MELiSSA
MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

UMONS
Université de Mons

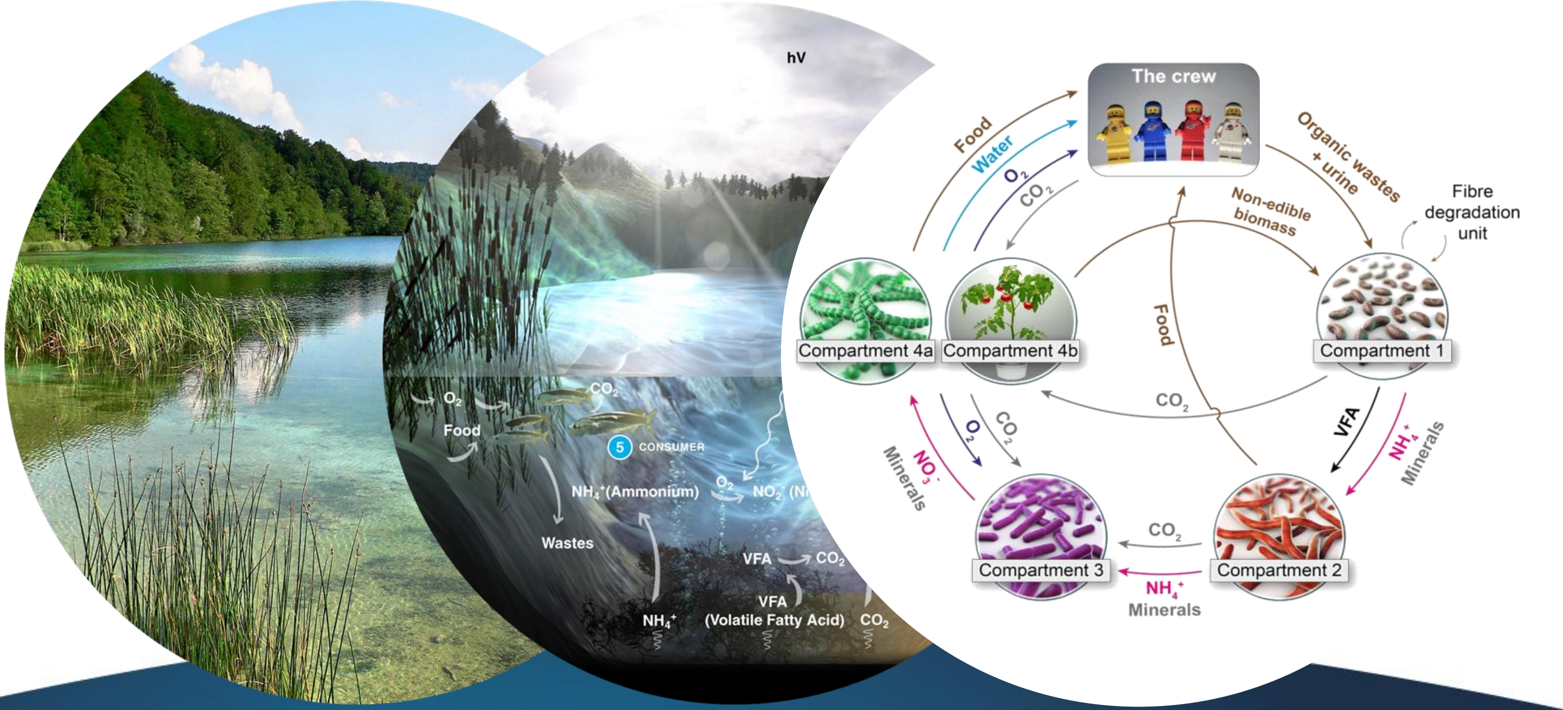
CREATING
A CIRCULAR
FUTURE

From a metabolic stoichiometry to a full MELiSSA metabolome

Baptiste Leroy
PROTMIC
UMONS

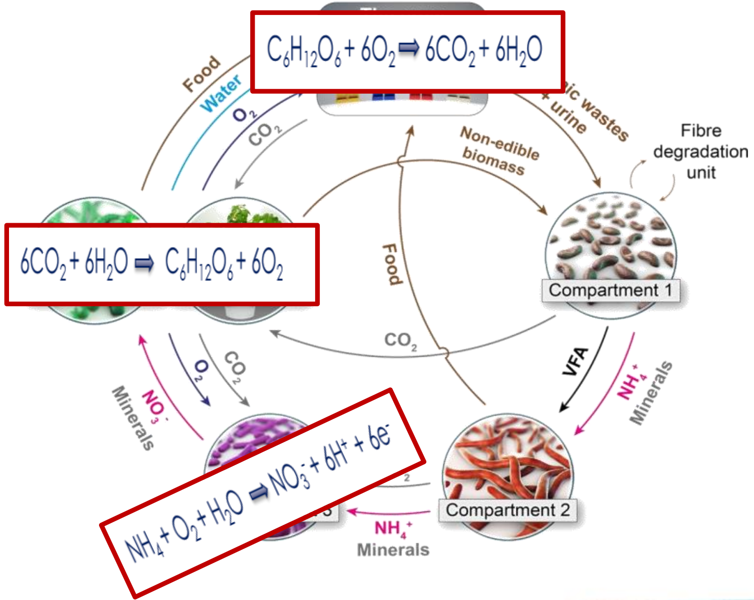
MELiSSA complexity is elastic

- Complexity reduction



MELiSSA complexity is elastic

- Stoechiometry based modelling reduce the complexity



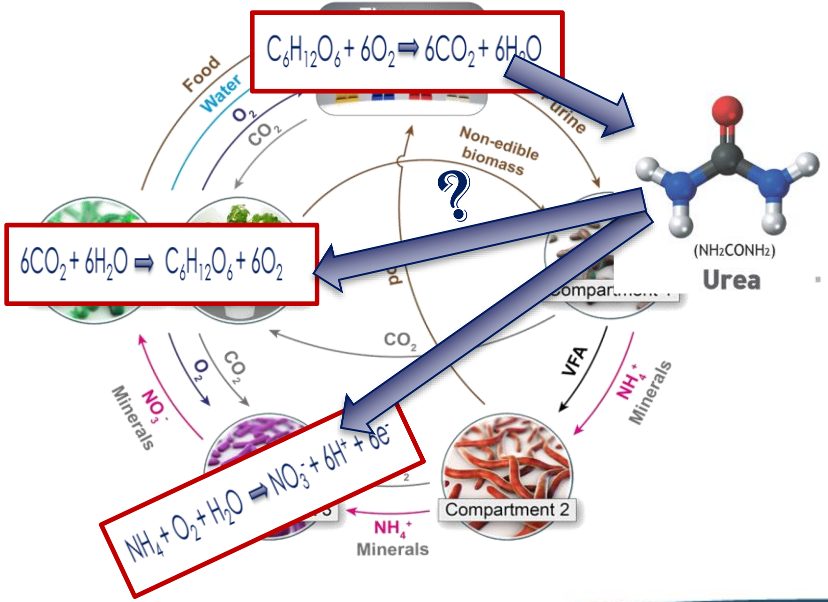
$C_6H_{12}O_6 + 6O_2 \Rightarrow 6CO_2 + 6H_2O$

