Modeling of bacterial induced changes in the root environment

Collin Timm, Jeremiah Henning, Sara Jawdy, Dale Pelletier, Dave Weston
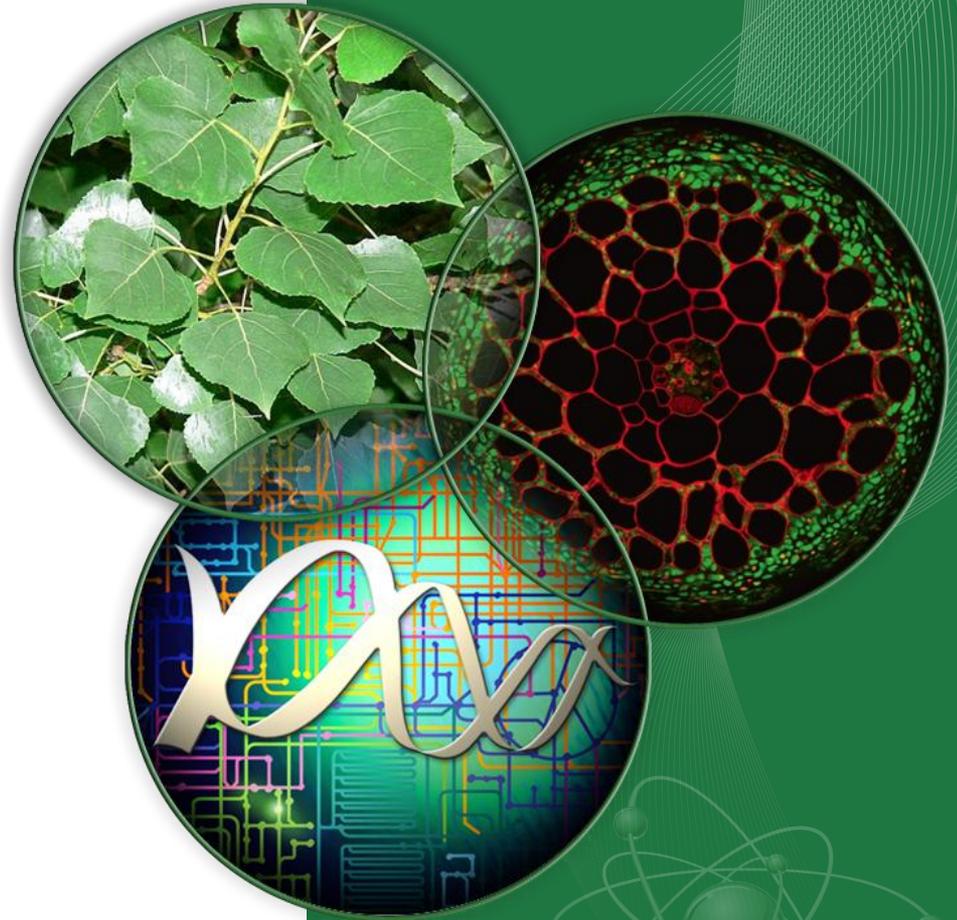
Biosciences Divison
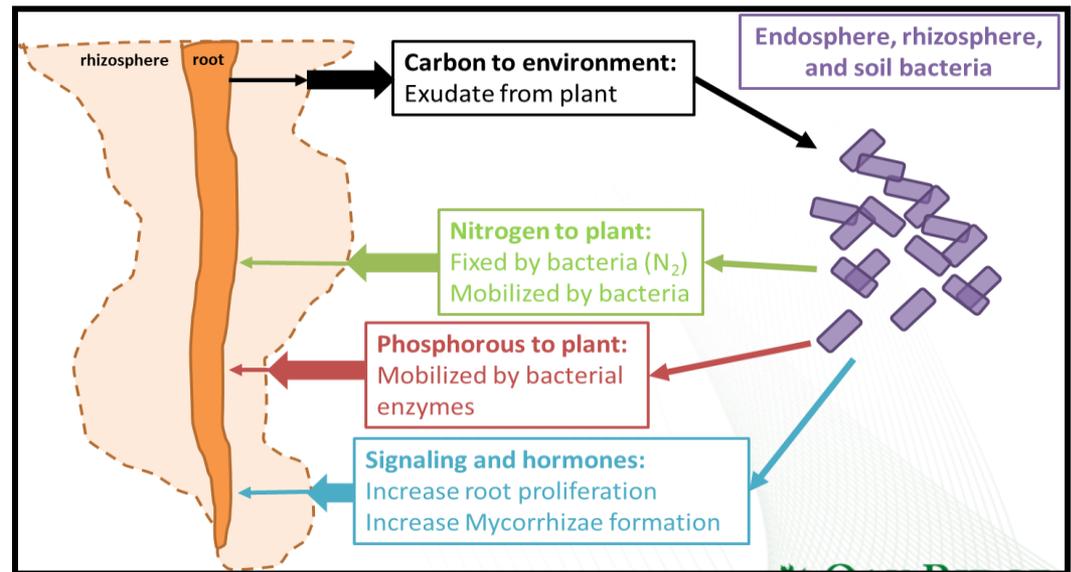
Oak Ridge National Laboratory, TN, USA

