UPDATE:
Understanding Nutrient Impacts and Sources at the Watershed Scale to Enhance Environmental Stewardship

January 7, 2016

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Proposal Objectives

- Analyze archived water samples for bovine, swine, and poultry source-specific DNA markers
- Identify associations between nutrients, MST markers, land use, land characteristics, agricultural practices, and climate
- Create maps identifying locations to focus mitigation strategies

Relationship to Corn Growers goals

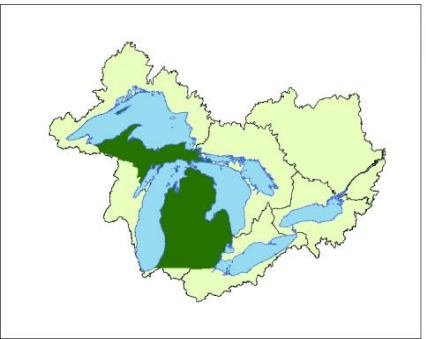
- “support non-proprietary, common good research to optimize economic returns and environmental stewardship for corn production”
- By linking microbial source tracking (MST) with nutrient loading, we will be able to identify which agricultural practices that degrade water quality

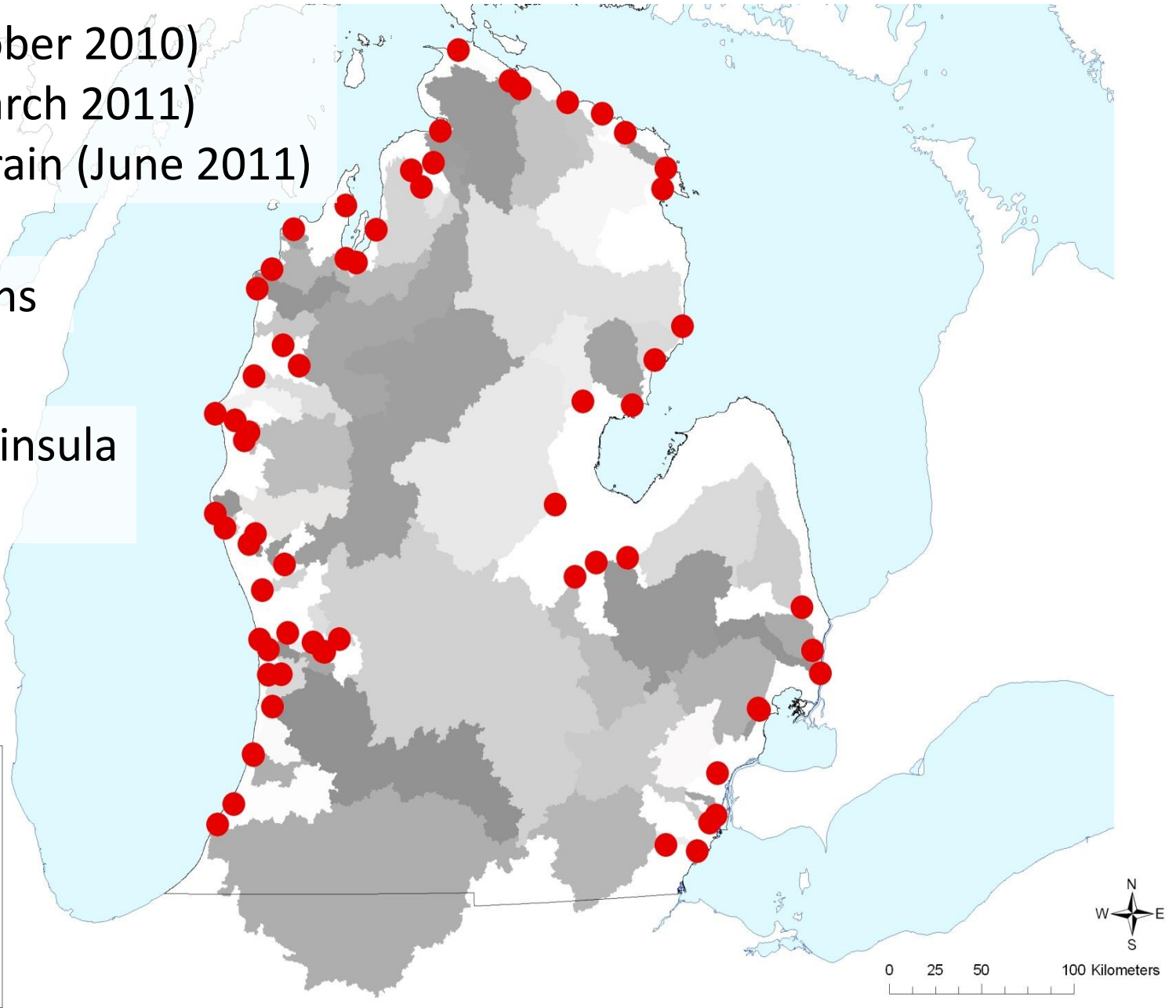
Sampling approach

Baseflow (October 2010)
Snow melt (March 2011)
Early summer rain (June 2011)

64 River systems

84% Lower Peninsula drainage area

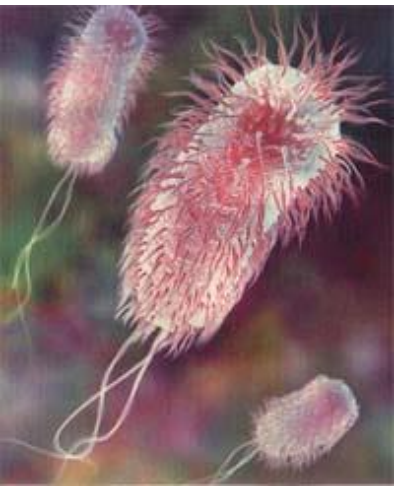


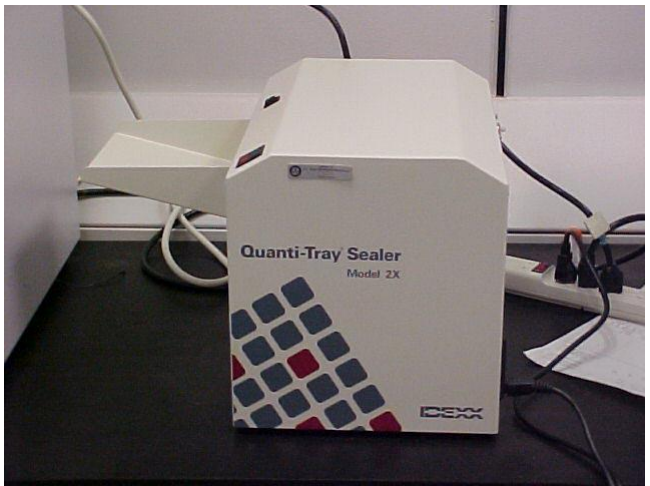


Approach – Sample analysis

Escherichia coli (*E. coli*)

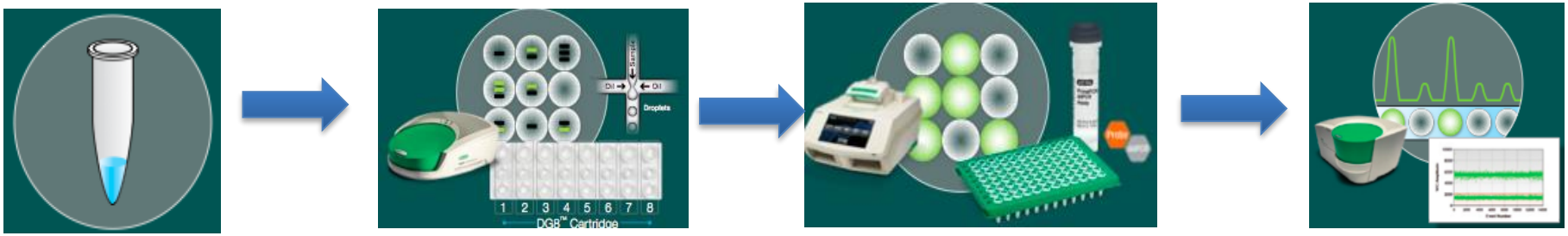
- General indicator of fecal contamination
- Linked to gastrointestinal illness through epidemiological studies (DuFour et al. 1982; Wade et al. 2006, 2008, 2010)
- USEPA recreational freshwater criterion: 2.5 log CFU/100 ml
- IDEXX Colilert® Quanti-Tray 2000®





Approach for MST Analysis

- droplet digital PCR (ddPCR)
  - Absolute quantification
  - High accuracy and precision
  - No standard curve
- Microbial Source Tracker (MST)



M2 Bovine Marker

- Bovine M2 Marker
- Specific to *Bacteriodales* bacteria in cows
- 100% Sensitivity
- >90% Specificity
- Tested by 5 separate Labs

Porcine Marker

- Pig2Bac
- Specific to *Bacteriodales* bacteria in pigs
- 100% Sensitivity
- 99% Specificity

Approach: Statistical Methods

- Basic stats: Correlation (Spearman Rank), Regression

- Classification And Regression Tree (CART)
  - Automated trial-and-error algorithm to classify data according to the influence of a potentially large number of independent variables (land use, nutrients, soils)
  - Sequentially splits dependent variable (bovine marker) into groups
  - Split variable and value selected that produces the least variance within groups
  - A *tree* is formed as subgroups are then split hierarchically
  - Final tree is *pruned* to include only significant splits