$6CO_2 + 6H_2O \Rightarrow C_6H_{12}O_6 + 6O_2$

$NH_4 + O_2 + H_2O \Rightarrow NO_3^- + 6H^+ + 6e^-$

MELiSSA complexity is elastic

- Improving flexibility… increase complexity



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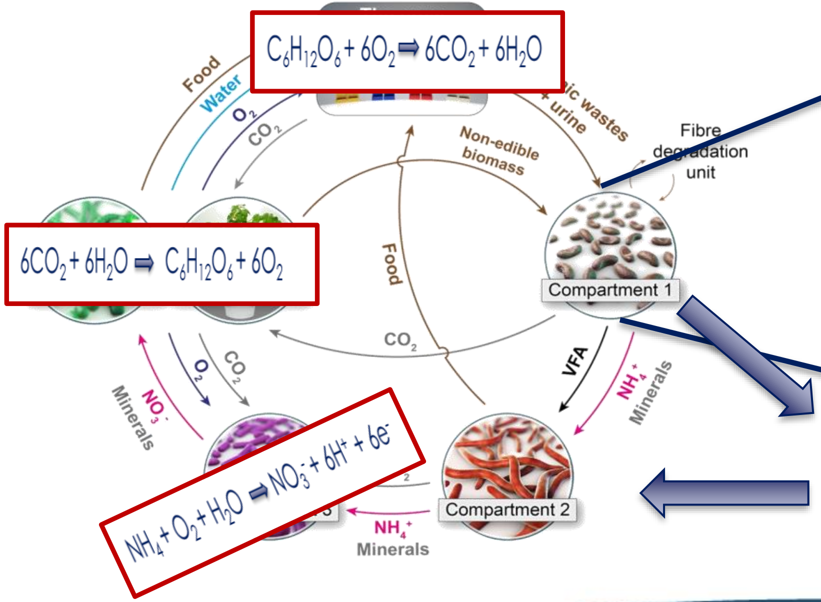
- Ureolytic heterotroph required
- Effect of salinity on nitrifier
- Effect of organic carbon on cyanobacteria
- Effect of salinity on cyanobacteria
- Effect of fluctuating nitrogen sources on cyanobacteria

MELiSSA complexity is elastic

- Temporal evolution increase complexity



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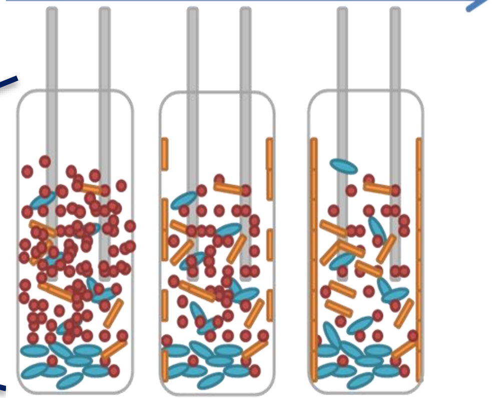
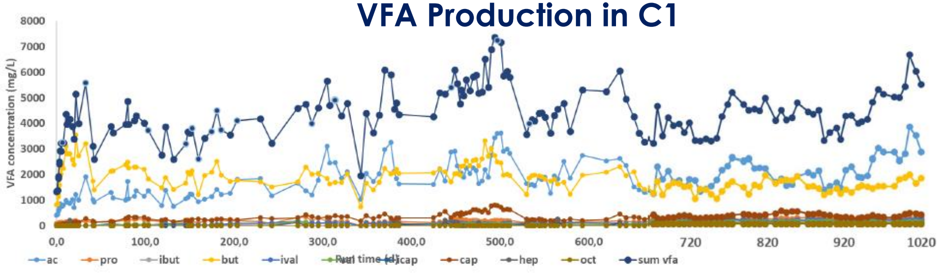
Temporal heterogeneity



VFA Production in C1



MELiSSA complexity is elastic

- Opening the (biological) black box is required

- Do we really care?
- Couldn't we just predict process outcome based on previous experiment?

Let's have a short video…

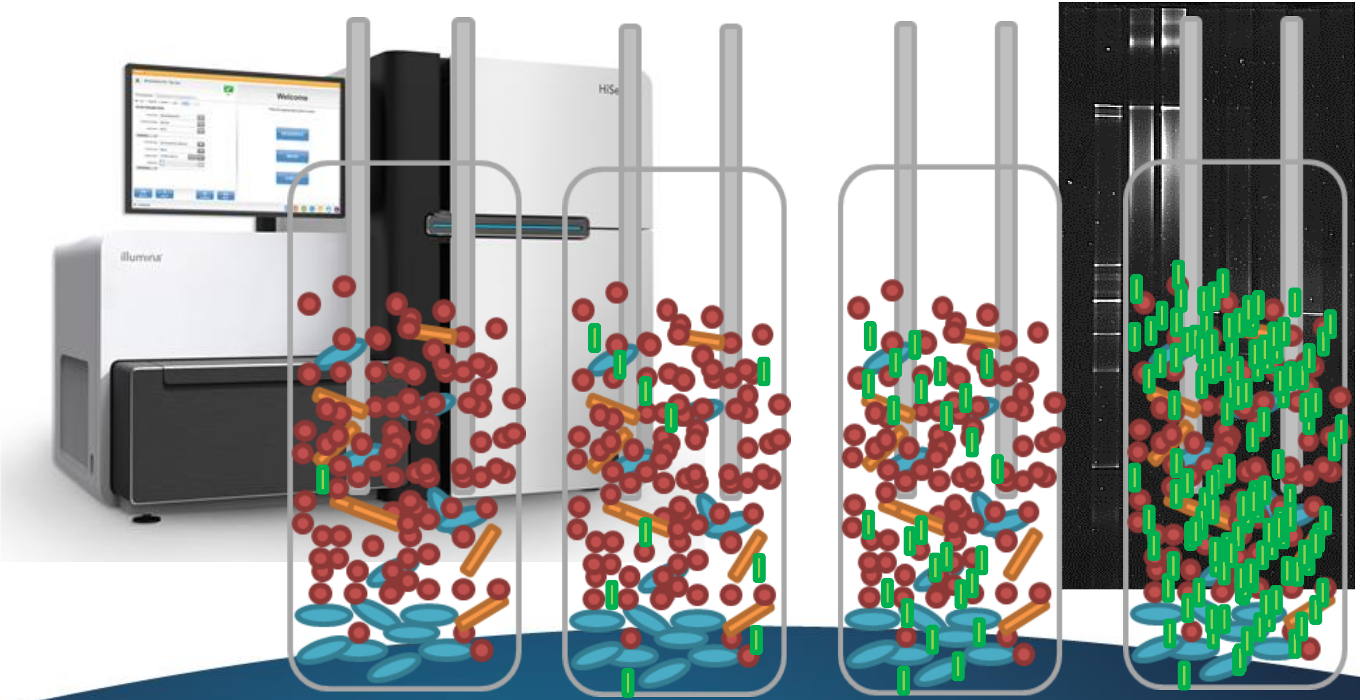

sina

MELiSSA Omics

- Omics are a major part of our molecular toolbox for dissecting MELiSSA metabolome
- (meta)genomic, transcriptomic, proteomic, metabolomics levels

