The background of the image is a wide-angle photograph of a Arctic tundra landscape. The foreground is filled with low-growing, yellowish-brown grasses and small white flowers. In the distance, rolling hills are visible under a vast sky filled with scattered, colorful clouds ranging from deep blues to bright yellows and oranges, suggesting a sunset or sunrise.

# The Metaproteomic Analysis of Arctic Soils with Novel Bioinformatic Methods

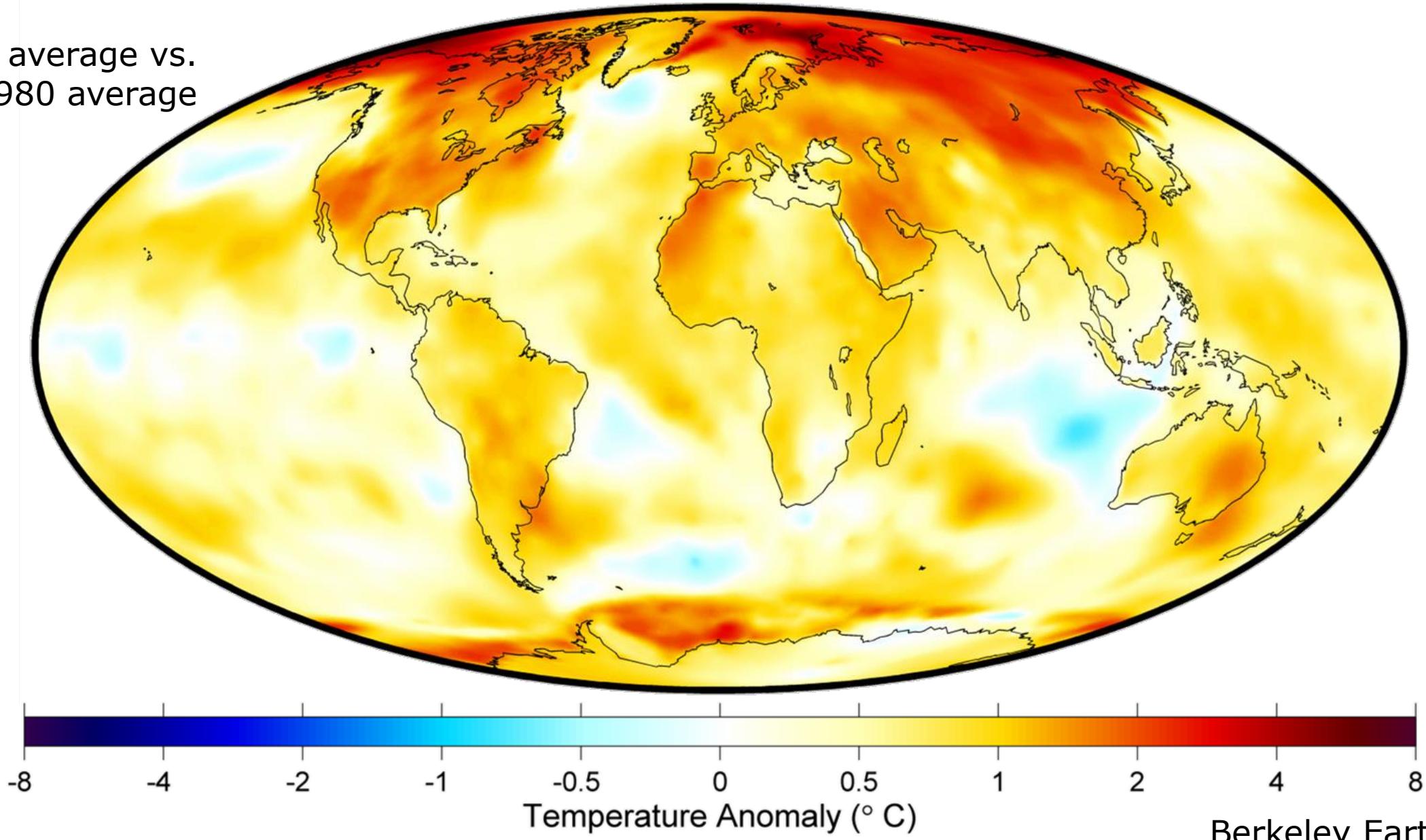
Samuel Miller  
University of Chicago  
Department of the Geophysical Sciences  
Dissertation Defense  
November 1, 2018

# Outline

- Introduction
  - Rapid Arctic warming has large effects on plants and soil
  - Models of soil biogeochemistry can be improved with a greater understanding of microbial processes
  - Direct study of proteins clarifies these processes
- Proteomic methods development
  - *Postnovo* improves protein sequence accuracy
  - *ProteinExpress* increases the amount of useful data recovered from complex metaproteomes
- Arctic soil analyses
  - Identification of key biogeochemical processes and which microbes are doing them

# Arctic warming

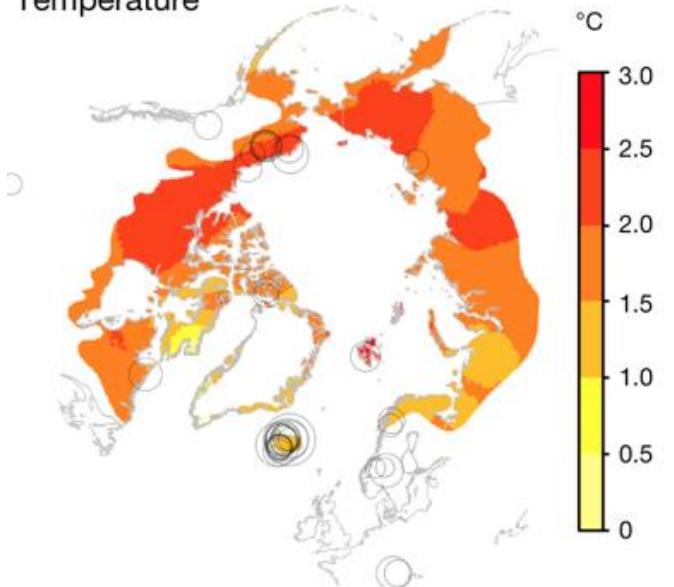
2017 average vs.  
1951-1980 average



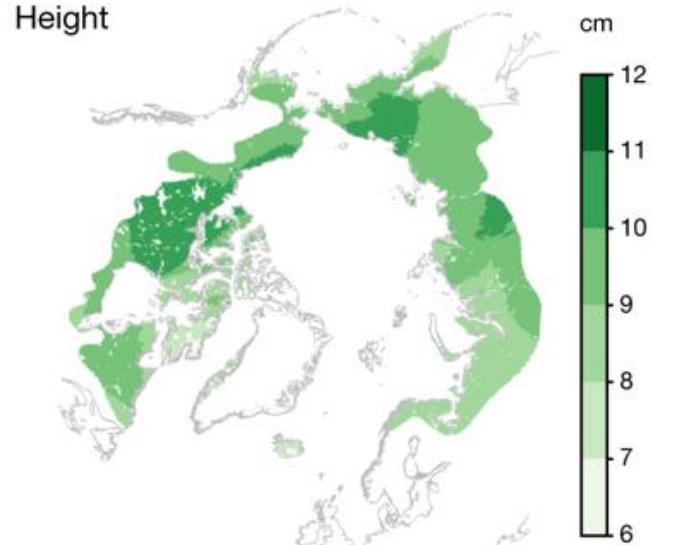
# Arctic greening

1979-2016

Temperature



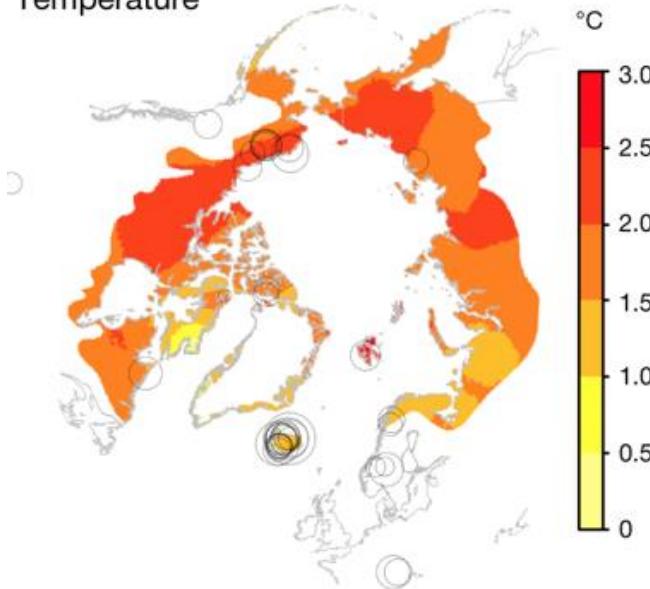
Height



# Arctic greening

1979-2016

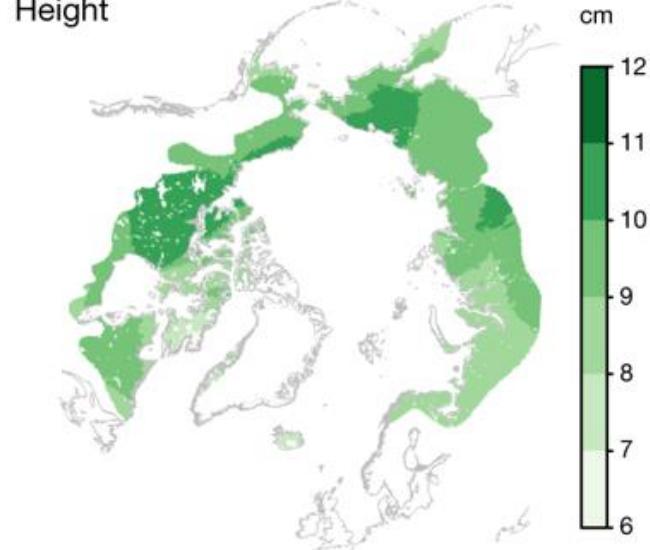
Temperature



°C

3.0  
2.5  
2.0  
1.5  
1.0  
0.5  
0

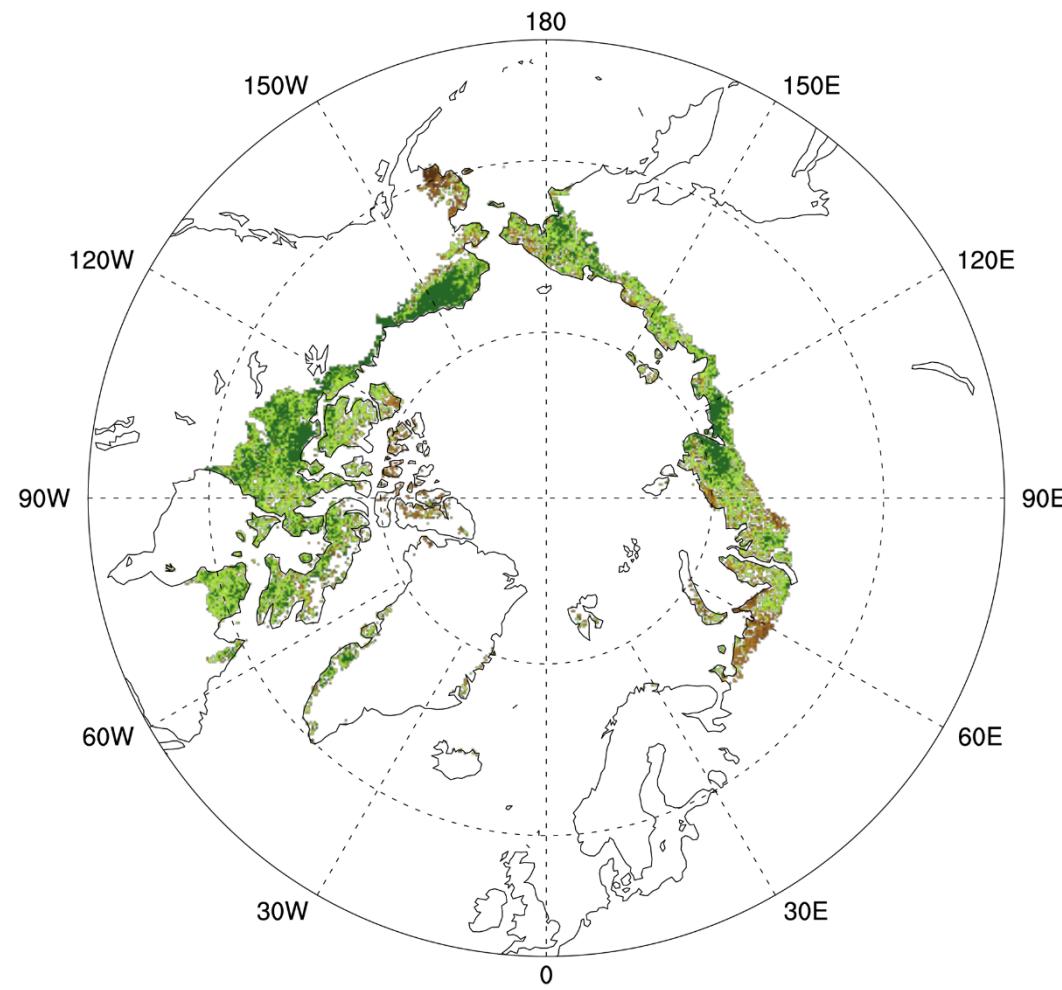
Height



cm

12  
11  
10  
9  
8  
7  
6

1982-2016



-0.16 -0.12 -0.08 -0.04 0 0.04 0.08 0.12 0.16

MaxNDVI

Bjorkman et al., 2018, *Nature*

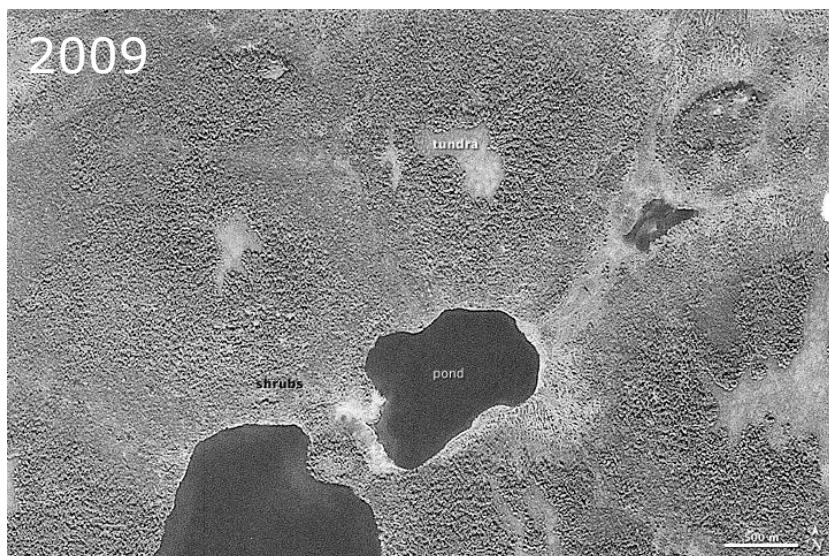
Epstein et al., 2017, NOAA Arctic Report Card