Updated analyses

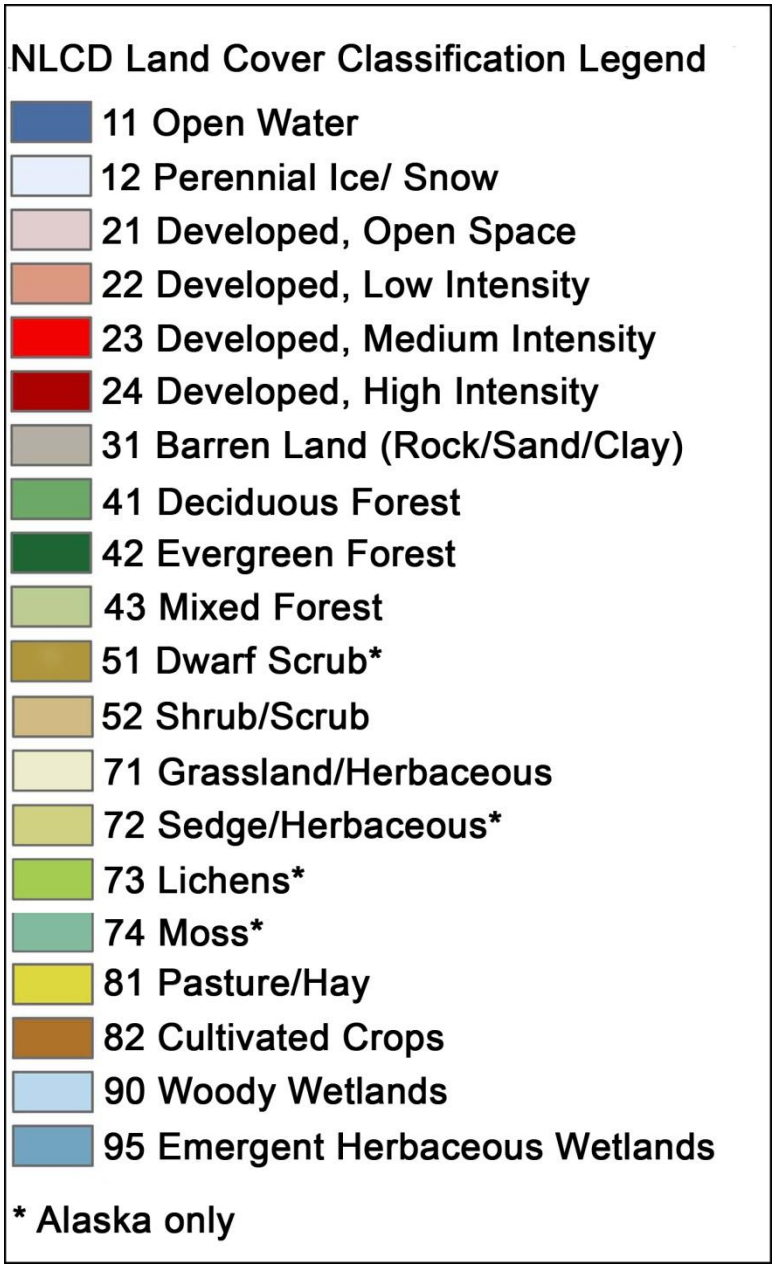
Baseflow,

Snowmelt and

Summer Rain

- New variables in CART analyses
  - % Cropland Data Layers (CDL from National Ag, Stat Services) provides crop types
  - National Land Cover Data (NLCD from USGS)
  - Annual mean concentration of N applied to the land from all animal manure
  - Ag fertilizer

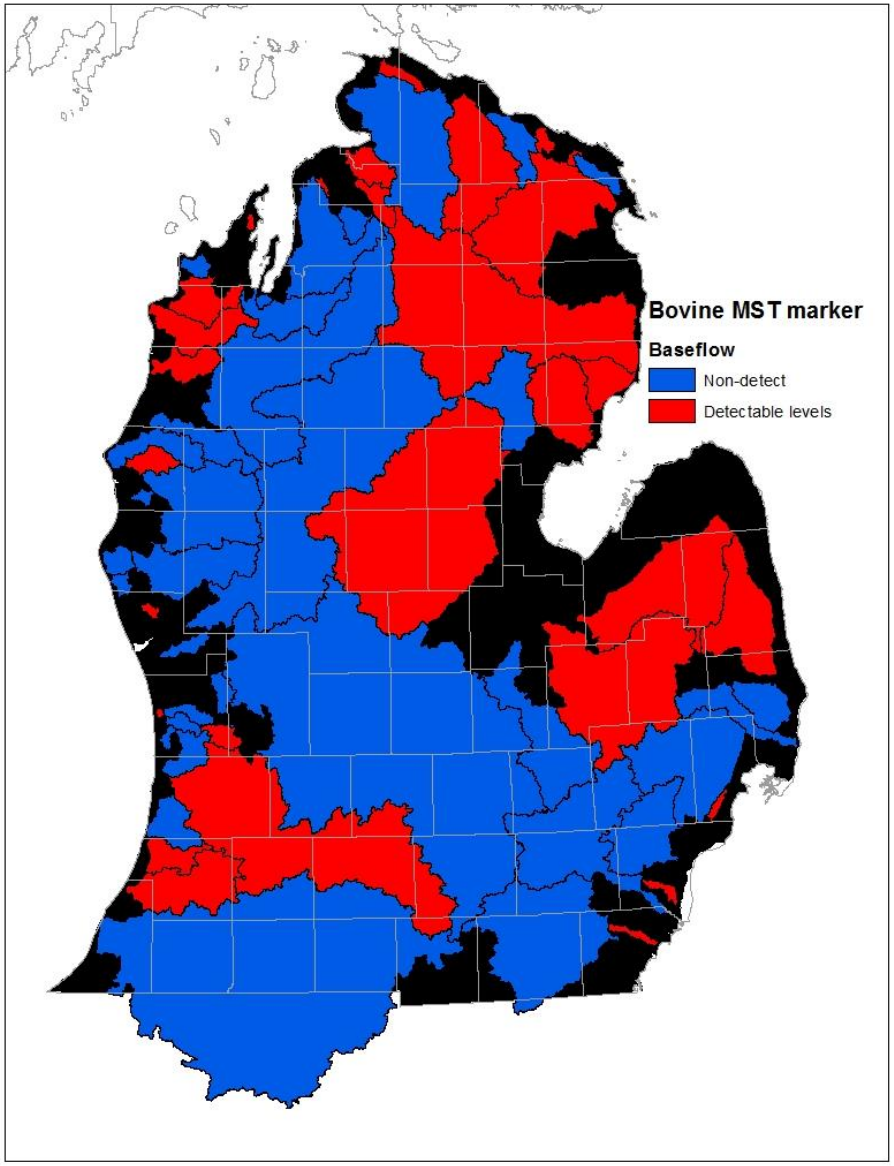
NLCD classifications

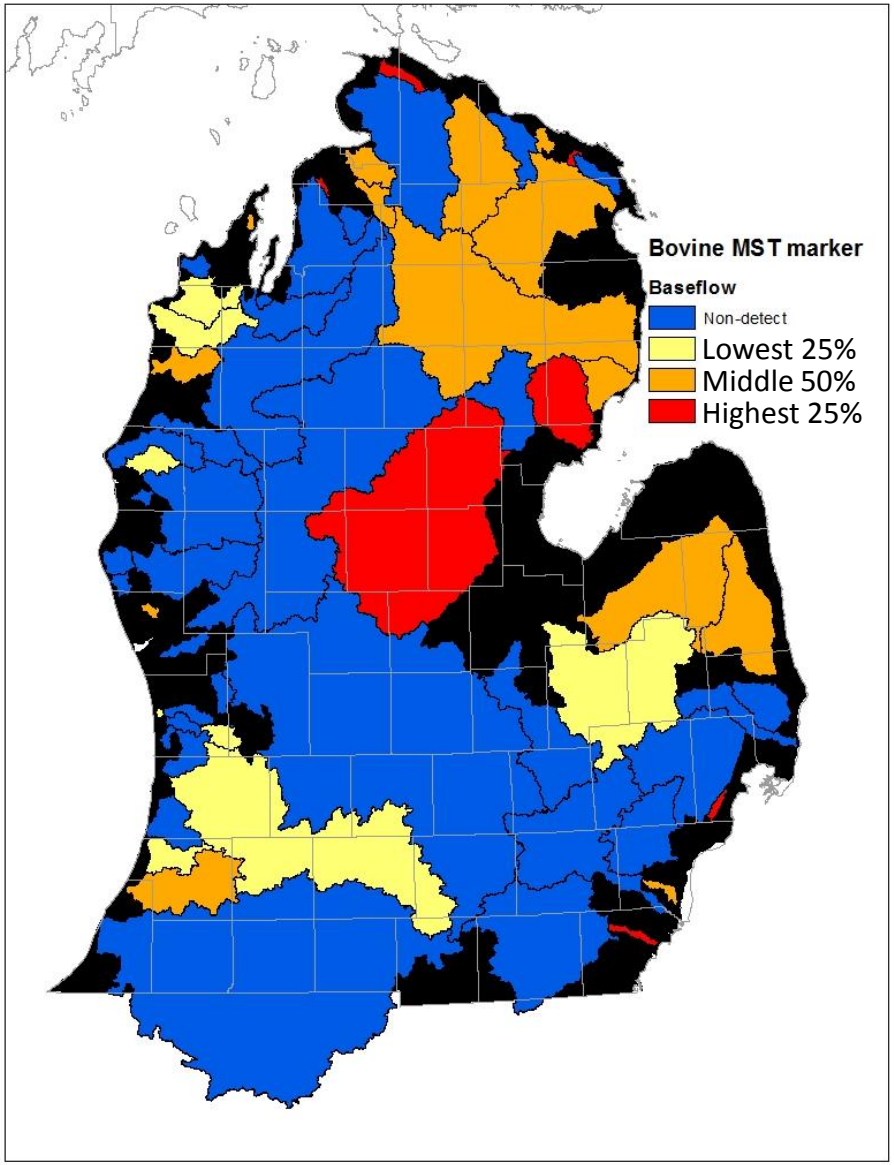


Results of the M2P Bovine Marker

	Baseflow (Fall)	Snow Melt (Spring)	Summer Rain
Percent Positive	45% (28/63)	62%(38/61)	75% (47/63)
Average Concentrations (CE/100ml)	35.7	21.2	31.2
Range of Concentrations (CE/100ml)	5.4 – 224	1.6-807	1.6 – 748.8