MELiSSA Omics

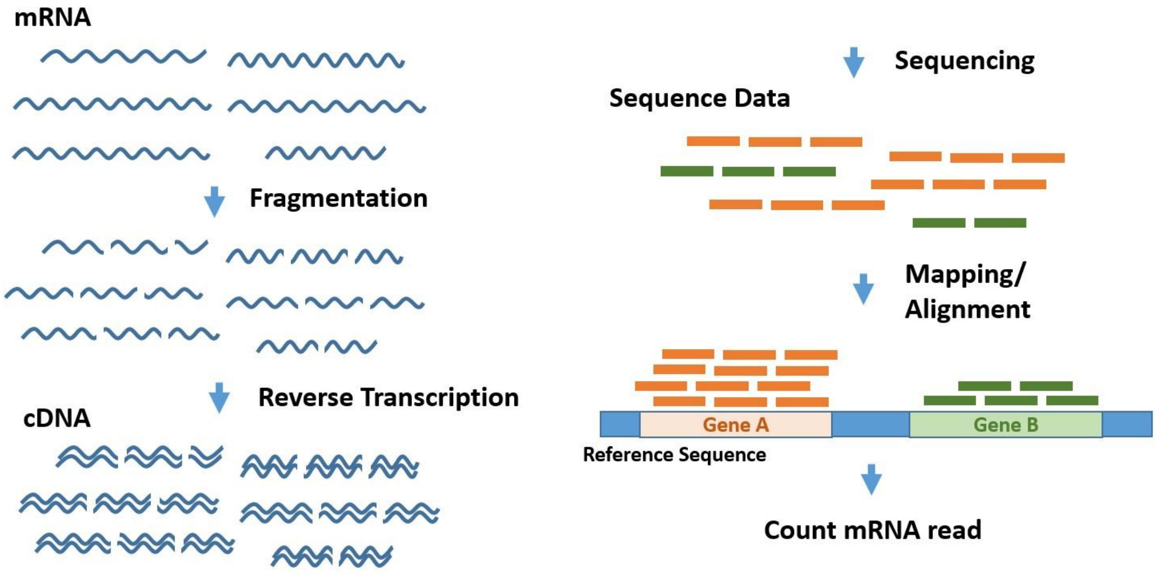
- (meta)Genomic analysis for consortium characterization

