



CHARACTERIZING BIOLOGICAL POLLUTANTS IN AGRICULTURAL RUNOFF AT COLORADO DAIRIES

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ROADMAP

- Background & Project Objectives
- Field Campaign
- Analytic Strategies
 - CSU Soils Lab Results
 - Antimicrobial Analysis
 - Next Generation Sequencing
- Joint Ventures and Future Funding



BACKGROUND

Contaminants generated during modern livestock production has raised concerns about environmental pollution and the potential public health impact of water-borne contaminants.

Objective: Characterize biological pollutants in agricultural runoff (bacteria, antibiotics, antibiotic resistant genes) at Eastern Colorado dairies.

- Test state-of-the-art techniques in exposure assessment and analysis of microbial communities



FIELD CAMPAIGNS

Sampling Locations



1. Upstream
2. Manure Slurry
3. Tap
4. Holding Pond
5. Runoff
6. Field Blank
7. Replicate
8. Downstream

(plus Lab Blank)

8



FIELD CAMPAIGNS



Manure Slurry



Runoff



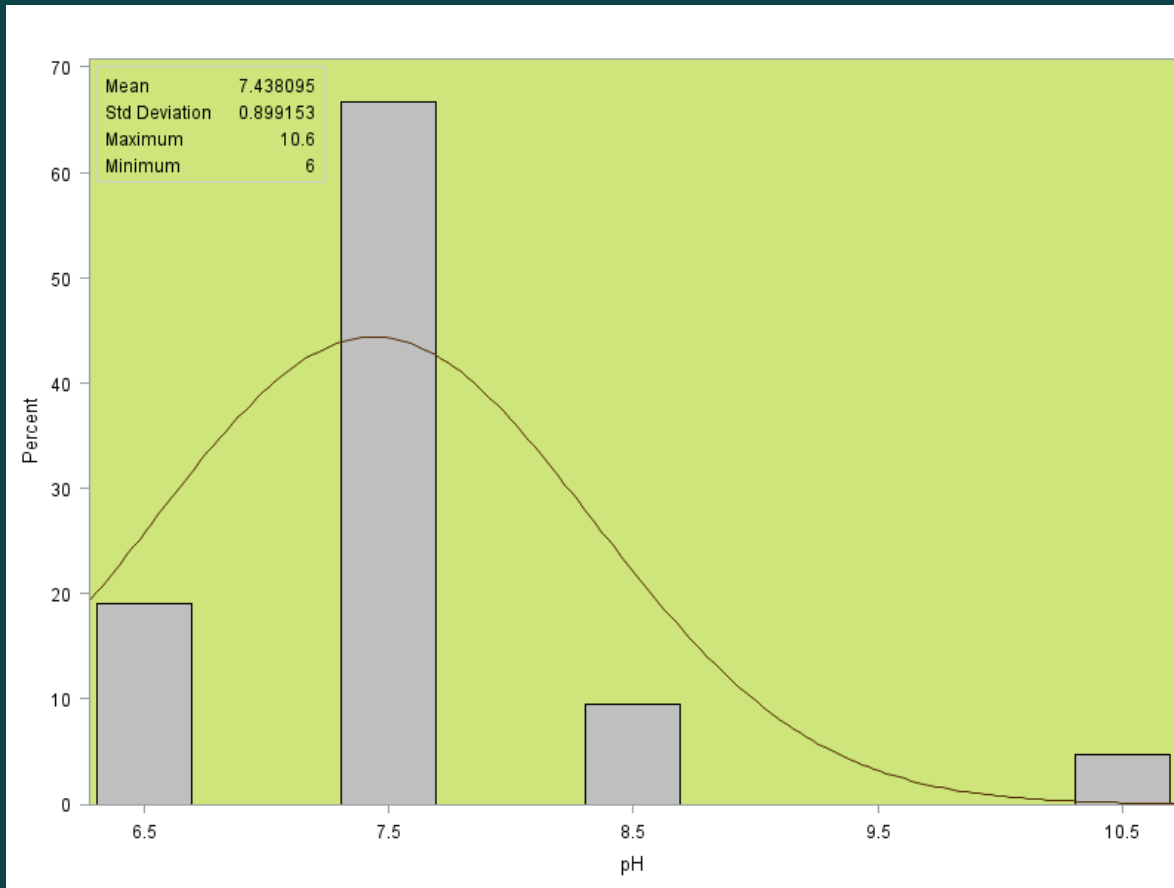
Downstream

Latitude and longitude for each sampling point collected



ANALYTIC STRATEGIES

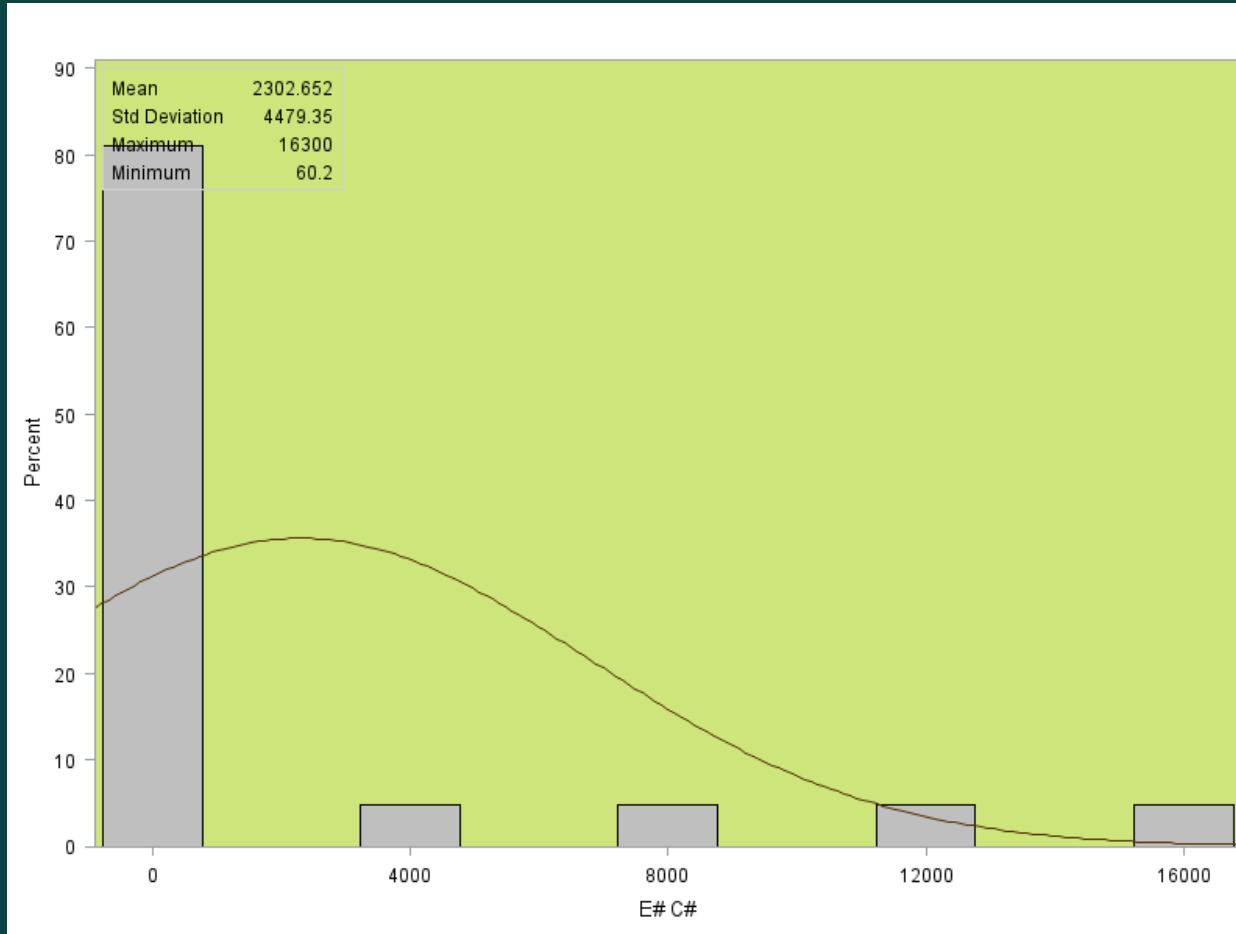
Soils Lab Analysis: pH





ANALYTIC STRATEGIES

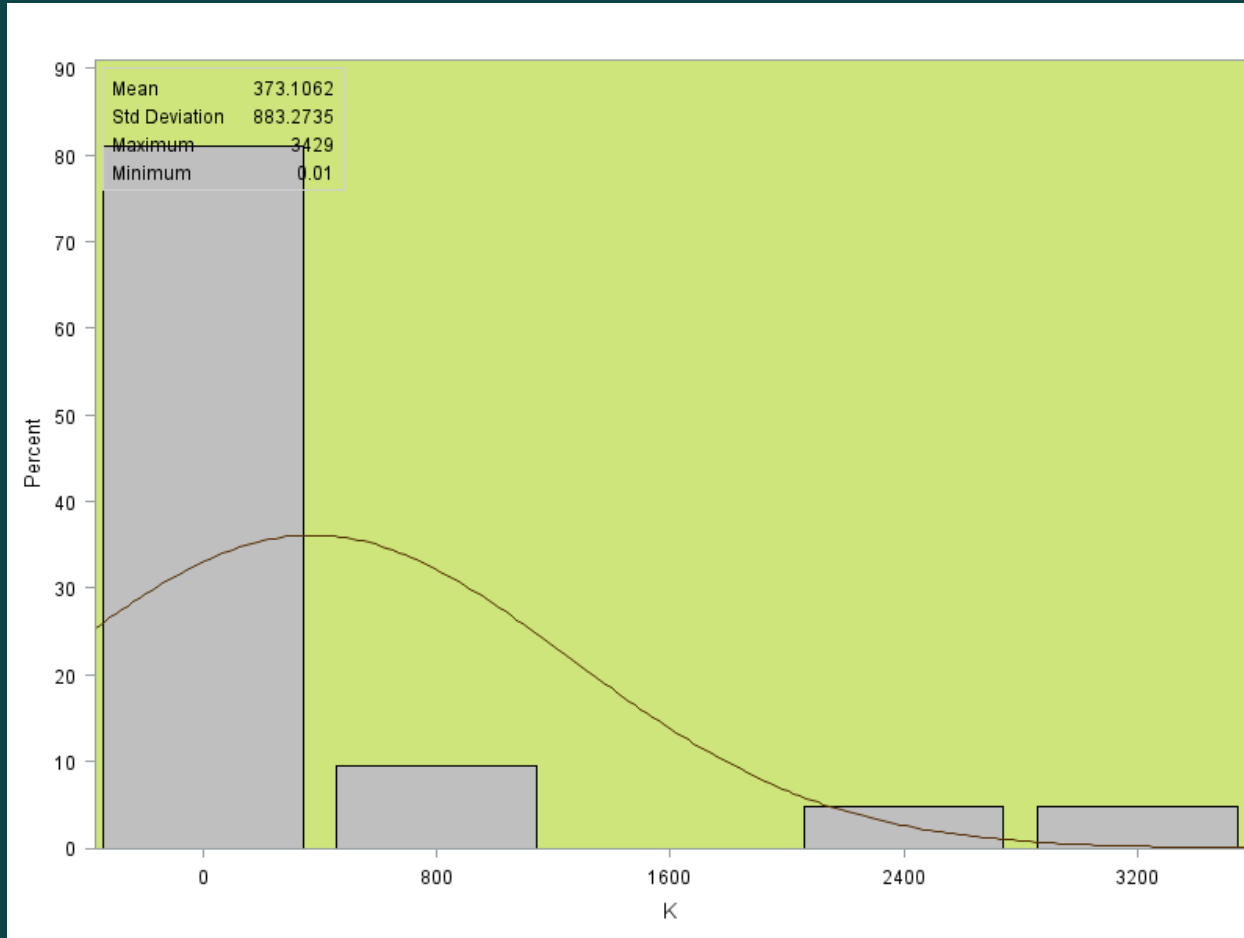
Soils Lab Analysis: Electrical conductivity (salts) ($\mu\text{mhos/cm}$)