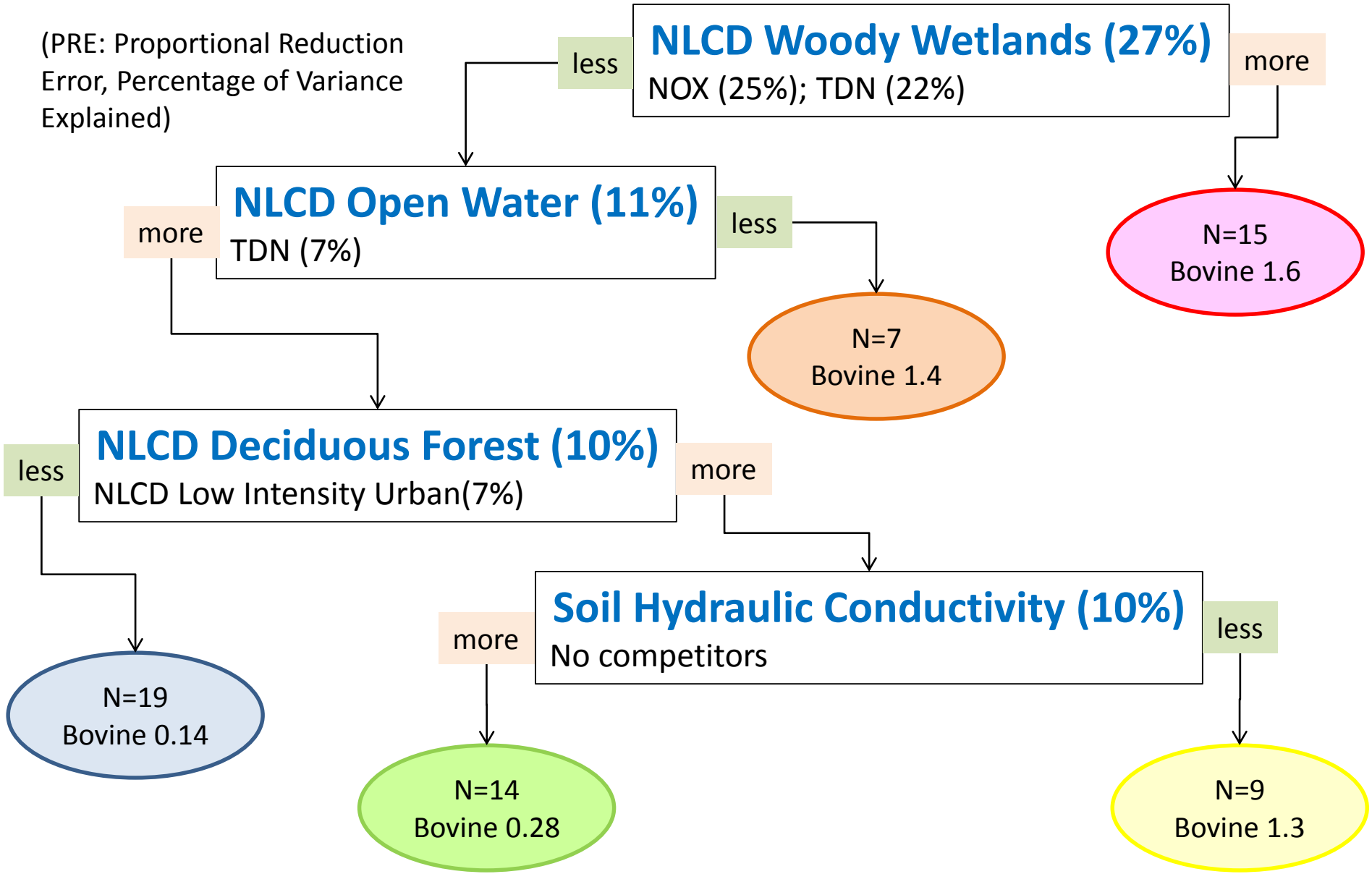
Baseflow - Bovine





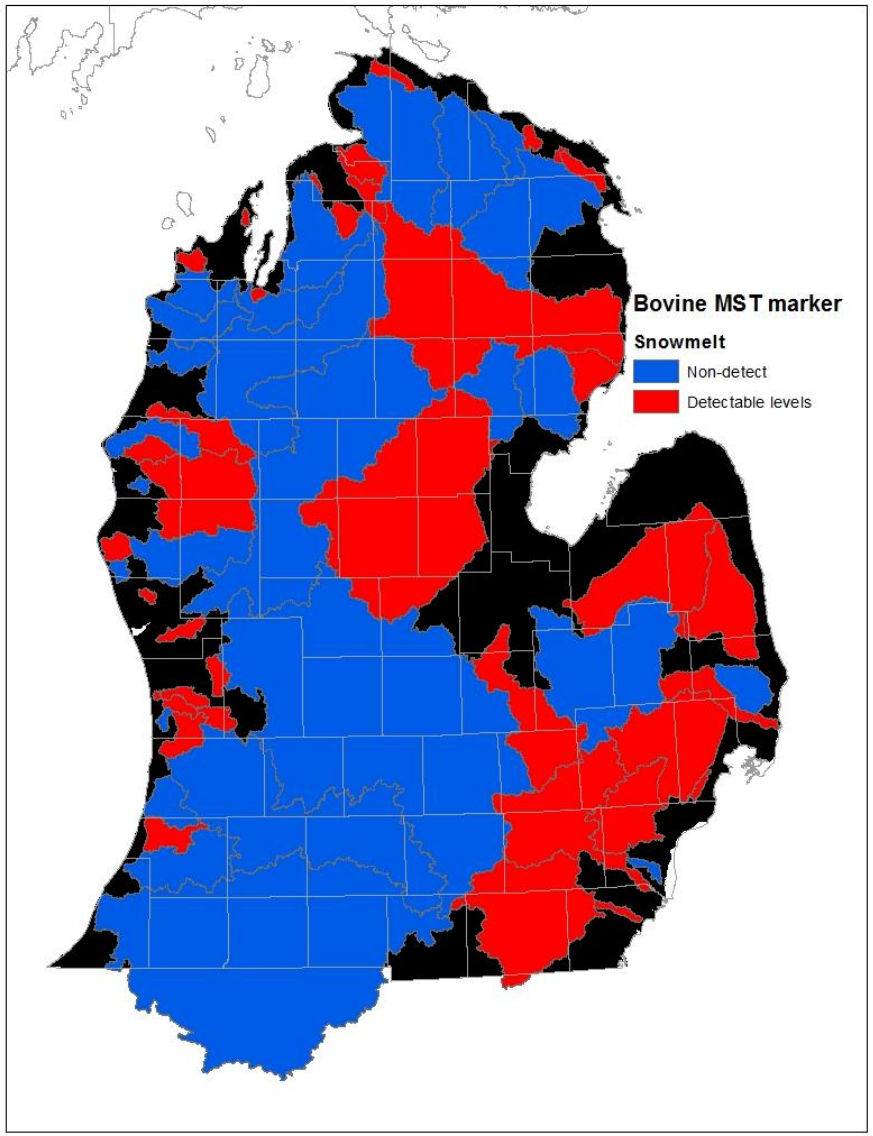
Baseflow – Bovine (PRE 58%)