ESA 2014

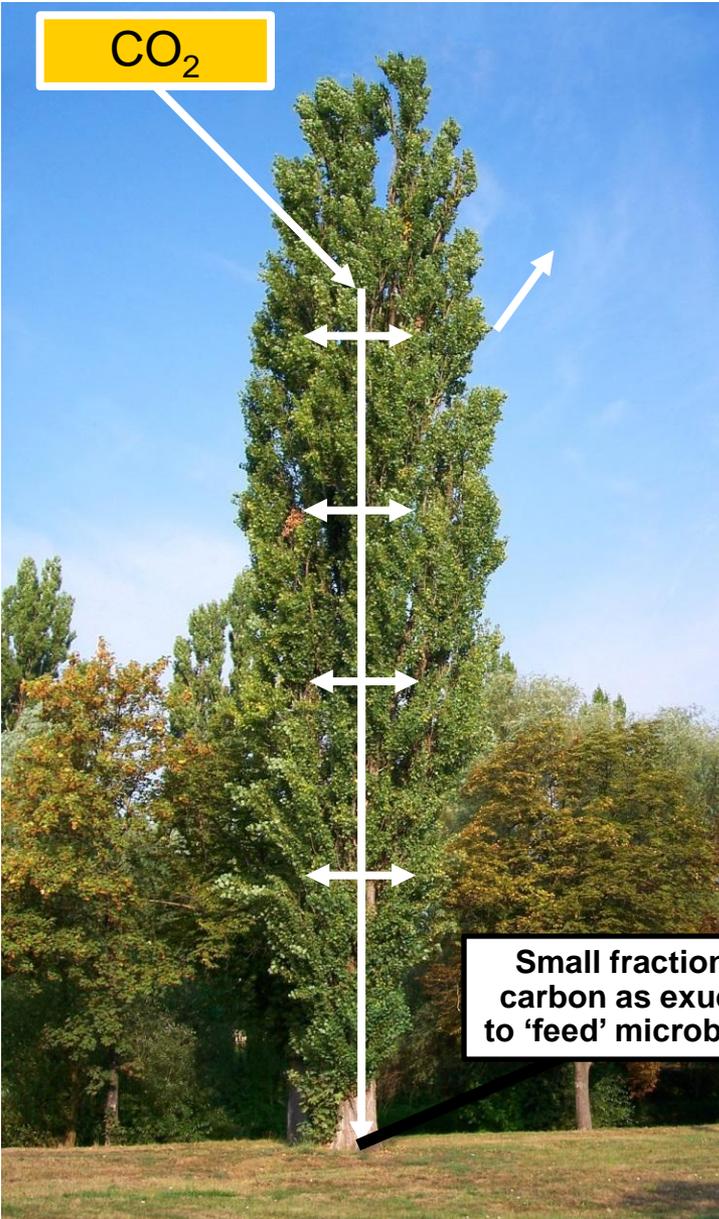OAK RIDGE
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Poplar and the microbiome

- Model temperate tree species
- Biofuel precursor
  - Grows quickly (2 meters per year)
  - Grows in poor soil
  - Does not compete with food crops
  - Genome sequence available

Carbon to environment: Exudate from plant

Endosphere, rhizosphere, and soil bacteria

Nitrogen to plant: Fixed by bacteria (N_2) Mobilized by bacteria

Phosphorous to plant: Mobilized by bacterial enzymes

Signaling and hormones: Increase root proliferation Increase Mycorrhizae formation

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Laboratory microcosm

side

top

Microbiome replacement

Count cells

Measure metabolites

Controlled stress

CO$_2$

Small fraction of carbon as exudate to 'feed' microbiome

rhizosphere · root

Carbon to environment: Exudate from plant

Endosphere, rhizosphere, and soil bacteria

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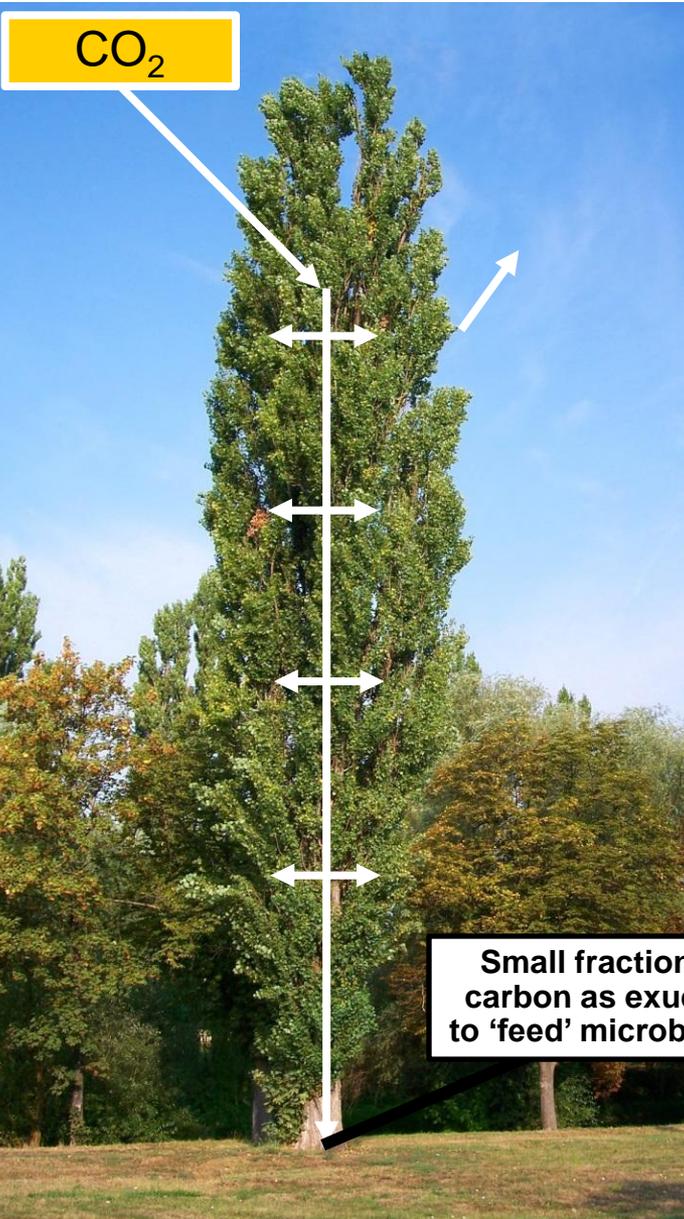Small fraction of carbon as exudate to 'feed' microbiome

Interactions are difficult to quantify in these systems:

How much carbon is allocated to exudate/microbiome? (mmol C/hr)

What are bacteria contributing to the plant? (mmol IAA/hr)

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CO$_2$

Laboratory microcosm

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Microbiome replacement

Count cells

Measure metabolites

Controlled stress

Small fraction of carbon as exudate to 'feed' microbiome

Interactions are difficult to quantify in these systems:

Genome-based modeling of bacterial metabolism

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Studying isolates from the poplar microbiome

