The Utility of Microbial Profiling for Identification of Trace Soil Samples

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Overview

- Background and Significance
  - Types of Evidence
  - Soil characterization & analysis techniques
- Research at Michigan State University
  - Meyers and Foran (2008)
  - Lenz and Foran (2010)
  - Smith and Foran (current)
- Conclusions

Background of Soil Analysis

- Used for over 100 years
  - Georg Popp in 1904
- Can associate person/object with location
- Can help determine possible locations for further investigation

Soil Analysis Techniques

- Physical/Chemical analysis
  - Color, texture, and particle size
  - Minerals, oxides, and elemental composition
  - pH and organic content
  - Animal and plant material (e.g. pollen)
- Advantages/Disadvantages
  - can be very discriminating
  - requires years of experience and training
  - can be time consuming

Soil Analysis Techniques

- Microbial analysis
  - Denaturing gradient gel electrophoresis
  - Amplified fragment length polymorphism
  - Terminal-restriction fragment length polymorphism
  - Real-time polymerase chain reaction (PCR)
- Advantages/Disadvantages
  - can also be very discriminating
  - not as much training required
  - can also be time consuming
  - sensitivity to temporal and spatial variability

Soil Research at MSU

- Specific aims of research
  - Interhabitat variability
  - Intra-habitat variability
    - Temporal Variability
    - Spatial Variability
- Daubert/Frye challenges

Meyers and Foran (2008)

- Soil collected at 5 habitats

Agricultural Field

Sandy Woodlot

Marsh

Woodlot

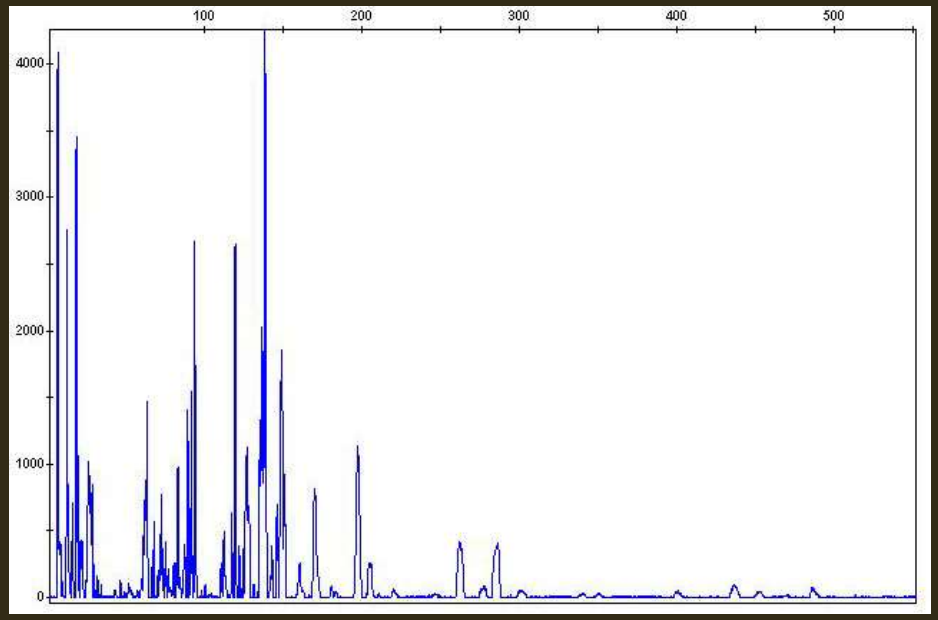
Yard

Meyers and Foran (2008)

- Sample collection
  - Main site every month Sep. 2004 – Aug. 2005
  - Samples10 ft from main site every 3 months
- Stored in -20^{0}C freezer
- Incomplete extraction using UltraClean® Soil DNA Isolation Kit (MoBio)
- Complete extraction using PowerSoil® DNA Isolation Kit (MoBio)

Meyers and Foran (2008)

- T-RFLP analysis
  - *16S rRNA* gene
  - Assayed all bacteria present
  - ANOVA and MANOVA on similarity indices



Meyers and Foran (2008)

