Use and Evaluation of New Genomic Techniques To Understand Microbes In Waterways

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BioTechnology Institute

Fecal Pollution

- Indicator organisms imply fecal pollution
- The fecal coliform bacteria, enterococci, and *E. coli* are primary indicators of fecal contamination
- Conventional microbiological methods cannot determine sources of fecal pollutants

Progress

- Problems Associated with Traditional Coliform Testing
- We now have a much better idea of what is there and where it is coming from but…

The problem of throughput!

Large Genetic Diversity in Indicator Bacteria require analysis of a large number of samples!

Overall Hypotheses

- Fecal indicator bacteria are most likely coming from human sources.

and

- Fecal indicator bacteria signal the possible presence of human pathogens.
- Human fecal bacteria are more harmful than those from other animals.
- But we have not really tested this directly (no undergrads or prisoners).

Can Metagenomics (more specifically high throughput 16S-based taxon analyses) save the day?

What is Metagenomics?

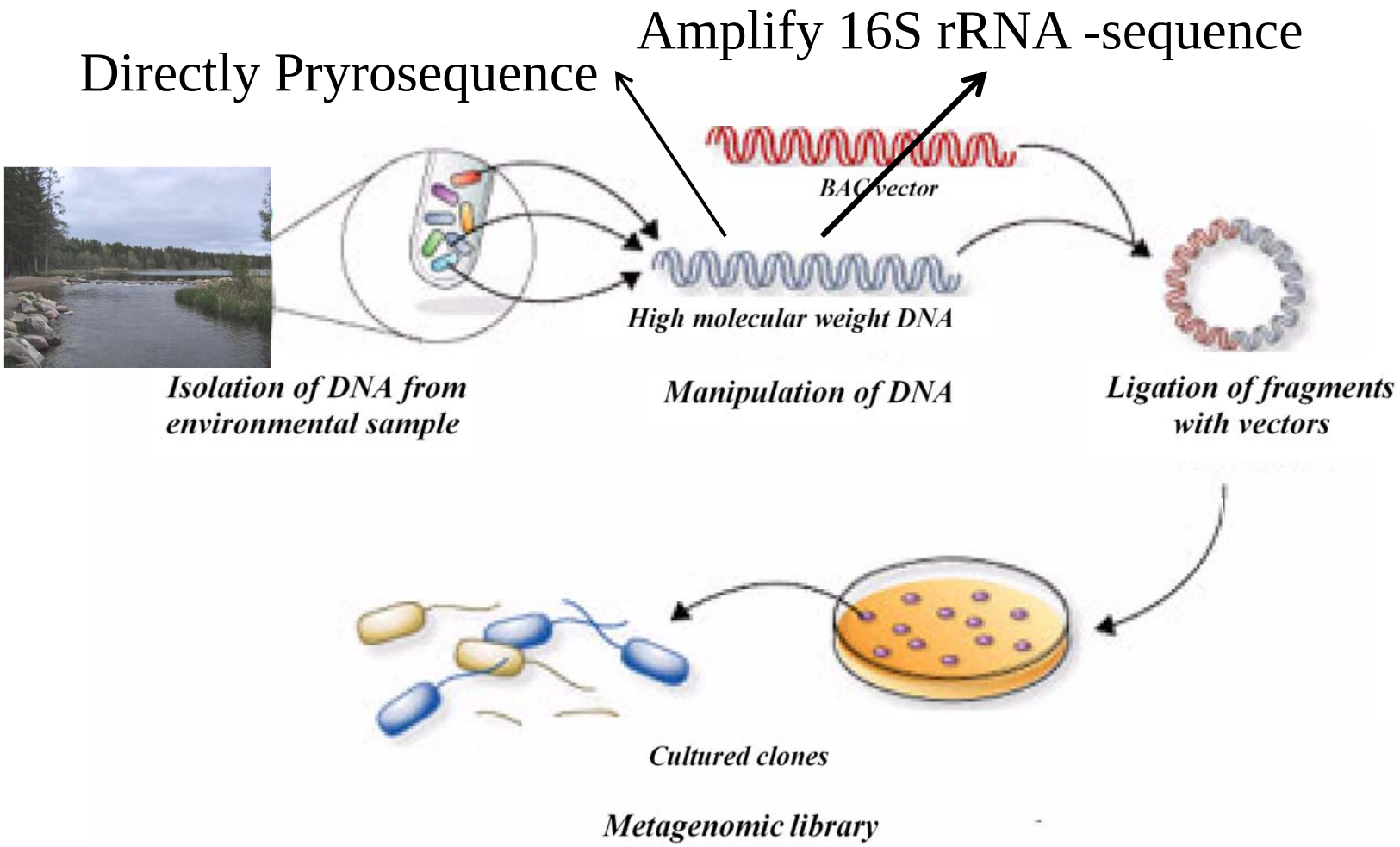
- The study of the totality of genetic material (genomes or their fragments) recovered directly from environmental samples.

- Many types of Metagenomic Analyses
  a. Diversity (16S rDNA)
  b. Microbial Community Analyses
  c. Functional Gene Discovery Analyses

Why use Metagenomic Analyses

- The majority of microorganisms in environmental and animal samples (estimated to be less than 1%) remain uncultured or non-culturable.
- *E. coli* and enteroocci grow in the environment.
- *E. coli* does not show up in most metagenomic analyses it is only 1 of a 1000 species of gut microbes.

General Approaches



DNA Sequencing

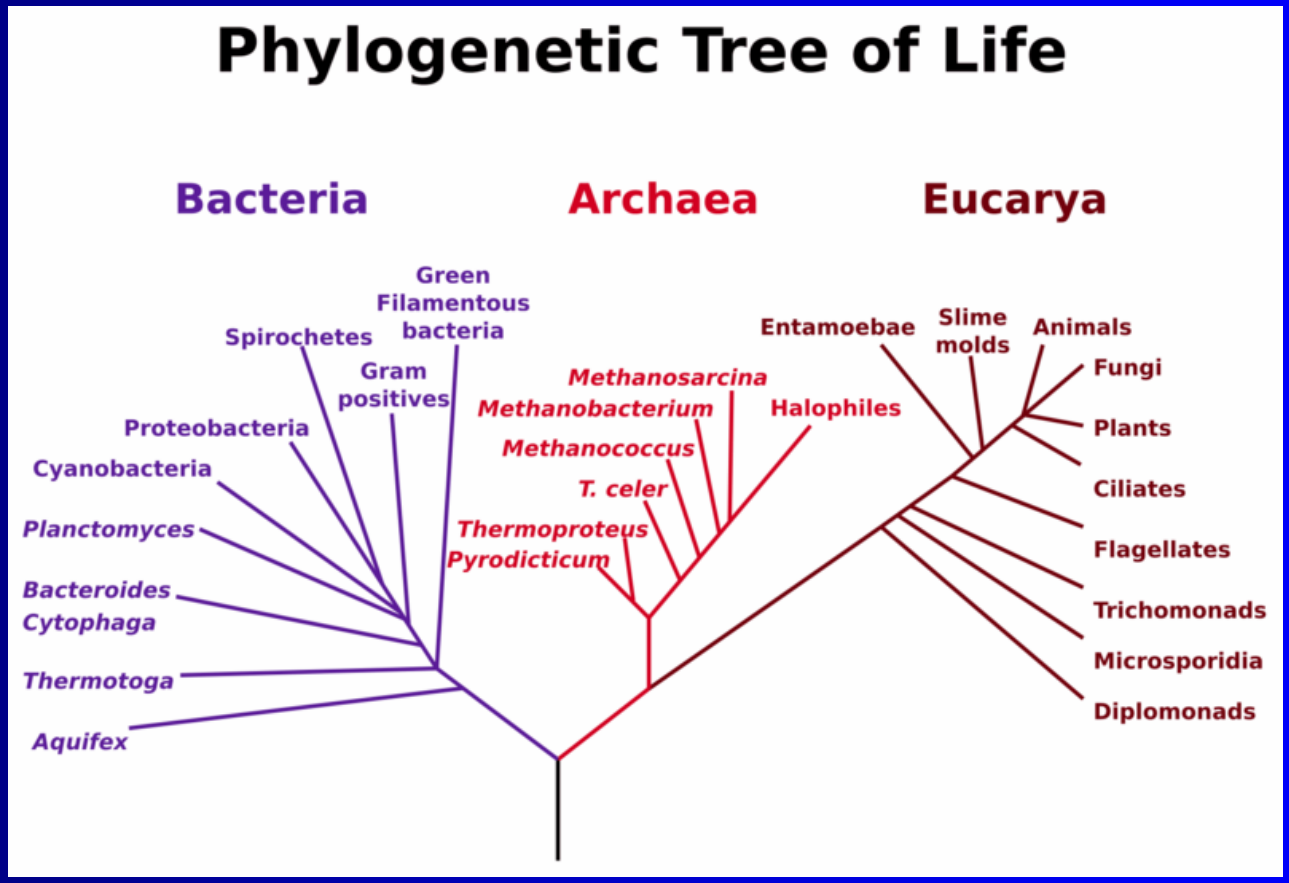
- High-Throughput, Large Scale, and Inexpensive DNA Sequencing Technology

Illumina Sequencing Platforms

- HiSeq
  - 2 x 250 bp read length; 170-200 million output
  - ~8-10 days ~ 80-120 samples
- MiSeq
  - 2 x 250bp read length; 4.5-7.1 million total output - ~24 hours
  -

Produces 100s of millions of DNA sequences

Microbes and The Tree of Life



Our studies are only targeting 1 domain of life – the bacteria

Minnesota Mississippi Metagenome Project

M3P

Lock and Dam Number 7, Wikimedia.org

The Itasca Headwaters are relatively pristine – but….

.. as is passes through the U.S. heartland, anthropogenic activity alters is structure and function.