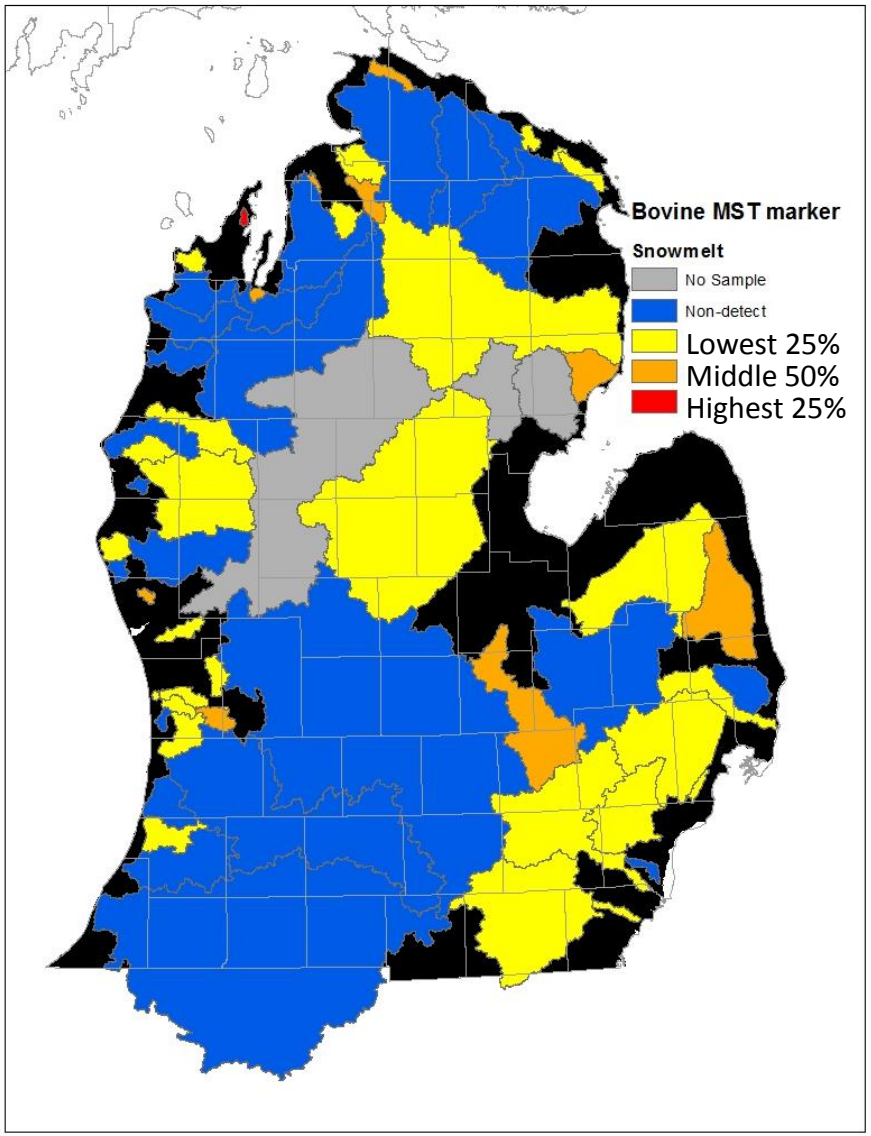
(PRE: Proportional Reduction Error, Percentage of Variance Explained)



* Bovine reported in log10. Detection limit = 0.11

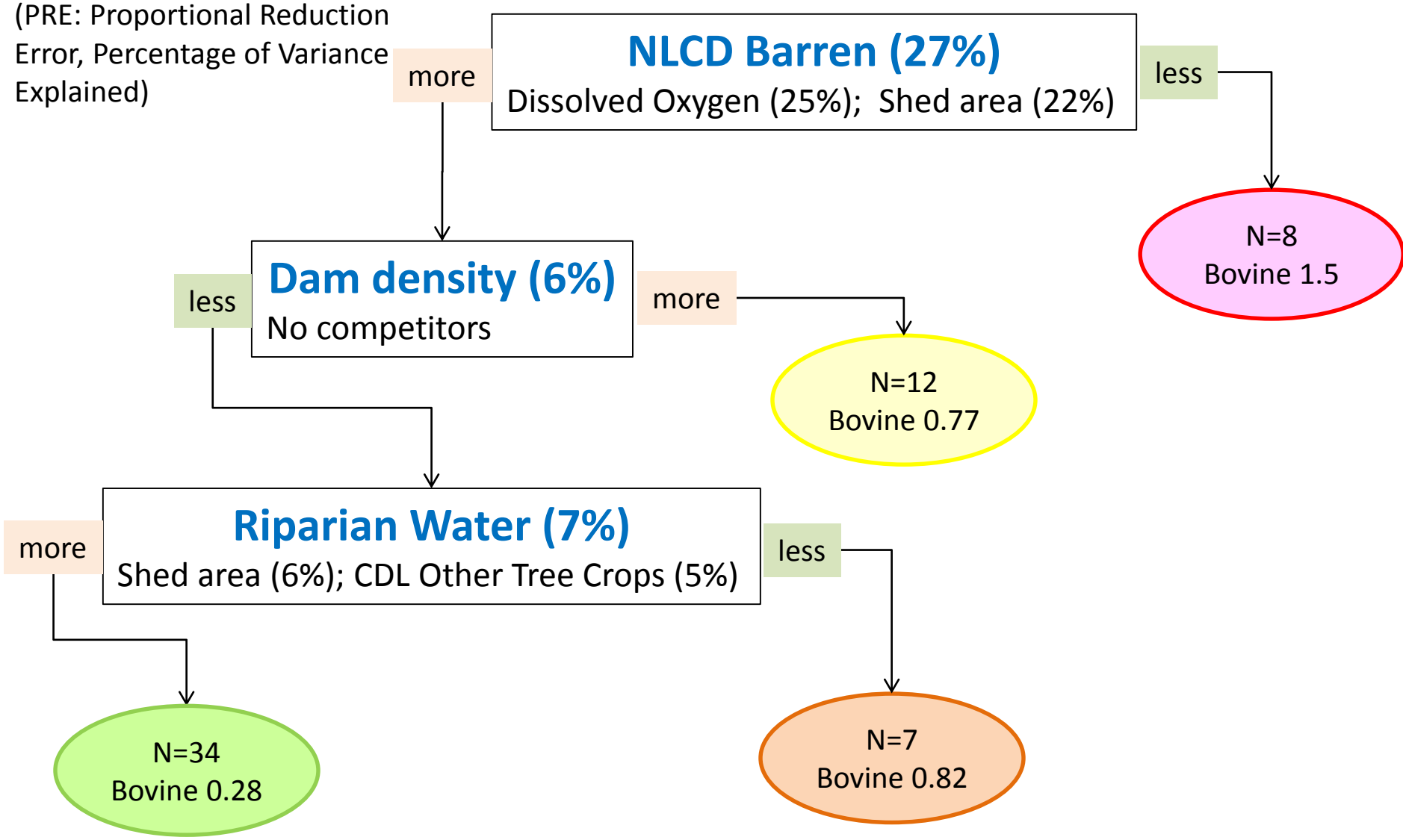
Snowmelt - Bovine





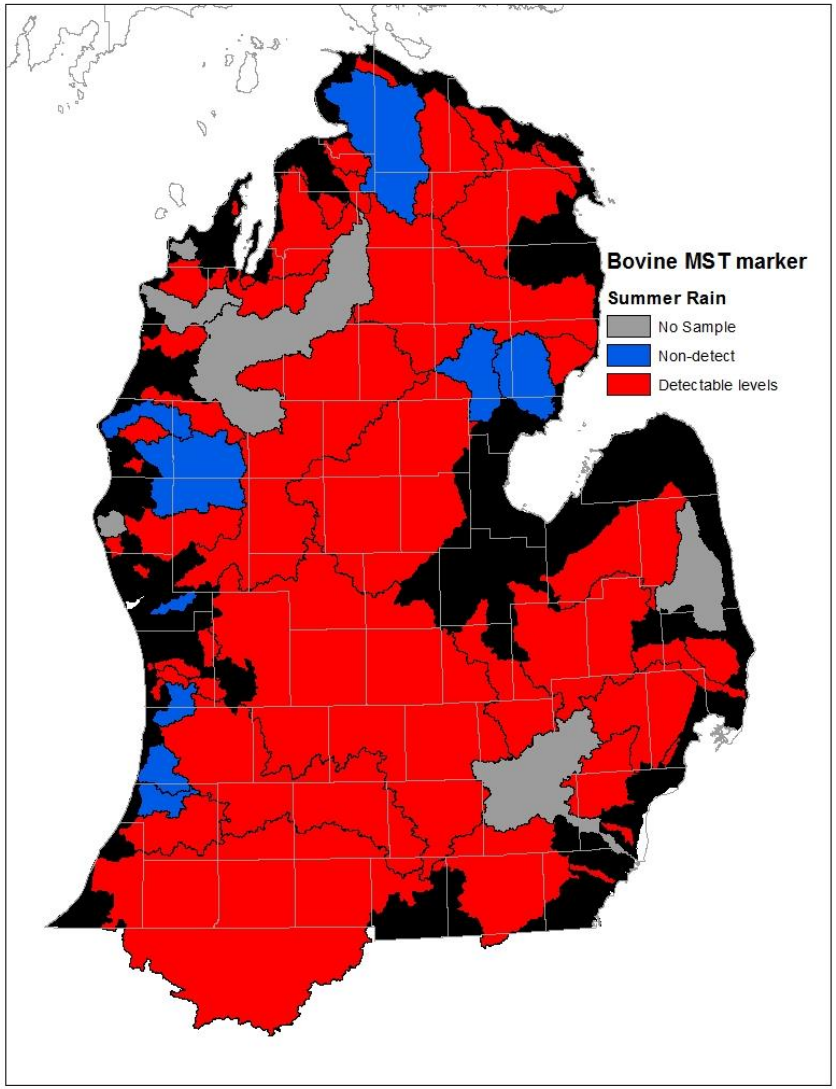
Snowmelt – Bovine (PRE 44%)

