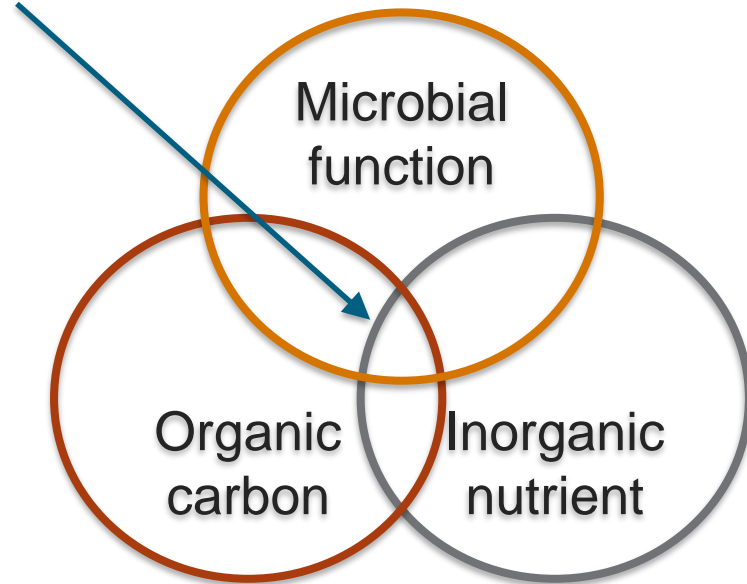


# Deciphering taxonomic carbon exchange between plants and microorganisms using proteomics coupled with $^{13}\text{C}$ tracers and spatially resolved protein extraction

JIM MORAN

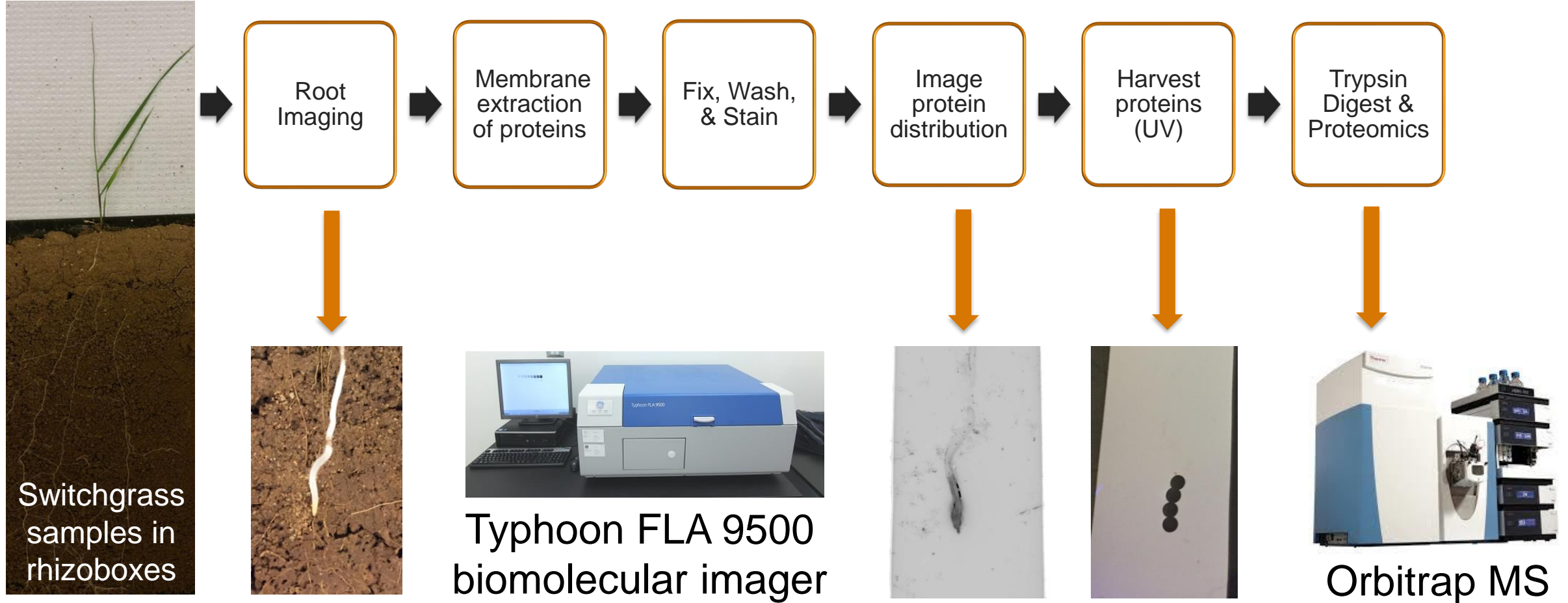
# Spatial heterogeneity drives nutrient relationships along the root-rhizosphere-soil continuum

Hotspot of  
nutrient  
exchange?



