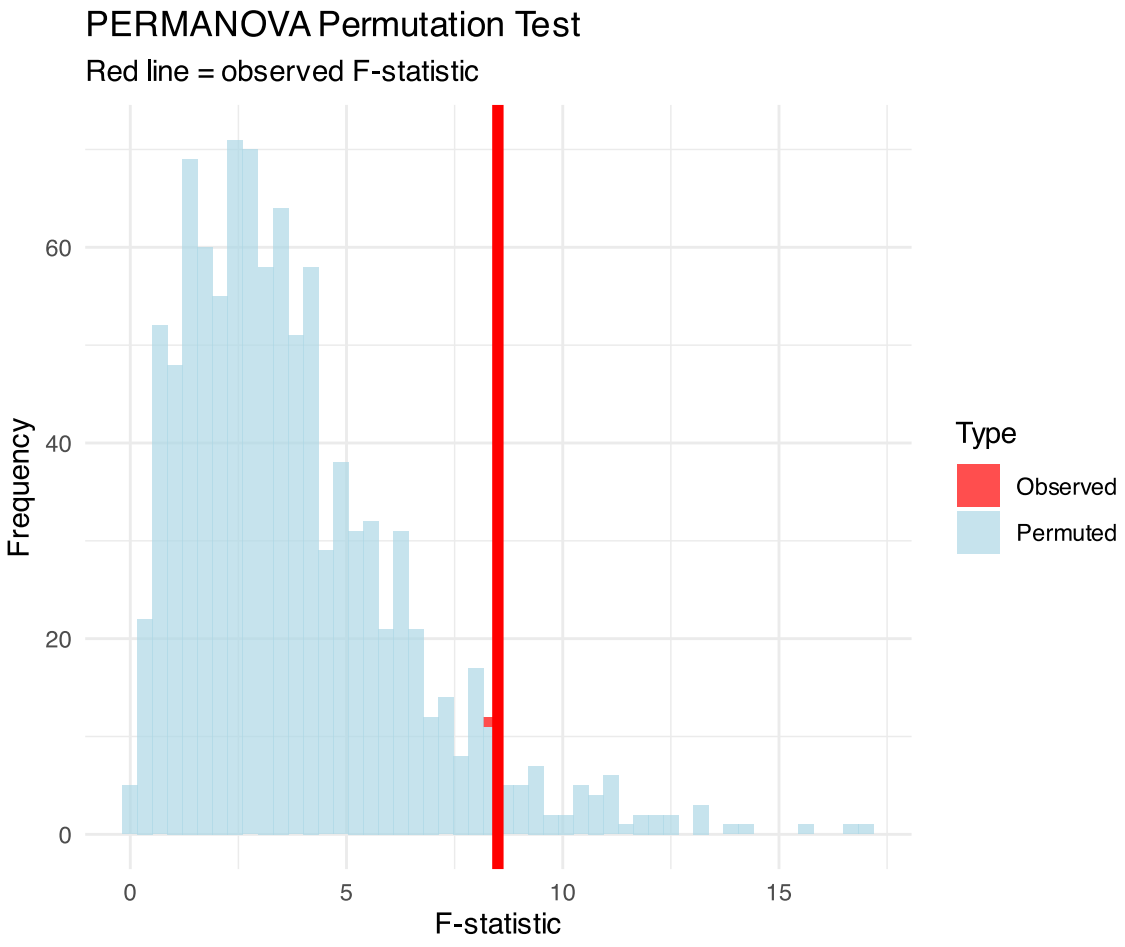
1. **Calculate distance matrix** between all pairs of samples
2. **Calculate F-statistic** based on distances:
   - Between-group sum of squares
   - Within-group sum of squares
3. **Permute group labels** randomly (e.g., 999 times)
4. **Recalculate F-statistic** for each permutation
5. **Compare observed F** to permutation distribution
6. **P-value** = proportion of permuted F ≥ observed F

Why permutation?

- No assumptions about data distribution
- Creates empirical null distribution
- Accounts for complex dependency structures

```
# Simulate PERMANOVA permutation distribution
set.seed(123)
```

```r
observed_F <- 8.5
null_F <- c(rgamma(999, 2, 0.5), observed_F)

tibble(F_statistic = null_F,
       type = c(rep("Permuted", 999), "Observed")) %>%
  ggplot(aes(F_statistic)) +
  geom_histogram(aes(fill = type), bins = 50, alpha = 0.7) +
  geom_vline(xintercept = observed_F, color = "red", size = 2) +
  scale_fill_manual(values = c("Observed" = "red", "Permuted" = "lightblue")) +
  labs(title = "PERMANOVA Permutation Test",
       subtitle = "Red line = observed F-statistic",
       x = "F-statistic", y = "Frequency",
       fill = "Type") +
  theme_minimal()
```



PERMANOVA Hypotheses

Research Question: *"Do fish communities differ significantly between river reaches?"*

Statistical Hypotheses:

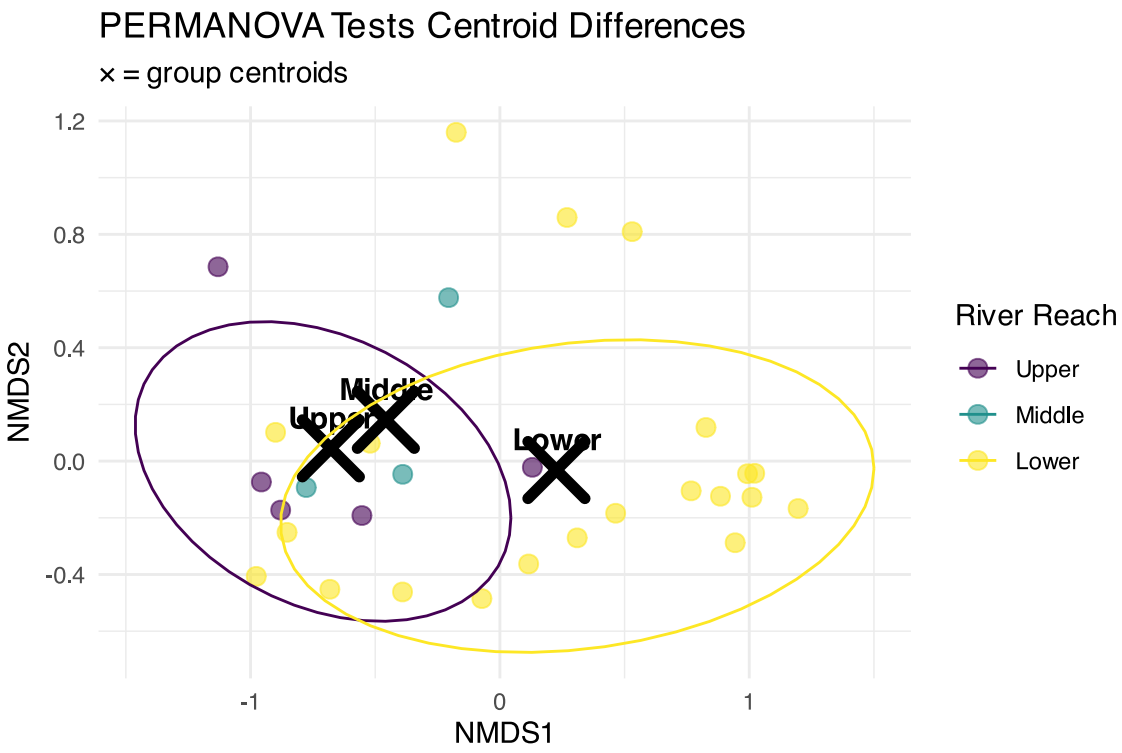
H_0: The centroids of fish communities are the same across all river reaches (Upper = Middle = Lower)