# Changing floras

1966



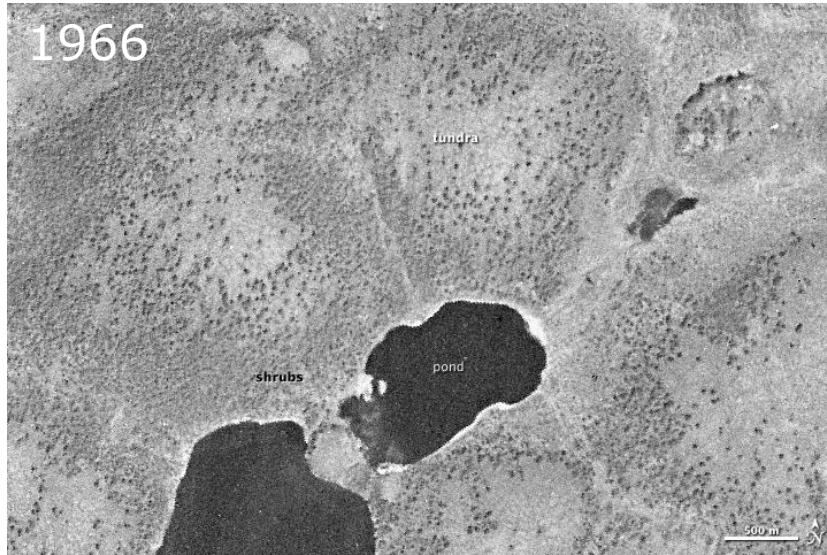
2009



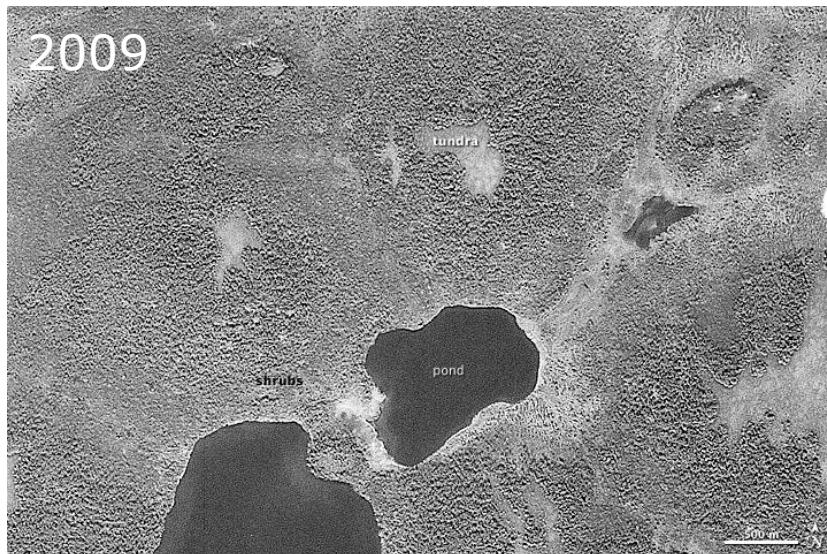
Frost, 2011,  
NOAA Arctic Report Card

# Changing floras

1966



2009



Tussock sedges  
Intertussock mosses/lichens

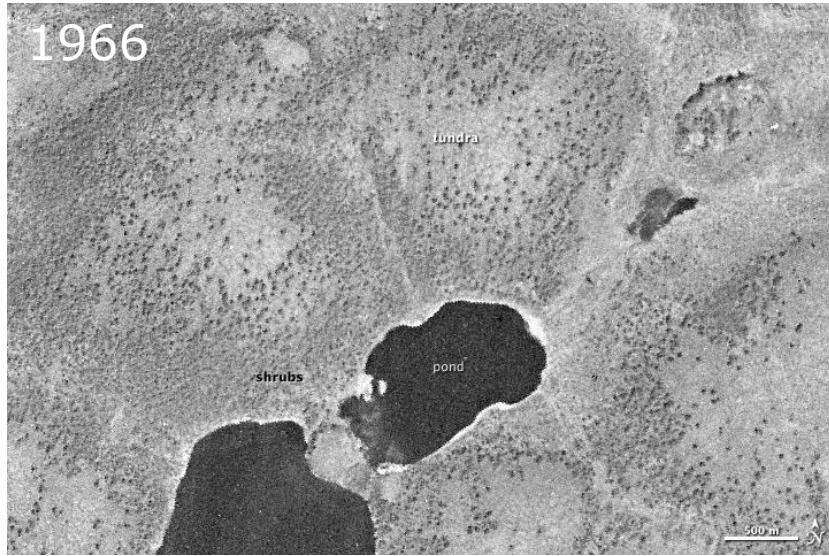


Frost, 2011,  
NOAA Arctic Report Card

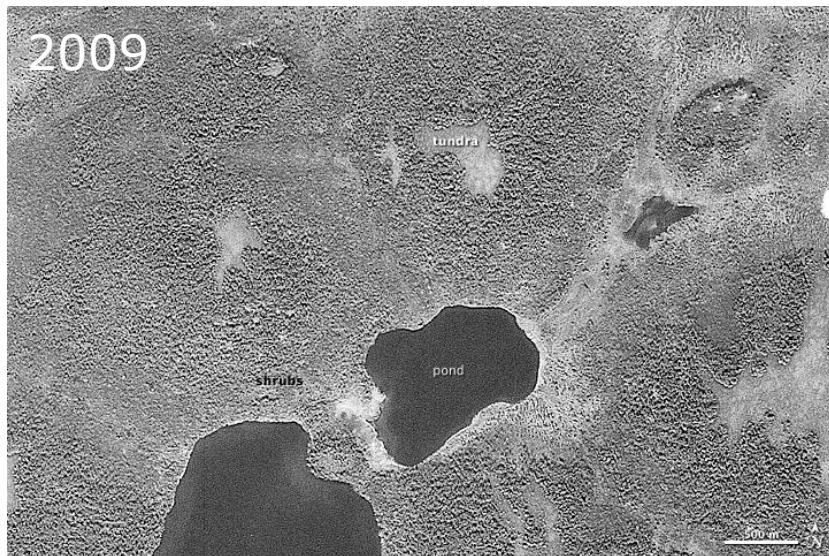
Myers-Smith, 2017, in Medium

# Changing floras

1966



2009



Tussock sedges

Intertussock mosses/lichens



Woody shrubs



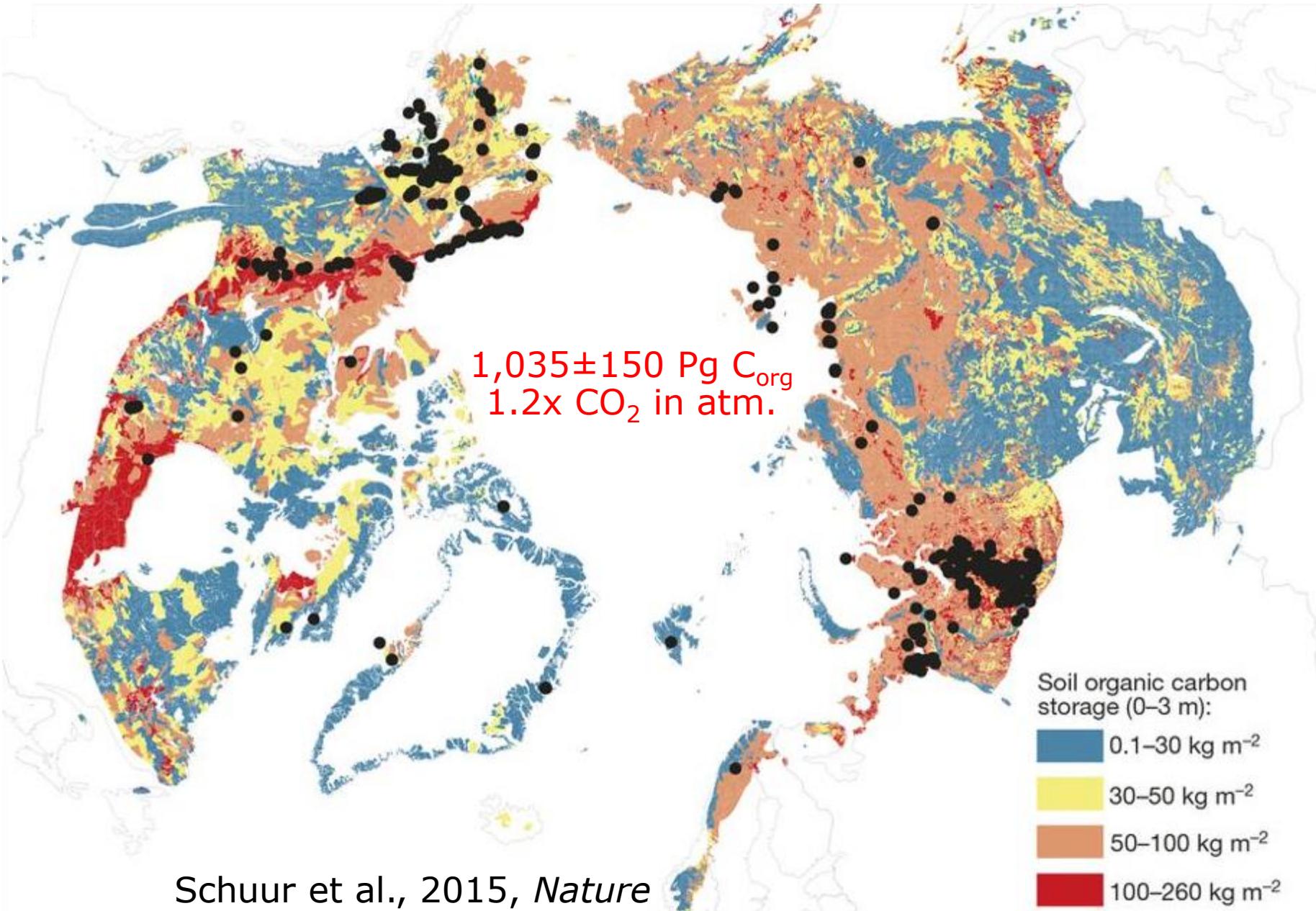
Frost, 2011,  
NOAA Arctic Report Card

Myers-Smith, 2017, in *Medium*

Walker,  
Arctic Geobotanical Atlas

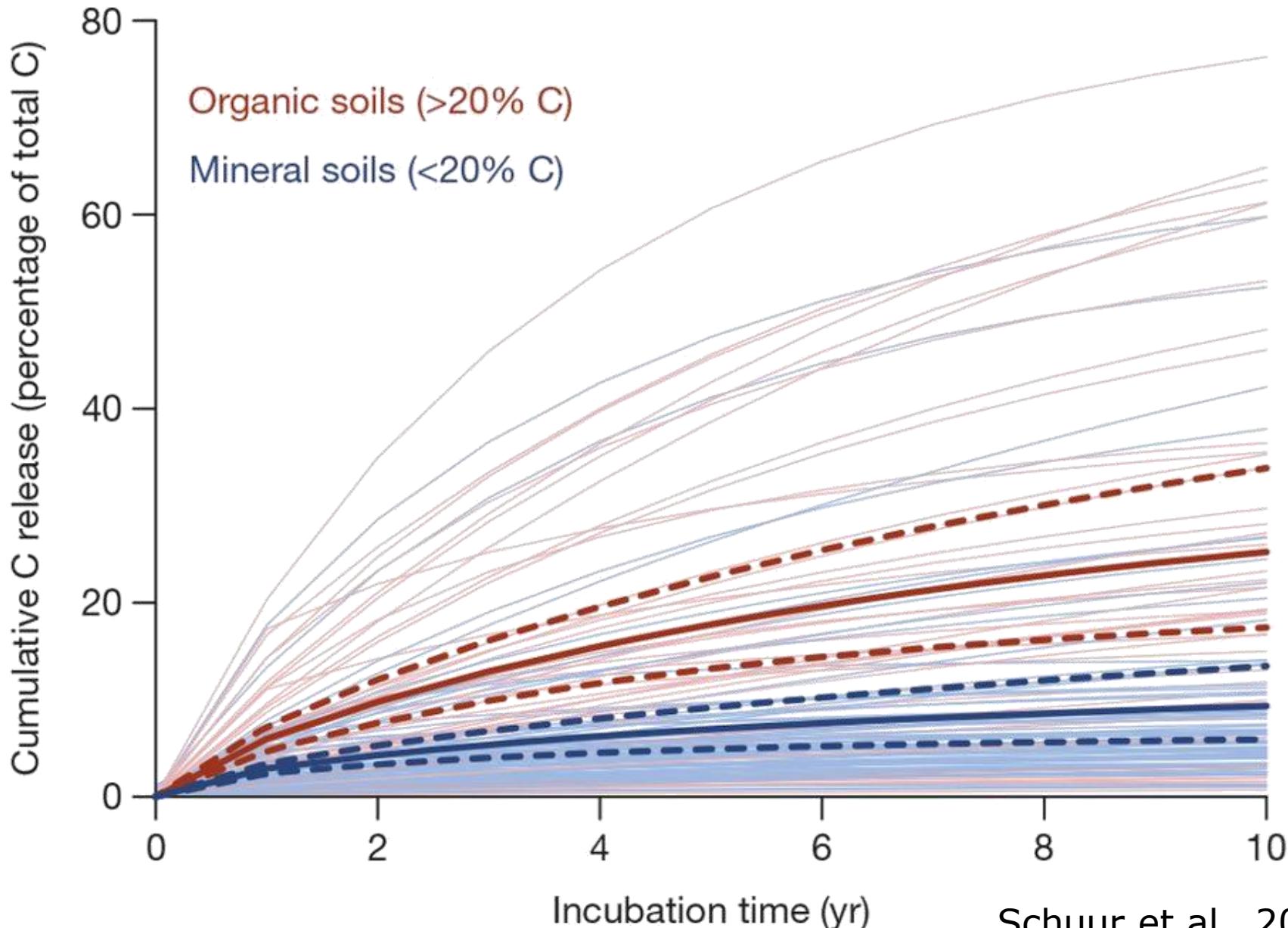


# Large stock of soil carbon



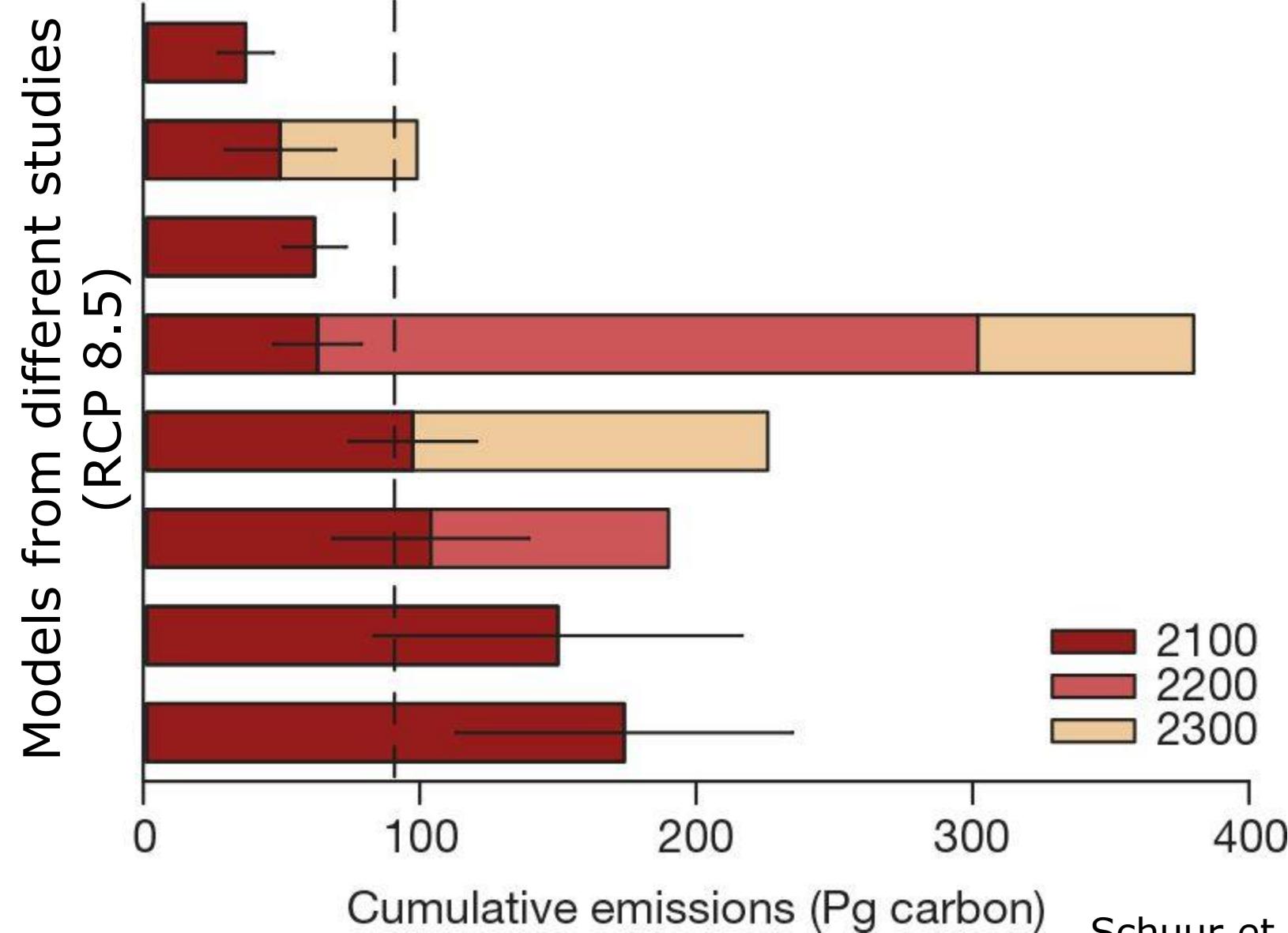


# Carbon loss in 5°C incubations



Schuur et al., 2015, *Nature*

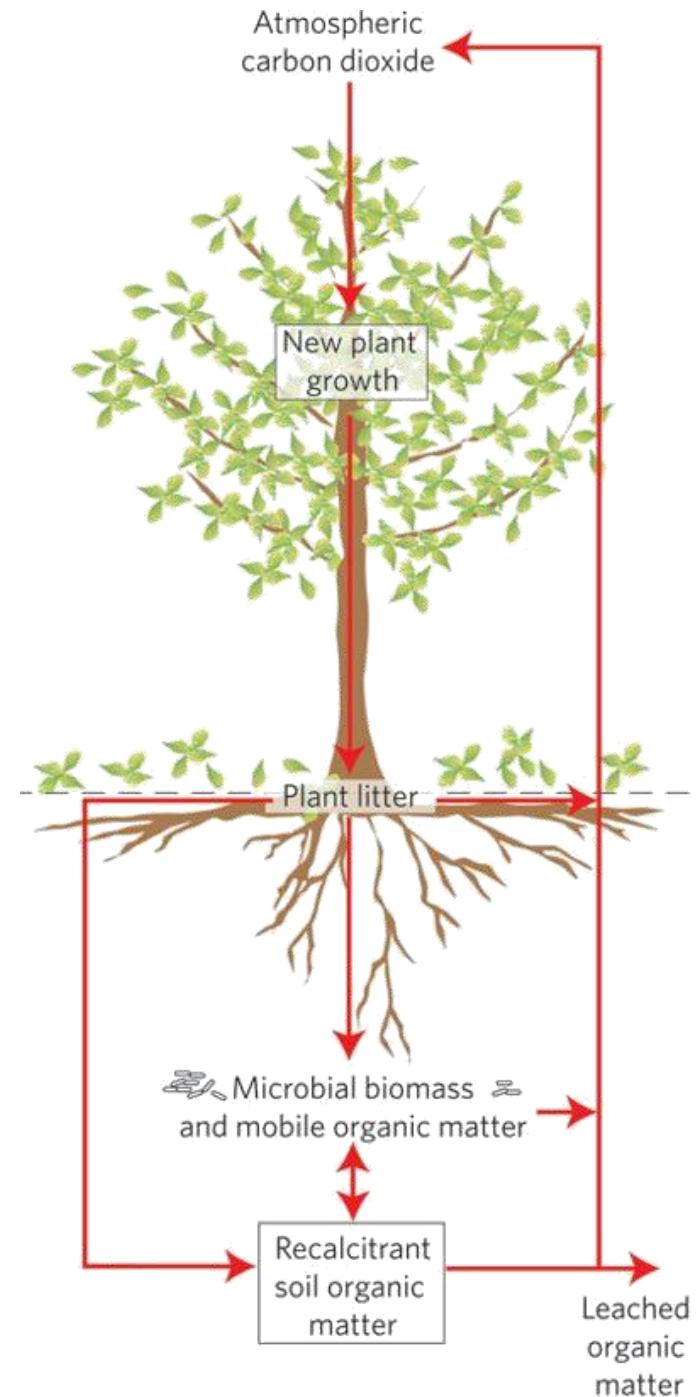
# Projected carbon loss



Schuur et al., 2015, *Nature*

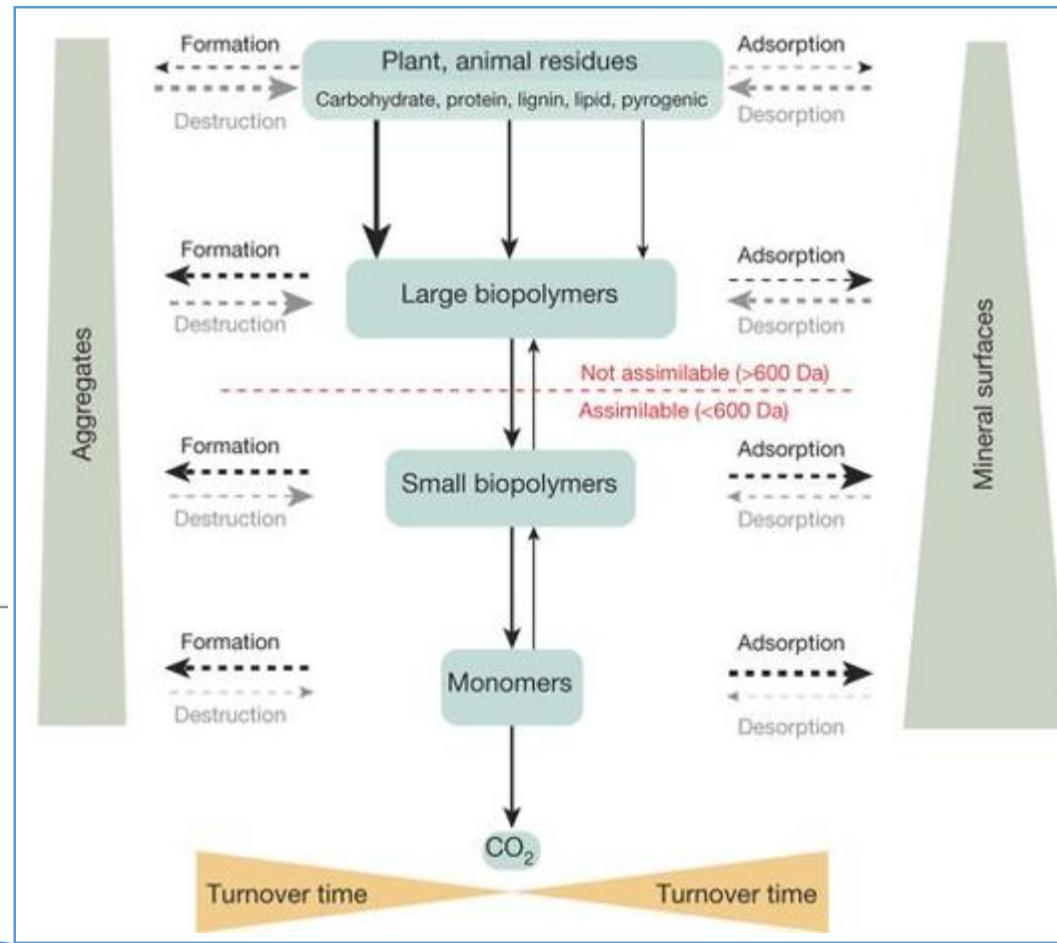
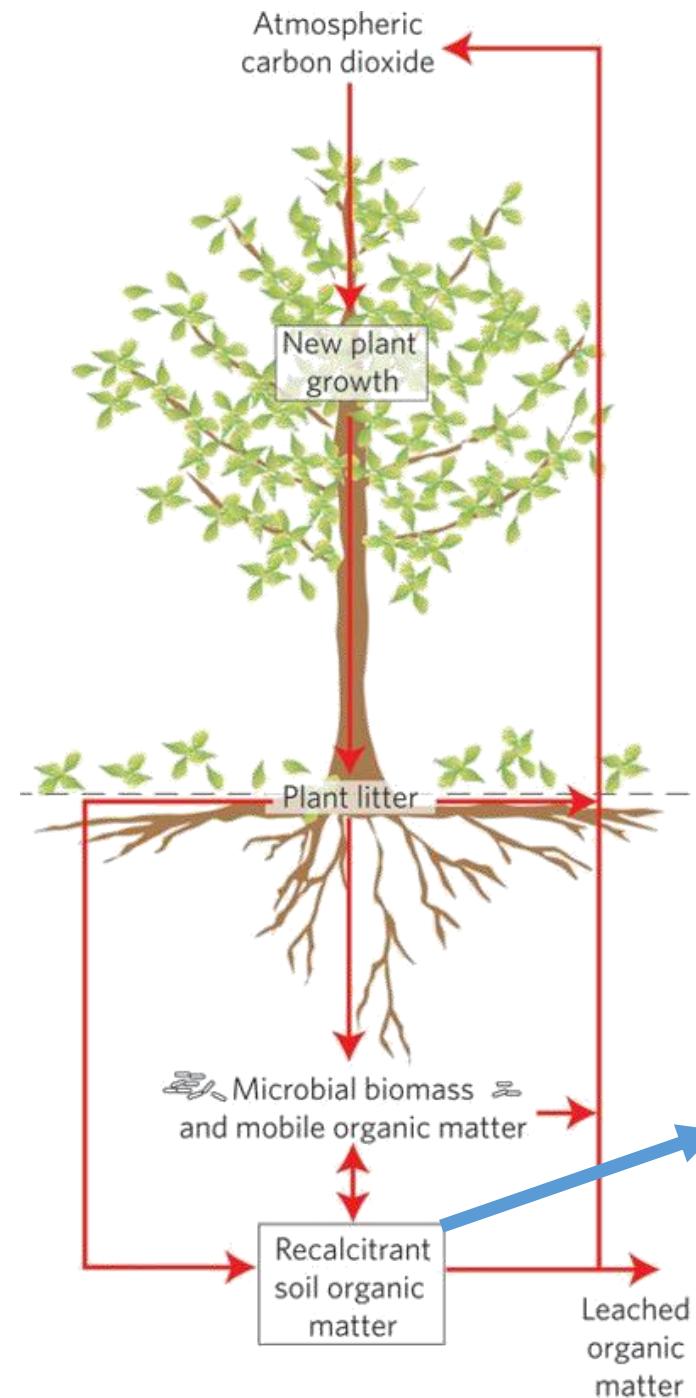


# Carbon gain and loss



Talbot and Treseder, 2011,  
*Nature Climate Change*

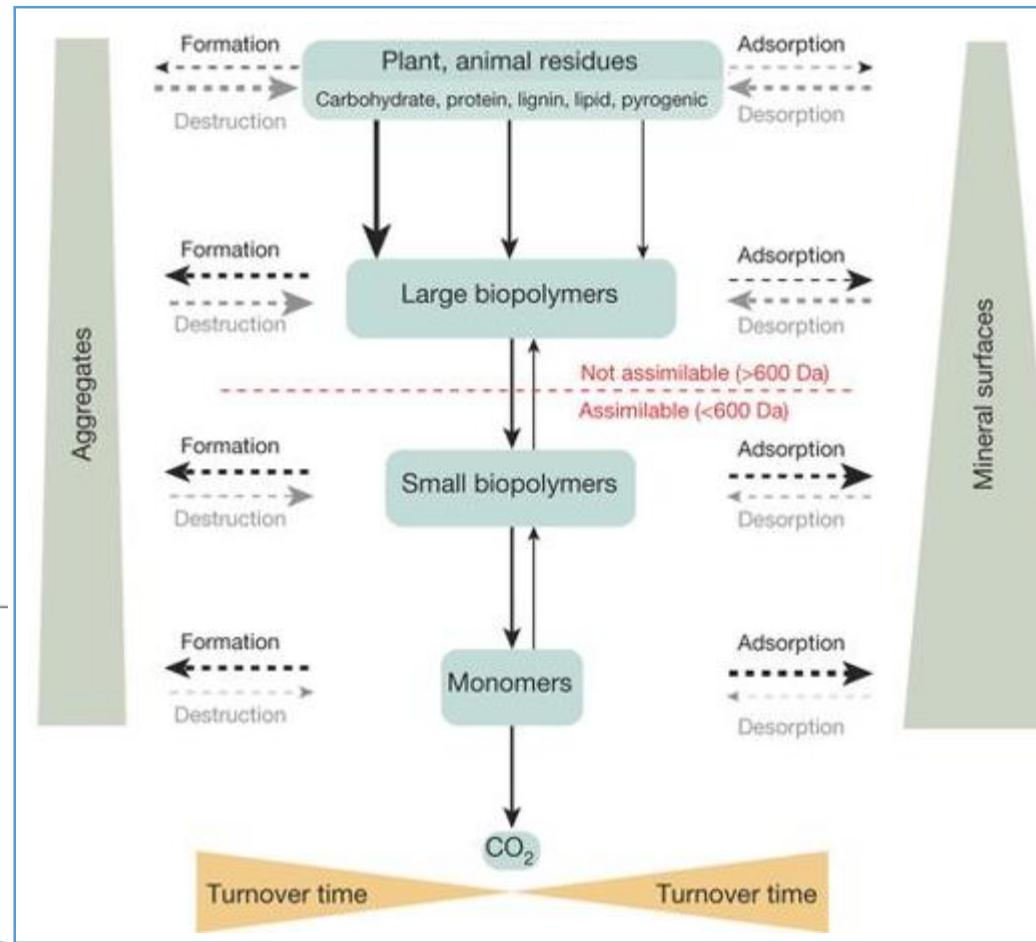
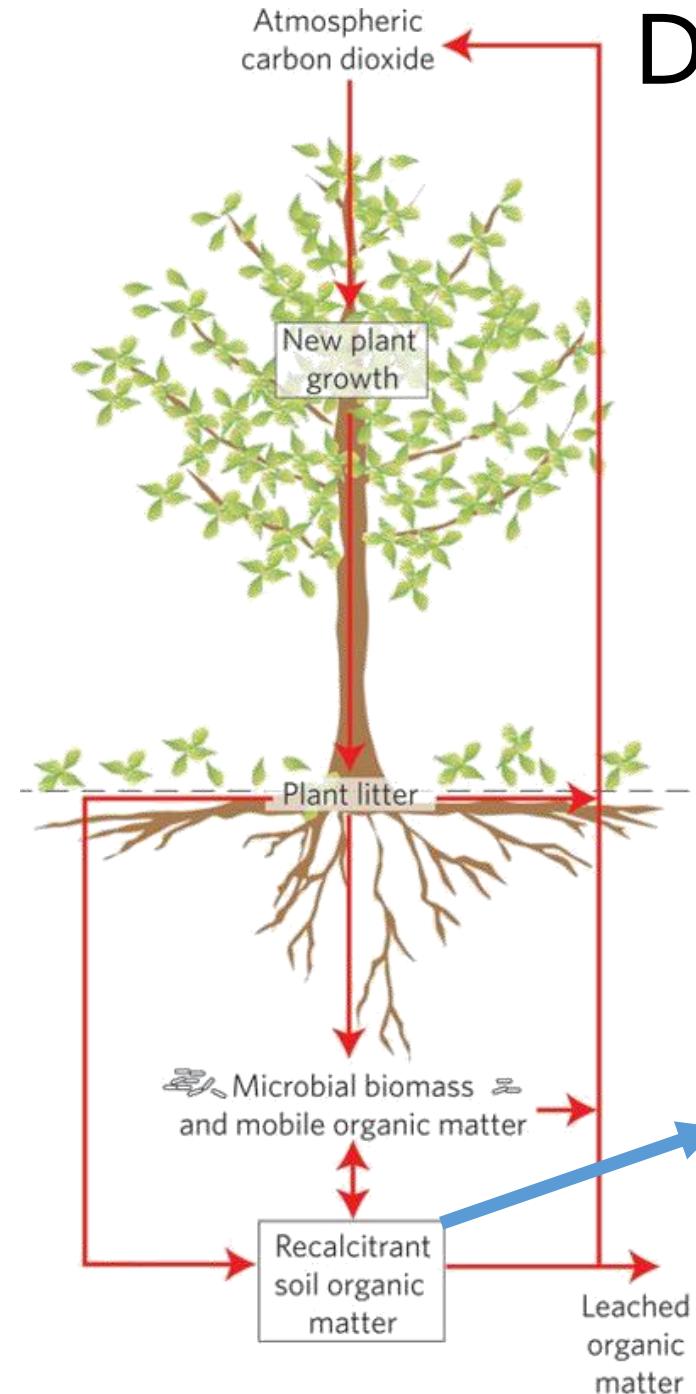
# Model of organic matter



Lehmann and Kleber, 2015, *Nature*

Talbot and Treseder, 2011,  
*Nature Climate Change*

# Decomposition mechanism



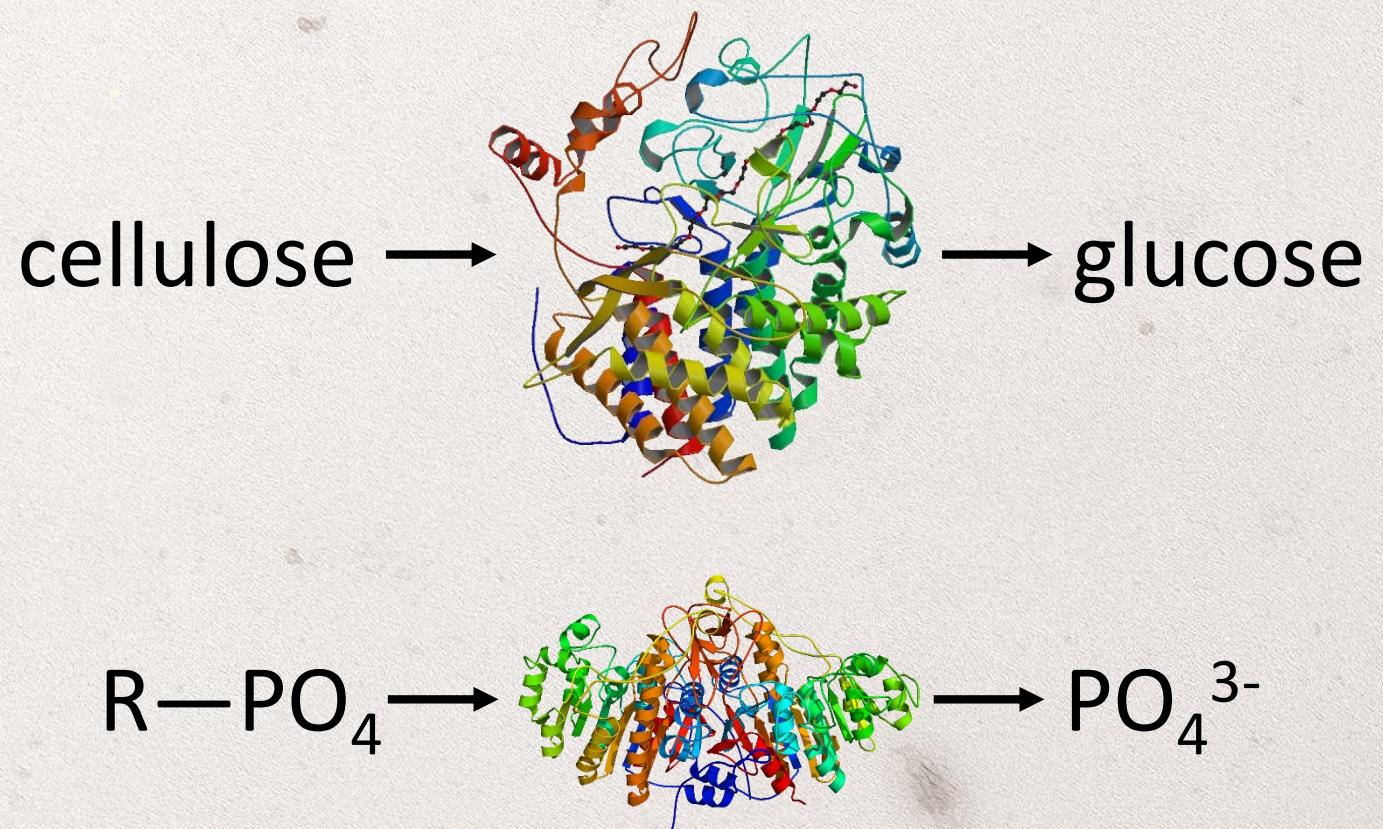
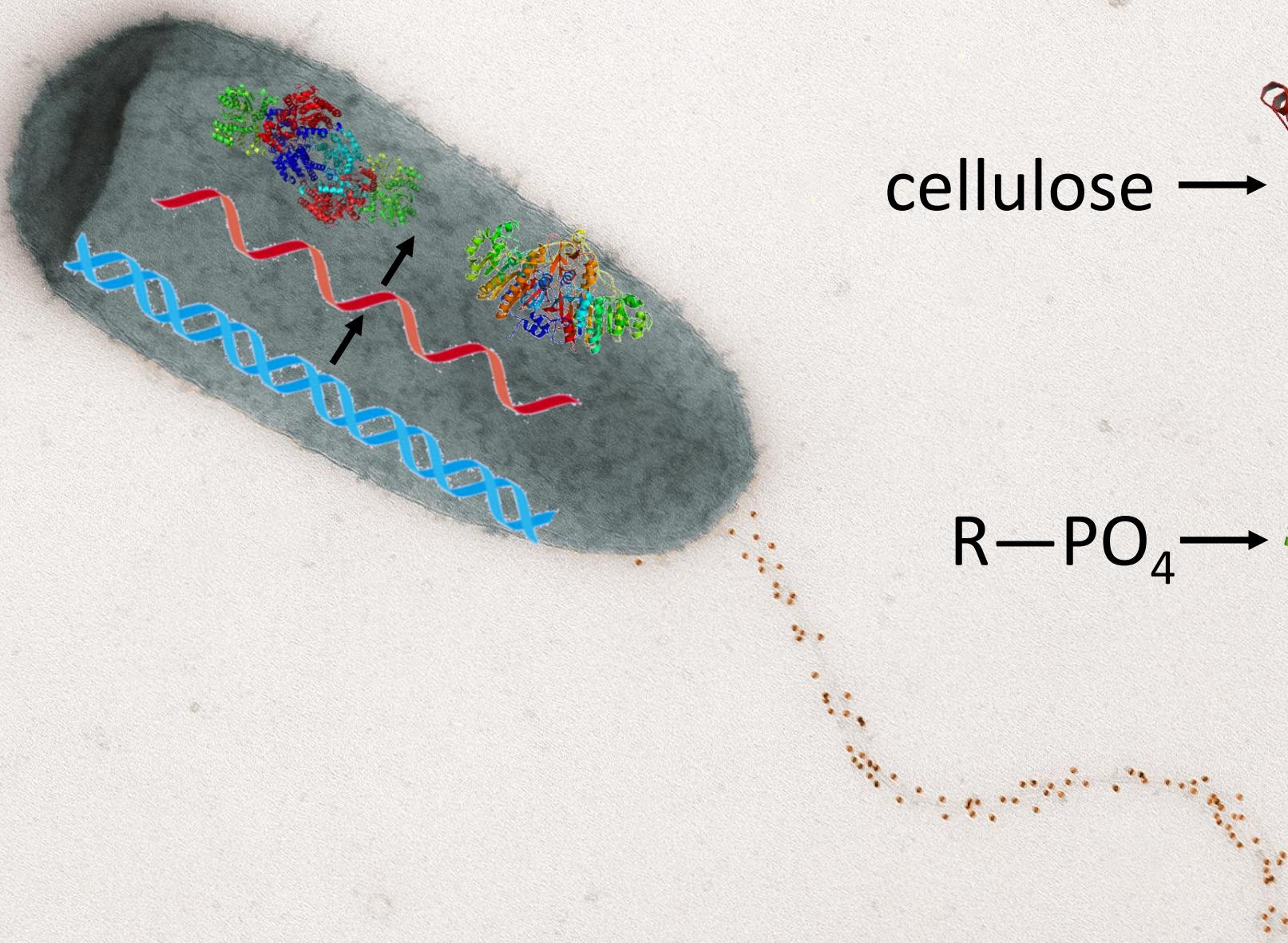
Lehmann and Kleber, 2015, *Nature*

Talbot and Treseder, 2011,  
*Nature Climate Change*

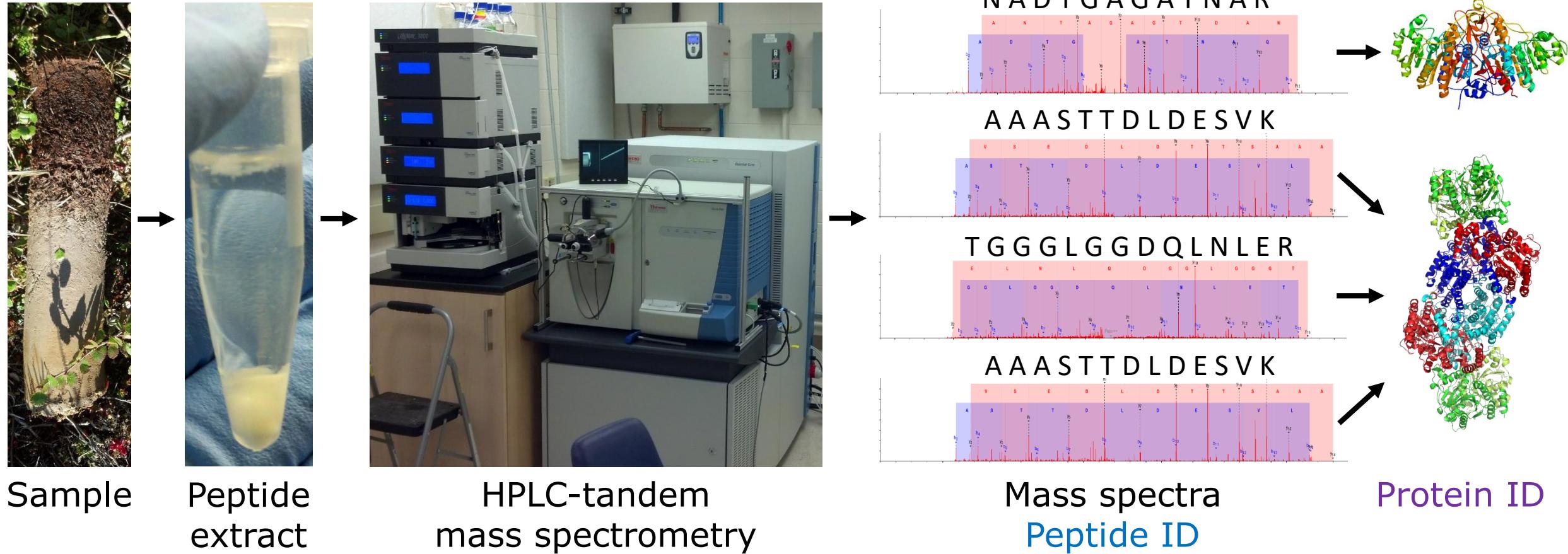
"[T]here are numerous individual processes and taxa associated with the metabolism of the thousands of organic compounds found in soil. This complexity makes it very difficult to predict soil function. If, for example, we want to know the fate of labile carbon compounds in soil (which is important in soil carbon models), information about what taxa are present in a given soil sample is unlikely to be useful."

