

# Community proteomics reveals ecological relationships in a model microbial consortium

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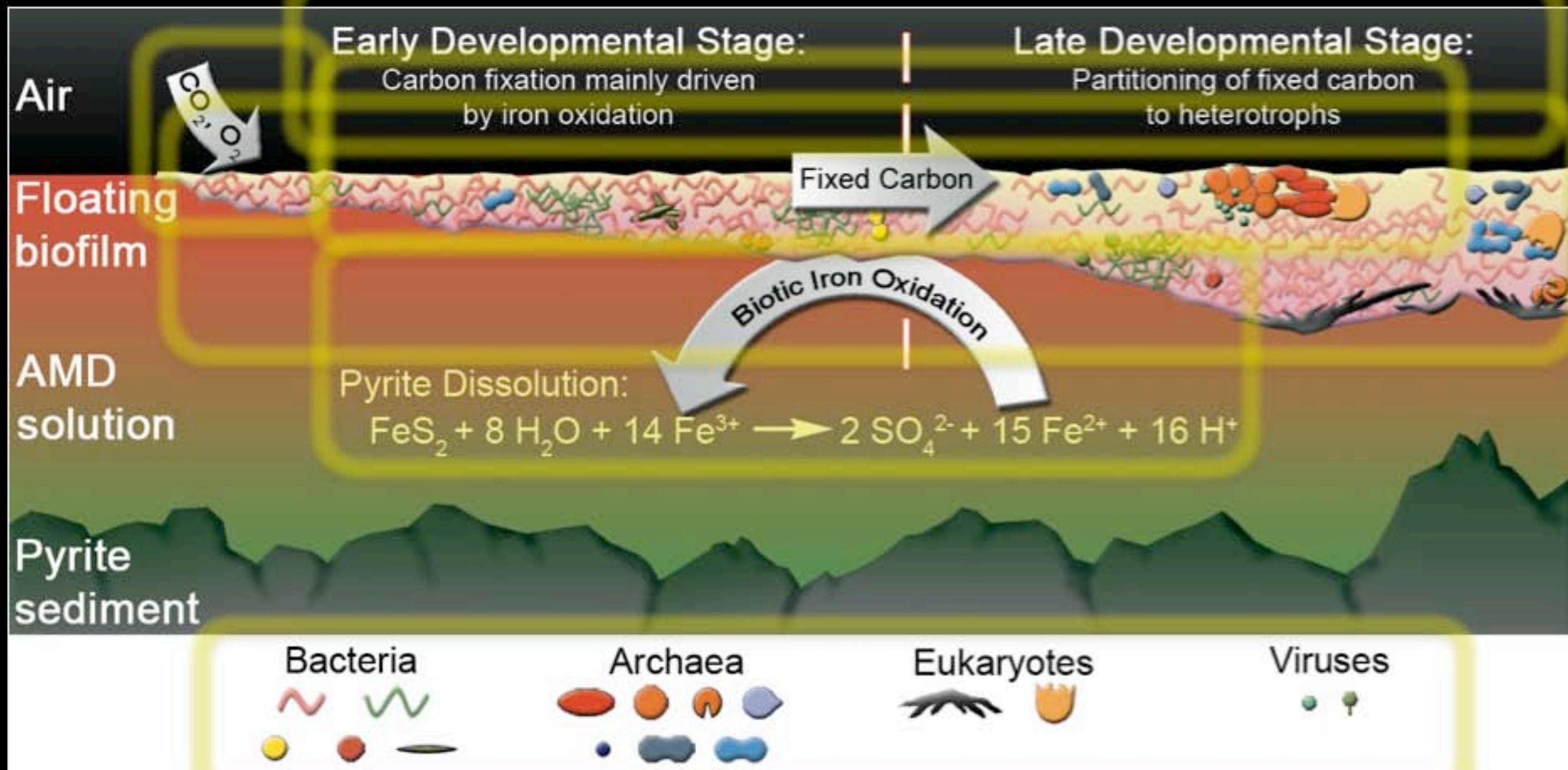
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Photo credits: NOAA Restoration Center & Damage Assessment and Restoration Program

# AMD biofilms are model communities



Main Players: *Leptospirillum* Group II    *G-plasma*    *Ferroplasma* Type 1  
*Leptospirillum* Group III    *A-plasma*    *Ferroplasma* Type 2

# AMD biofilms are model communities

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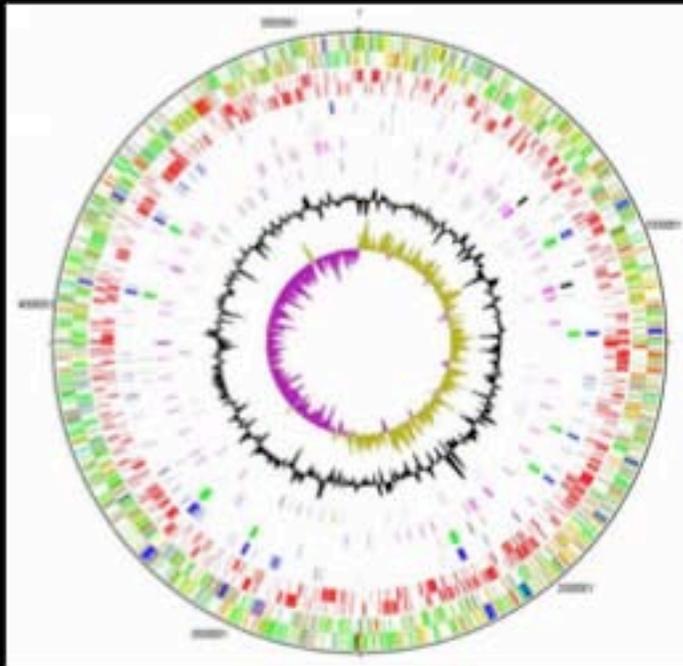
# Scientific question...

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Can we use proteomics to define ecological processes in these natural microbial communities?

# Comprehensive molecular analyses to examine ecological processes

Community Genomics



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Community Proteomics

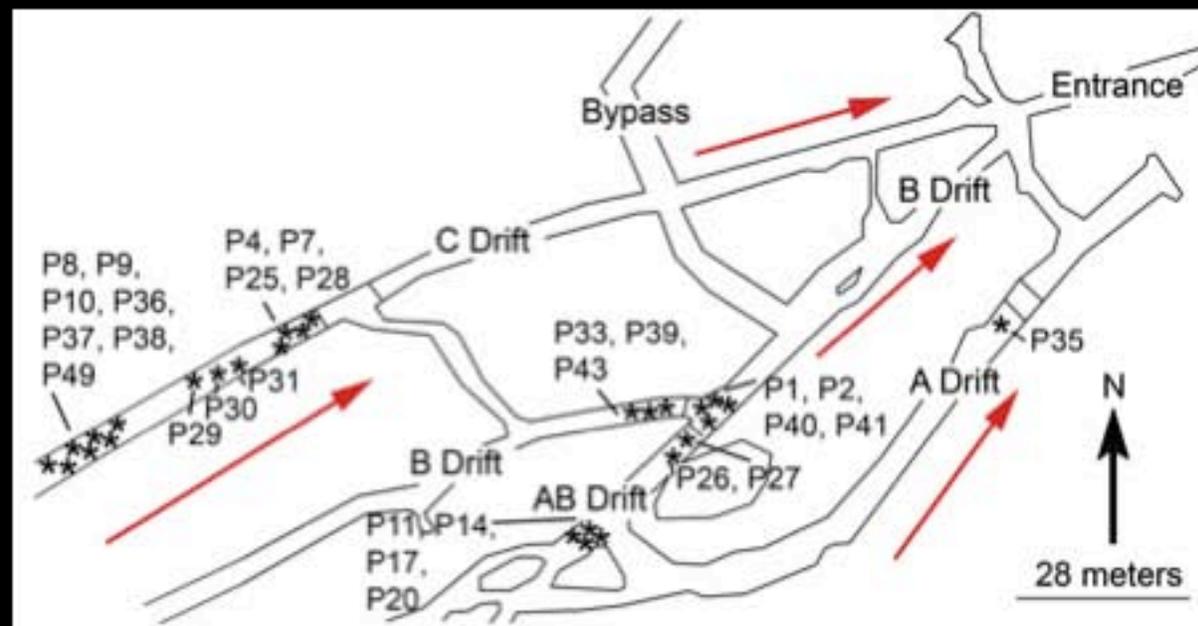


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Environmental Data Collection



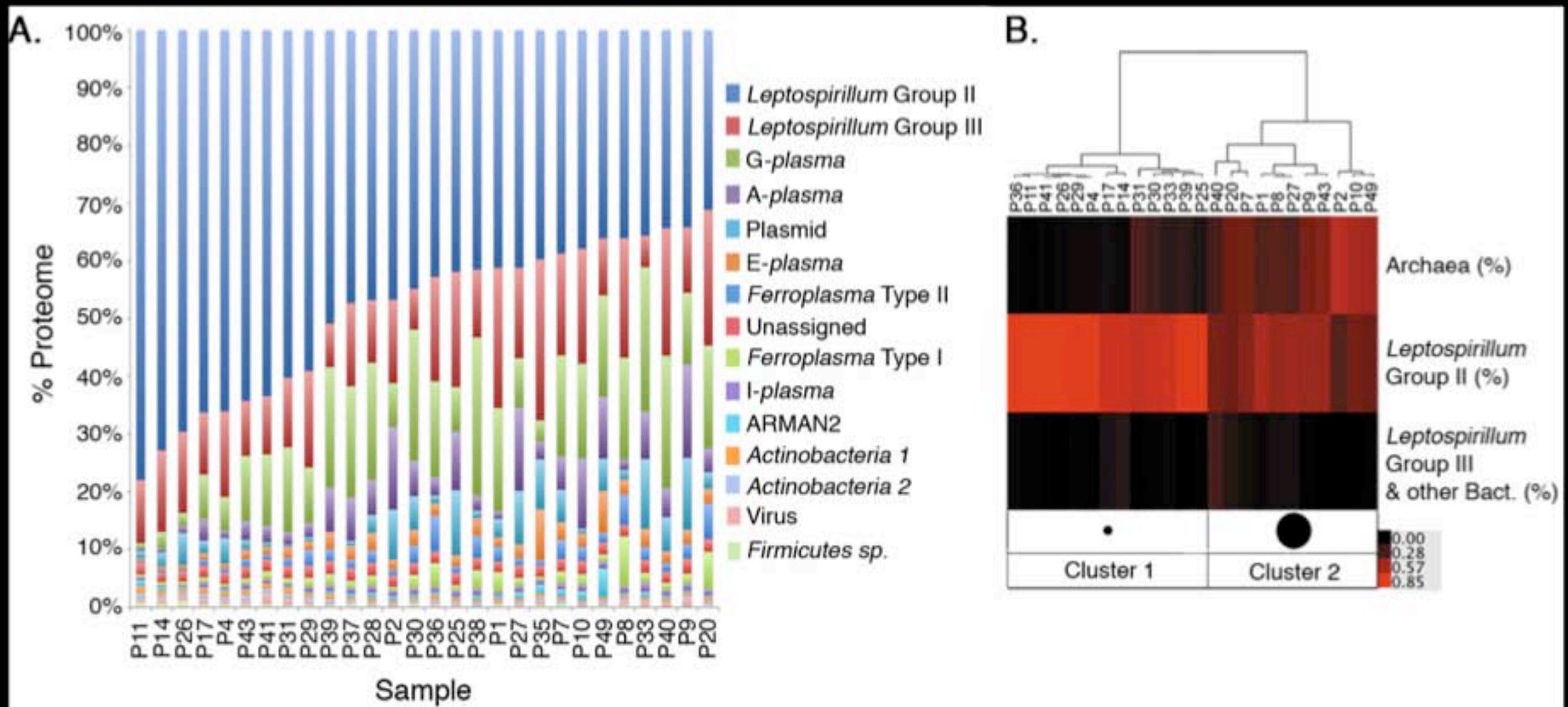
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Extensive & Long-term Sampling