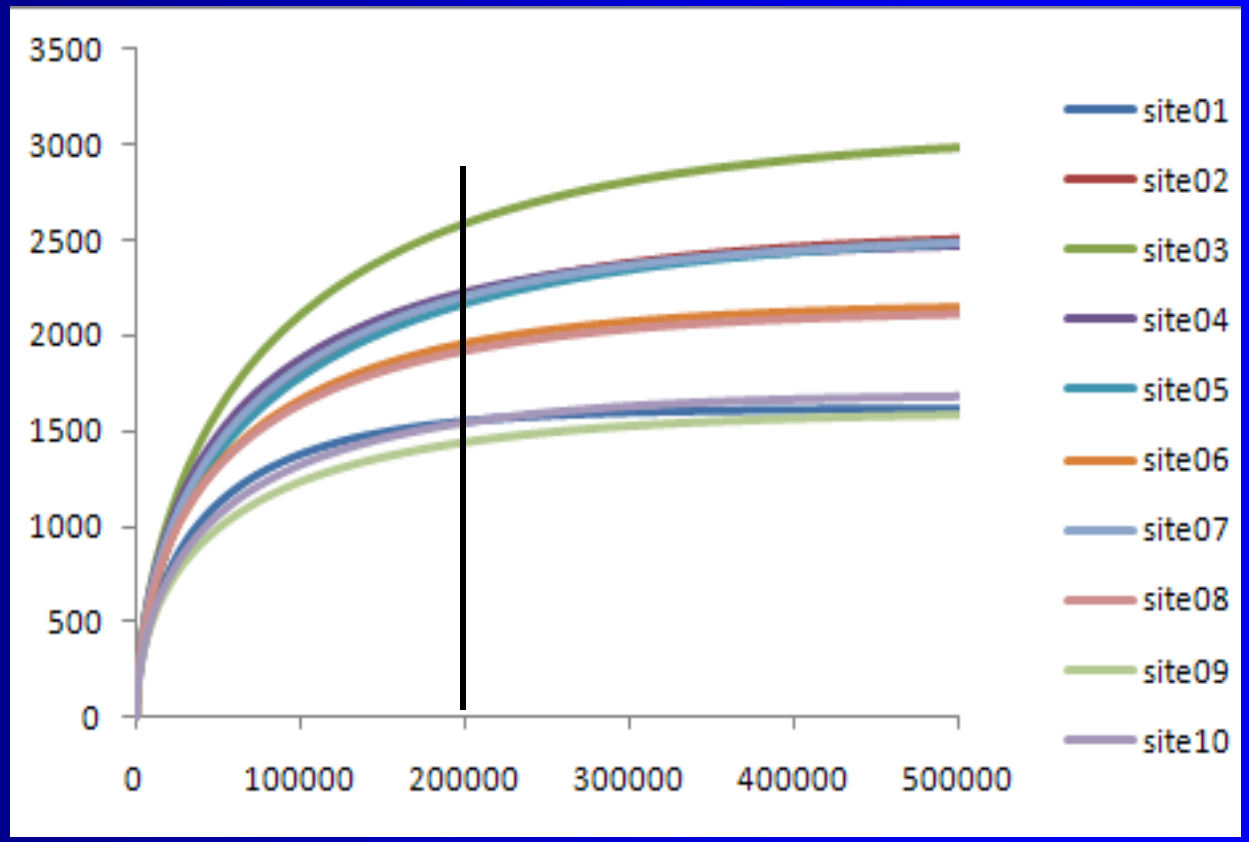
Confluence of other U.S. Rivers lead to further inputs into the Mississippi

Alterations in the Gulf of Mexico and Delta

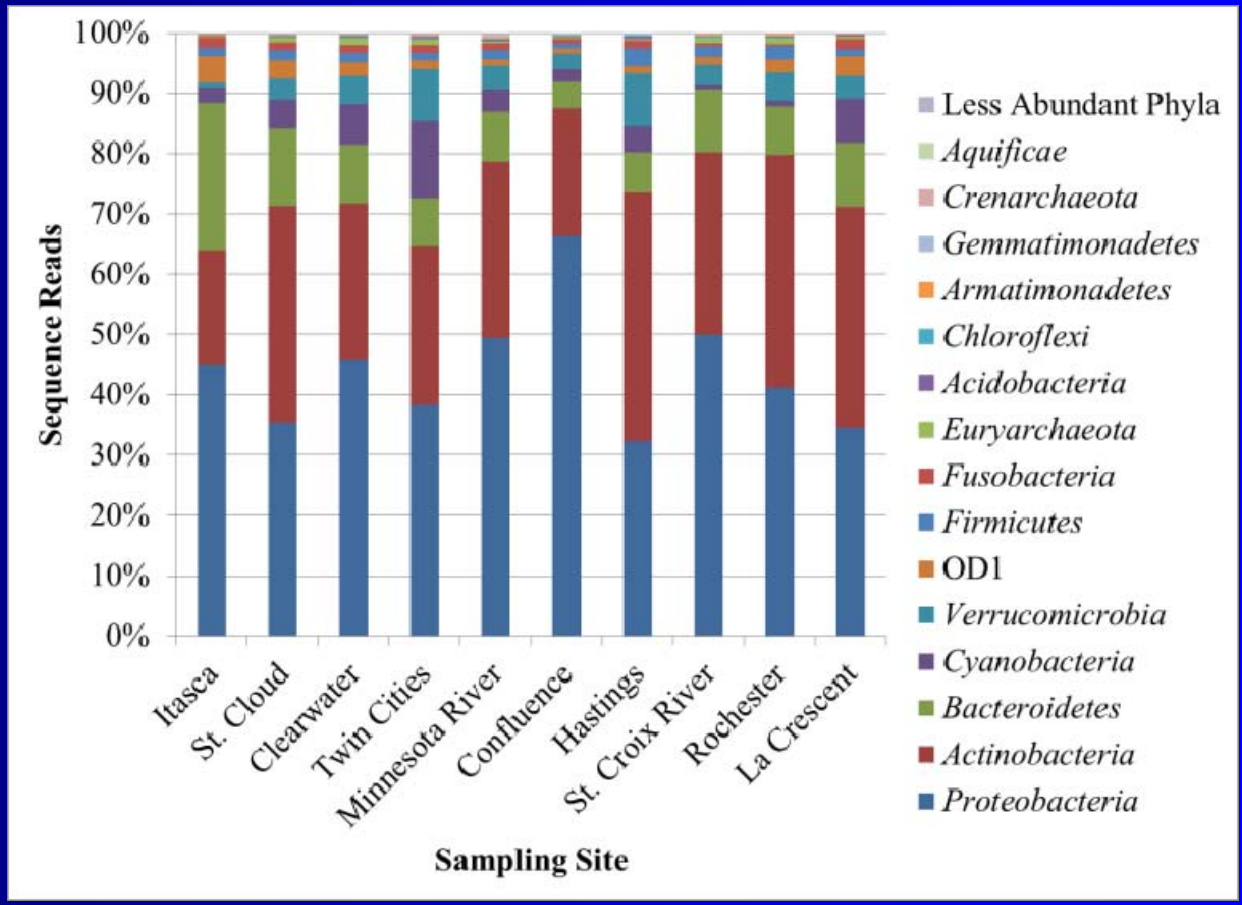
Summary of diversity indices among different sample volumes. S_{obs} is the number of OTUs/

Volume Filtered (L)	S_{obs}	Shannon	NP_Shannon	Simpson
0.33	9218	4.9	4.9	0.03
1.00	8990	4.8	4.8	0.04
2.00	8490	4.7	4.7	0.05
6.00	8432	4.6	4.6	0.05
Mean	8783	4.7	4.8	0.04
Standard Deviation	383	0.1	0.1	0.01