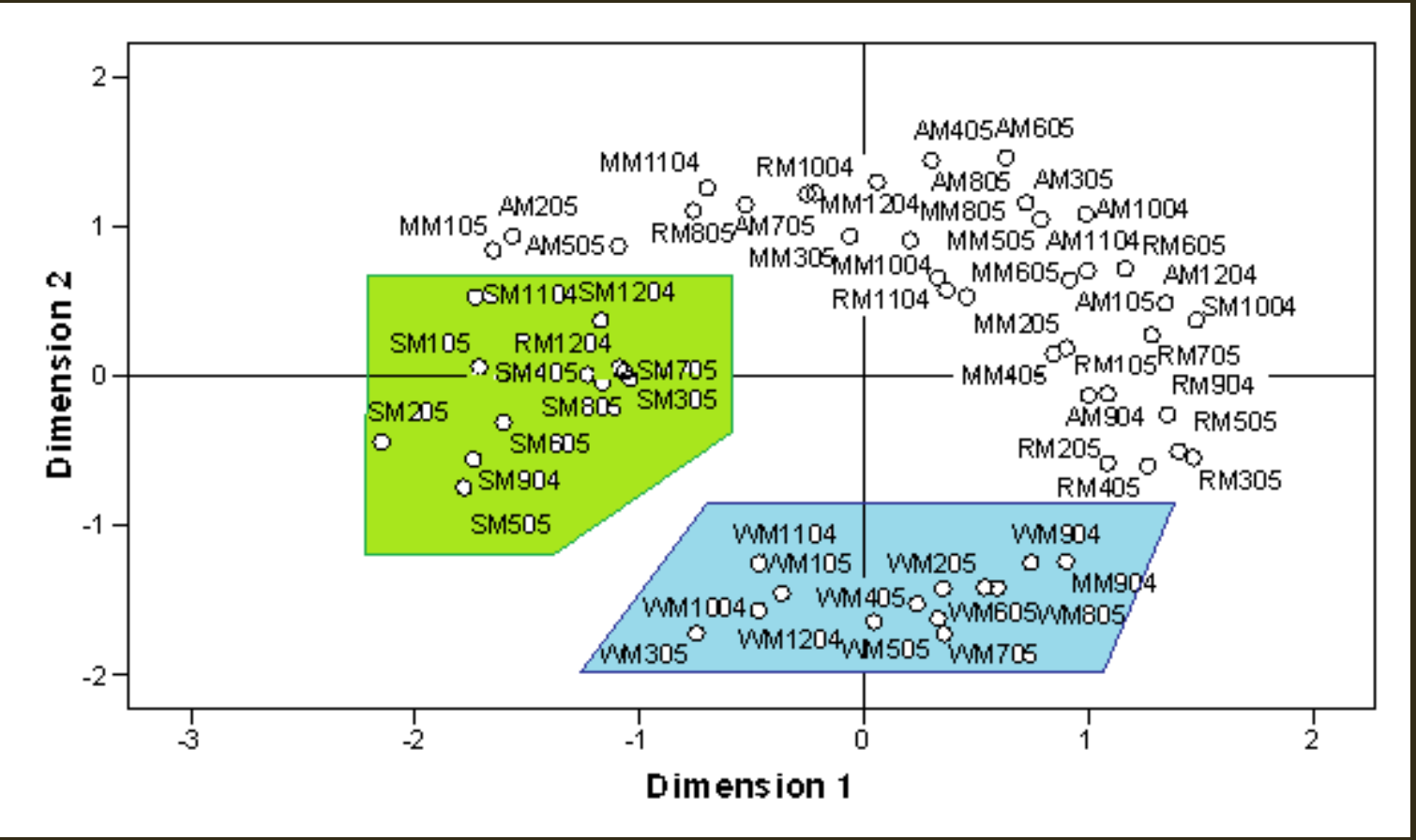
- Results
  - Interhabitat variability
    - Habitats most different in March and most similar in October
    - Similarity indices among habitats differed based on month
  - Intra-habitat temporal variability
    - More pronounced in the spring
    - Agricultural field had significant temporal variability
  - Intra-habitat spatial variability
    - No significant difference based on distance from main collection site

Lenz and Foran (2010)

- Used T-RFLP to analyze DNA extracts from Meyers and Foran (2008)
- Targeted *recA* gene of genus *Rhizobium*
- Non-metric multidimensional scaling (NMDS)

Lenz and Foran (2010)

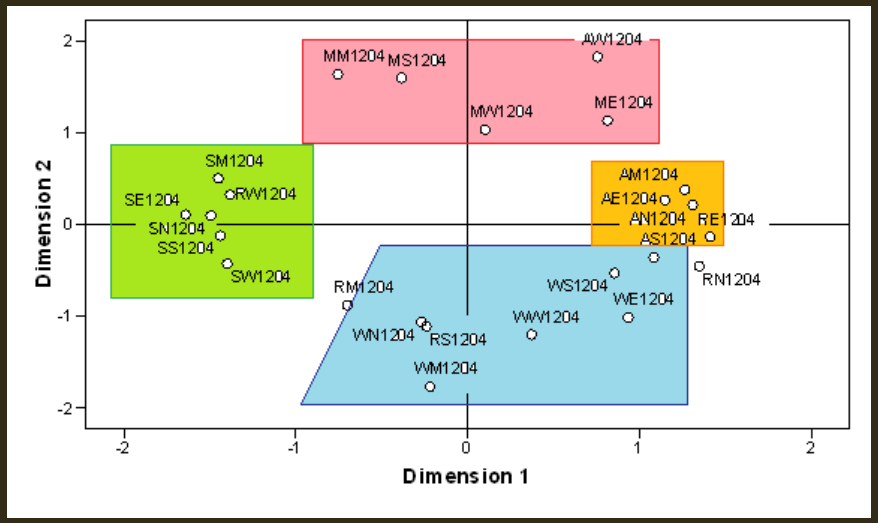
- Inter-habitat and temporal variability