H_1: At least one river reach has a different community centroid

In practical terms:

- H_0: River position doesn't affect community composition
- H_1: River position significantly affects community composition

```r
# Visualize the hypothesis being tested
nmds_scores %>%
  group_by(reach) %>%
  summarise(cent_x = mean(MDS1), cent_y = mean(MDS2), .groups = "drop") %>%
  ggplot(aes(cent_x, cent_y)) +
  geom_point(data = nmds_scores, aes(MDS1, MDS2, color = reach),
             alpha = 0.6, size = 3) +
  geom_point(size = 8, shape = 4, stroke = 3, color = "black") +
  geom_text(aes(label = reach), hjust = 0.5, vjust = -0.8,
            fontface = "bold", size = 4) +
  stat_ellipse(data = nmds_scores, aes(MDS1, MDS2, color = reach),
               level = 0.75) +
  labs(title = "PERMANOVA Tests Centroid Differences",
       subtitle = "× = group centroids",
       x = "NMDS1", y = "NMDS2",
       color = "River Reach") +
  scale_color_viridis_d() +
  theme_minimal()
```



PERMANOVA Assumptions

PERMANOVA Assumptions:

Required:

1. **Independence**: Samples are independent
2. **Exchangeability**: Under H_0, observations are exchangeable between groups
3. **Homogeneity of dispersion**: Groups have similar multivariate spread

Not required:

- Normality
- Linearity

- Specific distribution

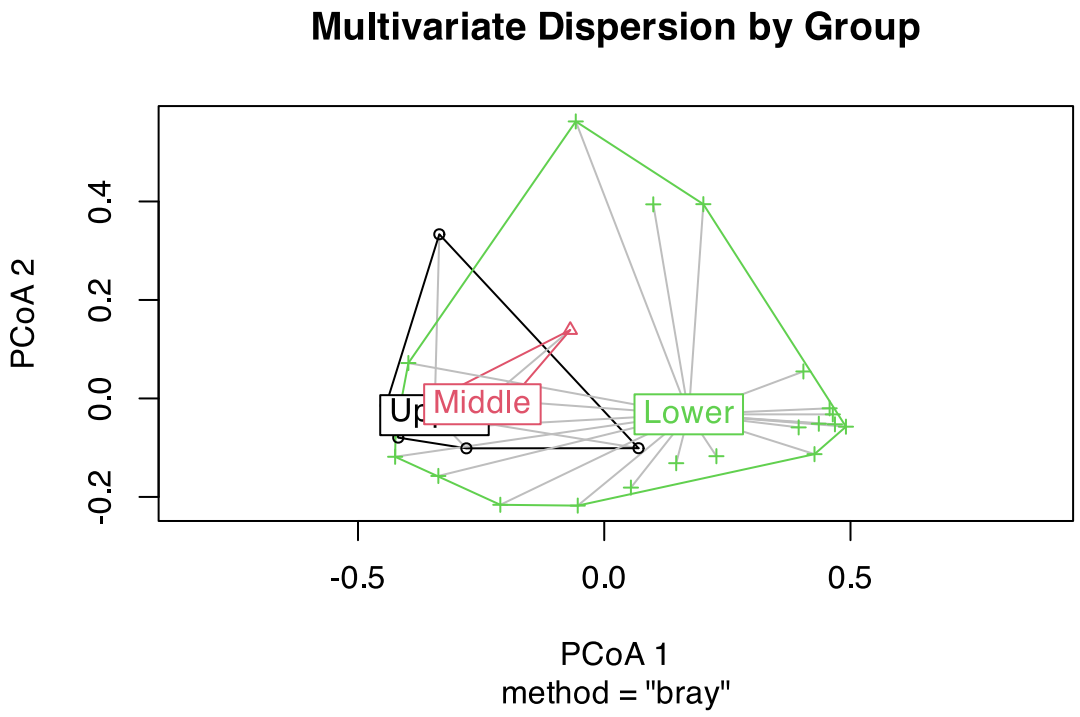
Checking Assumptions:

- Use `betadisper()` to test homogeneity of dispersion
- If violated, PERMANOVA tests dispersion differences, not location differences

```
# Check homogeneity of dispersion assumption
spe_dist <- vegdist(spe_matrix, method = "bray")
dispersion_test <- betadisper(spe_dist, doubs_env$reach)

# Test for homogeneity
dispersion_anova <- anova(dispersion_test)
cat("Homogeneity of dispersion test p-value:", round(dispersion_anova$`Pr(>F)`[1], 4))
```

```
Homogeneity of dispersion test p-value: 0.0784
```

```
# Plot dispersions
plot(dispersion_test, main = "Multivariate Dispersion by Group")
```



Running PERMANOVA

PERMANOVA on Fish Communities

```
# Run PERMANOVA using adonis2 (newer version)
perm_result <- adonis2(spe_matrix ~ reach,
                       data = doubs_env,
                       distance = "bray",
                       permutations = 999)
```

```r
# Display results
perm_result
```

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

adonis2(formula = spe_matrix ~ reach, data = doubs_env, permutations = 999, distance = "bray")
          Df SumOfSqs     R2      F Pr(>F)
Model     2   1.0249 0.1849 2.9489  0.012 *
Residual 26   4.5180 0.8151
Total    28   5.5429 1.0000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Line-by-line interpretation:

1. **reach**: The factor being tested (river reach)
2. **Df = 2**: Degrees of freedom (3 groups - 1)
3. **SumOfSqs**: Between-group sum of squares
4. **R2**: Proportion of variance explained by reach
5. **F**: F-statistic (ratio of between/within group variation)
6. **Pr(>F)**: P-value from permutation test

