# Engineering (and Understanding) Cell and Community Metabolism

**Orkun S Soyer**

**MELISSA Programme**
**Rome, 17 May 2018**

OSS LAB

WARWICK CENTRE FOR
**INTEGRATIVE SYNTHETIC BIOLOGY**

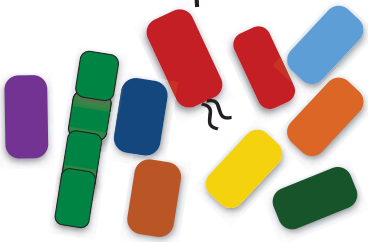
EPSRC & BBSRC Centre for Doctoral
Training in Synthetic Biology

SynBio CDT

WARWICK
THE UNIVERSITY OF WARWICK

# Closed ecosystems…

EcoSphere by Hanson J and Folsome C

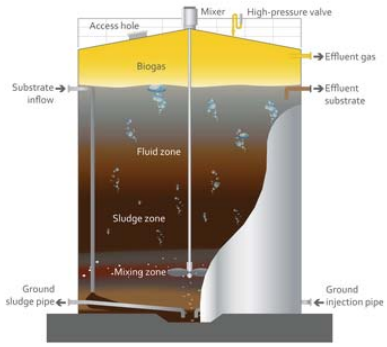


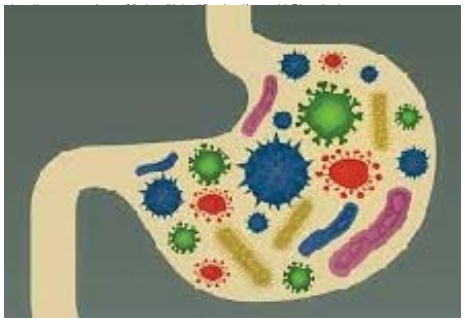
… are underpinned by microbial communities

# Microbial Communities

## High industrial and medical relevance

**BIOTECH | AGRI-TECH**
**WATER | MEDICAL**



## Scientific frontier with a multitude of open questions

How can we explain diversity in microbial communities?

What is the function (if any) in a given microbial community?

Does community stability relate to diversity?

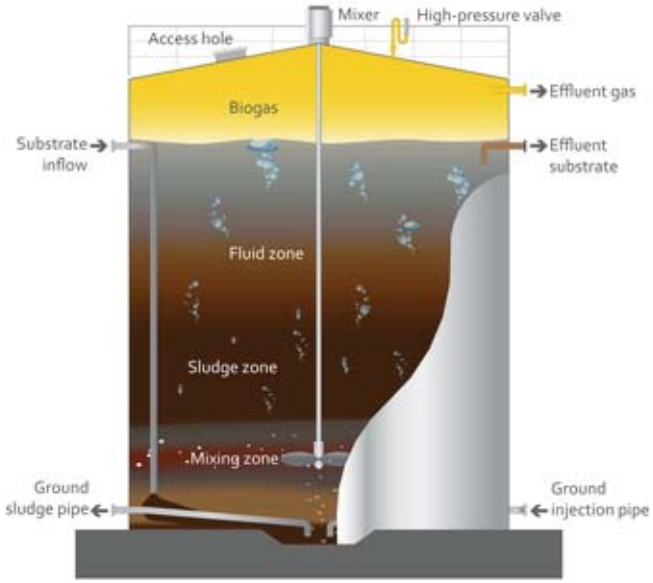
What are the key interactions in microbial communities?

What is the relation between ecological and evolutionary processes?

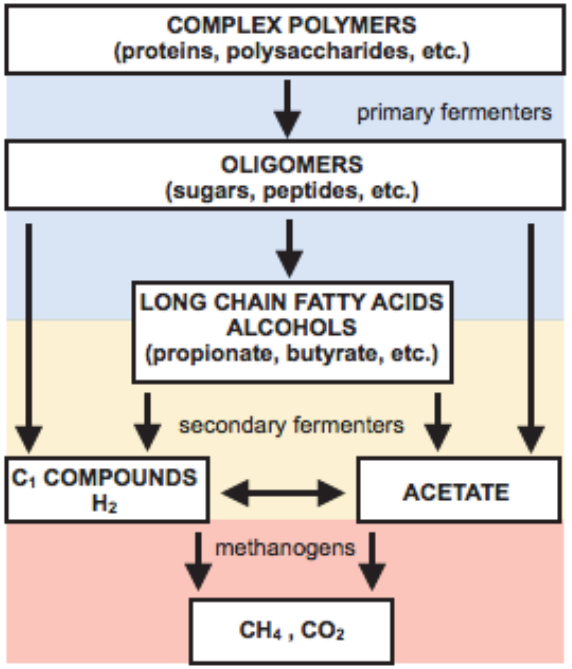
Widder S. *et al*, ***ISME J*** 10:11 (2016)

# A Functional/Tractable Microbial Community?

## Anaerobic Digestion (AD)




http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/




Schink B *Microbiol Mol Biol Rev* 61:2 (1997)

BBSRC bioscience for the future

*sLoLa*
*2013-2018*

WARWICK THE UNIVERSITY OF WARWICK

UNIVERSITY OF EXETER

Imperial College London

Newcastle University

TGAC The Genome Analysis Centre™

# Engineering Synthetic Microbial Communities for Biomethane Production

£3.86M

10 PIs
7 PDRAs
6 PhDs

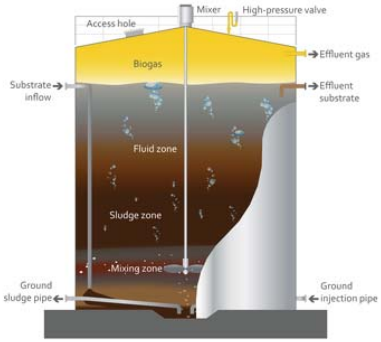
>22


4 sci.
2 ind.
2 videos

1 IPA
1 TRDF
1 Spin-out

# Our Approach

*Decipher complex, natural systems to identify general trends*

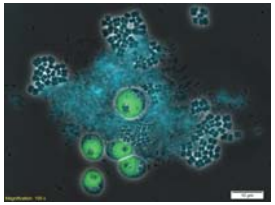



http://www.mannvit.com/Markets/UnitedKingdom/AnaerobicDigestion/

TOP-DOWN

? Insights and Applications ?

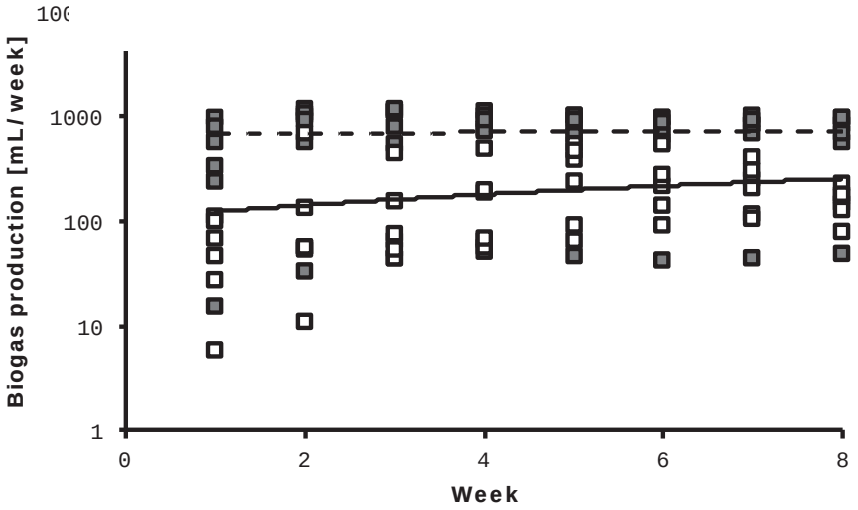
BOTTOM-UP

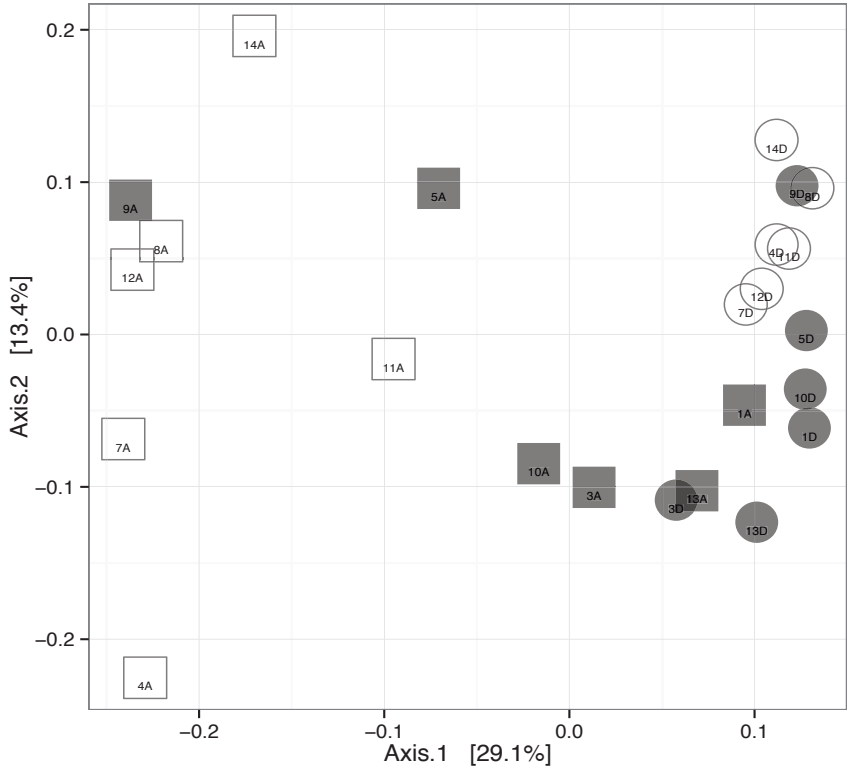


*Engineer minimal systems to learn about biochemical basis of communities*

# ‘Top-down insights’ from AD communities

Work led by Angus Buckling *et al*



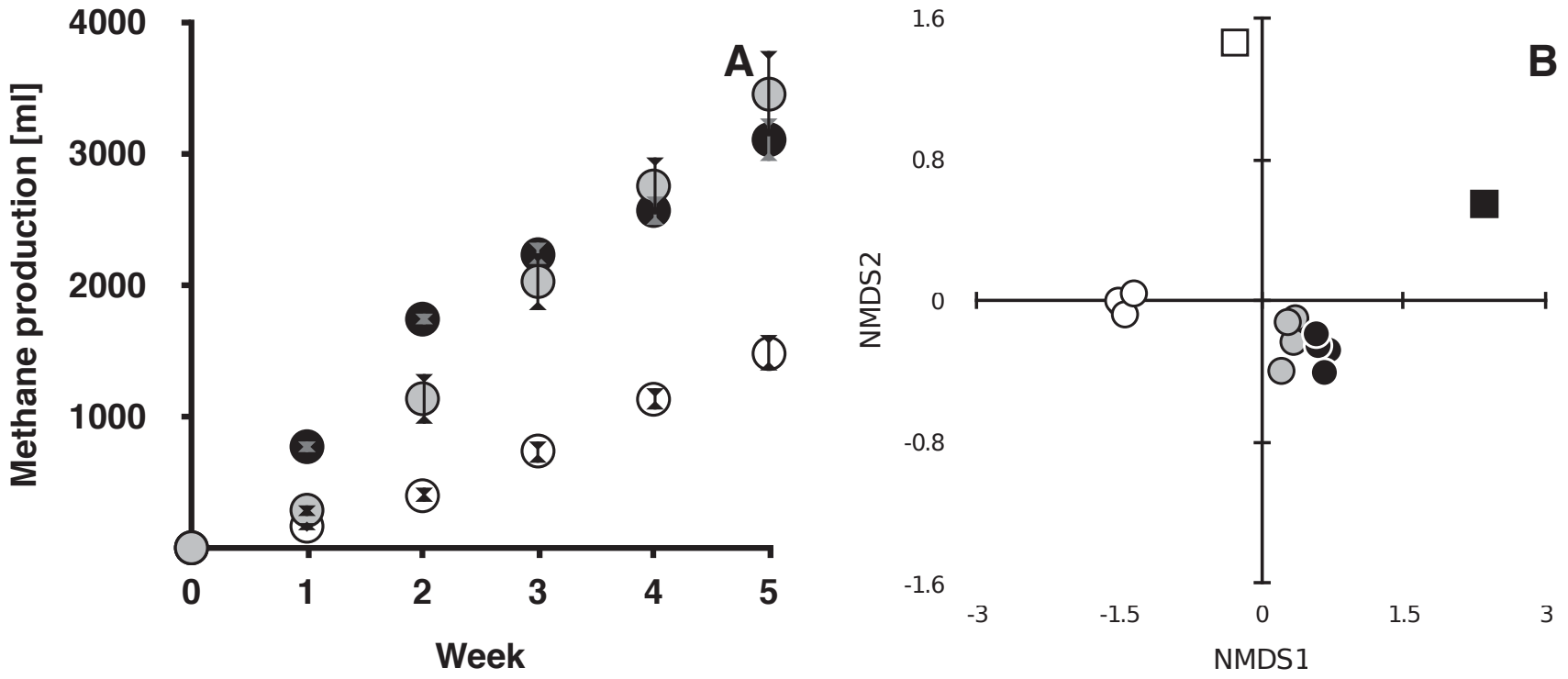

Degradation performance, ie biogas production differs among communities from AD reactors vs. natural

Community composition (and biogas production) converges upon adaptation to same feedstock

# ‘Top-down insights’ from AD communities

Work led by Angus Buckling *et al*

Communities’ mixtures are dominated by top performing community




Sierocinski P et. al. ***Curr. Biology*** (2017).