ANALYTIC STRATEGIES

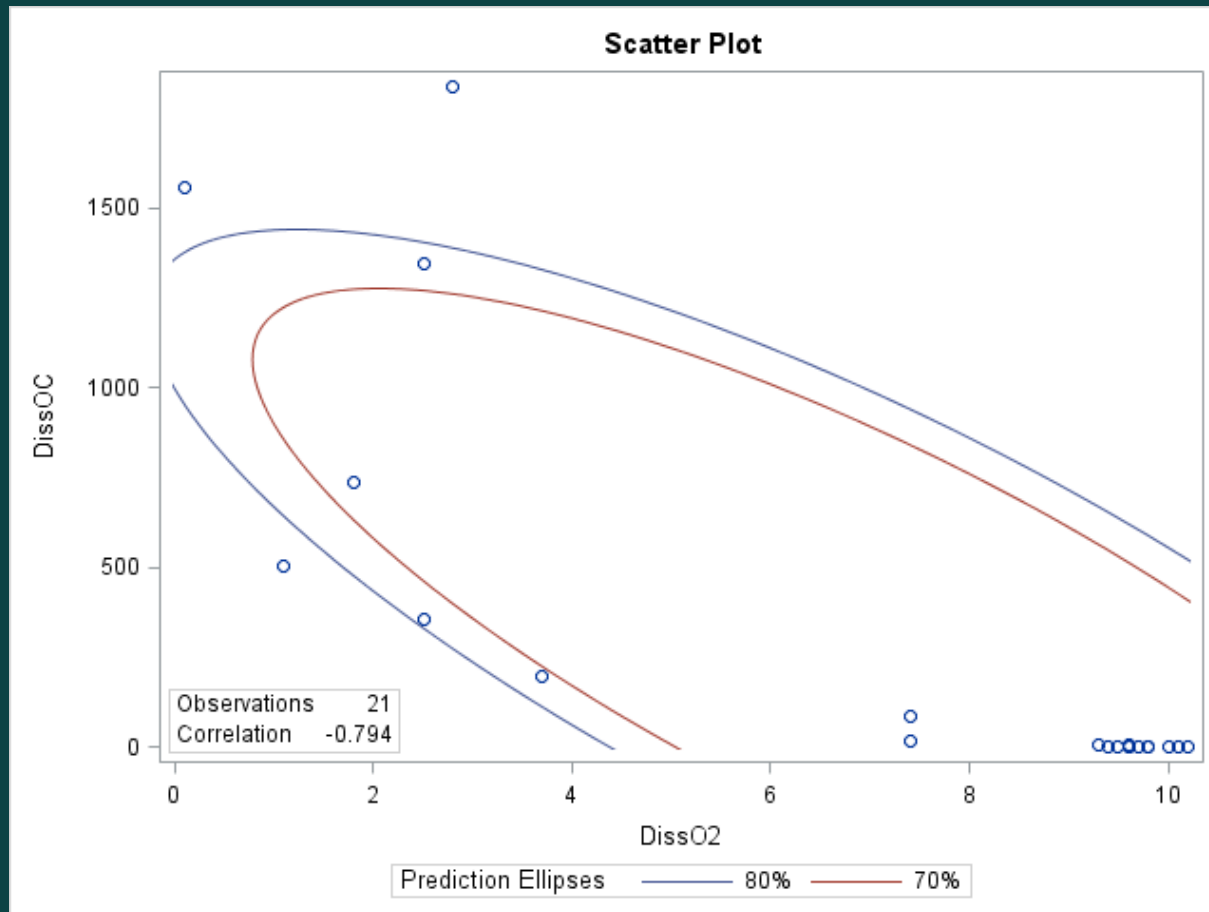
Soils Lab Analysis: Potassium (mg/L)





ANALYTIC STRATEGIES

Soils Lab Analysis: Dissolved Oxygen/Dissolved OC





ANALYTIC STRATEGIES

Antibiotic Analysis

- FDA-approved drugs for dairy cattle= 164
 - Source: Food Animal Residue Avoidance Databank (FARAD)
- Antibiotic Use
 - Therapeutic v. prophylactic
- Consultation with Veterinarians
 - Dr. Noa Roman-Muniz, CSU Extension
 - Dr. Craig McConnel, CVMBS, Dairy Population Health Management
- Focus on likely targets
 - Specificity v. generalizability



ANALYTIC STRATEGIES

Antibiotic Analysis

- Target compounds
 - Penicillin, oxytetracycline, ceftiofur
- Sample preservation
 - Amber glass bottles – prevents phototransformation
 - Acidify to pH 2 – prevents biotransformation
 - 2.5 g EDTA – minimizes metal chelation
- Sample preparation
 - Solid phase extraction – Oasis HLB cartridges
 - Spike with D-labeled isotopes – produces more accurate data
- Sample analysis
 - Liquid chromatography-tandem mass spectrometry



Robert Young



ANALYTIC STRATEGIES

Next Generation Sequencing

- Bacterial diversity and relative abundance
- Illumina Mi-Seq Desktop Sequencer
 - Target Amplicon Sequencing
 - Bridge amplification (DNA extracted according to EMP)
 - Barcoded primer set
 - 15 gigabases per run
 - 50-300 base pair reads
 - Paired end reading
 - High quality reads with <0.1 error rate
 - 25 million sequencing reads per run