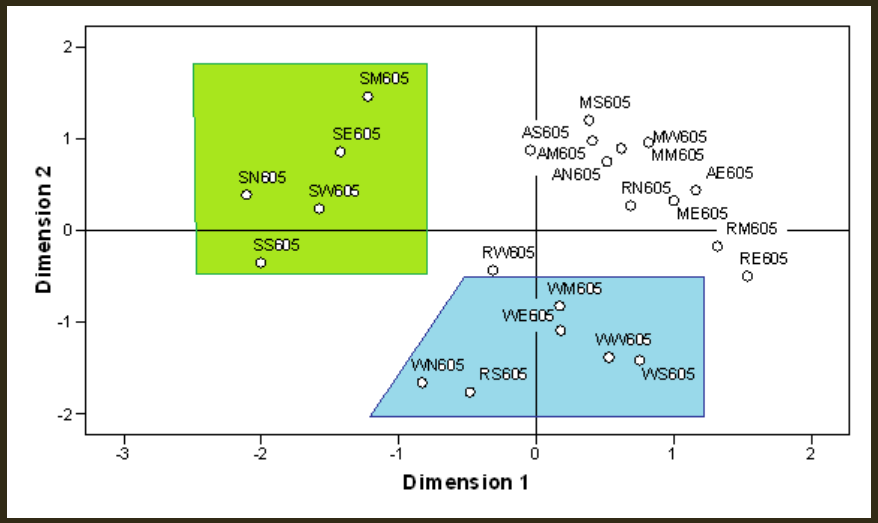
Lenz and Foran (2010)