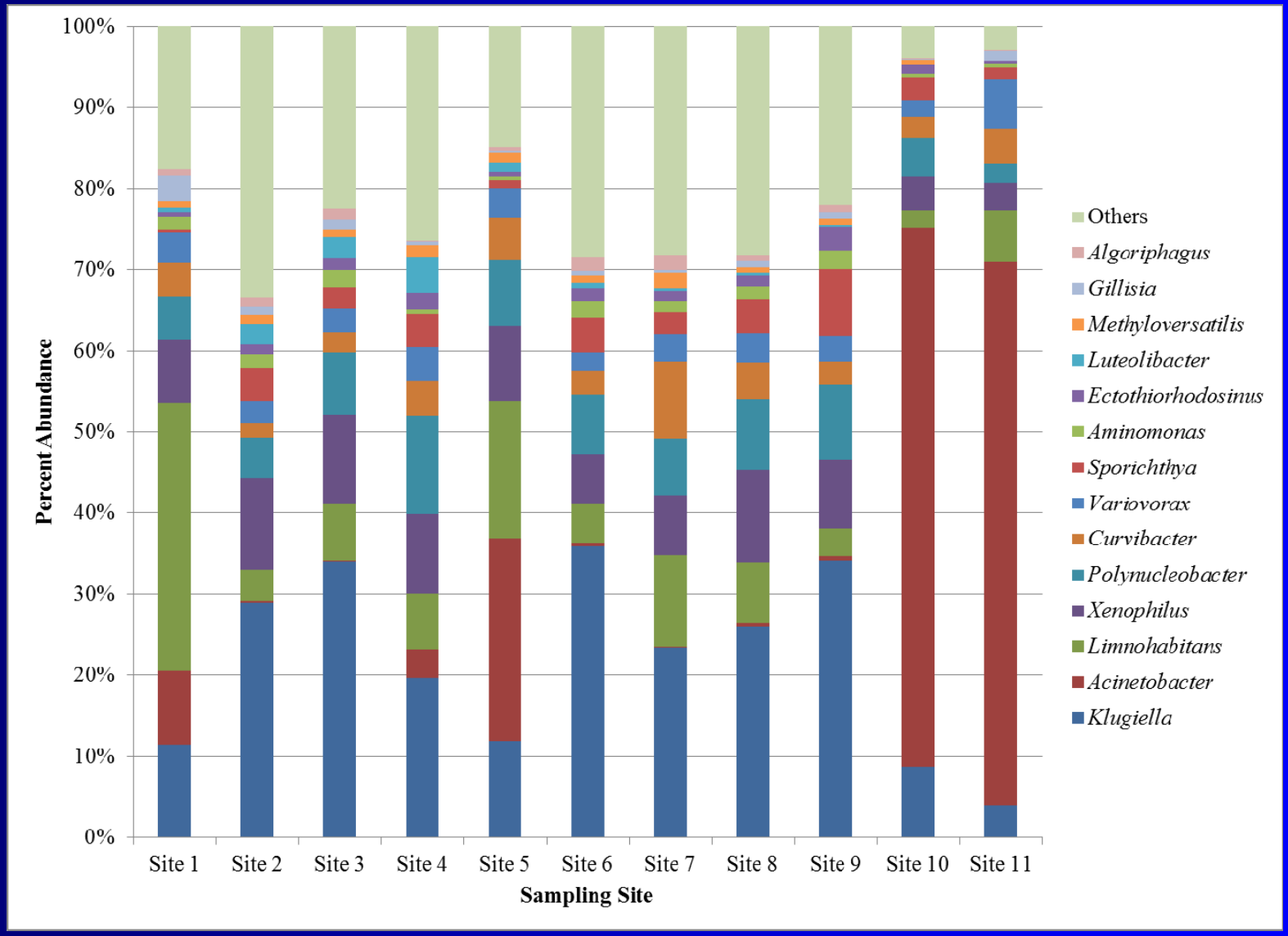
Rarefaction curves



Most Abundant Phyla

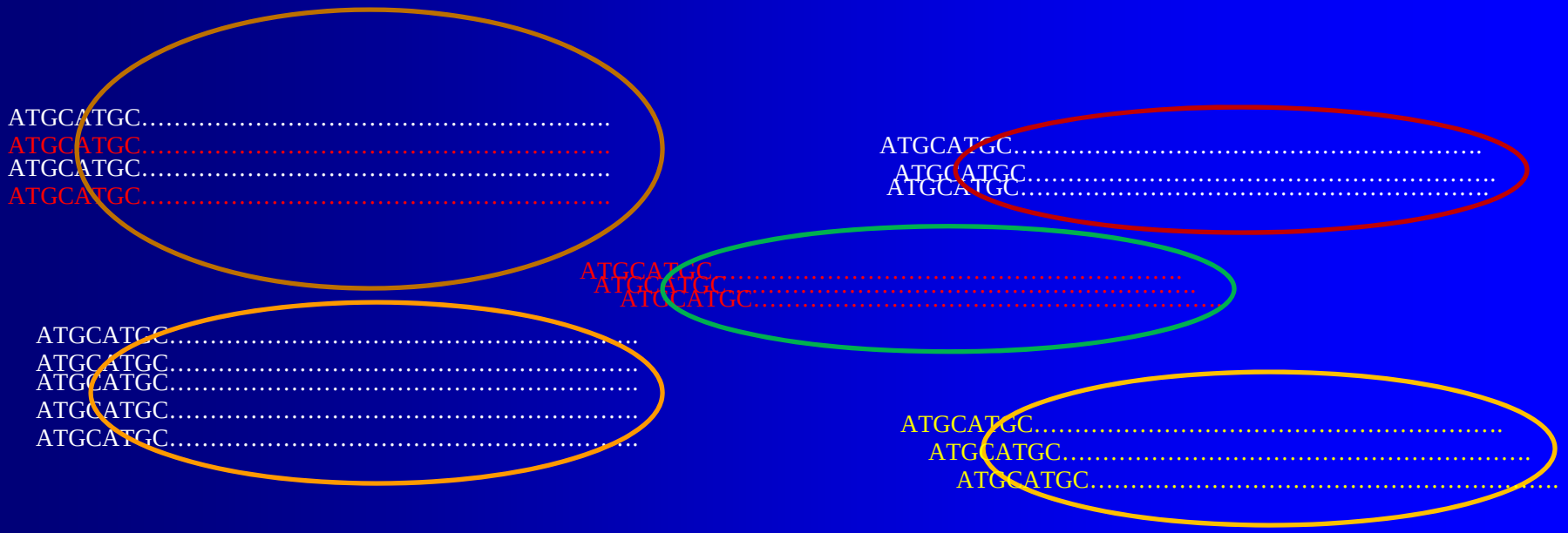


Genera of Classified Bacteria Recovered at Each Site

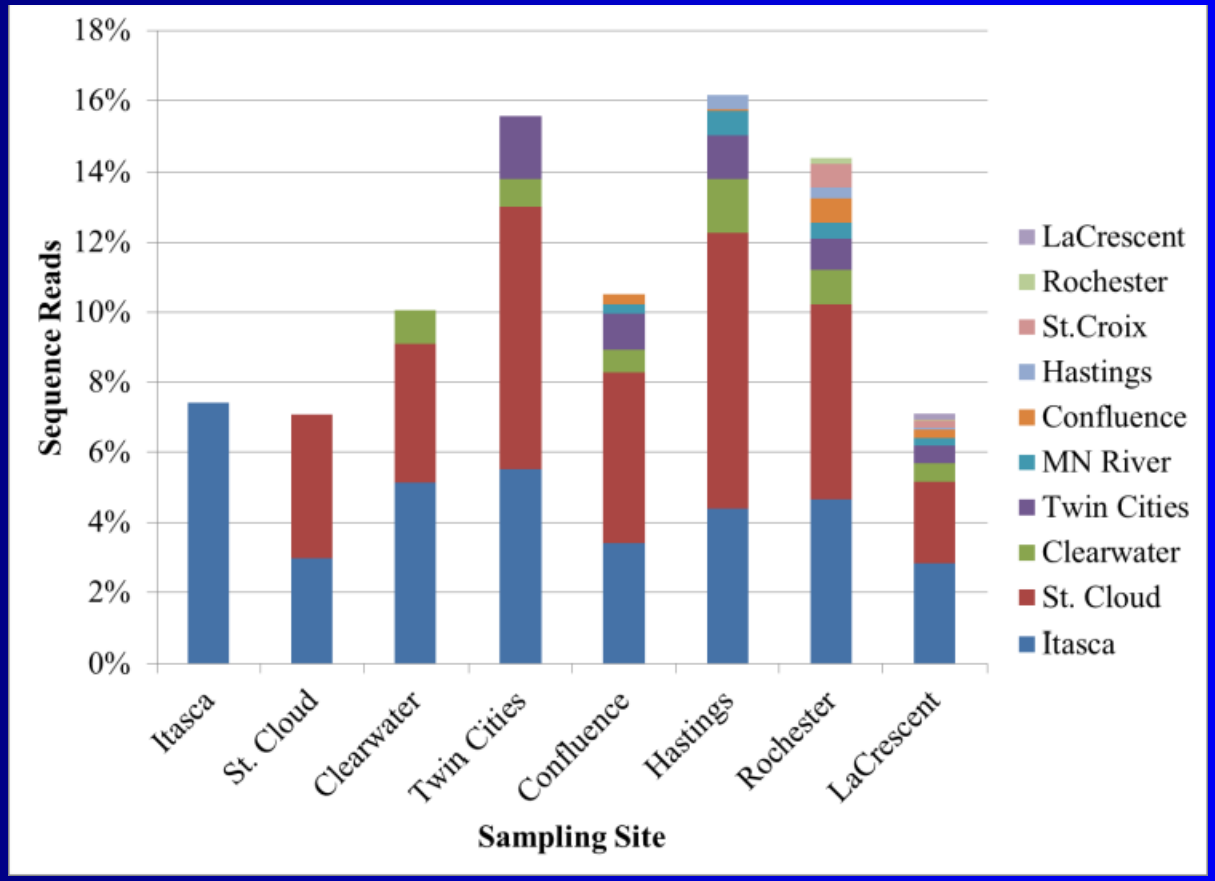


What are OTUs?

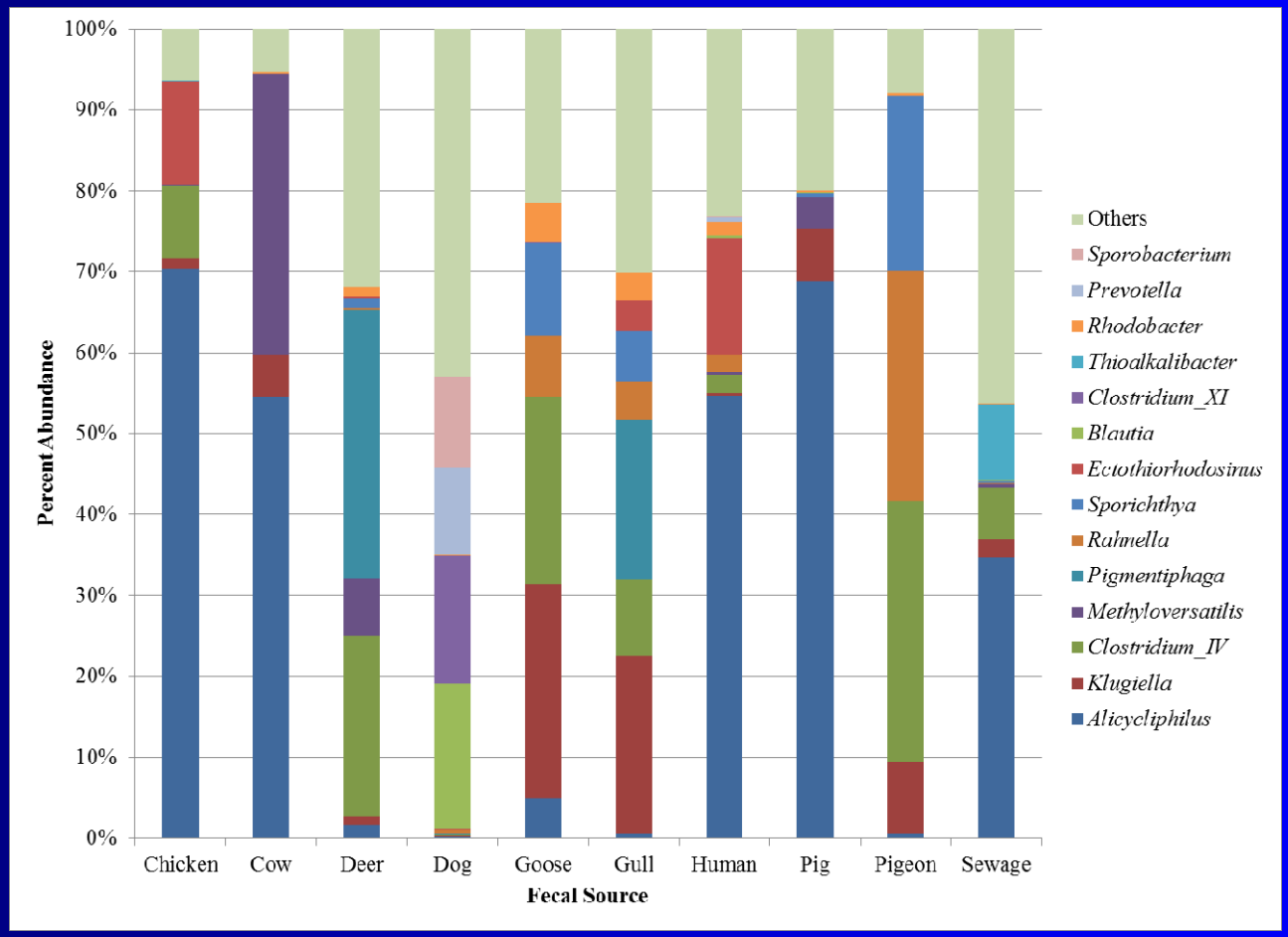
Operational taxonimic units – sequence data Independent of Taxonomy



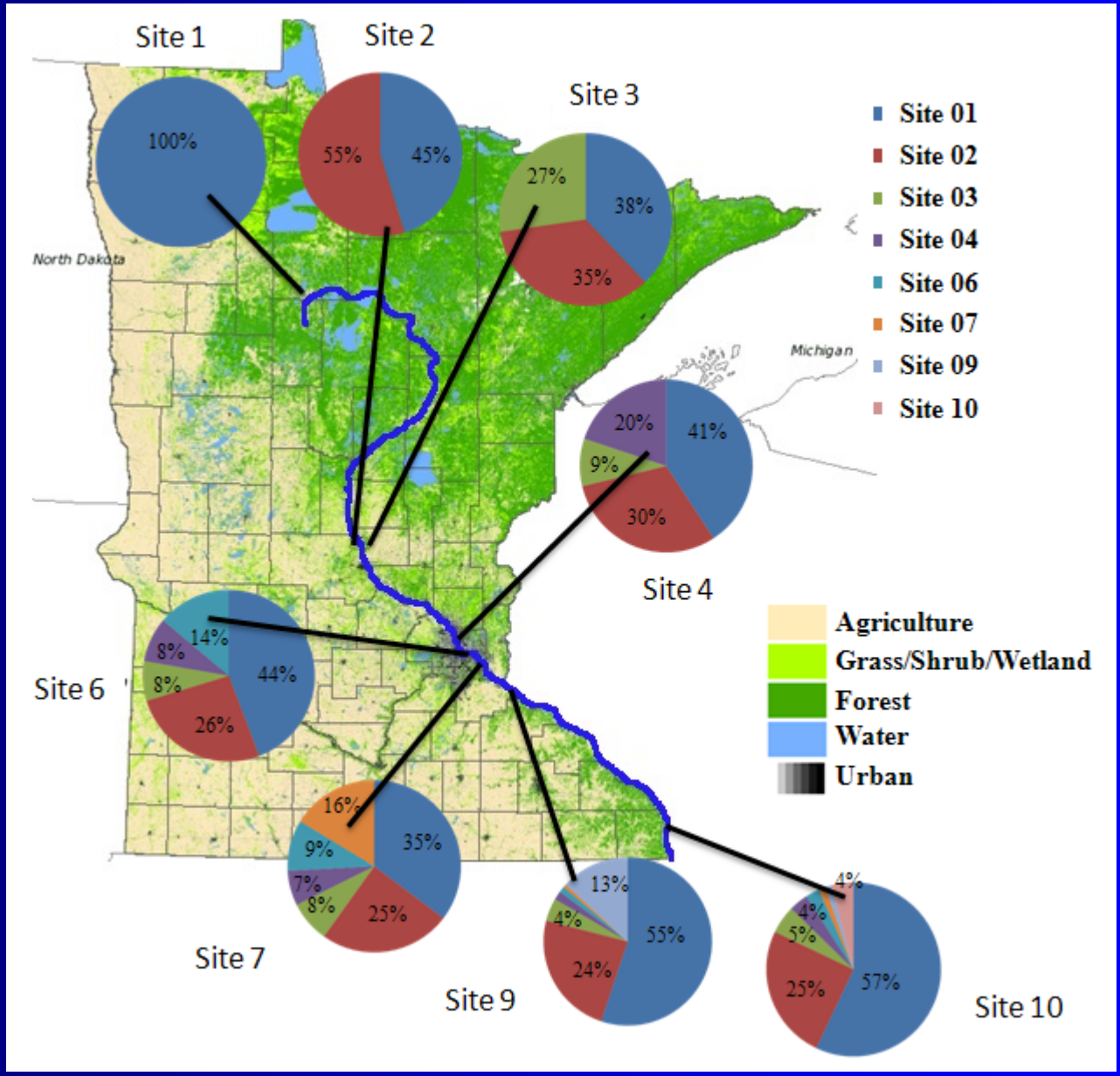
Unique OTUs


18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
Sequence Reads
Itasca
St. Cloud
Clearwater
Twin Cities
Confluence
Hastings
Rochester
LaCrescent
Sampling Site
LaCrescent
Rochester
St.Croix
Hastings
Confluence
MN River
Twin Cities
Clearwater
St. Cloud
Itasca