# Offshoot: Temporal analysis of industrial AD reactors

www.anaerodynamics.com



- Weekly samples over a year
- 16s (bacteria and archea) and metagenomics
- Metadata on methane, pH, feed, etc.

# Functional annotation of genomes and meta genomes

**MetQy**

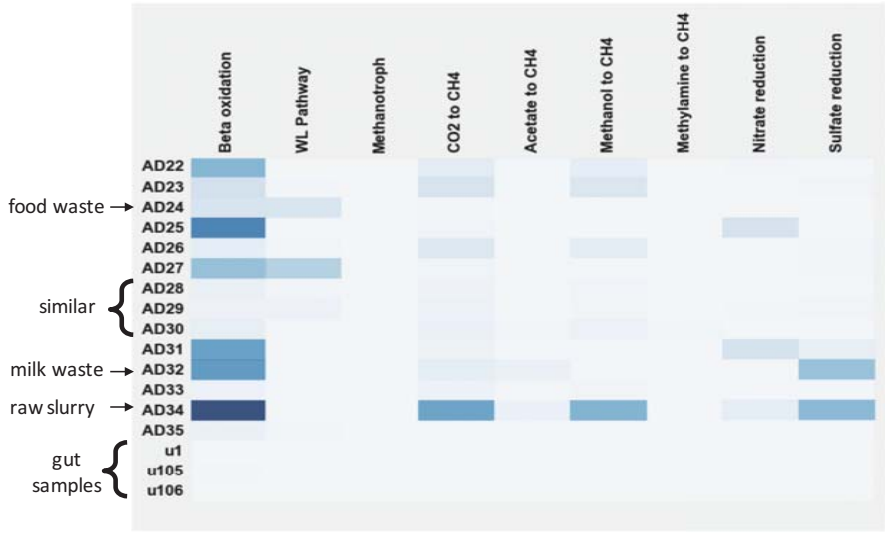
available to download

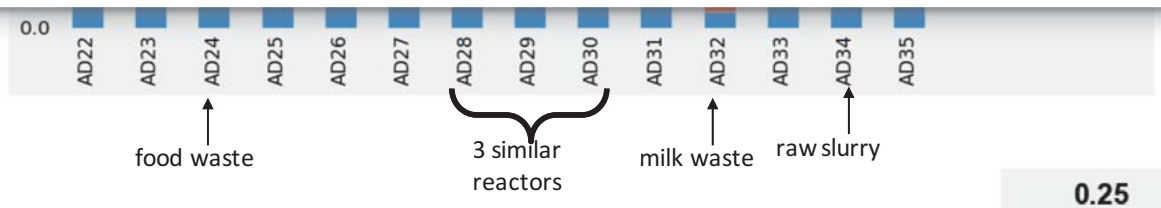
Andrea
Martinez-Vernon

**See Andrea's poster and bioRxiv manuscripts;**

https://www.biorxiv.org/content/early/2017/11/16/215525

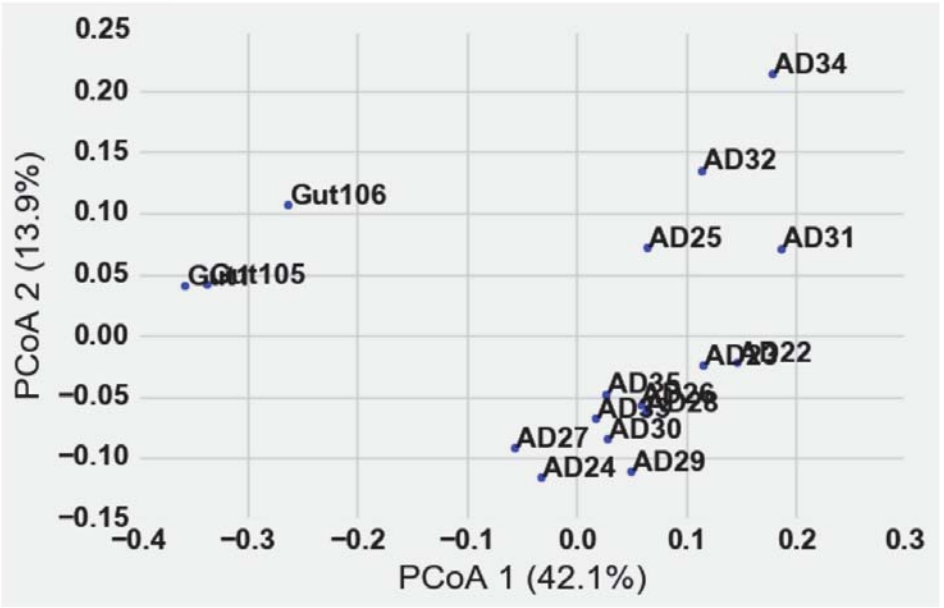
https://www.biorxiv.org/content/early/2018/04/25/307157





Different reactors, fed with different 'food' source show different microbiomes



# ‘Top-down insights’ from AD communities

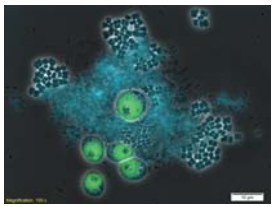
Microbiome composition/diversity is important for community function (methane production)

Members of functional communities tend to ‘stick’ together as a community

=> *interactions within communities are important and perhaps optimised through co-adaptations*

? Insights and Applications ?

BOTTOM-UP

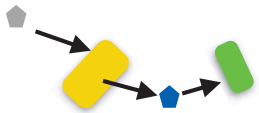


*Engineer synthetic communities to learn about biochemical basis of communities*

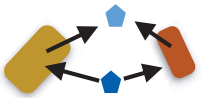
**design principles ??**

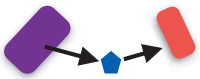
**modules**

Cross-Feeding



Metabolic Cycles

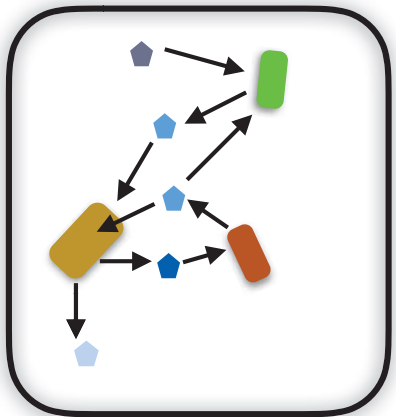




Auxotrophy

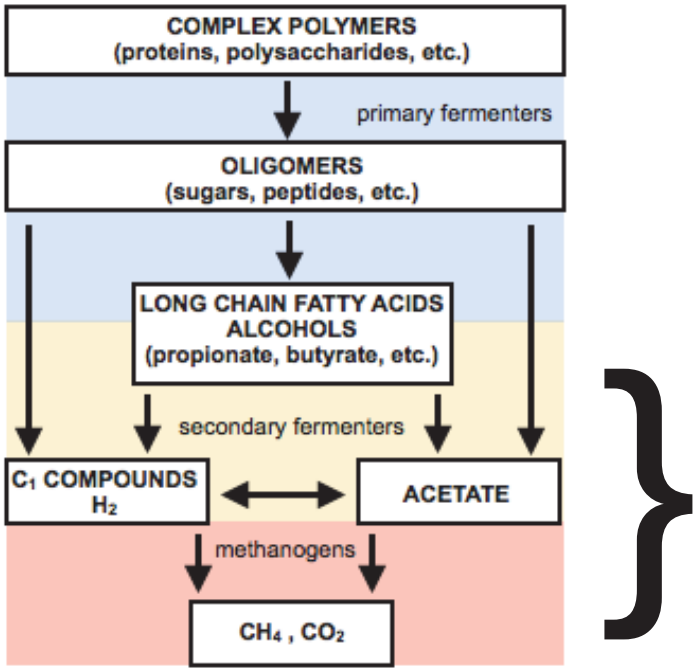
Syntrophy

**functional systems**

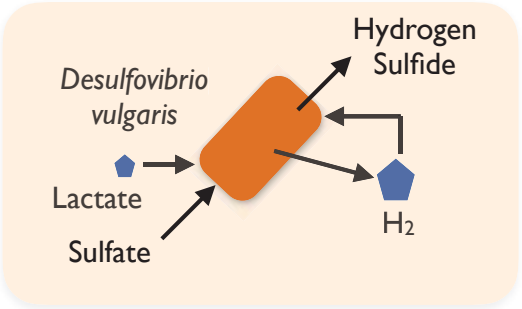


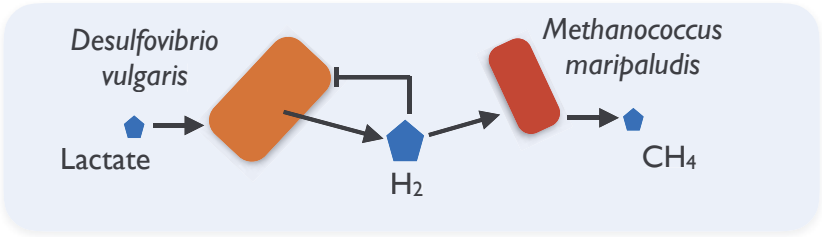
Grosskopf & Soyer, ***Curr. Op. Biotech*** (2014)

# Syntrophy: Crucial in AD systems




Schink B *Microbiol Mol Biol Rev* 61:2 (1997)




$$\text{Lactate} + 2\text{Sulfate} + 3H^+ \longrightarrow 3CO_2 + 2HS^- + 3H_2O \qquad \Delta G_0 = -259.09 \text{ kJ/mol}$$

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + 0.5CO_2 + 0.5CH_4 \qquad \Delta G_0 = -74.19 \text{ kJ/mol}$$

# Thermodynamics basis for genetic drivers of syntrophy

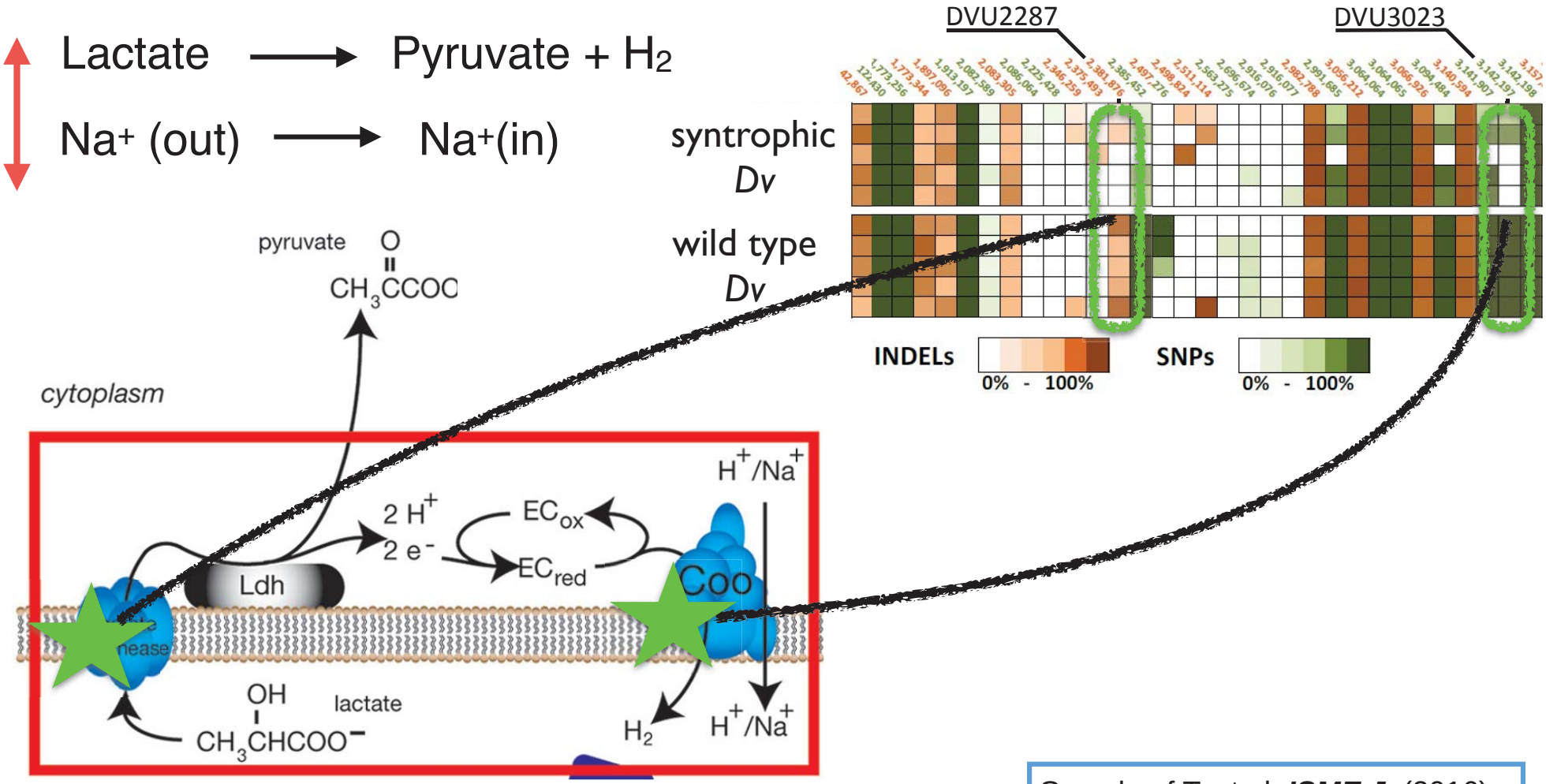
The syntrophy enabling mutation allows energy investment to overcome thermodynamic hurdle:

$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + CO_2 + 2H_2 \qquad \Delta G_0 = -8.79 \text{ kJ/mol}$$

?