Root growth stimulation

Sequence Genome

6150 genes

Interaction with other isolates

sucrose

Carbon from environment

Bacterial Metabolism
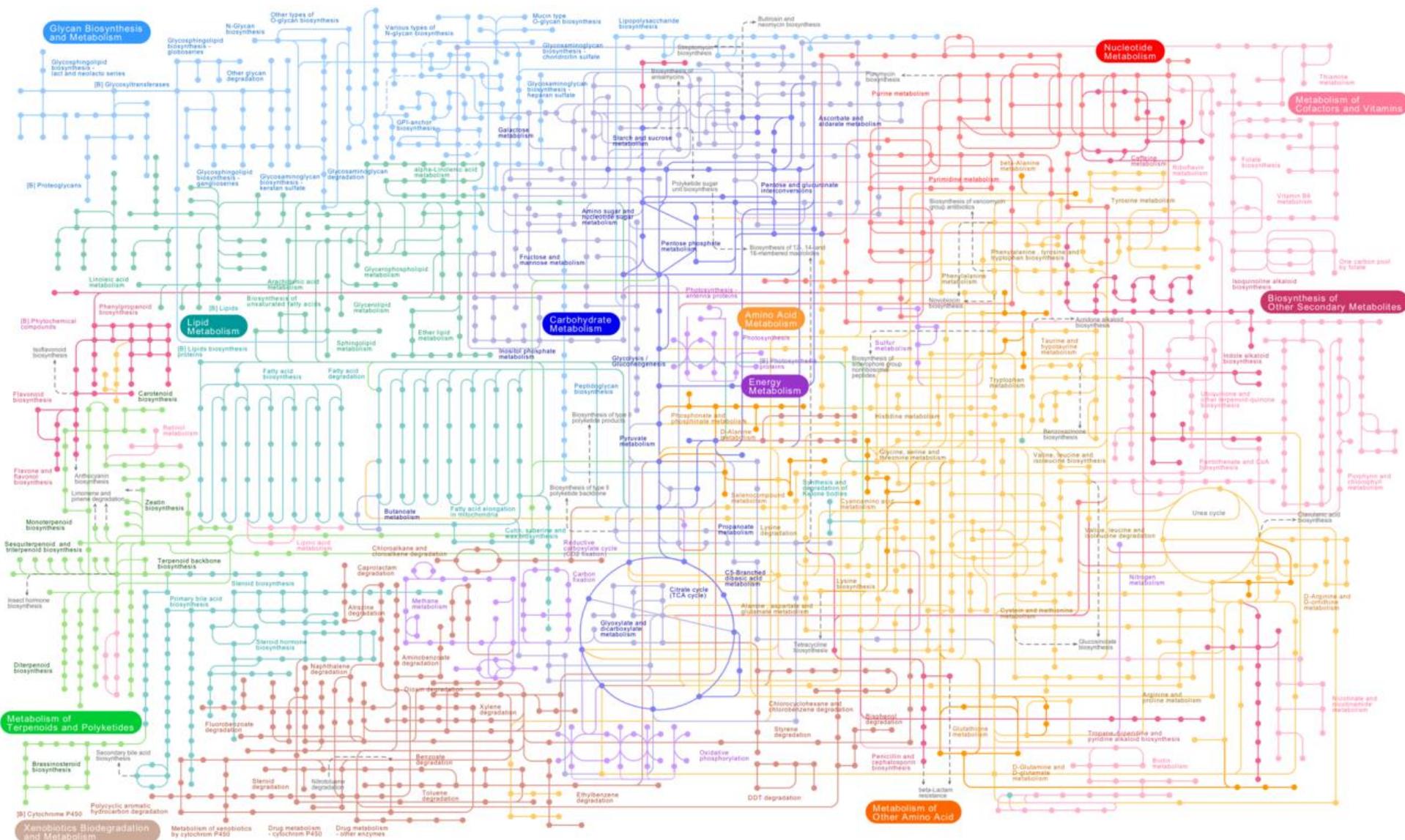
Cellular biomass
Auxin

- Collect bacterial isolates from poplar roots (~3000)