Genera of Classified Bacteria Identified in Source Fecal Samples



CS2


Site 1
Site 2
Site 3
Site 4
Site 6
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Site 10
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55%
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38%
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41%
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14%
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26%
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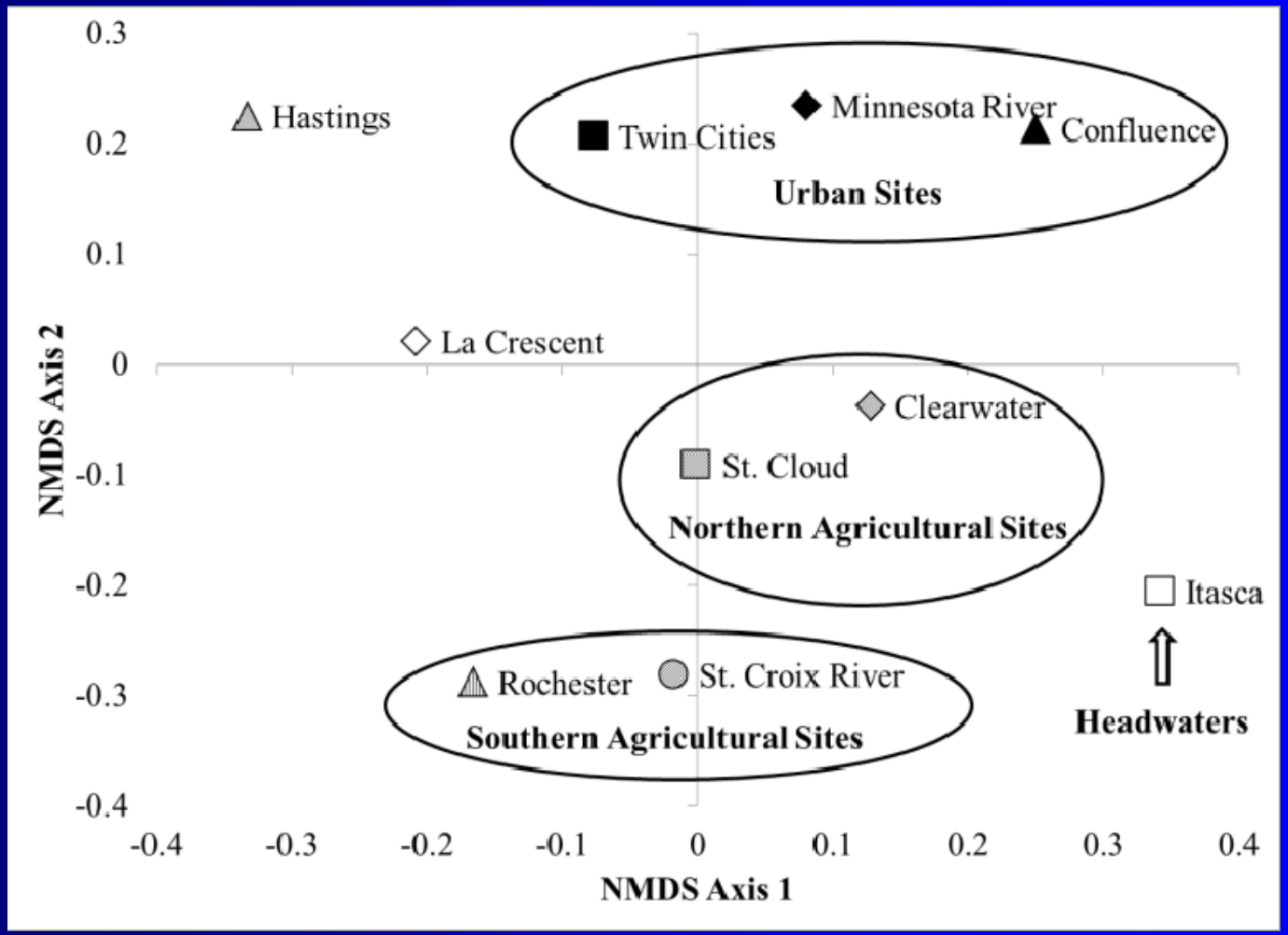
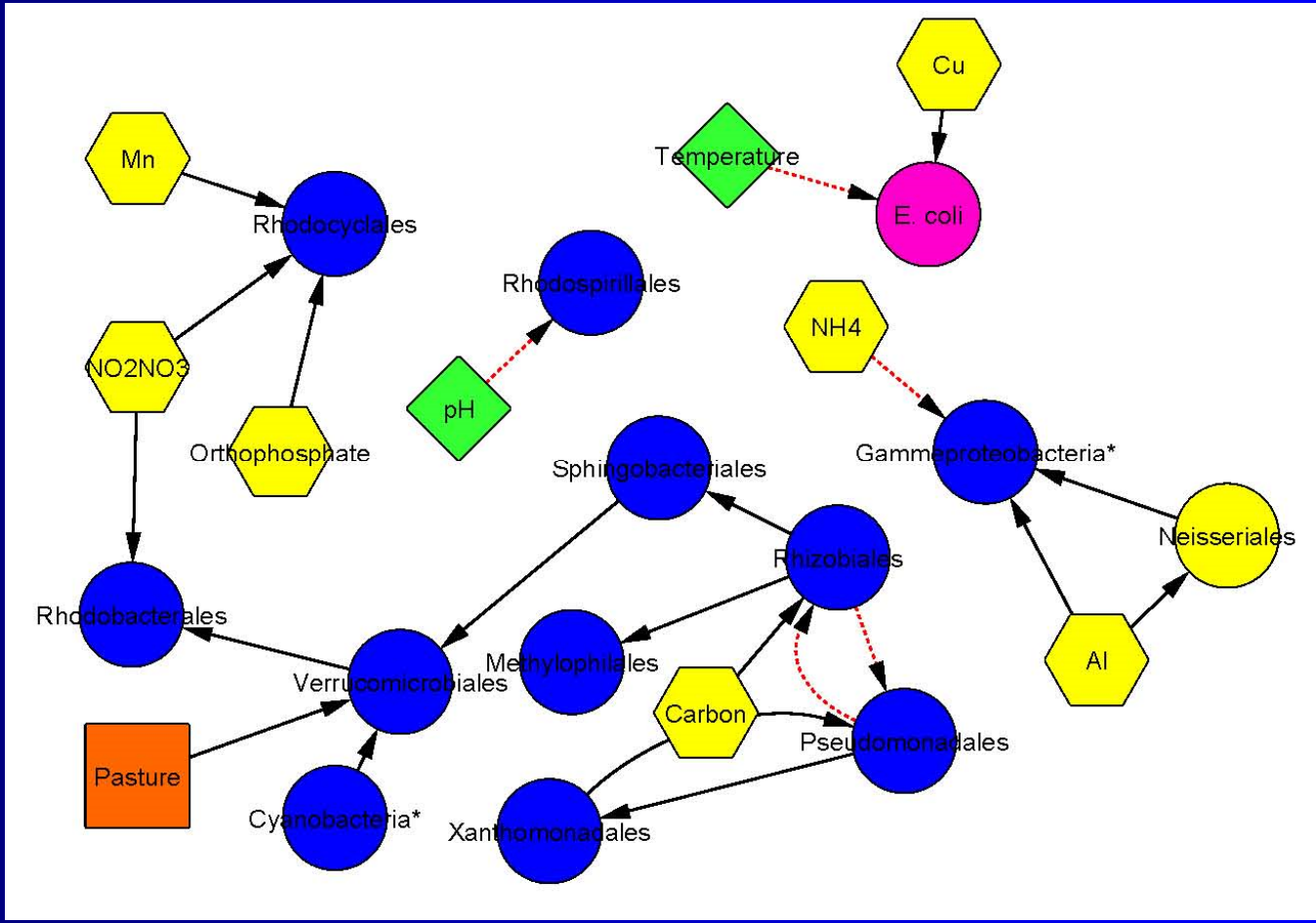
13%
4%
55%
24%
4%
4%
5%
25%
57%
North Dakota
Michigan
Site 01
Site 02
Site 03
Site 04
Site 06
Site 07
Site 09
Site 10
Agriculture
Grass/Shrub/Wetland
Forest
Water
Urban

Slide 26

CS2 Not sure this fits here. Maybe after the mothur pipeline.
ChristopherStaley, 10/12/2012

NMDS of Community Composition by Site





Staley, et al. Figure 6. Consensus inferred Bayesian network relating bacterial orders (blue circles), physicochemical parameters (green diamonds), chemicals (nutrients and ions; yellow hexagons), *E. coli* (pink circle), and land use (orange squares). Solid black lines indicate positive associations while dashed red lines indicate negative associations and arrows are directed from parent to child. Edge length is arbitrary. Asterisks (*) designate orders that are unclassified or designated *incertae sedis*.