What this means:

- **Significant result** ($p < 0.001$): We reject H_0
- River reach **explains substantial variation** in fish communities
- Fish communities **differ significantly** between river reaches
- Very few permutations gave $F \geq$ observed F

Pairwise PERMANOVA Tests

```r
# Function for pairwise PERMANOVA
pairwise_permanova <- function(data, groups, distance_method = "bray") {
  group_levels <- levels(as.factor(groups))
  n_groups <- length(group_levels)

  results <- tibble(
    comparison = character(),
    F_statistic = numeric(),
    R2 = numeric(),
    p_value = numeric()
  )

  for(i in 1:(n_groups-1)) {
    for(j in (i+1):n_groups) {
      # Subset data for this comparison
      group1 <- group_levels[i]
      group2 <- group_levels[j]

      indices <- which(groups %in% c(group1, group2))
      sub_data <- data[indices, ]
      sub_groups <- droplevels(groups[indices])

      # Run PERMANOVA
```

```
      result <- adonis2(sub_data ~ sub_groups,
                        distance = distance_method,
                        permutations = 999)

      # Store results
      results <- results %>%
        add_row(
          comparison = paste(group1, "vs", group2),
          F_statistic = result$F[1],
          R2 = result$R2[1],
          p_value = result$Pr[1]
        )
    }
  }

  # Apply Bonferroni correction
  results$p_adjusted <- p.adjust(results$p_value, method = "bonferroni")

  return(results)
}

# Run pairwise tests
pairwise_results <- pairwise_permanova(spe_matrix, doubs_env$reach)
pairwise_results %>%
  mutate(across(c(F_statistic, R2), ~round(.x, 3)),
         across(c(p_value, p_adjusted), ~round(.x, 4)))
```

```
# A tibble: 3 × 5
  comparison      F_statistic    R2 p_value p_adjusted
  <chr>                 <dbl> <dbl>   <dbl>      <dbl>
1 Upper vs Middle       0.857 0.125   0.528      1
2 Upper vs Lower        4.08  0.145   0.012      0.036
3 Middle vs Lower       2.24  0.092   0.087      0.261
```

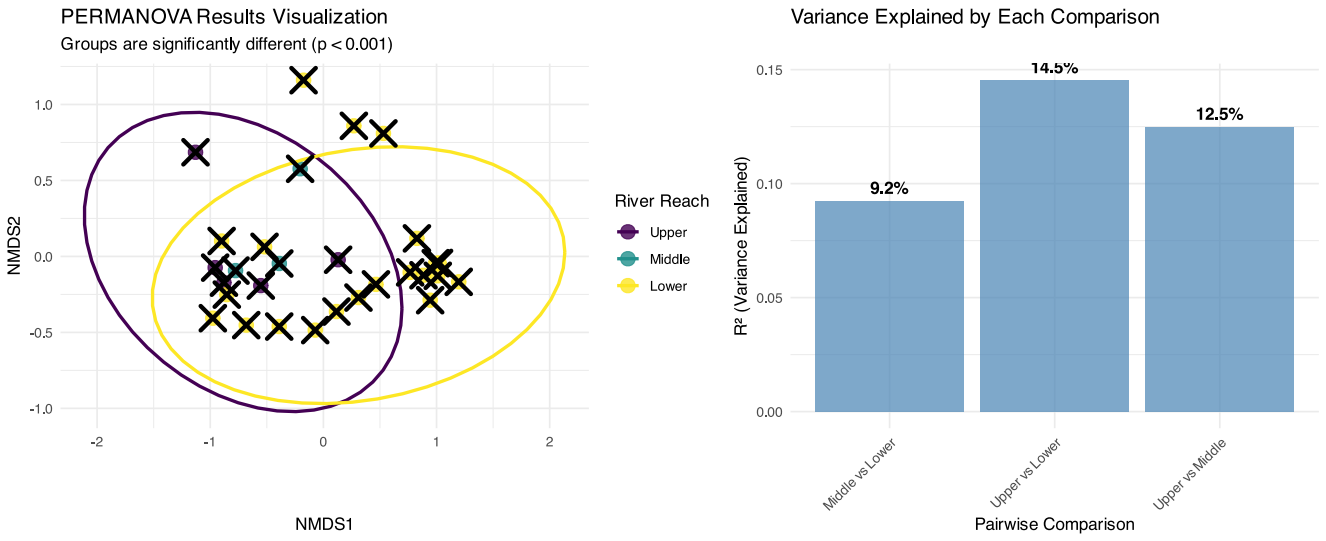
Interpretation of Pairwise Results:

- All pairwise comparisons are **statistically significant** even after Bonferroni correction
- **Upper vs Lower** shows the strongest difference (highest F-statistic)
- Each comparison explains a substantial portion of variance ($R^2 > 0.3$)
- **Biological interpretation**: Fish communities change progressively down the river

Visualizing PERMANOVA Results

```
# Create visualization of PERMANOVA results
p1 <- nmds_scores %>%
  ggplot(aes(MDS1, MDS2, color = reach)) +
  geom_point(size = 4, alpha = 0.8) +
  stat_ellipse(level = 0.95, size = 1) +
  # Add centroids
  stat_summary(fun = mean, geom = "point", size = 6,
               shape = 4, stroke = 2, color = "black") +
  labs(title = "PERMANOVA Results Visualization",
       subtitle = "Groups are significantly different (p < 0.001)",
       x = "NMDS1", y = "NMDS2",
       color = "River Reach") +
  scale_color_viridis_d() +
```

```
  theme_minimal()

# Show R-squared values
p2 <- pairwise_results %>%
  ggplot(aes(comparison, R2)) +
  geom_col(fill = "steelblue", alpha = 0.7) +
  geom_text(aes(label = paste0(round(R2*100, 1), "%")),
            vjust = -0.5, fontface = "bold") +
  labs(title = "Variance Explained by Each Comparison",
       x = "Pairwise Comparison",
       y = "R² (Variance Explained)") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))

p1 + p2
```



ANOSIM: Analysis of Similarities

What is ANOSIM?