# Proteomics defines community structure in AMD biofilms

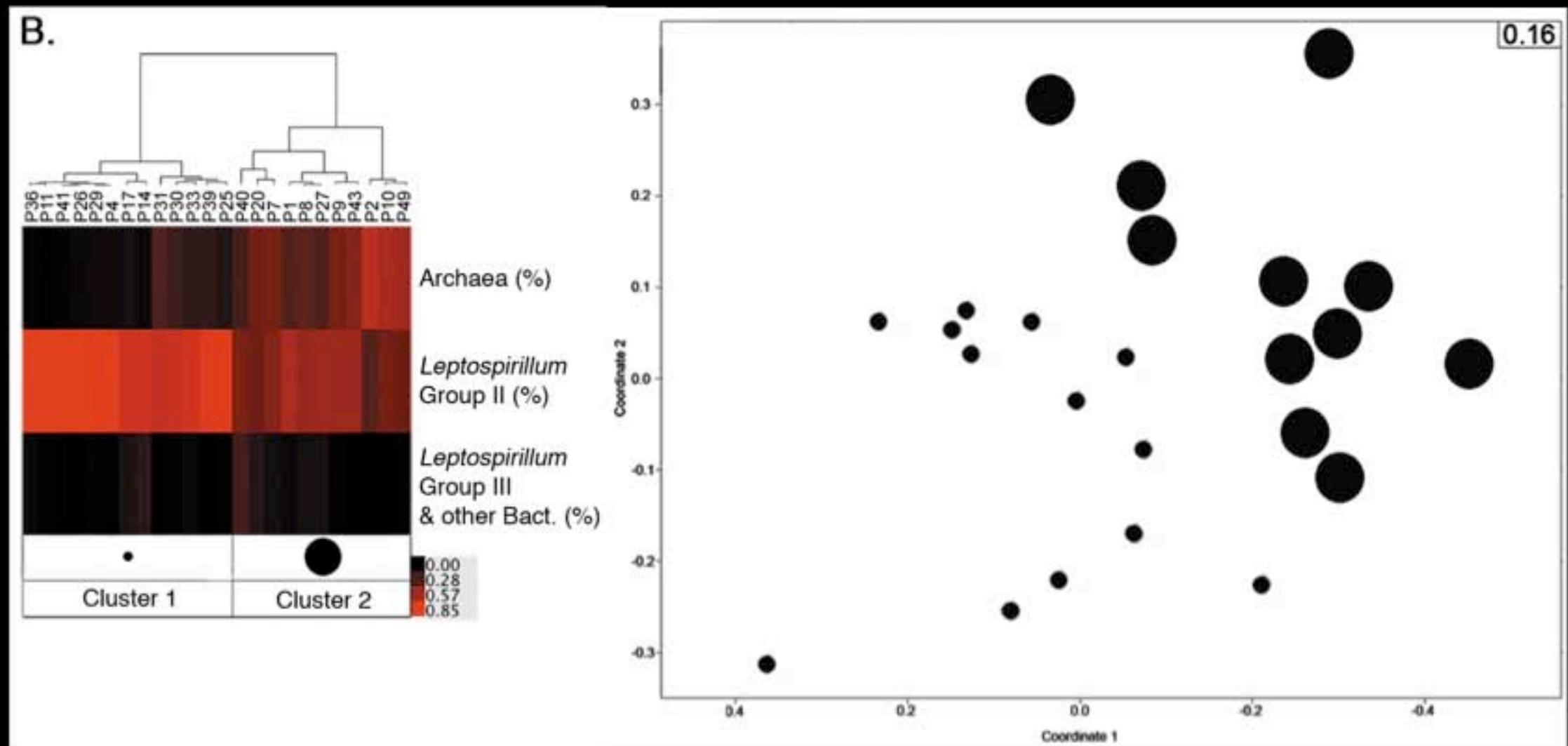
From 28 whole cell proteomes 6,297 unique proteins belonging to 15 different genomes were identified [ $2,182 \pm 411$  per proteome].



*Leptospirillum* Group II proteins dominate all proteomes

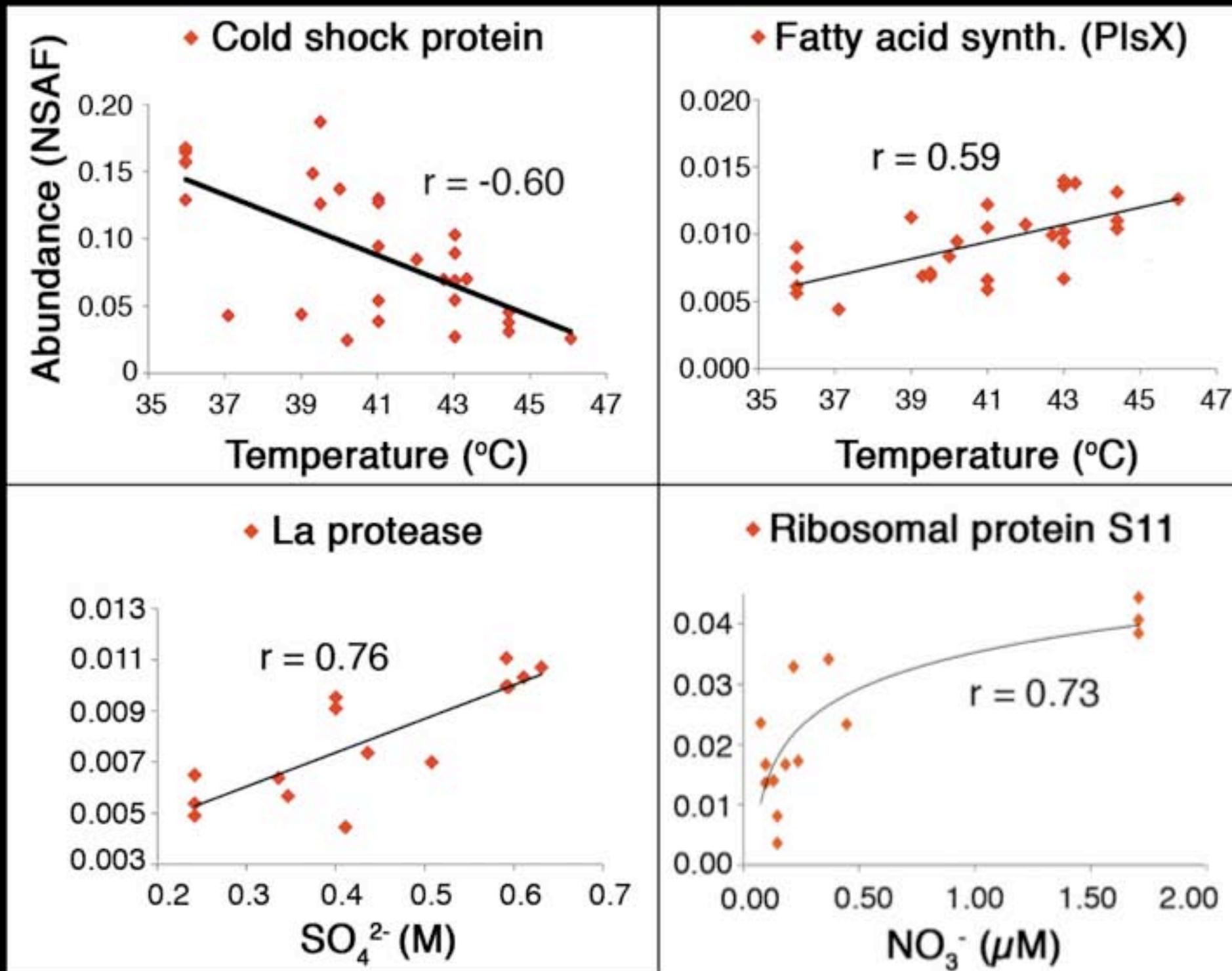
Cell counts also demonstrate this organism's dominance throughout ecological succession

# As communities diversify *Leptospirillum* Group II's proteomes change



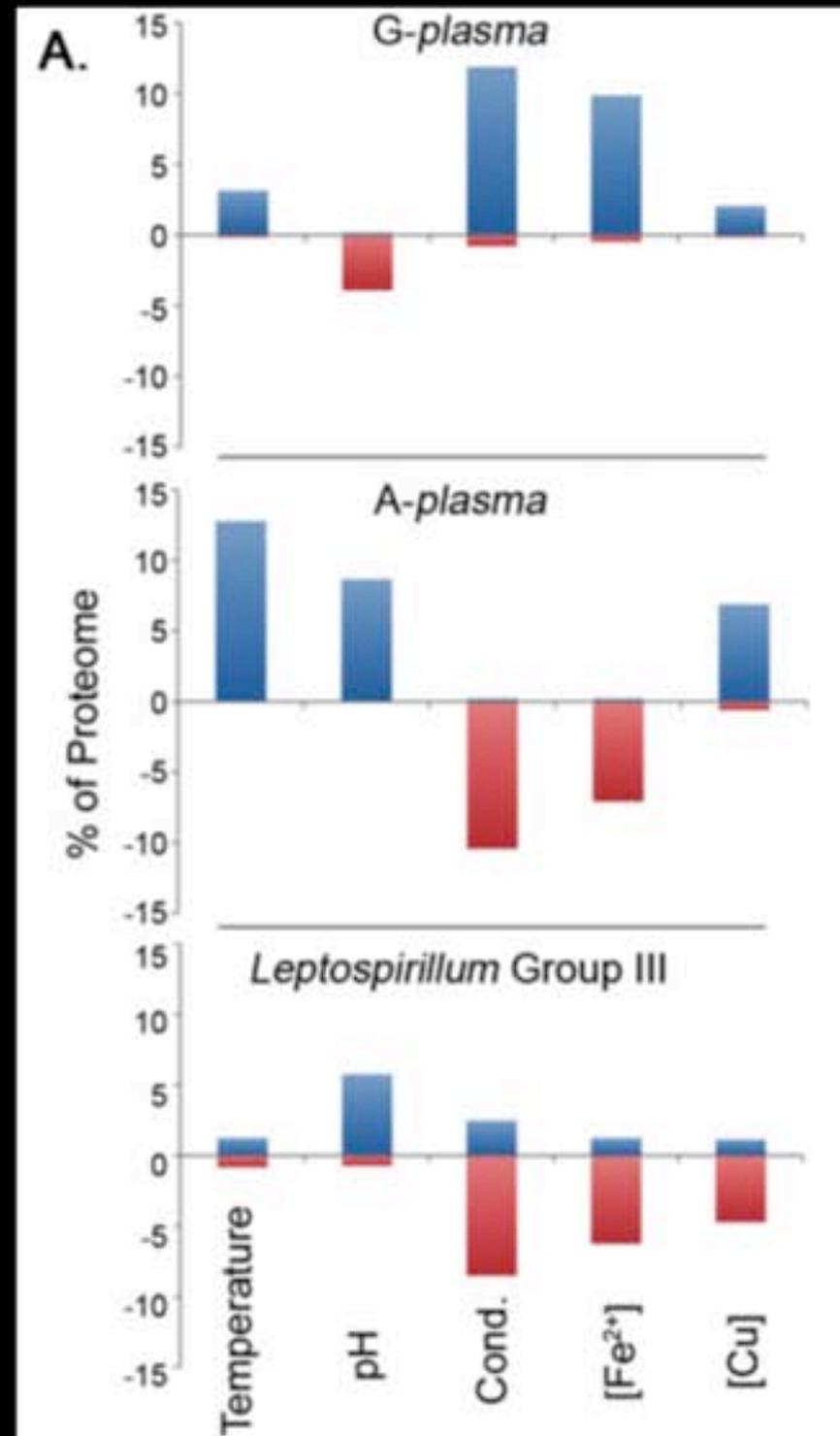
- Changes in community structure are the strongest correlating factor with changes in the proteomes of the *Leptospirillum* Group II (BIOENV test,  $r = 0.42$ ,  $p < 0.001$ ).