Understanding Metagenomes is of Primary Importance

Next Generation Fecal Taxon Libraries - FTL

- Contains all the taxonomic units and OTUs in pooled fecal samples from known animal sources.
- Gives information about fecal and environmental bacteria in samples.
- Can be used for Microbial Source Tracking

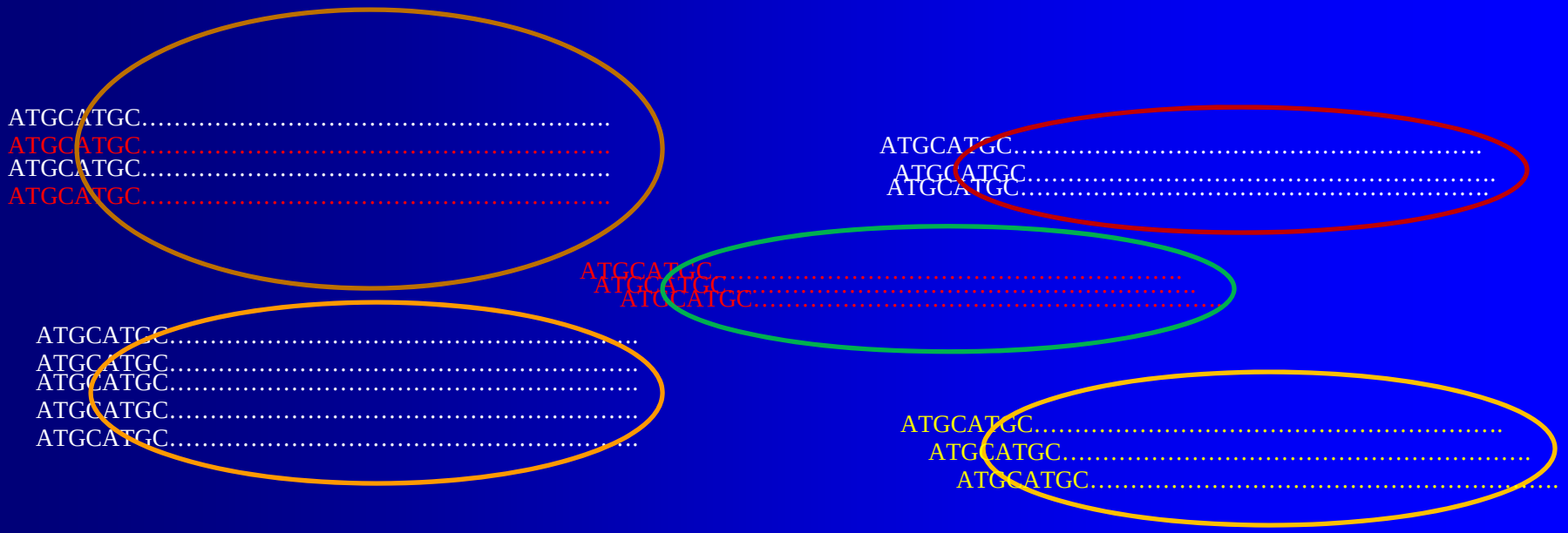
Overall Goals

Match DNA Sequences in Data Sets created from feces of known animals to those recovered in rivers samples.

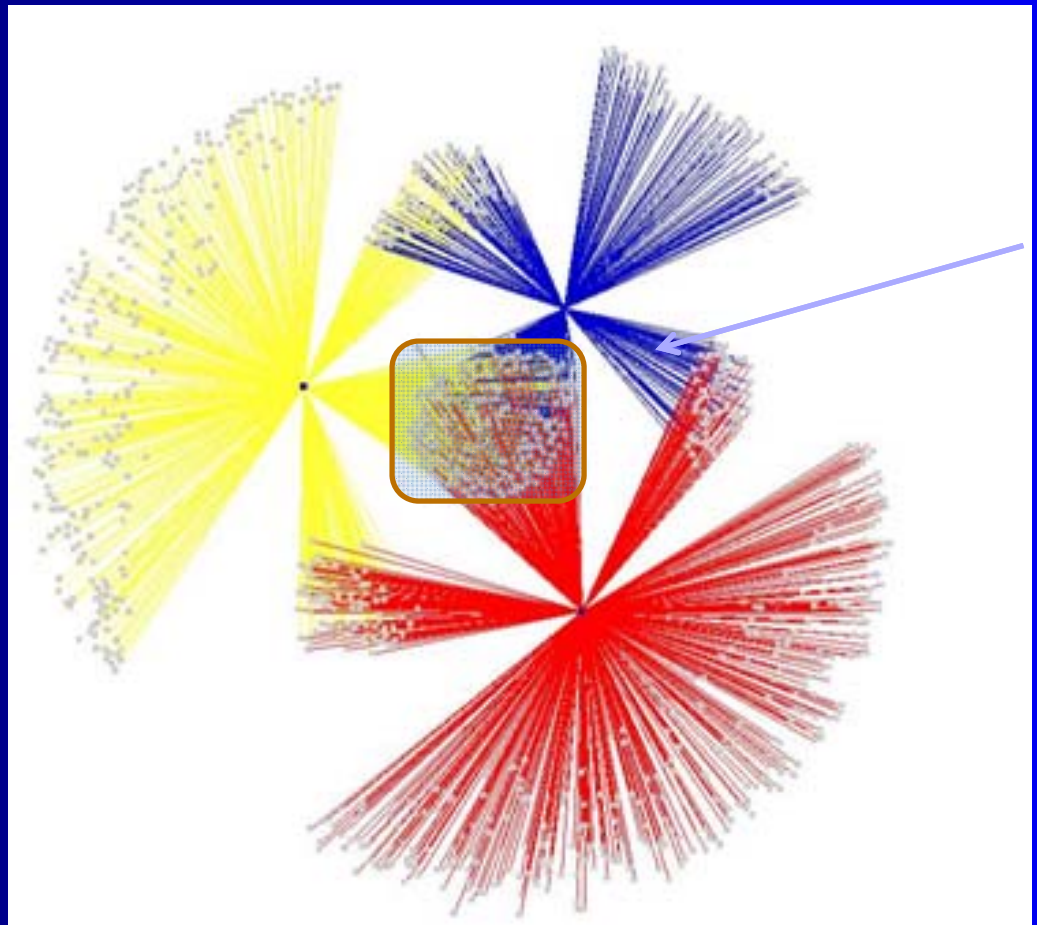
1. Shared OTUs – Taxonomy Independent
2. Shared Taxonomic Units - Genera

What are Shared OTUs?

OTUs from fecal and environmental DNA samples
Independent of Taxonomy



Network image of shared OTUs



OTU based network analysis of
sample A, sample B, AND sample C

New MST method with Next Generation Sequencing technique

Environ. Sci. Technol. **2010**, *44*, 7777–7782

Use of Barcoded Pyrosequencing and Shared OTUs To Determine Sources of Fecal Bacteria in Watersheds

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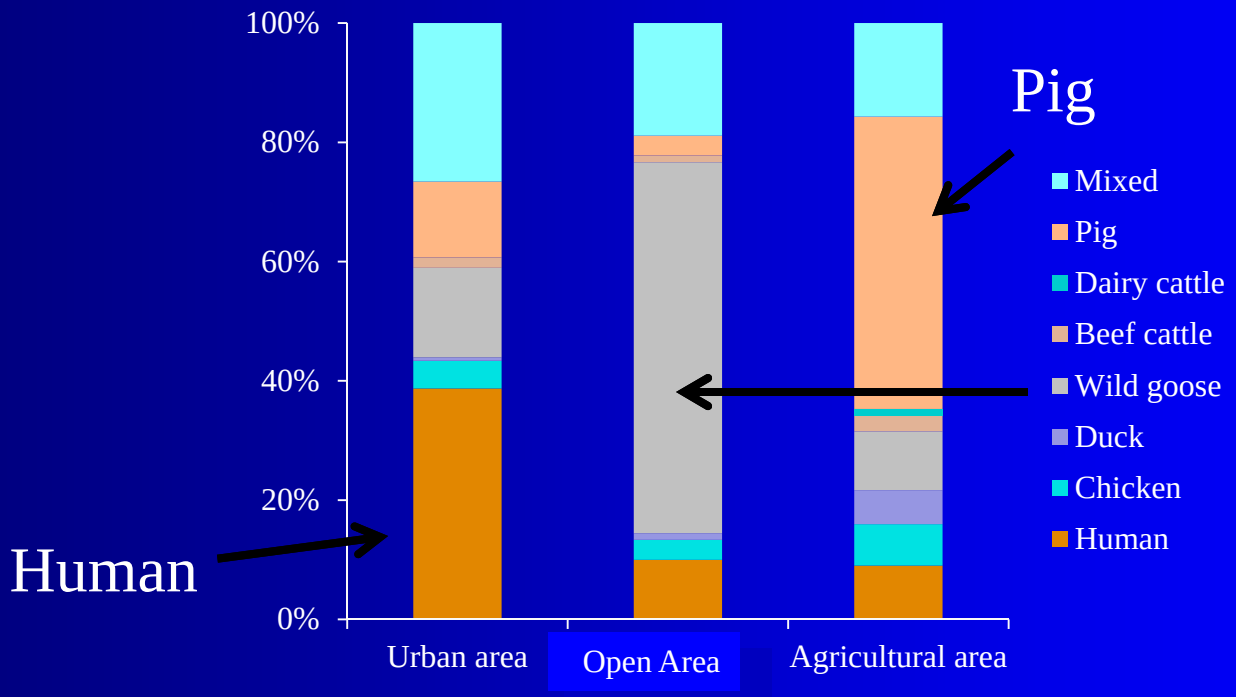
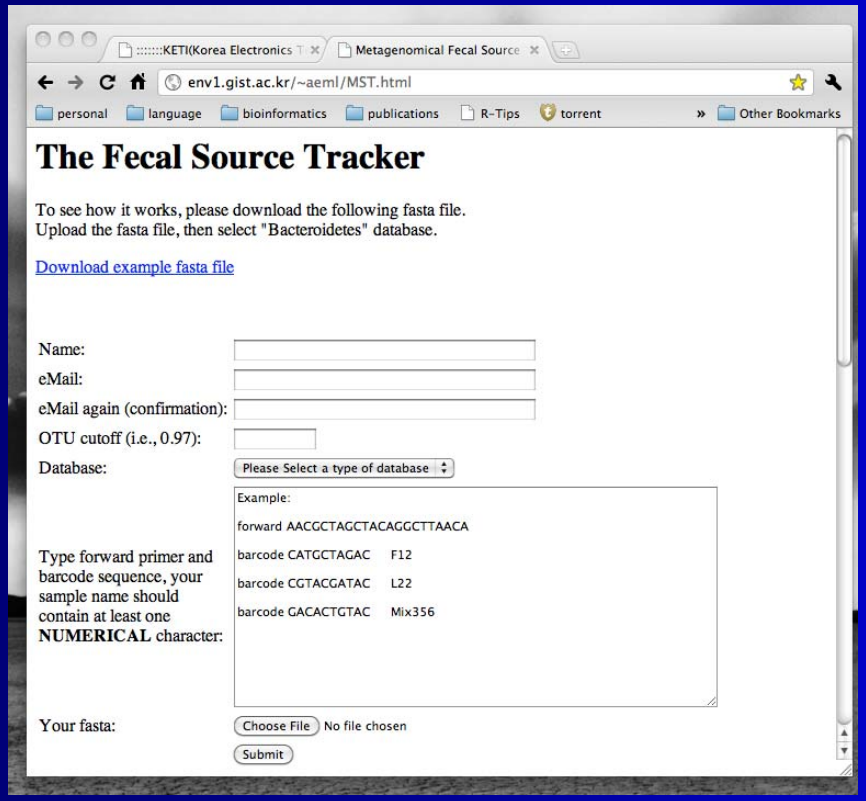
Department of Environmental Science and Engineering and International Environmental Research Center, Gwangju Institute of Science and Technology, Gwangju 500-712, Republic of Korea, Department of Soil, Water, and Climate and BioTechnology Institute, University of Minnesota, St. Paul, Minnesota 55108, and School of Biological Sciences, Seoul National University, NS70, 56-1 Daehak-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