- Intra-habitat spatial variability

December

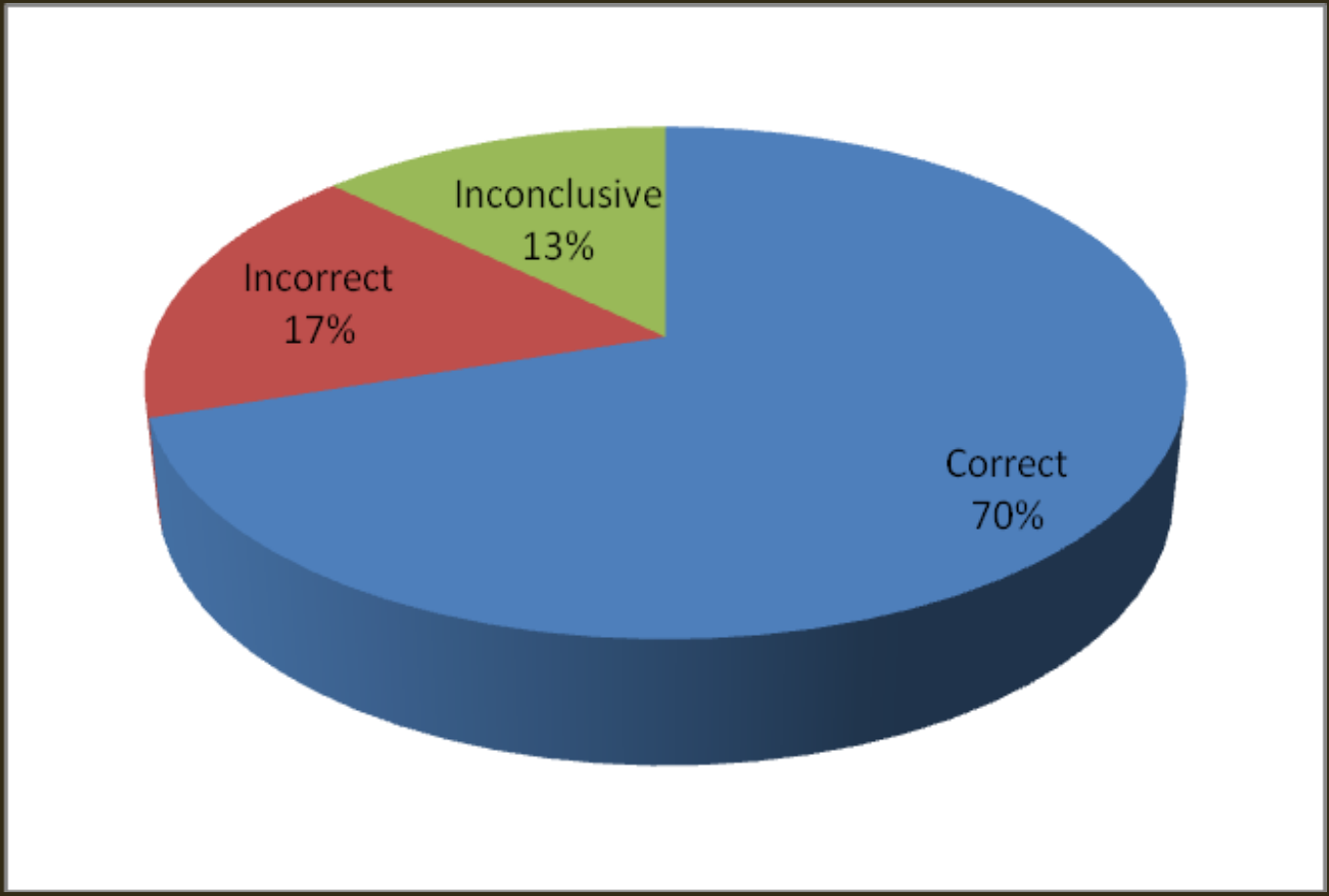


June



Lenz and Foran (2010)

- Pairwise comparisons and “unknowns”



Smith and Foran (current)

- Relative abundance using real-time PCR
  - Different based on soil type
  - Can help differentiate habitats
- Results with statistical confidence
- Real-time PCR used in most crime laboratories

Smith and Foran (current)

- Soil collected at 4 habitats

Agricultural Field

Marsh

Yard

Woodlot

Smith and Foran (current)

- Sample collection
  - Main site over a course of days, weeks, months
  - 10 inch core sample for depth study
- Stored in -20^{0}C freezer
- Extracted DNA using PowerSoil® DNA Isolation Kit (MoBio)

Smith and Foran (current)

- Screened for presence of bacterial groups targeting *16S rRNA* gene
  - Primers designed with *ARB* software
  - Tested against control DNA
- Real-time PCR with Bio-Rad iQ™5 thermocycler

Bacteria Amplified

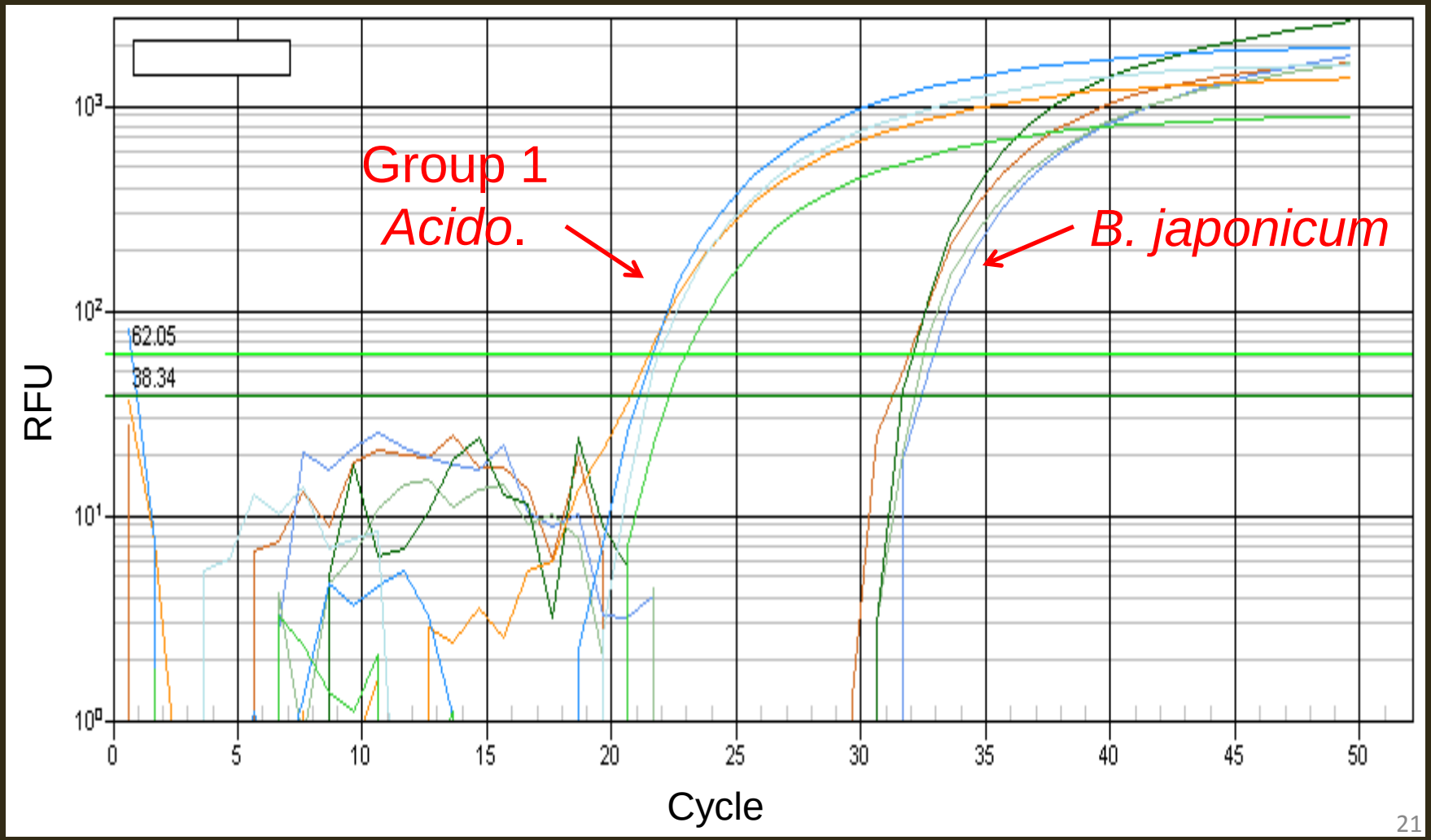
- *Bradyrhizobium japonicum*
  - Symbiote with legumes
- Group 1 *Acidobacteria*
  - Abundant in many types of soil and vary with pH
- Genus *Burkholderia*
  - Commonly found in ground water and soil
  - Very complex taxonomy
- Genus *Agrobacterium*
  - Within the same family as *Rhizobium*