-Noah Fierer, 2017,  
*Nature Rev. Microbiol.*

# Proteins control decomposition: study the proteins



# Metaproteomic workflow



**Postnovo:** Miller, Rizzo, Waldbauer, 2018, *J. Proteome Res.*

**ProteinExpress:** this work, in prep

# Peptide identification by database search

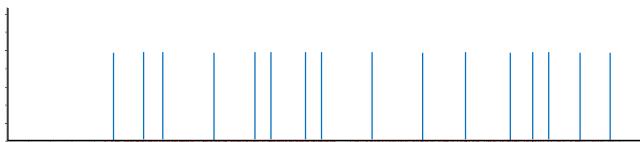
Database → Predicted protease cleavage/ fragment peaks → Compare predicted peptides to spectra with same precursor mass

>VIMSS208926 dnaA-1 chromosomal replication initiator protein DnaA (TIGR)  
MLQQALRQHLRQTCSEQR.HQWFDPDLDRDDAQQRLEVRFPHFARWFEAQALERFTA  
GVRDVVGTSVALVPPEGIKTTDRSTVQPPSQPPLASESVCAPGFAAFITDFAITNRKNQF  
PLAAAREAARANGHQRQTYNPVLCAGSNGKTHLLRALANELAALYGDADVFCGSAELHD  
RYNTEERLAMRRRTLCAHRLALLDDLHRLRALPDREELTALFDHFYDHGKQMAFAYAGRL  
SDLDLFLEAPLRSRLEGLIVDLKEPDLDVRVRYIHARCAALSQLAREHVLTLaQRCHF  
RHLSGLLLKVAAVYRDMVGRDILDRELEQILRNTDSGPVERAPDTIISAAAEEHFGVTPRD  
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MHALVAELKRRCLNHDG

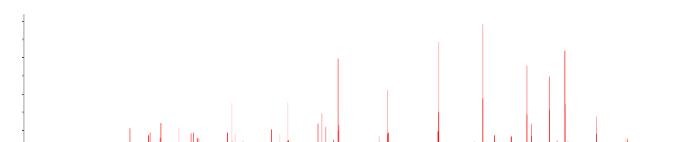
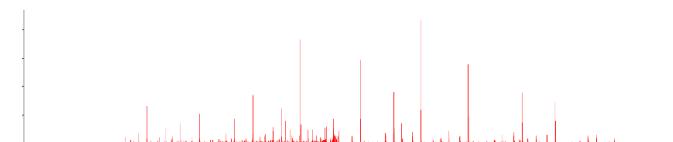
>VIMSS208928 dnaN DNA polymerase III, beta subunit (TIGR)  
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VTEPGLAVQGRAFVDLLRKLPAGEIVLQLDAEANVLRIEQGRRKYKLPVNPDVWFQNFS  
DFPADGAVVWSGDFLQELIDRIACFCISDEDAVEAIACLMKPVAEGRIEACGLNGHQFAM  
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SSYQYPDYMNFIAKLQGEGVSNLEVDRKEGMDALRQLQIFNSDNNRCTYFDLSGGEVVLT  
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TNQFSYETLRKFEELS YNRLN RGELEIFIDERSGESHTFFAEGGIRQFVKDLNSGETGIHP  
IVAGEKTDGVVVDFALQYNA GYKENVLT FANNIRTKEGGTHLAGFTALTRAINGYVG  
QADLT KKMKG TALSGDDVREGLTAVVSKLPQPQFEGQT KLG NSEIAGIVAGIVYDRL  
NVHFEENPKDVR LIIDKAVDAAR A RDAARRAKEL VRRKGAL SDN ALPGK LADC QSKDPAL  
SELFIVEGDSAGGS AKQGRDP STQ AILPLRGK ILNTERTRFDKML ANKEV KALIT AMGAG  
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HNSR MERFIKDDAELNNFLLTRV SEDVEVRTPGGCSFKGT DIVRLMRSIETVAARI RDVE  
TAGISRELFLGFLEYGERITPEWEFQHDNGNGLRPWLAGRGSYMTAELES GDEGDRTFVV  
ENTNGHRTIGA EFGSRM YRSSWDALEAIRSEC GGSLFTDRKGETV GEGDMFDLQRMV  
FEEARRGLNIQRYKG LGEMNPEQLWVTTMN PENRTL LQV SIED EEA SDAF EQL MGRV  
PRREFIVRNALAVQELDI

L H Q W F D P L D L R  
mass = 1438.736 Da



Experimental fragmentation spectra  
precursor masses ≈ 1438.736 Da



# Peptide identification by database search

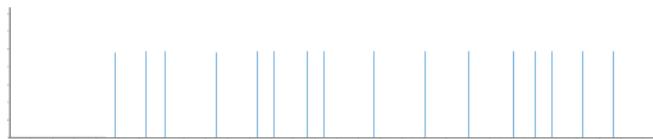
Database → Predicted protease cleavage/ fragment peaks → Compare predicted peptides to spectra with same precursor mass

>VIMSS208926 dnaA-1 chromosomal replication initiator protein DnaA (TIGR)  
MLQQALRQHLRQTCSEQR.HQWFDPDLDRDDAQQRLEVRFPHFARWFEAQALERFTA  
GVRDVVGTSVALVPPEGIKTTDRSTVQPPSQPPLASESVCAPGFAAFITNKRKNQF  
PLAAAREAARANGHQRQTYNPVLCGASGNGKTHLLRALANELAALYGDADVFCGSAELHD  
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SDLDLFLEAPLRSRLEGLIVDLKEPDLDVRVRYIHARCAALSQLAREHVLTLaQRCHF  
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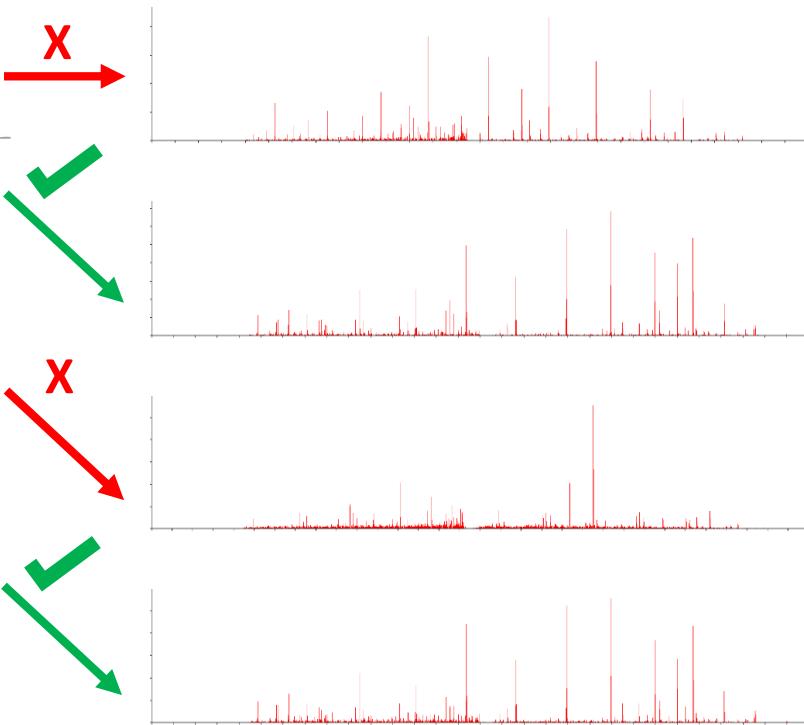
>VIMSS208928 dnaN DNA polymerase III, beta subunit (TIGR)  
MYLKVKEDVIEGLQKAANIIPAKTGAAYLRSIWLNAAADGTL SVMATDSNIEFRGTYTAE  
VTEPGLAVQGRAFVDLRLKPAGEIVLQLDAEANVLRIEQGRRKYKLPVNPDVWFQNFS  
DFPADGAVVWSGDFLQELIDRIACFISDEDAVEAIACLMKPVAEGRIEACGLNGHQFAM  
LRFLHDDLHAKLPAEGVLVQKKYLGEKKWLADEIENLISEKRMHLLRTGDGRETFSPL  
SSYQYPDMNFIAKLQGEGVSNLEVDRKEGM DALDRQLIFNSDNNRCTYFDLSGGEVVLT  
AQGQDVGSASESLEASYDGDIRRIFPTRLNIDIMNHYQSGLRLTLTGAEGPCGISGEE  
DPEYQVIVMPMKIVEETYYSEEEV

>VIMSS208929 gyrB DNA gyrase, B subunit (TIGR)  
MTTGNNYTADSITILEGLSAVRKRPAMYIGSTDARGLHHLVYEVVDNSIDEAMAGFCS  
KVVVKLHLDNSVTVDNGRGPVDMHPKEGRPAVEVVMTKLHAGGKFDNNAYKVSGGLHG  
VGVSCVNALESEWLEVRVKRDGQRYGQRYARGVPQEDLRVLGASEGHGTTVRFKPDEEIFE  
TNQFSYETLRKFEELS YNRGLEIEFIDERSGESHTFFAEGGIRQFVKDLNSGETGIHP  
IVAGEKGKTDGVVVDFALQYNAQYKENVLT FANNIRTKEGGTHLAGFTALTRAINGYVG  
QADLT KKMGTALS GDDVREGLTAVVSVKLPQPQFEGQT KTKLGNSEIAGIVAGIVYDRL  
NVHFEENPKDVR LIIDKAVDAAR A RDAARRAKEL VRRKGAL SDN ALPGK LADC QSKDPAL  
SELFIVEGDSAGGS AKQGRDP STQ AILPLRGK ILNTERTRFDKML ANKEV KALIT AMGAG  
IGEDDTDYDKL RYHKIVIMTADVDGAHIRTLLTFFF RQYQKLIESGYLYIAQ PPLYRA  
HNSR MERFIKDDAELNNFL LTRV SEDVE VRTPGGCSFK GTDIV RL MRSI ETVA AIRD VE  
TAGISRELFLGFLEYGERITPEWEFQHDNGNGLRPWLAGR GSMTAELES GDEG DRTFVV  
ENTNGH RTRIGA EFGSRM YRSSW DALE AIRSEC GGSLFT DRK GETV GEG DMFD LQRMV  
FEEARRGLNIQRYKG LGEM NPEQLW VTT MN PEN RTL LQVS IEDA EEA SDAF EQL MGDRVE  
PRREFIVRN ALA VQELDI

L H Q W F D P L D L R  
mass = 1438.736 Da



Experimental fragmentation spectra  
precursor masses ≈ 1438.736 Da



# Database search can't find unexpected sequences

>VIMSS208926 dnaA-1 chromosomal replication initiator protein DnaA (TIGR)  
MLQQALRQHLRQTCSEQR.HQWFDPDLR|DDAQQRLEVRFPHFARWFEAQALERFTA  
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RYNTEERLAMRRTLCAHRLALLDLHRLRALPDLREELTALFDHFYDHGKQMAFAYAGRL  
SDLDLFLEAPLRSRLEGLIVDLKEPDLDVRVRYIHARCAALSQLAREHVLTQARCHEF  
RHLSGLLLKVAAVYRDMVGRDILDRELEQILRNTDSGPVERAPDTIISAAAEEFGVTPRD  
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MHALVAELKRRCLNHDG

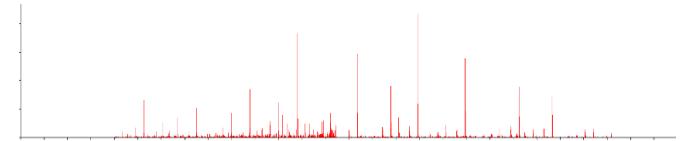
>VIMSS208928 dnaN DNA polymerase III, beta subunit (TIGR)  
MYLKVKEDVIEGLQKAANIIPAKTGAAYLRSIWLNAAADGTL SVMATDSNIEFRGTYTAE  
VTEPGLAGVQGRAFVDLLRKLPAGEIVLQLDAEANVLRIEQGRRKYKLPVNNDPVWFQNFS  
DFPADGAVVWSGDFLQELIDRIACFISDEDAVEAIACLMKPVAEGRIEACGLNGHQFAM  
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SSYQYPDMNFIAKLQEGVSNLEVDRKEGMDALDRQIFNSDNNRCTYFDLSGGEVVLT  
AQGQDVGSASESLEASYDGDIRRIFPTRNLIDIMNHYSQSGRLRLTGAEGPCGISGEE  
DPEYQVIVMPMKIVEETYYSEEEV

>VIMSS208929 gyrB DNA gyrase, B subunit (TIGR)  
MTTGNNYTADSITILEGLSAVRKRPAMYIGSTDARGLHHLYEVVDNSIDEAMAGFCS  
KVVVKLHLDNSVTVDNGRGPVDMHPKEGRPAVEVVMTKLHAGGKFDNNAYKVSGLHG  
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FEEARRGLNIQRYKGLGEMNPEQLWVTTMNPNENRTLLQVSIEDEAEASDAFEQLMGRVE  
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L H Q W F D P L D L R  
mass = 1438.736 Da

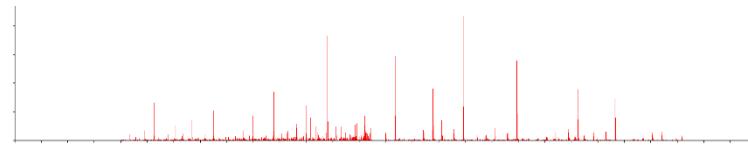
X →

Experimental fragmentation spectra  
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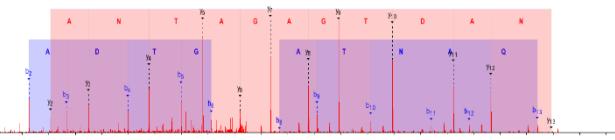


# An alternate approach: “de novo” sequencing

Spectra

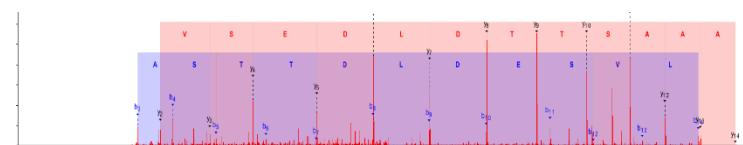
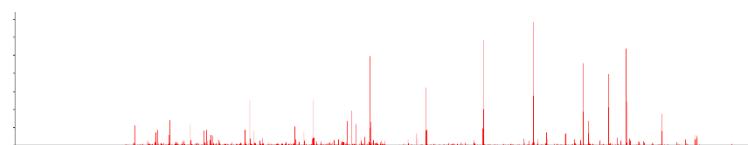


Fragment prediction

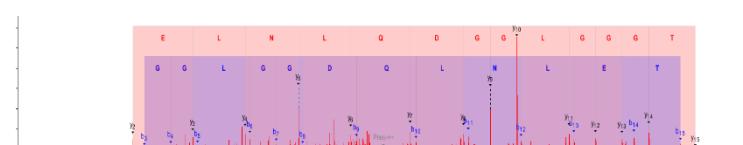


Peptide prediction

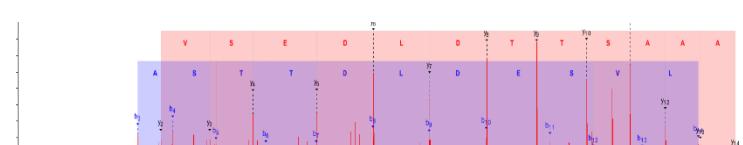
NADTGA...NAR



AAAS...DESVK

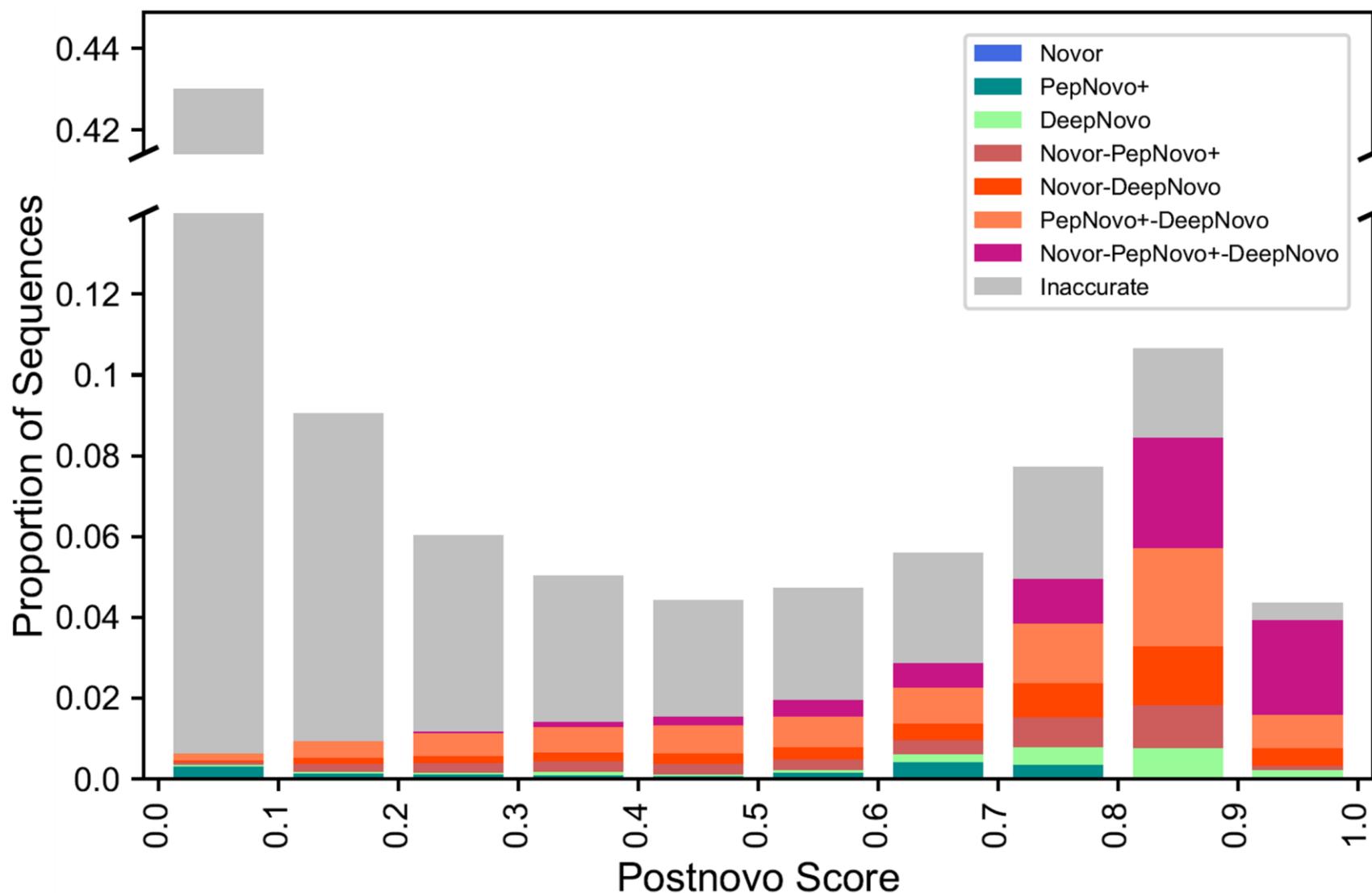


TGGGLGGDQLNLEK



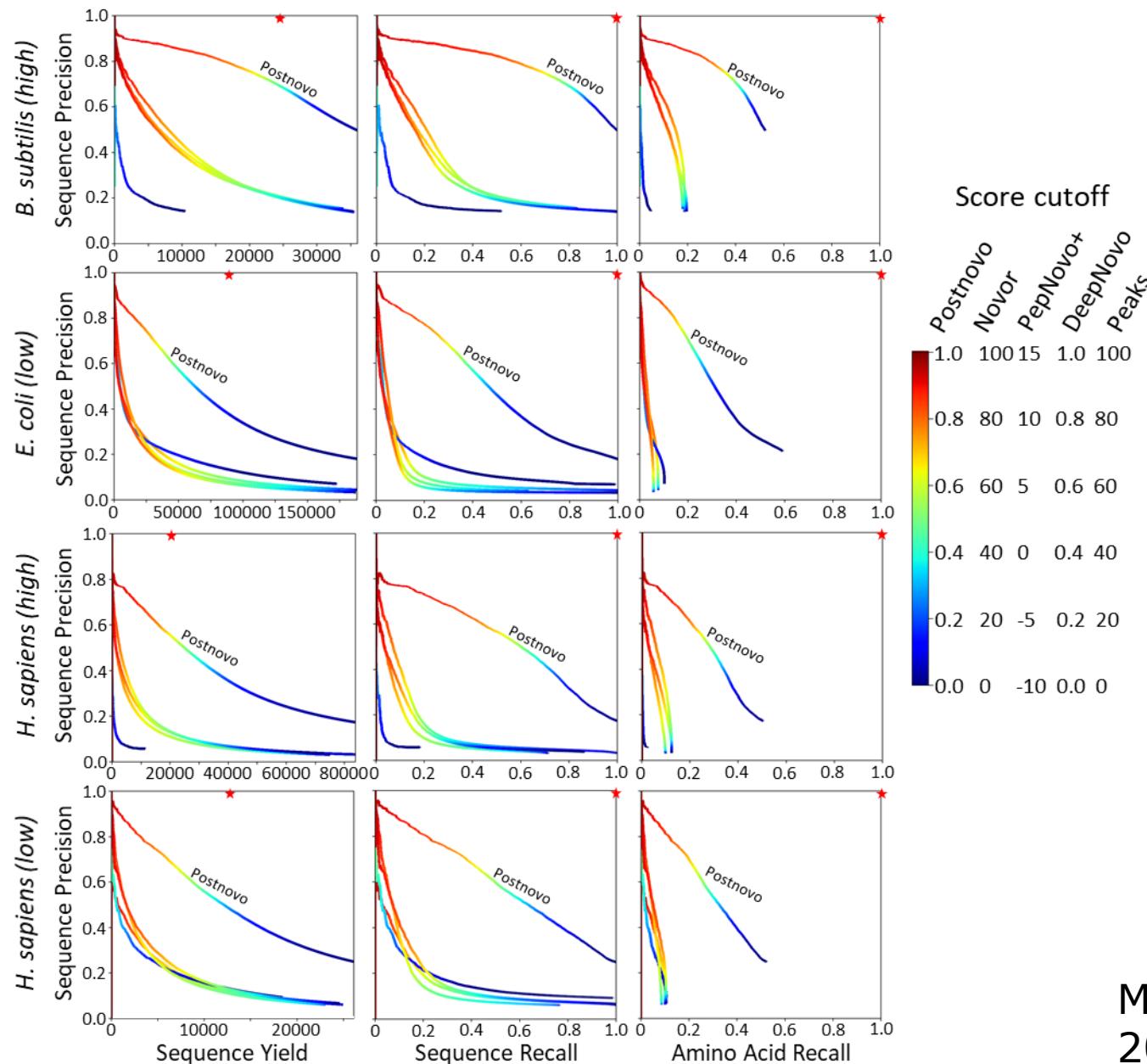
AAAS...DESVR

# *Postnovo refines de novo sequence predictions*



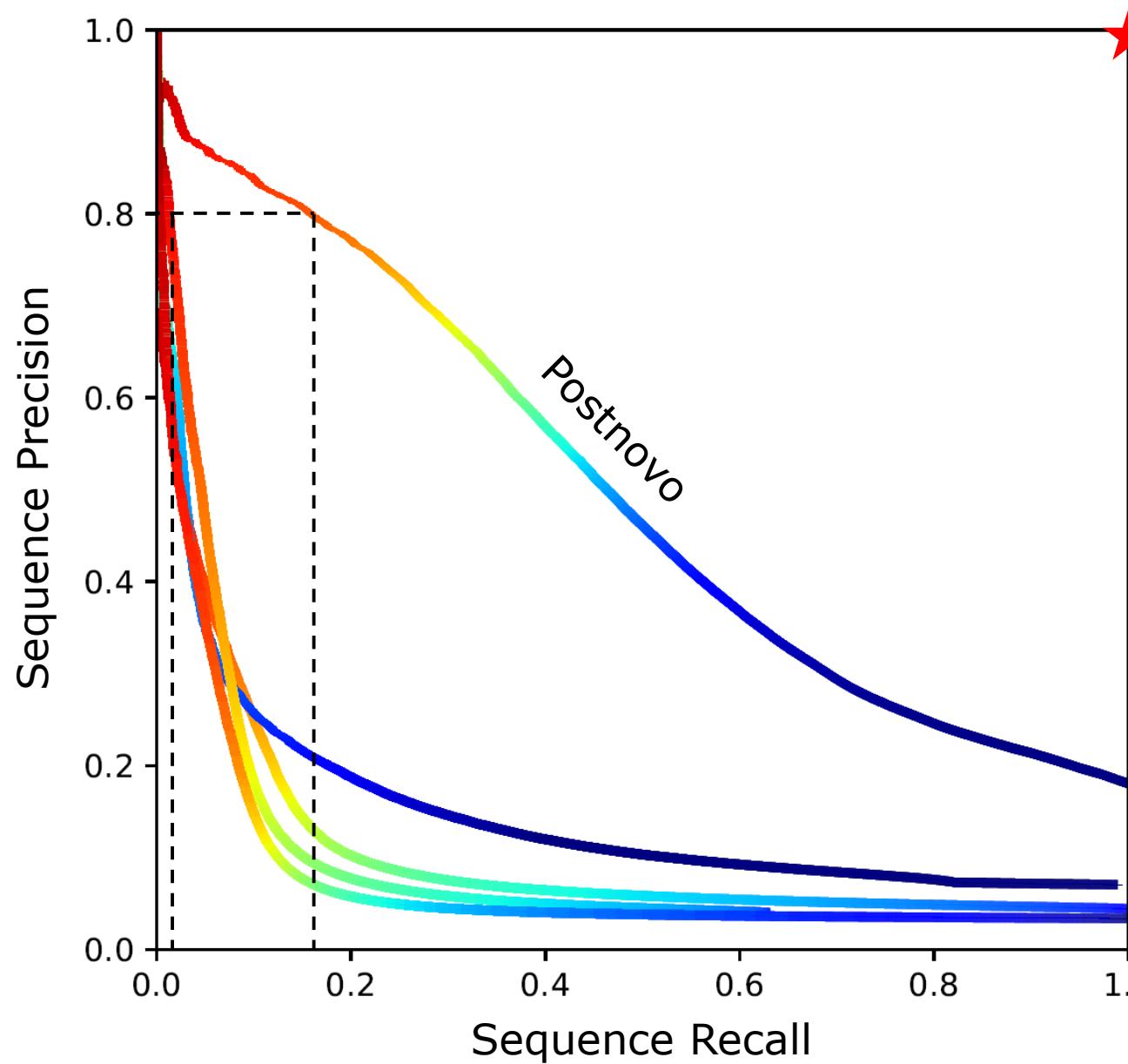
Miller, Rizzo, Waldbauer,  
2018, *J. Proteome Res.*

# Postnovo validation with simple datasets



Miller, Rizzo, Waldbauer,  
2018, *J. Proteome Res.*

# Ten-fold increase in yield of accurate sequences



Miller, Rizzo, Waldbauer,  
2018, *J. Proteome Res.*

# Additional bioinformatic advances

- Taxonomic/functional annotation of short de novo sequences
  - Very short de novo sequences cannot be linked to related sequences in large databases – obvious for a sequence of length 1
  - Sequences >11 amino acids with identical database sequences can be uniquely identified across a range of database sizes
  - Divergence between soil and database sequences has a relatively small effect on identification of database sequences related to soil sequences

# Additional bioinformatic advances (continued)

- *ProteinExpress* compares spectra to (pooled) DNA/RNA datasets, identifying metaproteomic peptides in protein coding sequences
  - DNA sequences from related organisms are binned
  - Identified proteins are similarity-searched against taxonomic bins to find which taxa likely express which proteins
  - Proteins are assigned to pathways and 141 functional groups that I defined, e.g., cellulases and amino acid transporters

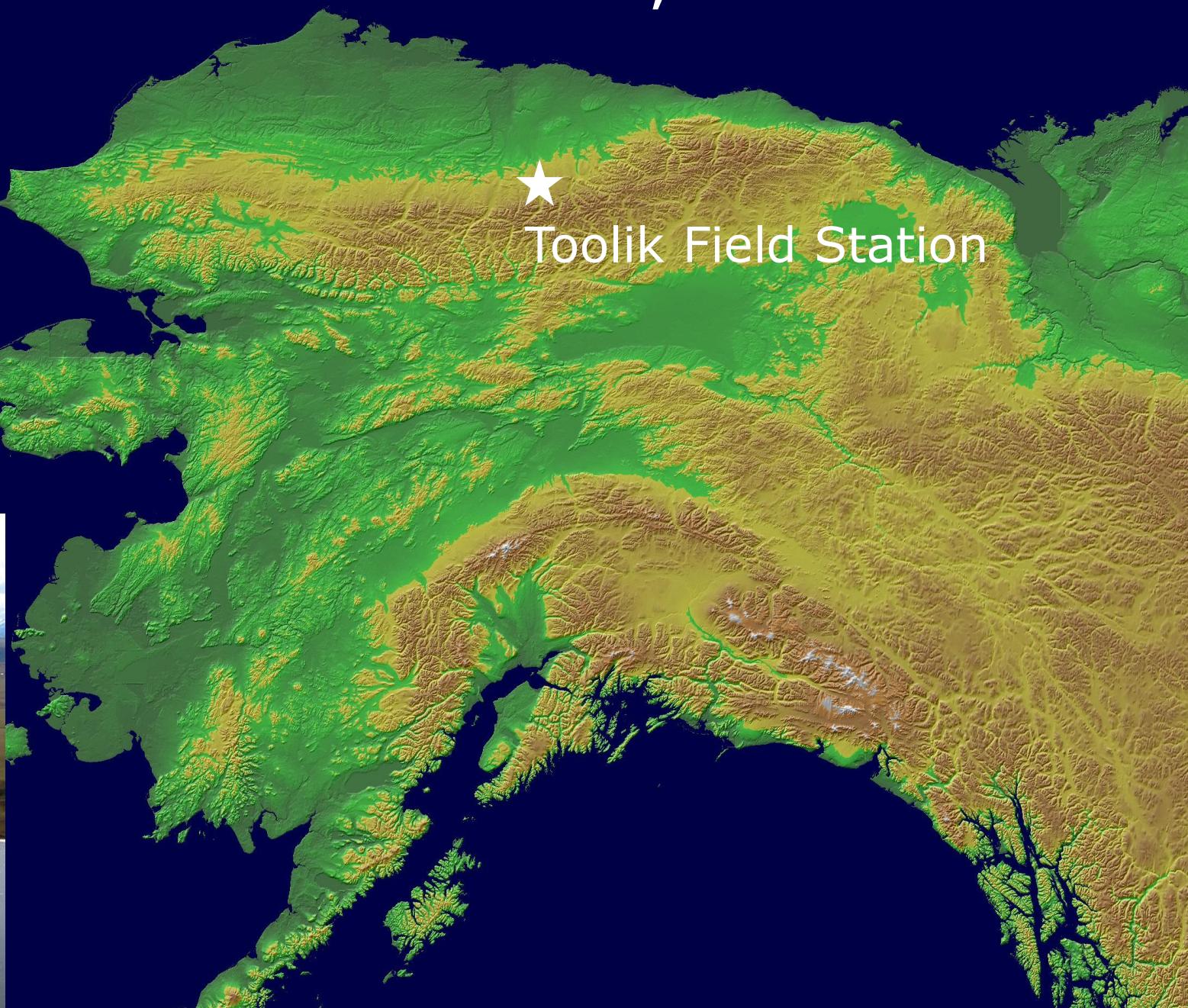
# Sampling at Toolik Field Station, Alaska



Brooks Range



Toolik Lake



Toolik Field Station

# Sampling at Toolik Field Station



# Sequence datasets

- 18 metaproteomes from Toolik and nearby Imnavait Valley
- Database search against 20 metagenomes and 8 metatranscriptomes from Imnavait Valley and Fairbanks area
- Relative abundances of proteins/functions calculated from spectral abundances