- Screen for phenotypes and sequence genomes

- Build a model of bacterial metabolism and link to host traits
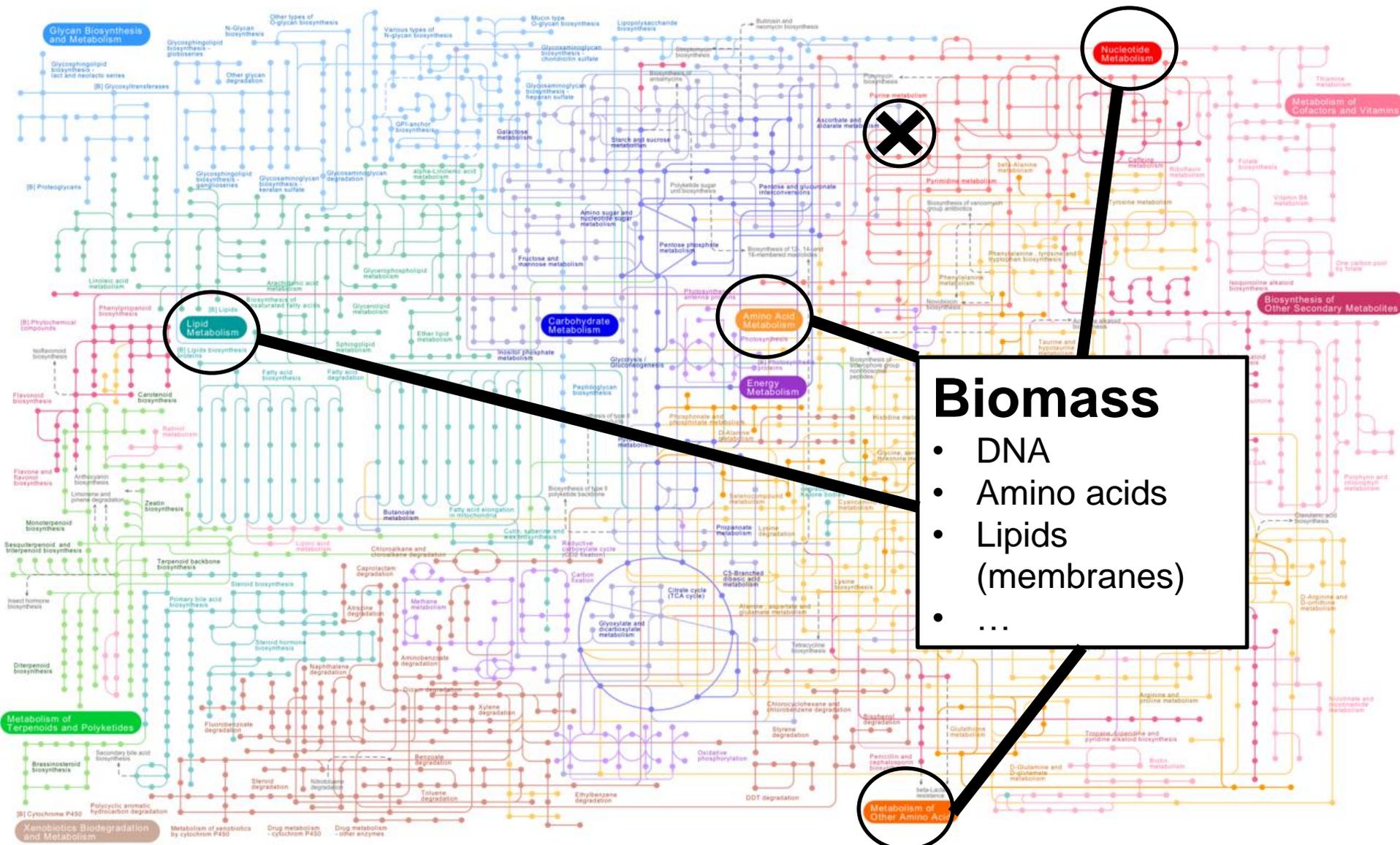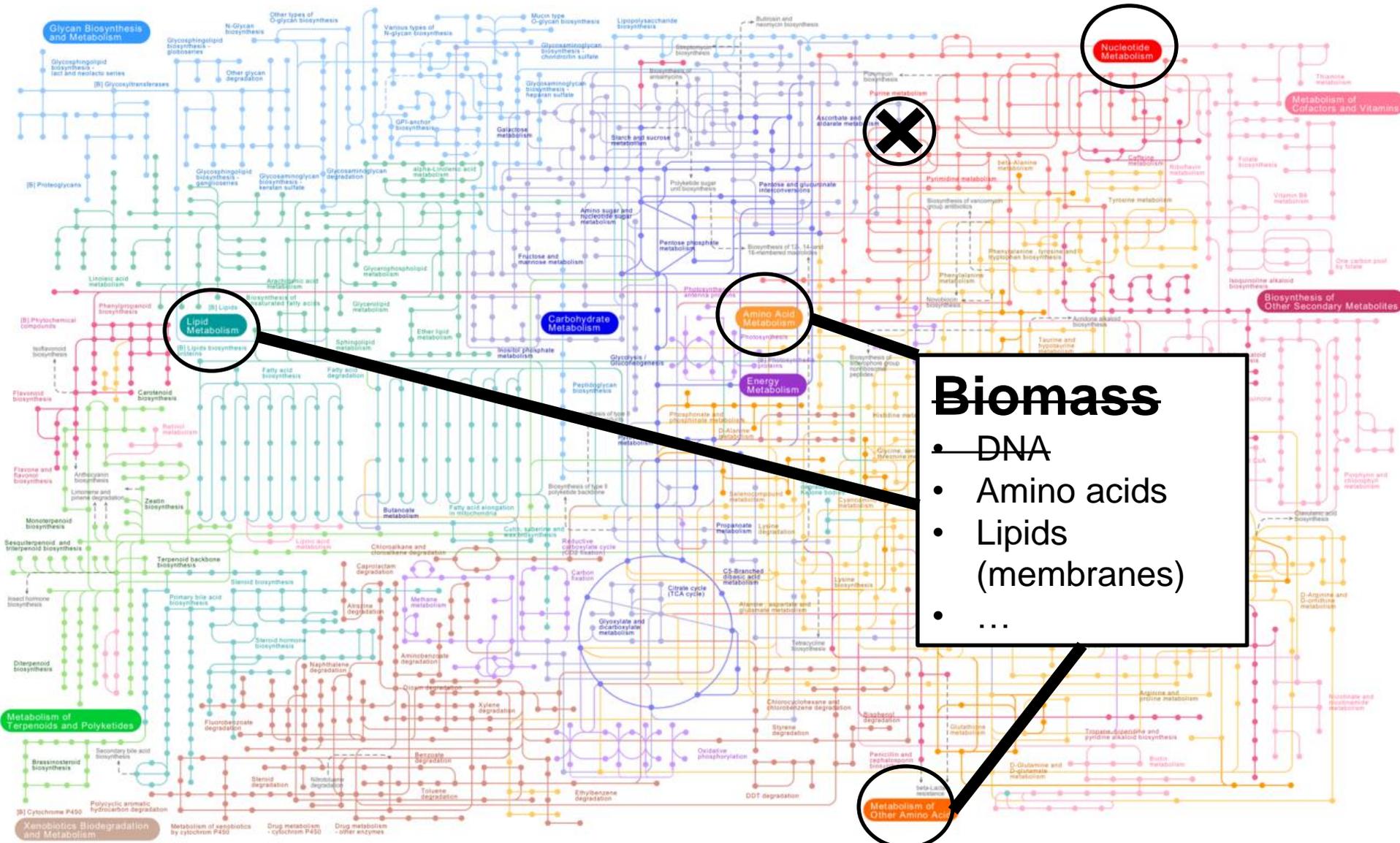
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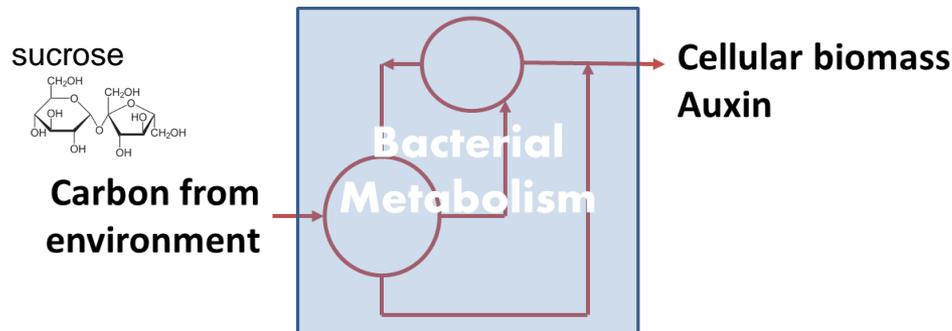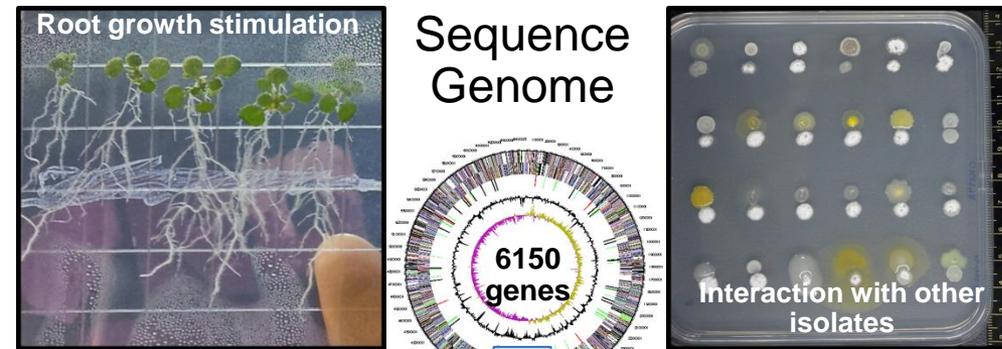
Metabolic models, biomass, and gaps