Lactate ⟶ Pyruvate + $H_2$

$Na^+$ (out) ⟶ $Na^+$(in)

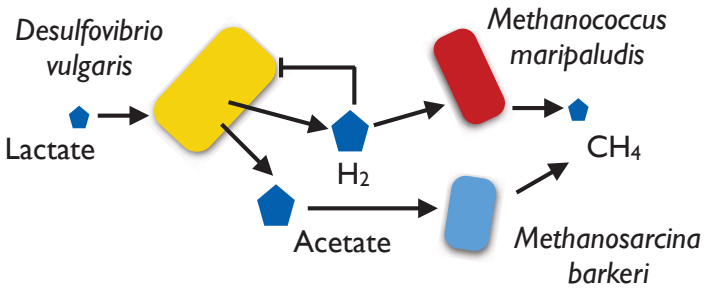


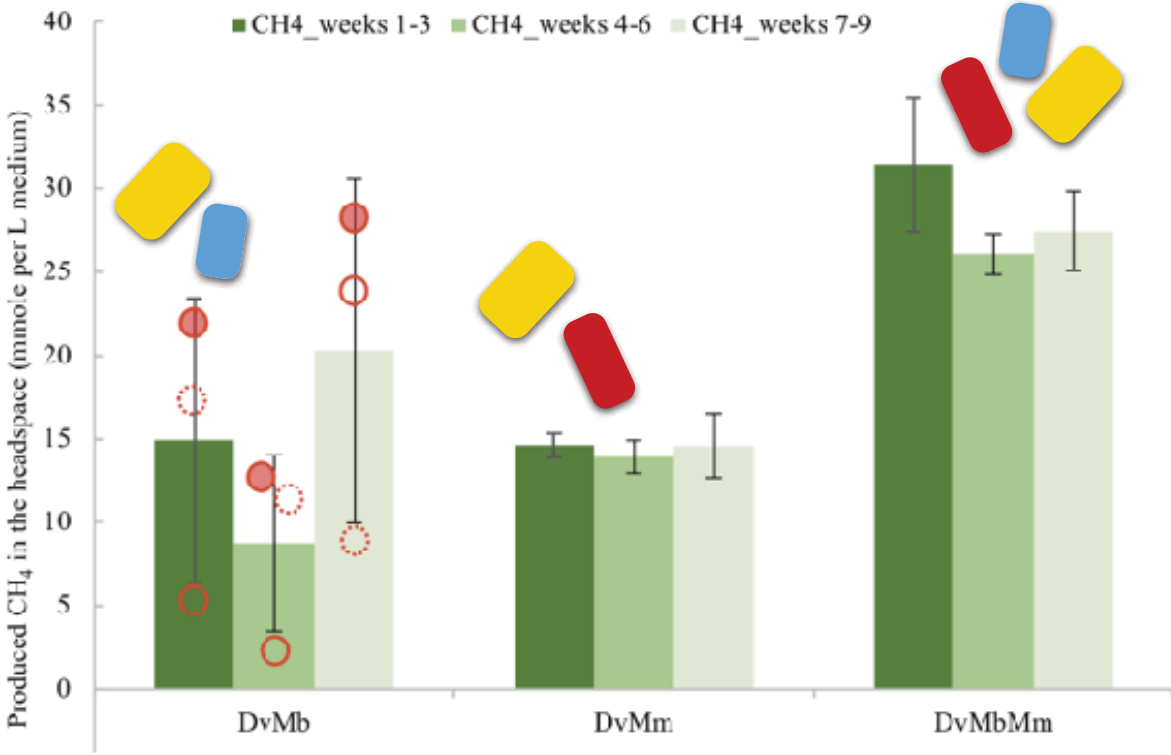

Grosskopf T. et al. ***ISME J.*** (2016).

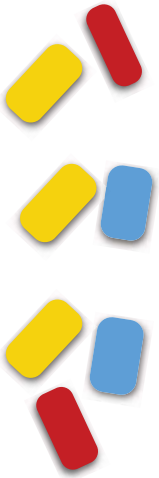
# Extending syntrophic interactions increases methane production from lactate

Jing Chen

## Extended Syntrophies



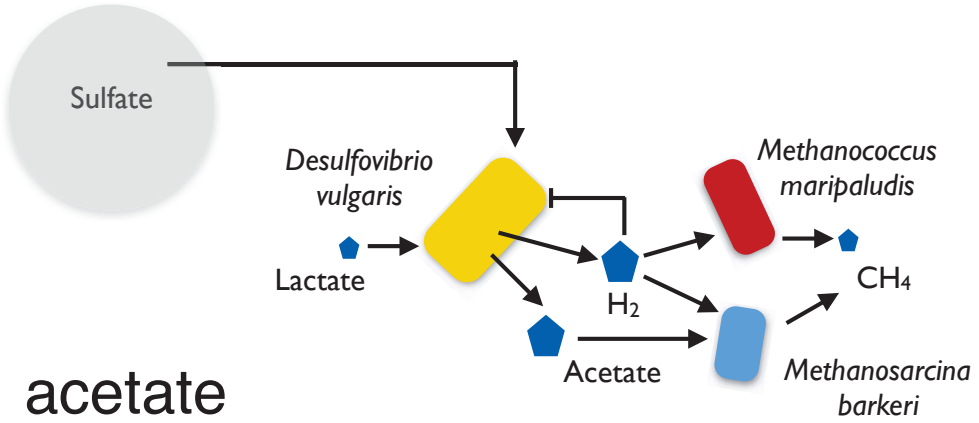
$$\text{Lactate} + H_2O \longrightarrow \text{Acetate} + 0.5CO_2 + H_2O + 0.5CH_4 \qquad \Delta G_0 = -74.19$$

$$\text{Lactate} + H^+ + H_2O \longrightarrow 2CO_2 + 2H_2 + 1CH_4 \qquad \Delta G_0 = -44.69$$

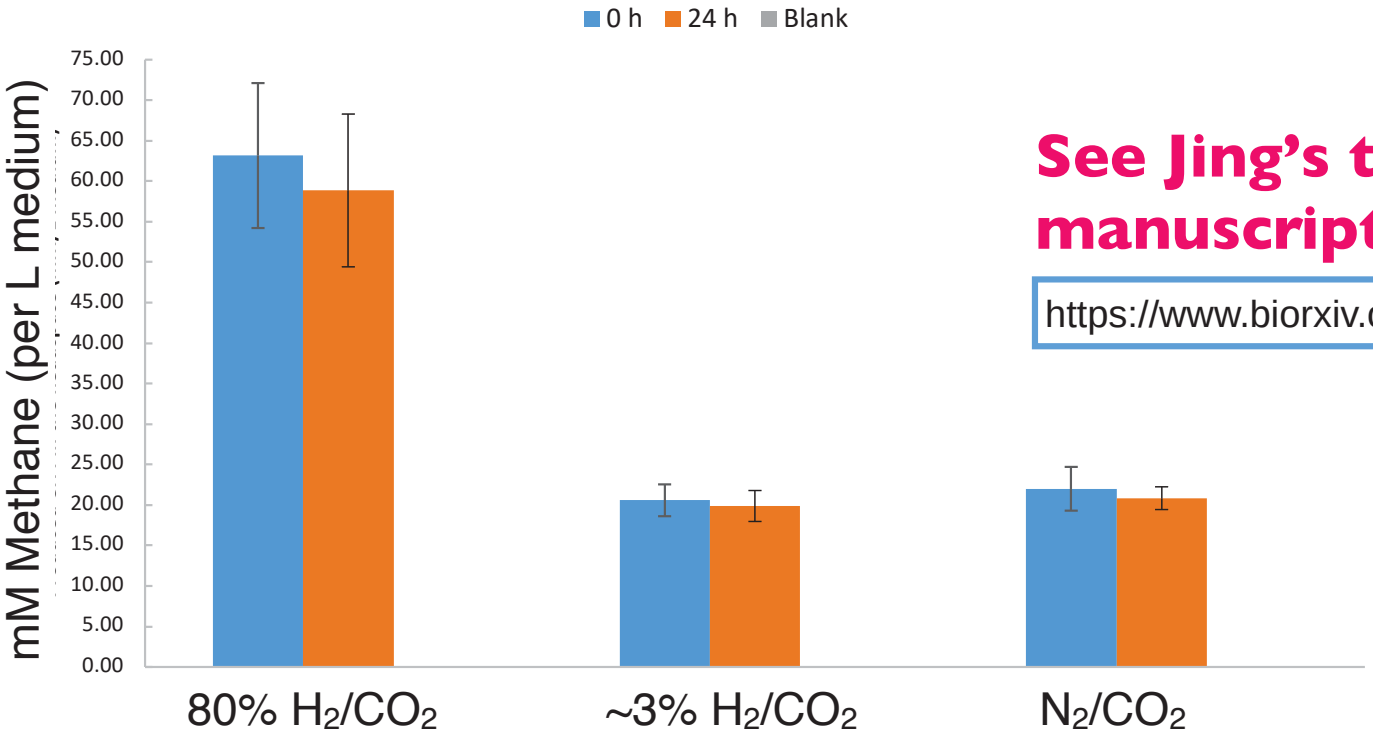
$$\text{Lactate} + H^+ \longrightarrow 1.5CO_2 + 1.5CH_4 \qquad \Delta G_0 = -110.09$$

unpublished results

# Syntrophic stability driven by competition for $H_2$



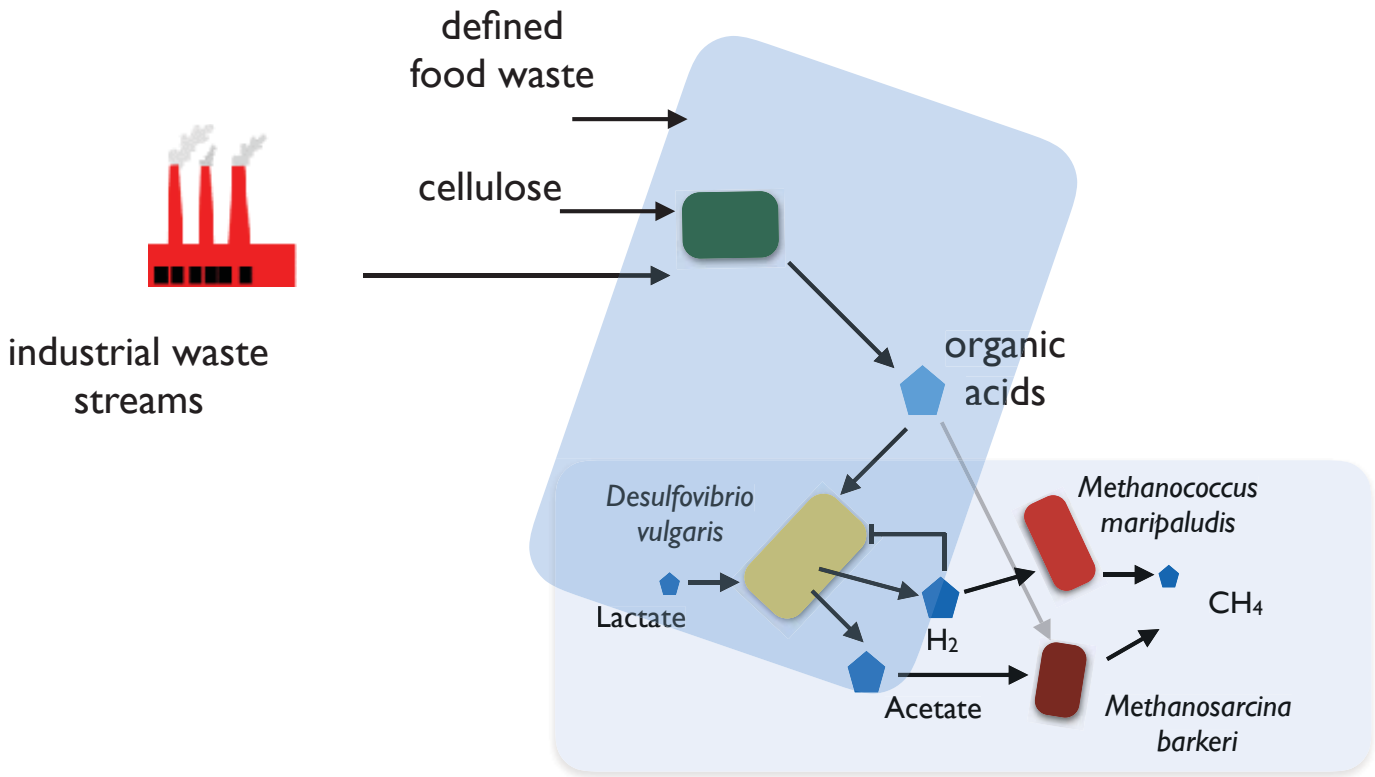

Mb monoculture on 30mM acetate with or without $H_2$




**See Jing's talk and bioRxiv manuscript;**

https://www.biorxiv.org/content/early/2018/04/24/307041

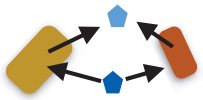
# Towards a minimal system for anaerobic degradation of defined wastes?