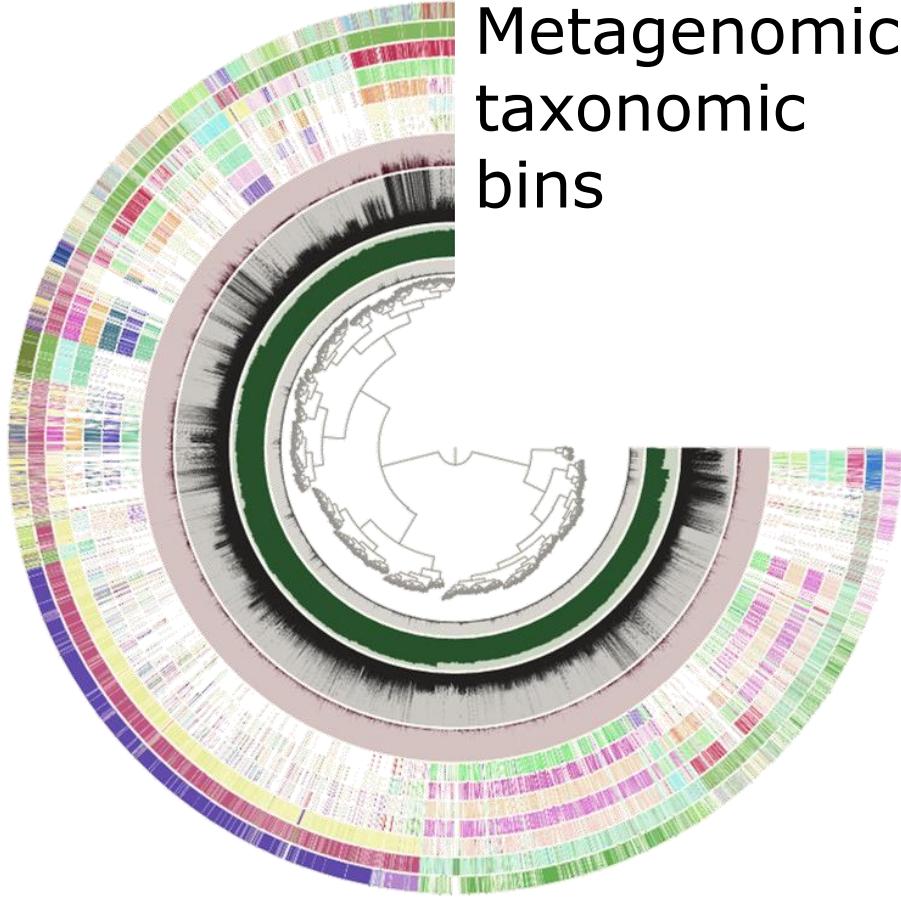


Myers-Smith, 2017, in *Medium*

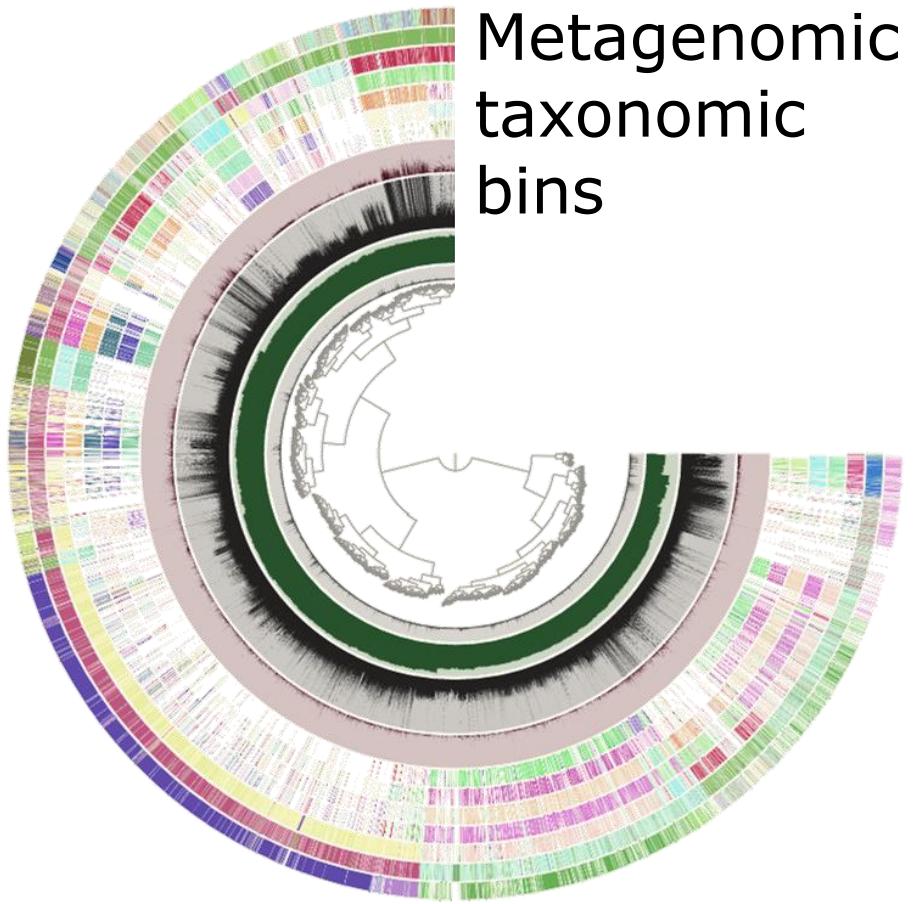


Walker,  
*Arctic Geobotanical Atlas*

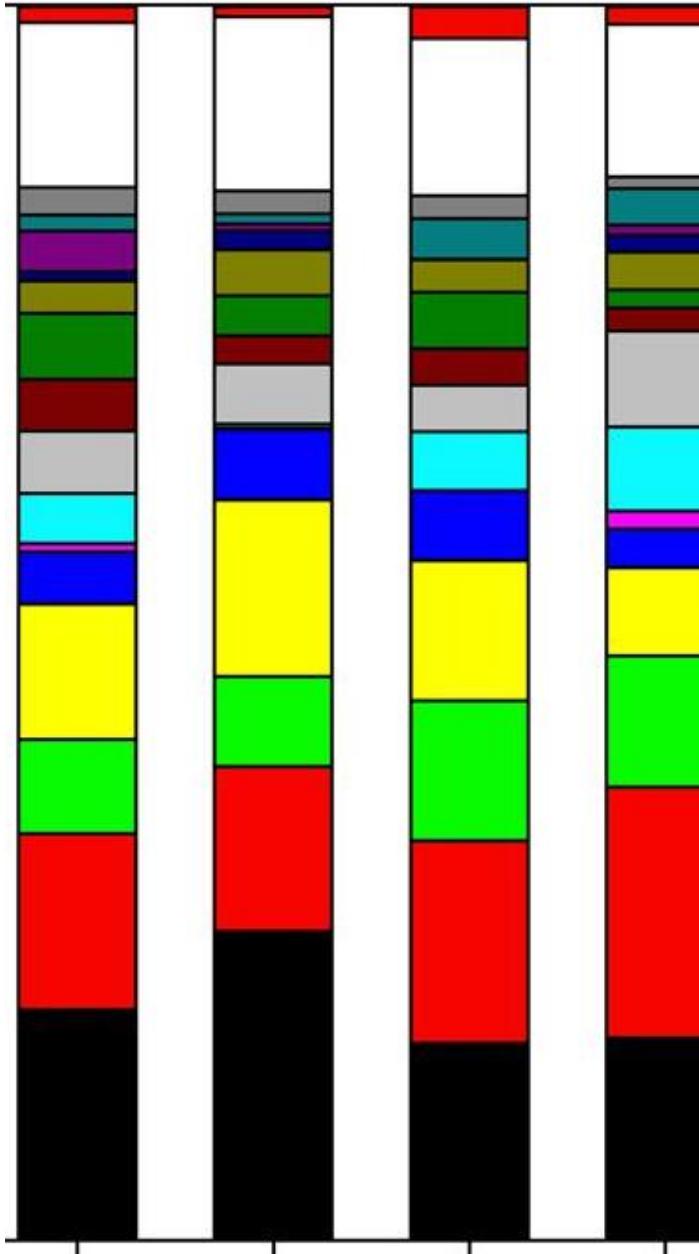
# Taxonomic diversity of soil bacteria



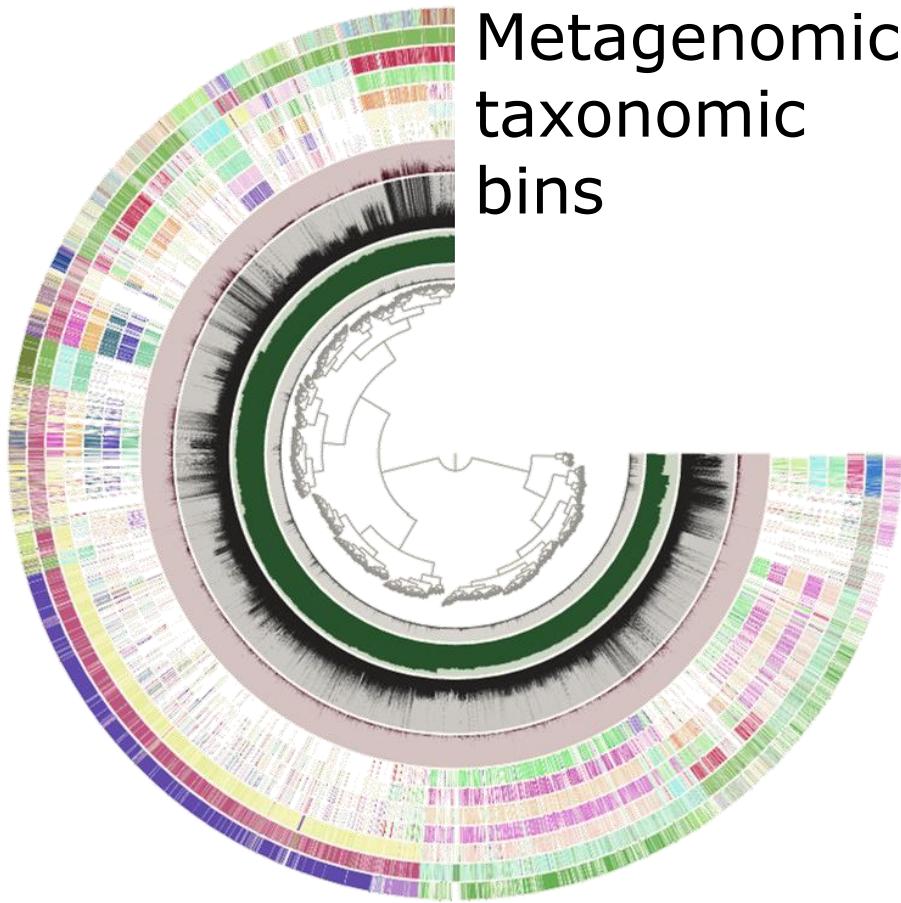
# Taxonomic diversity of soil bacteria



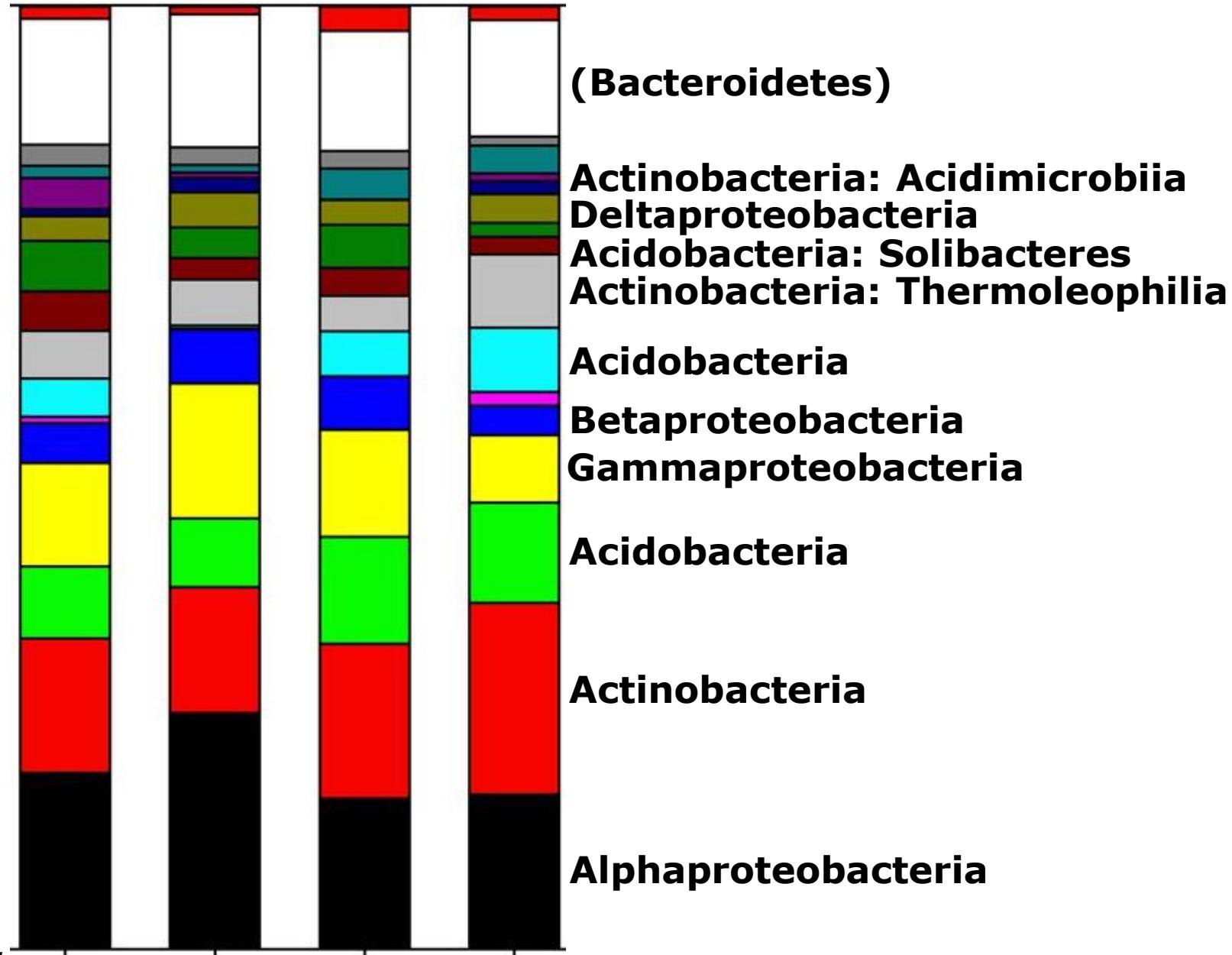
Metagenomic  
taxonomic  
bins



# Taxonomic diversity of soil bacteria

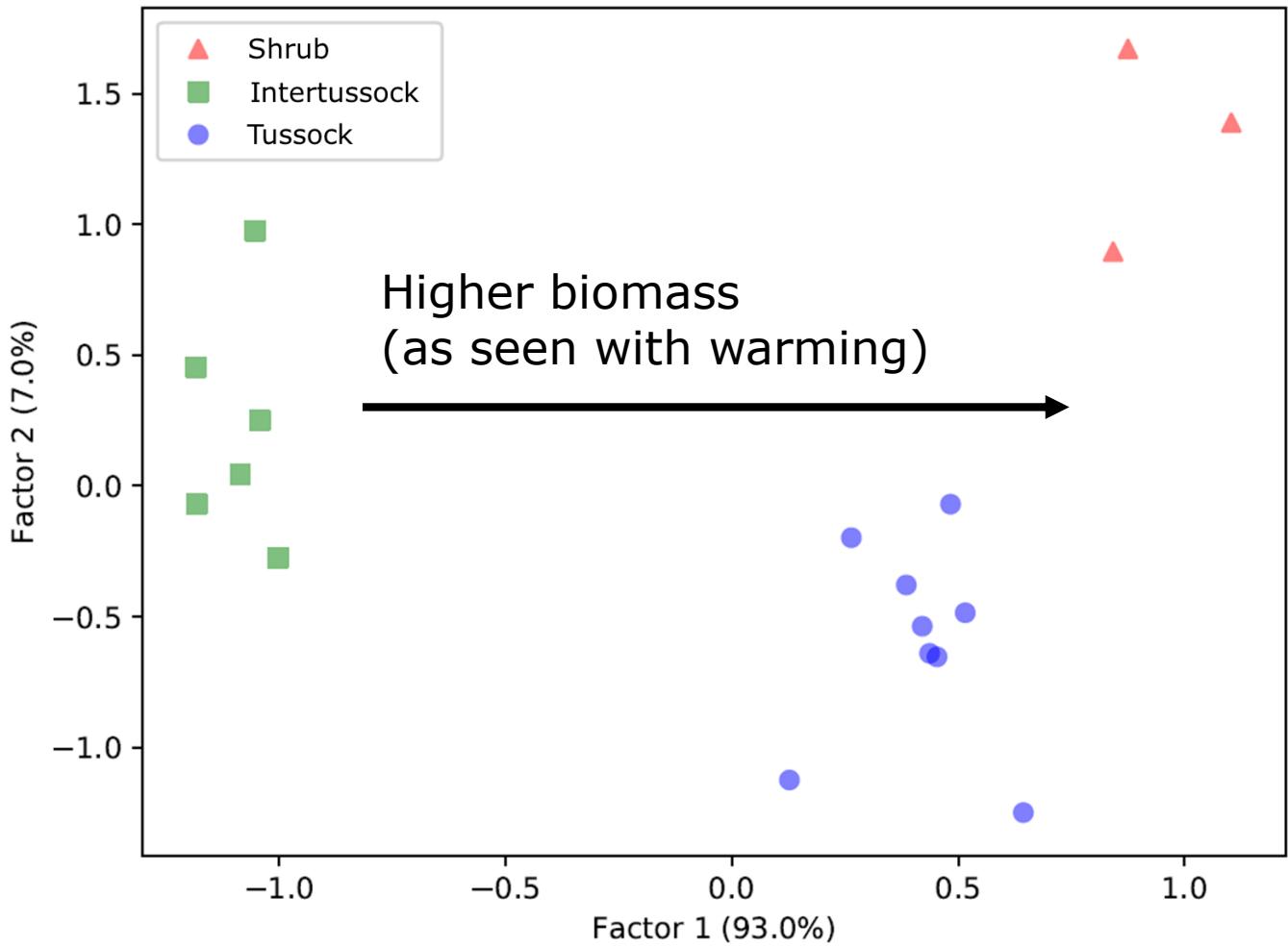


Metagenomic  
taxonomic  
bins

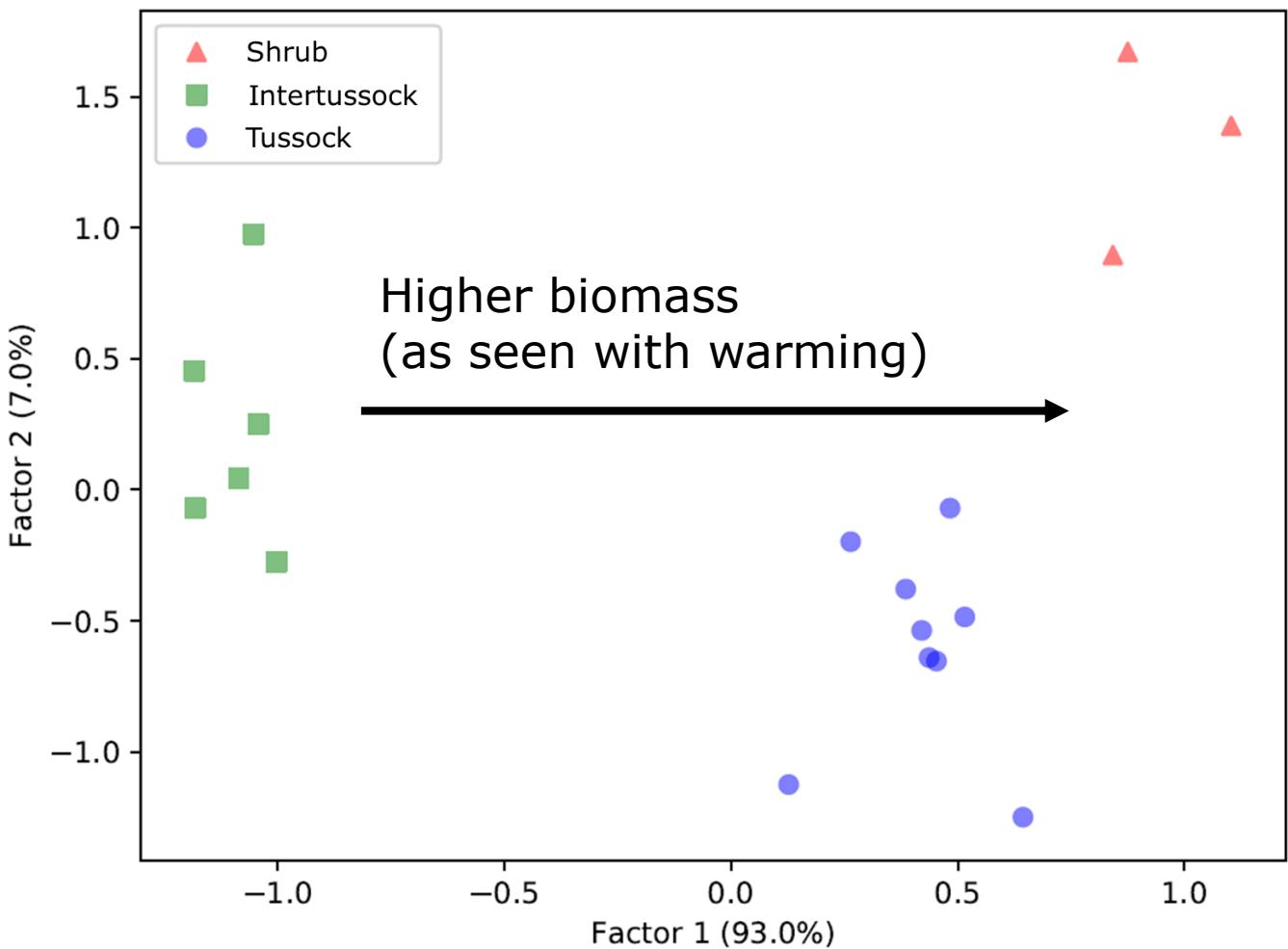


# Protein expression profile changes with flora

- Based only on the relative abundances of proteins in the 18 soil metaproteomic samples, the samples sort into low and high biomass floras



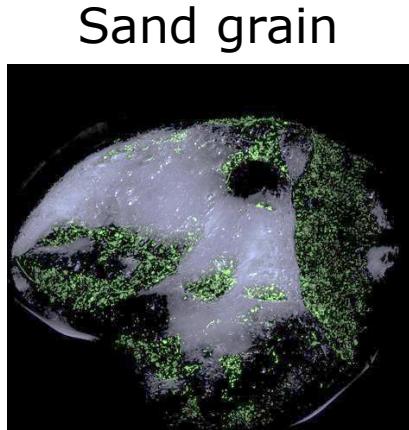
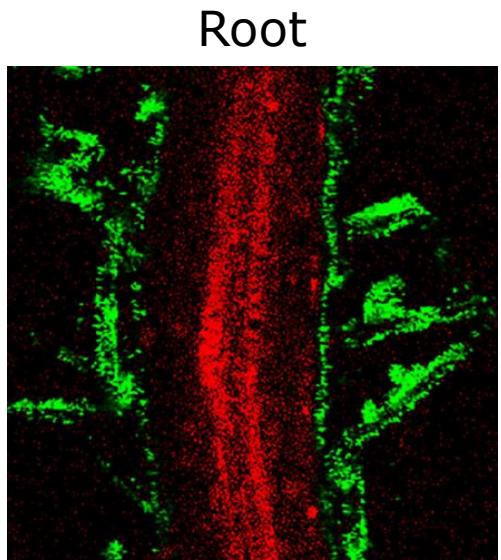
# Protein expression profile changes with flora



Functions associated with Factor 1 include  
sugar transporters and  
succinoglycan EPS (slime) production

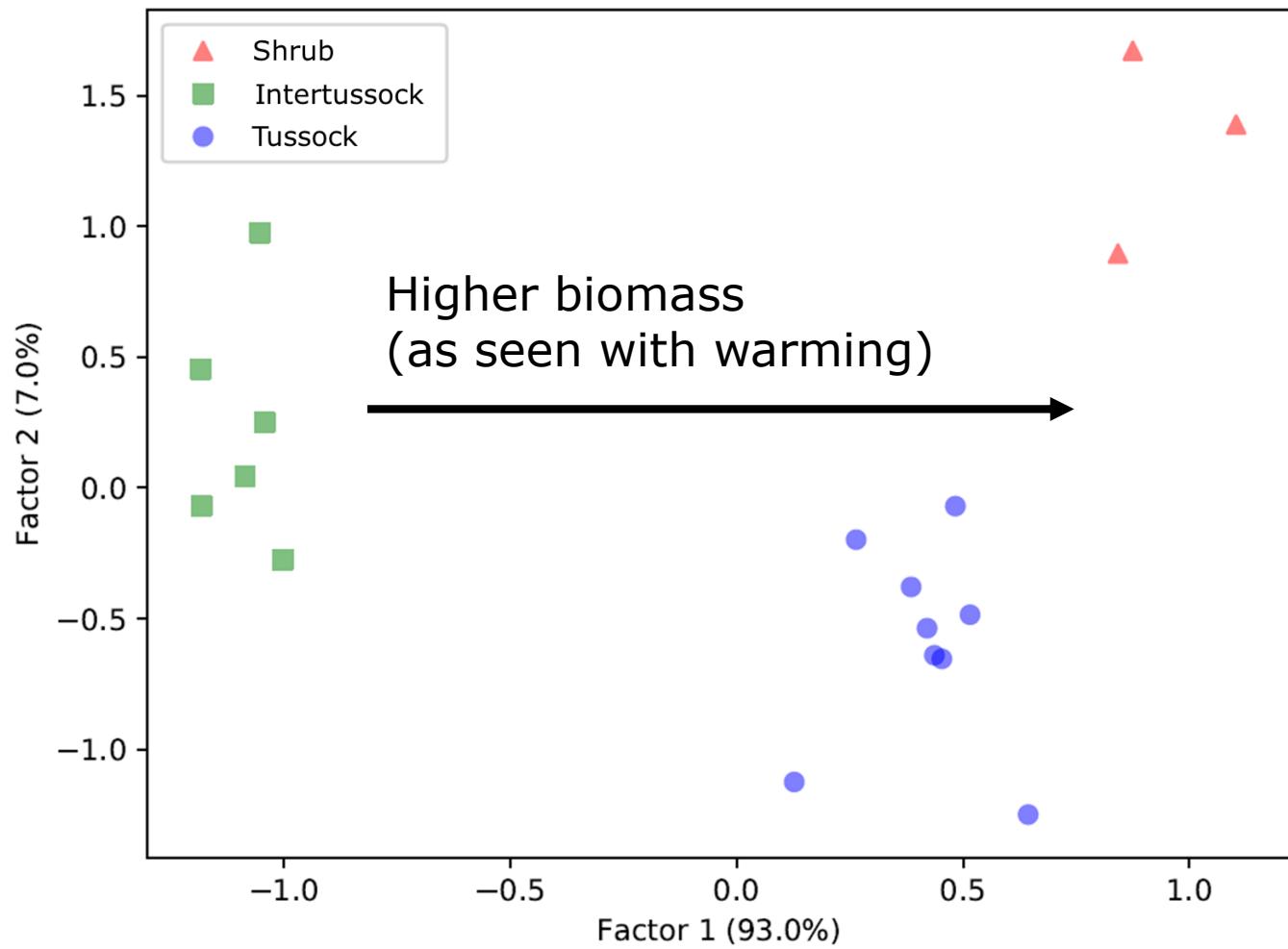
# Protein expression profile changes with flora

- Hypothesis: Greater plant biomass increases importance of microbe/plant interactions in biogeochemical cycling



Zhao et al., 2018,  
*Front. Microbiol.*

Probandt et al.,  
2018, *ISME J.*



Functions associated with Factor 1 include sugar transporters and succinoglycan EPS (slime) production

# Taxonomic protein expression profiles

Taxonomic “fidelity” =  
Overall abundance of function \*  
Relatedness of function to taxon (similarity score)

Fidelity is used as a measure of protein expression by different taxa

# Taxonomic protein expression profiles

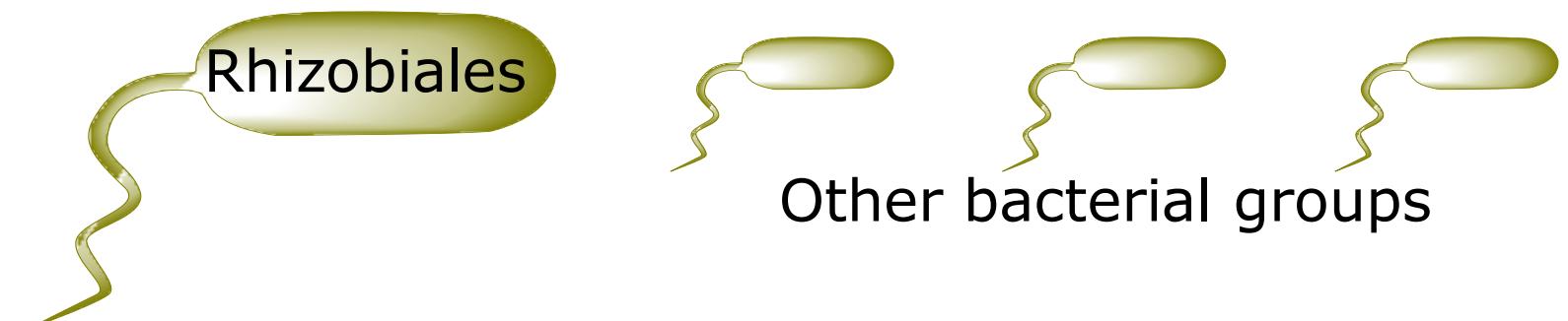
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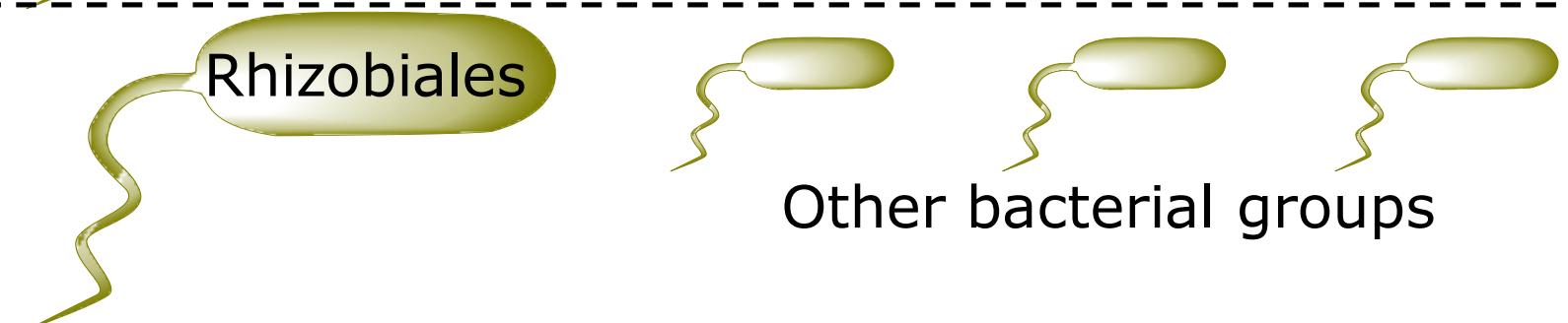
When fidelity scores for a function are **normalized across taxa**,  
they indicate the relative expression of each function by different taxa