- ▶ Heterogeneity in soil provides a huge number of choices to roots
- ▶ Assumption: plants invest resources (i.e., carbon) at spatially focused locations having the highest potential for investment returns (i.e., nutrients or water)
- ▶ **Driving hypothesis:** hotspots of nutrient exchange occur at the confluence of needed organic carbon, microbial function, and inorganic nutrient accessibility

# Developing process work flow for spatially resolved proteomics in rhizosphere and soil systems



# Combined staining and imaging enable spatially resolved proteomic sampling



Sampled  
root



Visual  
image



SYPRO  
stain



Extraction  
locations

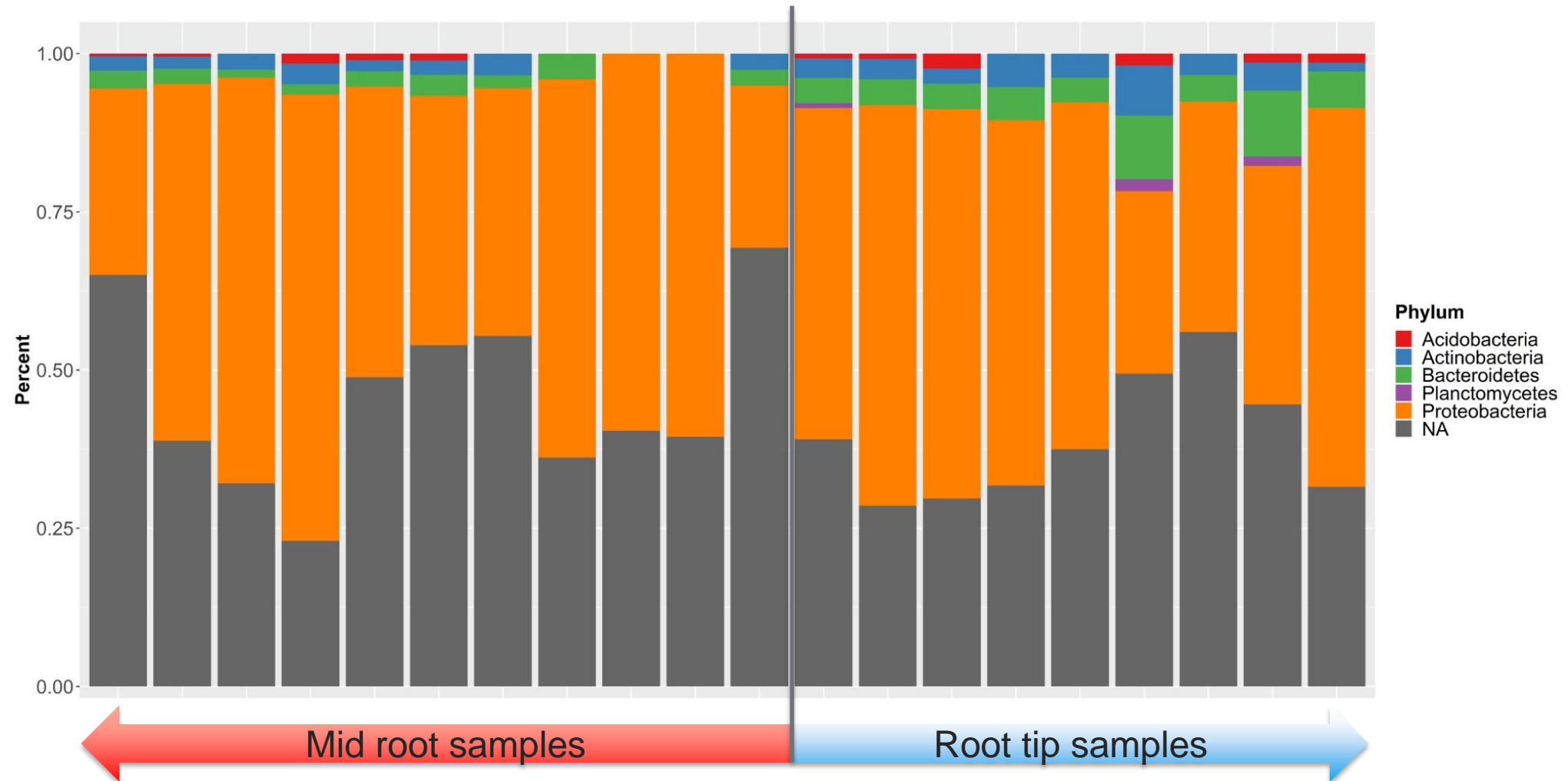


Samples  
from mature  
root

Samples  
from root tip

0.5 cm

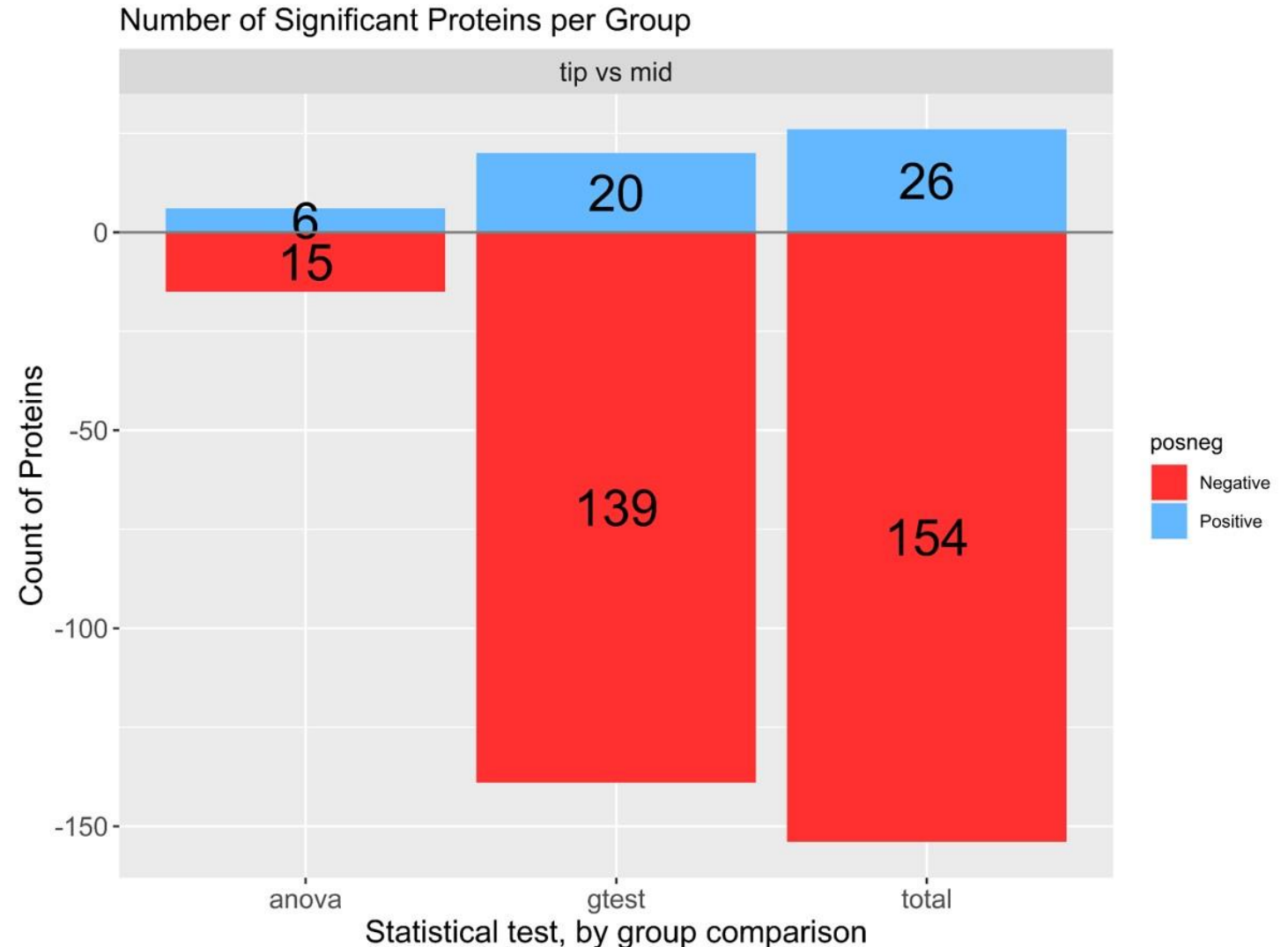
# Phylum level taxonomic distribution shows likely shift in community composition based on sampling location



