Los Alamos
NATIONAL LABORATORY

Microbes as Soil Indicators

A DNA based Approach

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Soil Microbiology Project

- The Goal
  - Develop microbial indicators of changing soil status (C sequestration, nutrient availability).
- Summary of Progress
  - Strong correlations seen between bacterial signals (taxonomic) and soil carbon and nitrogen content.

A DNA Sequence-Based Approach to Soil Microbial Communities

- Current Limitation: Soil microbial communities are too complex and populous.
- New Approach: DNA sequence-based approaches can allow us to focus investigations on functionalities within these microbial communities.
- Probe soil microbe DNA for sequences unique to the specific gene(s) encoding the target function(s).
- Advantages:
  - Focus your investigation and thus minimize the impacts of the complexity.
  - Investigate the “un culturable” 90+% of the soil microbes.
- One method is Terminal Restriction Fragment (TRF) patterns.

Microbes as Soil Indicators

- *Our goal:* Use the genetic diversity for soil microbe functions as indicators of changing soil status (C sequestration, nutrient availability).

- Vast community of soil microbes carry out an array of functions.
  - Decompose plant biomass to supply energy and metabolites to soil organisms.
  - Mobilize mineral nutrients (N, P, Fe, etc.) for plants.
  - Cycle C and N back to atmosphere.
  - Metabolize contaminants.

- These functions are carried out by genetically encoded enzymes.
  - Nitrogen cycling: fixation, ammonia oxidation and denitrification.
  - Carbon accumulation - possibly glomalin
  - Biomass decomposition: cellulase and lignase.

Detecting Signals in Microbial Communities

Terminal Restriction Fragment (TRF) Patterns

Microbial DNA is Extracted from Soil Sample

Target Gene is Amplified and tagged with a Fluorescent-Label

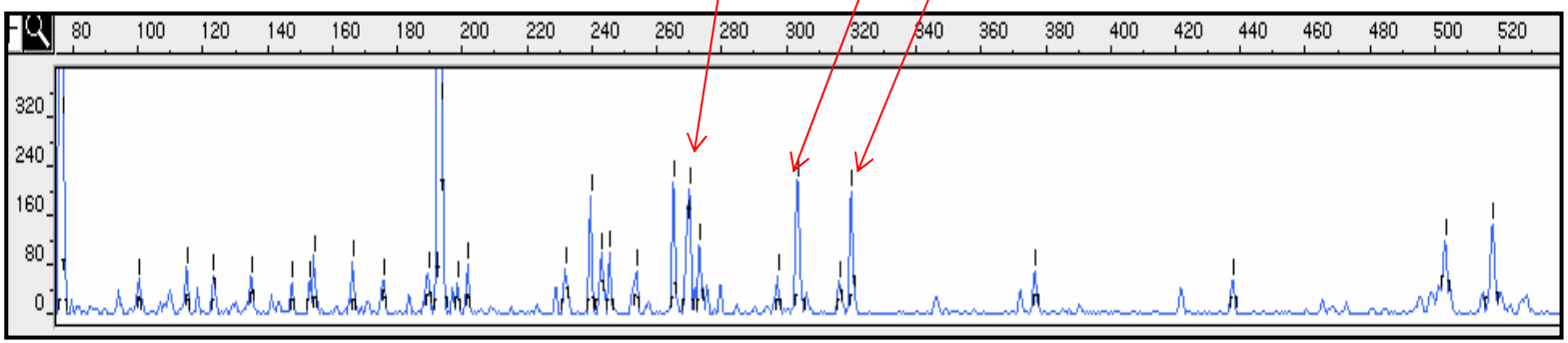
Labeled Target Gene is Cut into Fragments by a DNA sequence-specific enzyme