Bacteria Amplified

	Habitats			
Bacteria	Ag Field	Marsh	Woodlot	Yard
B. japonicum	+	+	+	+
Acidobacteria Group 1	+	+	+	+
Genus *Burkholderia*	+	+	+	+
Genus *Agrobacterium*	+	+	-	+

Real-time PCR profile

Marsh



Statistics

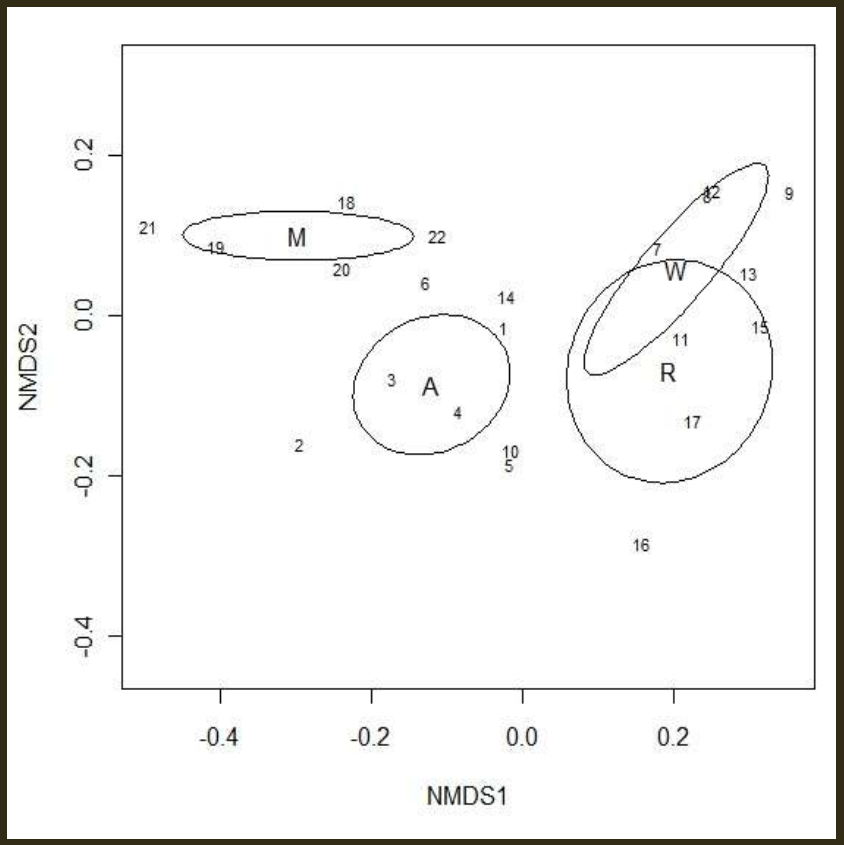
- ADONIS
  - Multivariate ANOVA based on dissimilarity
  - p-value based on permutation tests
- NMDS
  - 95% confidence ellipses