## Sugar transporters

Low plant biomass



High plant biomass



# Taxonomic protein expression profiles

Taxonomic “fidelity” =  
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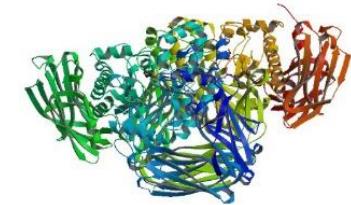
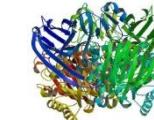
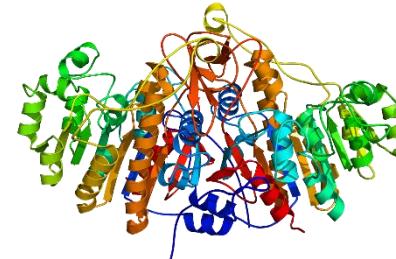
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When fidelity scores for a taxon are **normalized across functions**,  
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## Rhizobiales

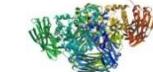
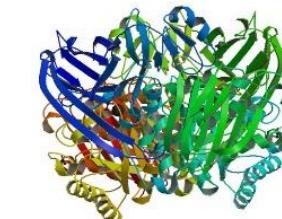
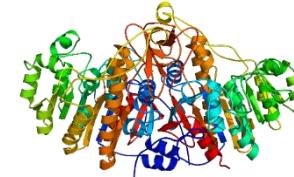
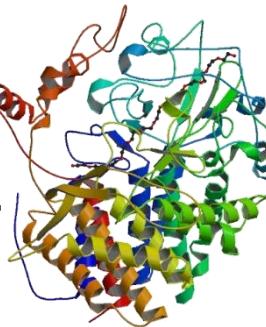
Low plant biomass

Sugar  
transporters

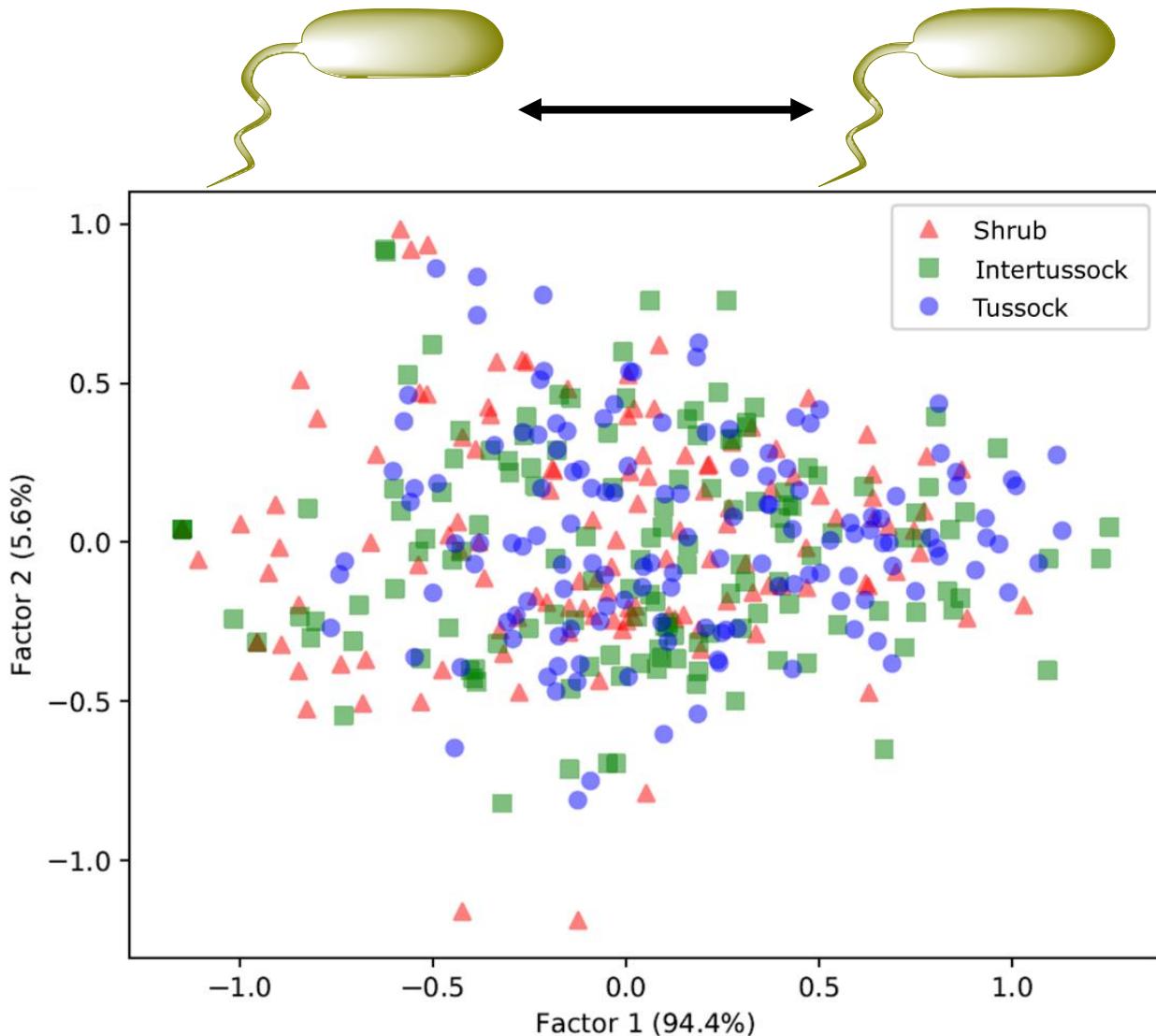
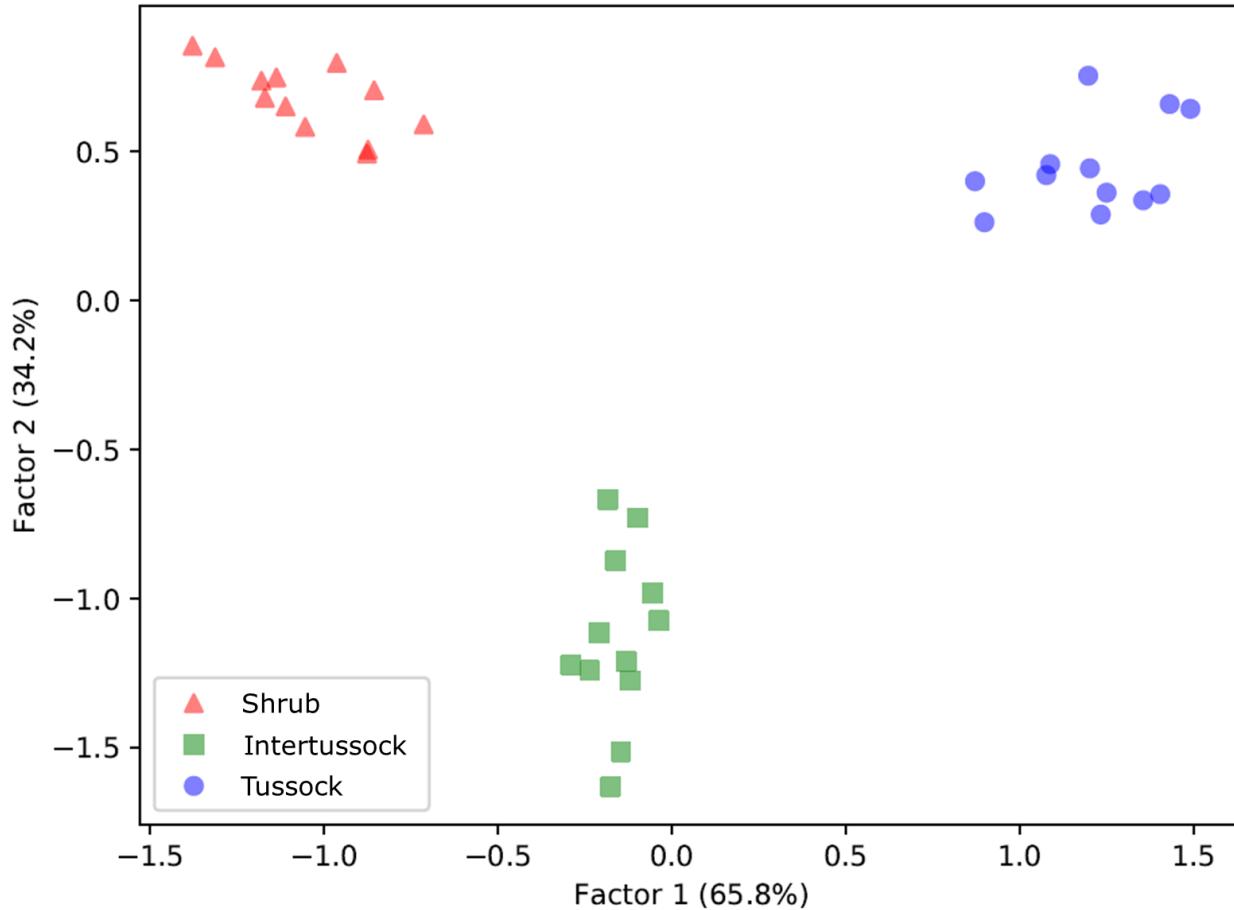
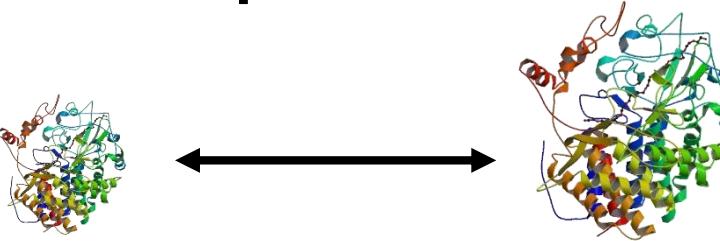


High plant biomass

Sugar  
transporters

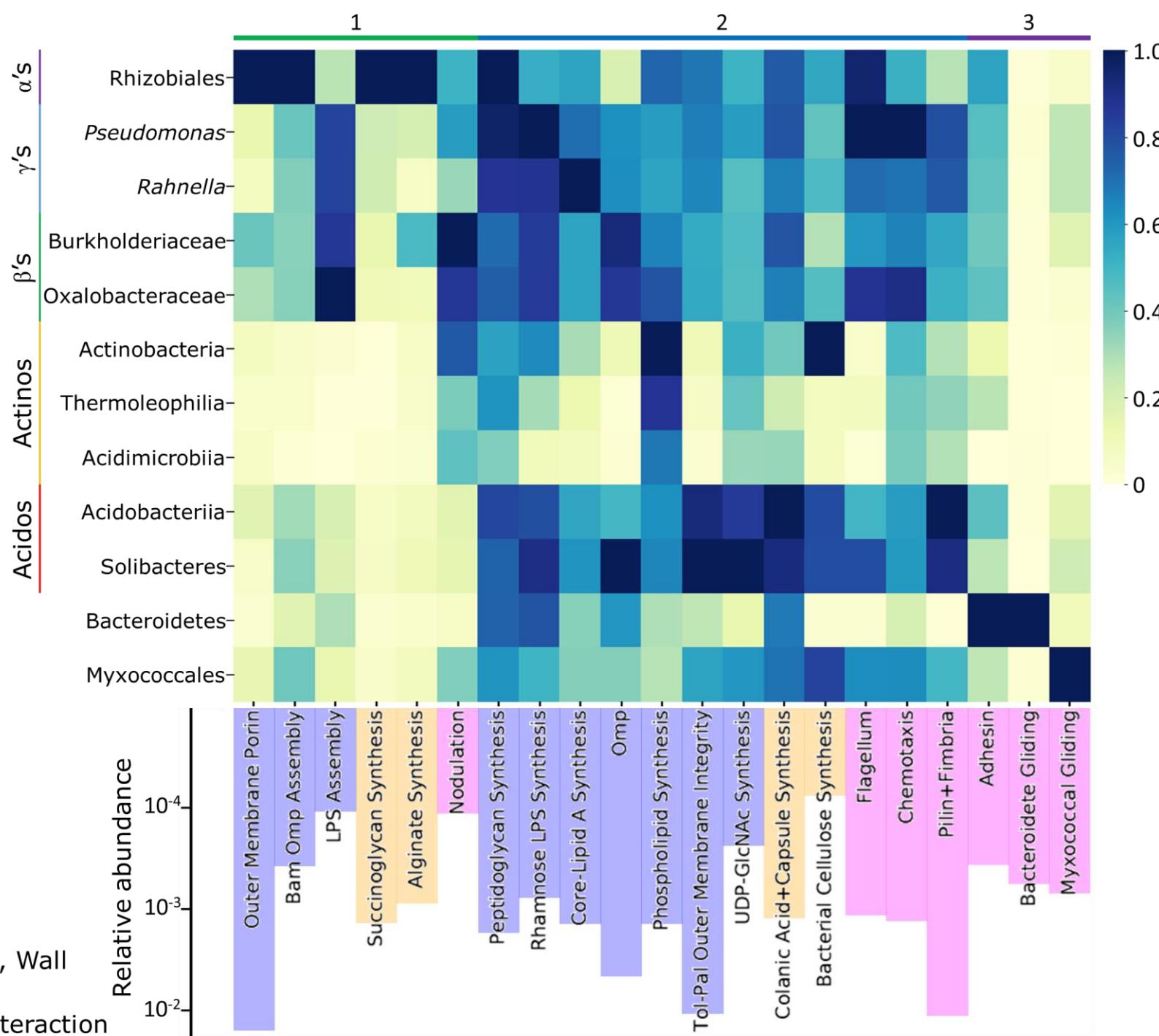


# Flexible expression of functions by the same taxa



# Heatmap: Taxonomic fidelity

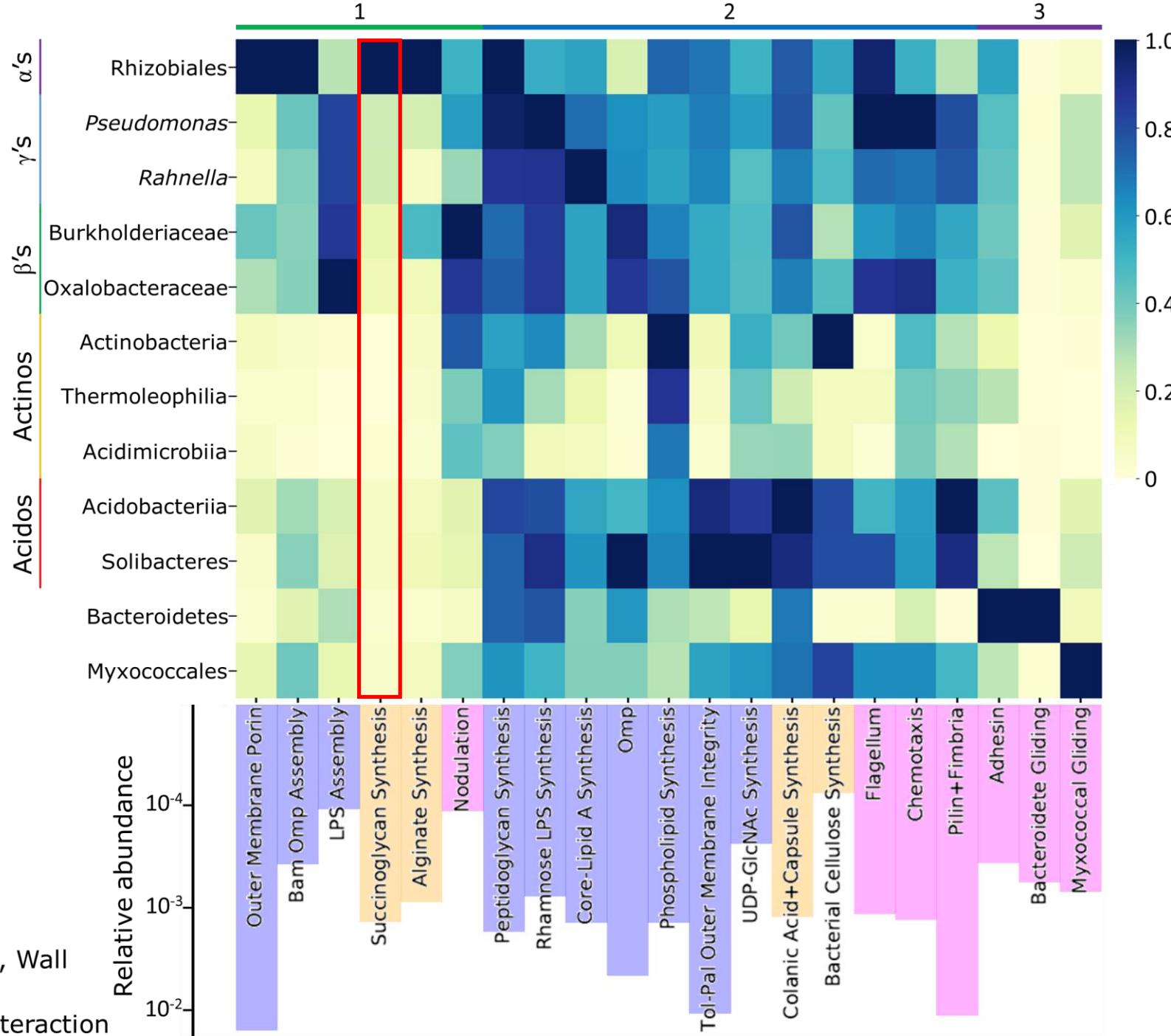
Bars:  
Overall abundance



# Heatmap: Taxonomic fidelity

Bars:  
Overall abundance

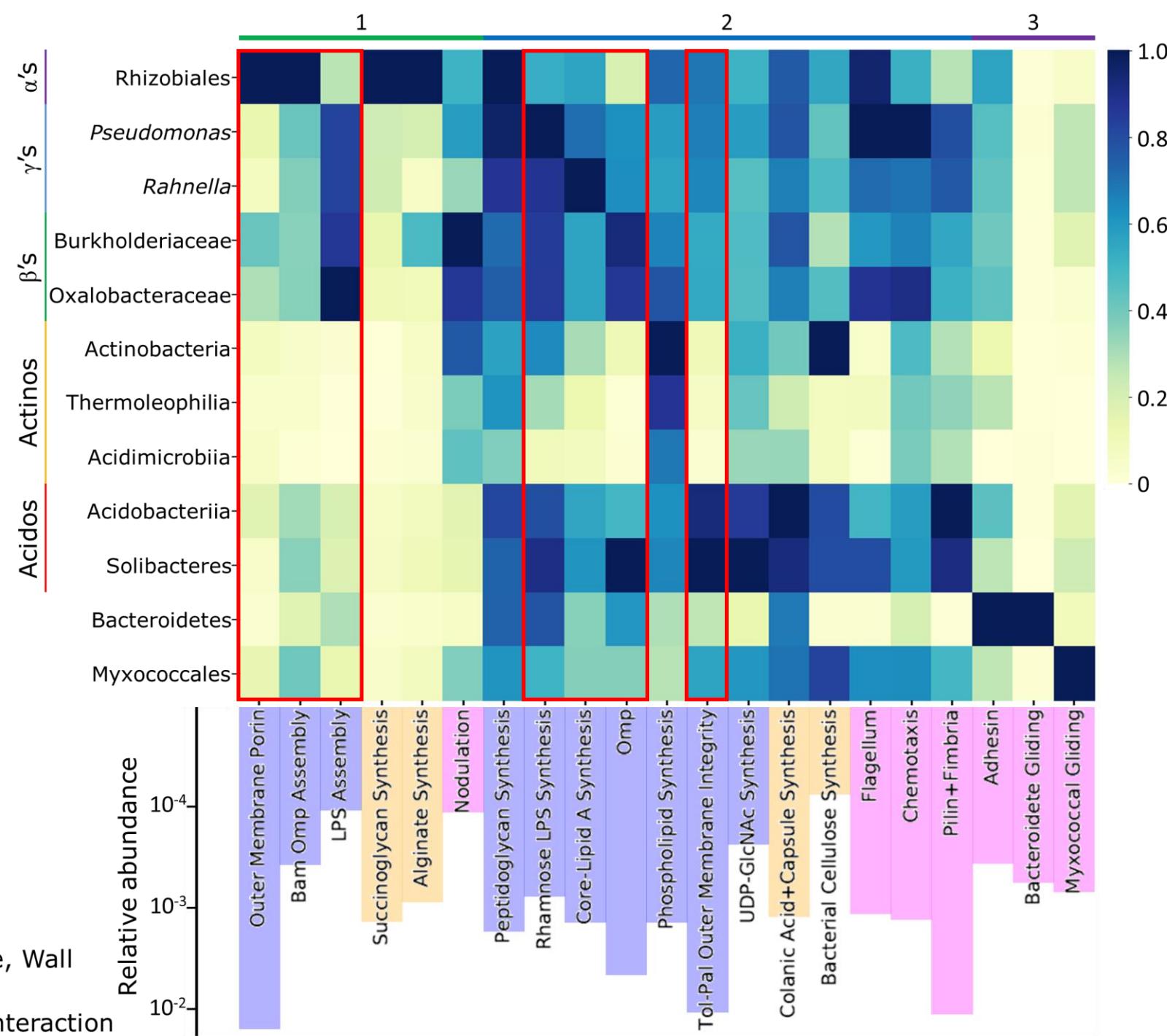
## Cell Envelope



# Validation of similarity score as a measure of taxonomic relatedness for calculation of fidelity

Outer membrane-related proteins have very low scores in Gram-positives

## Cell Envelope

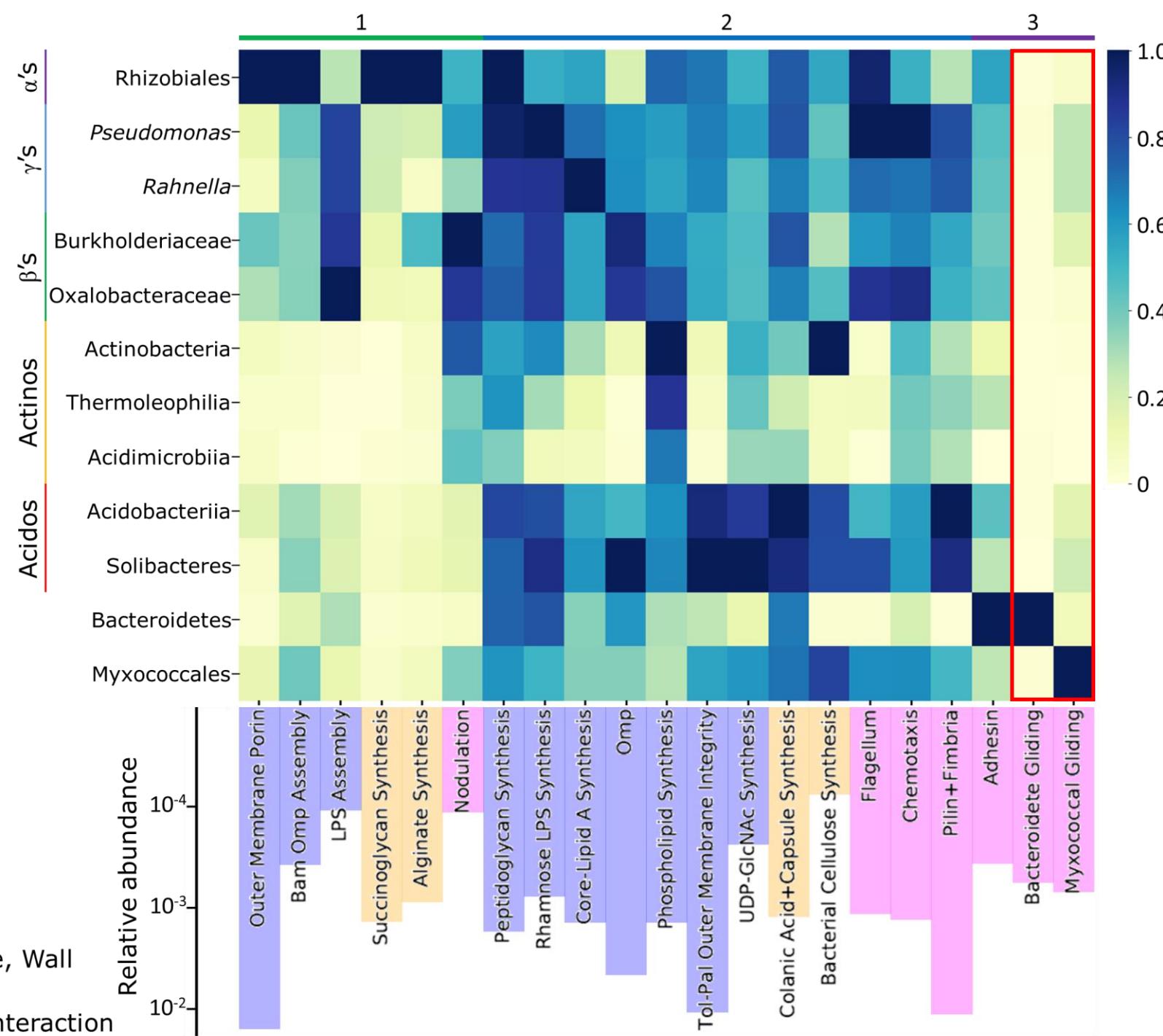


# Validation of similarity score as a measure of taxonomic relatedness for calculation of fidelity

Outer membrane-related proteins have very low scores in Gram-positives

Bacteroidete and myxococcal gliding proteins have very high scores in Bacteroidetes and Myxococcales

## Cell Envelope



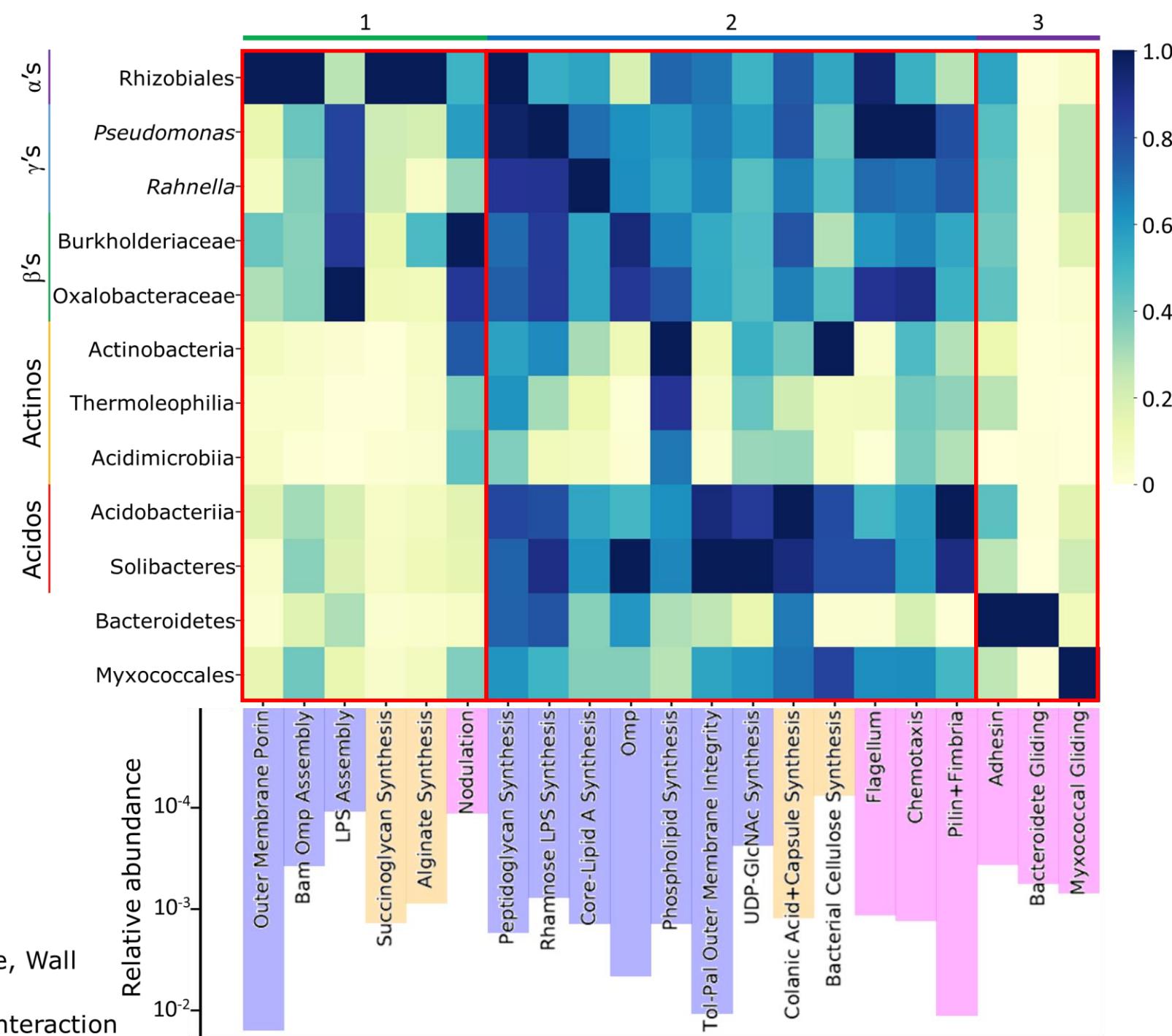
# Three functional clusters

Cluster 1:  
High Proteobacteria,  
Low Non-Proteobacteria

Cluster 2:  
Relatively even  
Proteobacteria and  
Non-Proteobacteria

Cluster 3:  
Low Proteobacteria,  
High Non-Proteobacteria

## Cell Envelope



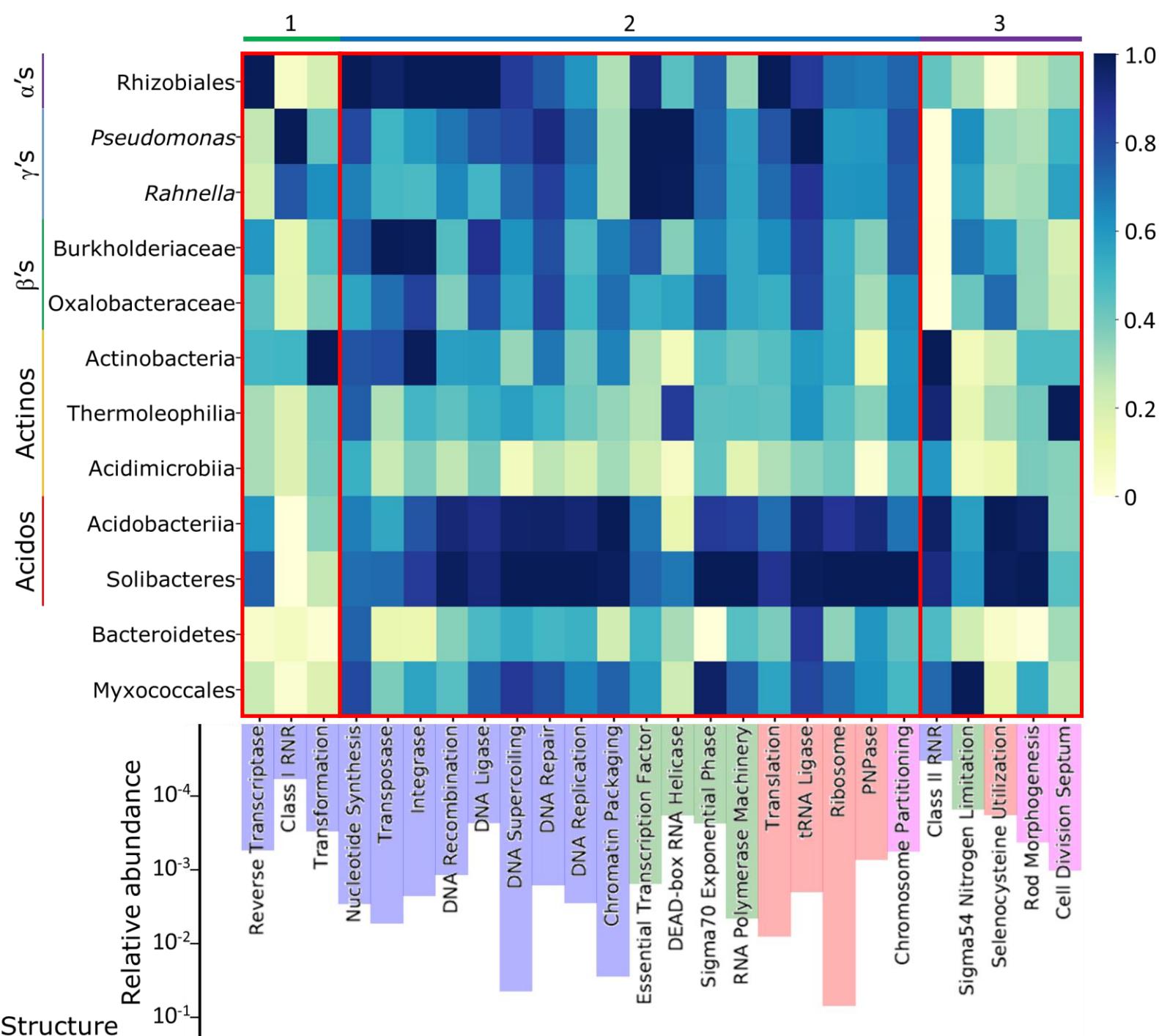
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## Core Cellular Functions