Metabolic models, biomass, and gaps
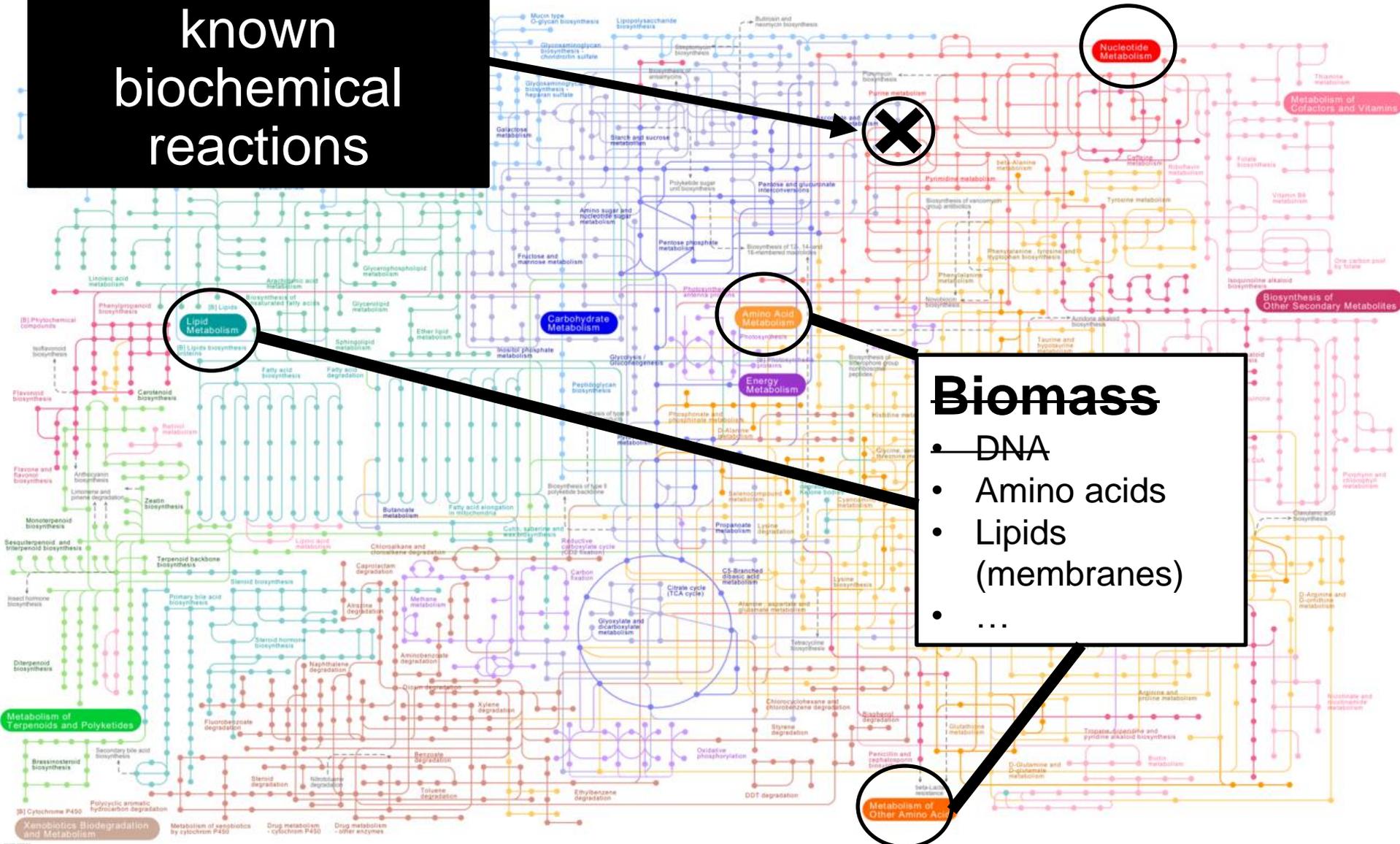
Biomass
- DNA
- Amino acids
- Lipids (membranes)
- …