Josh Schaeffer



ANALYTIC STRATEGIES

Targeted Amplicon

- 16S ribosomal RNA in Bacterial Genomes:
 - Highly conserved (universally distributed)
 - Weakly affected by horizontal gene transfer
 - 9 “hypervariable regions” within conserved stretches
 - Demonstrate sequence diversity among bacterial species
- Quantitative Insights Into Microbial Ecology
 - Data pipeline used for comparison and analysis of microbial communities

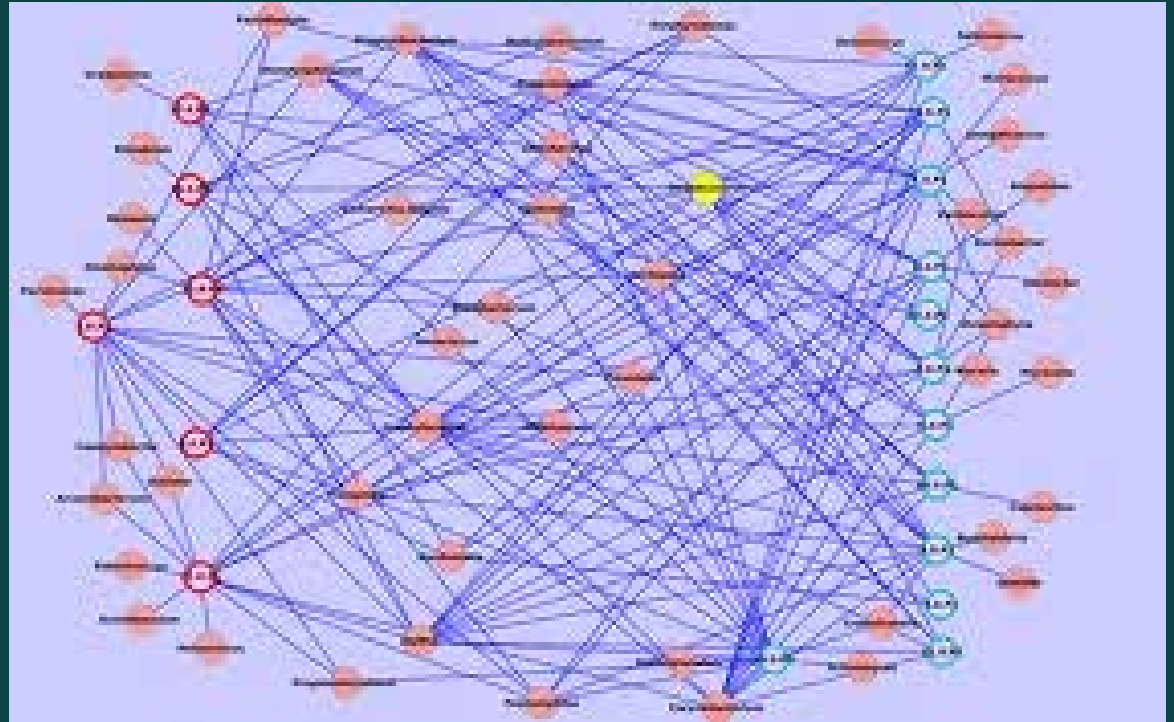


ANALYTIC STRATEGIES

Quantitative Insights Into Microbial Ecology (QIIME)



Chloe Stenkamp-Strahm



[Source: microbiota.com]

Taxonomic assignment, phylogenetic trees + visualization



JOINT VENTURES

- Amazon Elastic Compute Cloud (EC2) funding for QIIME analysis
- Simultaneous sampling campaign with ongoing exposure assessments
 - Dairy bioaerosols (PI: Reynolds)
 - Calf and cow sampling (PI: McConnel)
 - CVMBS CRC for longitudinal exposure assessment (submitted)
- Development of *Collaborative Health Research on the Microbiome and the Environment (CHROME)* Project
[<http://csu-cvmb.colostate.edu/academics/erhs/agricultural-health-and-safety/Pages/microbiome.aspx>]



FUTURE FUNDING

NIH/National Institute for Environmental Health Sciences: Investigator-Initiated R01

USDA: Agriculture and Food Research Initiative – Food Safety

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THANK YOU

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Our Dairy Hosts & CSU Water Center

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