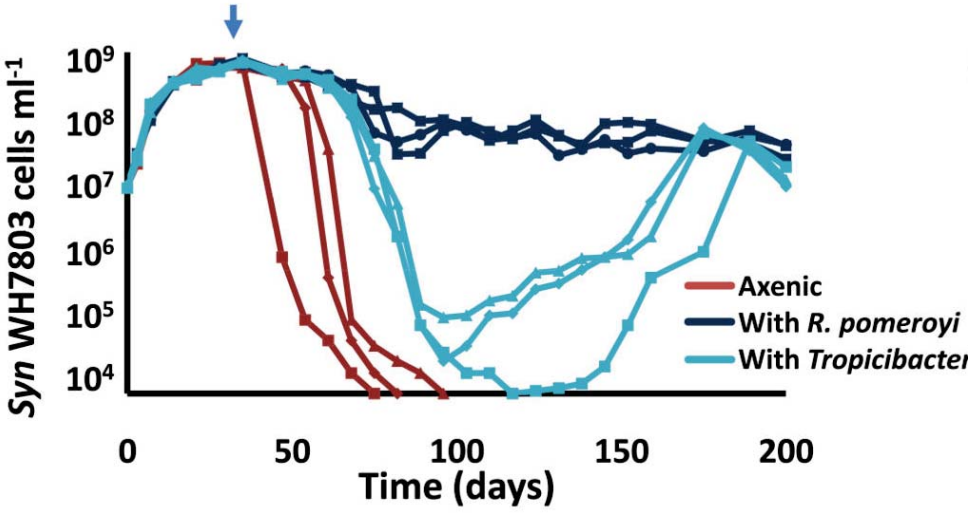
# Metabolite cycling: Crucial in marine systems?

Christian Zerfass

Metabolic Cycles

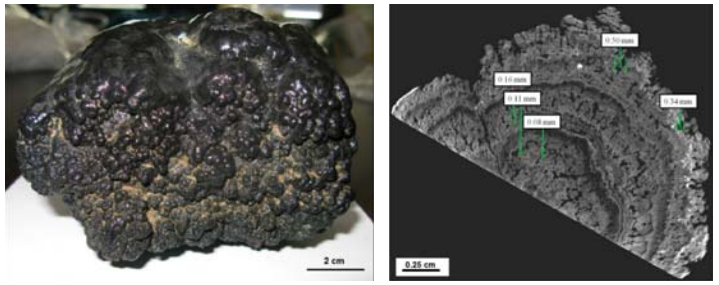


## Helper heterotrophs?




*Nat Microbiol.* ; 2: 17100. doi:10.1038/nmicrobiol.2017.100.

## Mineral provision for phototrophs?



Polymetallic (Mn-rich) nodules of biogenic origin.


Blöthe et al., *Environ. Sci. Technol.* **2015,** 49, 7692.

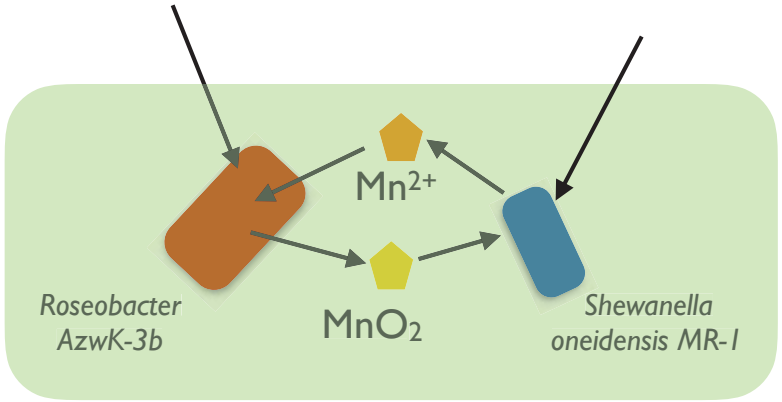
Tebo et al., *Annu.Rev.EarthPlanet.Sci.* **2004**, 32 (1), 287.

Remucal CK, Ginder-V. M, *Environ.Sci.: Processes Impacts* **2014**, 16, 1247.

Li et al., *Current Biology* **2016**, 26, 950.

Rhoads et al., *Environ. Sci. Technol.* **2005**, 39 (12), 4666.

# Establishing a mineral re-cycling motif around Manganese and Manganese-oxide(s)

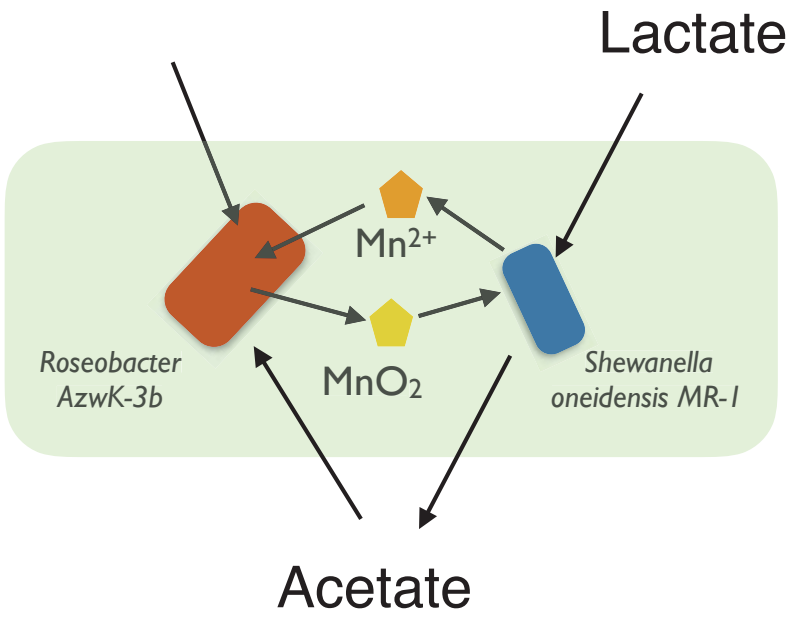


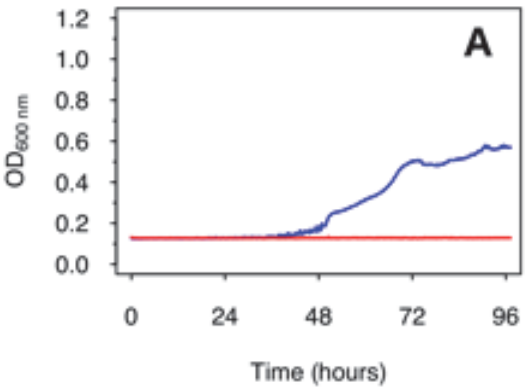

A co-culture media?

| | ***Shewanella oneidensis*** | ***Roseobacter sp.* AzwK-3b** |
|---|---|---|
| **Salinity (NaCl)** | Low – Intermediate | Intermediate – High |
| **$NH_4^+$** | Required | Required |
| **$Ca^{2+}$** | Induces Aggregation | Required |
| **Vitamins** | - | 5 Required |
| | | |
| **Growth on** | | |
| -Lactate | YES | NO |
| -Acetate | NO (negligible) | YES |

# A natural cross-feeding interaction

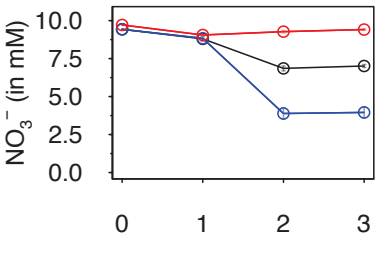
Cross-feeding allowing direct interaction in a bi-culture

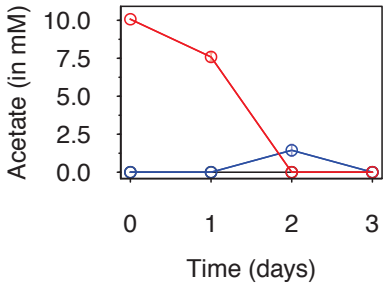


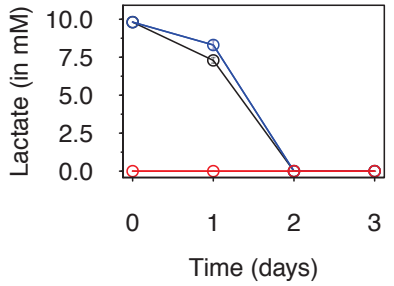



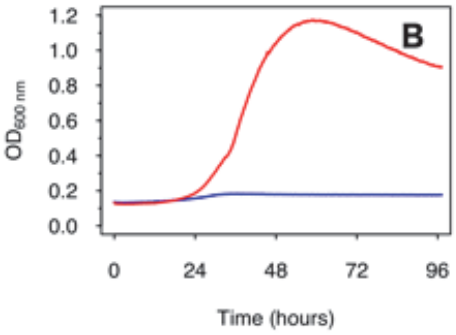

Sw.

Rb.



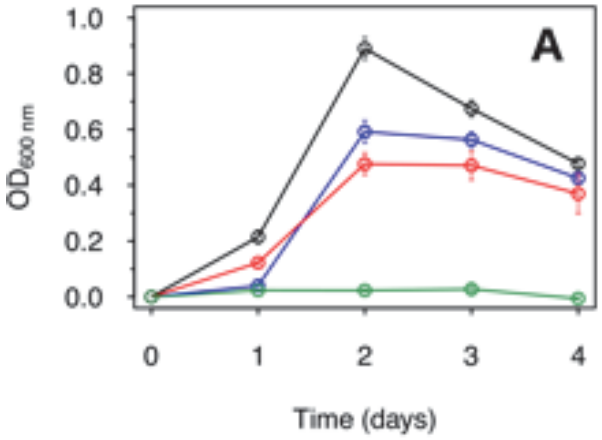




Rb.

Sw.