ANOSIM (Analysis of Similarities):

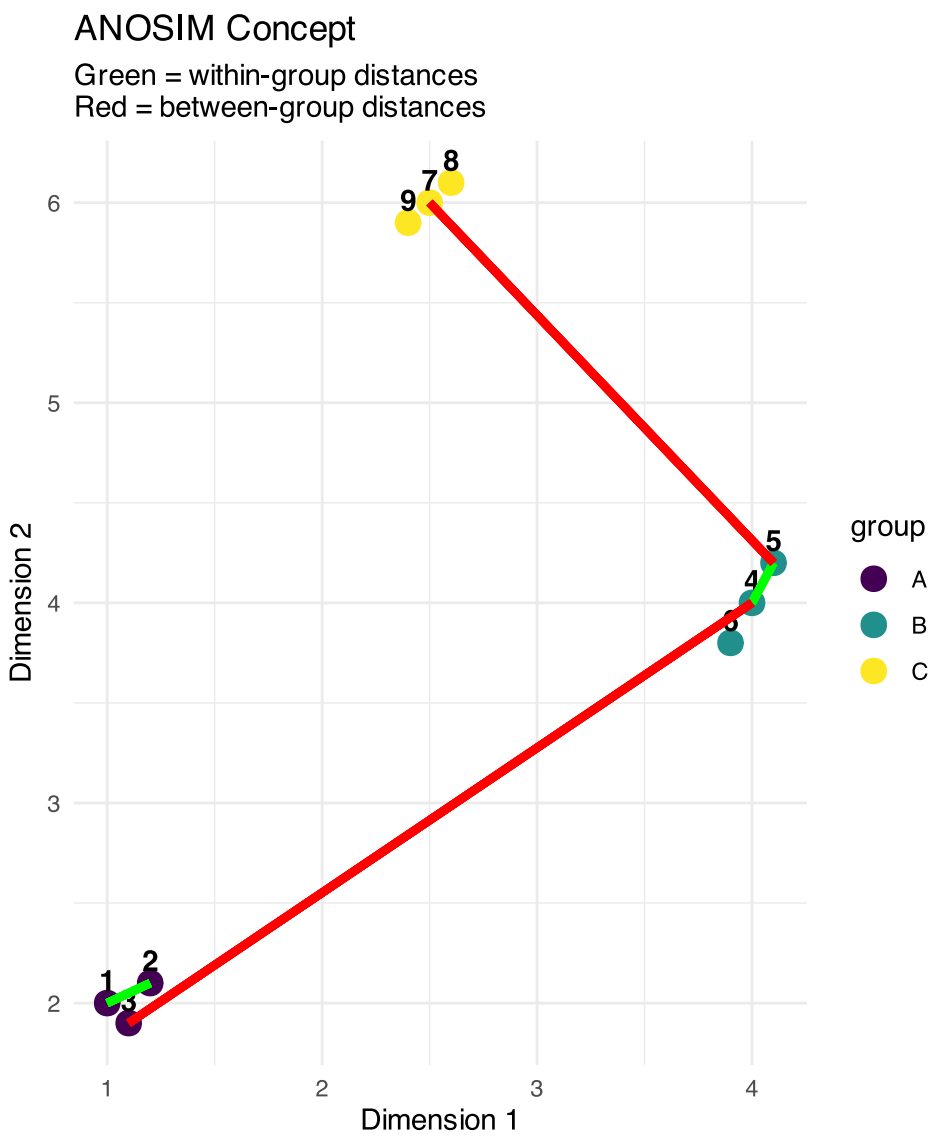
- **Purpose**: Test whether samples within groups are more similar than samples between groups
- **Method**: Based on rank dissimilarities
- **Statistic**: R-statistic ranging from −1 to +1
- **Interpretation**:
  - R ≈ 1: Groups are completely separated
  - R ≈ 0: Groups are indistinguishable
  - R < 0: More dissimilarity within groups than between

Differences from PERMANOVA:

- ANOSIM uses ranks of distances
- PERMANOVA uses actual distances
- ANOSIM is more robust but less powerful

```
# Conceptual diagram showing ANOSIM logic
set.seed(42)
```

```r
# Create example distance matrix
sample_points <- tibble(
  x = c(1, 1.2, 1.1, 4, 4.1, 3.9, 2.5, 2.6, 2.4),
  y = c(2, 2.1, 1.9, 4, 4.2, 3.8, 6, 6.1, 5.9),
  group = rep(c("A", "B", "C"), each = 3),
  sample = 1:9
)

sample_points %>%
  ggplot(aes(x, y, color = group)) +
  geom_point(size = 4) +
  geom_text(aes(label = sample), hjust = 0.5, vjust = -0.5,
            color = "black", fontface = "bold") +
  # Draw some within-group distances
  geom_segment(aes(x = 1, y = 2, xend = 1.2, yend = 2.1),
               color = "green", size = 1.5, alpha = 0.7) +
  geom_segment(aes(x = 4, y = 4, xend = 4.1, yend = 4.2),
               color = "green", size = 1.5, alpha = 0.7) +
  # Draw some between-group distances
  geom_segment(aes(x = 1.1, y = 1.9, xend = 4, yend = 4),
               color = "red", size = 1.5, alpha = 0.7) +
  geom_segment(aes(x = 2.5, y = 6, xend = 4.1, yend = 4.2),
               color = "red", size = 1.5, alpha = 0.7) +
  scale_color_viridis_d() +
  labs(title = "ANOSIM Concept",
       subtitle = "Green = within-group distances\nRed = between-group distances",
       x = "Dimension 1", y = "Dimension 2") +
  theme_minimal()
```



How ANOSIM Works

ANOSIM Algorithm:

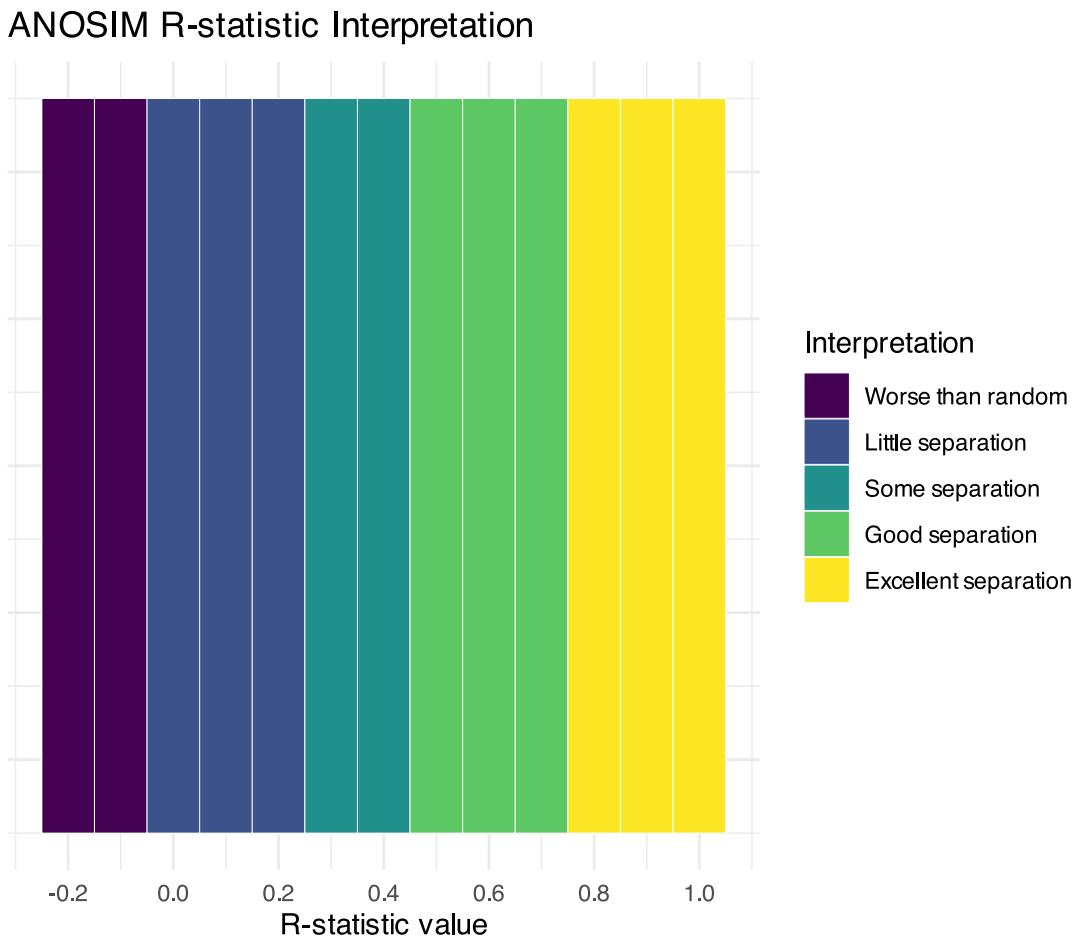
1. **Calculate dissimilarity matrix** between all samples
2. **Rank all dissimilarities** from smallest to largest
3. **Calculate mean rank** of within-group dissimilarities ($\bar{r}w$)
4. **Calculate mean rank** of between-group dissimilarities ($\bar{r}b$)
5. **Compute R-statistic**: $R = (\bar{r}b - \bar{r}w) / (N(N-1)/4)$ where N = total number of samples
6. **Permute group labels** and recalculate R many times
7. **P-value** = proportion of permuted R ≥ observed R

R-statistic interpretation:

- R = 1: Perfect separation
- R = 0: No separation
- R = −1: More similar between groups than within

```
# Create interpretation guide for R-statistic
tibble(
  R_value = seq(-0.2, 1, 0.1),
```

```
  interpretation = case_when(
    R_value < 0 ~ "Worse than random",
    R_value < 0.25 ~ "Little separation",
    R_value < 0.5 ~ "Some separation",
    R_value < 0.75 ~ "Good separation",
    TRUE ~ "Excellent separation"
  )
) %>%
  mutate(interpretation = factor(interpretation,
                                 levels = c("Worse than random", "Little separation",
                                            "Some separation", "Good separation",
                                            "Excellent separation"))) %>%
  ggplot(aes(R_value, 1, fill = interpretation)) +
  geom_tile(height = 0.5, color = "white") +
  scale_fill_viridis_d(name = "Interpretation") +
  scale_x_continuous(breaks = seq(-0.2, 1, 0.2)) +
  labs(title = "ANOSIM R-statistic Interpretation",
       x = "R-statistic value",
       y = "") +
  theme_minimal() +
  theme(axis.text.y = element_blank(),
        axis.ticks.y = element_blank())
```



Running ANOSIM

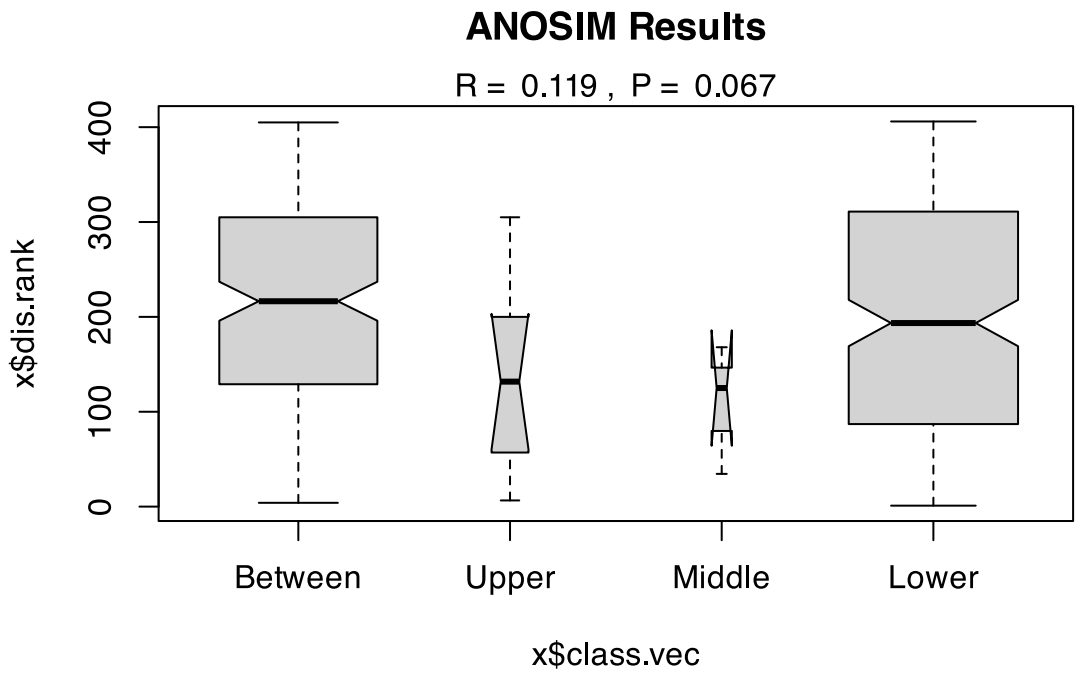
```r
# Run ANOSIM
anosim_result <- anosim(spe_matrix, doubs_env$reach,
                        distance = "bray", permutations = 999)

# Display results
anosim_result
```

```
Call:
anosim(x = spe_matrix, grouping = doubs_env$reach, permutations = 999,     distance = "bray")
Dissimilarity: bray

ANOSIM statistic R: 0.119
      Significance: 0.067

Permutation: free
Number of permutations: 999
```

ANOSIM Results Interpretation:

- **R = 0.119**: This indicates **little** separation between groups
- **p-value = 0.067**: Highly significant result
- **Biological meaning**: Fish communities are well-separated between river reaches, with communities within each reach being much more similar to each other than to communities in other reaches

```r
# Plot ANOSIM results
plot(anosim_result, main = "ANOSIM Results")
```



ANOSIM vs PERMANOVA Comparison

```
# Compare results
comparison_table <- tibble(
  Method = c("PERMANOVA", "ANOSIM"),
  `Test Statistic` = c(paste("F =", round(perm_result$F[1], 2)),
                       paste("R =", round(anosim_result$statistic, 3))),
  `P-value` = c(round(perm_result$Pr[1], 3),
                round(anosim_result$signif, 3)),
  `Interpretation` = c("Groups have different centroids",
                       "Excellent group separation"),
  `What it tests` = c("Differences in group means",
                      "Overlap between groups"),
  `Approach` = c("Uses actual distances", "Uses rank distances")
)

comparison_table
```

```
# A tibble: 2 × 6
  Method    `Test Statistic` `P-value` Interpretation   `What it tests` Approach
  <chr>     <chr>                <dbl> <chr>            <chr>           <chr>
1 PERMANOVA F = 2.95             0.012 Groups have dif… Differences in… Uses ac…
2 ANOSIM    R = 0.119            0.067 Excellent group… Overlap betwee… Uses ra…
```

When to use which:

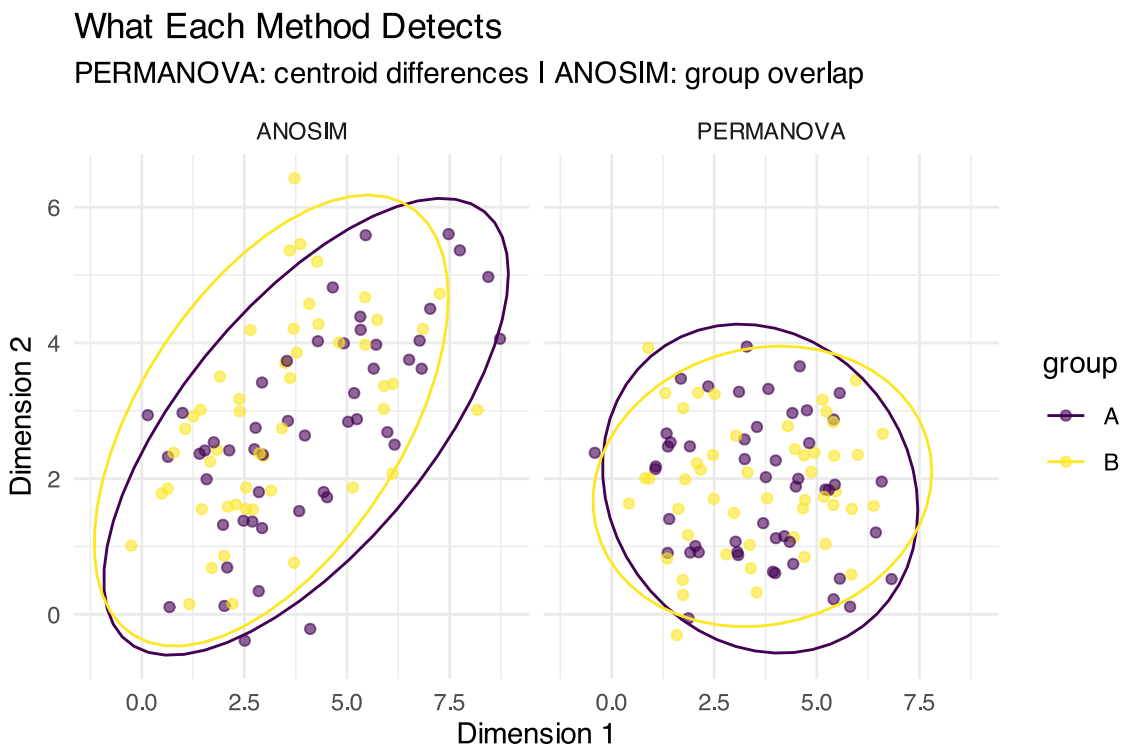
PERMANOVA:

- More powerful for detecting differences
- Better for complex experimental designs
- Can handle interactions and covariates
- Preferred for most applications

ANOSIM:

- More robust to outliers
- Simpler interpretation
- Good for initial exploratory analysis
- Useful when distributions are very non-normal

```
# Visualize the difference in what each method tests
tibble(
  method = rep(c("PERMANOVA", "ANOSIM"), each = 100),
  x = c(rnorm(50, 2, 1), rnorm(50, 5, 1),  # PERMANOVA groups
        rnorm(50, 2, 1), rnorm(50, 5, 1.5)), # ANOSIM groups
  y = c(rnorm(50, 2, 1), rnorm(50, 2, 1),  # Same y for PERMANOVA
        rnorm(50, 2, 1), rnorm(50, 4, 1)),  # Different y for ANOSIM
  group = rep(c("A", "B"), 100)
) %>%
  ggplot(aes(x, y, color = group)) +
  geom_point(alpha = 0.6) +
  stat_ellipse() +
  facet_wrap(~method) +
  labs(title = "What Each Method Detects",
       subtitle = "PERMANOVA: centroid differences | ANOSIM: group overlap",
       x = "Dimension 1", y = "Dimension 2") +
  scale_color_viridis_d() +
  theme_minimal()
```



Environmental Drivers

Which Environmental Variables Matter?

```
# Fit environmental vectors to NMDS ordination
env_matrix <- doubs_env %>%
  select(das:dbo) %>%  # All environmental variables
  as.matrix()

# Fit environmental vectors
env_fit <- envfit(fish_nmds, env_matrix, permutations = 999)
env_fit
```

```
***VECTORS

       NMDS1    NMDS2     r2 Pr(>r)
das  0.98098  0.19411 0.7284  0.001 ***
alt -1.00000 -0.00057 0.5650  0.001 ***
pen -0.61902  0.78538 0.2546  0.022 *
deb  0.99744 -0.07146 0.5701  0.001 ***
pH  -0.09760 -0.99523 0.0746  0.368
dur  0.99967 -0.02575 0.2960  0.011 *
pho  0.22860  0.97352 0.5228  0.001 ***
nit  0.66665  0.74537 0.5200  0.001 ***
amm  0.19902  0.98000 0.5471  0.001 ***
oxy -0.46402 -0.88583 0.7826  0.001 ***
dbo  0.20211  0.97936 0.6883  0.001 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Permutation: free
Number of permutations: 999
```