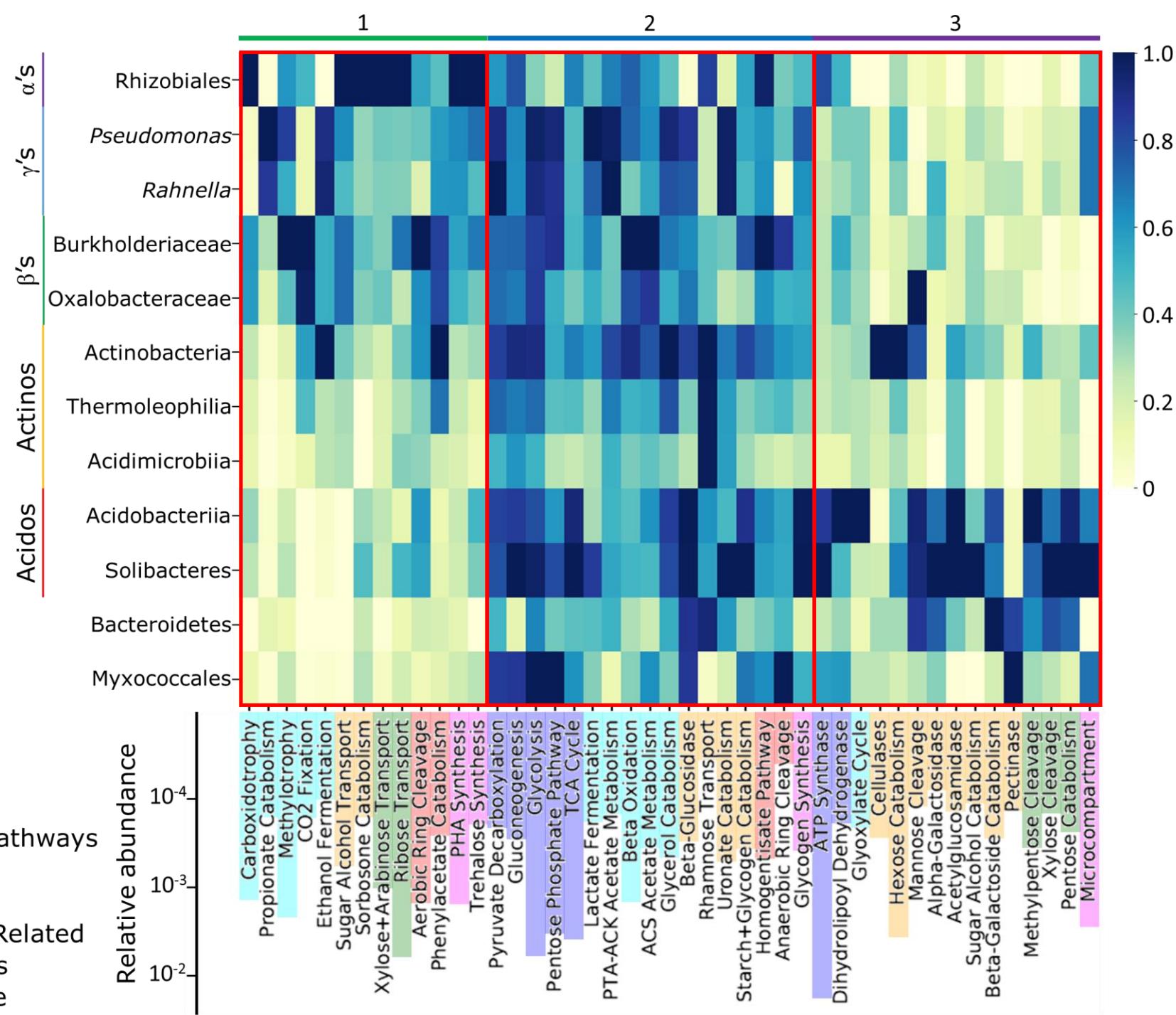
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High Non-Proteobacteria

## Carbon Metabolism



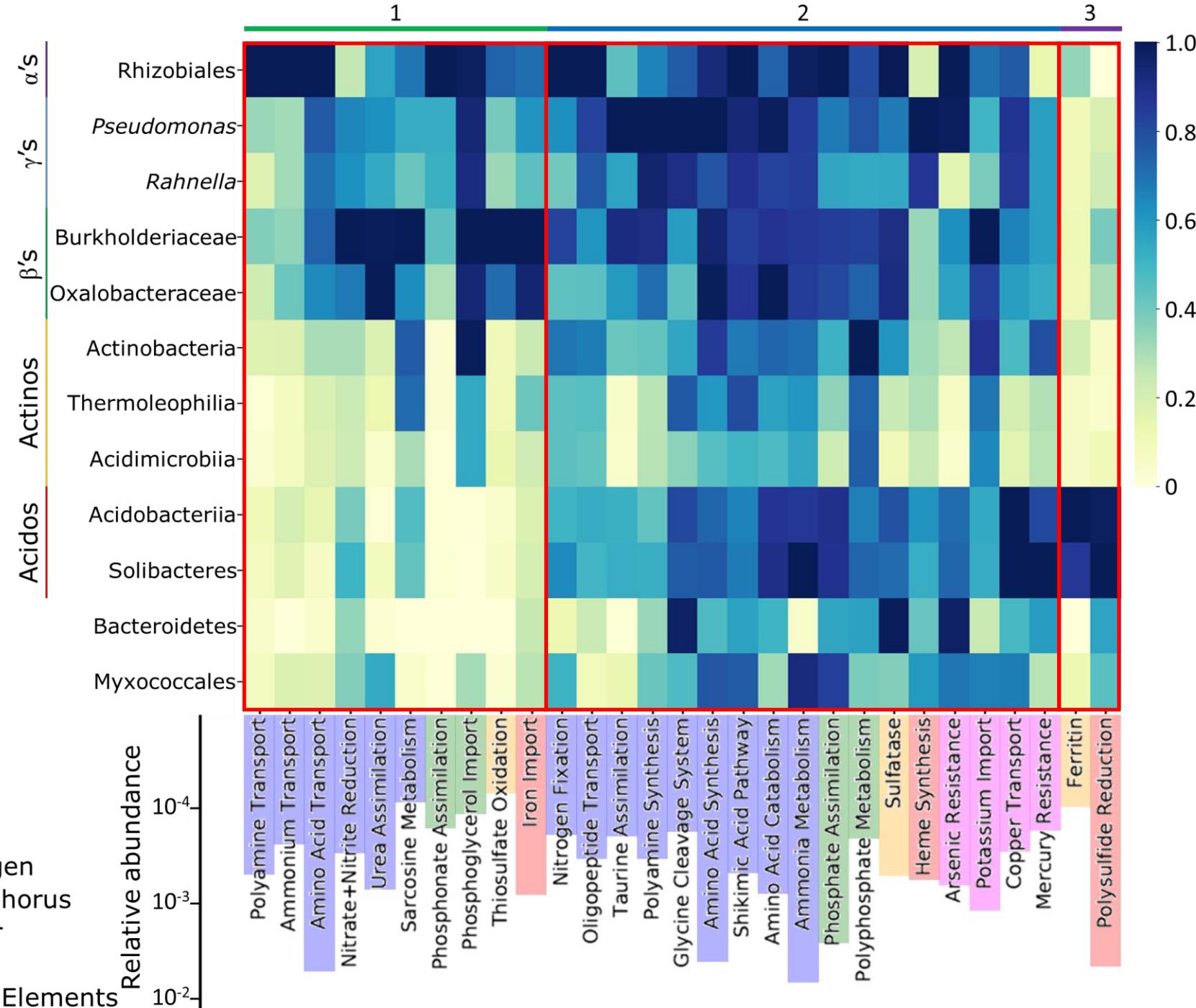
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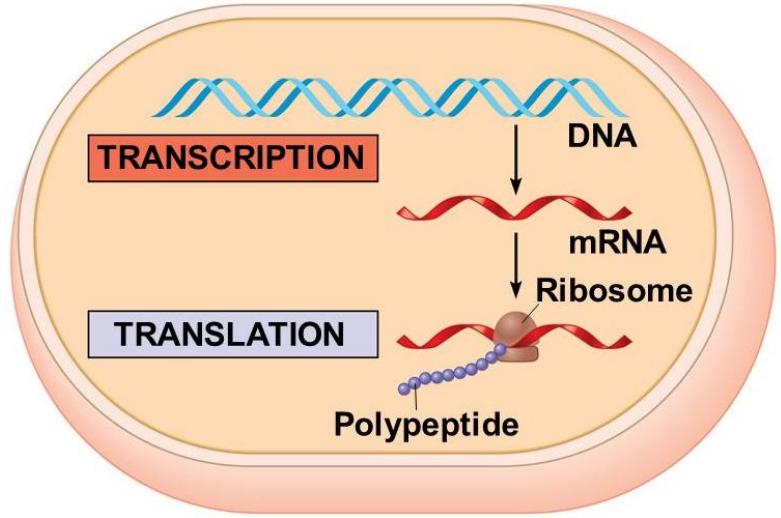
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Low Proteobacteria,  
High Non-Proteobacteria

## Nutrient Metabolism



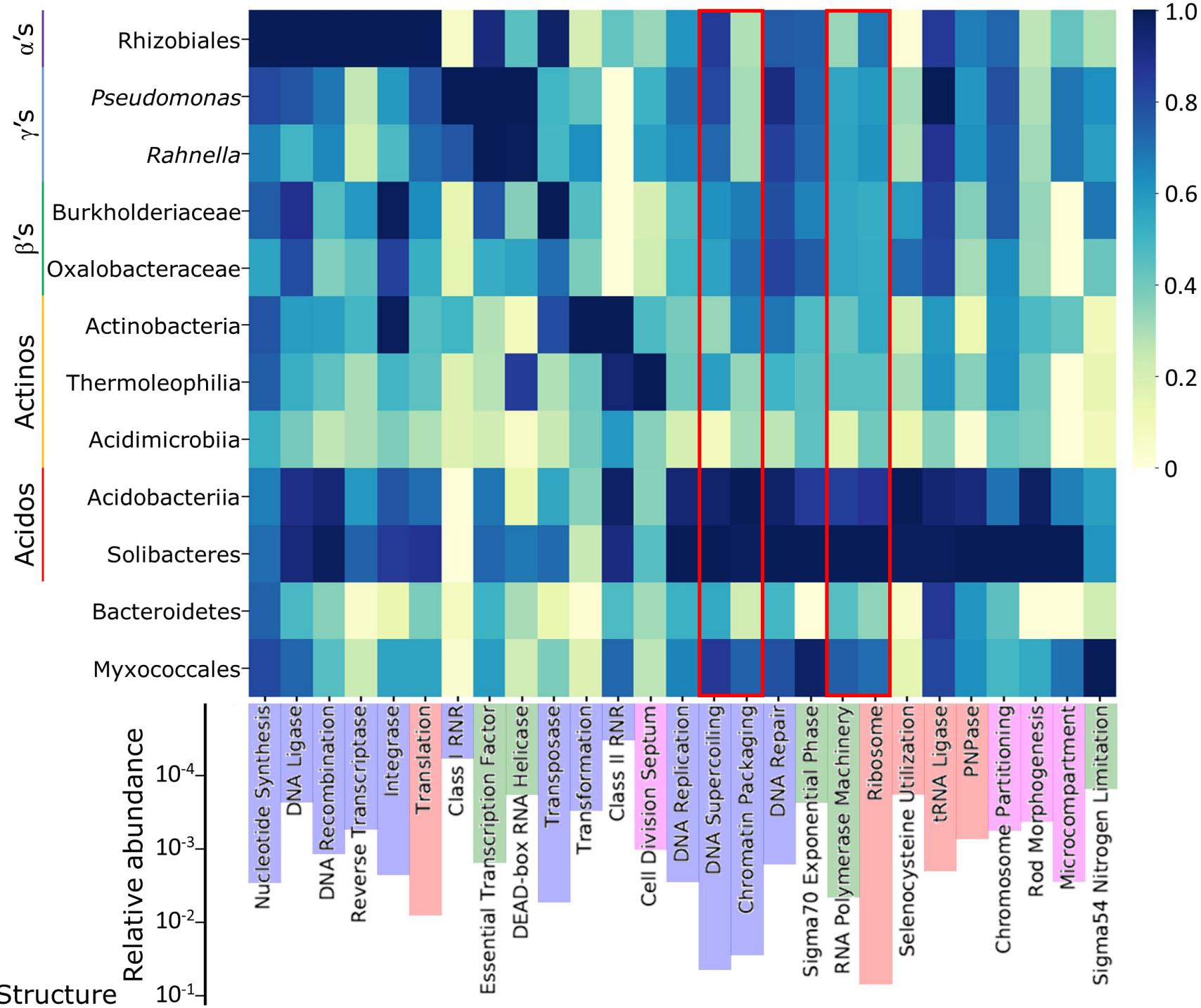
# Acidobacteria are the most active group, contrary to expectation from DNA abundance



Pearson Education

## Core Cellular Functions

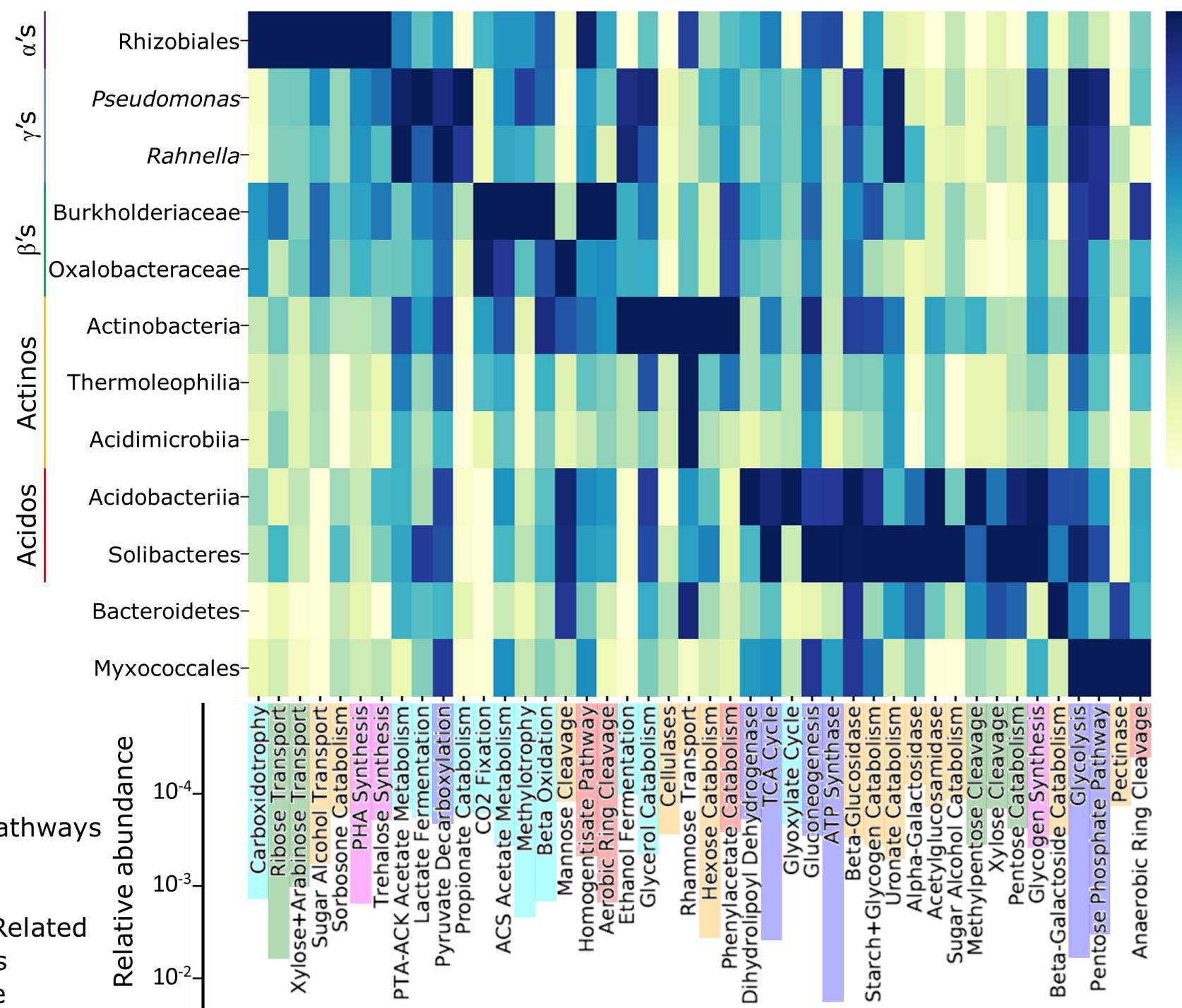
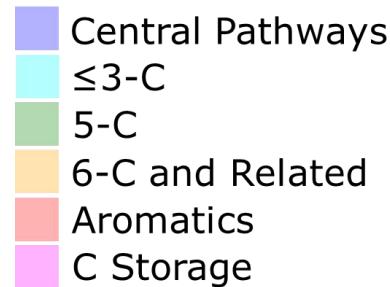
DNA  
RNA  
Protein  
Division, Structure



"[T]here are numerous individual processes and taxa associated with the metabolism of the thousands of organic compounds found in soil. This complexity makes it very difficult to predict soil function..."

**Hypothesis:** Pathways of organic matter decomposition are expressed by taxa in proportion to overall activity. (No resource partitioning)

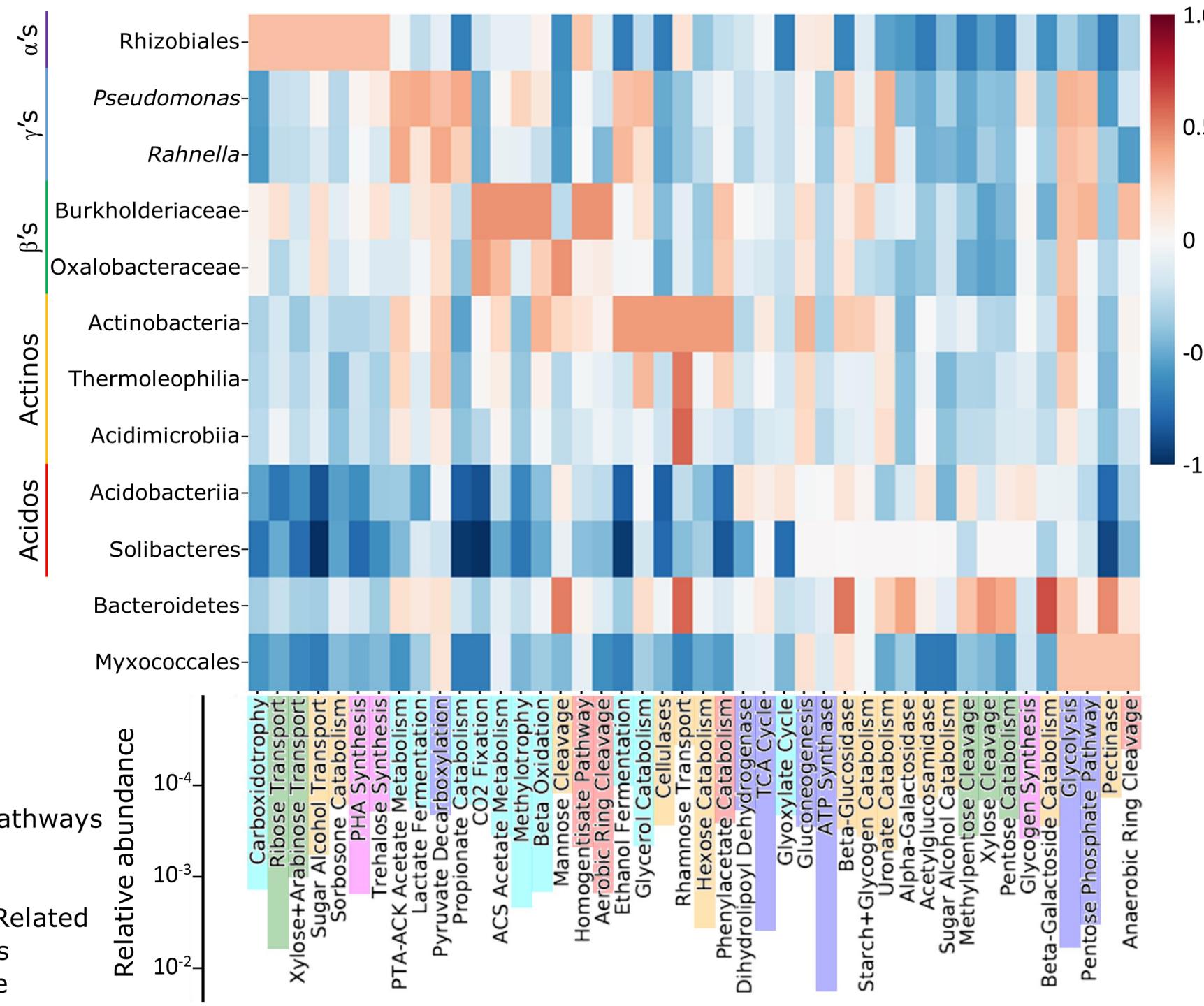
## Carbon Metabolism



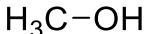
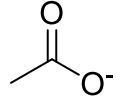
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**Hypothesis:** Pathways of organic matter decomposition are expressed by taxa in proportion to overall activity. (No resource partitioning) **FALSE**

# **Carbon Metabolism**

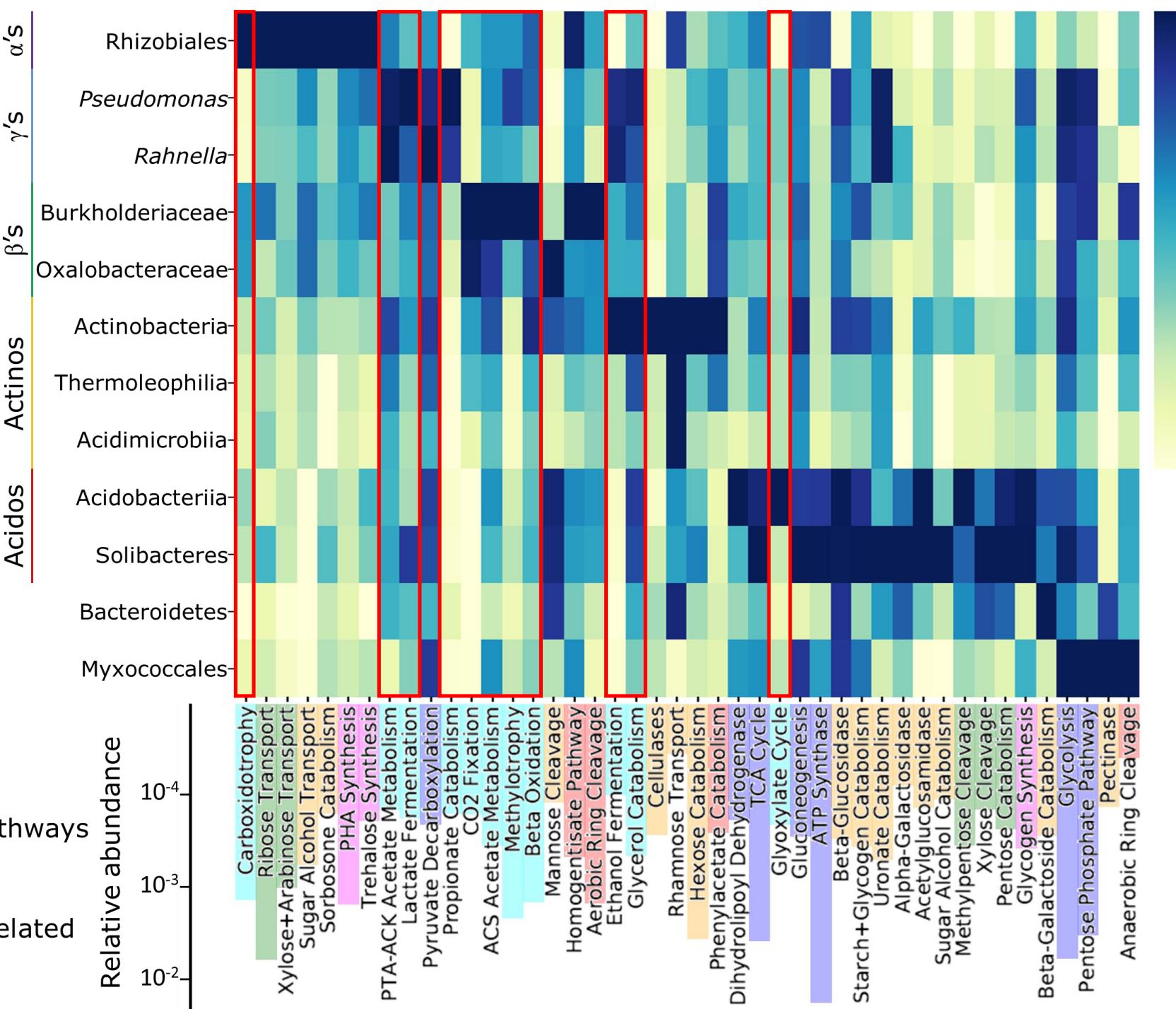


Proteobacteria dominate  
the metabolism of small  
compounds



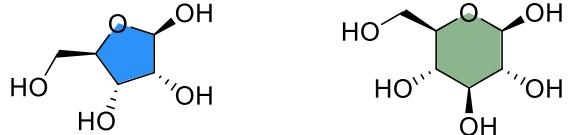
## Carbon Metabolism

- █ Central Pathways
- █  $\leq 3\text{-C}$
- █ 5-C
- █ 6-C and Related
- █ Aromatics
- █ C Storage