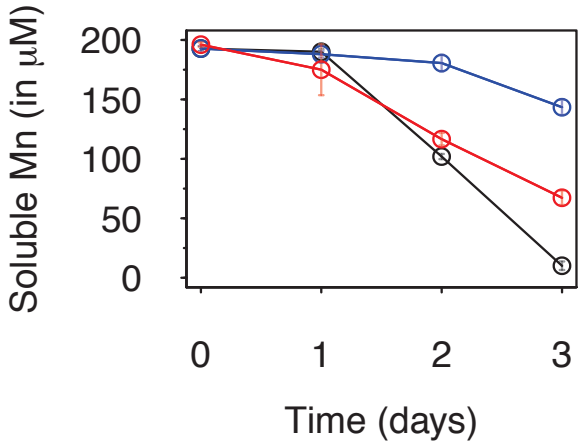



Individual growth in bi-culture further confirmed by selective plating

unpublished results

# Mn dynamics in the bi-culture

## Mn-oxidation dynamics:

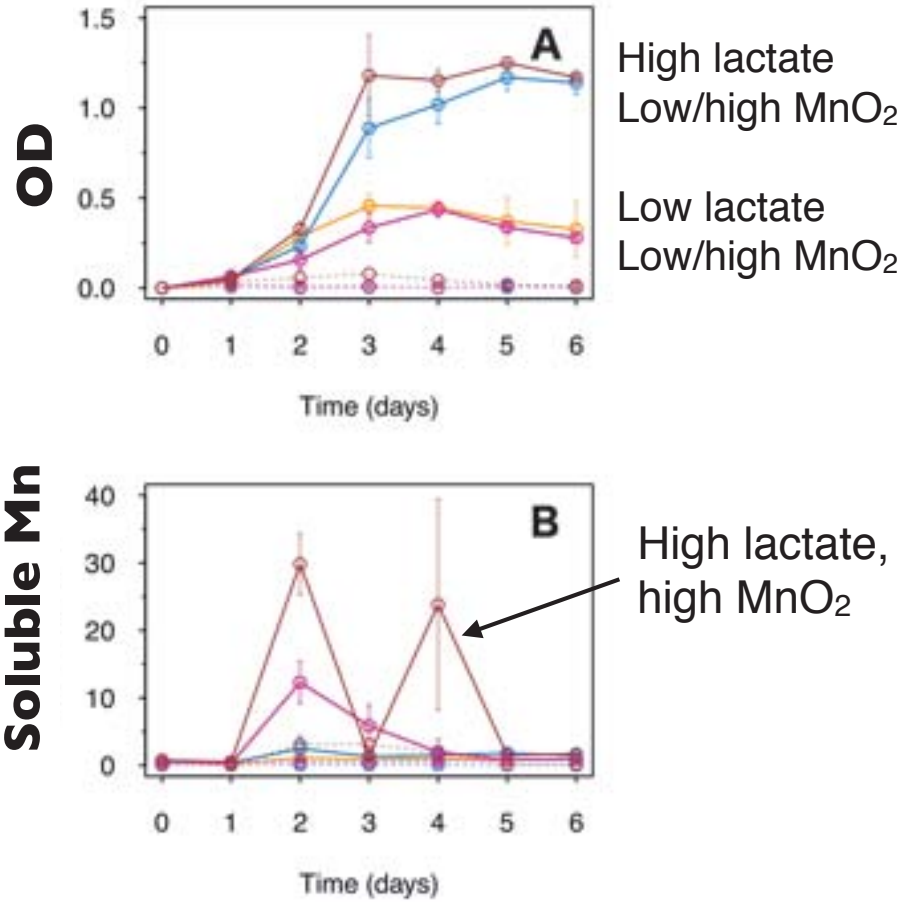



Lac - Biculture

Lac - *S. oneidensis*

Ac - AzwK-3b

*Sw* - mediated $MnO_2$ reduction through enhancing local anaerobic conditions




High lactate
Low/high $MnO_2$

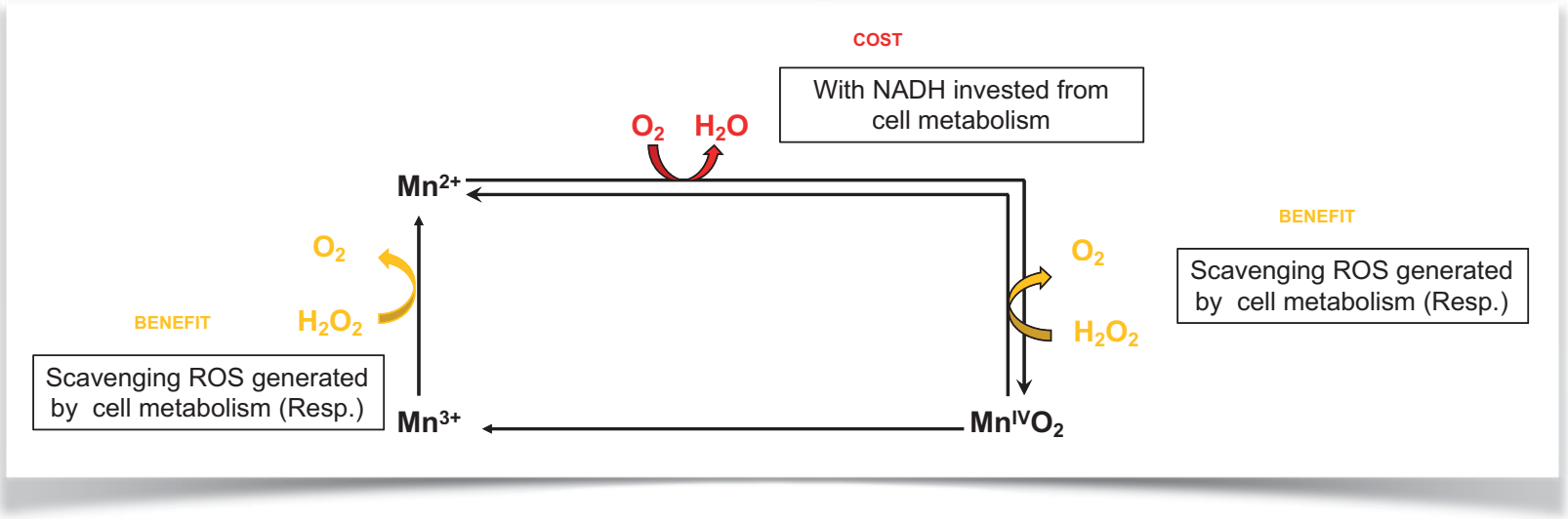
Low lactate
Low/high $MnO_2$

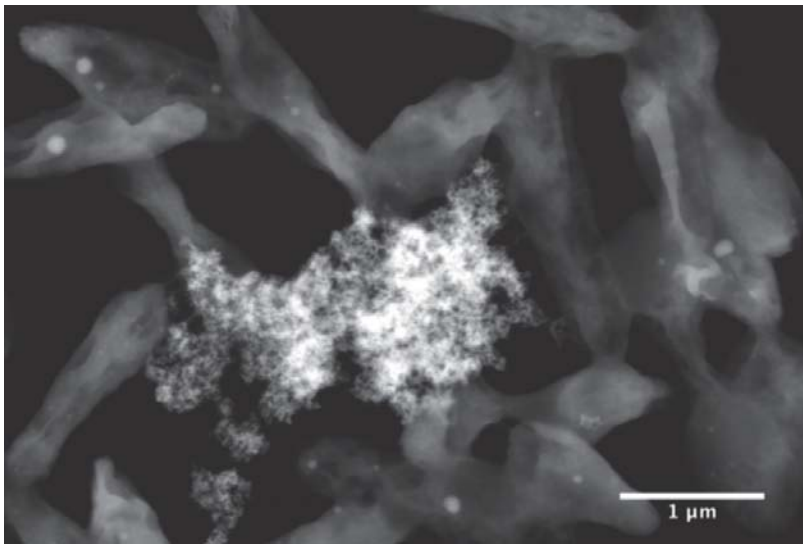
High lactate,
high $MnO_2$

unpublished results

# What about Mn oxidation? Why does it occur?

Mn oxidation requires excreted exoenzymes and NADH investment



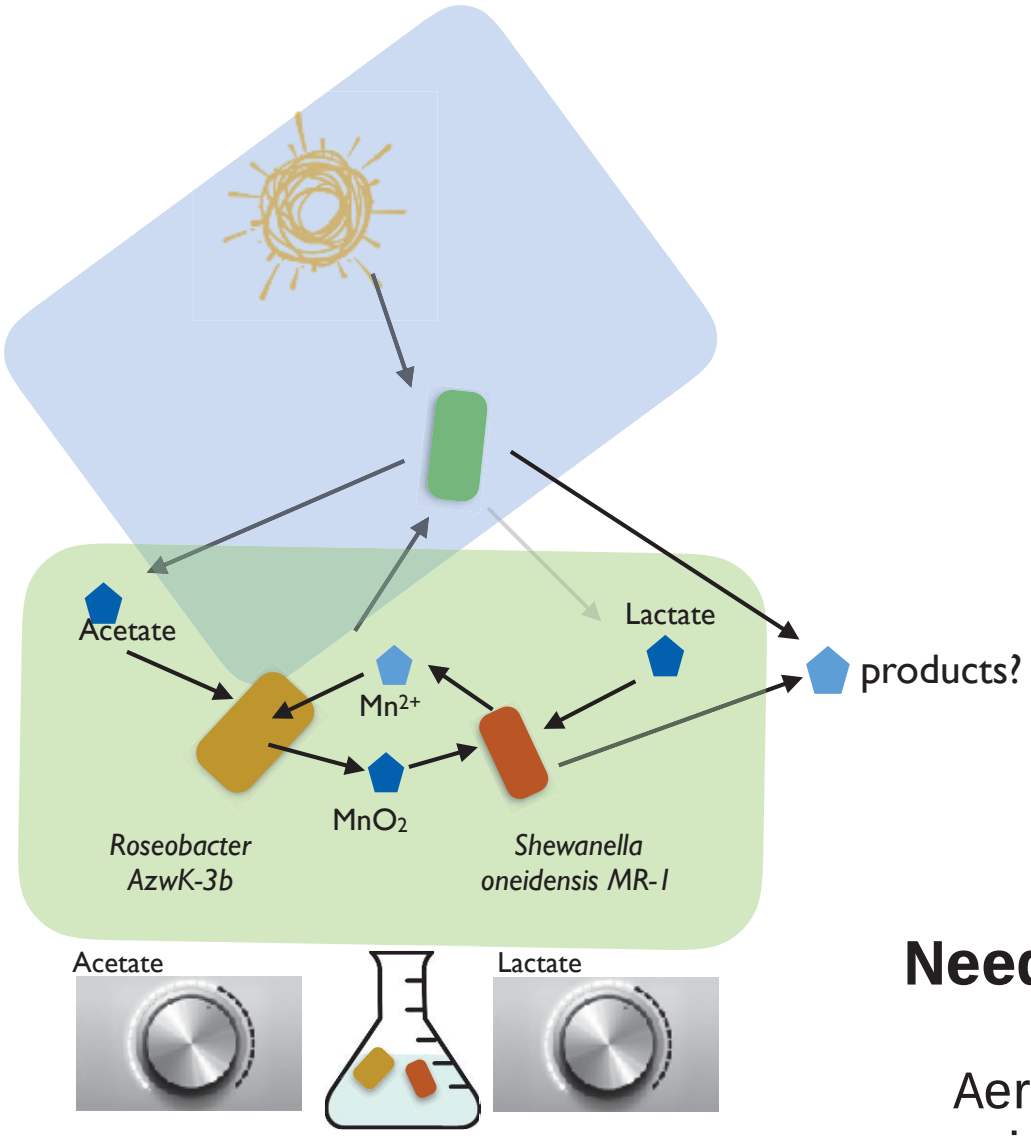

Mn oxidation as cooperative trait?

Impact on radical chemistry?

**See Christian's poster/talk and bioRxiv manuscript;**

https://www.biorxiv.org/content/early/2018/04/04/294975

# Towards a closed microbial ecosystem?







Self-sustaining production from sunlight??

**Need for a spatially engineered system?**

Aerobic, liquid phase for acetate and Mn oxidation

Anaerobic phase devoid of terminal electron acceptors, so that MnOx reduction is favoured

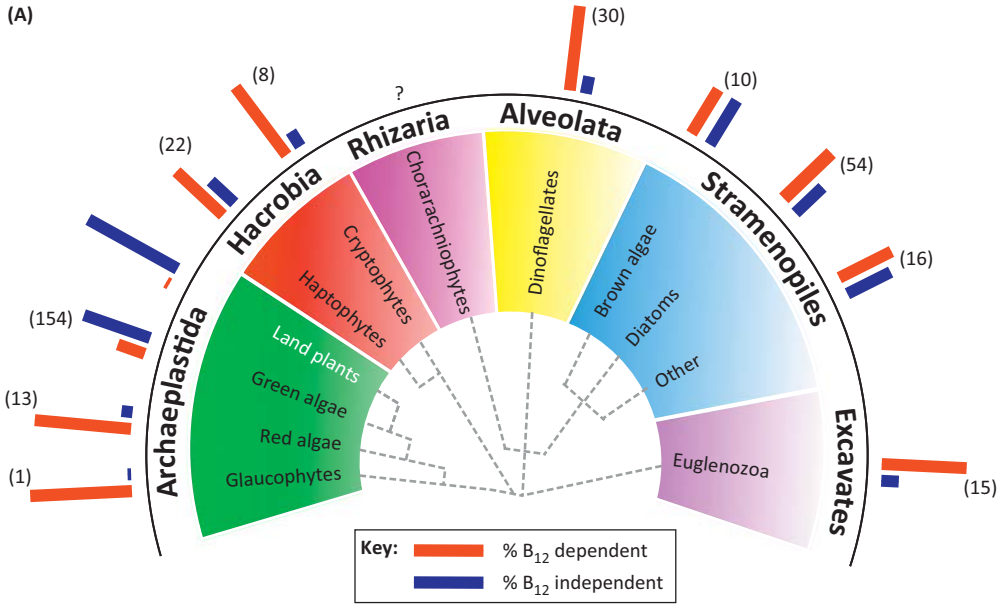
# Auxotrophy: Common in algae (and fungi?)


**Xue Jiang**

Functional losses due to stable environmental provisions??

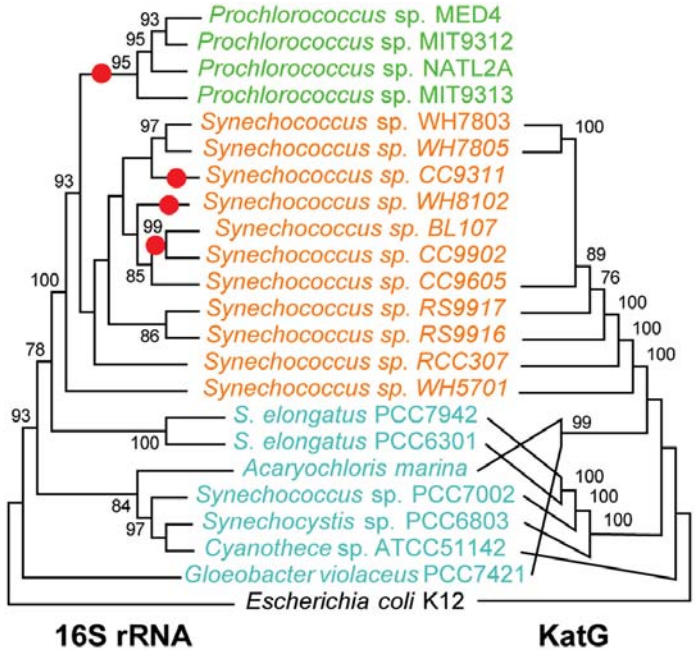
Morris JJ et al. ***mBio*** (2012)

## B12 dependency in algae




Heliwell KE et al. ***Trends in Genetics*** (2013)

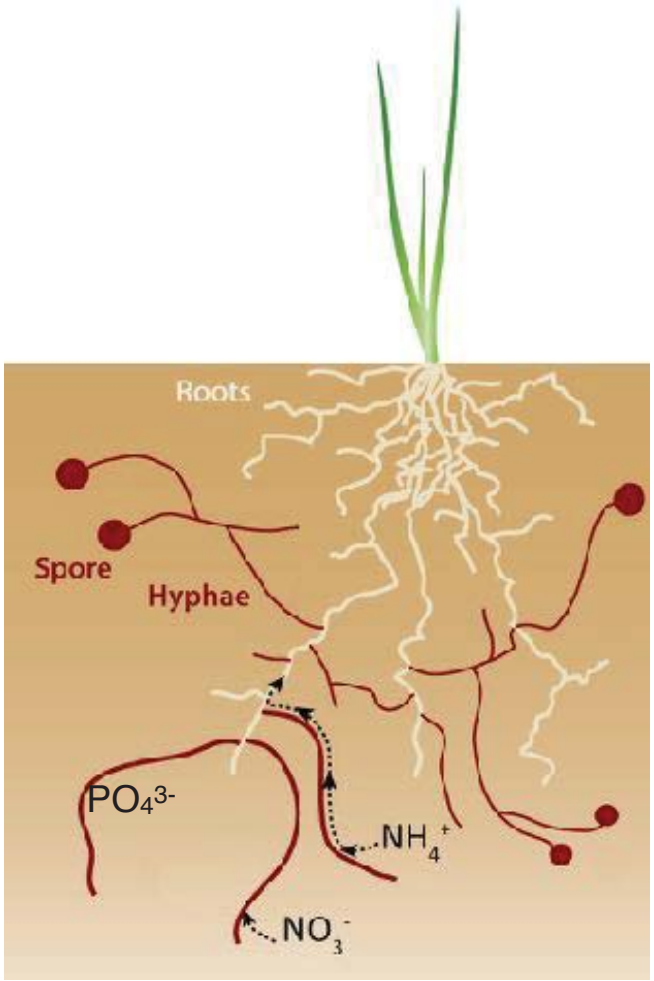
## Catalase loss in cyanobacteria




Morris JJ et al. ***mBio*** (2012)

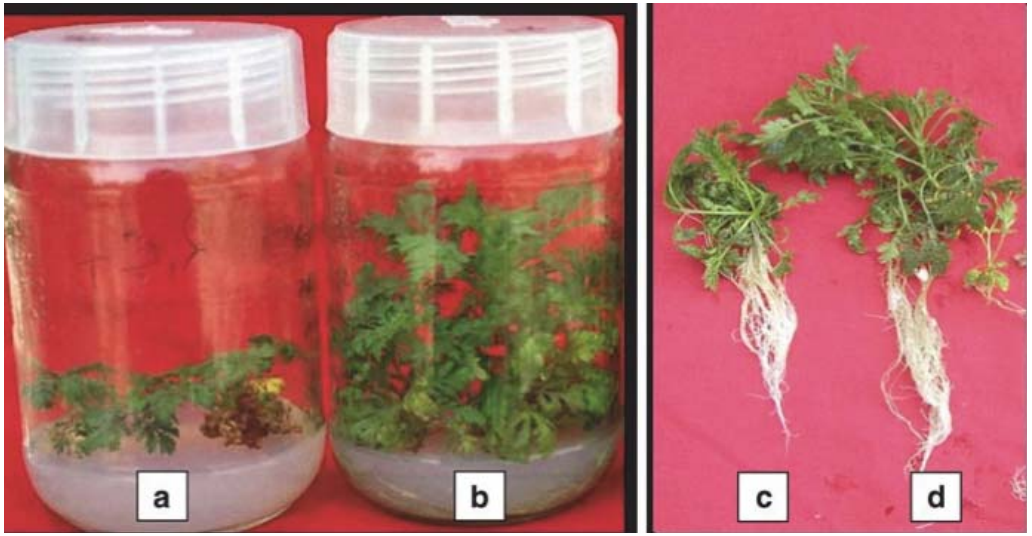
# A endophytic fungi that can also live on its own(?)