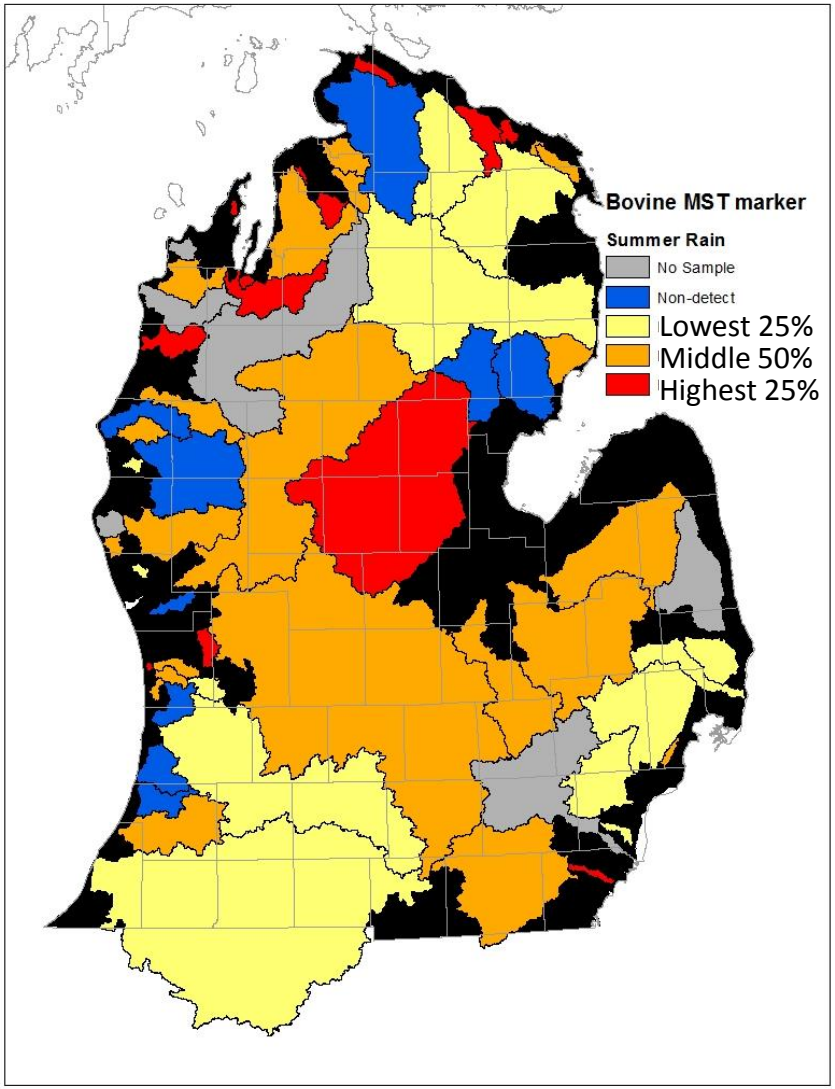
(PRE: Proportional Reduction Error, Percentage of Variance Explained)



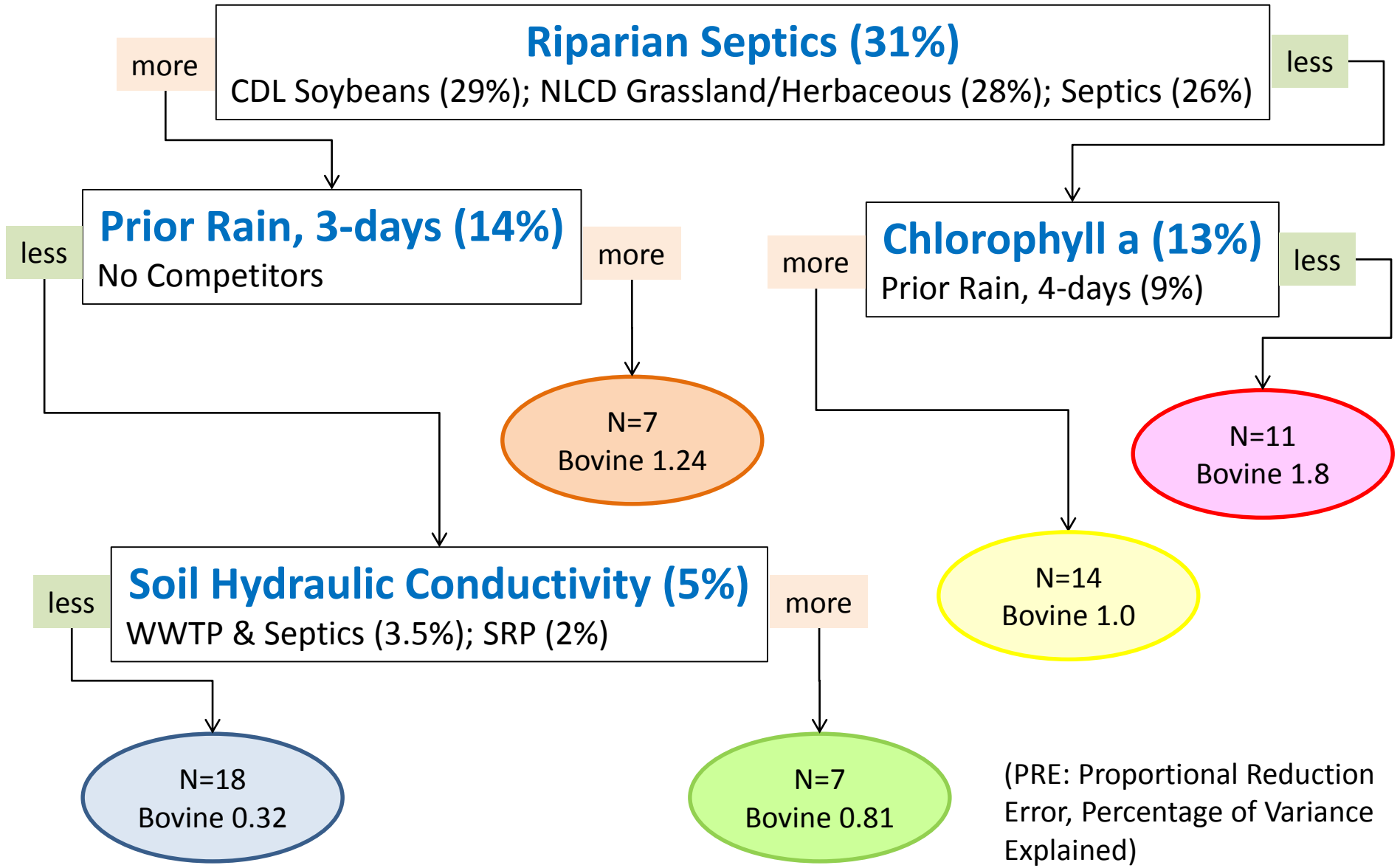
* Bovine reported in log10. Detection limit = 0.11

Summer Rain - Bovine





Summer Rain – Bovine (PRE 63%)

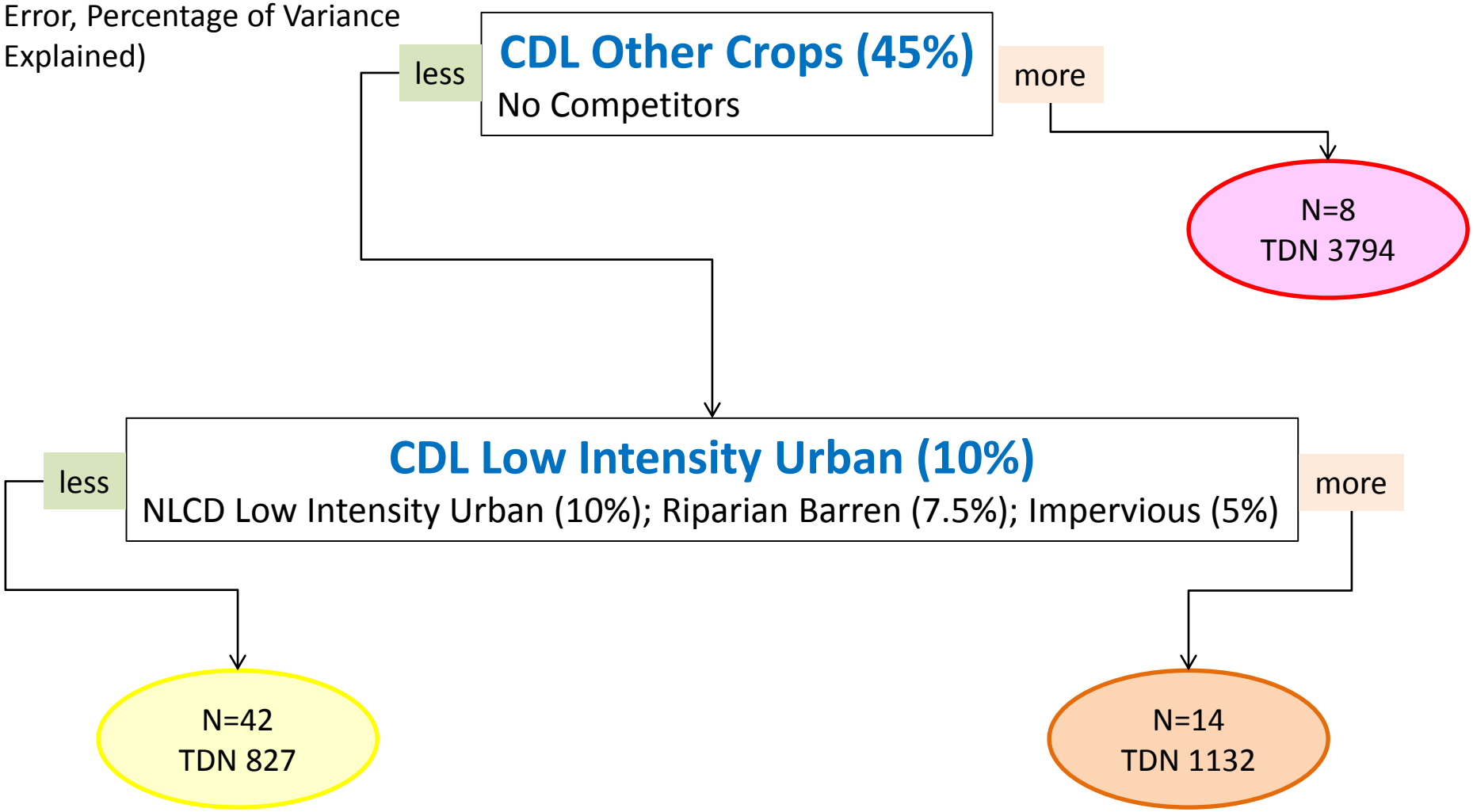


(PRE: Proportional Reduction Error, Percentage of Variance Explained)