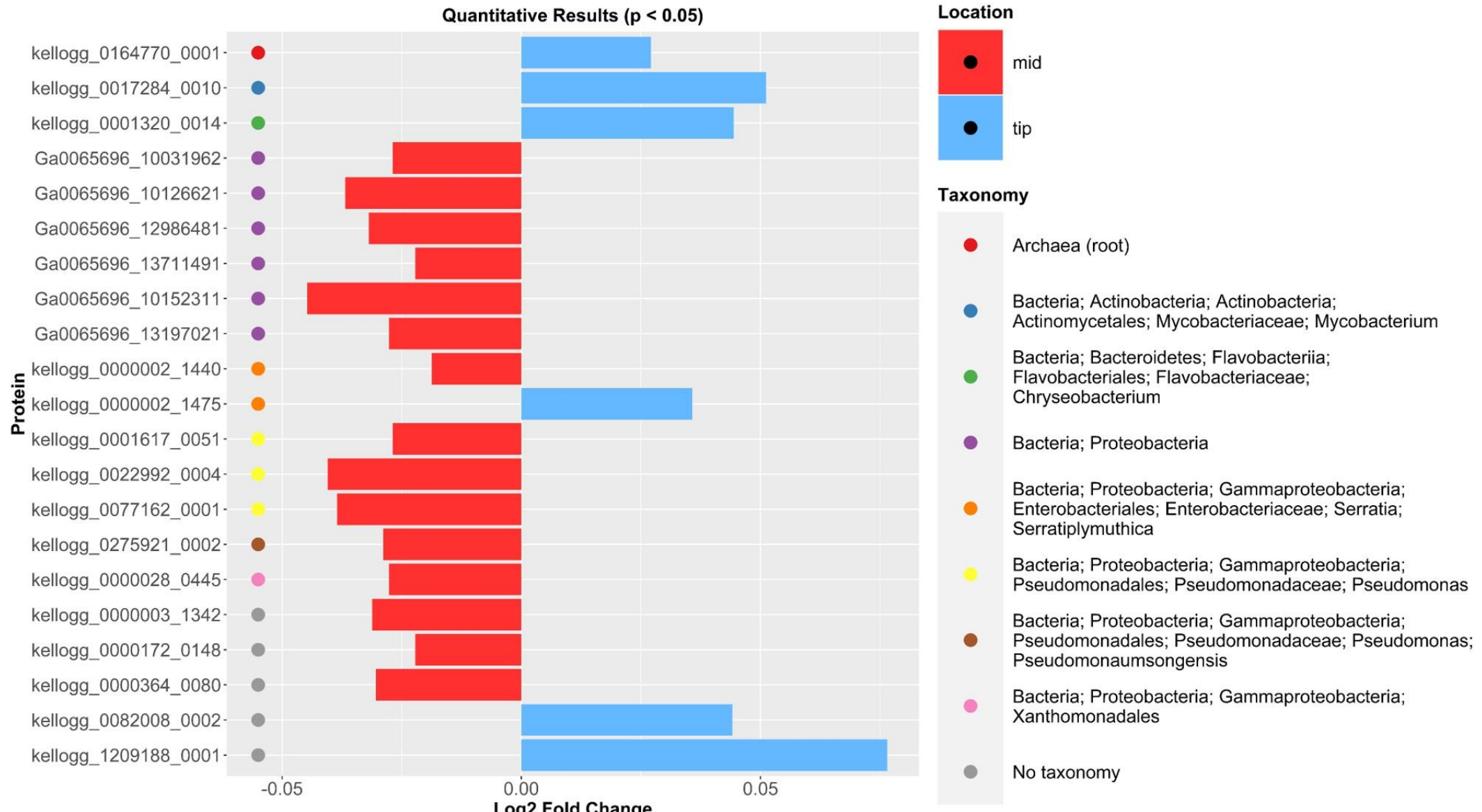


# Proteomic analysis reveals statistically significant variation in spatial abundance of specific proteins

- ▶ 987 distinct proteins were identified
- ▶ Of identified proteins, 180 showed a level of preference for root location