Species A

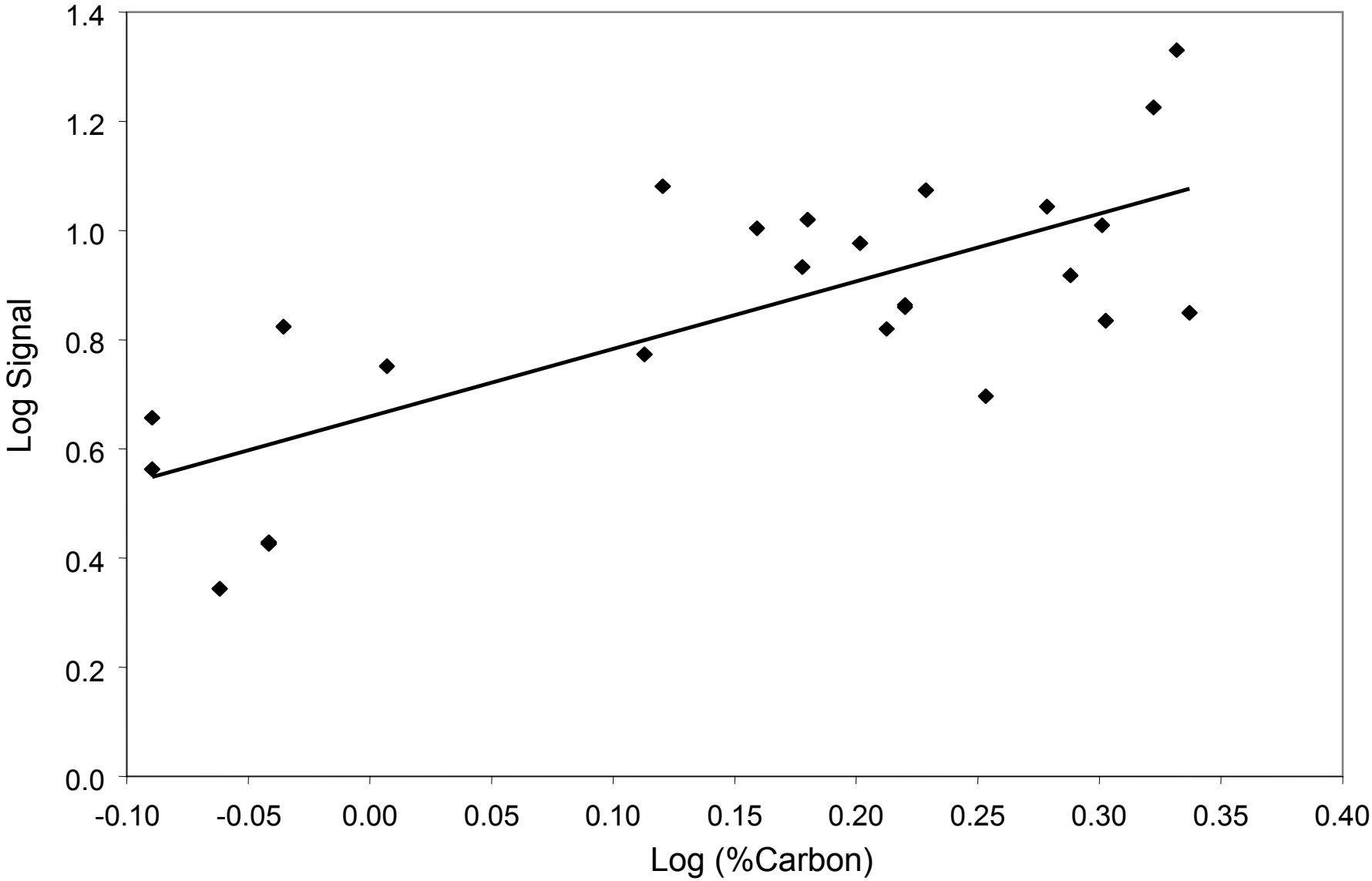
Species B

Species C

Fluorescent tag allows the fragments to be detected after separation by size.