Interhabitat Variability

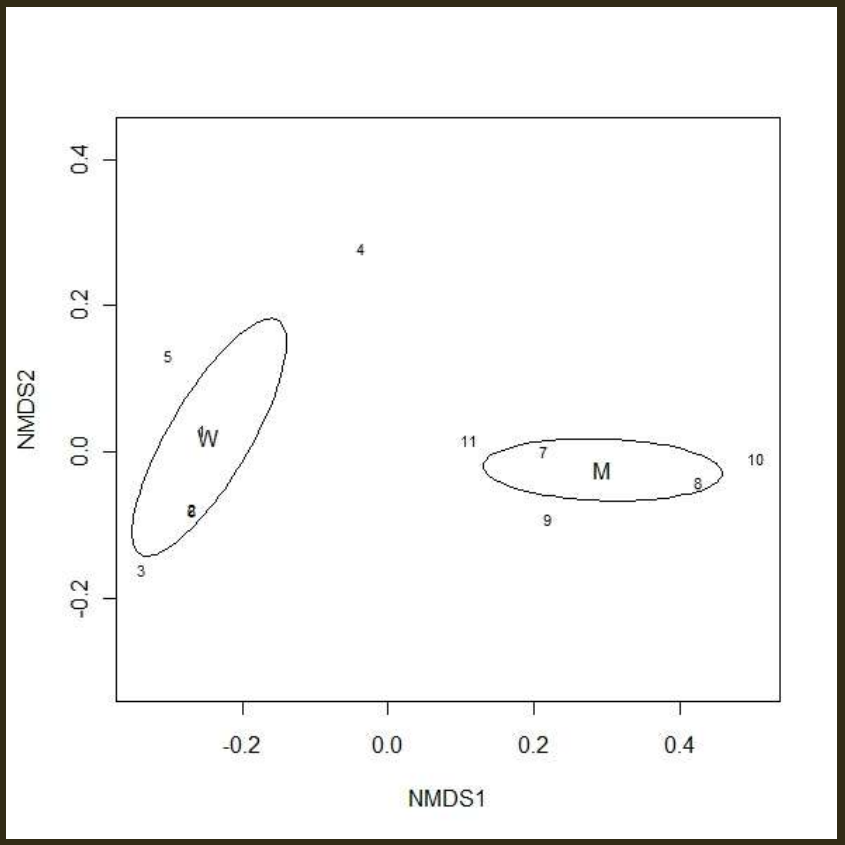
- ADONIS
  - Habitat as differentiating parameter
  - $p < 0.05$