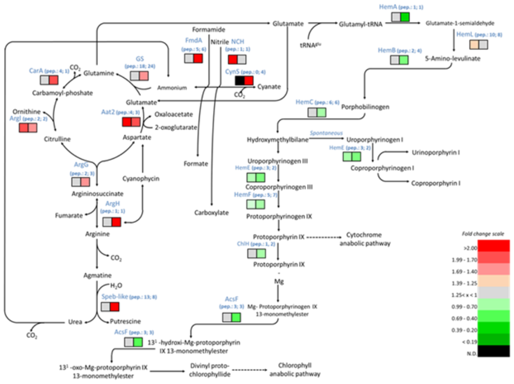


MELiSSA Omics

- Transcriptomic analysis for metabolic pathway identification

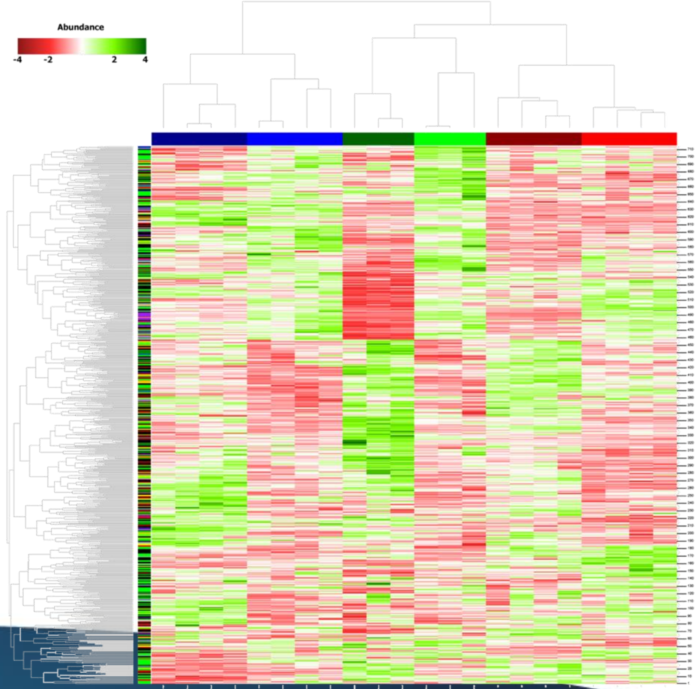
RNA-Seq Work flow





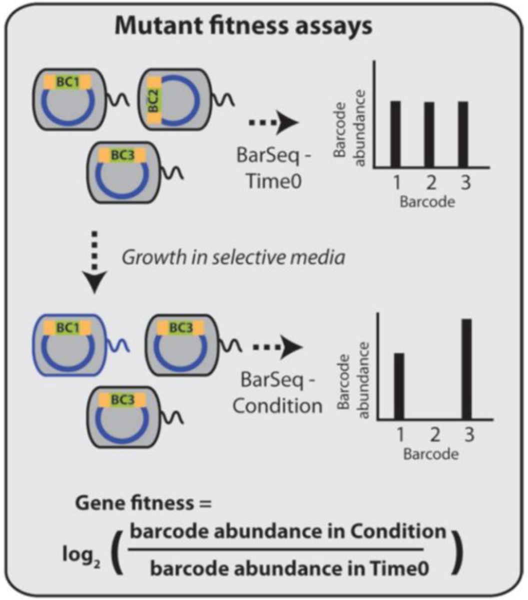
MELiSSA Omics

- Proteomic analysis for metabolic pathway identification



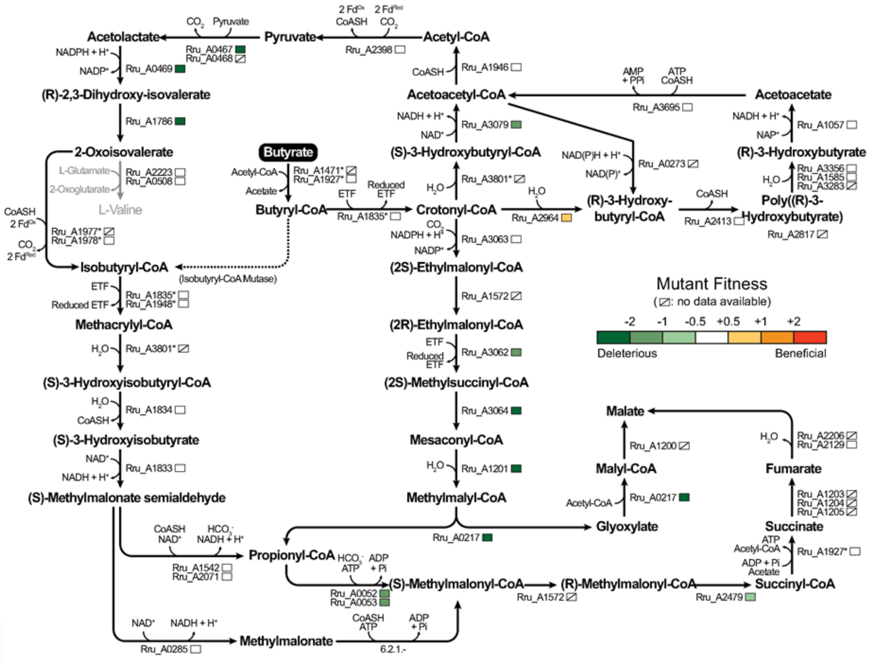
MELiSSA Omics

- Genome wide mutant library for metabolic pathway validation



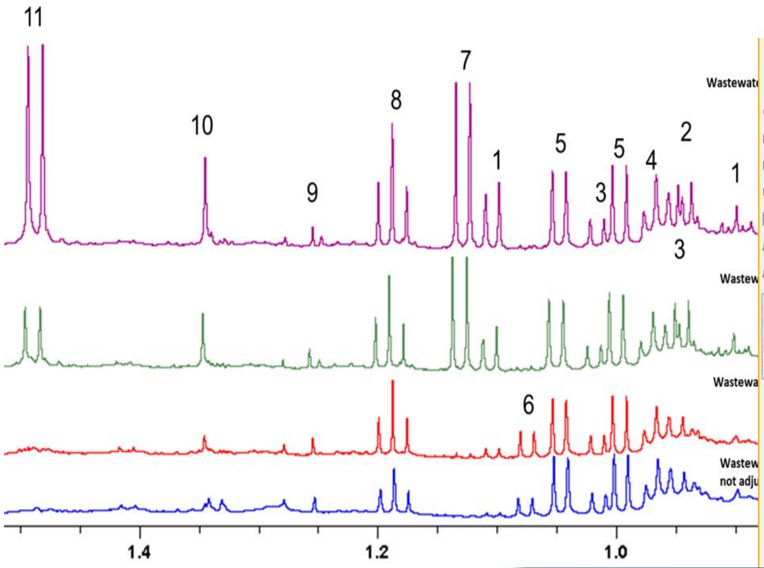
Gene fitness =

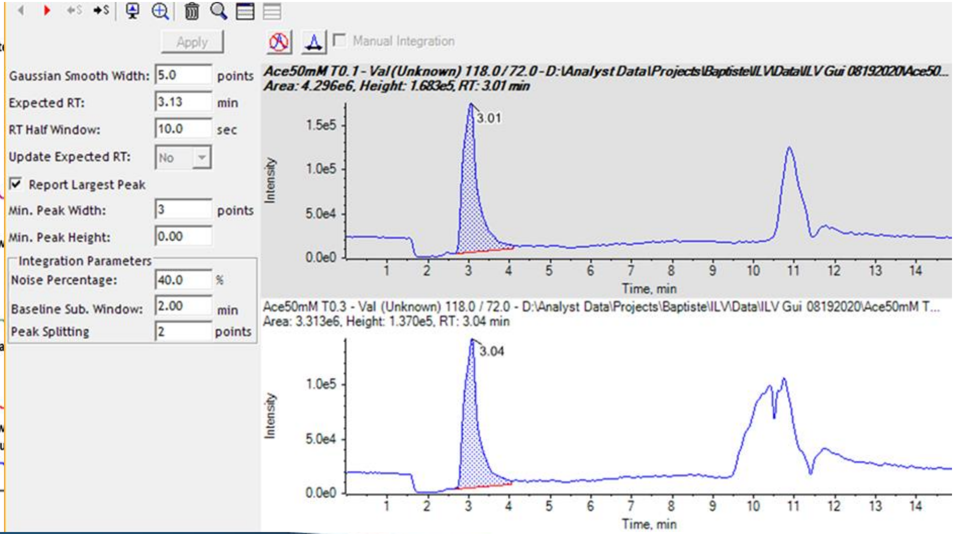
$$\log_2\left(\frac{\text{barcode abundance in Condition}}{\text{barcode abundance in Time0}}\right)$$



MELiSSA Omics

- Metabolomic through NMR and LC/GC MS





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Expected RT: 3.13 min
RT Half Window: 10.0 sec
Update Expected RT: No
☑ Report Largest Peak
Min. Peak Width: 3 points
Min. Peak Height: 0.00

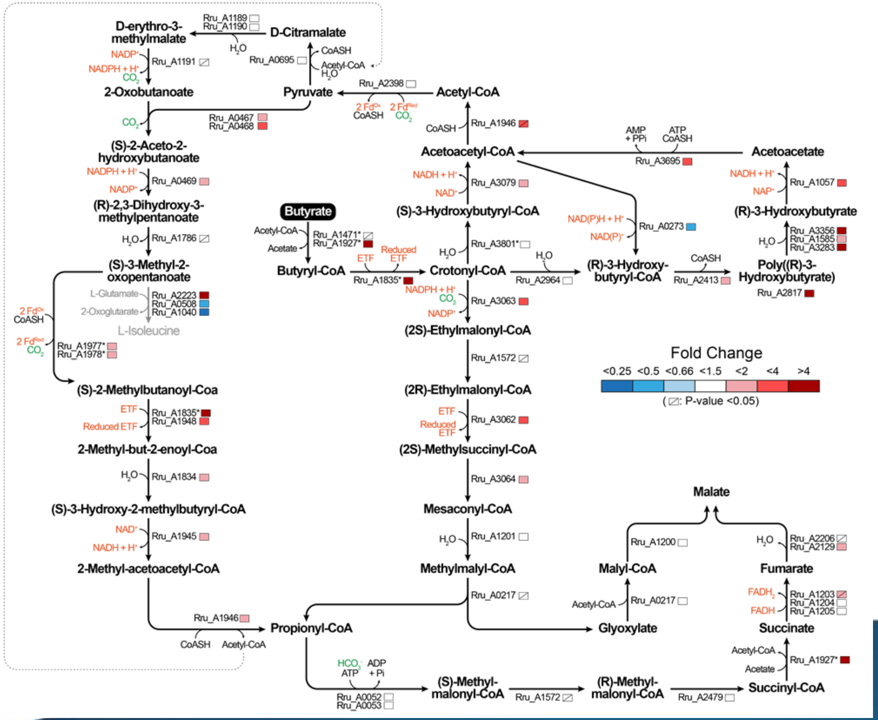
Integration Parameters
Noise Percentage: 40.0 %
Baseline Sub. Window: 2.00 min
Peak Splitting 2 points