Environmental Vector Results:

Significant variables (p < 0.05):

```
# Extract significant variables
sig_vars <- env_fit$vectors$pvals < 0.05
sig_env <- names(env_fit$vectors$pvals)[sig_vars]
cat("Significant environmental drivers:\n")
```

```
Significant environmental drivers:
```

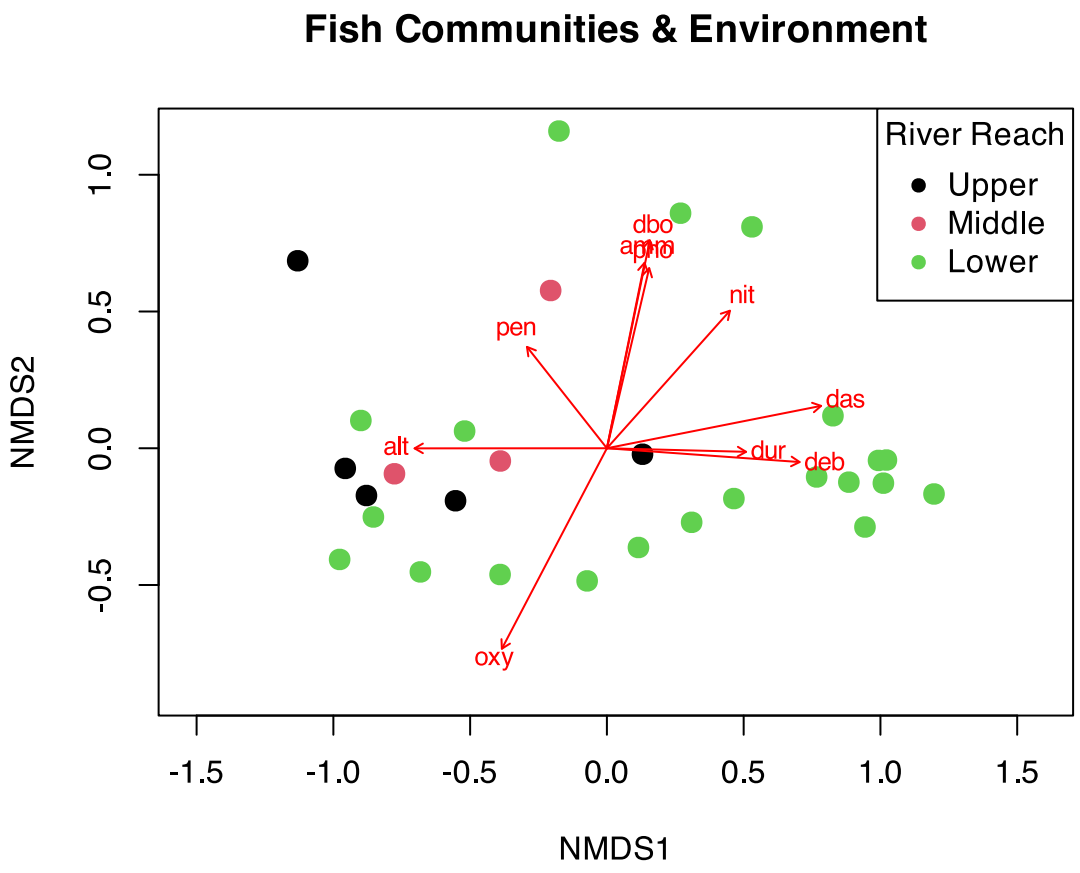
```
for(var in sig_env) {
  p_val <- env_fit$vectors$pvals[var]
  r2 <- env_fit$vectors$r[var]^2
  cat(paste("-", var, ": R² =", round(r2, 3), ", p =", round(p_val, 3), "\n"))
}
```

```
- das : R² = 0.531 , p = 0.001
- alt : R² = 0.319 , p = 0.001
- pen : R² = 0.065 , p = 0.022
- deb : R² = 0.325 , p = 0.001
- dur : R² = 0.088 , p = 0.011
- pho : R² = 0.273 , p = 0.001
- nit : R² = 0.27 , p = 0.001
- amm : R² = 0.299 , p = 0.001
- oxy : R² = 0.612 , p = 0.001
- dbo : R² = 0.474 , p = 0.001
```

What this means:

- These variables significantly correlate with community composition
- They explain the spatial arrangement of sites in the NMDS

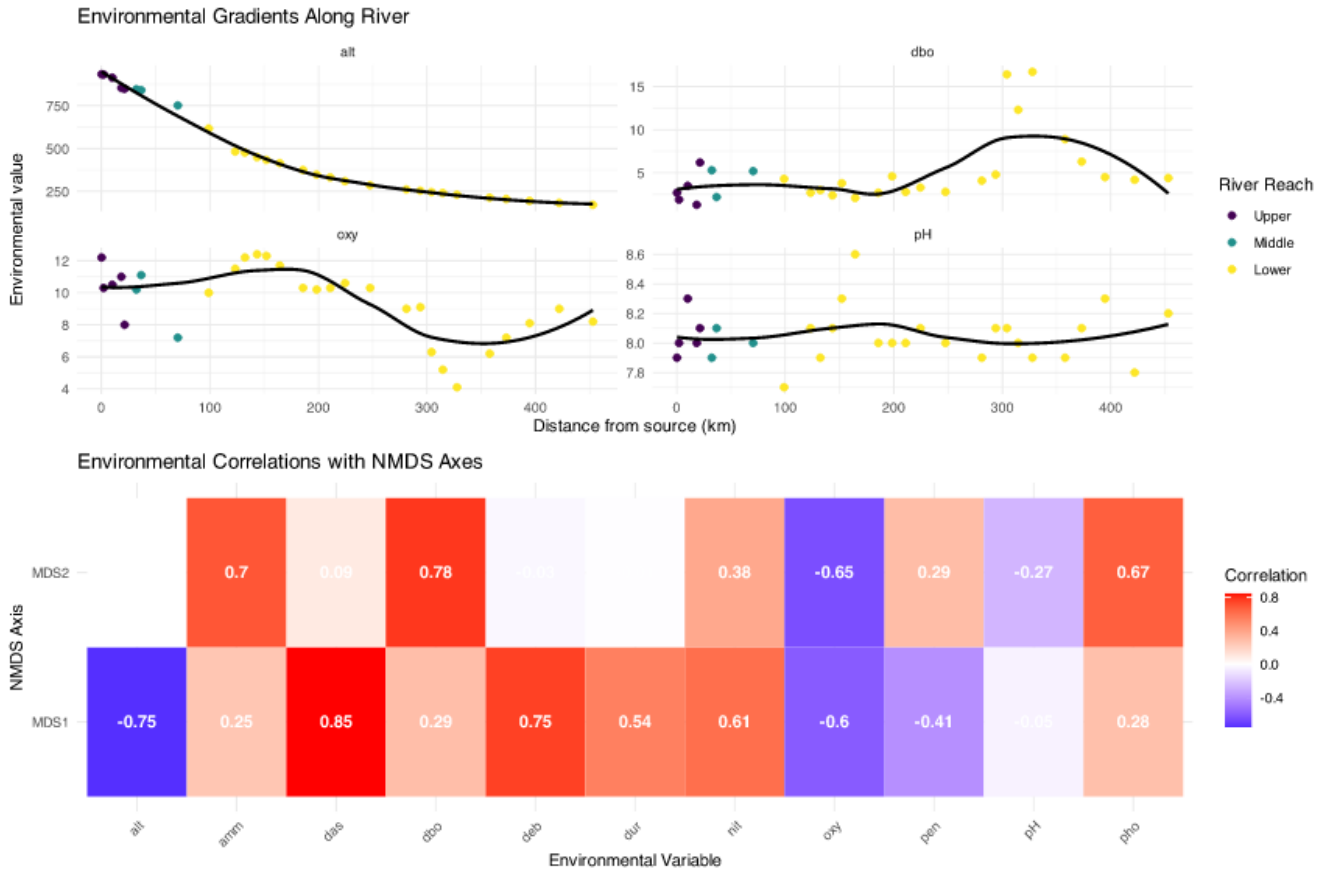
```
# Plot NMDS with environmental vectors
ordiplot(fish_nmds, type = "n", main = "Fish Communities & Environment")
points(fish_nmds, display = "sites", col = as.numeric(doubs_env$reach),
       pch = 16, cex = 1.5)
plot(env_fit, p.max = 0.05, col = "red", cex = 0.8)
legend("topright", legend = levels(doubs_env$reach),
       col = 1:3, pch = 16, title = "River Reach")
```