NMDS: Interhabitat Variability

All Habitats



Woodlot vs. Marsh

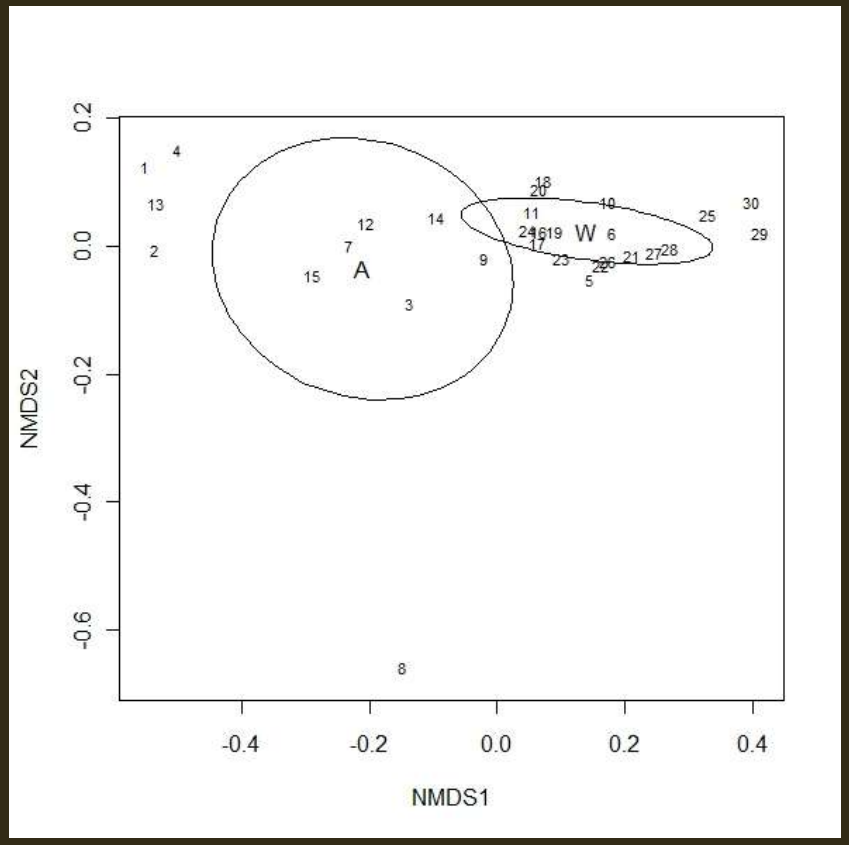


Intra-habitat Spatial Variability

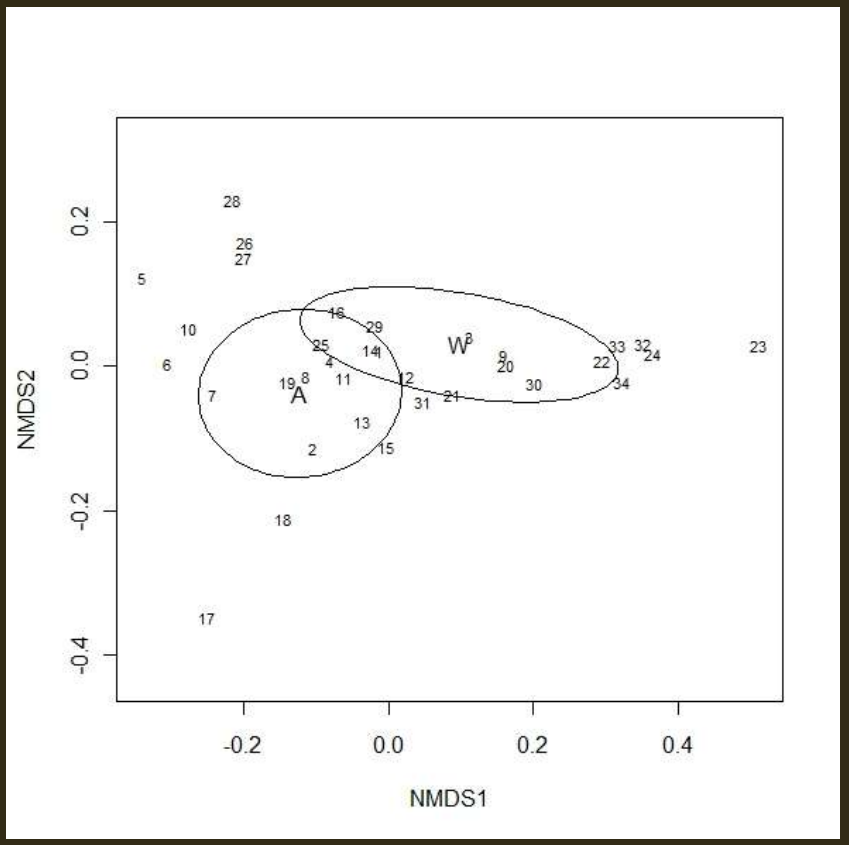
- ADONIS
  - Depth as differentiating parameter
  - No significant p-values for Ag Field or Woodlot