# Metabolic fine-tuning by *Lepto*. Group II as environmental conditions change



# Abiotic factors may influence the activities of lower abundance organisms

- Proteomes changes may reflect the environmental niches of sub-dominant populations.
- *A-plasma* and *G-plasma* have different correlation patterns.
- *Leptospirillum* Group III may prefer low-stress niches.



High temp.,  
Low pH,  
High cond.,  
High Fe<sup>2+</sup>,  
High Cu

High temp.,  
High pH,  
Low cond.,  
Low Fe<sup>2+</sup>,  
High Cu

High pH,  
Low cond.,  
Low Fe<sup>2+</sup>,  
Low Cu

# Conclusions I: Proteome changes across environmental gradients

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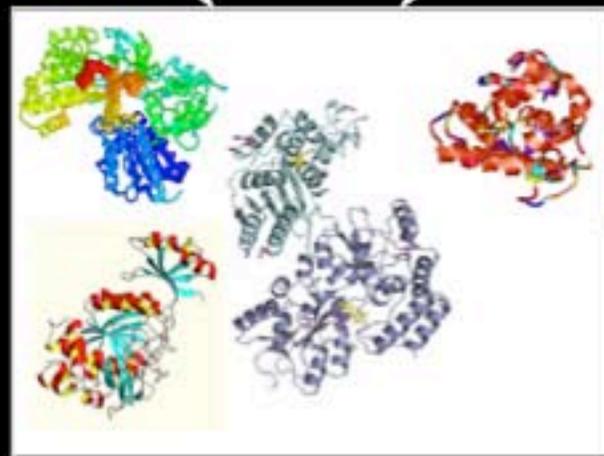
- **Community diversification is the strongest correlating factor with changes in the proteomes of the dominant community member (*Leptospirillum* Group II)**
- Subsets of proteins from *Lepto.* II correlate with abiotic changes in LII and may represent environmental fine-tuning
- Different abiotic factors may drive niche selection in lower abundance organisms

# Quantitative proteomics on biological replicates from a mixed developmental stage biofilm

Collection of 3 biological replicates from different developmental stages of same biofilm



Growth of communities in bioreactor (N<sup>15</sup>-labeled media)

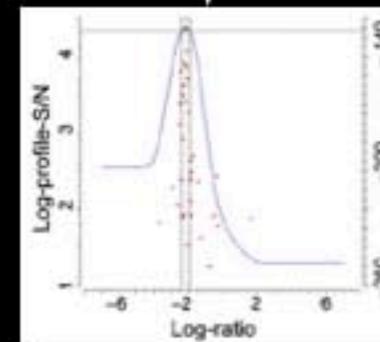


Combine equal amounts of labeled and unlabeled biofilm (~25 mg each) and extract membrane and soluble fractions

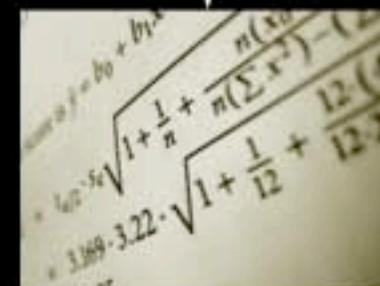


2D-LC MS/MS:

- 2 fractions
- 2 technical replicates
- 3 biological replicates
- 3 developmental stages
- 36 total runs



Calculate the relative N<sup>15</sup>/N<sup>14</sup> abundance ratios for proteins detected in field and bioreactor samples (MS Prorata)



Statistical analyses for pattern detection and signal extraction

# Summary statistics of proteomics experiments

Approximately **6,000** unique proteins were identified across all samples and fractions.

An average of ~ 2,500 soluble and ~ 1,300 membrane proteins were identified from each sample.

Organism	Soluble		
	GS0	GS1	GS2
<i>Leptospirillum</i> Group II	1458	1514	1648
<i>Leptospirillum</i> Group III	163	177	223
<i>Actinobacteria</i> 1	3	8	17
<i>Actinobacteria</i> 2	12	13	16
<i>Firmicutes</i> sp.	11	14	14
<i>A-plasma</i>	6	8	6
ARMAN-2	35	60	61
<i>E-plasma</i>	4	6	8
<i>Ferroplasma</i> Type 1	26	38	40
<i>Ferroplasma</i> Type 2	28	65	63
<i>G-plasma</i>	28	70	77
<i>I-plasma</i>	97	144	231
Plasmid	10	13	18
Unassigned	258	323	387
Virus	1	1	2
<b>Sum</b>	<b>2141</b>	<b>2455</b>	<b>2808</b>

Organism	Membrane		
	GS0	GS1	GS2
<i>Leptospirillum</i> Group II	757	784	656
<i>Leptospirillum</i> Group III	127	133	125
<i>Actinobacteria</i> 1	7	8	6
<i>Actinobacteria</i> 2	7	11	6
<i>Firmicutes</i> sp.	1	3	1
<i>A-plasma</i>	23	43	40
ARMAN-2	3	4	4
<i>E-plasma</i>	13	18	24
<i>Ferroplasma</i> Type 1	19	26	36
<i>Ferroplasma</i> Type 2	21	36	43
<i>G-plasma</i>	75	99	153
<i>I-plasma</i>	4	7	9
Plasmid	4	4	6
Unassigned	187	228	218
Virus	3	4	1
<b>Sum</b>	<b>1250</b>	<b>1410</b>	<b>1328</b>

# Variability in Complex Natural Samples is Low

Intra-replicate correlation of natural samples is high compared to non-replicate samples

Early Growth Stage (GS)

$$\rho_c \approx 0.91$$

Intermediate GS

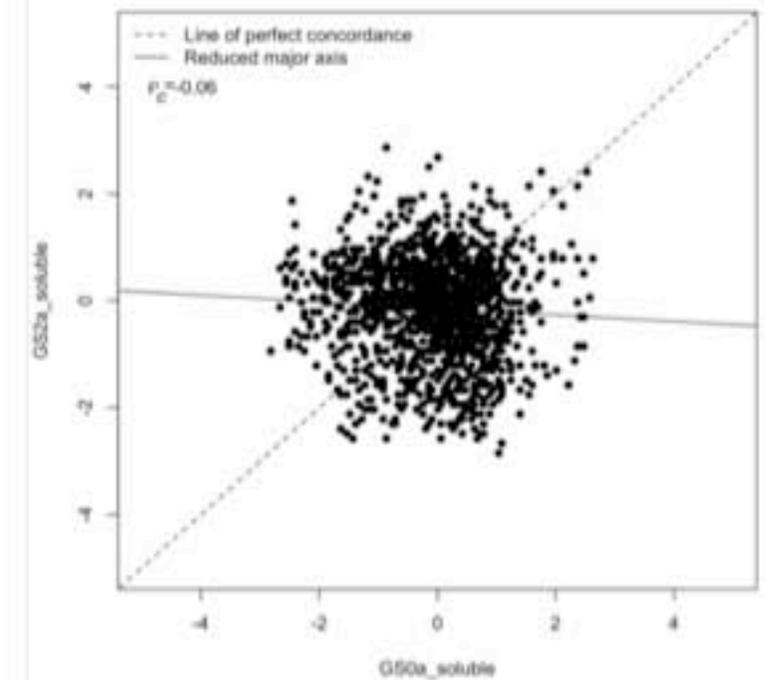
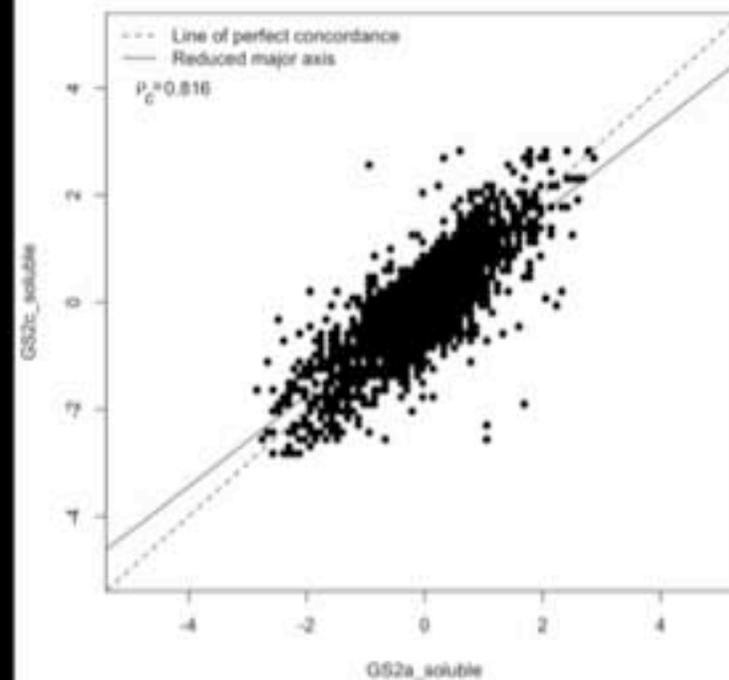
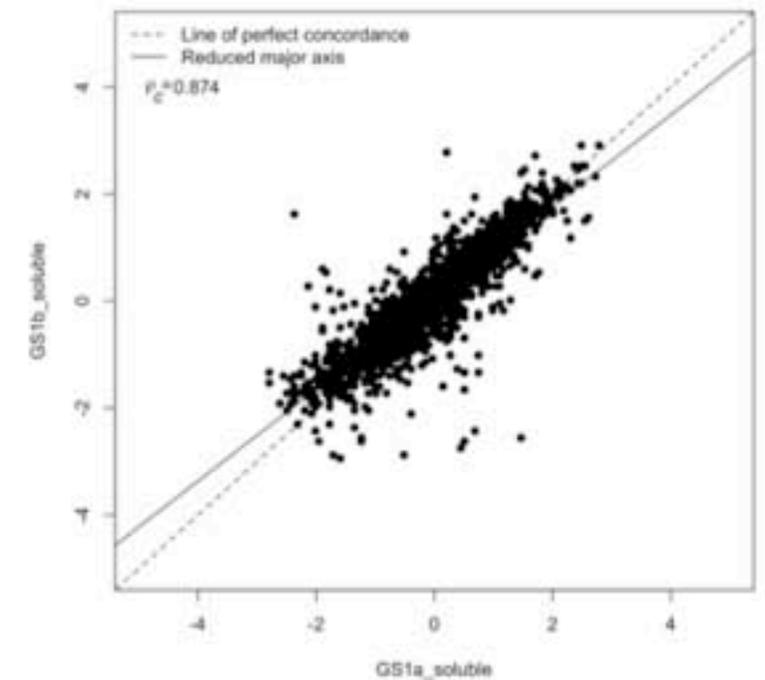
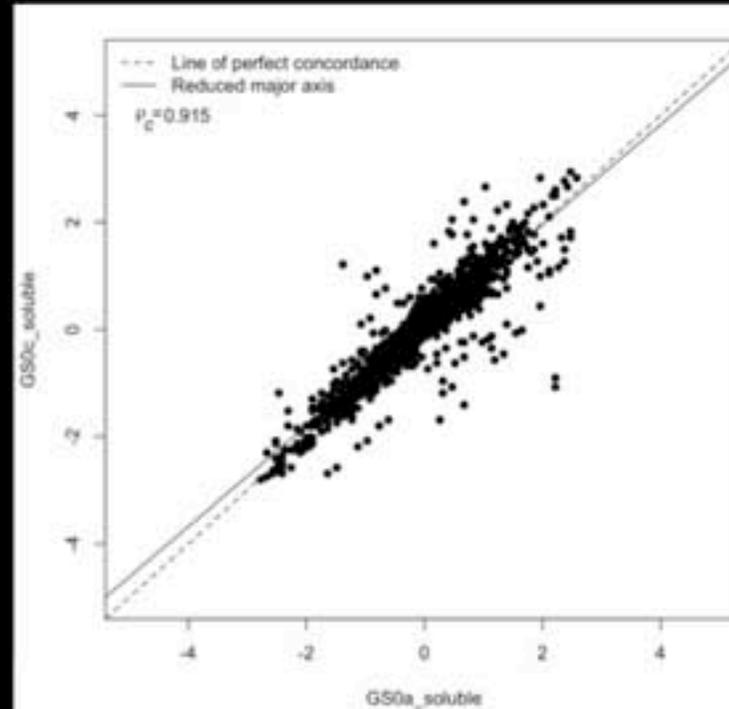
$$\rho_c \approx 0.87$$