Ace50mM T0.1 - Val (Unknown) 118.0 / 72.0 - D:\Analyst Data\Projects\Baptiste\ILV\Data\ILV Gui 08192020\Ace50...
Area: 4.296e6, Height: 1.683e5, RT: 3.01 min

Ace50mM T0.3 - Val (Unknown) 118.0 / 72.0 - D:\Analyst Data\Projects\Baptiste\ILV\Data\ILV Gui 08192020\Ace50mM T...
Area: 3.313e6, Height: 1.370e5, RT: 3.04 min

The MELiSSA metabolome

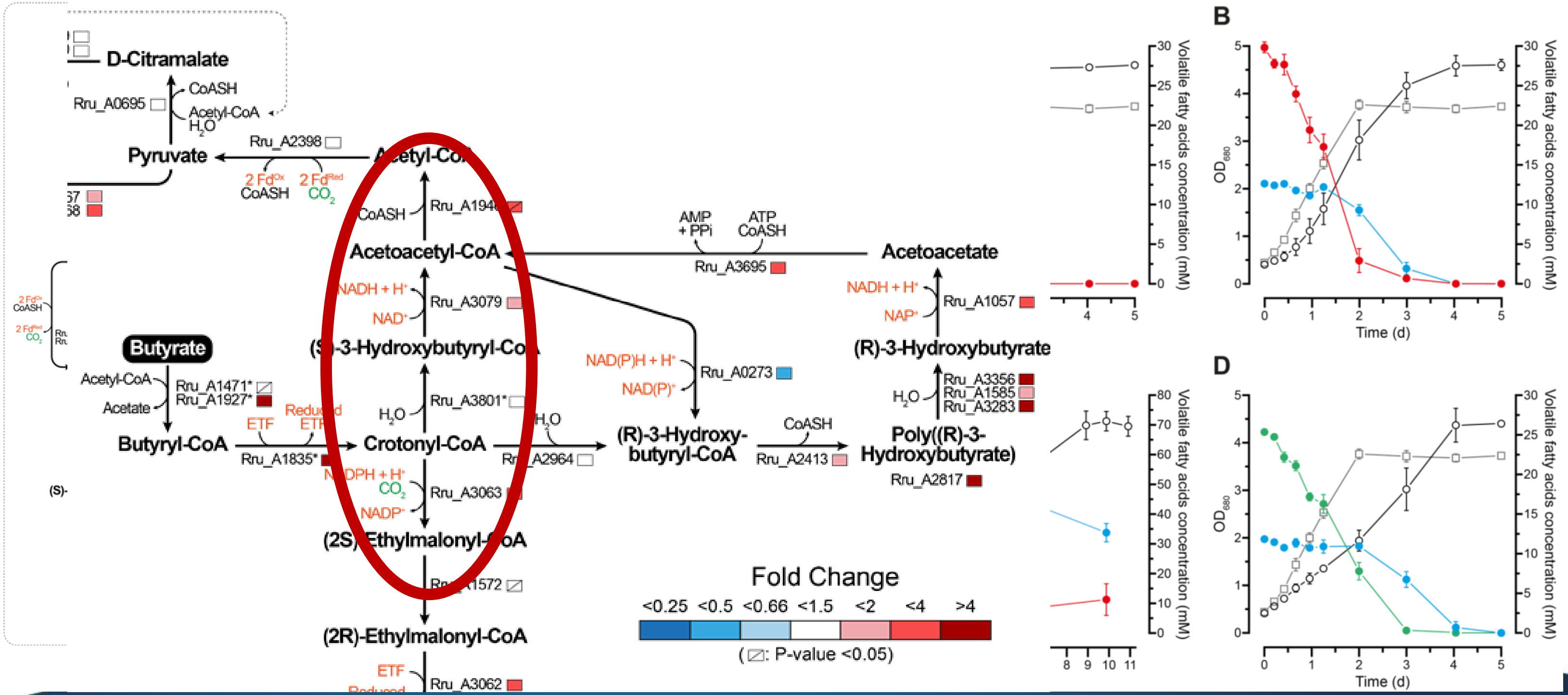
- Photoheterotrophic VFA assimilation by *Rs. rubrum*



- Characterization of assimilation pathways for acetate, propionate, butyrate, valerate, hexanoate
- Discovery of new assimilation pathway and new electron sinking mechanism (MBC pathway)

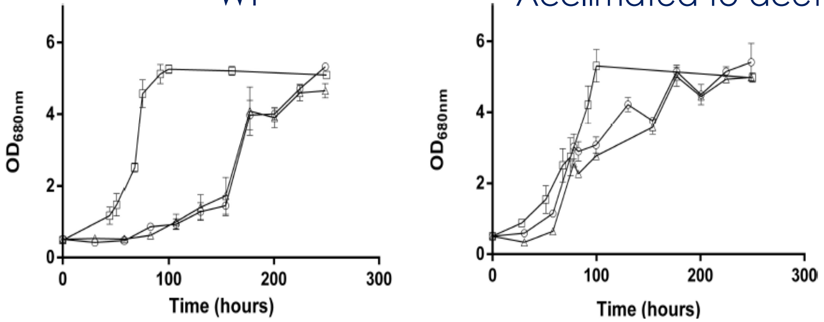
The MELiSSA metabolome

- Assimilation of mixture of VFAs by *Rs. rubrum*



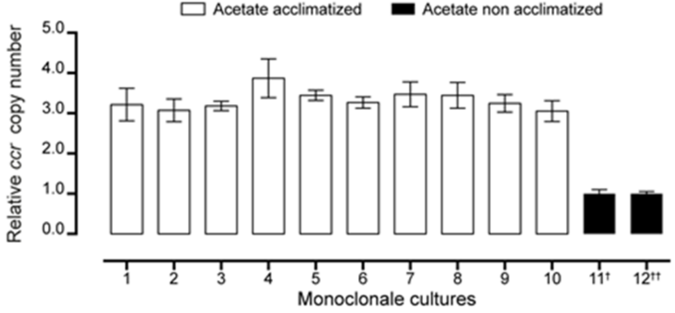
The MELiSSA metabolome

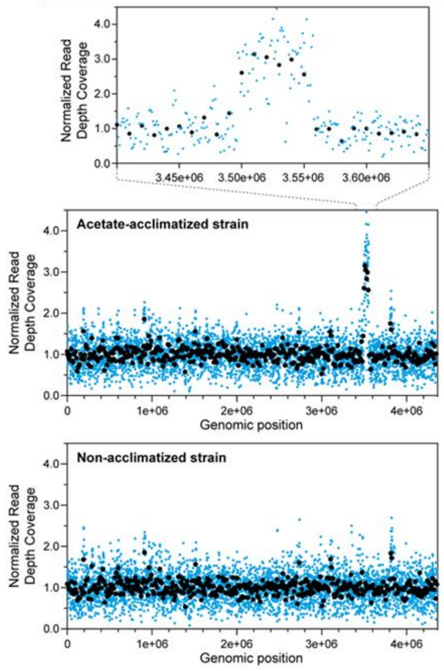
- Genetic adaptation to the environmental conditions