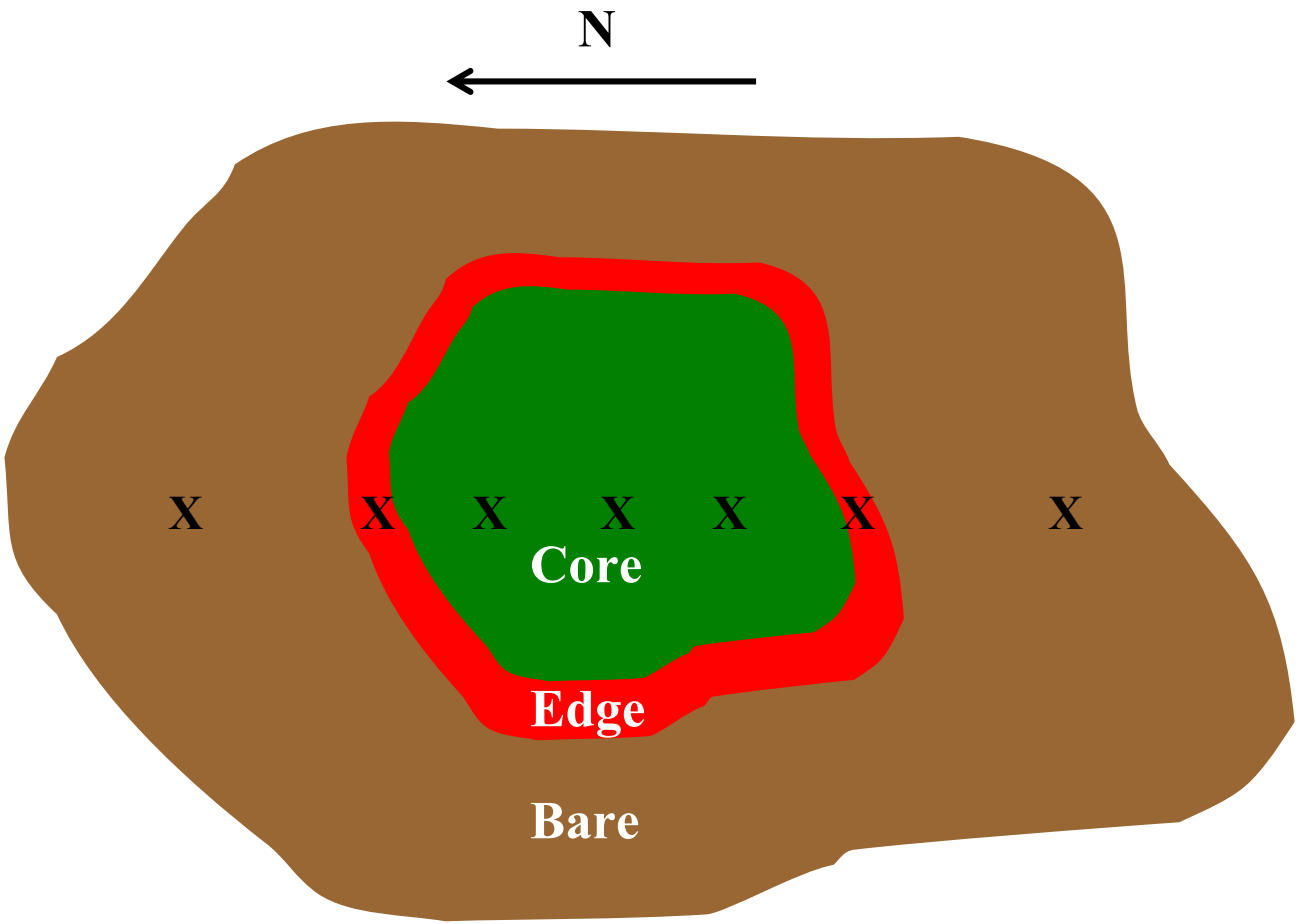


A Bacterial Signal of Soil Carbon Content