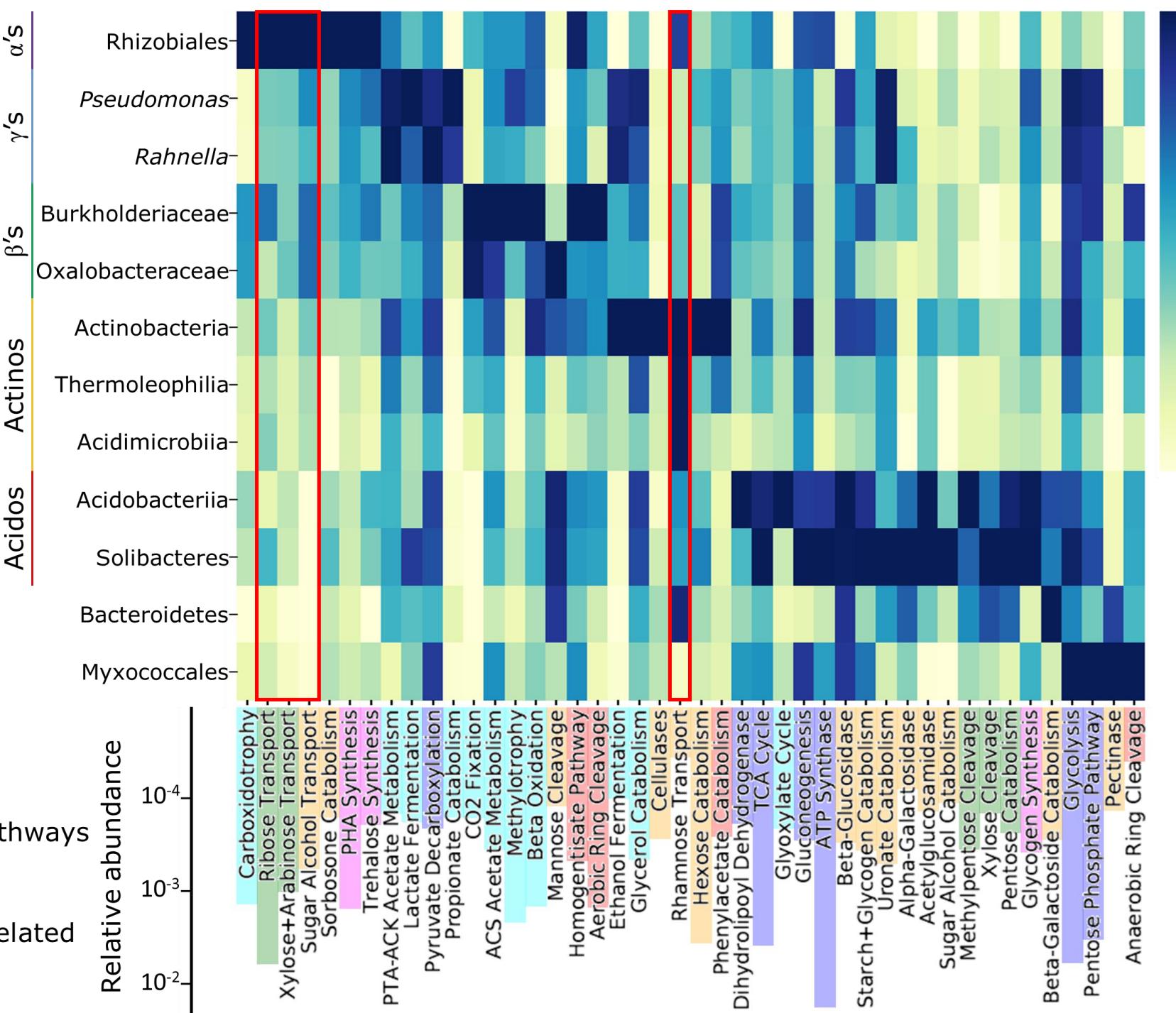
# Proteobacteria dominate the metabolism of small compounds

Proteobacteria, especially Rhizobiales, dominate the expression of simple sugar transporters



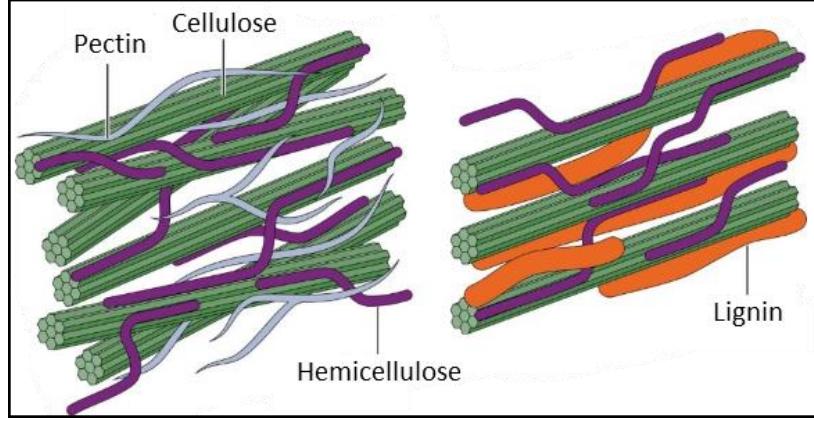
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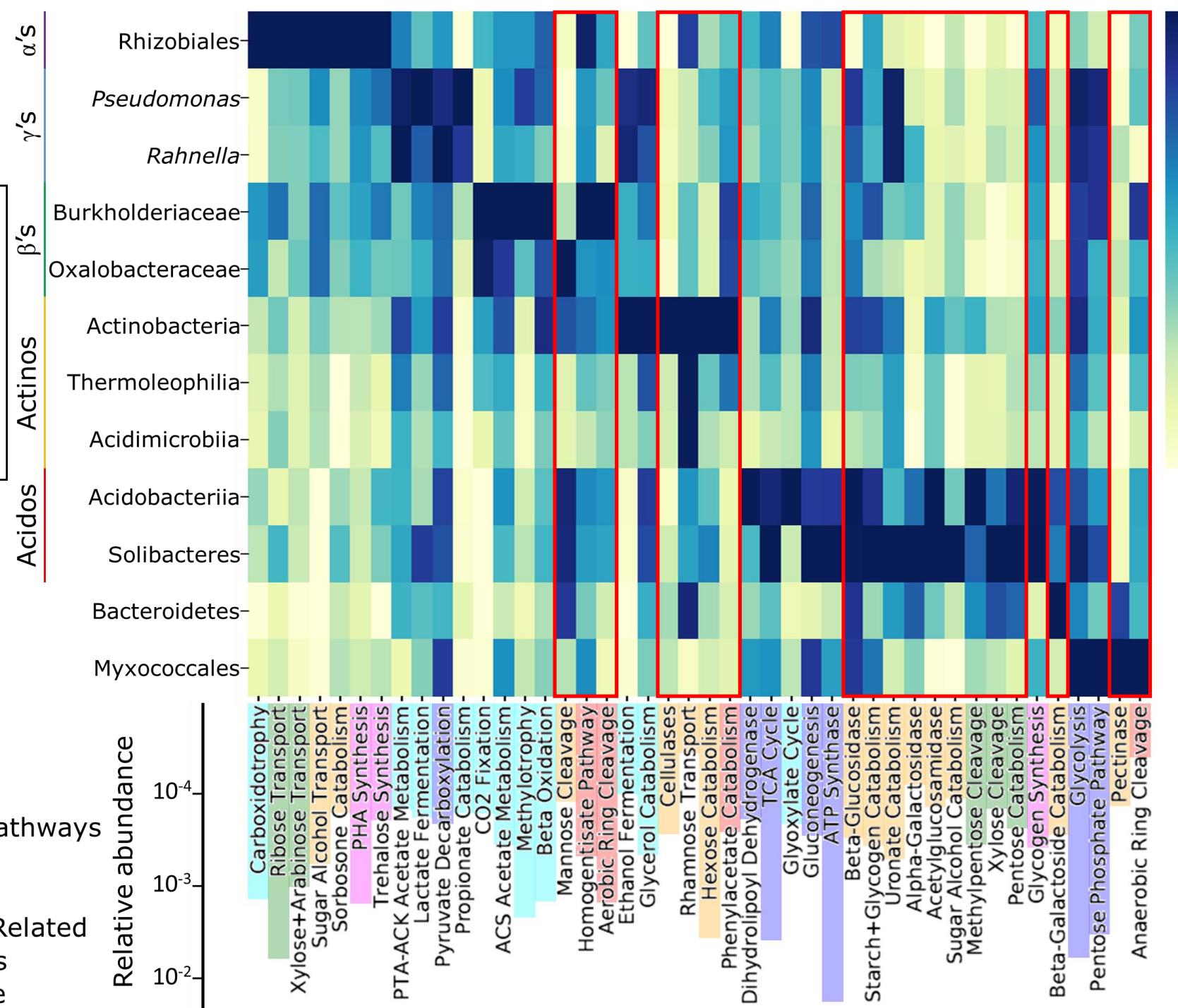


# Non-Proteobacteria dominate polymer degradation

Plant cell wall detritus



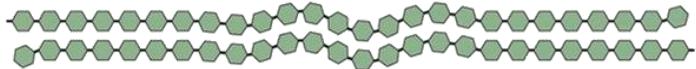
## Carbon Metabolism



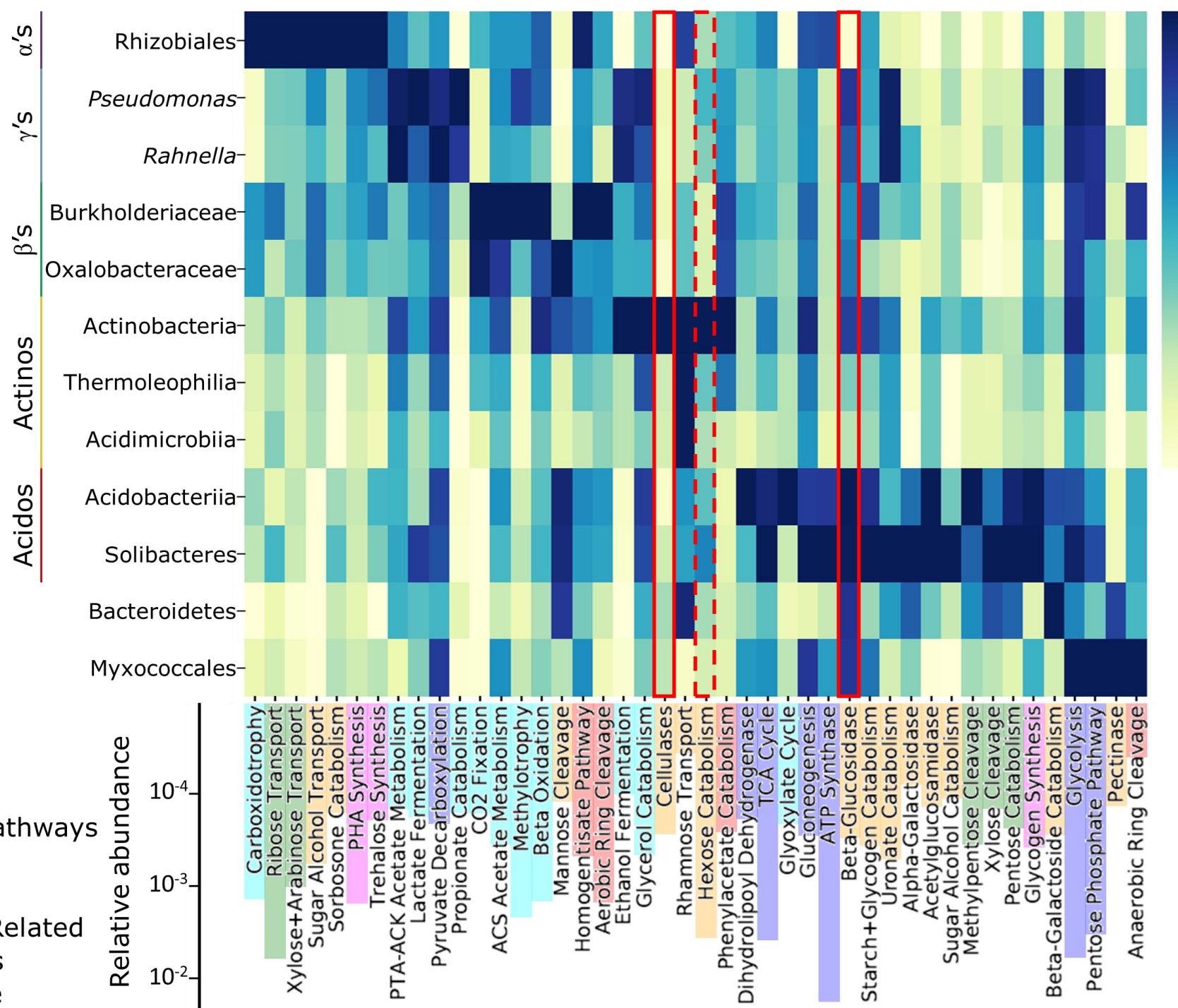
Non-Proteobacteria dominate polymer degradation

Actinobacteria dominate cellulose degradation

## Carbon Metabolism



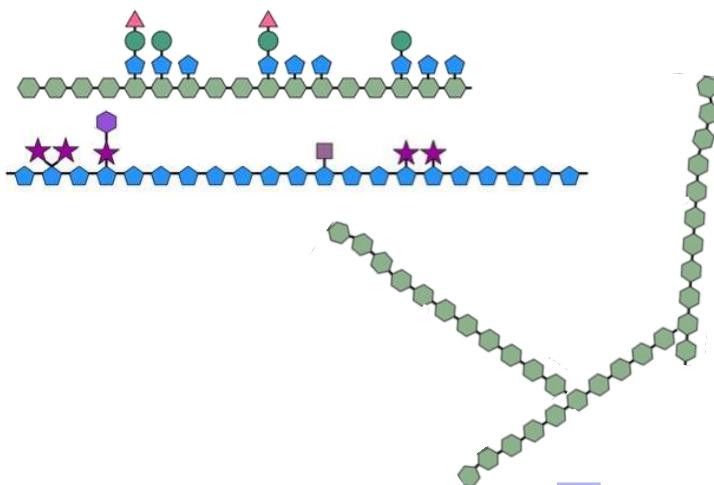
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- Aromatics
- C Storage



Non-Proteobacteria dominate polymer degradation

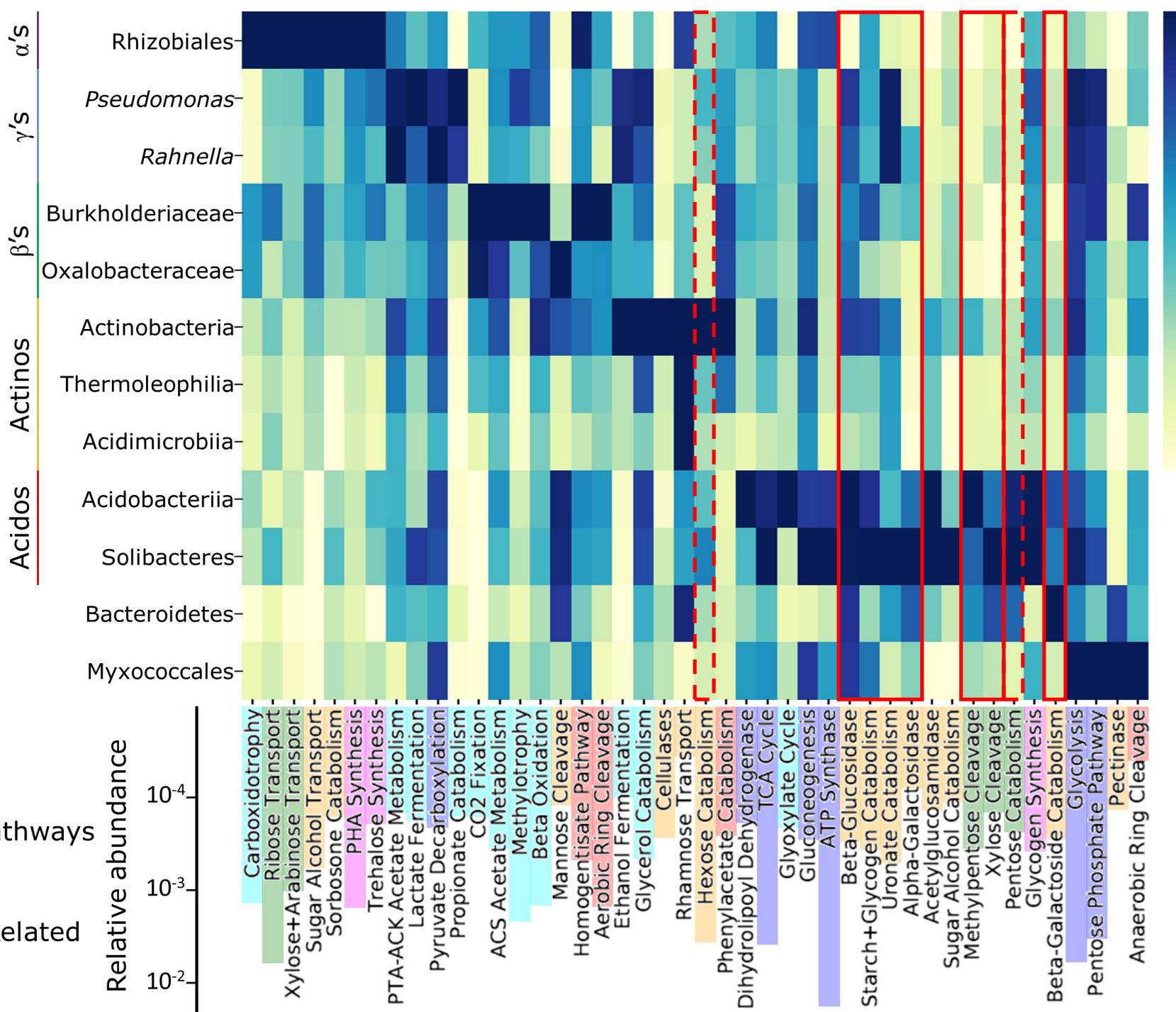
Actinobacteria dominate cellulose degradation

Acidobacteria degrade hemicelluloses, starch, and glycogen



## Carbon Metabolism

- Central Pathways
- $\leq 3\text{-C}$
- 5-C
- 6-C and Related
- Aromatics
- C Storage

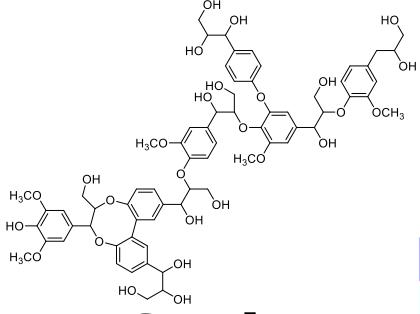


Non-Proteobacteria dominate polymer degradation

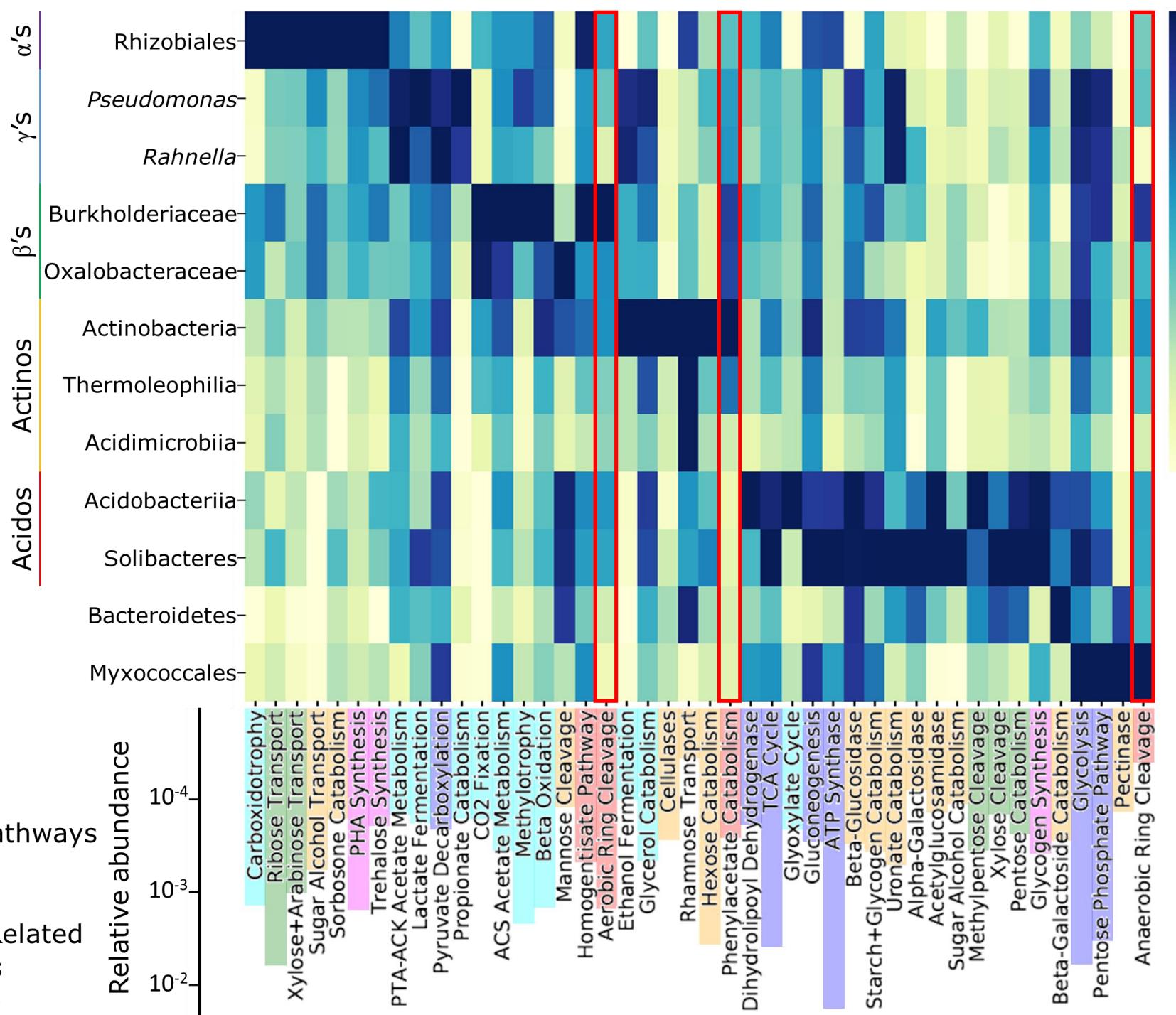
Actinobacteria dominate cellulose degradation

Acidobacteria degrade hemicelluloses, starch, and glycogen

Burkholderiaceae and Actinobacteria dominate lignin degradation



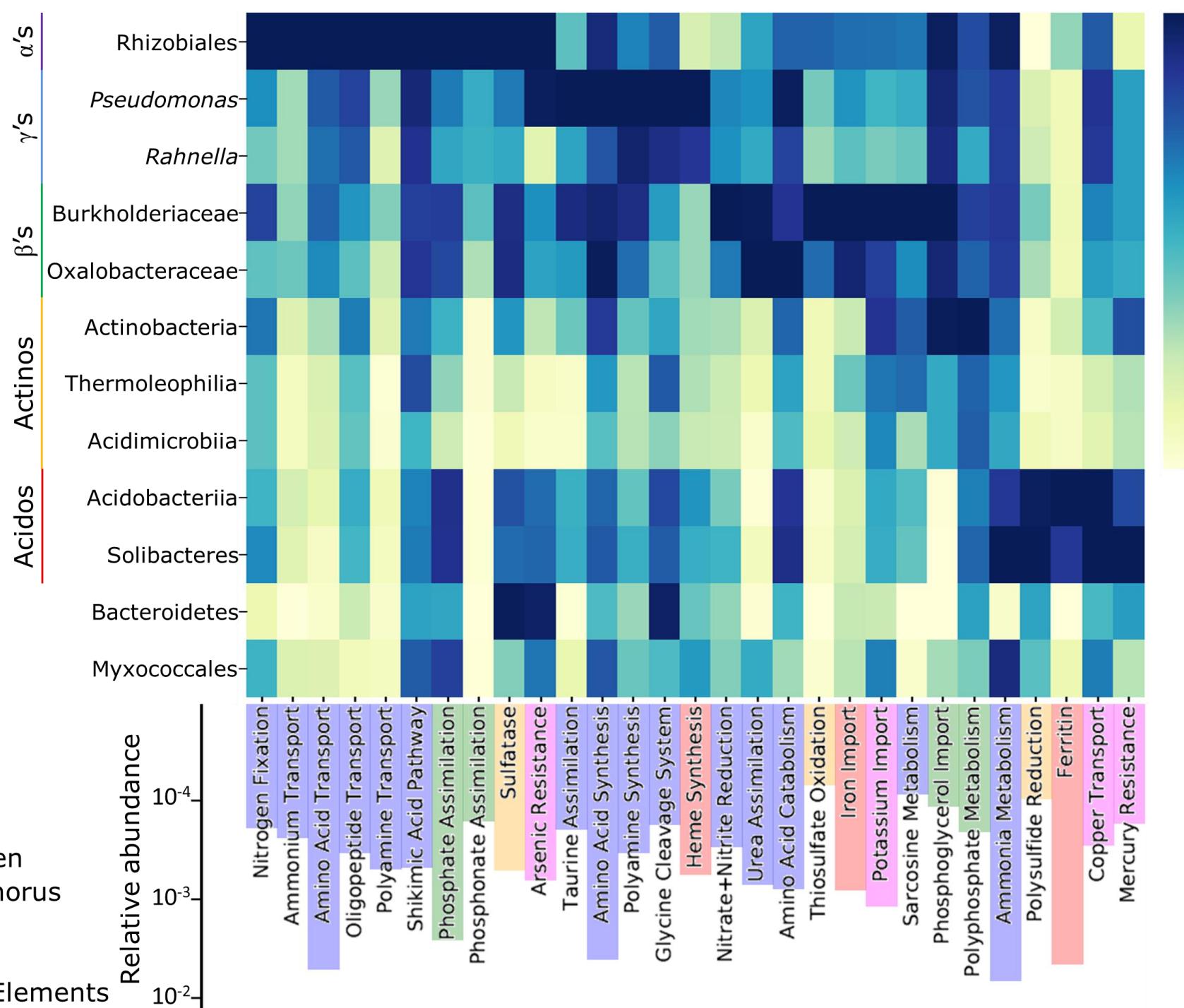
## Carbon Metabolism



**Hypothesis:** Proteins involved in nutrient usage are expressed by taxa in proportion to overall activity, especially for the common limiting nutrient, nitrogen. (No resource partitioning)

## Nutrient Metabolism

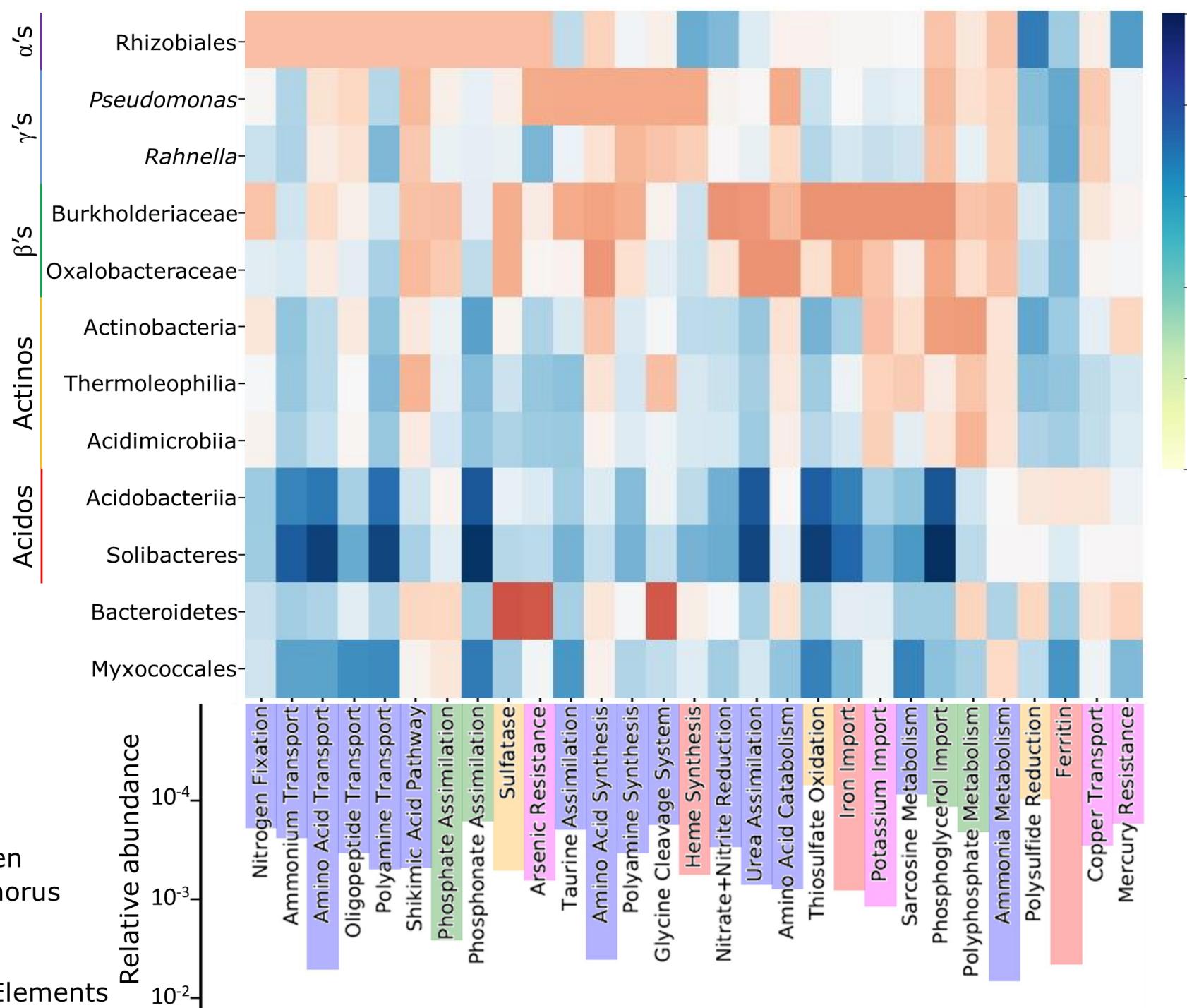
- Nitrogen
- Phosphorus
- Sulfur
- Iron
- Trace Elements



**Hypothesis:** Proteins involved in nutrient usage are expressed by taxa in proportion to overall activity, especially for the common limiting nutrient, nitrogen. (No resource partitioning) **FALSE**