Metabolic models, biomass, and gaps

Biomass
- DNA
- Amino acids
- Lipids (membranes)
- …

Metabolic models, biomass, and gaps

Biomass
- ~~DNA~~
- Amino acids
- Lipids (membranes)
- …

Metabolic models, biomass, and gaps

Database of known biochemical reactions

Biomass
- DNA
- Amino acids
- Lipids (membranes)
- …

Metabolic models, biomass, and gaps

Database of known biochemical reactions

Biomass
- DNA
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- …

Metabolic models, biomass, and gaps

Database of known biochemical reactions

Nucleotide Metabolism

Lipid Metabolism

Amino Acid Metabolism

Carbohydrate Metabolism

Energy Metabolism
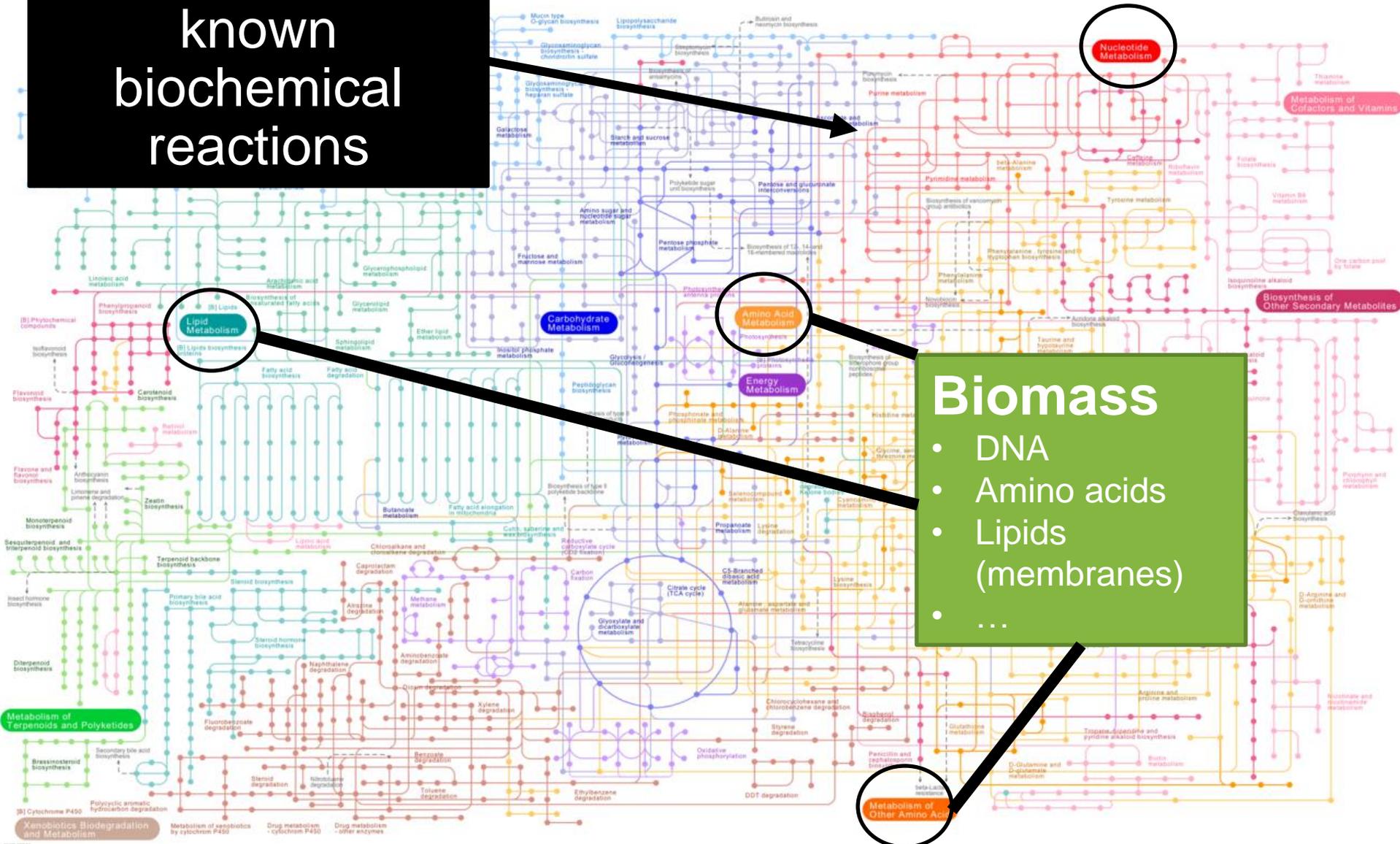
Biomass
- DNA
- Amino acids
- Lipids (membranes)
- …