WT

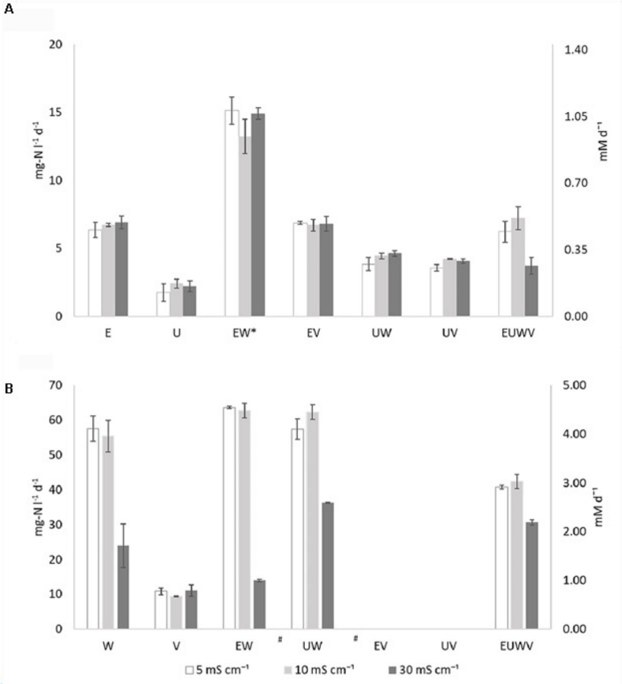
Acclimated to acetate

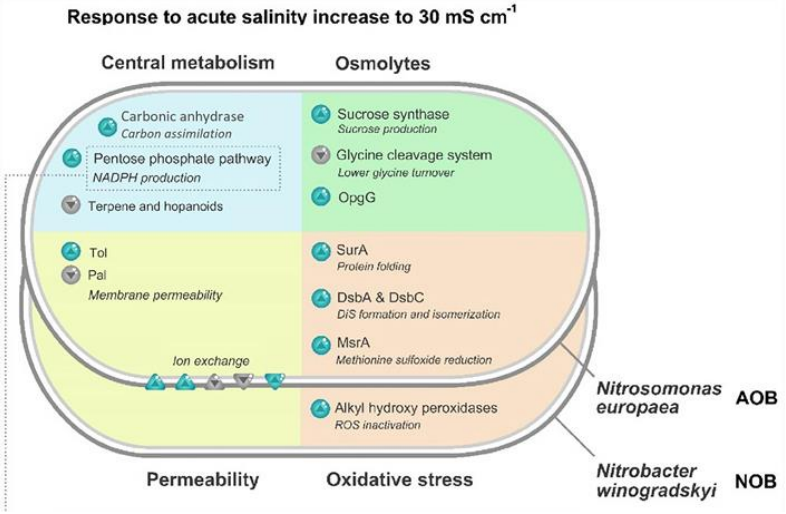




The MELiSSA metabolome

- Salinity adaptation of nitrifiers

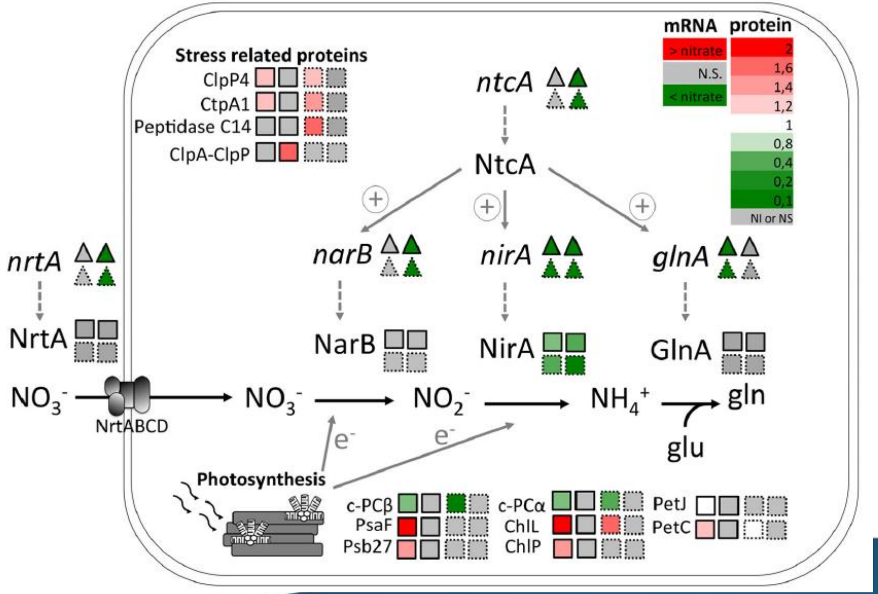




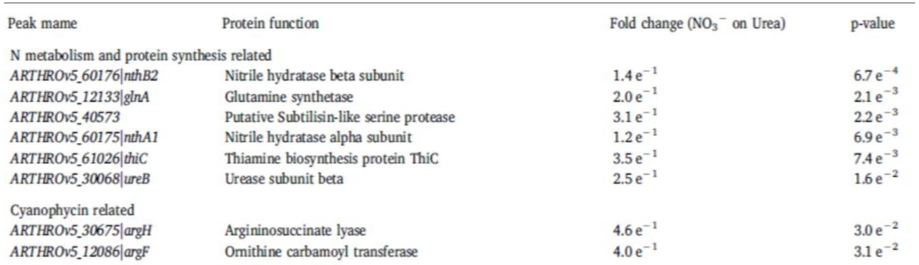
The MELiSSA metabolome

- Response to fluctuation of nitrogen sources in cyanobacteria

Ammonium assimilation



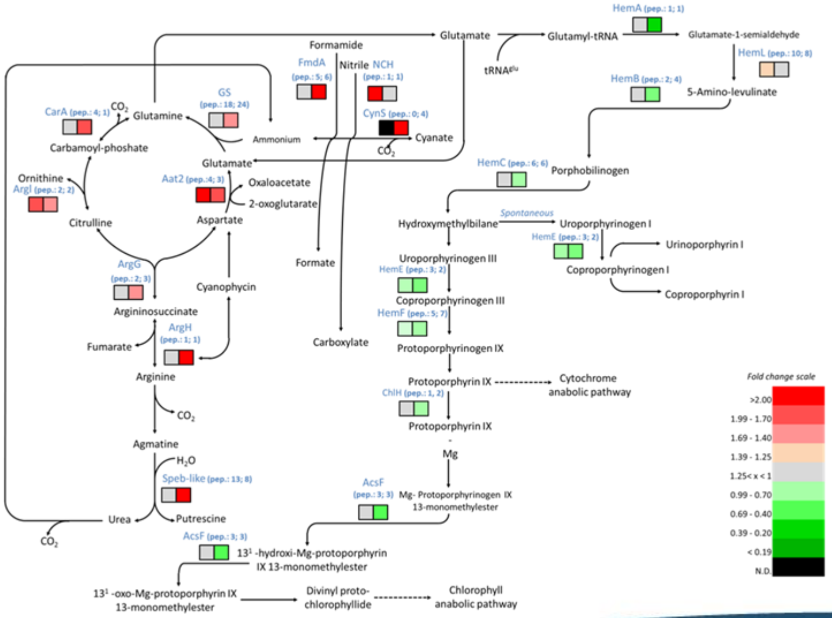
Urea assimilation

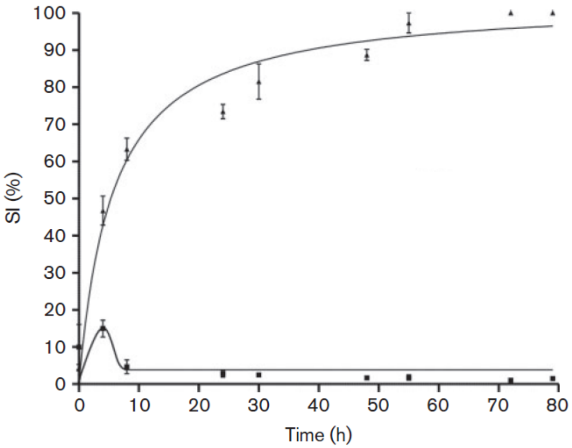


Peak mame	Protein function	Fold change (NO_3^- on Urea)	p-value
N metabolism and protein synthesis related			
ARTHROv5_60176|nthB2	Nitrile hydratase beta subunit	$1.4\ e^{-1}$	$6.7\ e^{-4}$