Result: Number of shared OTUs



Development of automated MST system



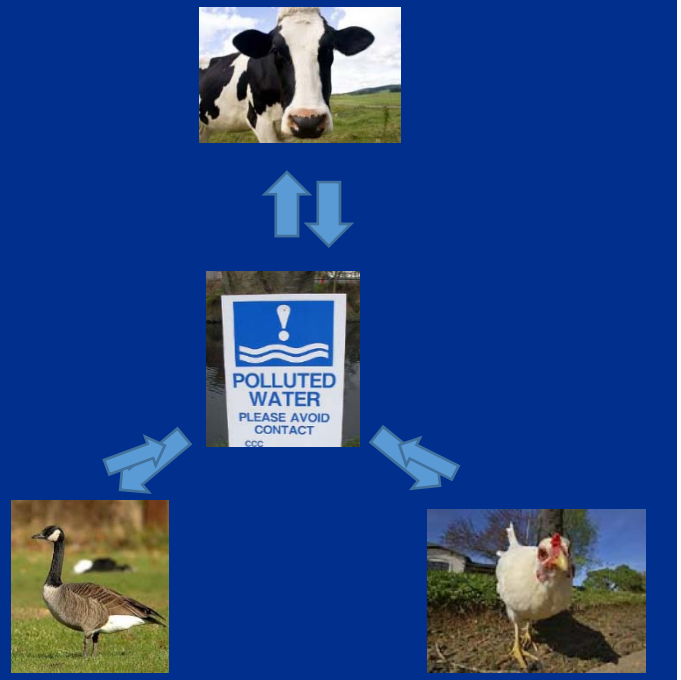
Applying batch program to automate pyrosequencing analysis

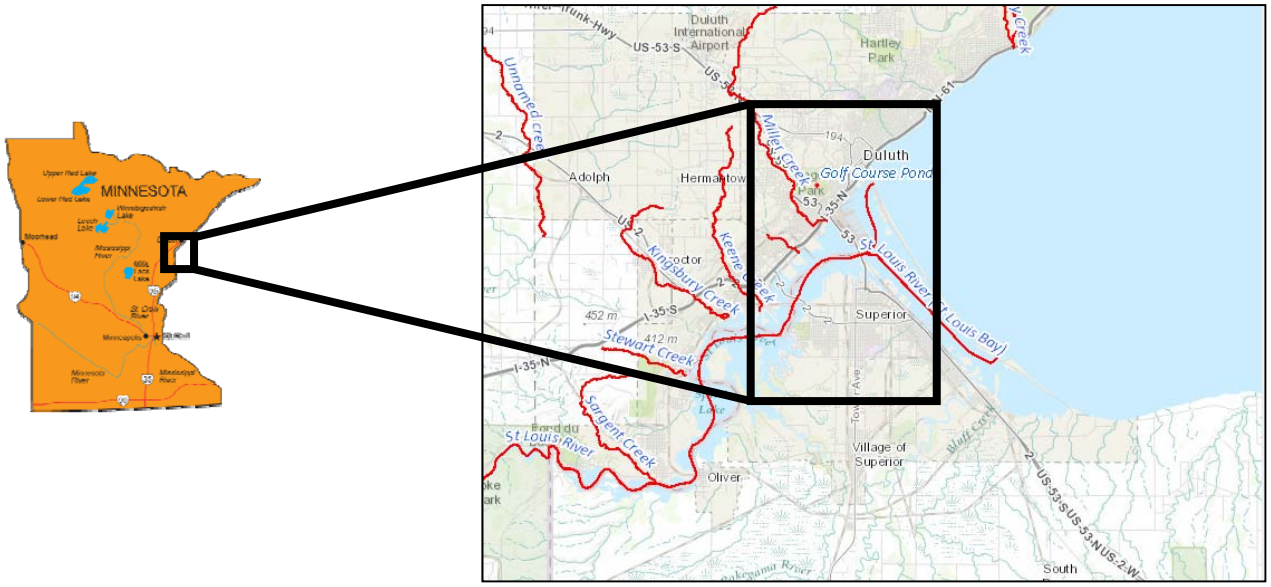
Integrated Online System for a Pyrosequencing-Based Microbial Source Tracking Method that Targets Bacteroidetes 16S rDNA.

Tatsuya Unno, Doris Di, Jang Jeonghwan, Yaeseul Suh, Michael Sadowsky and Hor-Gil Hur . Environ Sci Technol 46, 93-98.

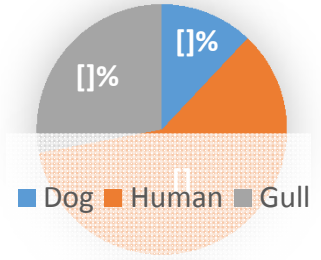
Research Questions

1. Are bacterial communities in environmental and fecal samples similar?
2. Are the bacterial composition of different animals distinguishable from each other?
3. Can the fecal microbiomes of different animals be differentiated using a NGS MST method?

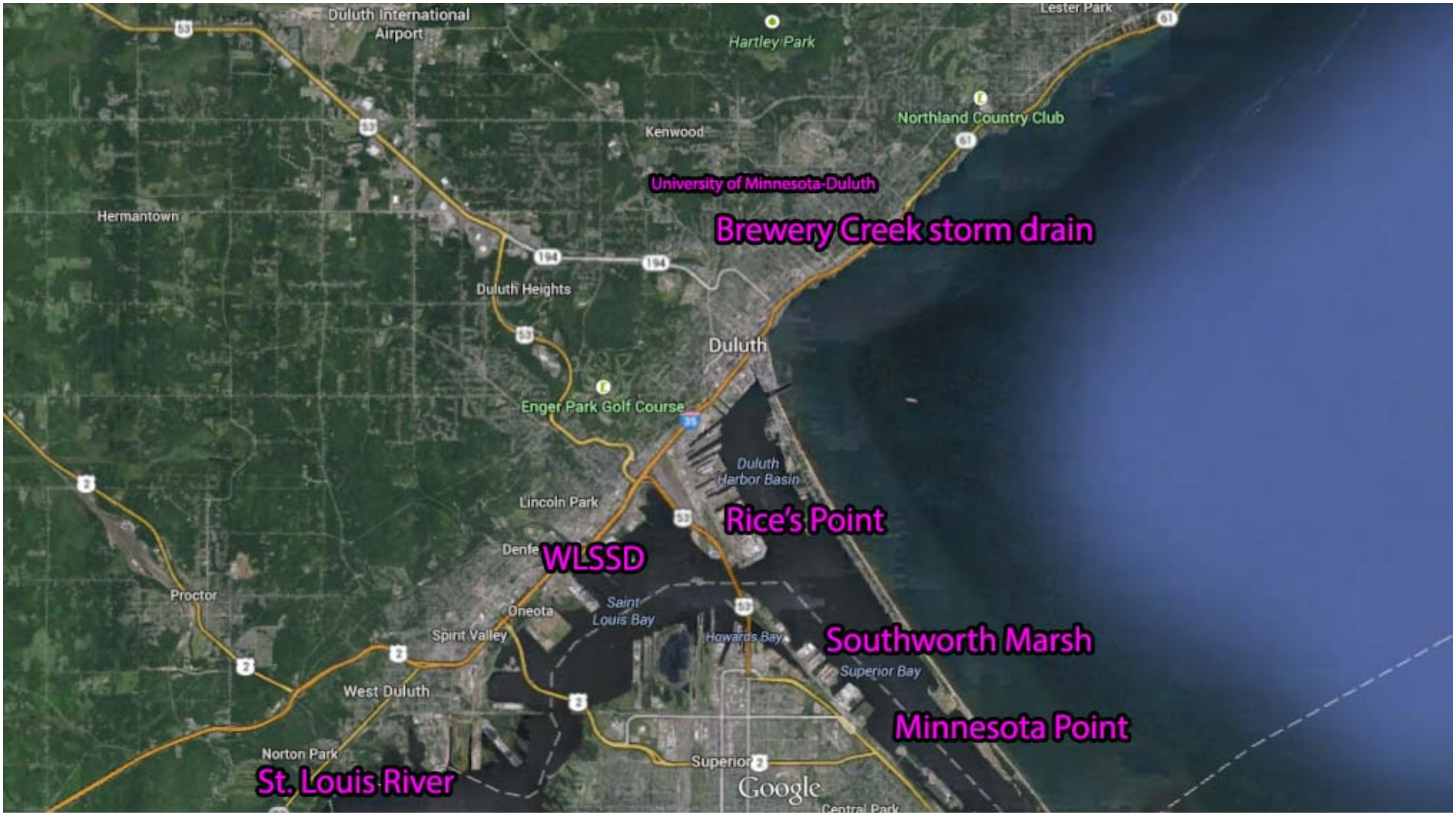




U.S. E.P.A. (2012) National Water Quality Inventory Report (Section 303(d) of the Clean Water Act;



Sample Collection → Sample Processing → DNA Isolation → Next Generation Sequencing → Data Analysis


Duluth International Airport
Hartley Park
Lester Park
Northland Country Club
Kenwood
University of Minnesota-Duluth
Brewery Creek storm drain
Hermantown
Duluth Heights
Duluth
Enger Park Golf Course
Duluth Harbor Basin
Lincoln Park
Rice's Point
Denfeld
WLSSD
Proctor
Oneota
Saint Louis Bay
Spirit Valley
Howards Bay
Southworth Marsh
Superior Bay
West Duluth
Minnesota Point
Norton Park
Superior
St. Louis River
Google
Central Park