Late GS

$$\rho_c \approx 0.82$$

Non-replicate

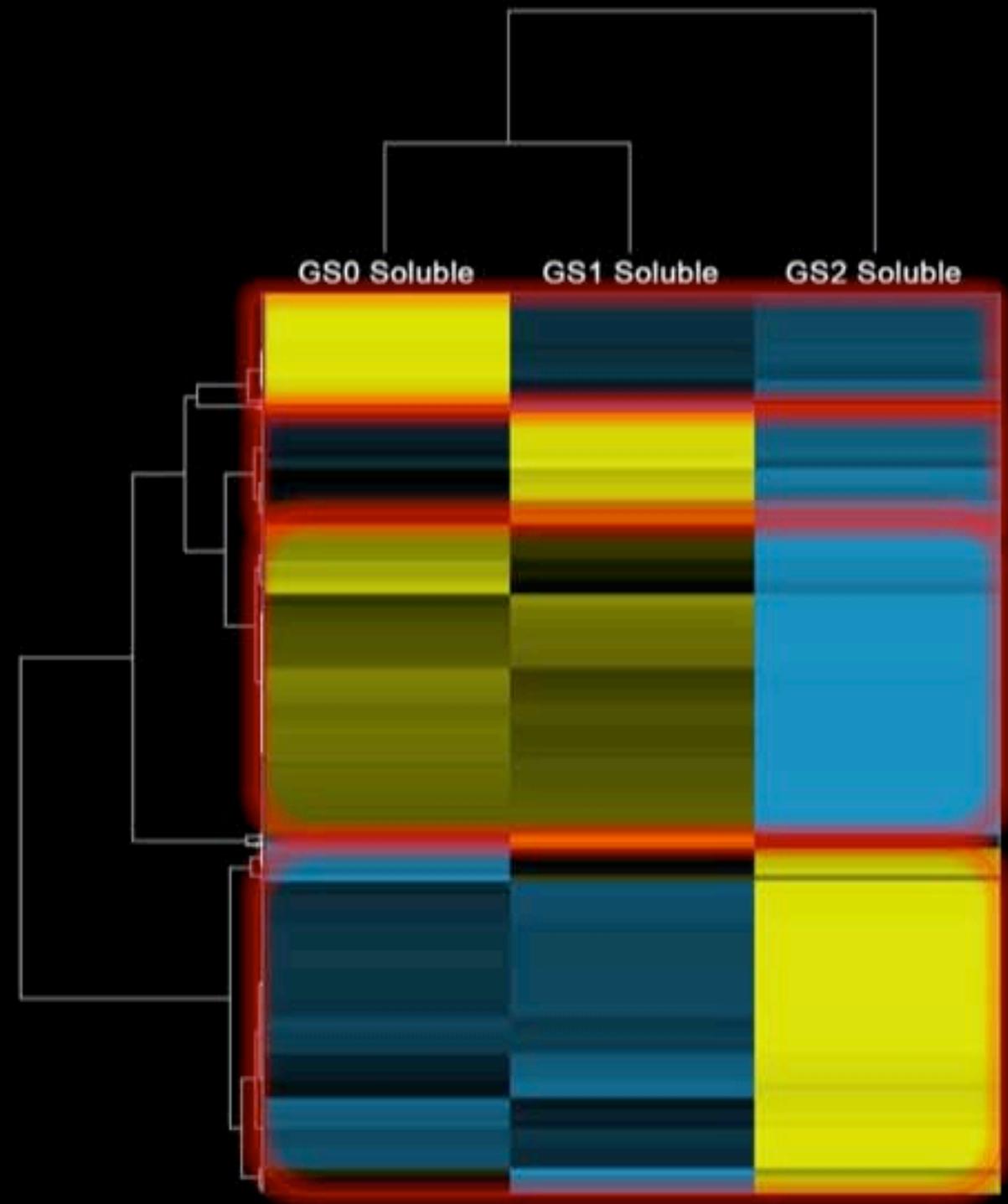
$$\rho_c \approx 0.25$$



# Distinct proteome differences exist between biofilm growth stages

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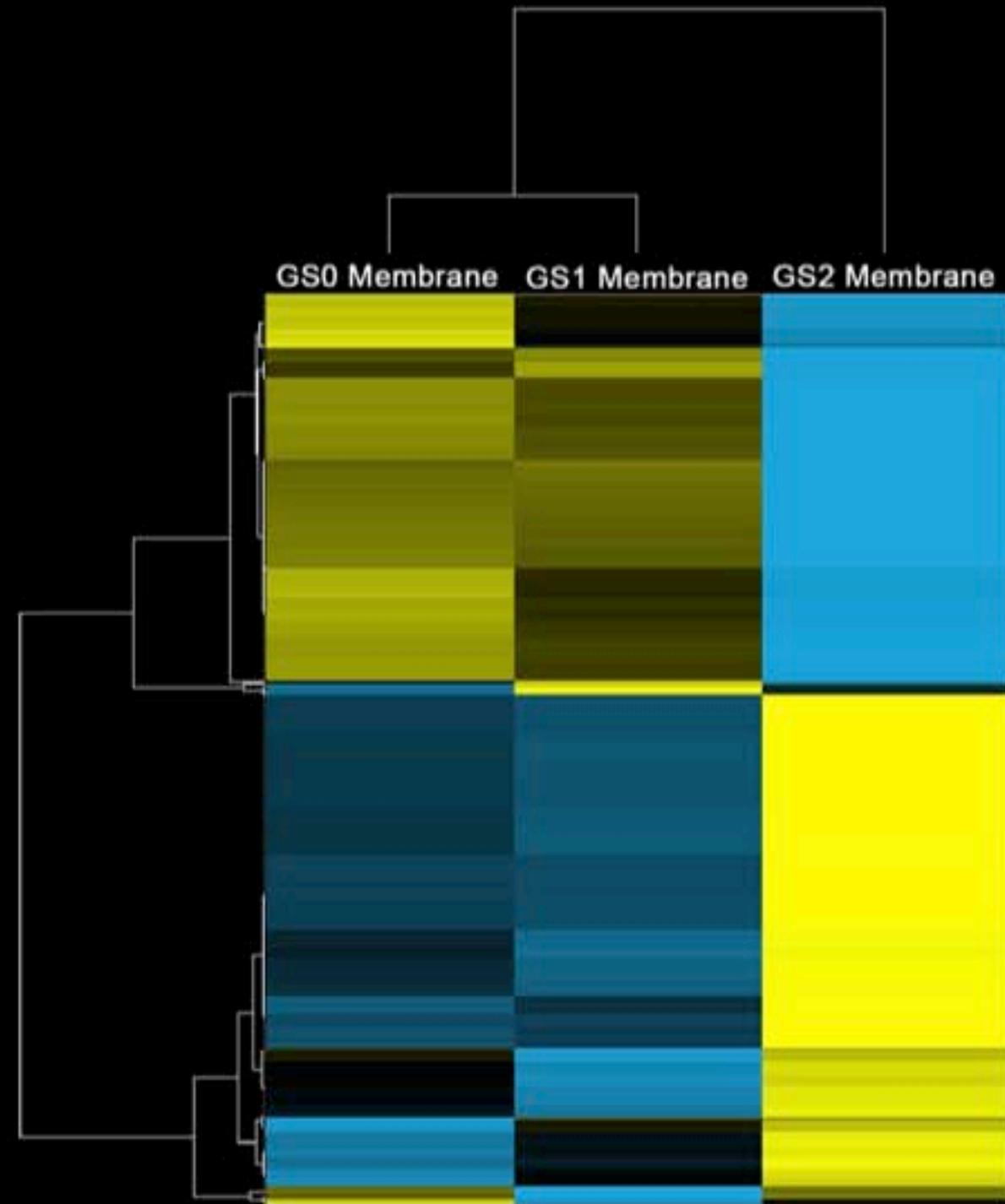
**1,599 soluble proteins** exhibit differences in abundance between developmental stage samples.



# Distinct proteome differences exist between biofilm growth stages

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**518 membrane proteins** exhibit differences in abundance between developmental stage samples.



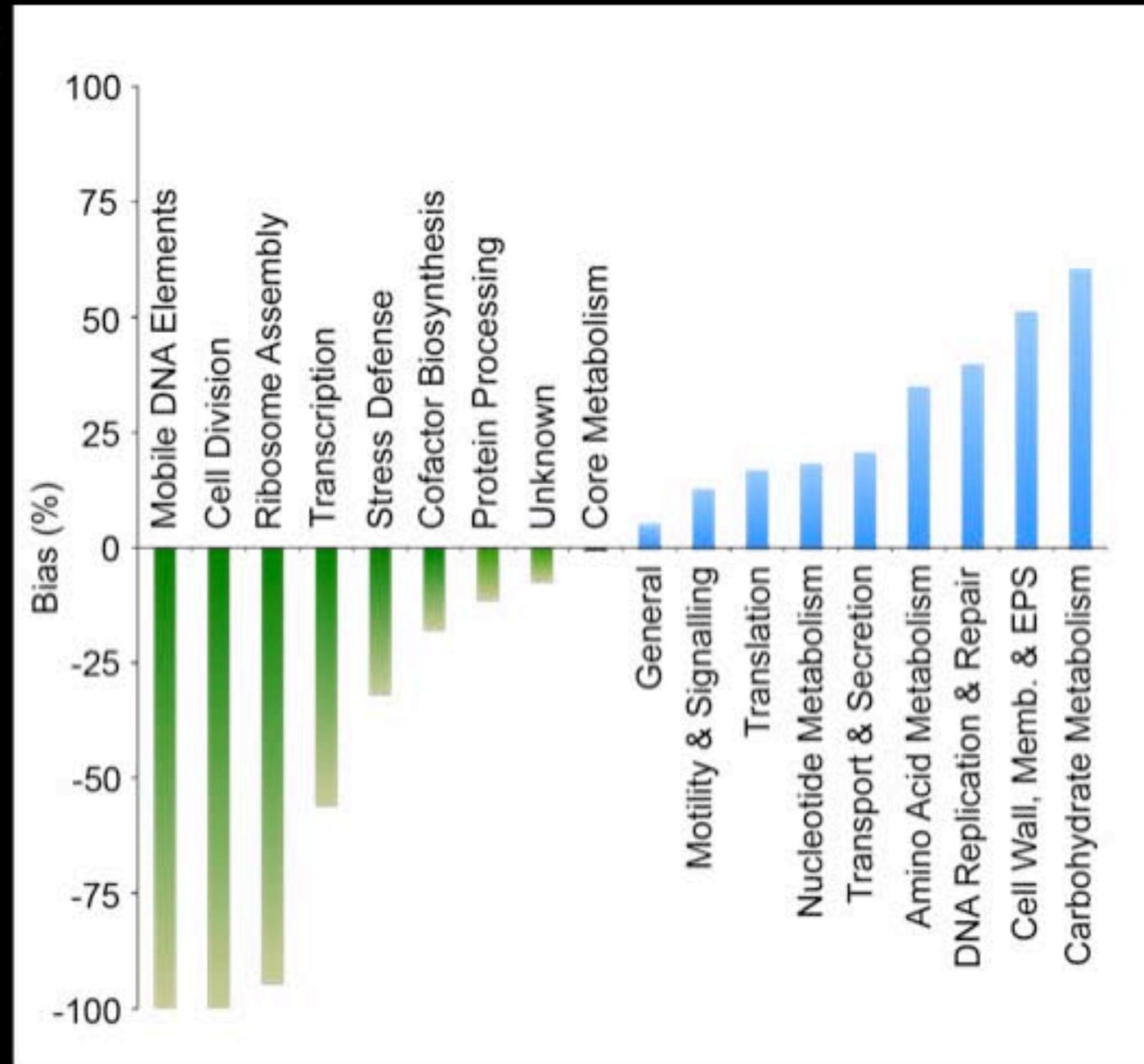
# Functional biases are apparent for *Lepto*. Group II between developmental stages