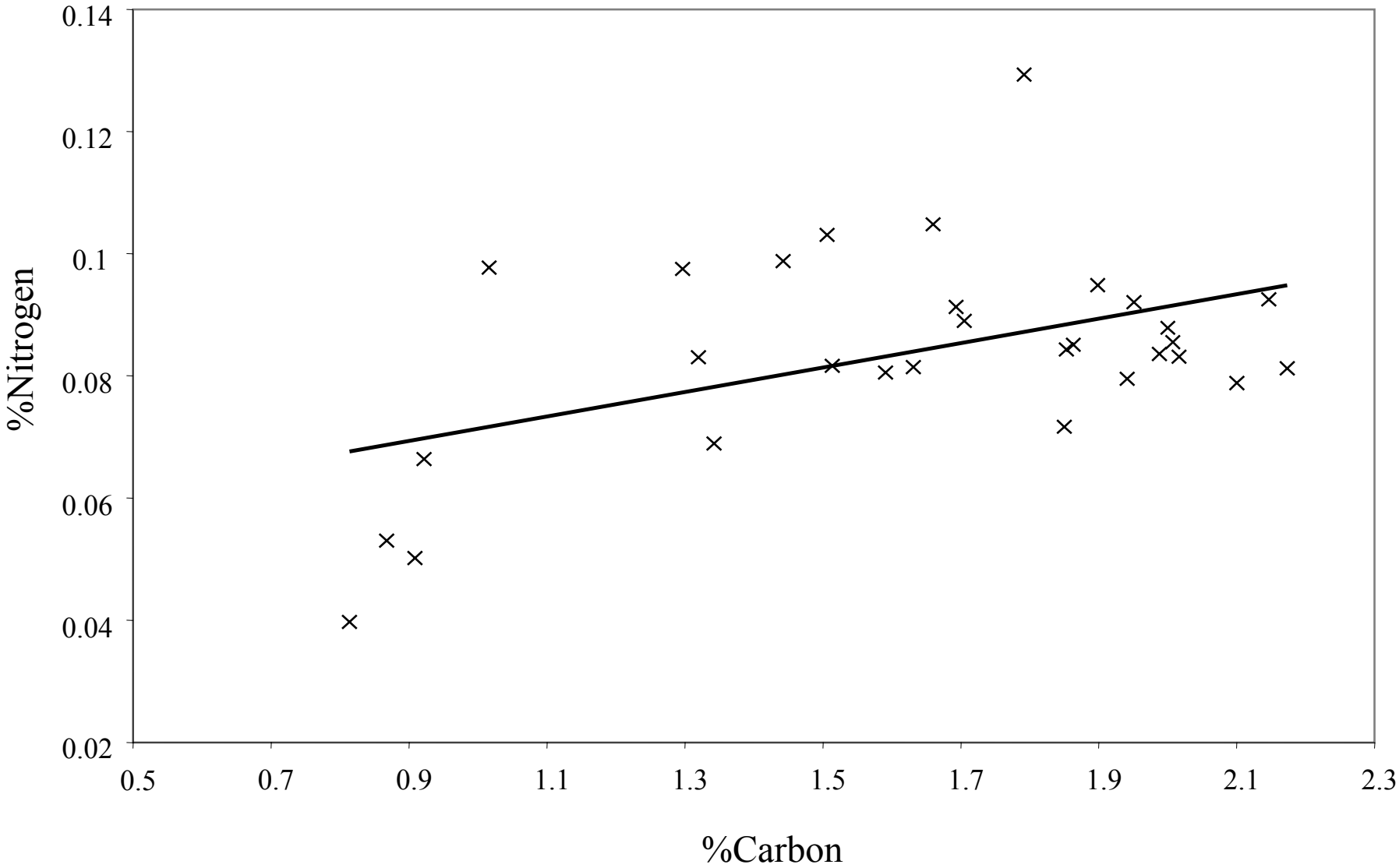


Grass Patch Sampling Scheme

(Designed to maximize nutrient differences)