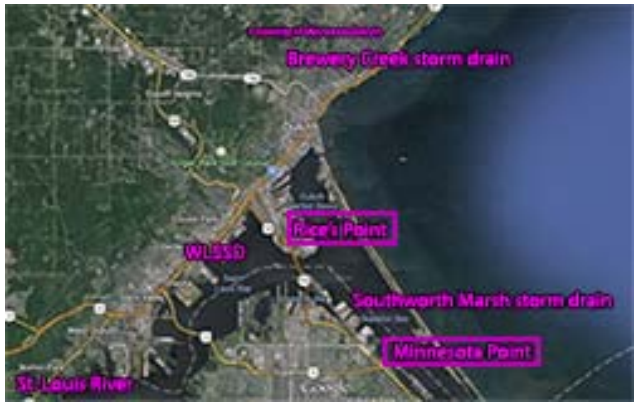
Southworth Marsh storm drain

St. Louis River

Brewery Creek storm drain

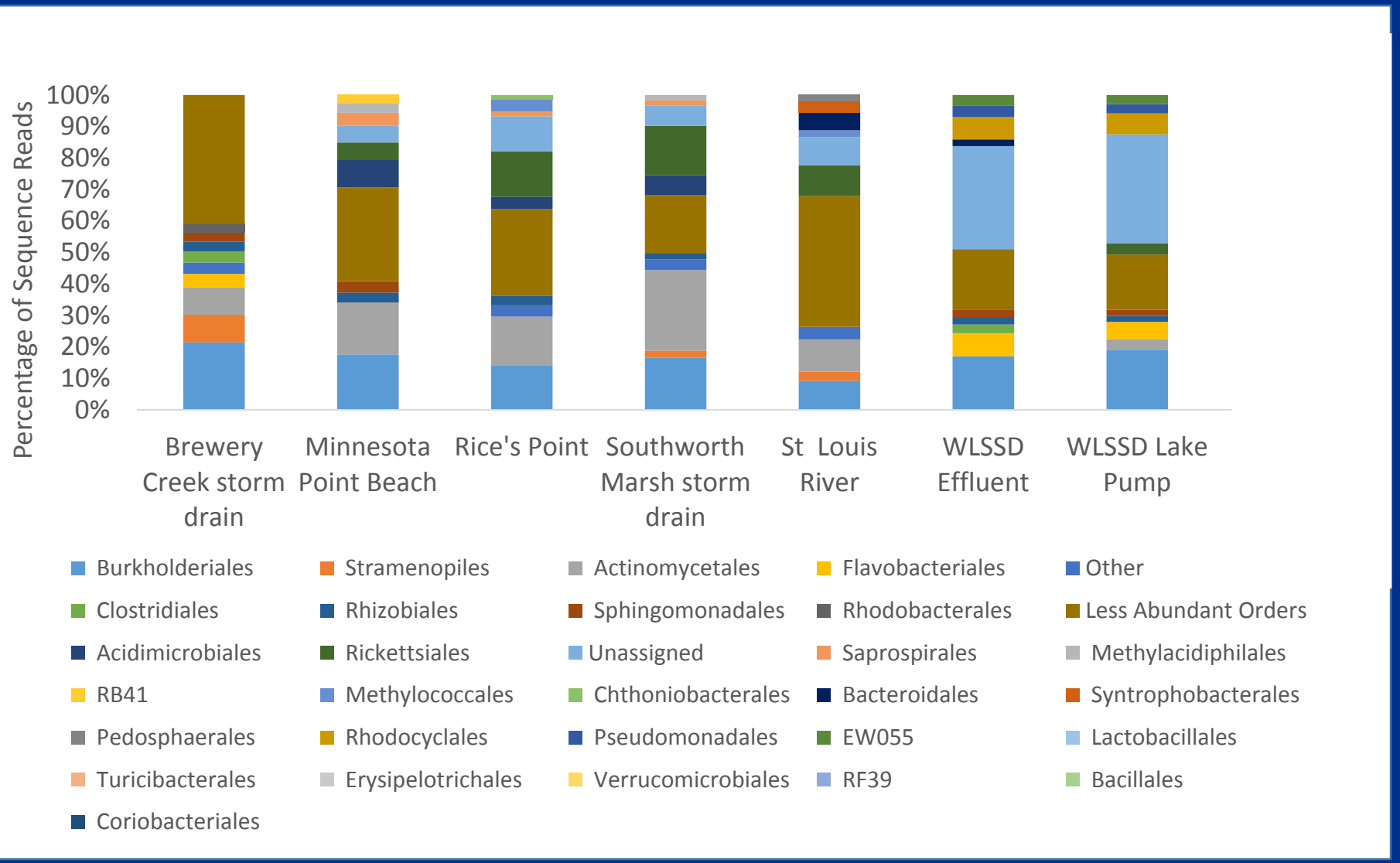
Storm drains are known microbial contaminant sources

Had some of the highest levels of *E.coli* across summer



Rice's Point

Minnesota Point Beach


Percentage of Sequence Reads
100%
90%
80%
70%
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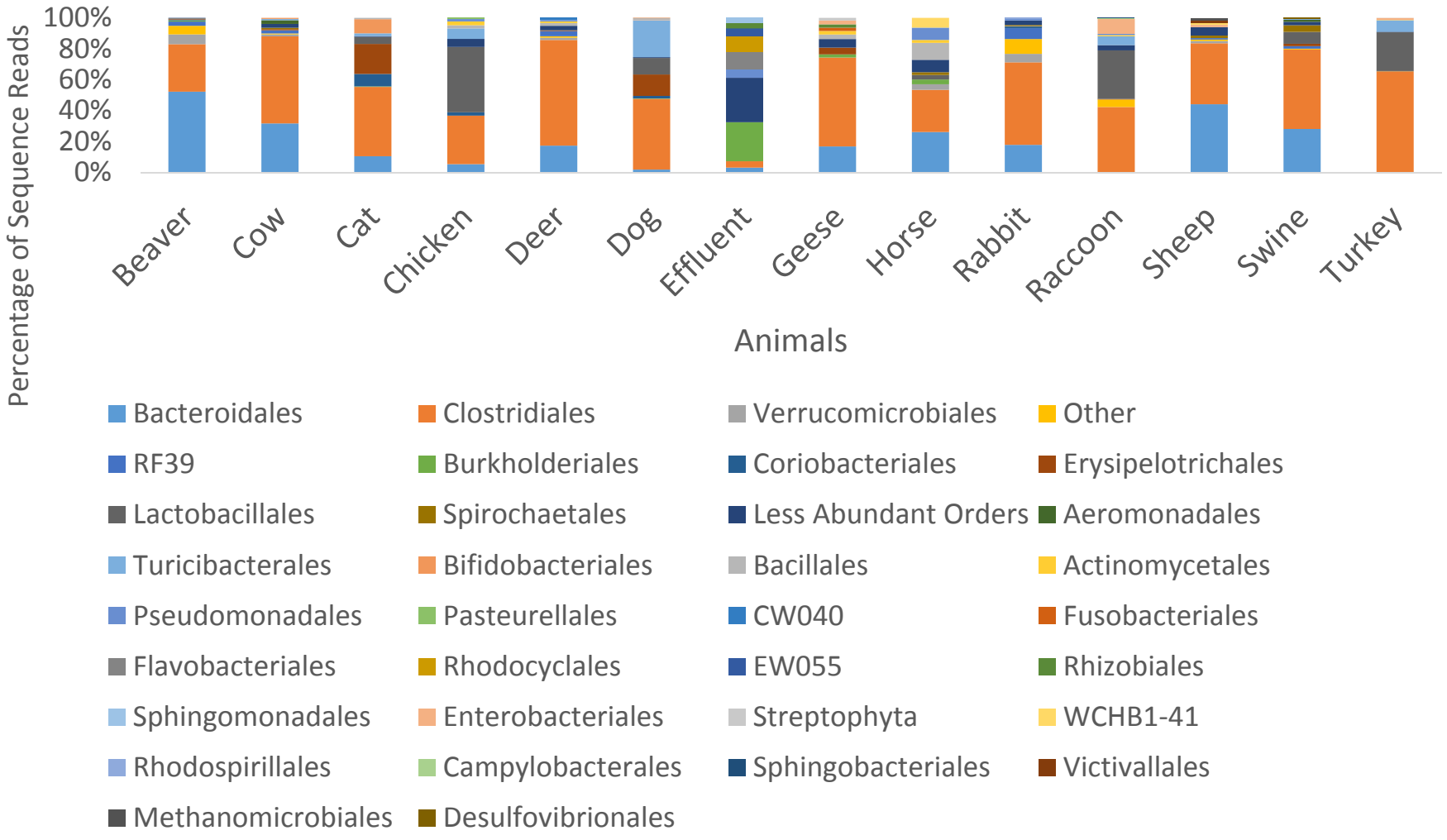
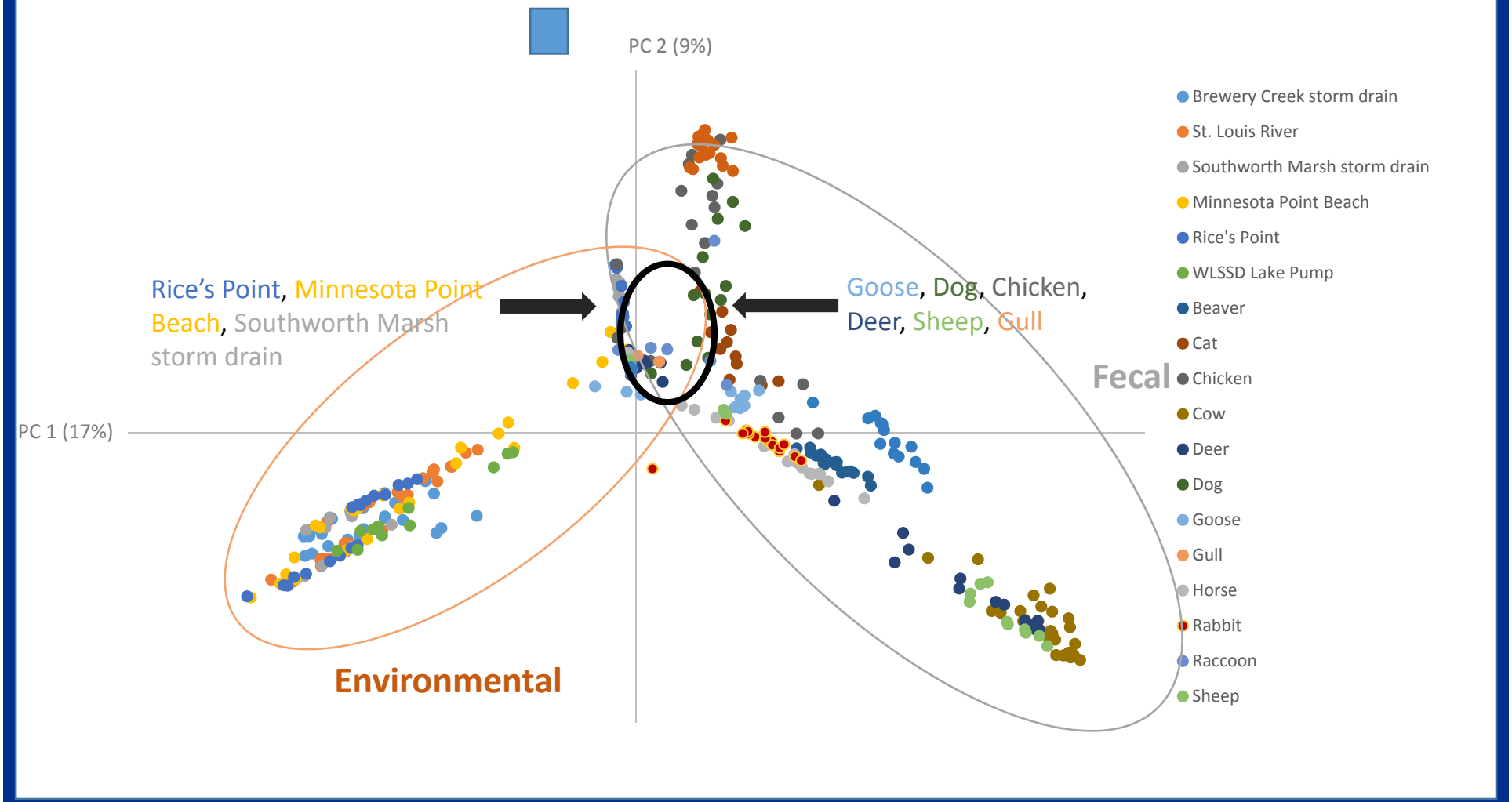
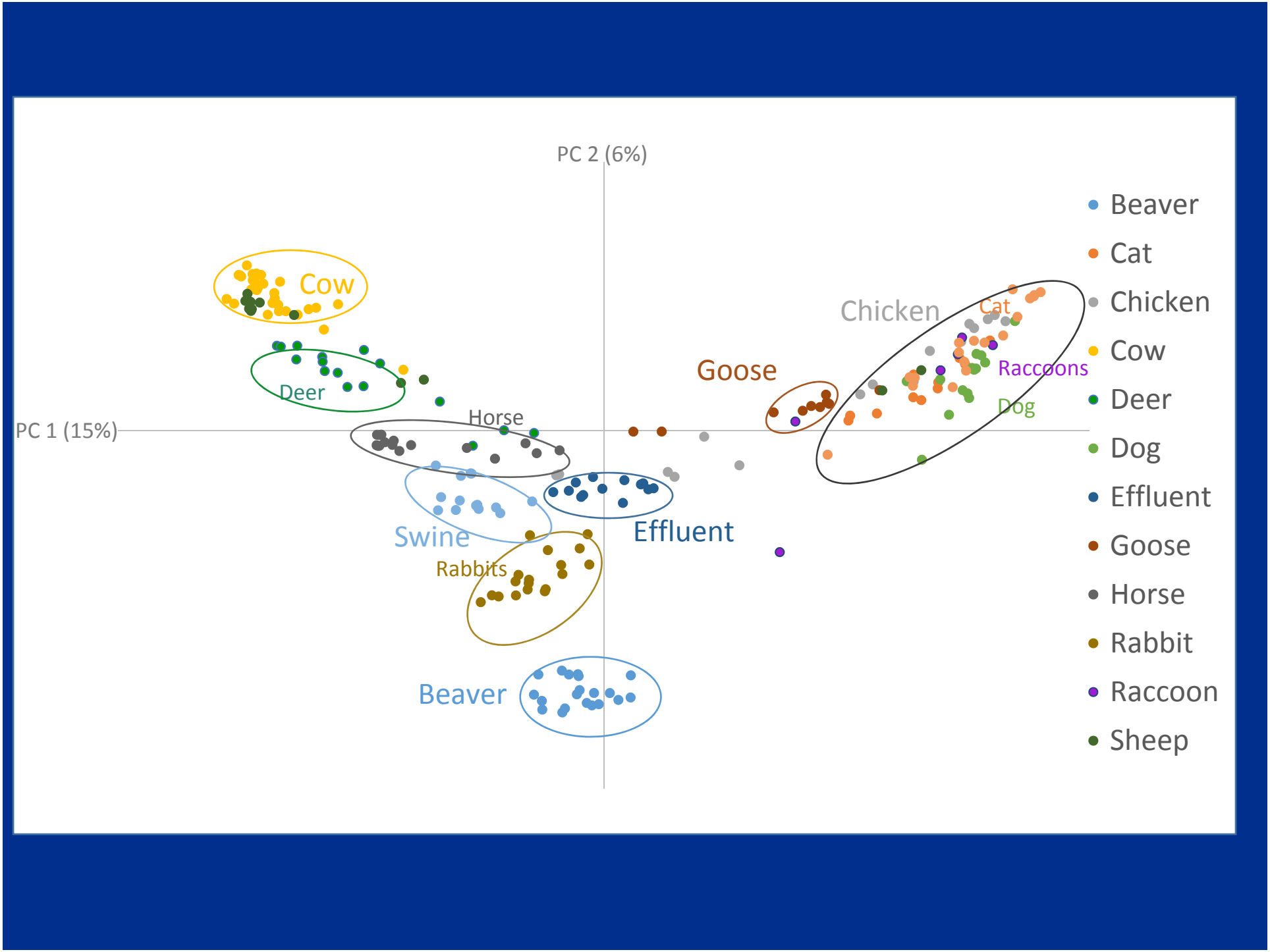
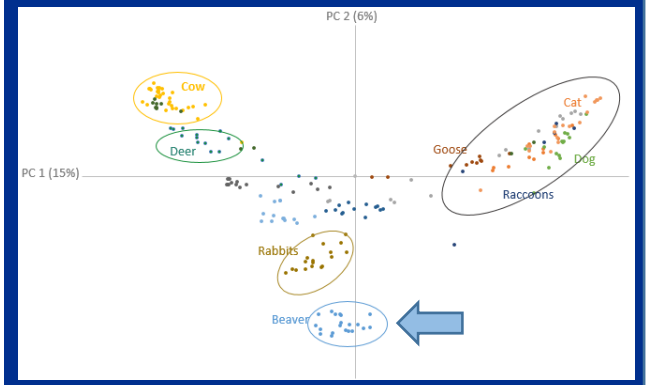
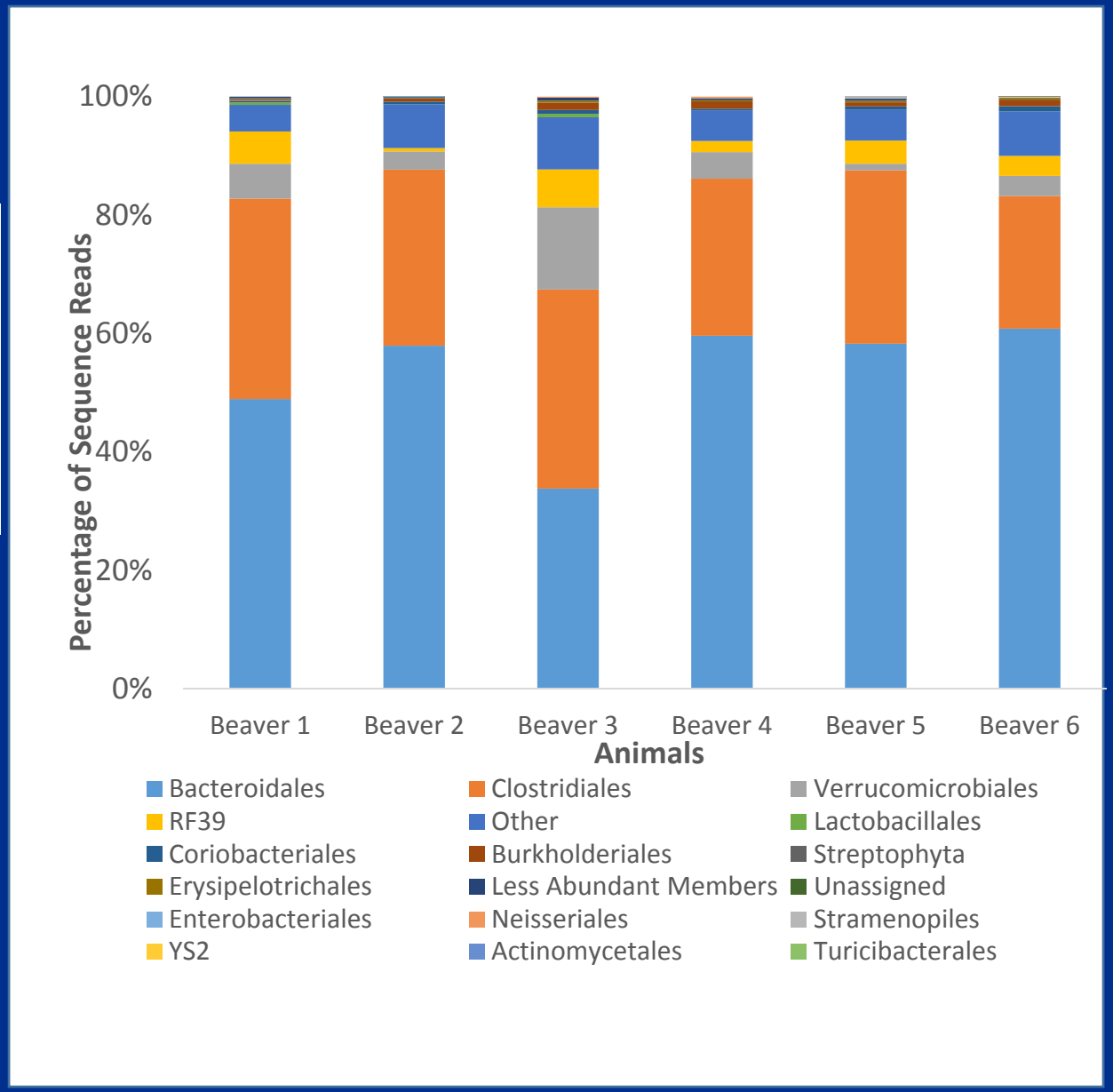
50%
40%
30%
20%
10%
0%
Brewery Creek storm drain
Minnesota Point Beach
Rice's Point
Southworth Marsh storm drain
St Louis River
WLSSD Effluent
WLSSD Lake Pump
Burkholderiales
Stramenopiles
Actinomycetales
Flavobacteriales
Other
Clostridiales
Rhizobiales
Sphingomonadales
Rhodobacterales
Less Abundant Orders
Acidimicrobiales
Rickettsiales
Unassigned
Saprospirales
Methylacidiphilales
RB41
Methylococcales
Chthoniobacterales
Bacteroidales
Syntrophobacterales
Pedosphaerales
Rhodocyclales
Pseudomonadales
EW055
Lactobacillales
Turicibacterales
Erysipelotrichales
Verrucomicrobiales
RF39
Bacillales
Coriobacteriales