## Early Growth Stage Bias:

- Mobile DNA elements
- Ribosome assembly
- Cell division
- Stress defense
- Unknown function
- Transcription

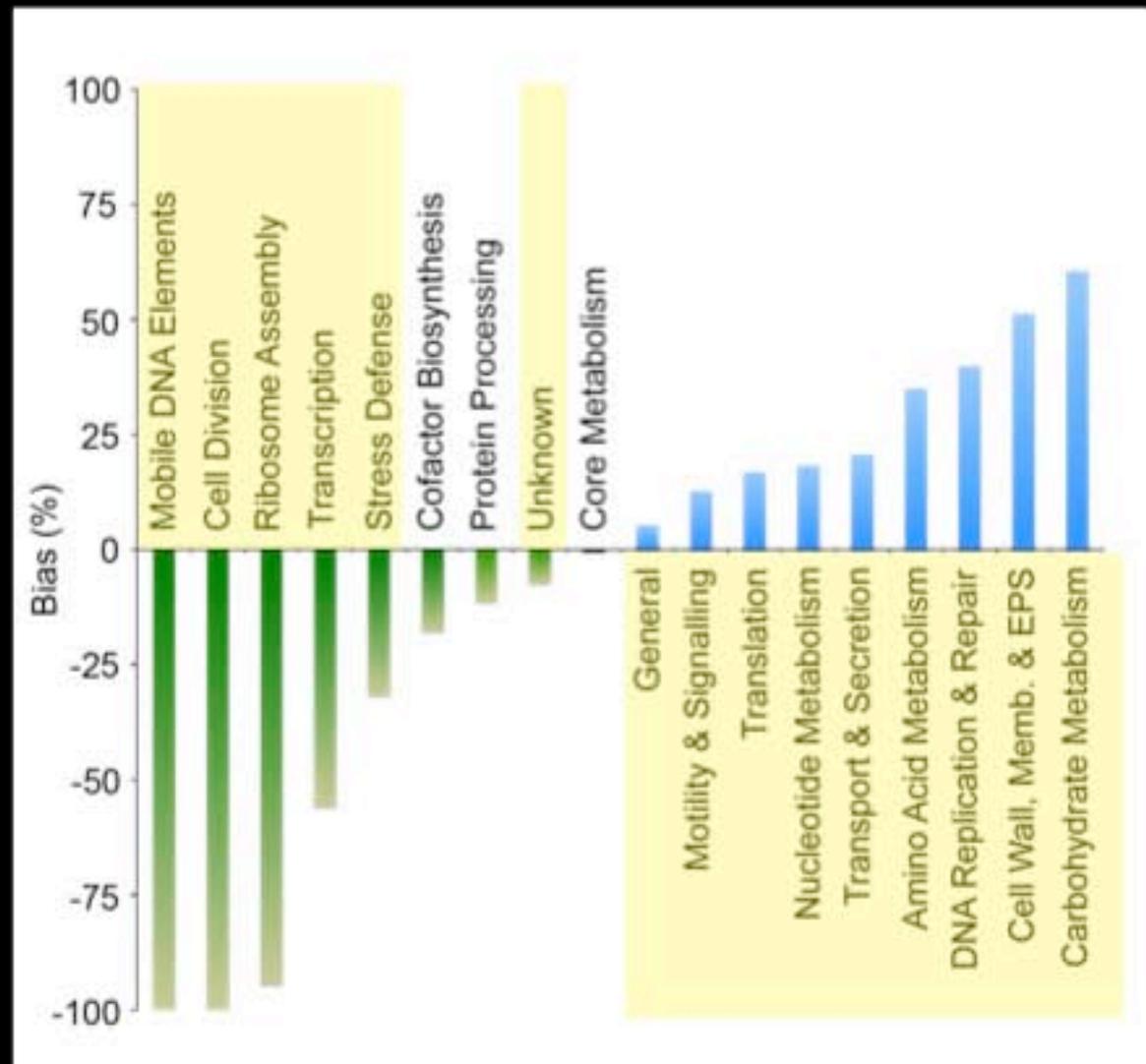
## Late Growth Stage Bias:

- CHO, AA, nucleotide, EPS, and cell wall biosynthesis
- Transport & secretion
- Motility & signaling
- Translation
- DNA replication