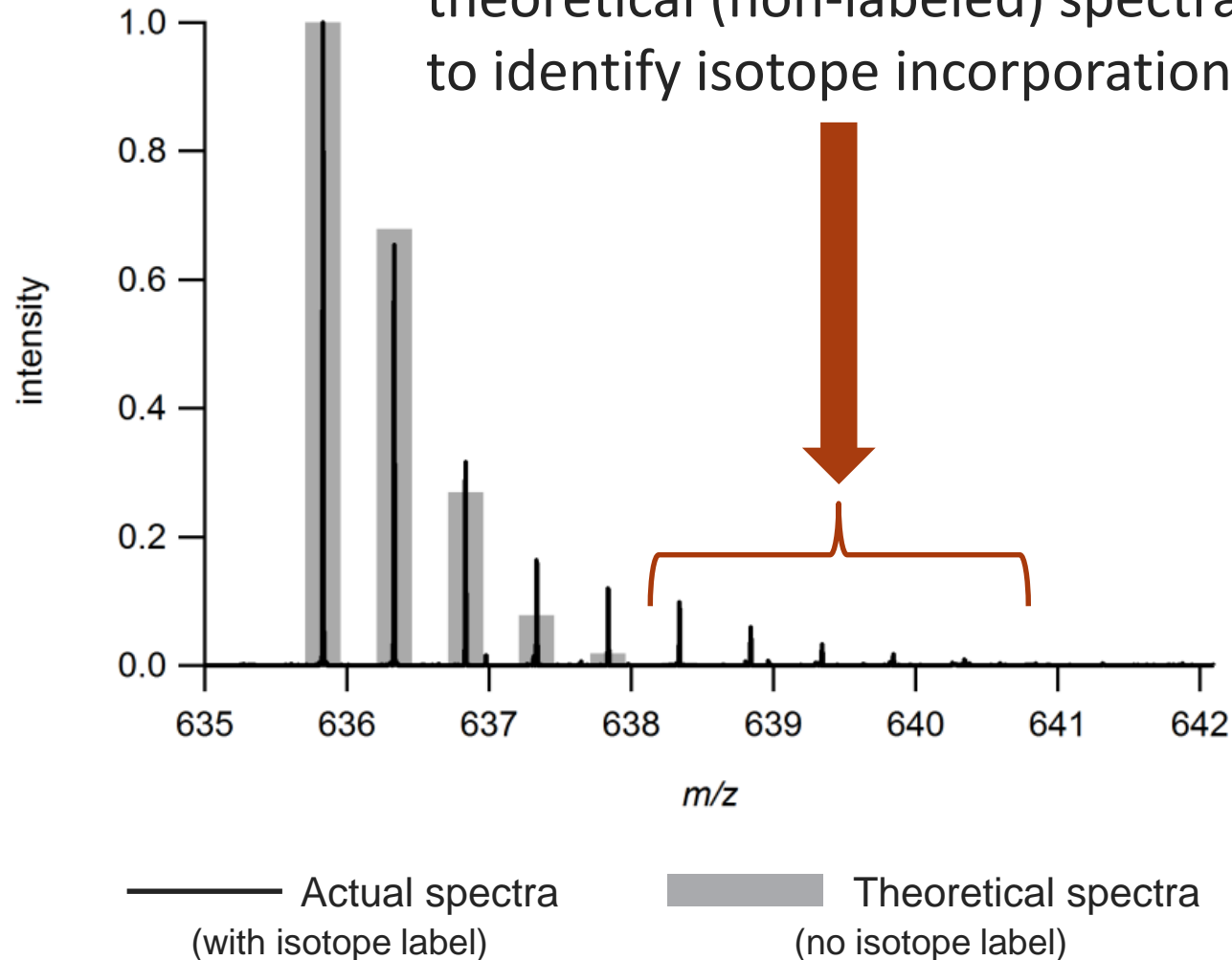


# Preliminary results identify specific proteins with higher abundance at the root tip and increased prevalence of Gamaproteobacteria at the at mid root