Metabolism of Other Amino Acids
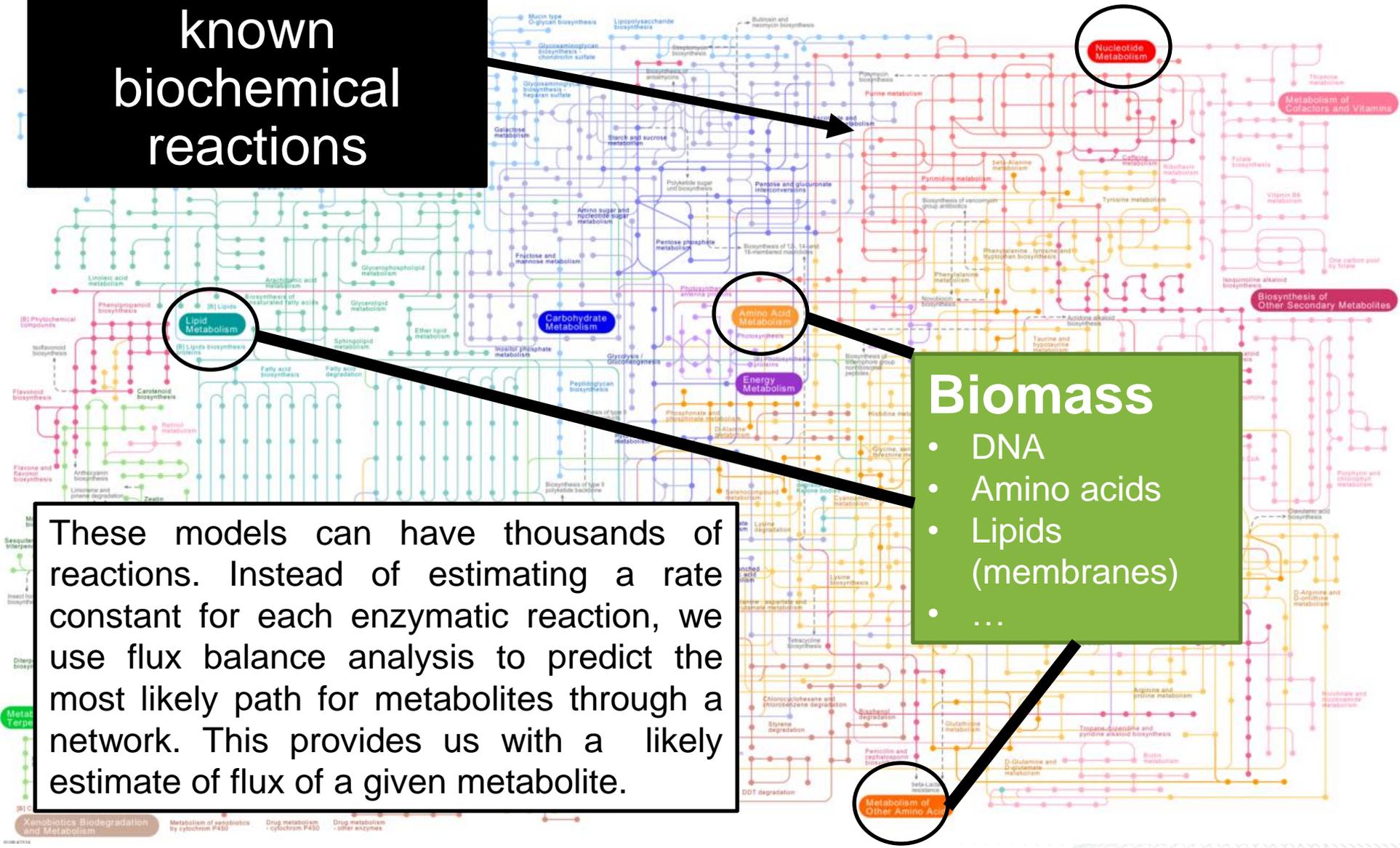
These models can have thousands of reactions. Instead of estimating a rate constant for each enzymatic reaction, we use flux balance analysis to predict the most likely path for metabolites through a network. This provides us with a likely estimate of flux of a given metabolite.
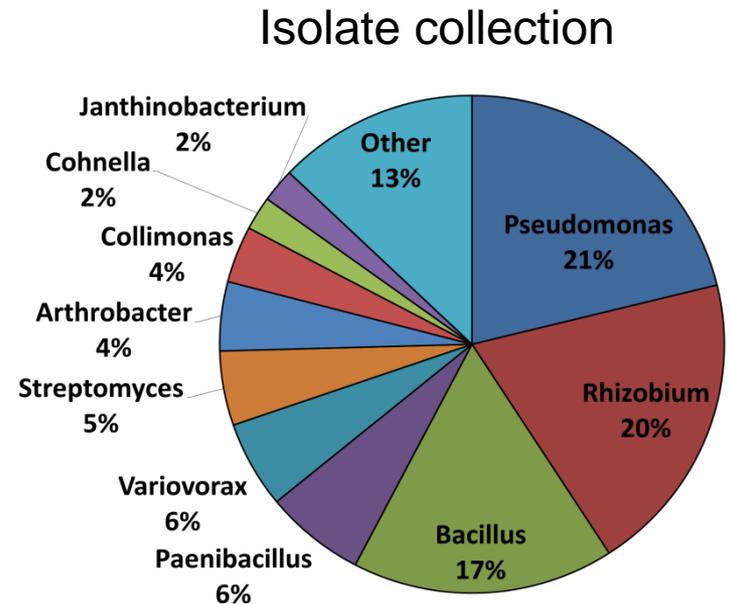
Poplar isolate with important implications to host plant

Pseudomonas fluorescens GM41

- One of 21 sequenced Pseudomonas isolates

- Mycorrhizae helper bacteria
  – Enhances mycorrhizae formation

- Can produce auxin

- Broad carbon substrate usage
  – 89/190 tested

- Genetically tractable
  – Transposon mutant library

Isolate collection

Pie chart labels:
- Pseudomonas 21%
- Rhizobium 20%
- Bacillus 17%
- Other 13%
- Paenibacillus 6%
- Variovorax 6%
- Streptomyces 5%
- Arthrobacter 4%
- Collimonas 4%
- Cohnella 2%
- Janthinobacterium 2%

*Mike Robeson and Chris Schadt

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Initial model results for GM41 metabolism

Genome:
6.6Mb
6174 genes (predicted)

Model:
1359 reactions
1270 compounds
<u>42</u> gapfill reactions

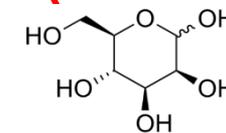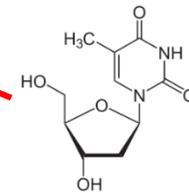|	Model	
Data	**+**	**-**
+	35	54
-	3	70

65% accuracy

Biolog substrate utilization plates (PM1 and PM2)
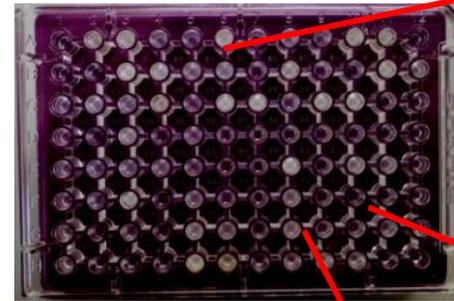
sucrose
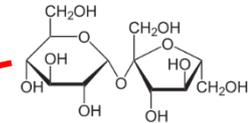
thymidine

mannose

False Negative Example

myoinositol: growth, proteomics
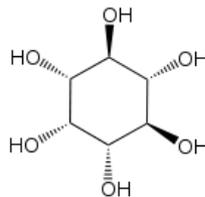
Can we use exudation rate to predict cell abundance?
How do different carbon sources affect growth (eg. auxin production)?