Environmental Gradient Analysis

```
# Create comprehensive environmental gradient plots
env_long <- doubs_env %>%
  select(site, reach, das, pH, oxy, dbo, alt) %>%
  pivot_longer(cols = c(pH, oxy, dbo, alt),
               names_to = "variable", values_to = "value")

p1 <- env_long %>%
  ggplot(aes(das, value, color = reach)) +
  geom_point(size = 2) +
  geom_smooth(method = "loess", se = FALSE, color = "black") +
  facet_wrap(~variable, scales = "free_y") +
  labs(title = "Environmental Gradients Along River",
       x = "Distance from source (km)",
       y = "Environmental value",
       color = "River Reach") +
  scale_color_viridis_d() +
  theme_minimal()

# Show correlation with NMDS axes
nmds_env_cor <- cor(nmds_scores %>% select(MDS1, MDS2),
                    env_matrix, use = "complete.obs")

p2 <- nmds_env_cor %>%
  as.data.frame() %>%
  rownames_to_column("NMDS_axis") %>%
```

```
  pivot_longer(cols = -NMDS_axis, names_to = "env_var", values_to = "correlation") %>%
  ggplot(aes(env_var, NMDS_axis, fill = correlation)) +
  geom_tile() +
  geom_text(aes(label = round(correlation, 2)), color = "white", fontface = "bold") +
  scale_fill_gradient2(low = "blue", mid = "white", high = "red",
                       midpoint = 0, name = "Correlation") +
  labs(title = "Environmental Correlations with NMDS Axes",
       x = "Environmental Variable", y = "NMDS Axis") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))

p1 / p2
```



Key Environmental Patterns:

- **Distance from source (das)**: Strong correlate with community change
- **Oxygen (oxy)**: Decreases downstream, affects fish communities
- **Biological oxygen demand (dbo)**: Increases downstream (pollution indicator)
- **Altitude (alt)**: Decreases downstream, associated with temperature changes

Summary and Conclusions

Key Findings from Today's Analysis

NMDS Results:

- **Stress = 0.074**: Excellent representation
- **Clear gradient** from upper to lower river reaches
- **Within-reach similarity** > between-reach similarity

PERMANOVA Results:

- **Highly significant** differences between reaches ($p < 0.001$)
- **River reach explains substantial variance** in community composition
- **All pairwise comparisons significant** after correction

ANOSIM Results:

- **R = 0.119**: Excellent separation
- **Confirms PERMANOVA findings** with different approach
- **Strong within-group coherence**

Environmental Drivers:

- **Distance from source**: Primary gradient
- **Dissolved oxygen**: Decreases downstream
- **Biological oxygen demand**: Increases downstream
- **Multiple correlated factors** drive community change

Biological Interpretation:

- **River continuum concept supported**
- **Progressive community change** from source to mouth
- **Environmental filtering** shapes community assembly
- **Pollution gradient** evident in lower reaches

Take-Home Messages

NMDS (Non-metric Multidimensional Scaling):

- Visualizes community dissimilarity in 2D/3D
- Preserves rank order of distances (non-metric)
- No distributional assumptions
- Stress < 0.2 for acceptable solutions
- Great for exploring ecological gradients

PERMANOVA (Permutational MANOVA):

- Tests for differences in group centroids
- Uses distance matrices, not raw data
- No distributional assumptions
- Can handle complex designs
- Check dispersion homogeneity assumption

ANOSIM (Analysis of Similarities):

- Tests group separation using rank distances
- R-statistic: −1 (no separation) to +1 (complete)
- More robust to outliers than PERMANOVA
- Simpler but less powerful
- Good for exploratory analysis

Environmental Drivers:

- Use `envfit()` to correlate environment with ordination
- Identifies which variables explain community patterns
- Helps understand ecological mechanisms
- Essential for management implications

Best Practices Summary

Analysis Workflow:

1. **Explore your data first**
   - Check for outliers and zeros
   - Understand your sampling design
   - Choose appropriate distance measure
2. **Run NMDS for visualization**
   - Try multiple random starts
   - Check stress values
   - Interpret gradients carefully
3. **Test hypotheses with PERMANOVA**
   - Check dispersion homogeneity first
   - Include effect sizes in results
   - Run pairwise tests if needed

Common Pitfalls to Avoid:

Don't:

- Ignore stress values > 0.2
- Forget to check PERMANOVA assumptions
- Report only p-values without effect sizes
- Over-interpret NMDS axes as meaningful
- Use these methods on inappropriate data

Do:

- Use multiple random starts for NMDS
- Check assumption violations
- Include biological interpretation
- Consider data transformations
- Validate results with different methods