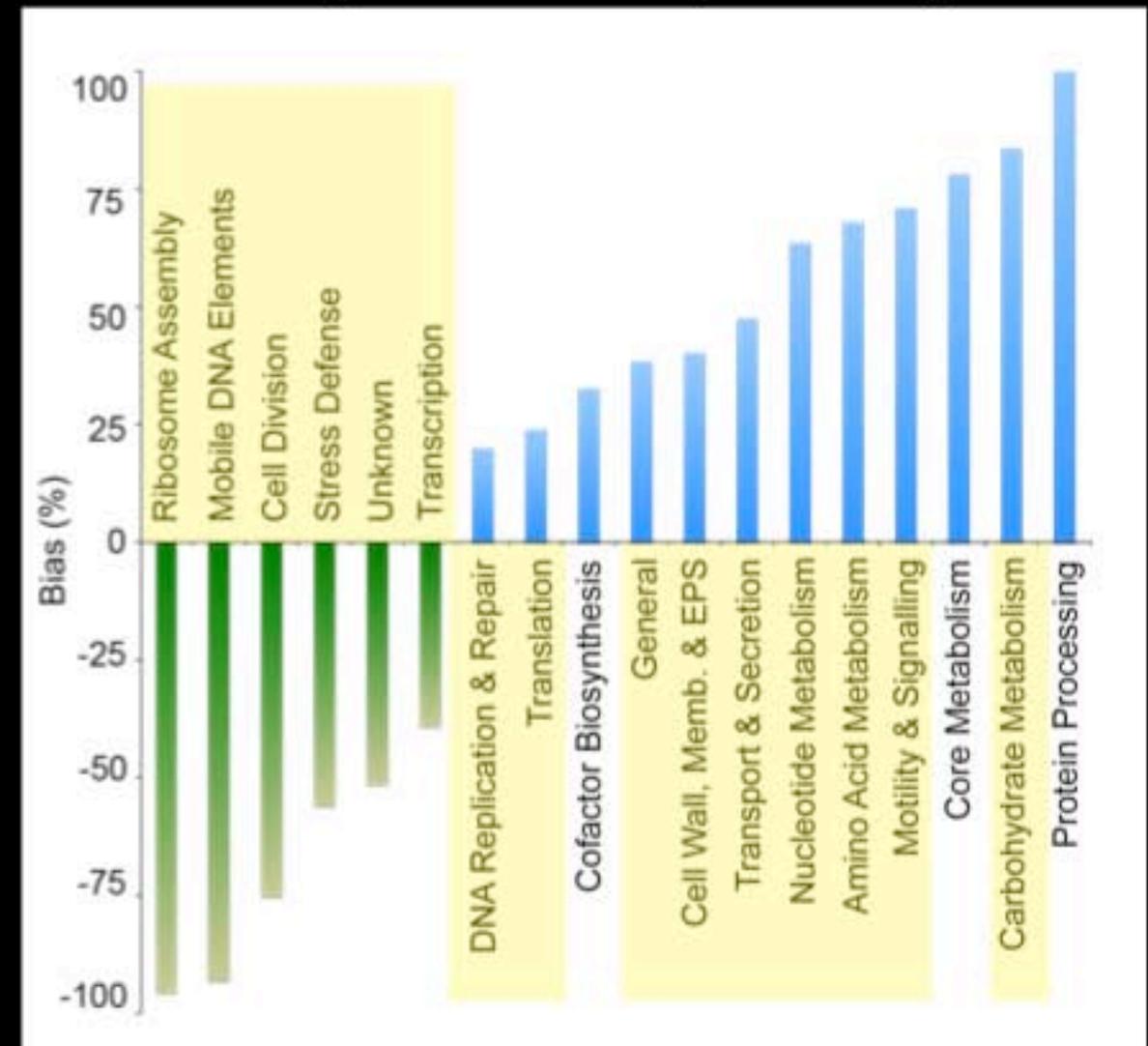


# Quantitative experiments agree with semi-quantitative results

## Quantitative Experiment



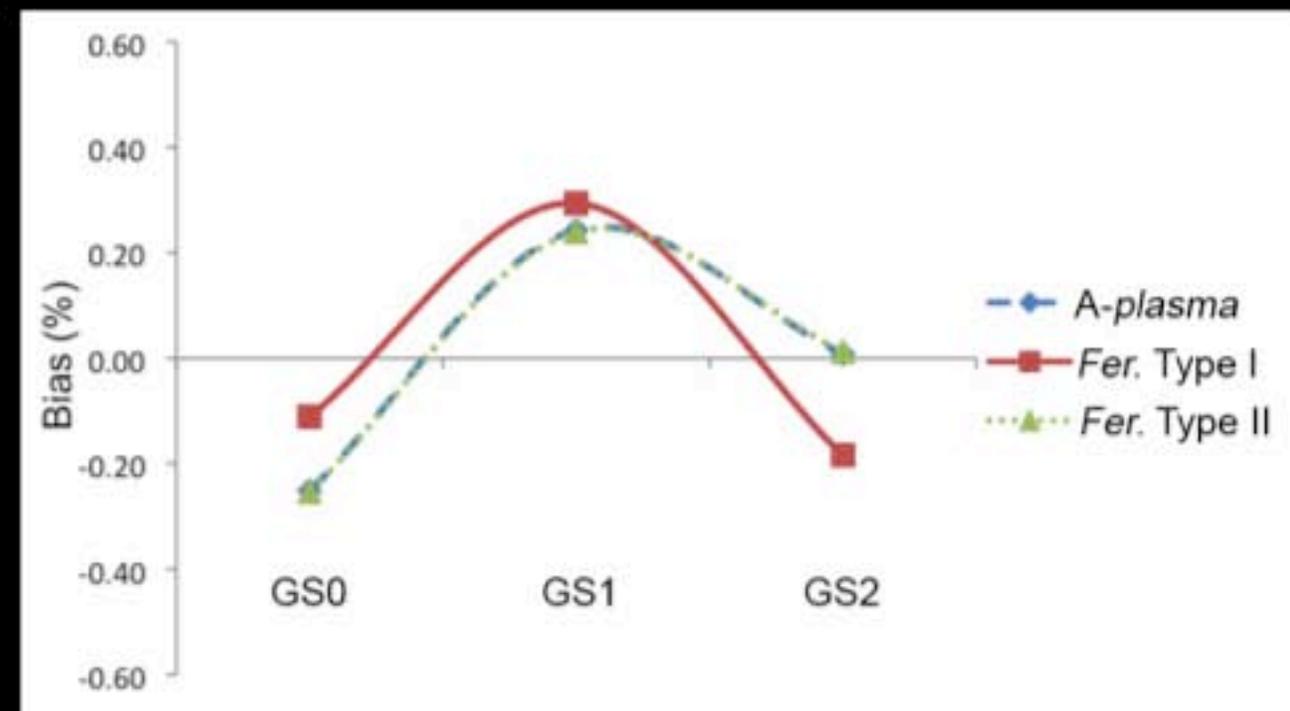
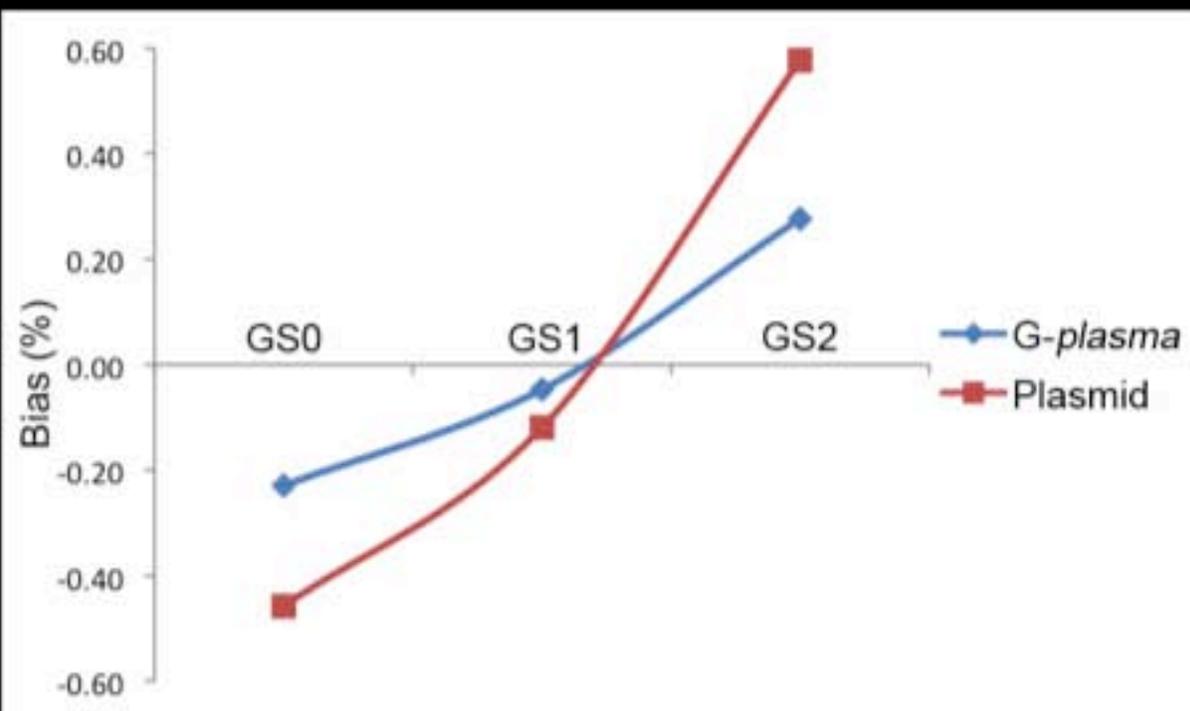
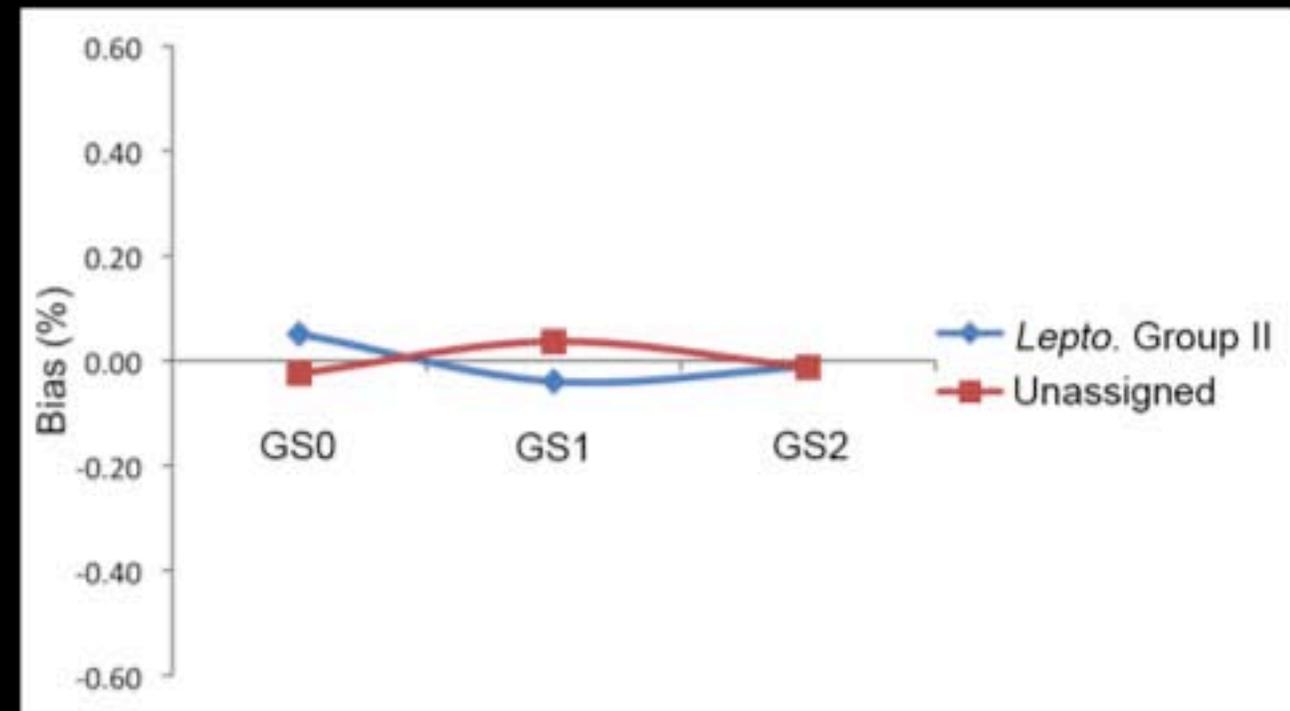
## Semi-quantitative Experiment (NSAF)



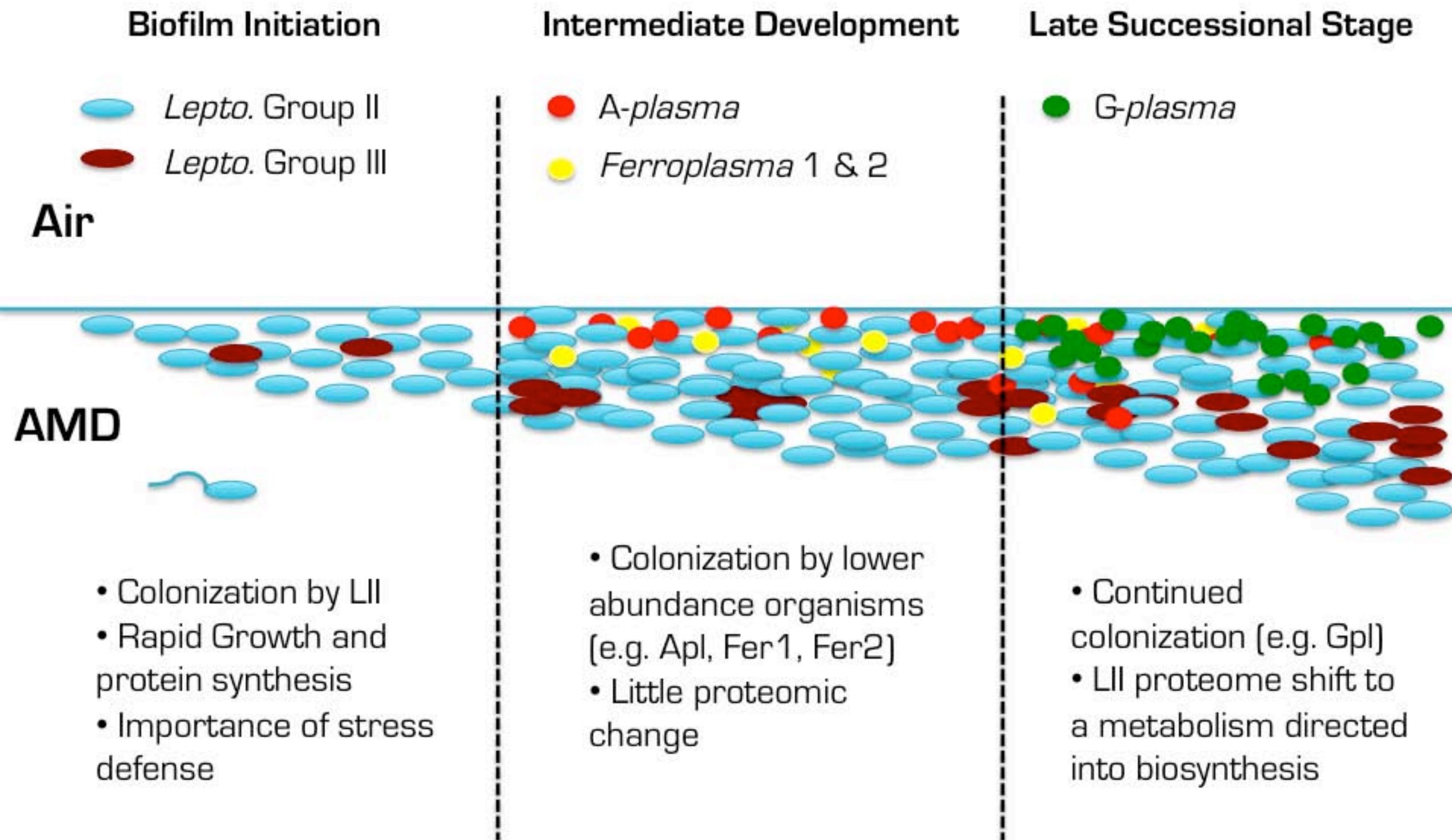
# The activity of low abundance organisms changes as ecological succession proceeds

Proteomes from specific organisms demonstrated greater than expected numbers of proteins associated with different growth stages.

[ $p < 0.05$ , Bonferroni Corrected,  $\chi^2$ -test]

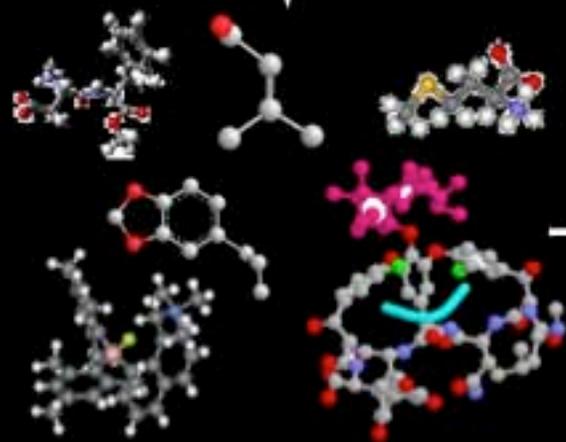


# Conclusions II: Proteome changes as biofilms mature



# Metabolomics on biological replicates from a mixed developmental stage biofilm

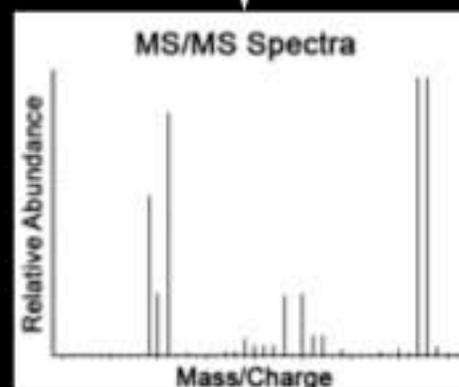
Collection of 3 biological replicates from different developmental stages of same biofilm (B-drift)



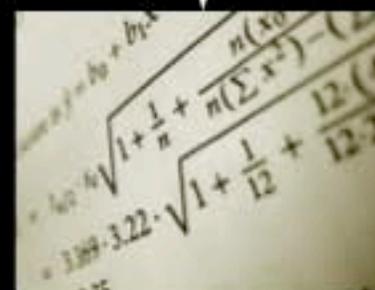
Extraction of metabolites from biological replicates using a suitable extraction technique (targeting specific metabolites, e.g. lipids)



- C18; Diamond Hydride
- MS: AGILENT 6520 Q-ToF
- ESI-ToF operated in positive and negative mode (profile and targeted mode)