*Serendipita indica* (formerly *Piriformaspira indica*)



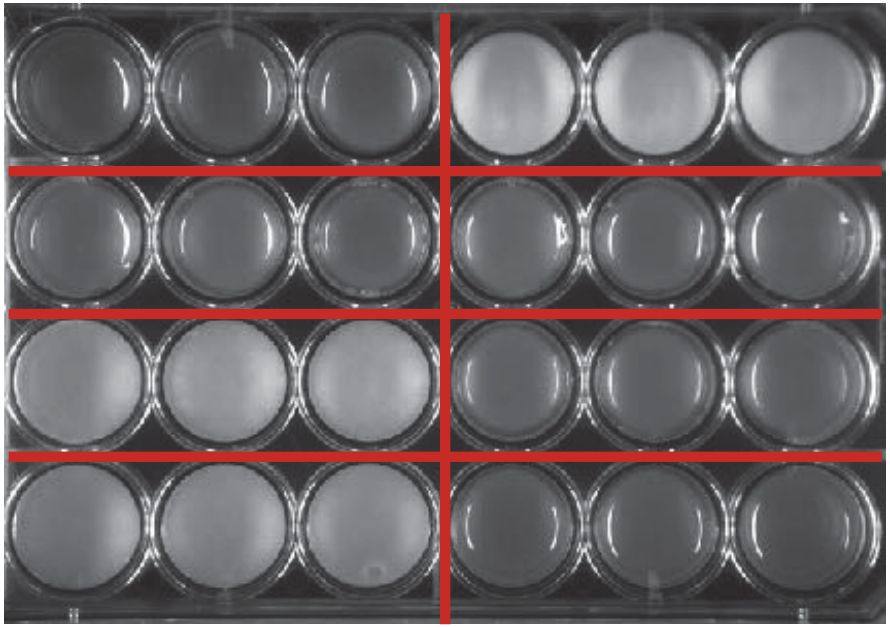

Sanders, I. R., & Croll, D. (2010).

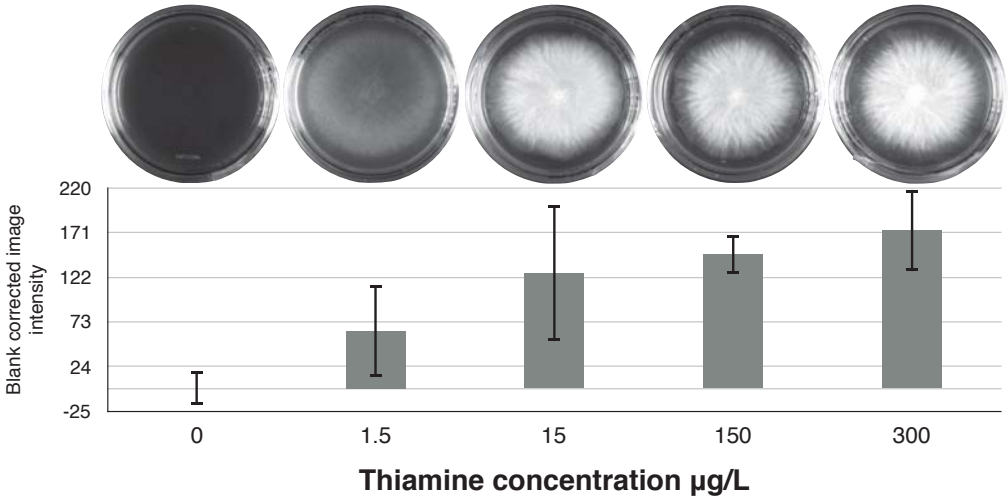
Promote plant growth
Associate wild range of plants
Can grow without host

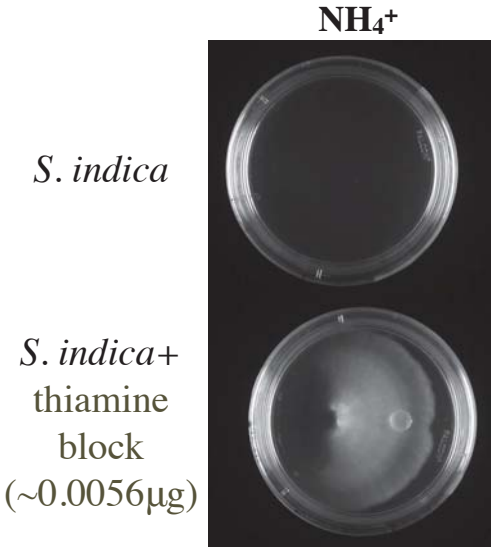



Kumari (2005)