# Ongoing development of an automated algorithm to identify peptides containing a $^{13}\text{C}$ tracer

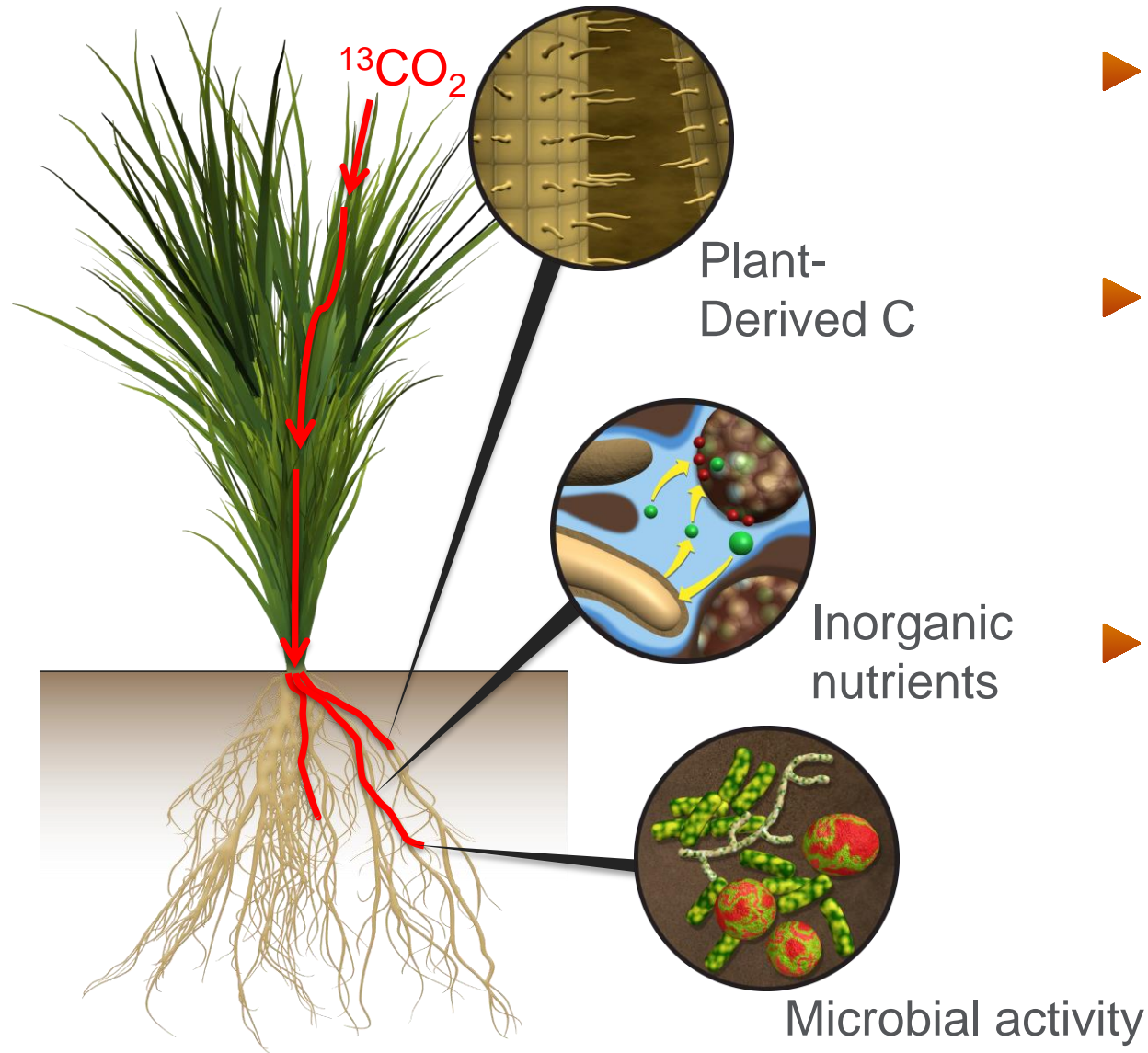
Overlay the actual and the theoretical (non-labeled) spectra to identify isotope incorporation



- ▶ Algorithms currently in development
- ▶ Identifies increased abundance of heavier masses, indicating label incorporation
- ▶ Should identify relatively low levels of  $^{13}\text{C}$  ( $\geq 5-7\%$ ) to enable short incubation times
- ▶ Potentially be adaptable to  $^{13}\text{C}$  and  $^{15}\text{N}$  labels



# Proteomic analysis will complement other methods for spatially tracking carbon exchange within the rhizosphere



- ▶ Previous work allows us to track carbon entry and elemental nutrient distribution within the rhizosphere
- ▶ Proteomic analysis will enable understanding of taxonomic consumption root exudates and spatial distribution of organisms in the rhizosphere
- ▶ Next steps:
  - Continued sensitivity improvements for spatial proteomic approach
  - Incorporation of isotope-tracer linked proteomics



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