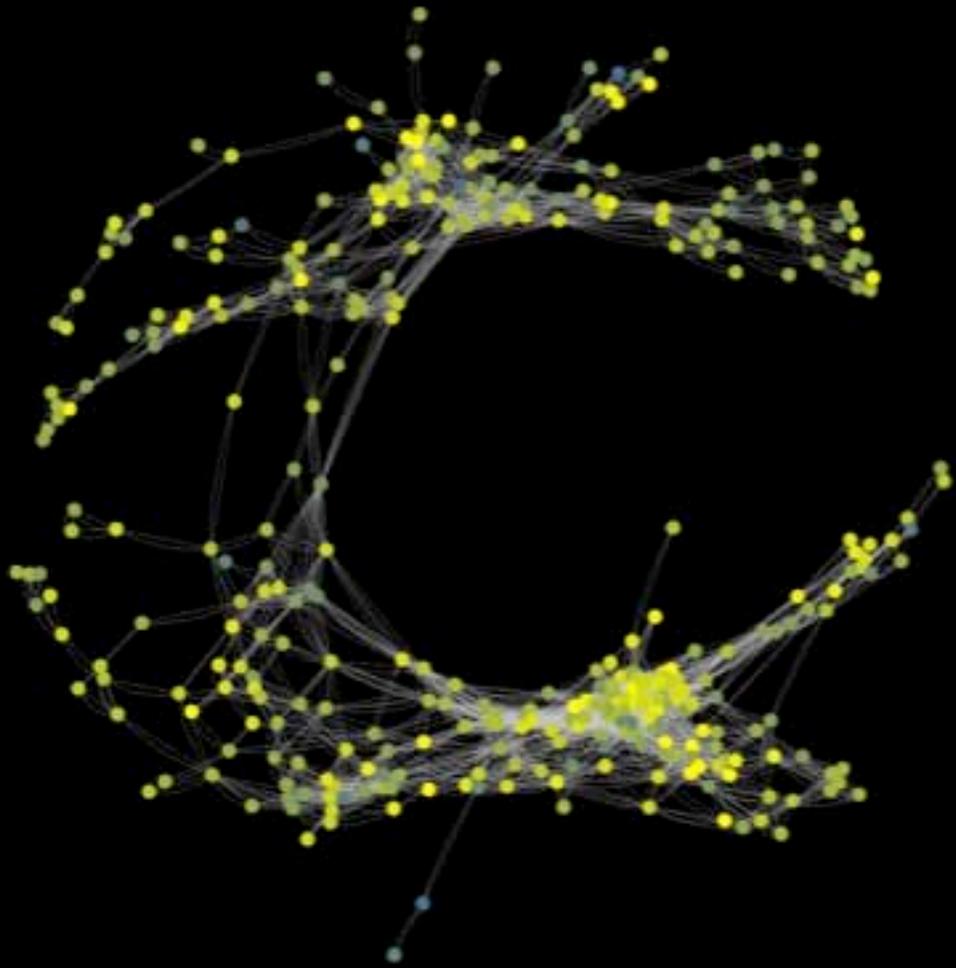
Metabolite identification and calculation of the relative abundances for metabolites detected in samples



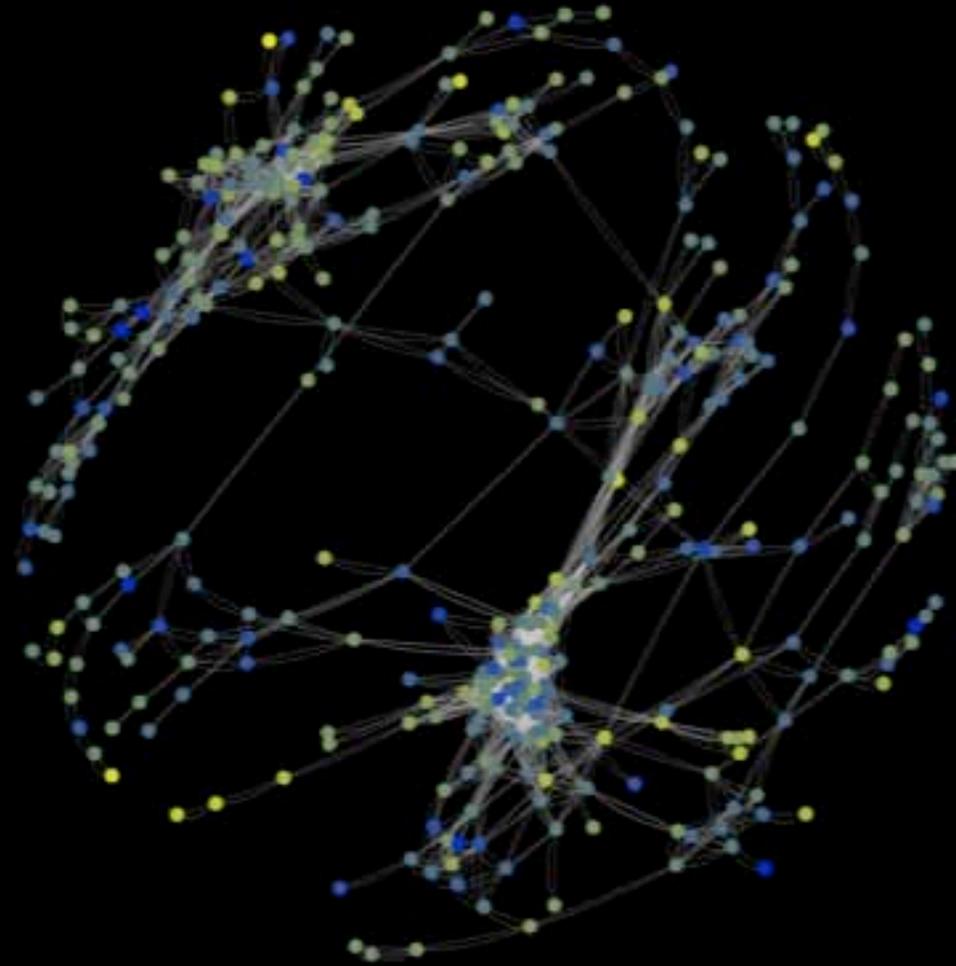
Statistical analyses for pattern detection and signal extraction

# Early and intermediate growth stage proteomes cluster together

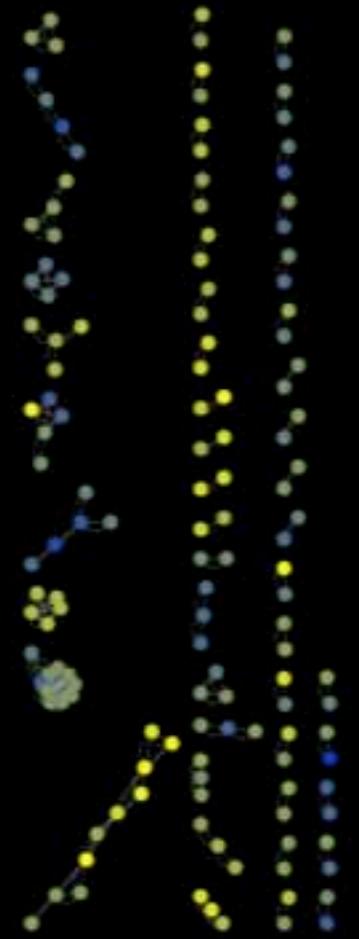
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Soluble proteins highly abundant in **early and intermediate** growth stages