## Nutrient Metabolism

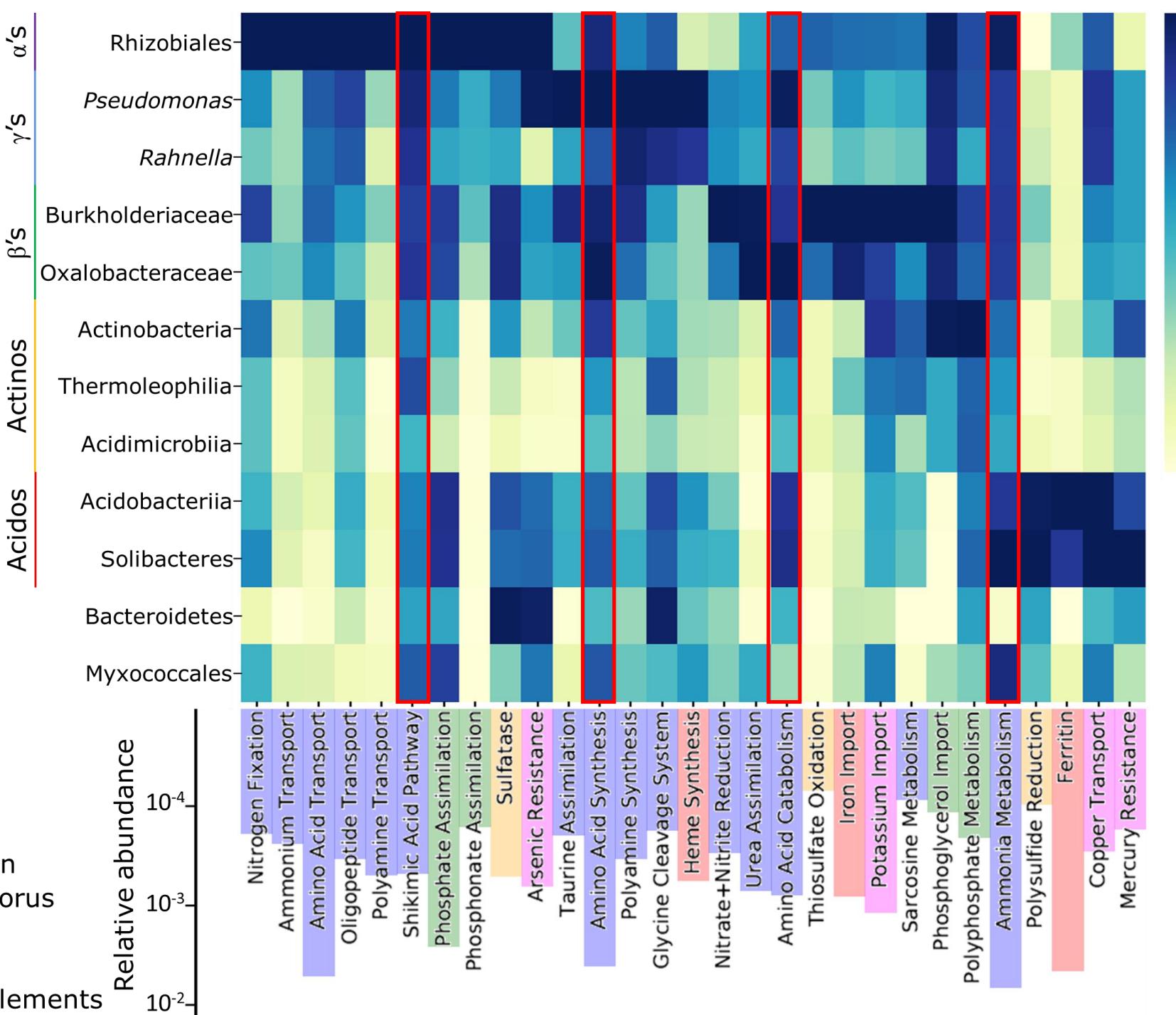
- Nitrogen
- Phosphorus
- Sulfur
- Iron
- Trace Elements



Key proteins for intracellular N cycling are relatively even across taxa

## Nutrient Metabolism

- Nitrogen
- Phosphorus
- Sulfur
- Iron
- Trace Elements

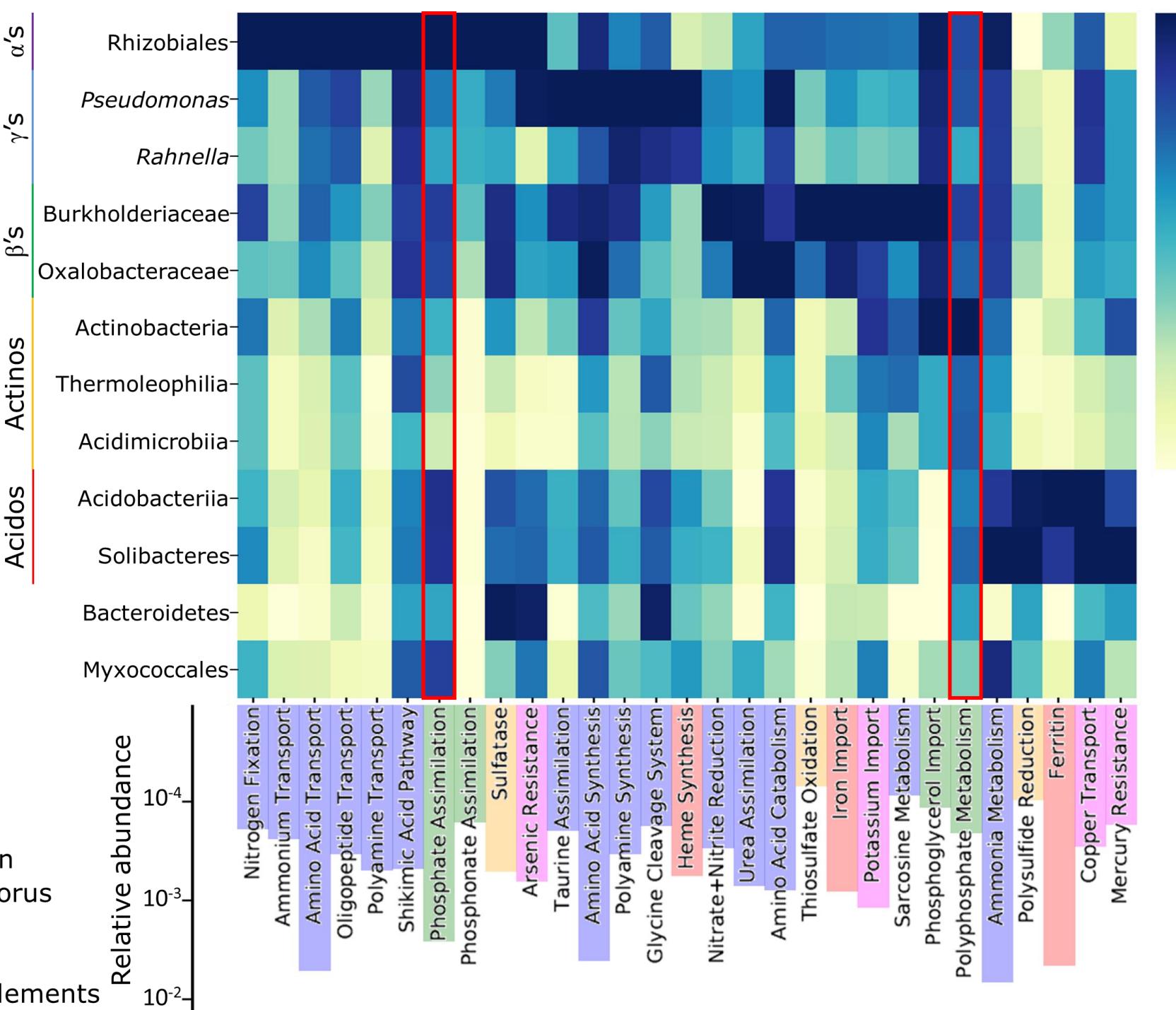


Key proteins for intracellular N cycling are relatively even across taxa

Key proteins for phosphate assimilation are relatively even across taxa

## Nutrient Metabolism

Nitrogen  
Phosphorus  
Sulfur  
Iron  
Trace Elements

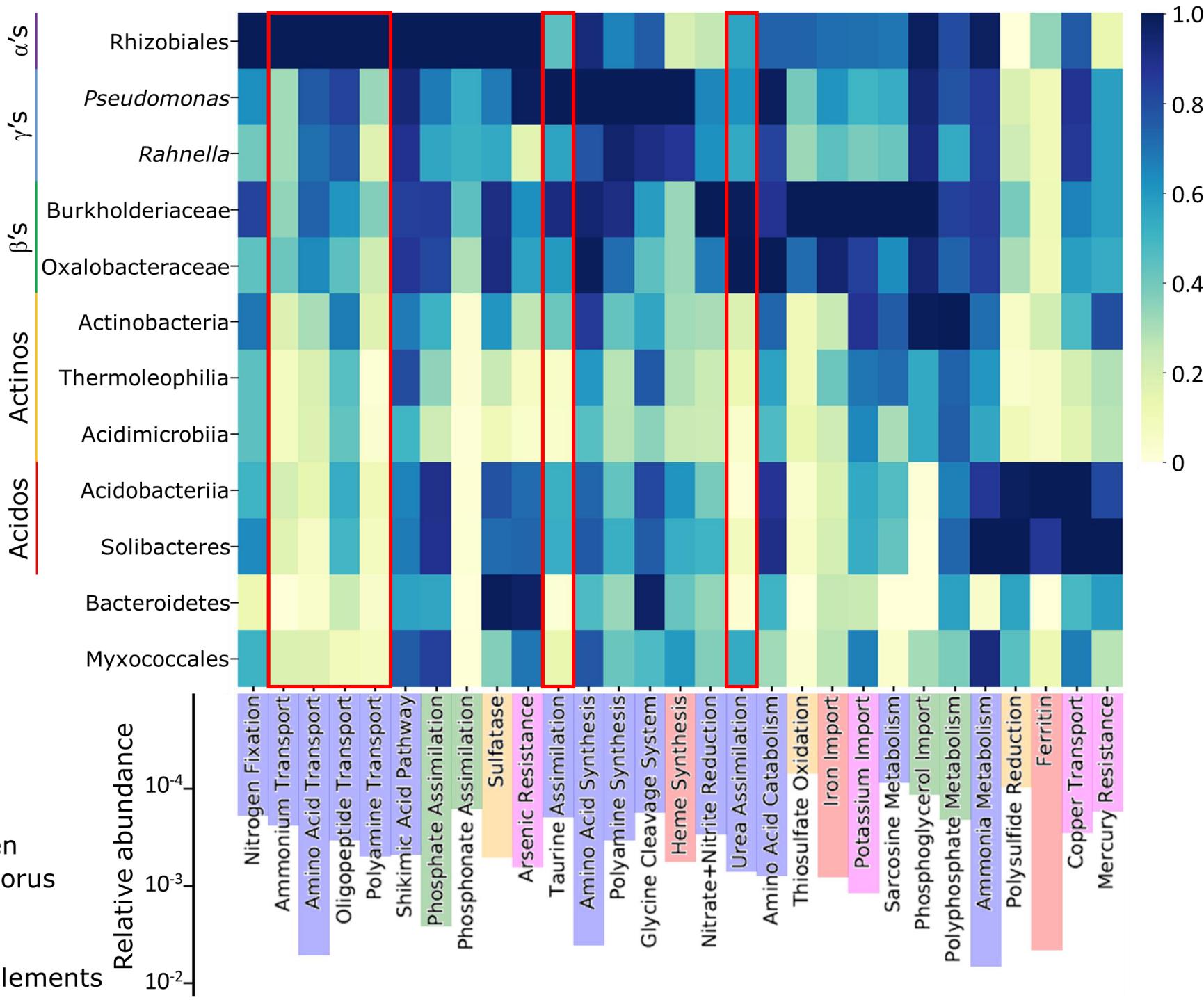


Key proteins for intracellular N cycling are relatively even across taxa

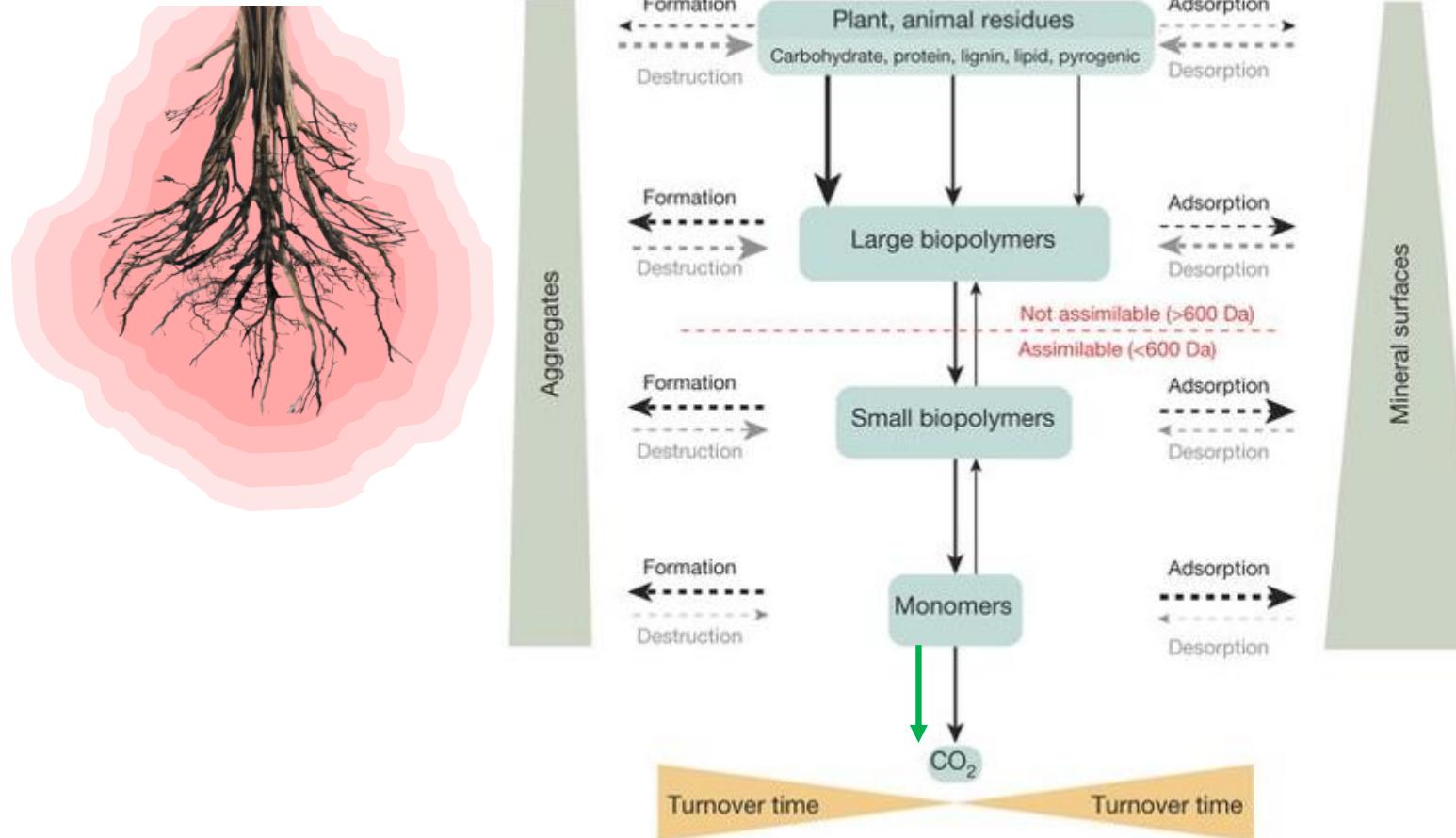
Key proteins for phosphate assimilation are relatively even across taxa

Proteobacteria – especially Rhizobiales – dominate the expression of N transporters, which mostly target organic N

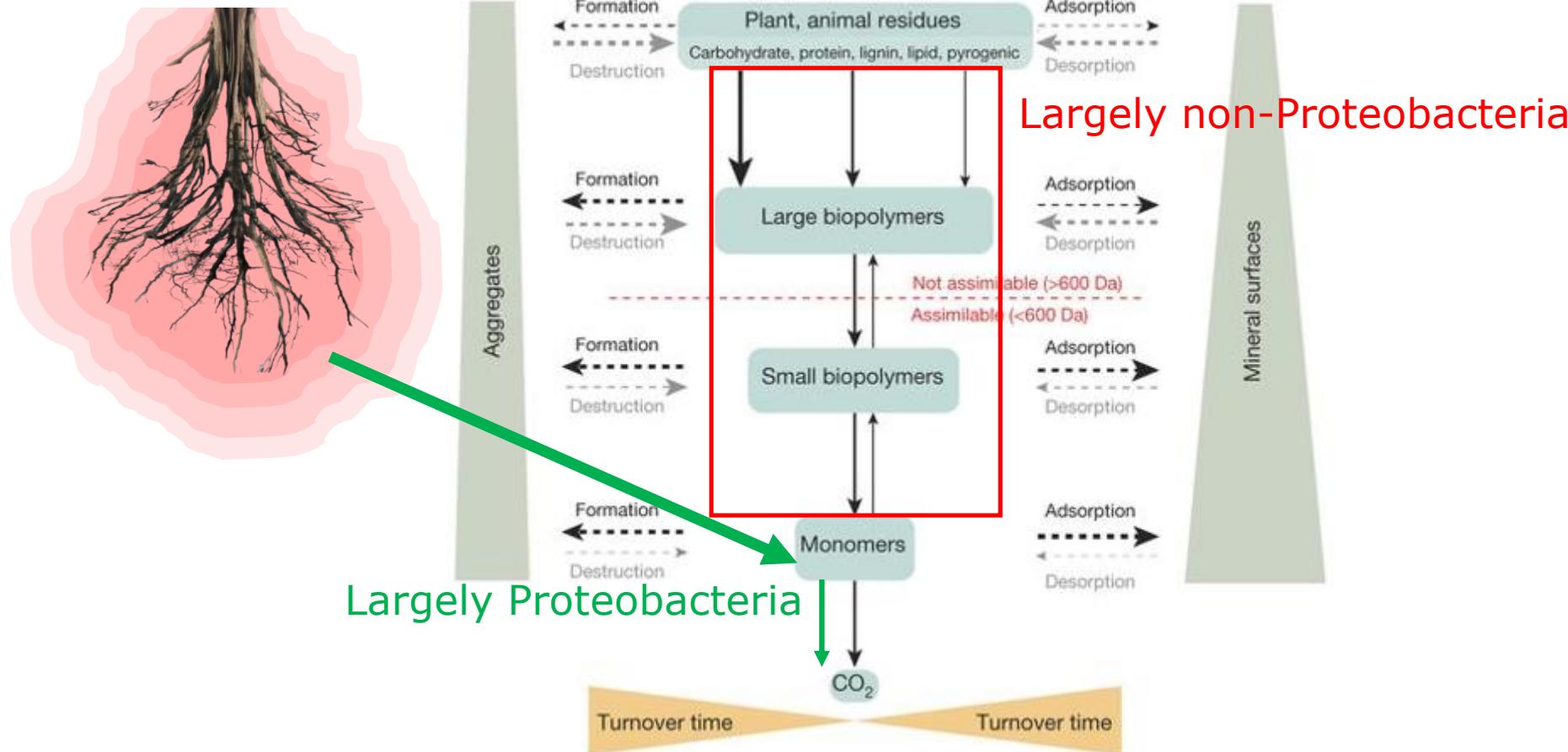
# Nutrient Metabolism



# Greater N limitation around roots



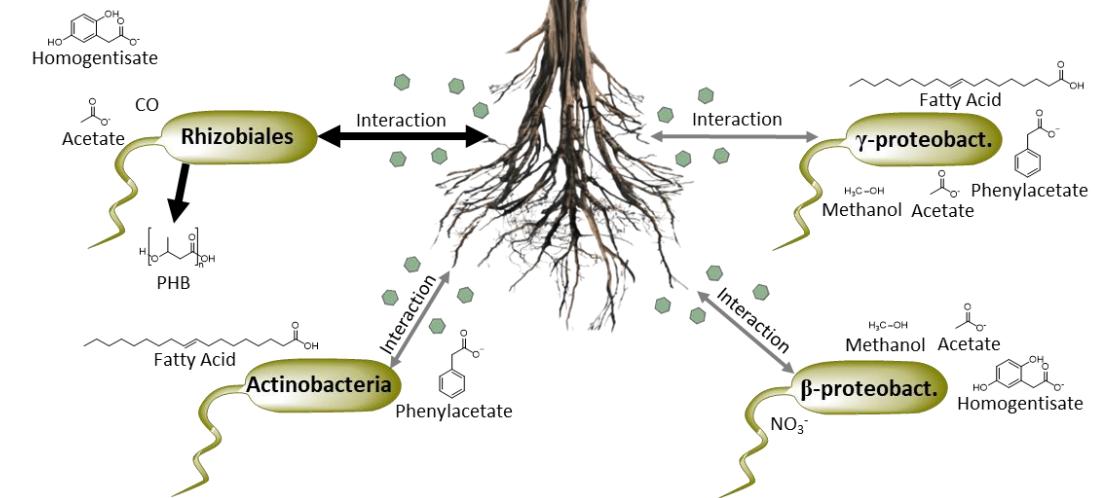
# Carbon resource partitioning



# Taxonomic niche partitioning relatively invariant among floras

Proteobacteria consume small solutes

Rhizobiales functions increase with  
greening, likely involved in exudate  
consumption and root biofilms

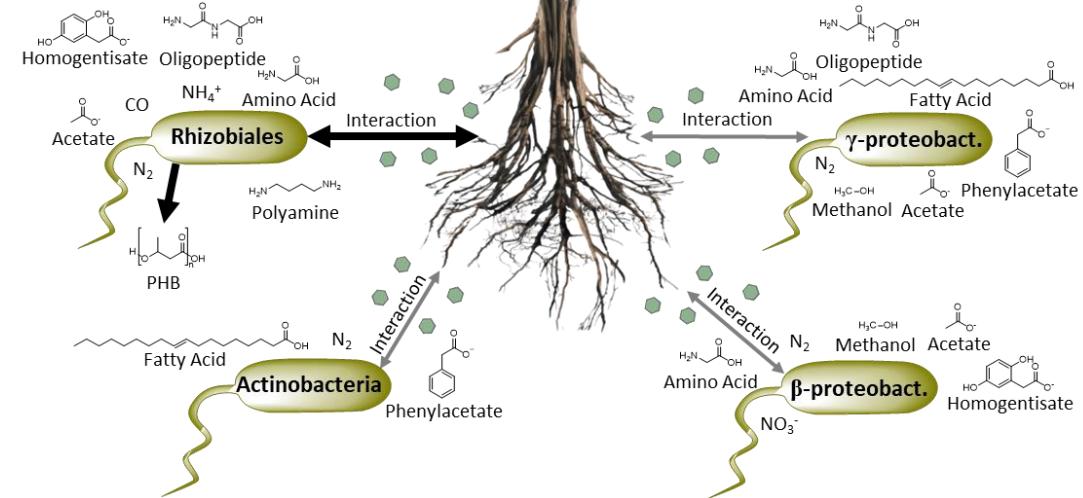


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Proteobacteria compete with plants  
for scarce N, mainly in organic forms



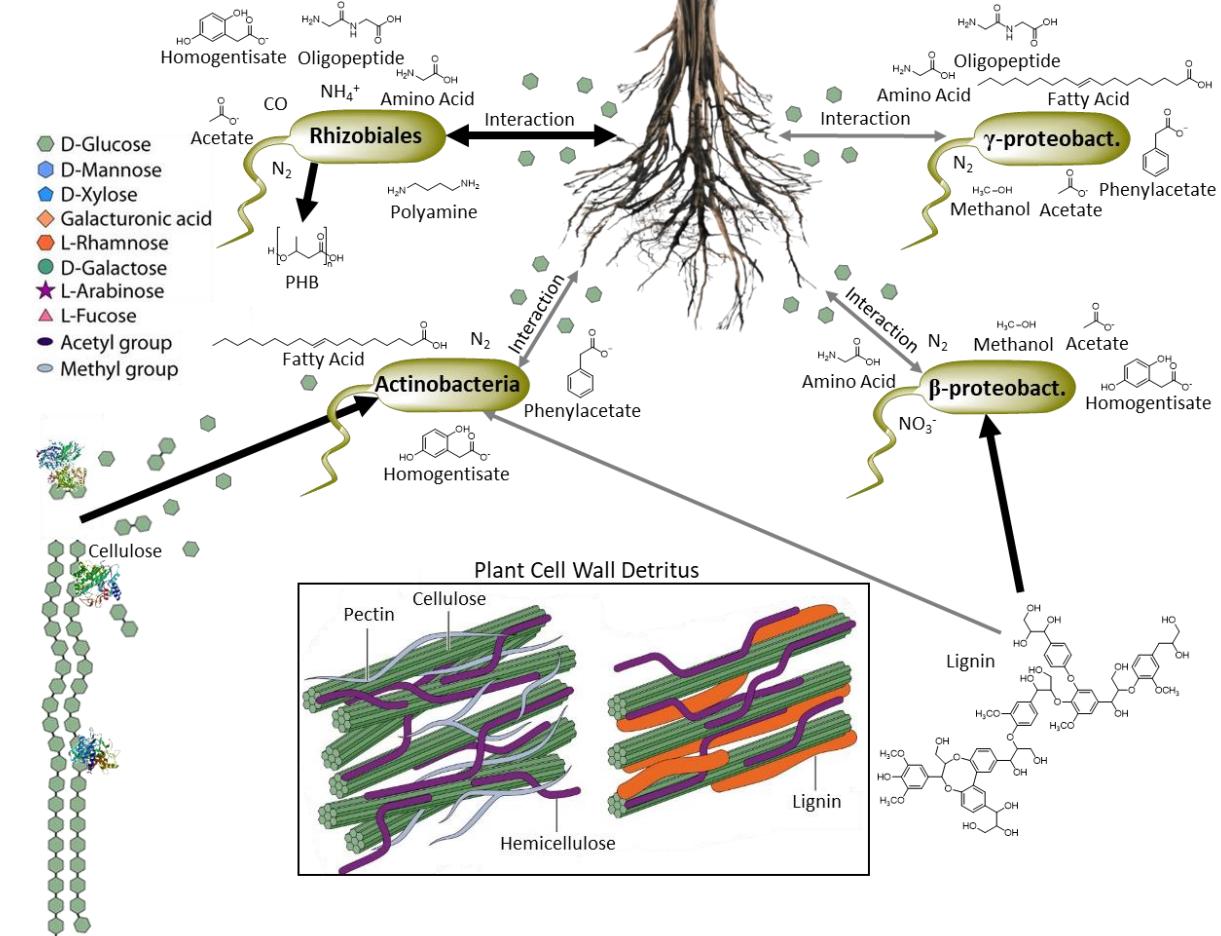
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Actinobacteria and Burkholderiaceae  
( $\beta$ ) span rhizosphere and bulk soil,  
degrading cellulose and lignin



# Taxonomic niche partitioning relatively invariant among floras

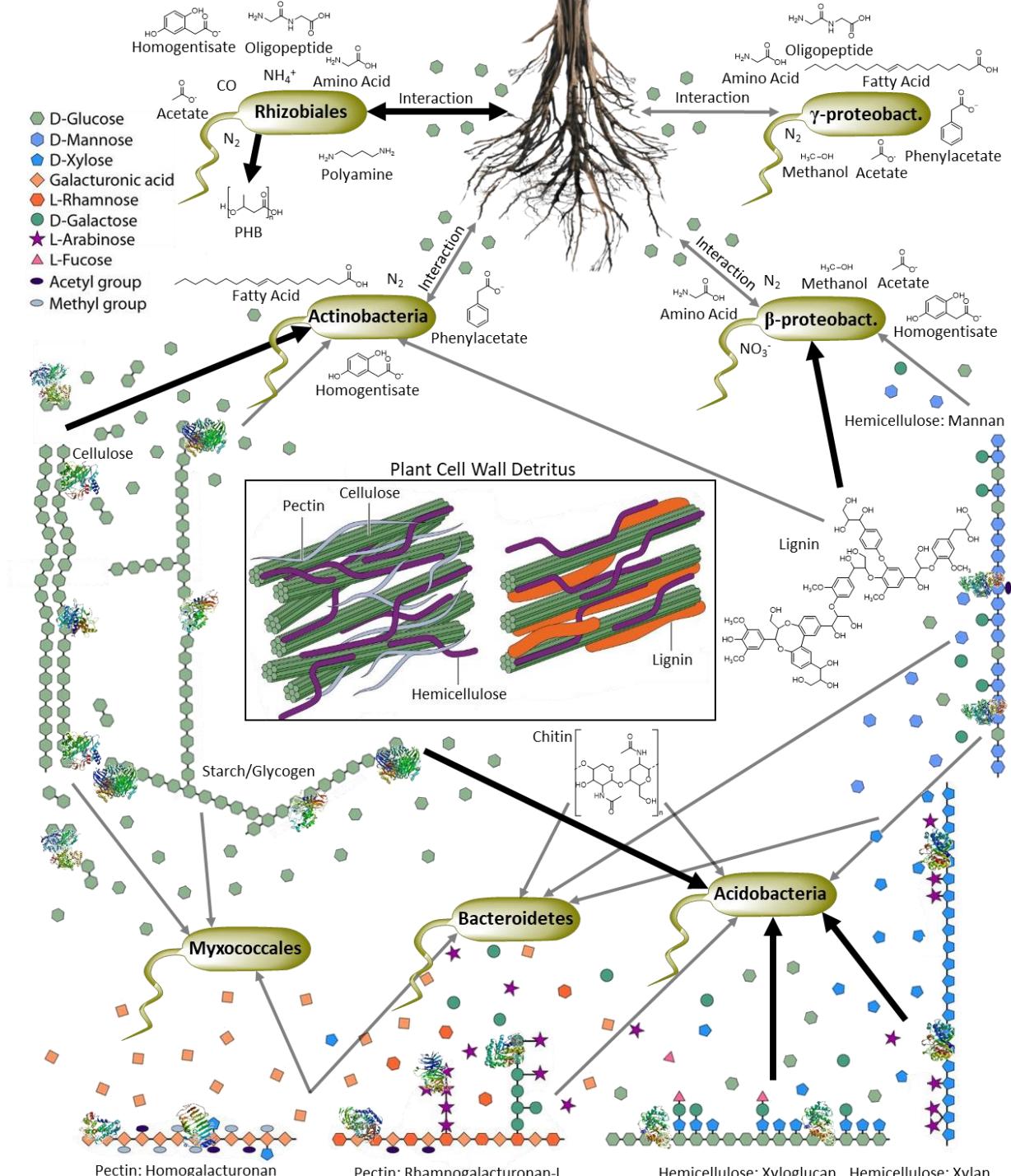
Proteobacteria consume small solutes

Rhizobiales functions increase with greening, likely involved in exudate consumption and root biofilms

Proteobacteria compete with plants for scarce N, mainly in organic forms

Actinobacteria and Burkholderiaceae ( $\beta$ ) span rhizosphere and bulk soil, degrading cellulose and lignin

Acidobacteria are most active, degrading labile polysaccharides



# Conclusions and Future Directions

- *Postnovo* extends the application of de novo sequencing
- *ProteinExpress* leverages a reference database of pooled DNA/RNA sequences for complex metaproteomes
- Taxonomic “fidelity” metric is useful for understanding which taxa express which functions
- Metaproteomics should now be pursued further in Arctic soils and other complex environments
- How does activity change seasonally and with secular warming?
- Do patterns of niche partitioning hold in other soils?

# Acknowledgments

Jacob Waldbauer, Maureen Coleman, Michael Foote, David Archer

Waldbauer/Coleman Lab Groups, Adriana Rizzo, Lichun Zhang,  
Waldbauer Lab Funding Sources (Moore and Simons Foundations)

Gerard Olack, Albert Colman, Mark Anderson

Alissa Sherman Miller, Jan and Richard Miller, Mary and Gene  
Sherman