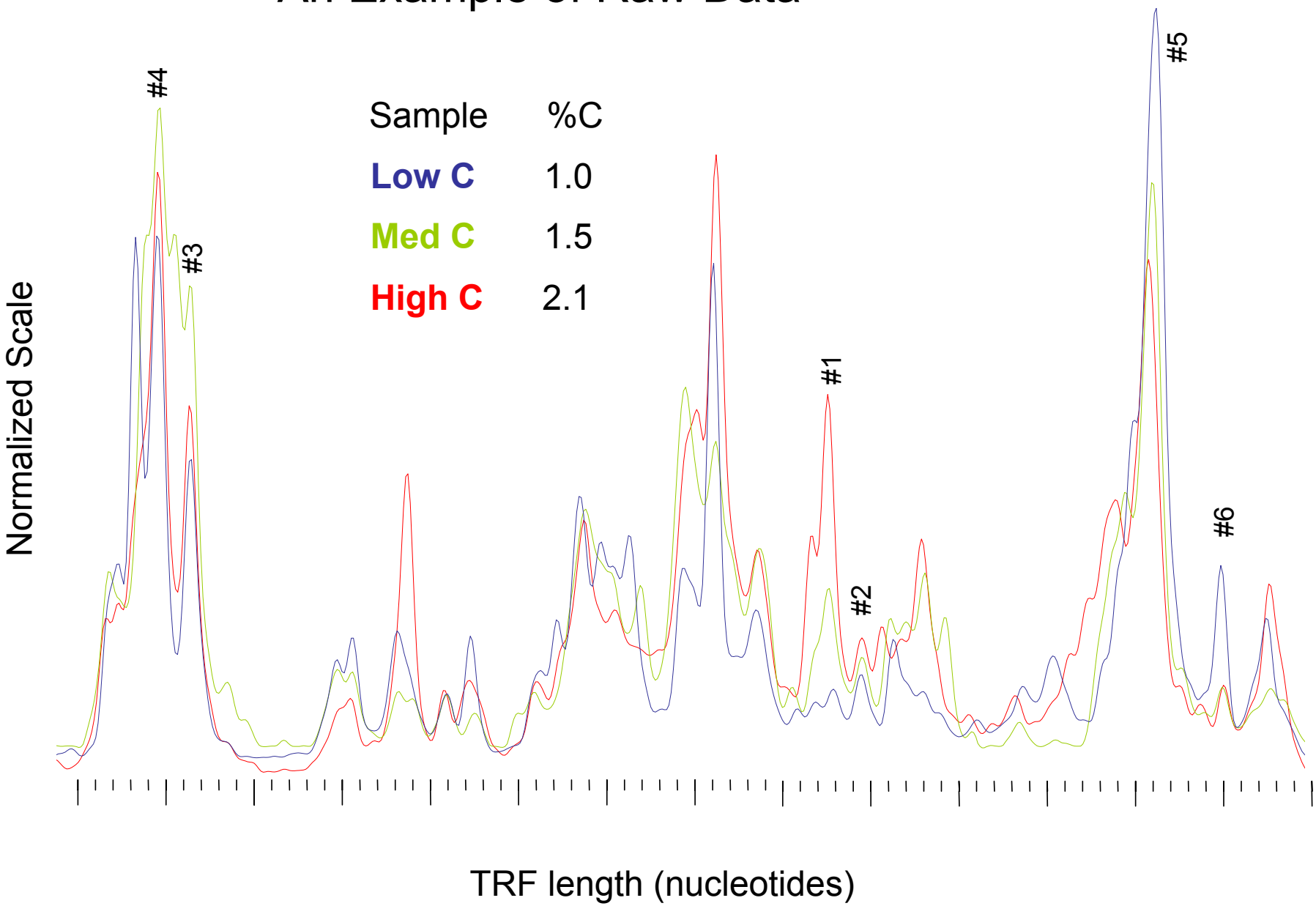
A Range of Carbon and Nitrogen Results



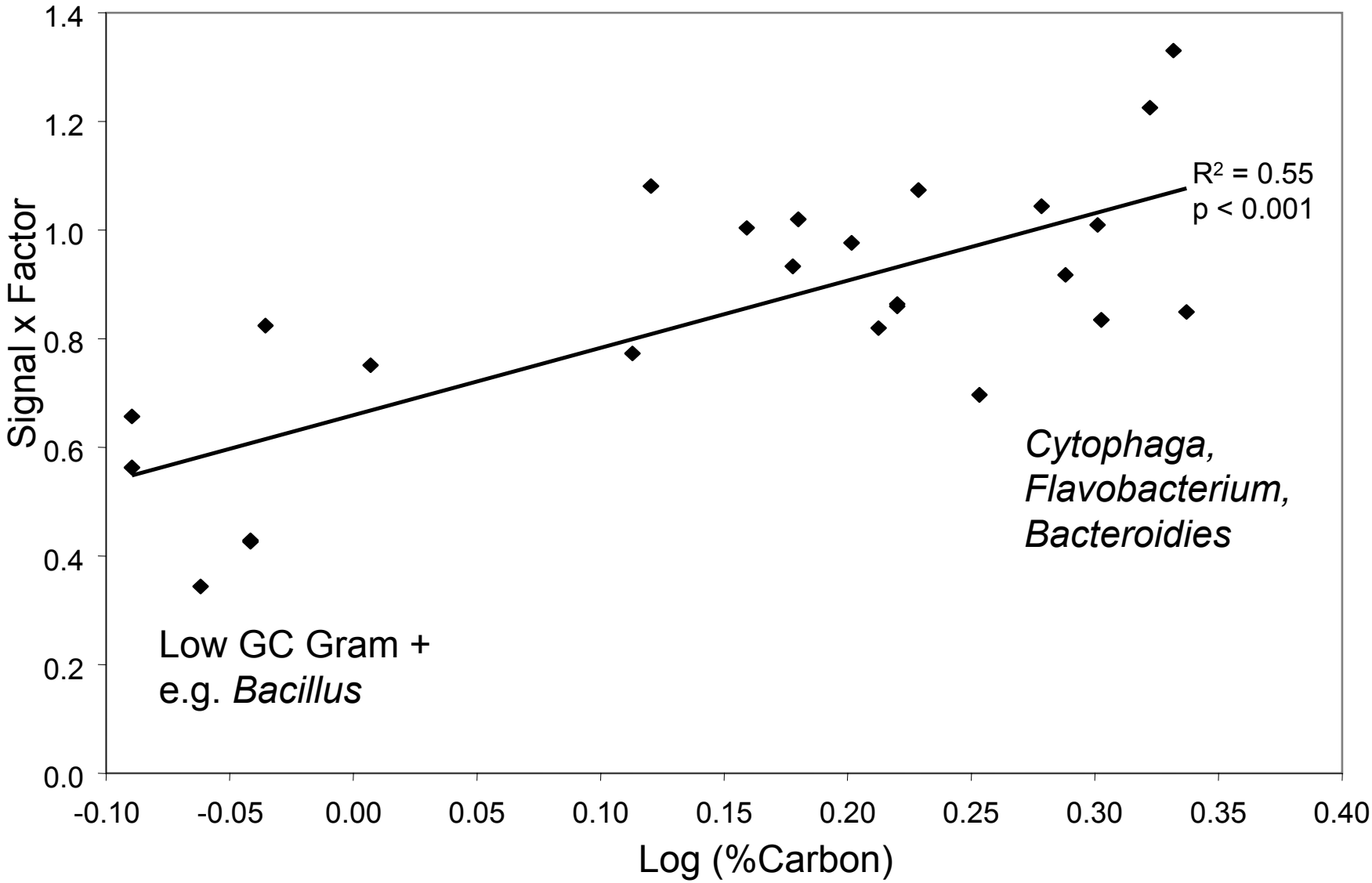
TRF Signal Correlations

TRF Signal #	Correlation to Percent Nitrogen	Correlation to Percent Carbon
1	0.55	0.70
2	0.67	0.67
3	0.56	0.63
4	0.54	0.57
5	-0.50	-0.36
6	-0.39	-0.64