Percentage of Sequence Reads
100%
80%
60%
40%
20%
0%
Beaver
Cow
Cat
Chicken
Deer
Dog
Effluent
Geese
Horse
Rabbit
Raccoon
Sheep
Swine
Turkey
Animals
Bacteroidales
Clostridiales
Verrucomicrobiales
Other
RF39
Burkholderiales
Coriobacteriales
Erysipelotrichales
Lactobacillales
Spirochaetales
Less Abundant Orders
Aeromonadales
Turicibacterales
Bifidobacteriales
Bacillales
Actinomycetales
Pseudomonadales
Pasteurellales
CW040
Fusobacteriales
Flavobacteriales
Rhodocyclales
EW055
Rhizobiales
Sphingomonadales
Enterobacteriales
Streptophyta
WCHB1-41
Rhodospirillales
Campylobacterales
Sphingobacteriales
Victivallales
Methanomicrobiales
Desulfovibrionales


PC 2 (9%)
PC 1 (17%)
Rice's Point, Minnesota Point Beach, Southworth Marsh storm drain
Goose, Dog, Chicken, Deer, Sheep, Gull
Fecal
Environmental
Brewery Creek storm drain
St. Louis River
Southworth Marsh storm drain
Minnesota Point Beach
Rice's Point
WLSSD Lake Pump
Beaver
Cat
Chicken
Cow
Deer
Dog
Goose
Gull
Horse
Rabbit
Raccoon
Sheep


PC 2 (6%)
PC 1 (15%)
Cow
Deer
Horse
Swine
Rabbits
Beaver
Effluent
Goose
Chicken
Cat
Raccoons
Dog
Beaver
Cat
Chicken
Cow
Deer
Dog
Effluent
Goose
Horse
Rabbit
Raccoon
Sheep


PC 2 (6%)
PC 1 (15%)
Cow
Deer
Goose
Cat
Dog
Raccoons
Rabbits
Beaver


100%
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Percentage of Sequence Reads
Beaver 1
Beaver 2
Beaver 3
Beaver 4
Beaver 5
Beaver 6
Animals
Bacteroidales
Clostridiales
Verrucomicrobiales
RF39
Other
Lactobacillales
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Burkholderiales
Streptophyta
Erysipelotrichales
Less Abundant Members
Unassigned
Enterobacteriales
Neisseriales
Stramenopiles
YS2
Actinomycetales
Turicibacterales

Conclusions

- Bacteria in animal fecal samples and environmental samples are different but some cluster together.
- Community structure is different between animals.
- Differences in the less abundant bacterial members in feces may allow differentiation of animals.

The most sensitive source tracking tool of all is

Acknowledgments

College of Biological Sciences
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LCCMR

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Clairessa Brown and Chanlan Chun
Brian Gibbens
Chris Staley
Jane Phillips
Trevor Gould
Tatsu Unno
Bruce Jarvis
James B. Cotner

Thank you for the Opportunity to Speak with you.

Thanks for your attention!