ARTHROv5_12133|glnA	Glutamine synthetase	$2.0\ e^{-1}$	$2.1\ e^{-3}$
ARTHROv5_40573	Putative Subtilisin-like serine protease	$3.1\ e^{-1}$	$2.2\ e^{-3}$
ARTHROv5_60175|nthA1	Nitrile hydratase alpha subunit	$1.2\ e^{-1}$	$6.9\ e^{-3}$
ARTHROv5_61026|thiC	Thiamine biosynthesis protein ThiC	$3.5\ e^{-1}$	$7.4\ e^{-3}$
ARTHROv5_30068|ureB	Urease subunit beta	$2.5\ e^{-1}$	$1.6\ e^{-2}$
Cyanophycin related			
ARTHROv5_30675|argH	Argininosuccinate lyase	$4.6\ e^{-1}$	$3.0\ e^{-2}$
ARTHROv5_12086|argF	Ornithine carbamoyl transferase	$4.0\ e^{-1}$	$3.1\ e^{-2}$

The MELiSSA metabolome

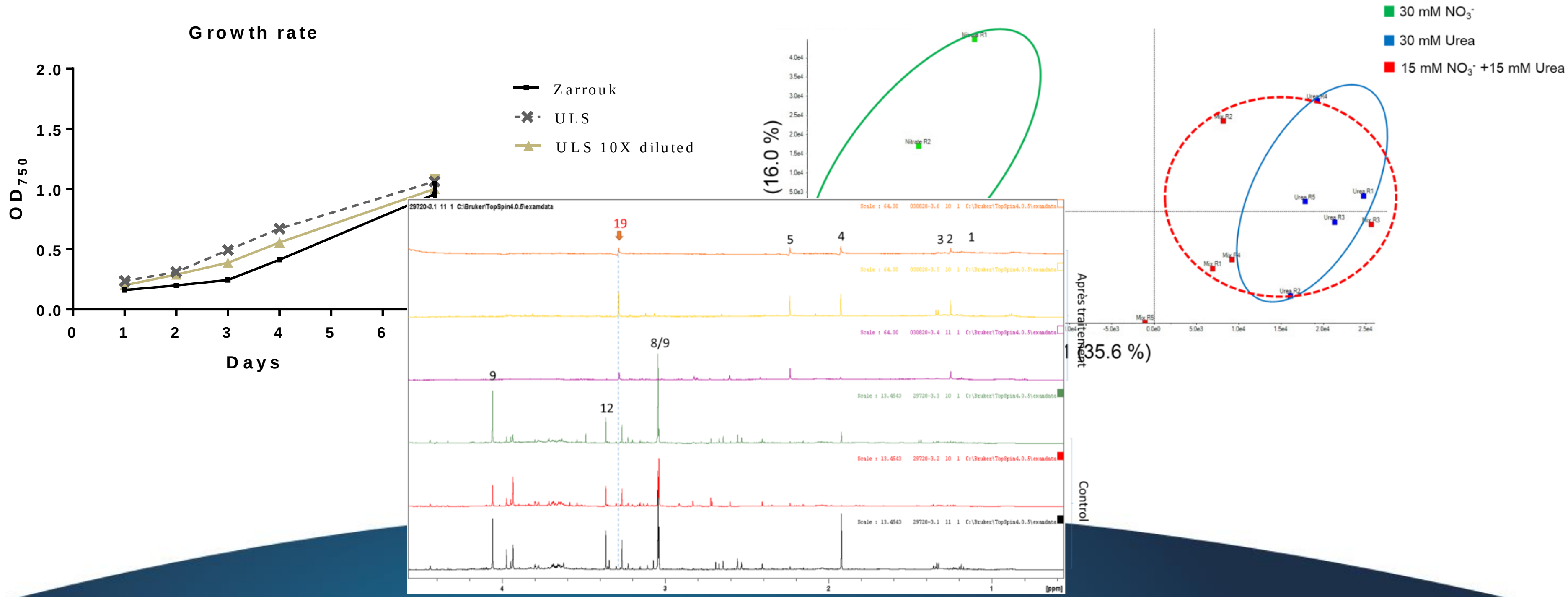
- Response to nitrogen deprivation in cyanobacteria