# *S. indica* is auxotrophic for thiamine…



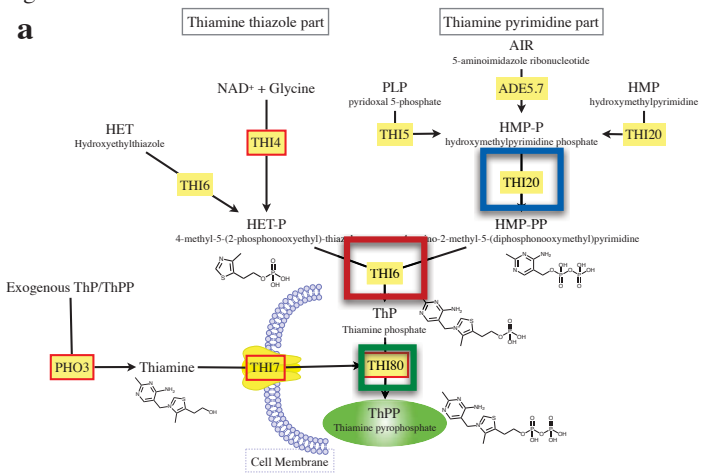



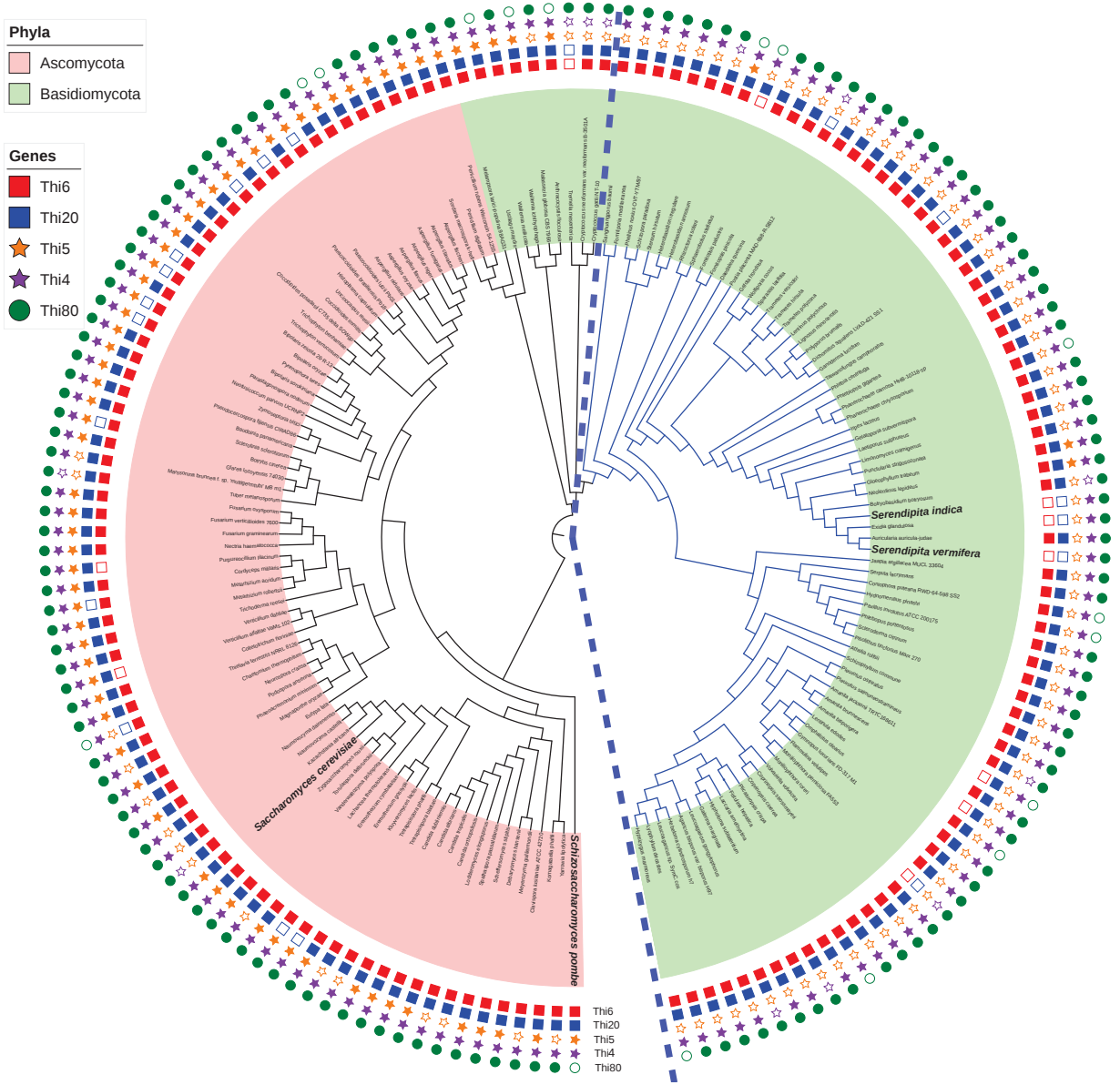



# Thiamine auxotrophy in fungi…

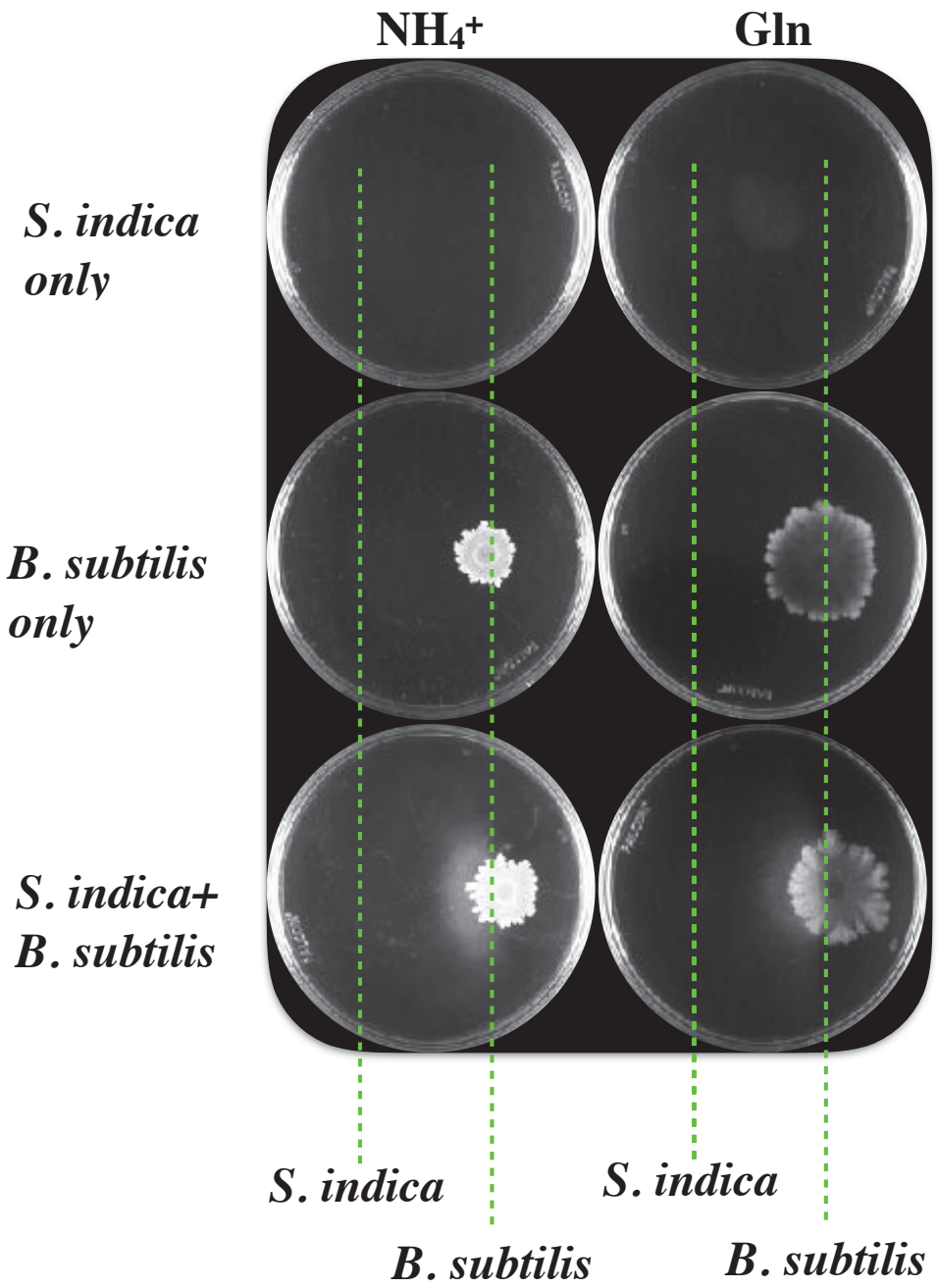
…seems confined to Basidomycota

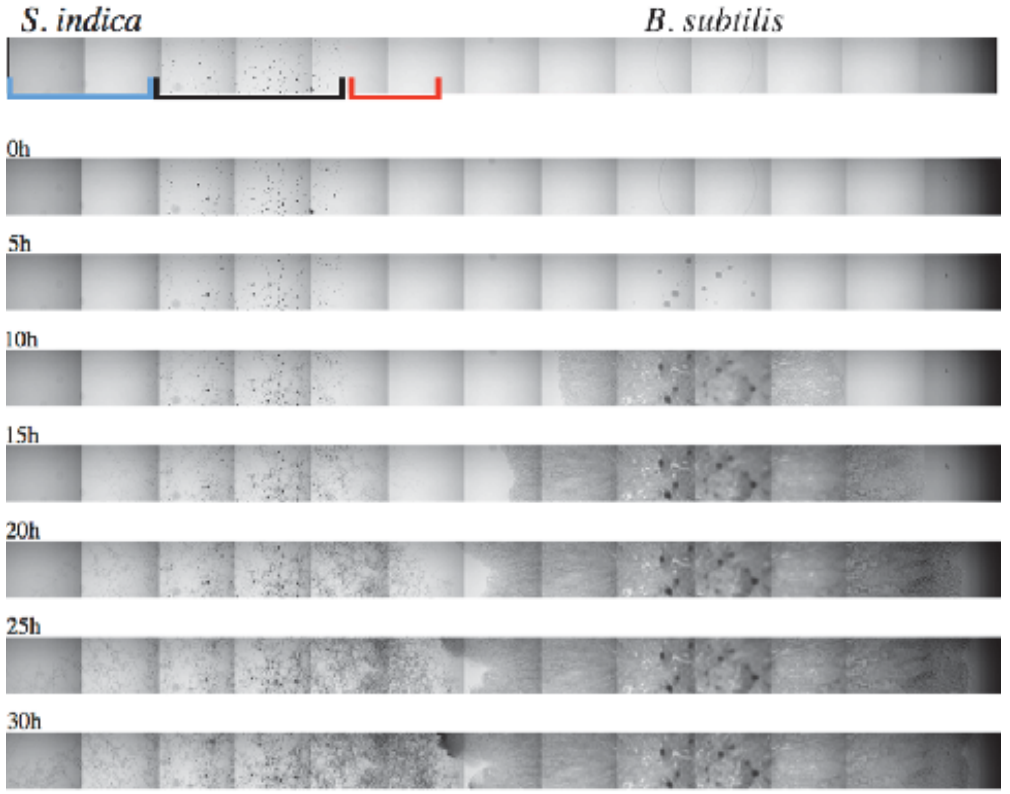
Figure 2a

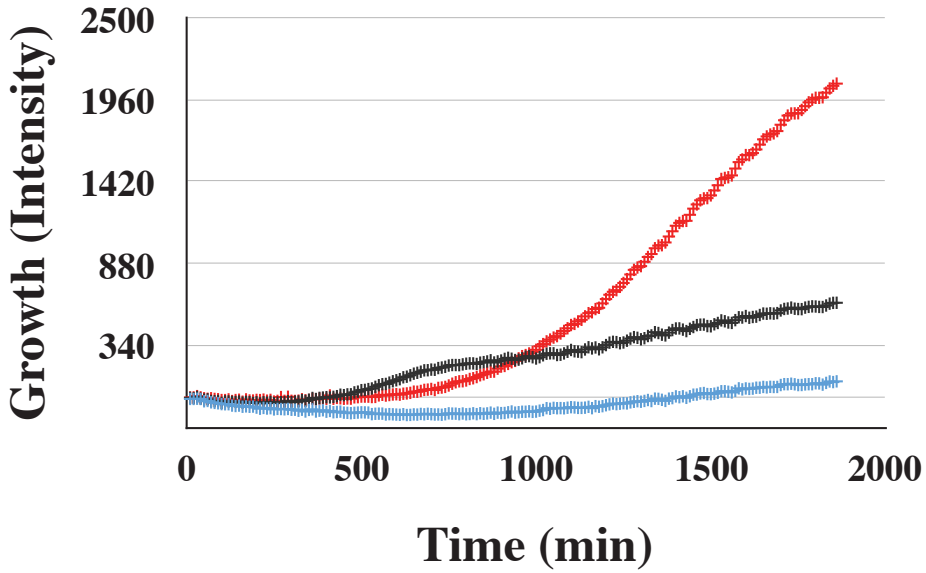


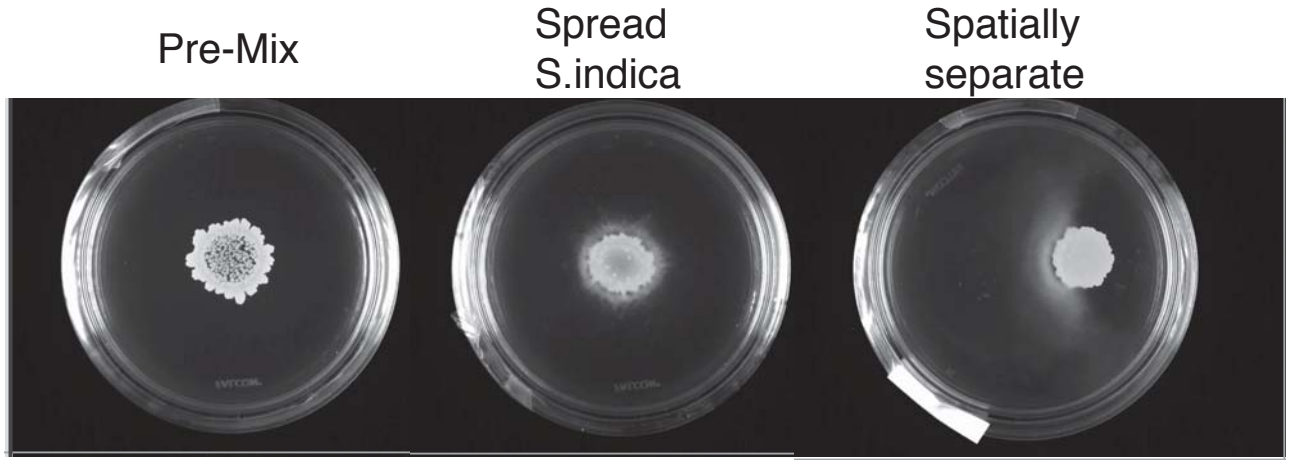
# …can be satisfied by soil bacteria *Bacillus subtilis*

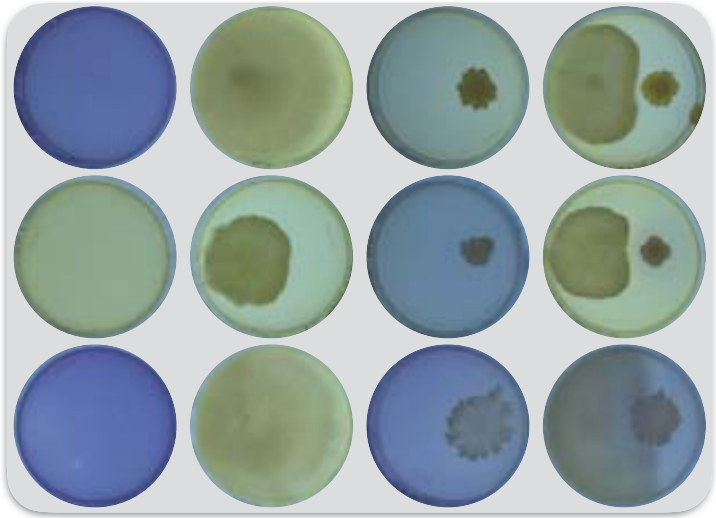


# The impact of spatial / temporal separation on establishing an auxotrophic interaction



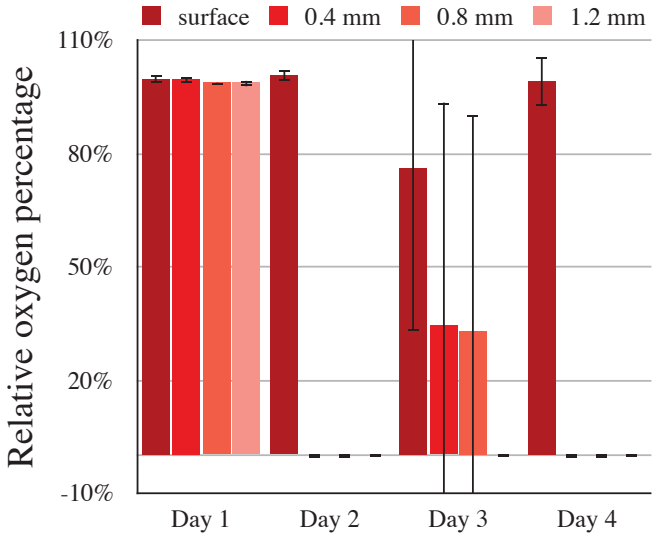



pH5

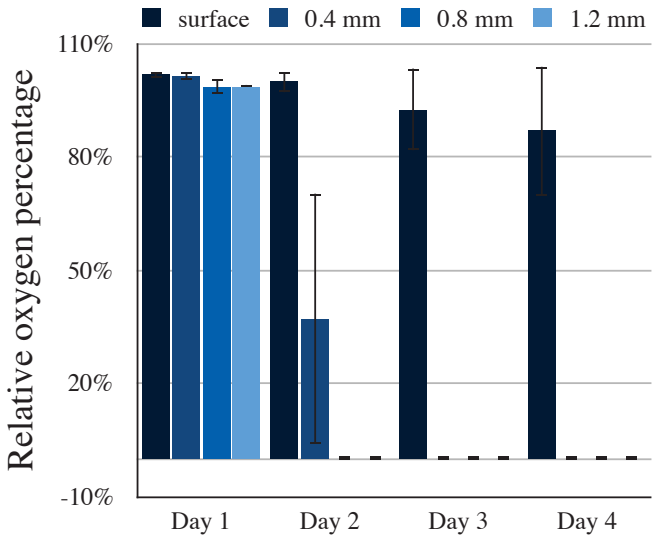
pH7

*S. indica* *B. subtilis*

***B. subtilis* colony**

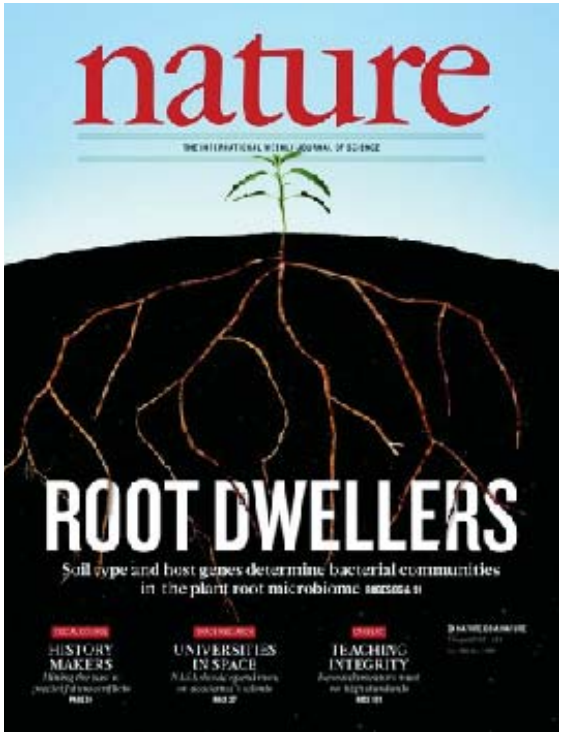



**Mixed culture inoculation point**



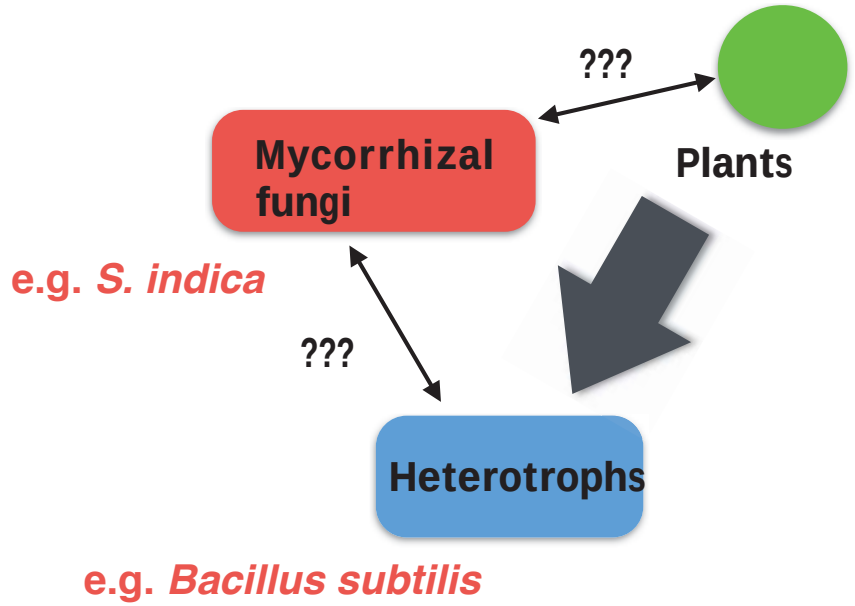

Jiang X et. al. ***ISME Journal*** (2018).