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Effect of microbial dissociation on Sphagnum productivity

*Anthony Walker
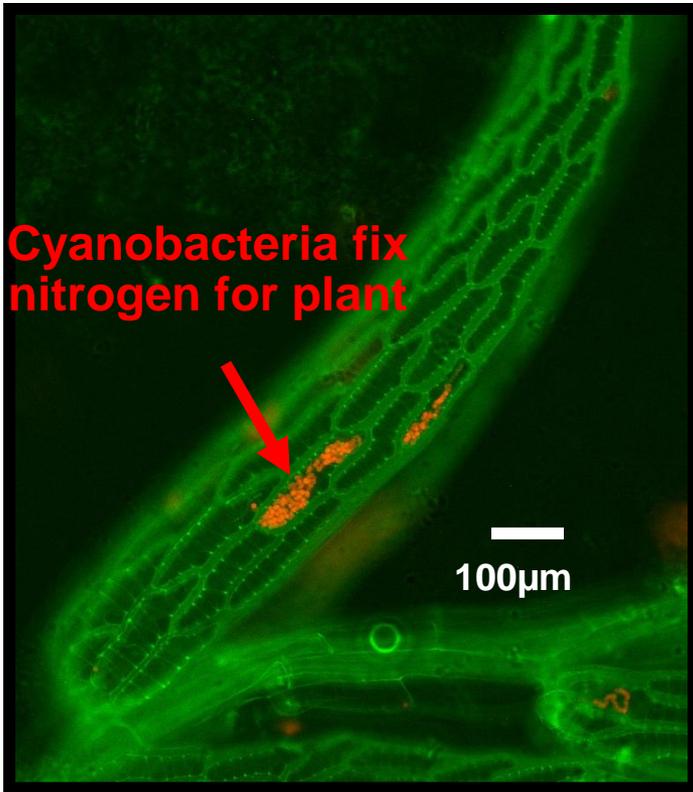

Cyanobacteria fix nitrogen for plant

100μm

Figure 3: Micrograph of axenic *S. magellanicum* leaf colonized with *Nostoc luscorum 1037 (source)*. Chlorophyllous cells fluoresce green, and the Nostoc cells fluoresce in the red spectrum This composite image shows Nostoc located within the hyaline cells.
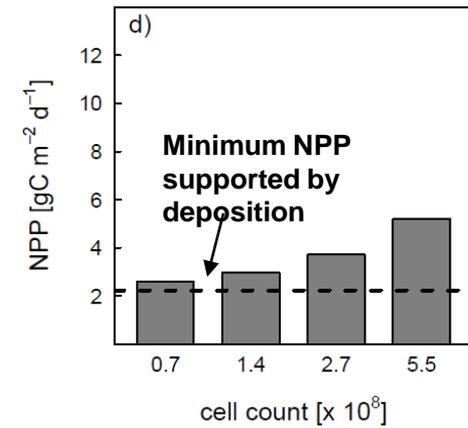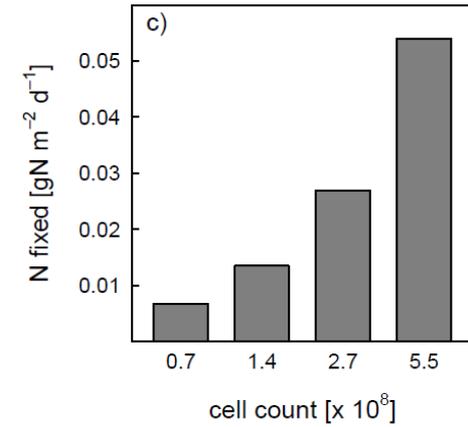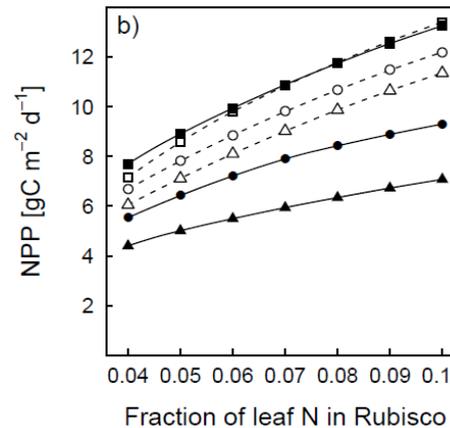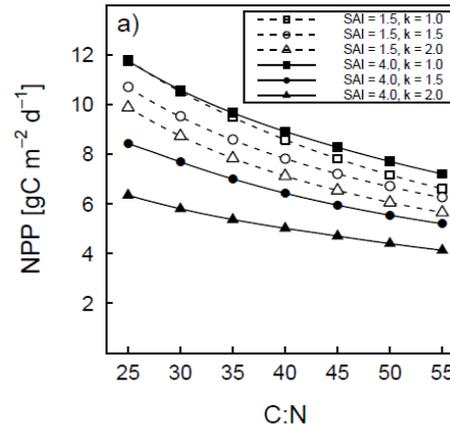
Figure 4: Simulated NPP variation in response to Sphagnum C:N ratios (a) and the fraction of leaf N in Rubisco (FLNR; b). The influence of varying shoot area index (SAI) from 1.5 (dashed lines and open symbols) to 4 (solid lines and closed symbols) is shown combined with the influence of the light extinction coefficient (k; 1 – squares, 1.5 – circles, and 2 – triangles). C) biological fixation of nitrogen as a function of cell number d) potential impact of dissociation on sphagnum productivity

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Weston et al., Plant Cell & Environment, 2014 *in press*

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Summary

- Bacteria are important in root processes

- We can build models of bacterial metabolism using genomes

- We are coupling bacterial models to plant models to understand the host-microbe interactions

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  - kbase.us

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Questions

The rhizosphere is a complex environment

High carbon
Buffered from dehydration
Host defense

rhizosphere

High carbon
Low pH
High competition

soil

Low carbon
Neutral pH
Subject to dehydration

organic acids

salts

mobile ions (phosphorous)

0   <5mm

distance from root

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