* Bovine reported in log10. Detection limit = 0.11

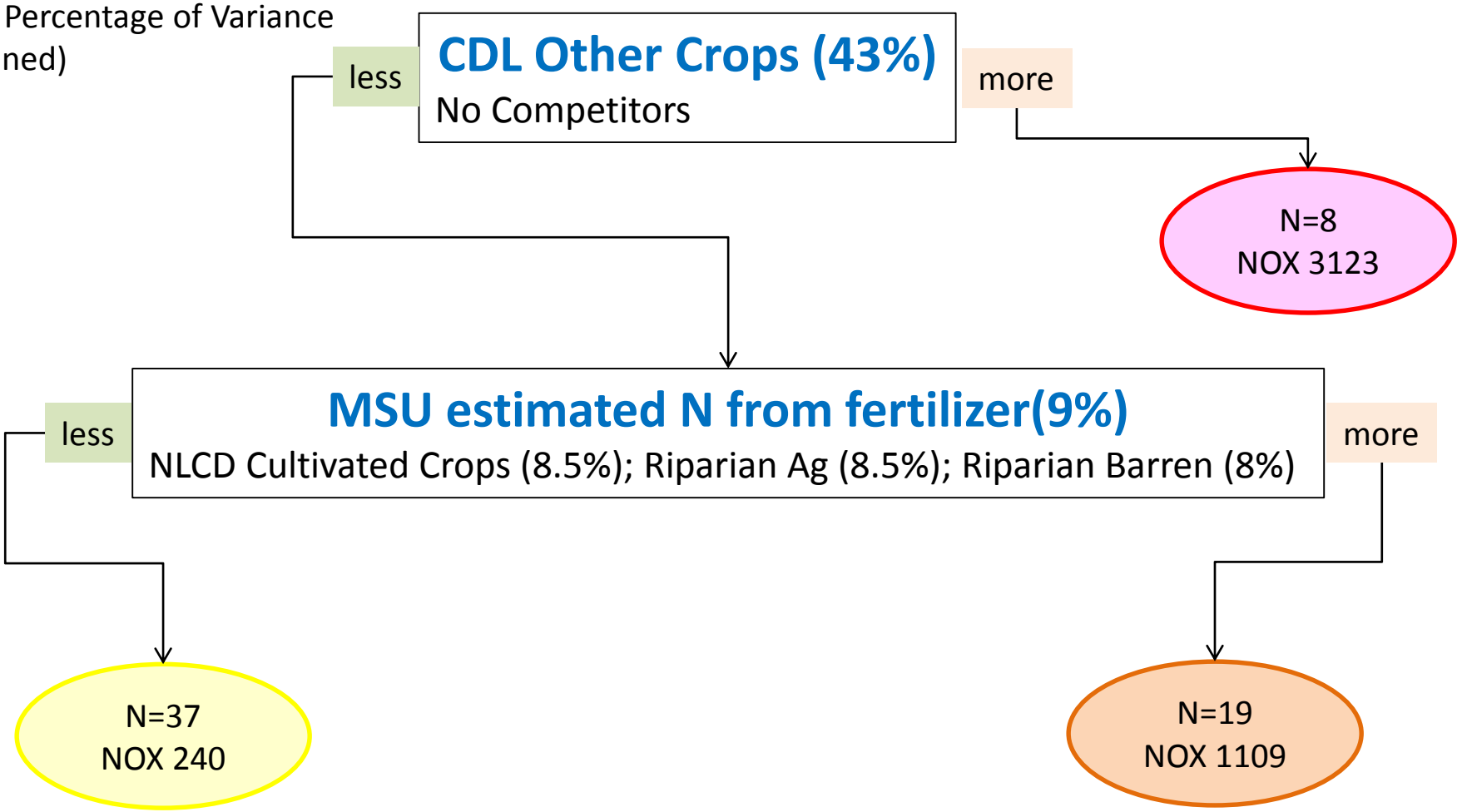
Baseflow – TDN (PRE 58%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)

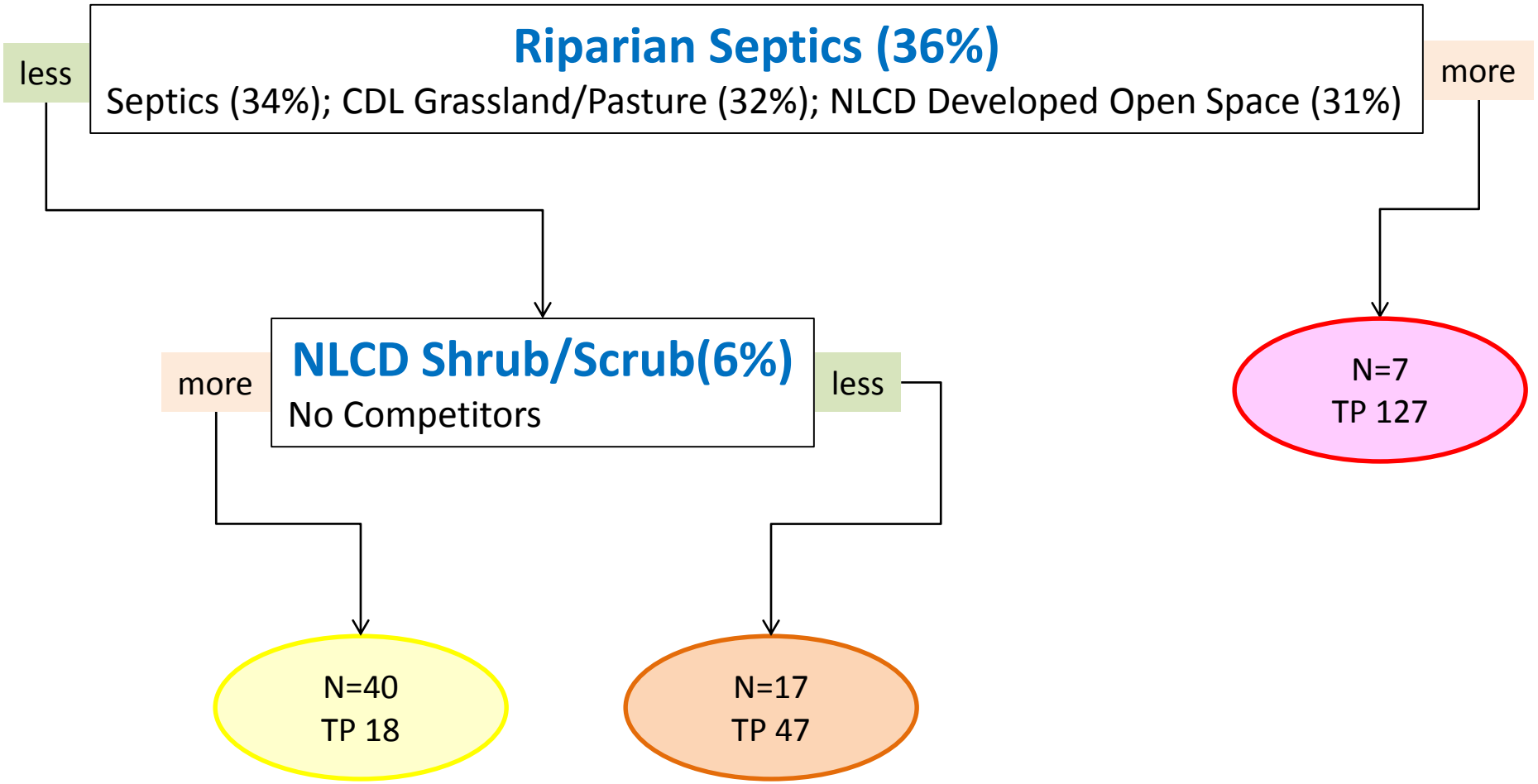


Baseflow – NOX (PRE 52%)

(PRE: Proportional Reduction Error, Percentage of Variance Explained)



Baseflow – TP (PRE 42%)



(PRE: Proportional Reduction Error, Percentage of Variance Explained)

Baseflow CART Results top variables explaining the dependent variables.

Bovine marker

- Woody wetlands
- NOX &TDN (inverse relationship)
- Less open water (inverse relationship)
- Grassland/pasture

TDN

- Other crops
- Low intensity urban
- Impervious surfaces

NOX

- Other crops
- N from fertilizer

TP

- Total septic tanks in 60 m buffer (riparian zones)
- Less shrubs (inverse relationship)

Snow melt CART Results top variables explaining the dependent variables.