NMDS: Spatial Variability

September



March

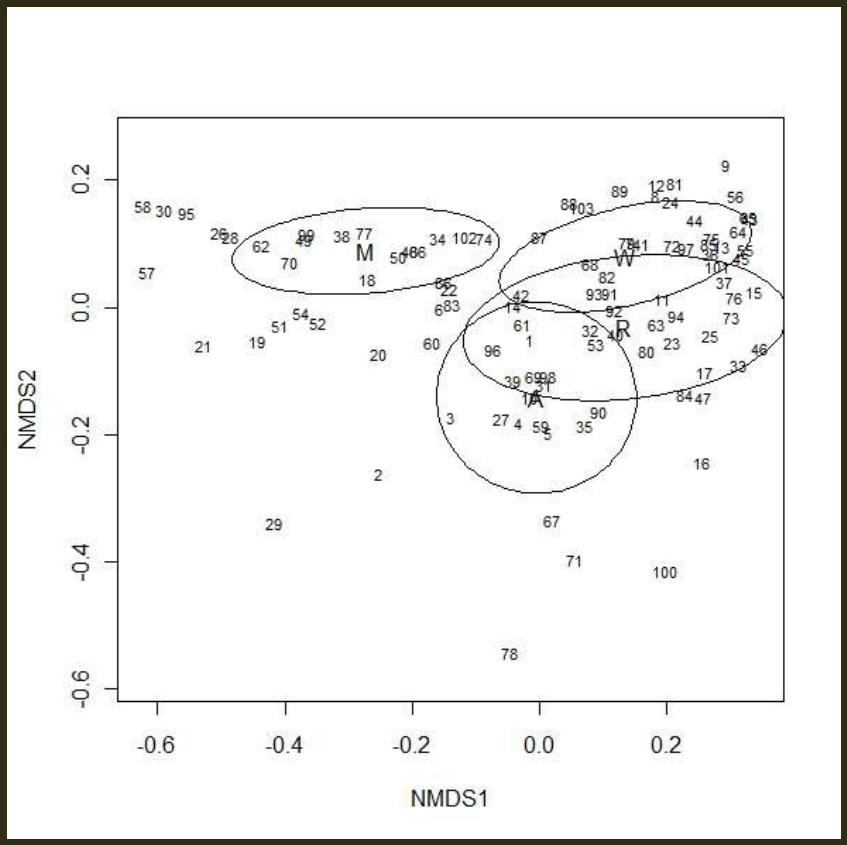


Intra-habitat Temporal Variability

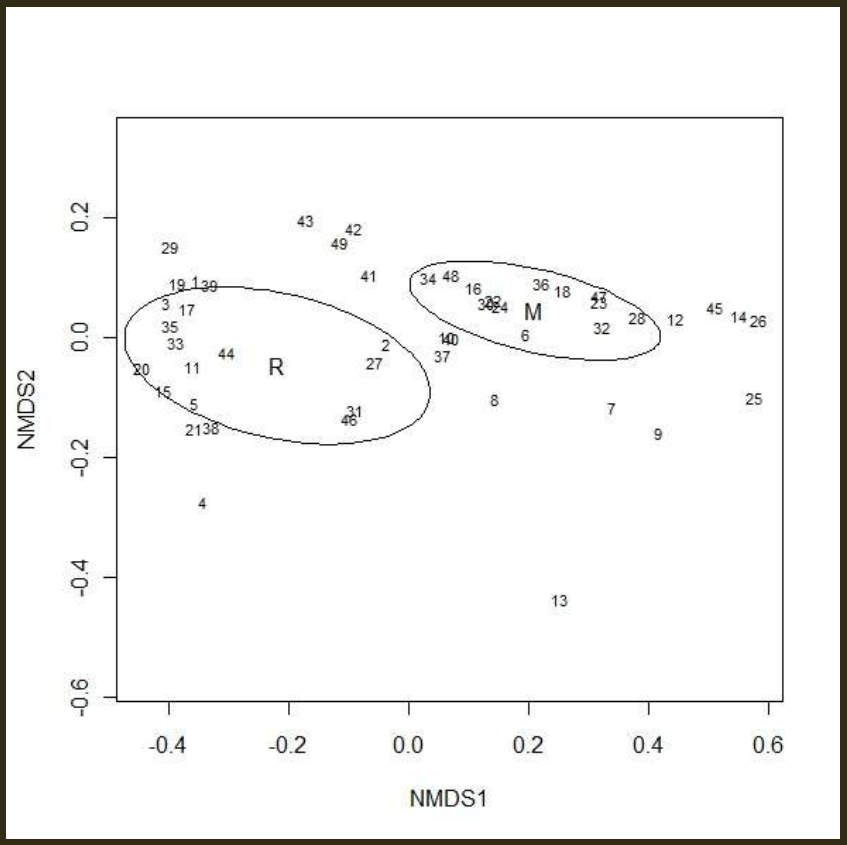
- ADONIS
  - Ag Field: $p < 0.05$
    - Could reflect rotation crop
  - Marsh: $p < 0.05$
    - Wet environment sensitive to weather

NMDS: Temporal Variability

All Habitats



Yard vs. Marsh



Smith and Foran (current)

- Complete separation in pairwise comparison

Interhabitat	Spatial	Temporal
83%	50%	66%

Conclusions of Soil Research

- Meyers and Foran (2008)
  - samples from same habitat were more similar
- Lenz and Foran (2010)
  - visual representation using NMDS
  - pairwise comparisons
- Smith and Foran (present)
  - statistical significance with ADONIS and confidence ellipses
  - pairwise comparisons

Acknowledgements

Contibuting Authors/Researchers

Melissa Meyers

Erin Grammer (Lenz)

Dr. David Foran

Forensic Biology Lab at MSU

Stephen Gicale

Cheryl Schaeper

Jade McDaniel

Beth Shattuck

Shannon Peters

Alicya Orlando

Keiauda Tennant

Sarah Thomasma

Amanda Buszek

NIJ/FSF

Contributors to each project

Family and friends for their support

This project was supported by Award No. 2008-DN-BX-K216 from the FSF awarded by the National Institute of Justice Programs, U.S. Department of Justice.

The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.