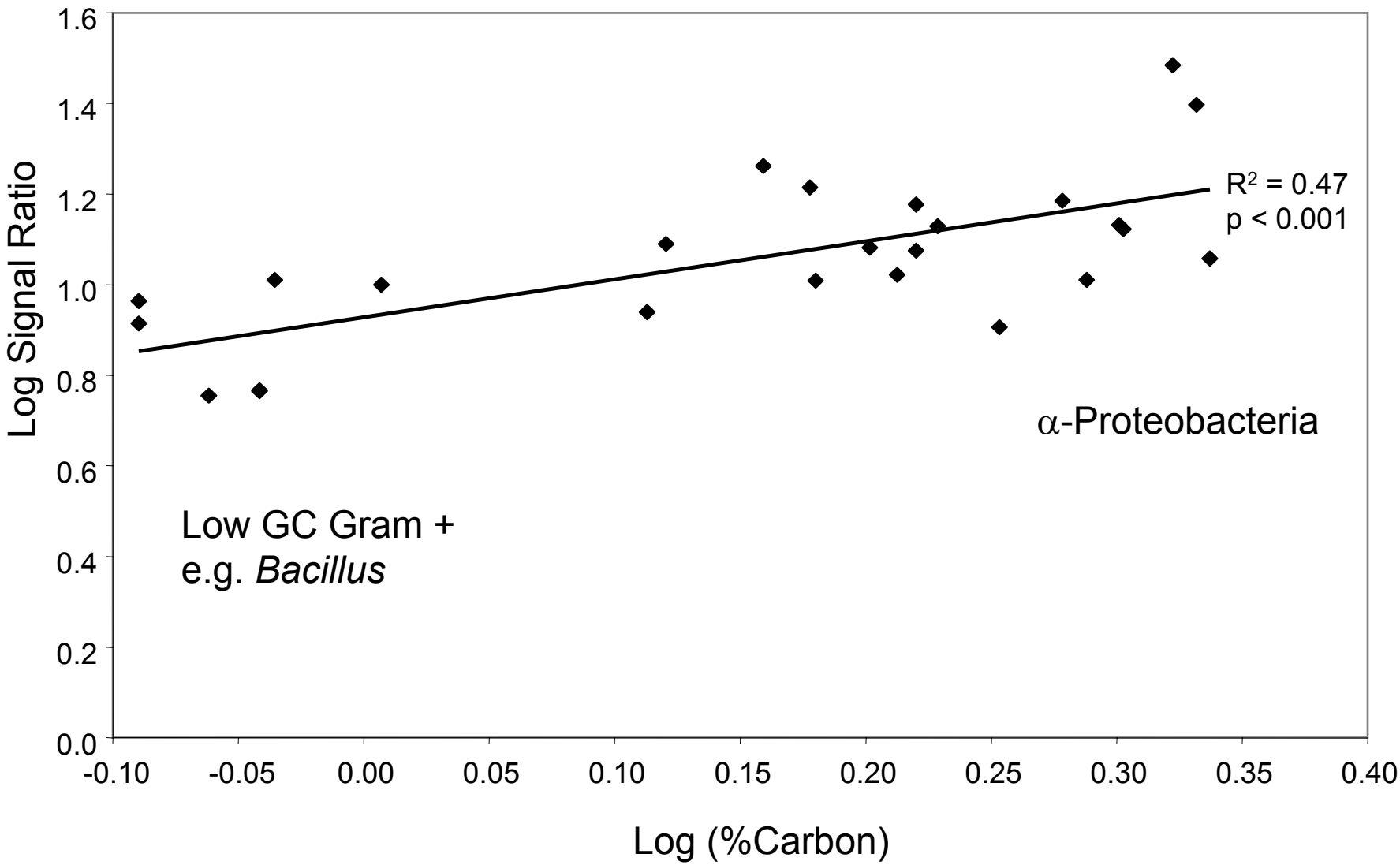
An Example of Raw Data



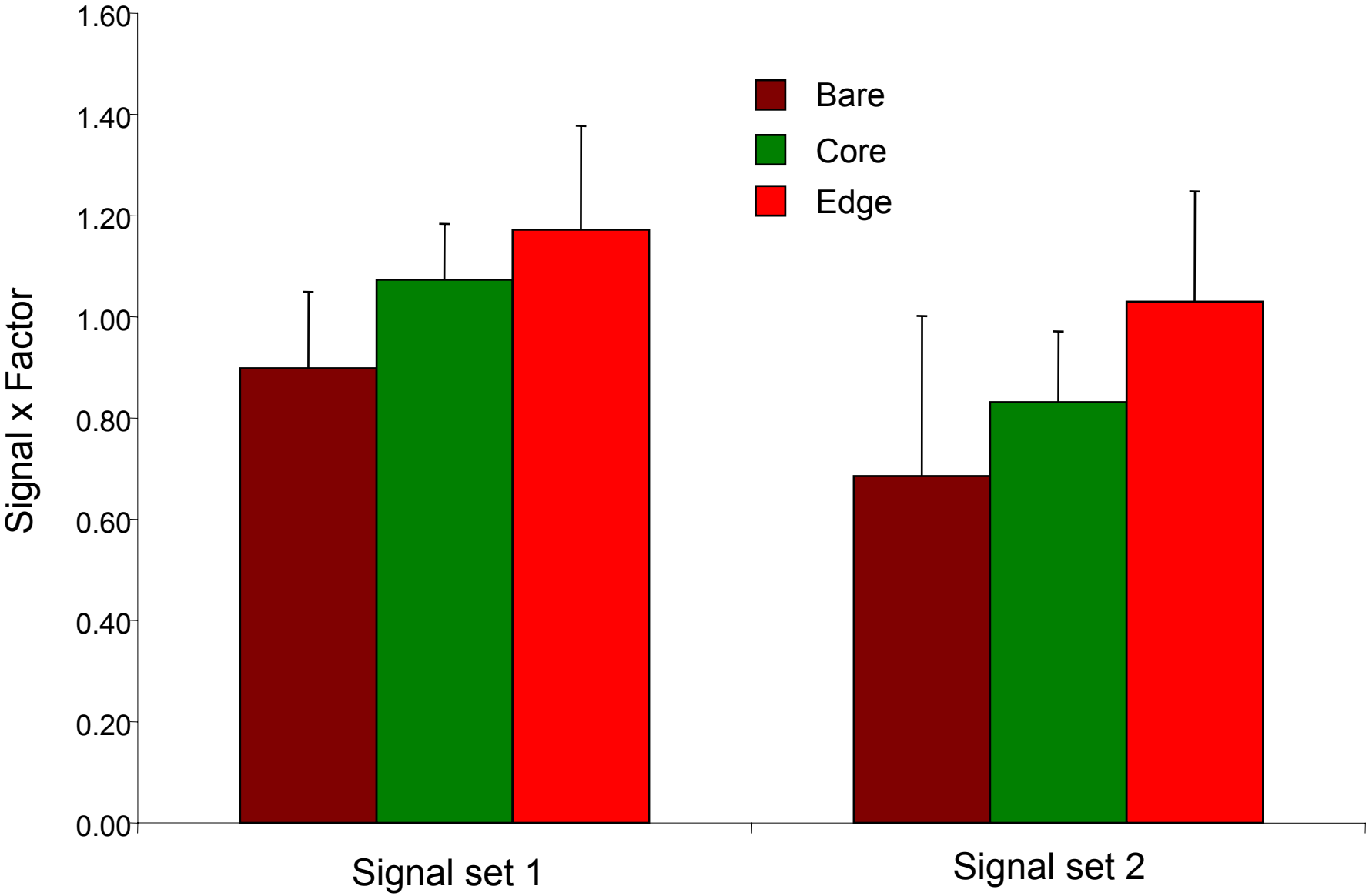
Signals x Factor Correlates with % Carbon



Second Signal Also Correlates



The Highest Signal is in the Transition Zone



Summary

- The abundance of certain bacterial types changes predictably with soil status.
  - Groups of bacteria respond differently.
    - Some groups increase as soil C and N increase.
    - Others decrease as soil C and N increase.
- Signal x factor for these bacterial types can be used as an indicator.
  - More robust than simple abundance.
  - Multiple signals may ensure applicability to different soil types.

Future Directions

- Confirmation of bacterial signal.
  - Confirm signal efficacy for a variety of soil types and carbon ranges.
  - Confirm signal response to changing soil status (time series study).