Bovine marker

- Less barren soils (inverse relationship)
- Lower DO (inverse relationship)
- Smaller watersheds
- More dams
- Less riparian areas

(inverse relationship)

TDN

- N-fertilizer with AG
- Potassium
- Corn

NOX

- Corn
- Magnesium
- Cultivated crops

TP

- Winter wheat
- Specific conductance
- Less Calcium (inverse relationship)

Summer Rain CART Results top variables explaining the dependent variables

Bovine marker

- Soy and grass lands
- Less Septic tanks
- Less Chlorophyll a (inverse relationship)
- Total precipitation in the prior 3 days

TDN

- Total precipitation in the prior 4 days

NOX

- Less barren land (inverse relationship)
- Dry beans

TP

- Less Open land, grasses (inverse relationship)
- Corn
- Total precipitation in the prior 8 days in small watersheds

Impact*	River Name	Watershed Area (km2)	Counties
HIGH	Little Pigeon Creek	14	Ottawa
HIGH	Belangers Creek	25	Leelanau
HIGH	Monroe Creek	27	Antrim, Charlevoix
HIGH	Little Trout River	28	Presque Isle
HIGH	Silver Creek	41	Muskegon
HIGH	Harrington Drain	53	Macomb, Wayne
HIGH	Trout River	82	Presque Isle
HIGH	Sandy Creek	82	Monroe
HIGH	Carp River	119	Cheboygan, Emmet
HIGH	Rush Creek	152	Kent, Ottawa
HIGH	Boyne River	199	Antrim, Charlevoix, Otsego
HIGH	Lincoln River	215	Mason
HIGH	Tawas River	403	Iosco
HIGH	Black River	1250	Lapeer, St. Clair, Sanilac
HIGH	Cass River	2174	Genesee, Huron, Lapeer, Saginaw, Sanilac, Tuscola
HIGH	Au Sable	5287	Alcona, Crawford, Iosco, Kalkaska, Montmorency, Ogemaw, Oscoda, Otsego, Roscommon
HIGH	Tiltabawasee River	6211	Arenac, Bay, Clare, Gladwin, Gratiot, Isabella, Mecosta, Midland, Montcalm, Ogemaw, Osceola, Roscommon

Impact*	River Name	Watershed Area (km2)	Counties
Low	Macatawa River	292	Allegan, Ottawa
Low	North Branch Black River	398	Allegan, Van Buren
Low	Pine River	440	Macomb, St. Clair
Low	Big Sable River	476	Lake, Manistee, Mason
Low	Boardman	716	Grand Traverse, Kalkaska
Low	Rifle	858	Arenac, Gladwin, Ogemaw, Roscommon
Low	White River	1049	Muskegon, Newaygo, Oceana
Low	Elk-Torch	1308	Antrim, Charlevoix, Grand Traverse, Kalkaska, Otsego
Low	Pere Marquette	1790	Lake, Mason, Newaygo, Oceana
Low	Cheboygan	2317	Charlevoix, Cheboygan, Emmet, Otsego
Low	Manistee	3559	Antrim, Benzie, Crawford, Grand Traverse, Kalkaska, Lake, Manistee, Missaukee, Osceola, Otsego, Wexford
Low	Muskegon	6418	Clare, Crawford, Kalkaska, Lake, Mecosta, Missaukee, Montcalm, Newaygo, Osceola, Roscommon, Wexford
Low	St. Joseph	11061	Berrien, Branch, Calhoun, Cass, Hillsdale, Kalamazoo, St. Joseph, Van Buren
Low	Grand	12854	Allegan, Barry, Calhoun, Clinton, Eaton, Gratiot, Hillsdale, Ingham, Ionia, Isabella, Jackson, Kent, Lenawee, Livingston, Mecosta, Montcalm, Muskegon, Newaygo, Ottawa, Shiawassee, Washtenaw

Key Findings

- ddPCR lacks the shortfalls of qPCR and is quite useful technique for quantitative assessment of the markers
- The bovine marker is related to the land use and crops, indirectly related to source of nutrients.
- During baseflow inverse relationship between bovine marker and nitrogen (TN, TDN and NOX), where more low intensity urban and septic tanks were associated with the nutrients.
- Nutrients are associated with crops including corn, estimated fertilizer applications.
- The bovine marker is found when it rains and coming in from runoff, nutrients are also related to rain.

Discussion

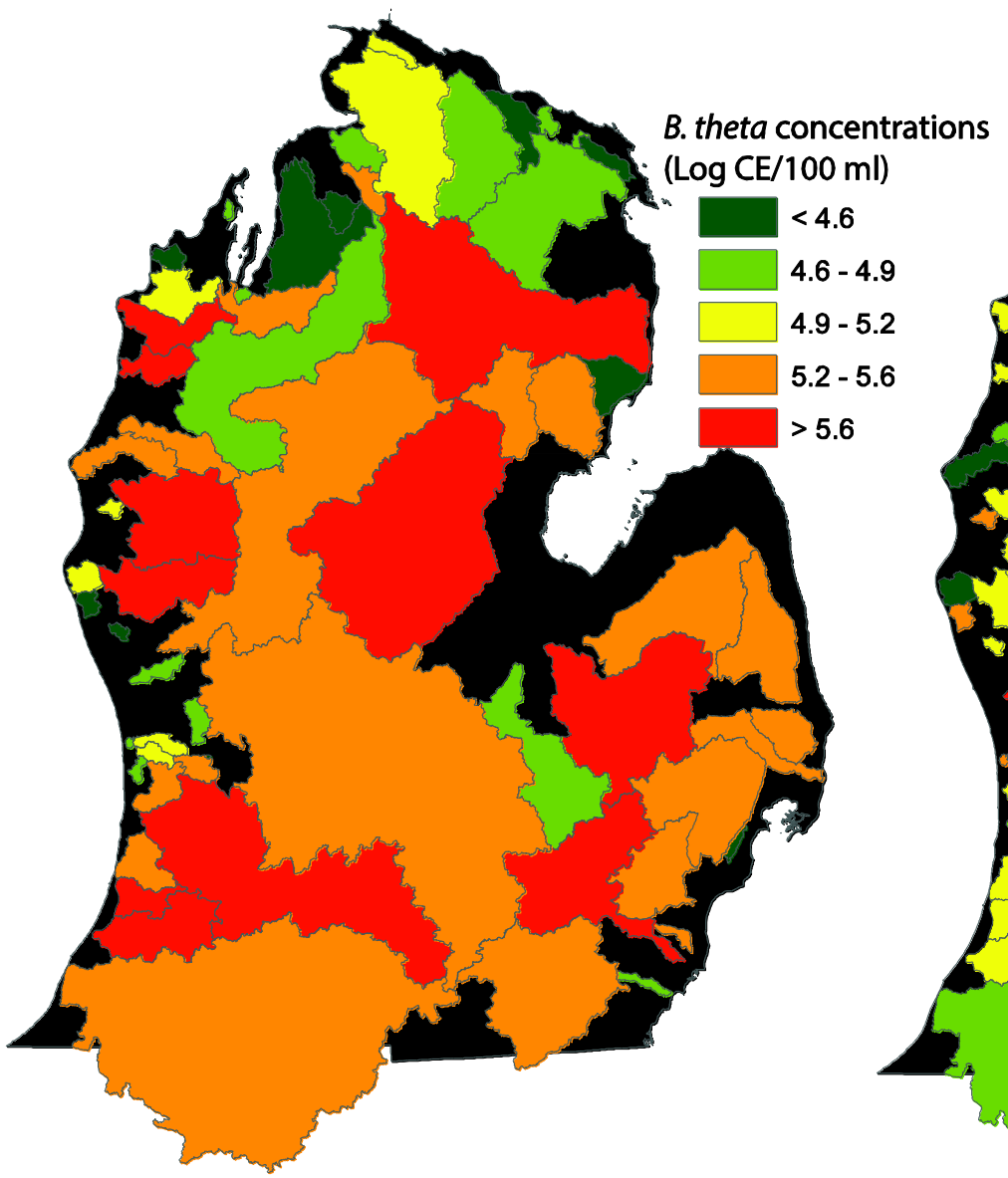
- We are beginning to understand the sources of fecal pollution in relationship to the landscape and climate factors (rain).
- The bovine marker could come from both manure and free ranging cattle, the pig marker may help elucidate this.
- *While E.coli* did have a relationship to phosphorous suggesting some relationship to fecal sources, the bovine marker was indirectly related to the nutrients.
- The transport of nutrients needs to be further evaluated in regard to the transport of the bacteria. Is accumulation in soils and/or sediments and later release delay the timing of the nutrient observations in the water column.
- We have identified 17 watersheds where the bovine marker was always present and 14 watersheds that did not have the marker or where is was present only once. These watersheds can be used to identify the impacts of agricultural management practices on water quality.
- Distinguish water quality impacts of fertilizer from those derived from human waste and animal waste/manure used on crop land
  - Focus efforts for water quality improvement
  - Elucidate relationships between agricultural characteristics, nutrient loading, and sources at the watershed scale to resulting water quality across the entire lower peninsula of Michigan.