# Towards a minimal plant supporting system?




Rhizobial ecosystem

??

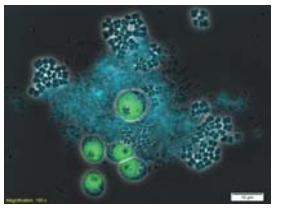



Synthetic cross-kingdom community

Possible applications in hydroponic plant growth systems, seed coatings, etc.

? Insights and Applications ?

BOTTOM-UP

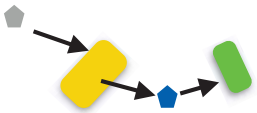


*Engineer synthetic communities to learn about biochemical basis of communities*

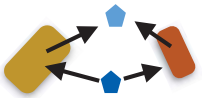
**design principles ??**

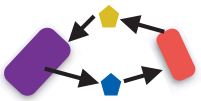
**modules**

Cross-Feeding



Metabolic Cycles

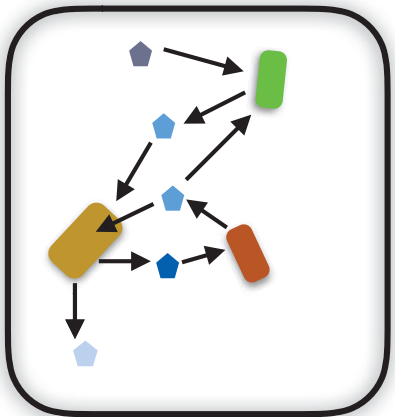




Complementary Auxotrophy

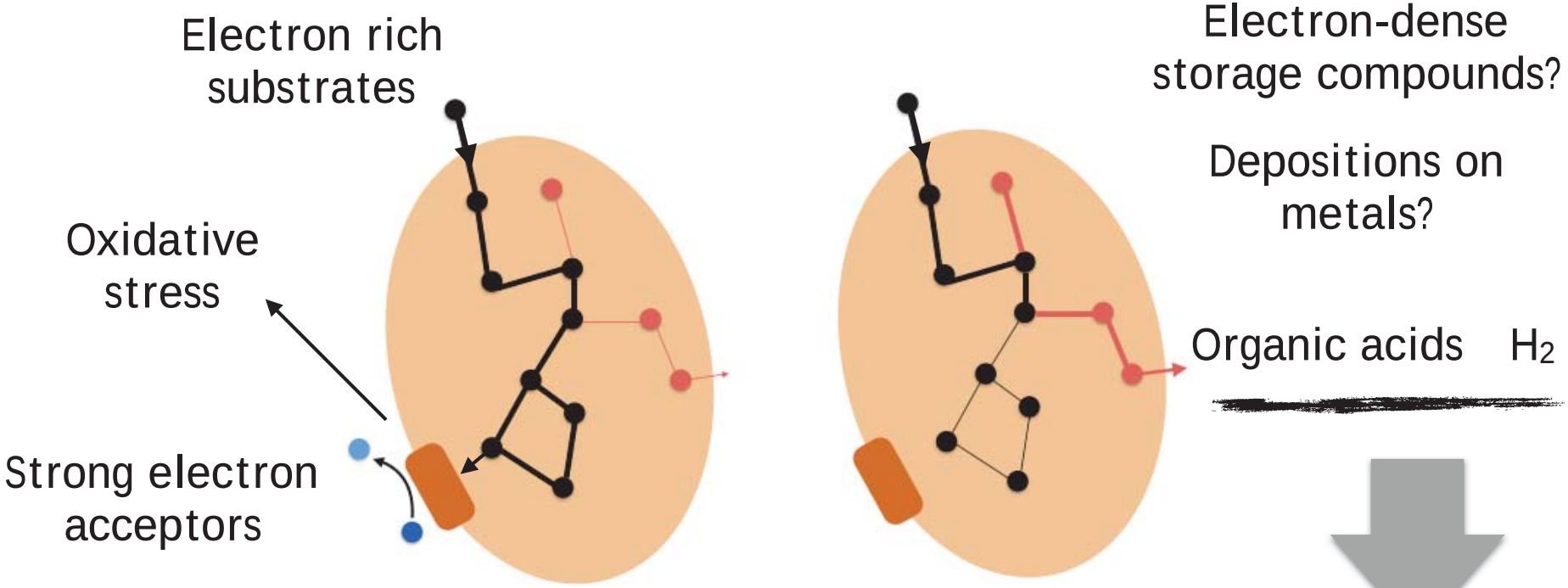
Syntrophy

**functional systems**



Grosskopf & Soyer, ***Curr. Op. Biotech*** (2014)

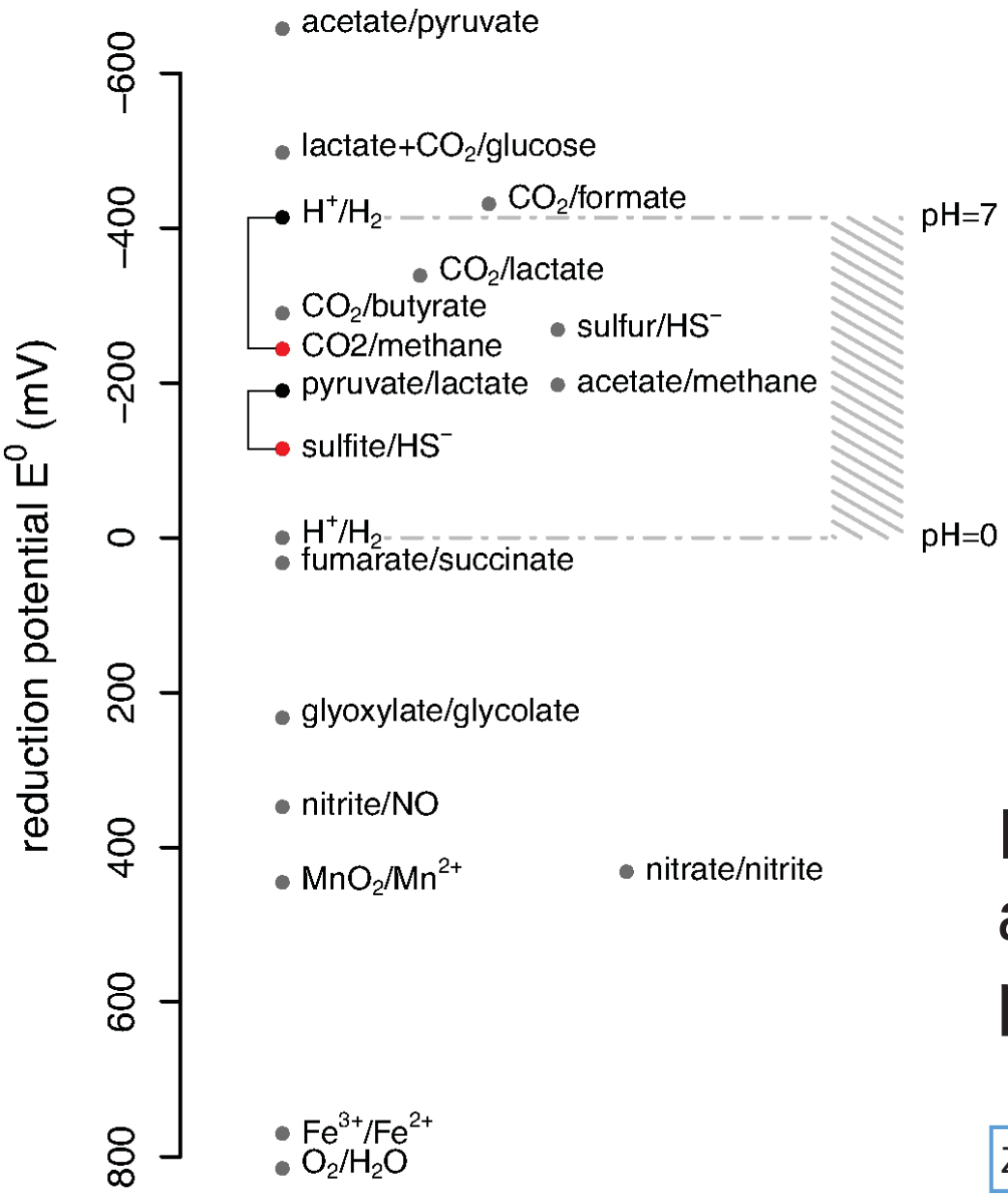
# Metabolism is about electron flow

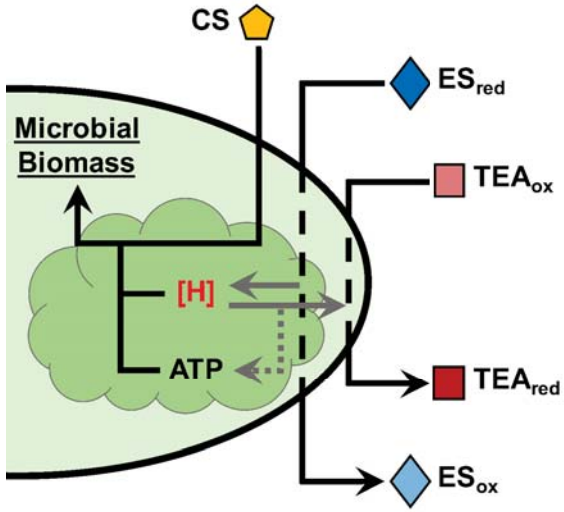



**Cellular trade-offs**

**Biophysical / Environmental drivers**

# Redox ladder and thermodynamic inhibition




Is 'electronic' control of cell and community metabolism possible?

Zerfass C, et al. ***Curr Op Biotech*** 2017

# BIO-ELECTRICAL ENGINEERING (BEE)
# 30/31 May 2018, Warwick

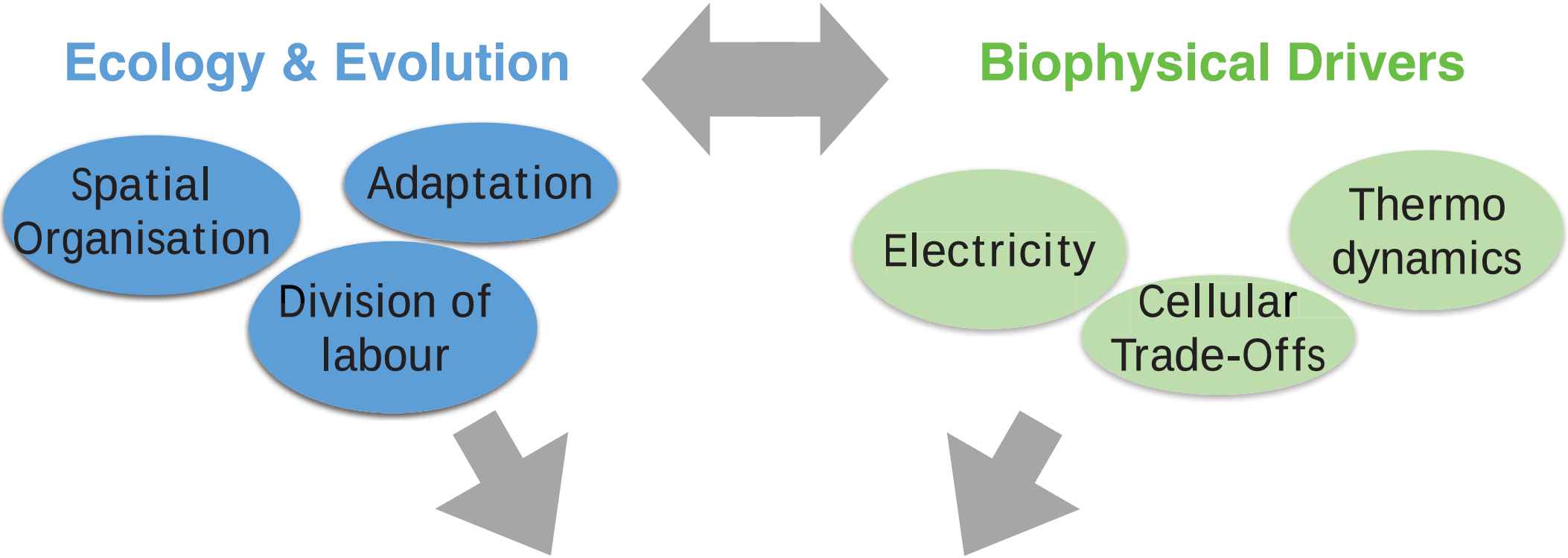
**Electricity and plants**

**Electrical interfaces to cells**

**Microbial electricity and electro-fermentation**

**Biological organisations through electrical fields**

**Bio-electrochemistry and electrical measurements**




# Towards a Theory of Metabolism For Cellular and Community Engineering

# THANK YOU

OSS LAB

http://osslab.lifesci.warwick.ac.uk

Kalesh Sasidharan
**Christian Zerfass**
**Jing Chen**
**Xue Jiang**
Clare Hayes
**Andrea Martinez-Vernon**
Sean Aller

**Open PhD and PDRA positions**

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BBSRC bioscience for the future

EPSRC Pioneering research and skills

WARWICK THE UNIVERSITY OF WARWICK

[dstl]

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Dominique Schneider (University of Grenoble)

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EPSRC & BBSRC Centre for Doctoral Training in Synthetic Biology

SynBio CDT

# THANK YOU

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