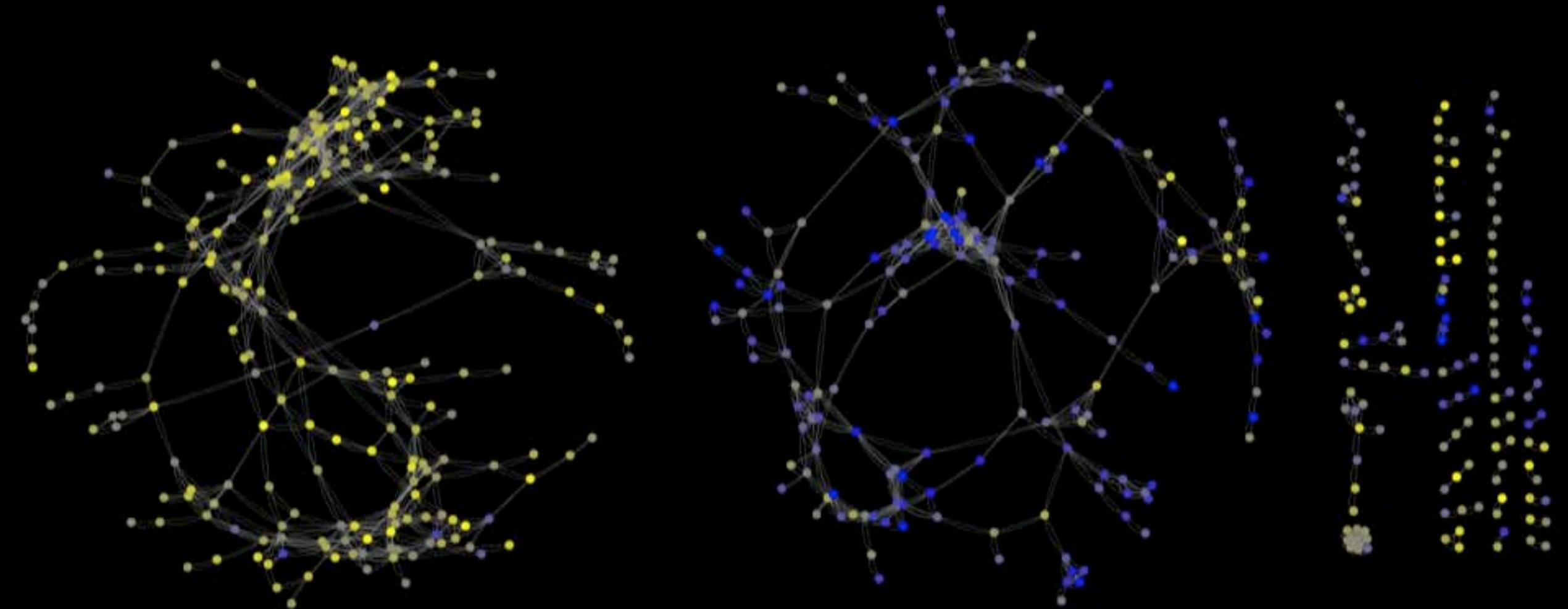


Soluble proteins highly abundant in **late** growth stages



# Early and intermediate growth stage proteomes cluster together

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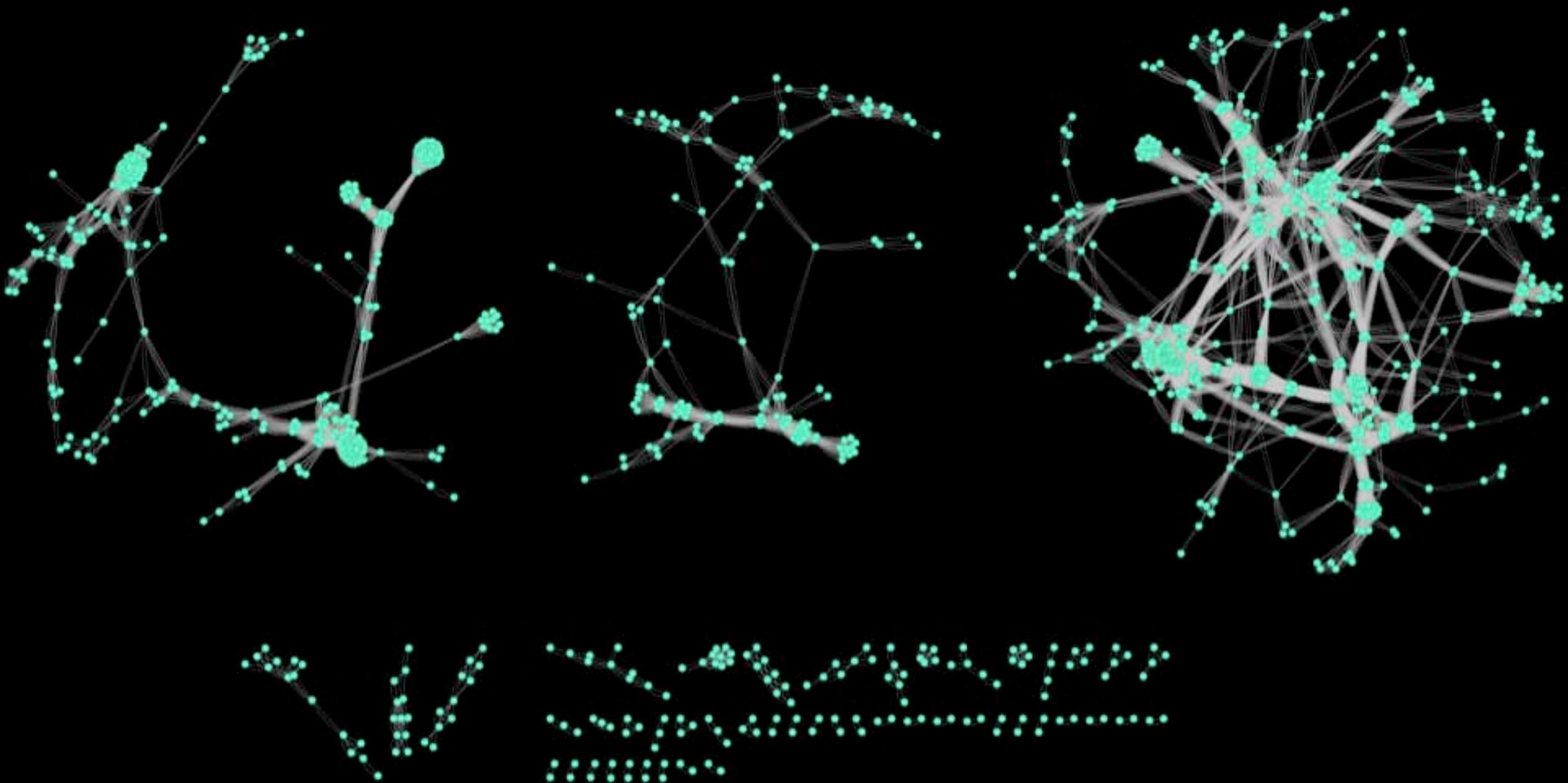


Membrane proteins highly abundant in **early and intermediate** growth stages

Membrane proteins highly abundant in **late** growth stages

# Metabolites form three clusters corresponding to each growth stage

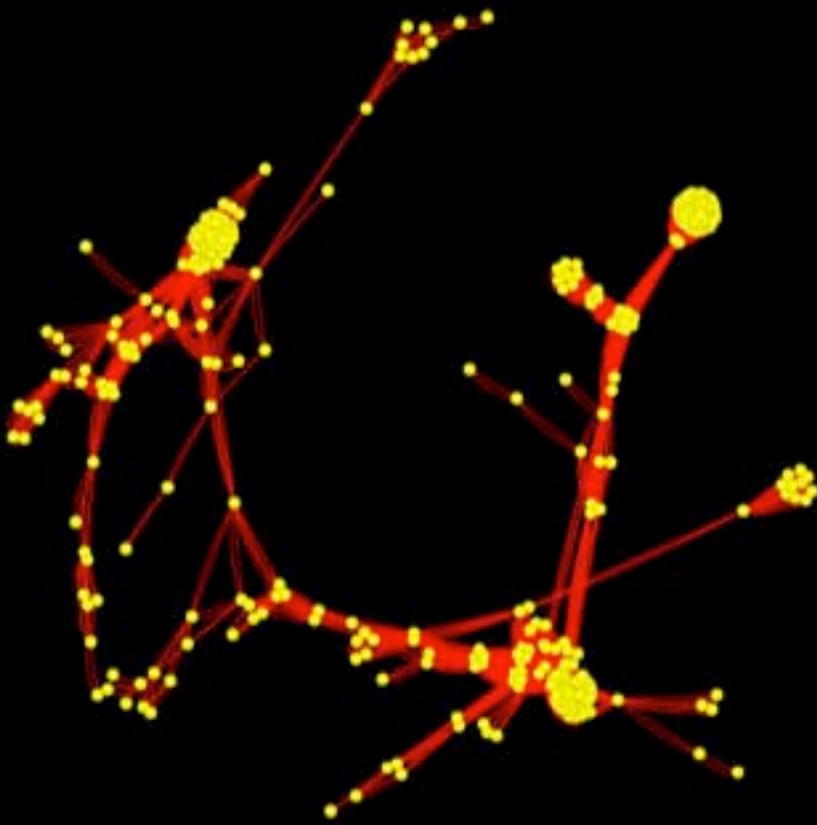
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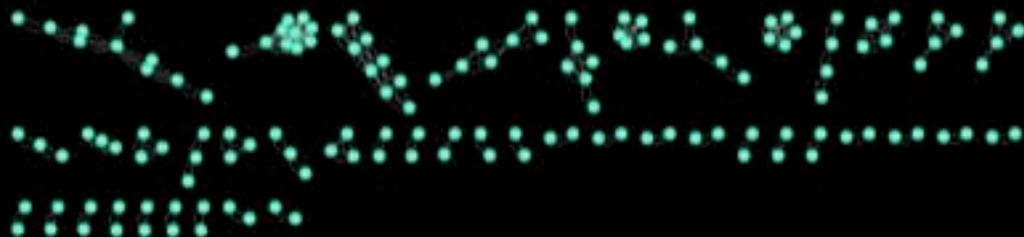
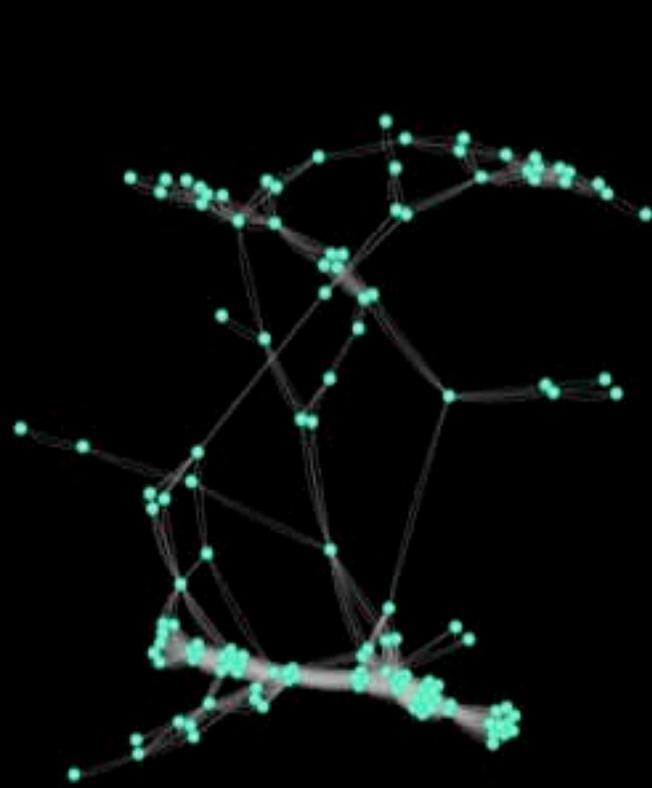
Of 8,660 filtered metabolites discovered in all experiments, the abundances of 1,396 demonstrate high covariance patterns with protein abundances

# Metabolites form three clusters corresponding to each growth stage

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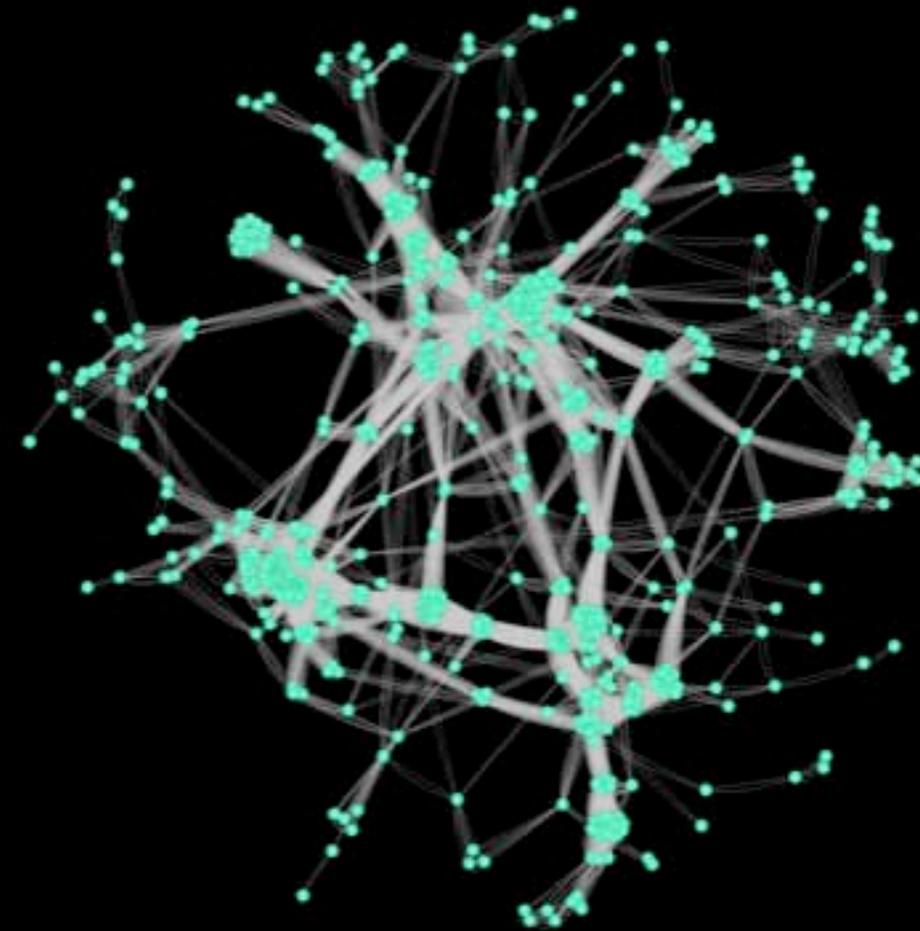
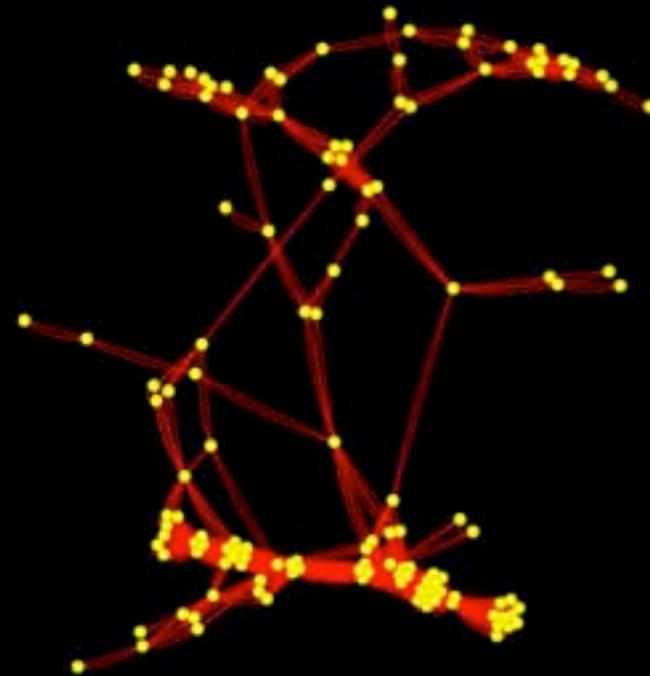


Early growth stage



# Metabolites form three clusters corresponding to each growth stage

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Intermediate growth stage