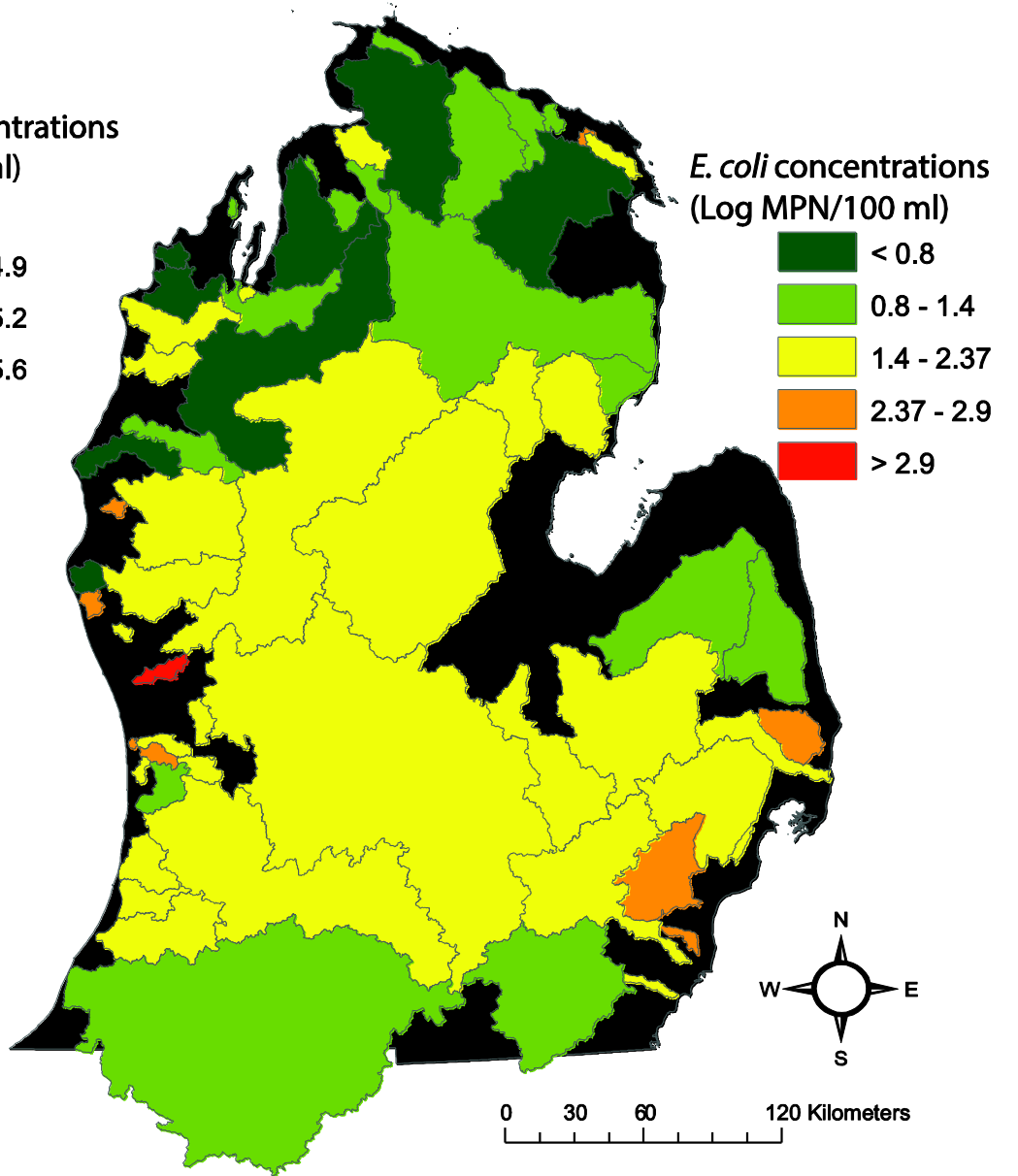
Next Steps

- Porcine MST marker will be completed in the next few months
- Bird marker analysis will be completed by May
- Further ion analysis will be ongoing to address manure versus fertilizer
- Key counties and watersheds with various BMPs will be identified for future studies.

THANK YOU

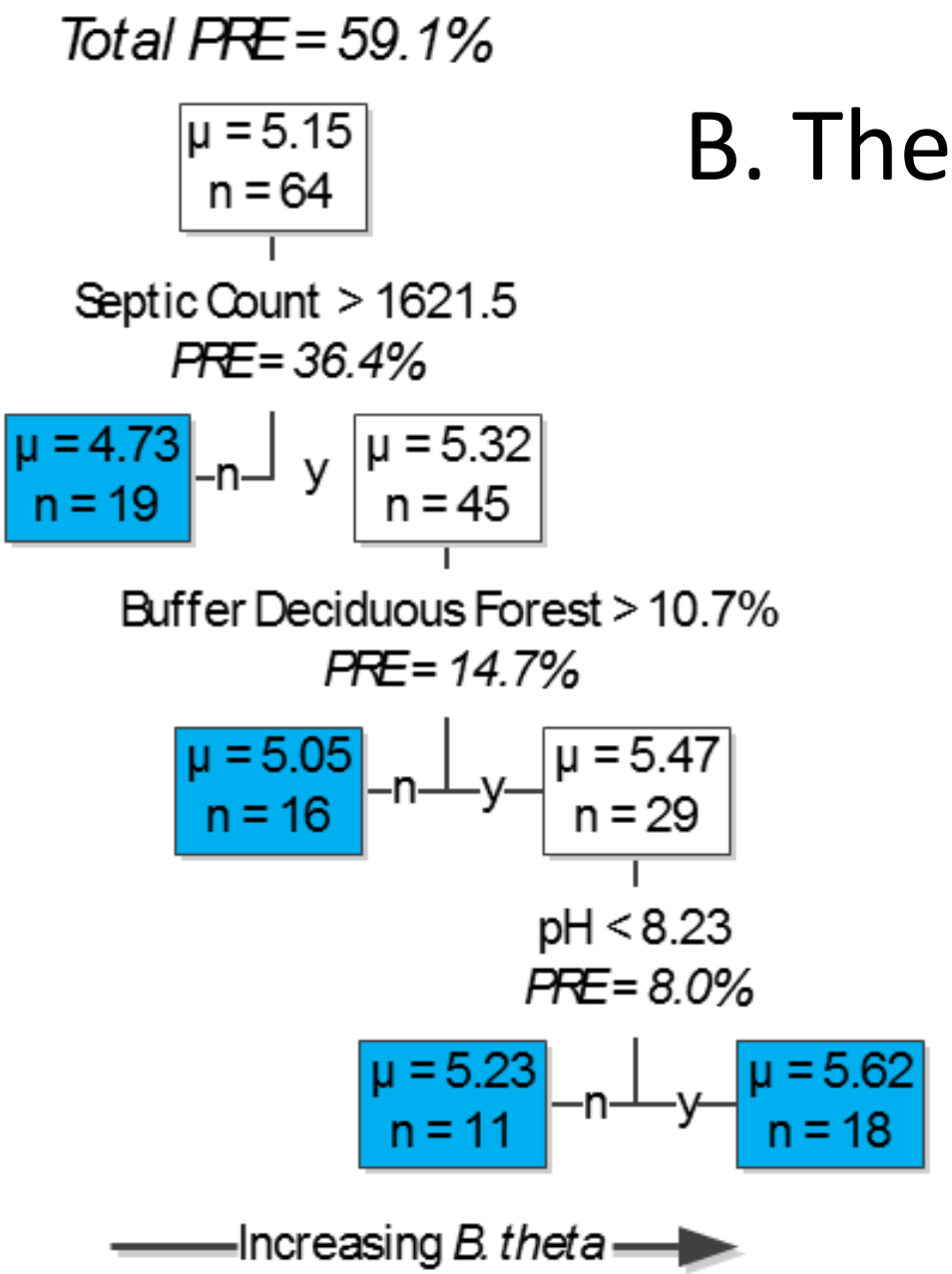
Previous Results – *B. theta* and *E.coli* concentrations during base flow





Baseflow

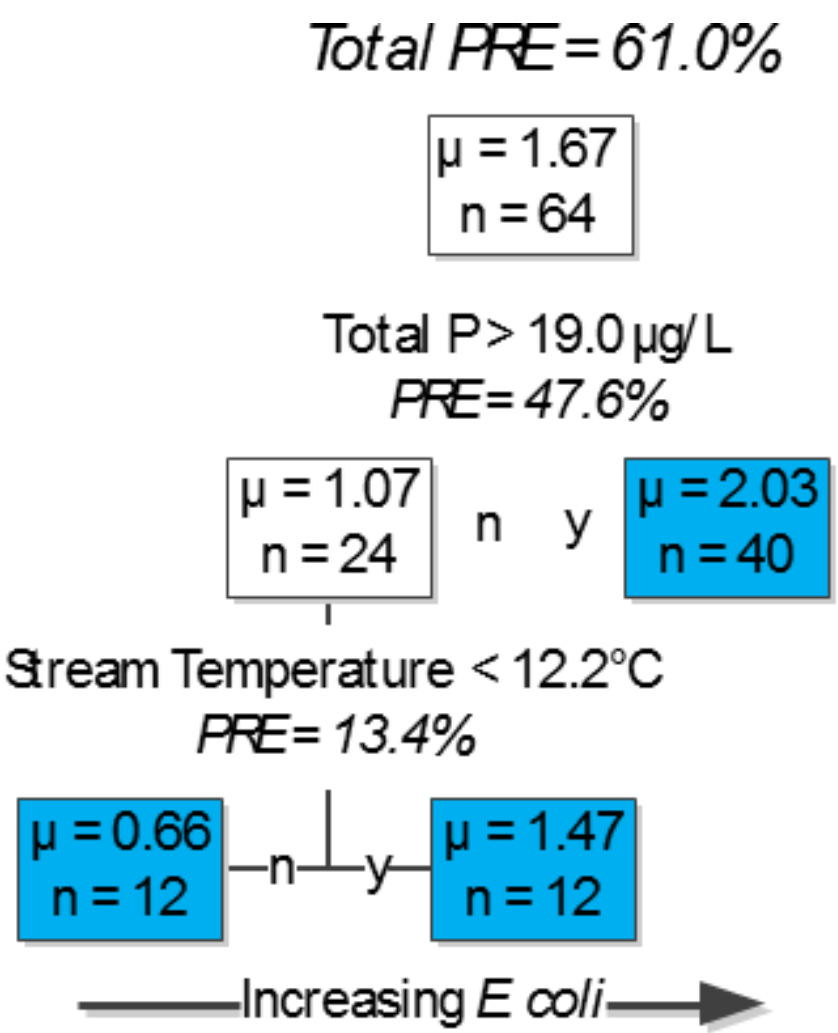
B. Theta CART statistics



- B.theta related to increasing septic tank counts
- Then Buffer deciduous forest
- Then pH

PRE: Proportional Reduction Error, Percentage of Variance Explained

Previous Results
Baseflow CART– *E. coli*



- *E.coli* related to Total phosphorous
- Then stream temperature

PRE: Proportional Reduction Error, Percentage of Variance Explained