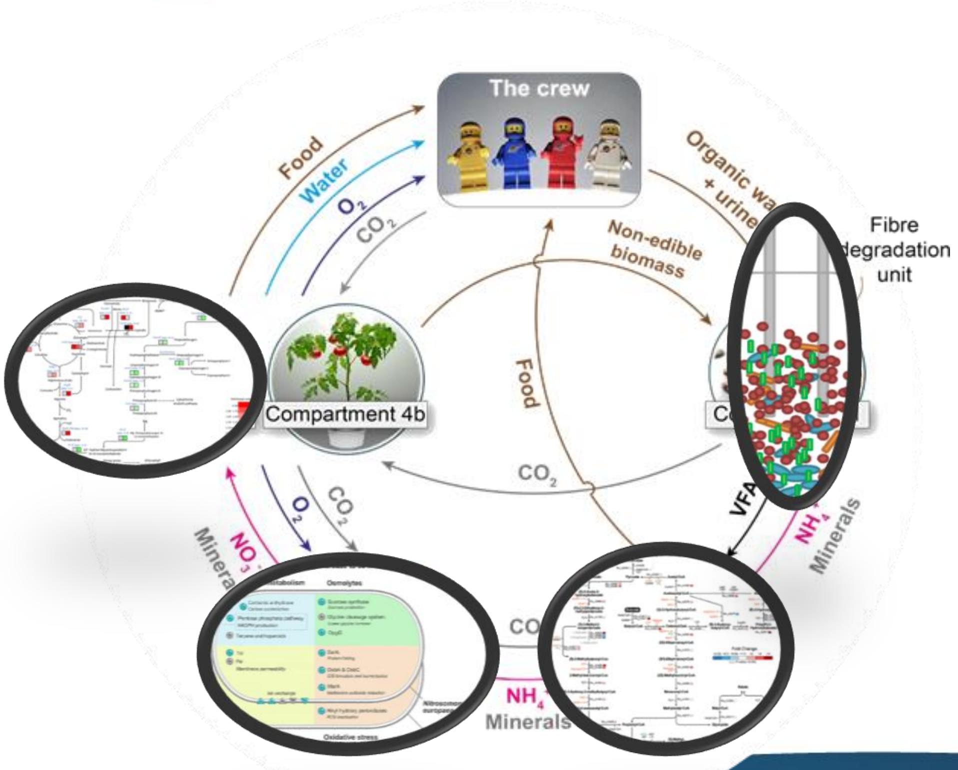

Biomass sedimentation

The MELiSSA metabolome

- Using (partially) nitrified urine as nitrogen source for cyanobacteria



The MELiSSA metabolome

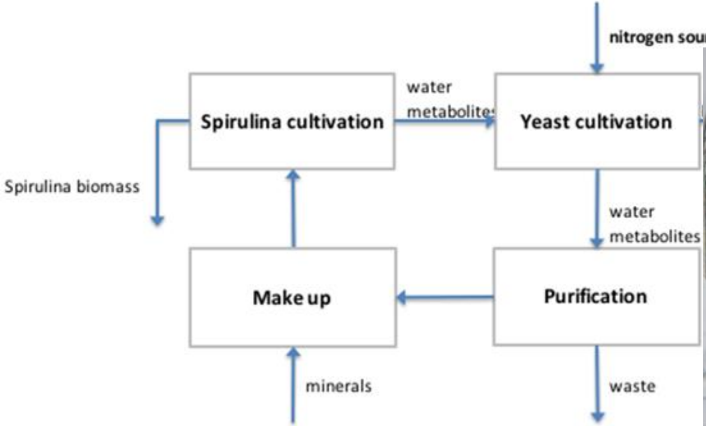


- Not to be exhaustively included in modelling!

- Better understanding of bioprocesses to allow :

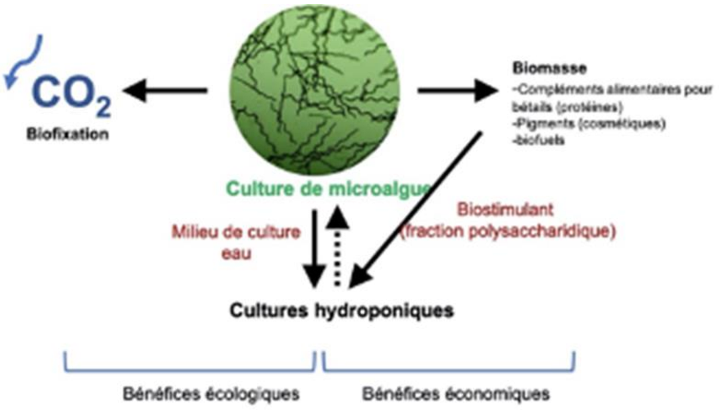
- Better definition of operational conditions
- Margins of the operational conditions
- Alternative scenario of operation
- Monitoring point

The MELiSSA metabolome …on earth



The MELiSSA metabolome …on earth

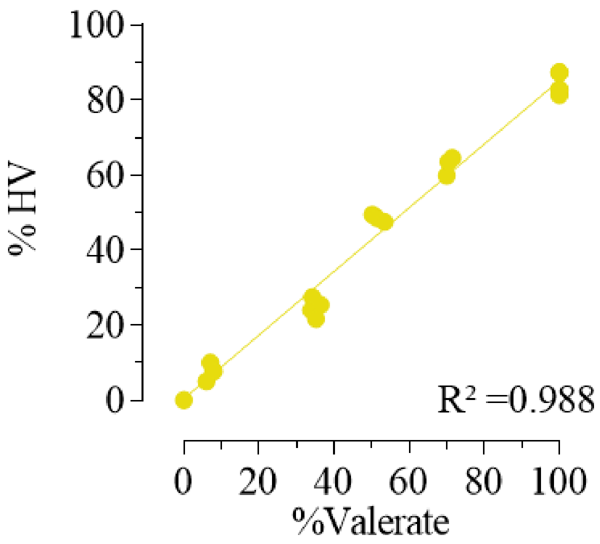
- ✓ MARS : Architecture, Energy and CO2/urea capture in a UMONS Living Lab (submitted)

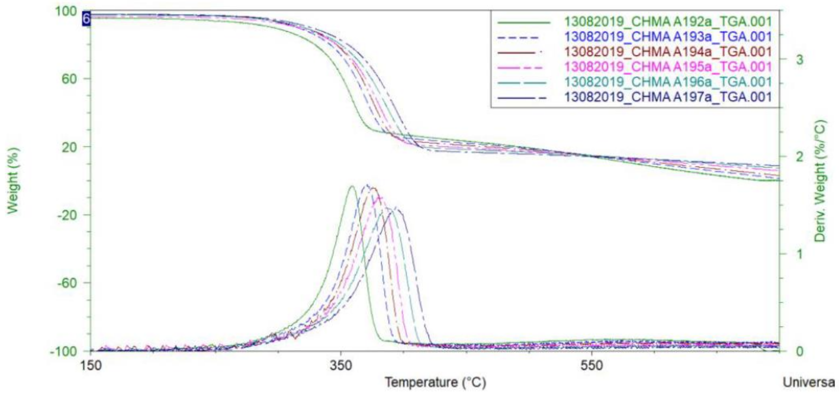


Credit: Chlorella blog/ ASU LightWorks

The MELiSSA metabolome ...on earth

- ✓ Bioplastic production (PHA)





The MELiSSA metabolome …on earth

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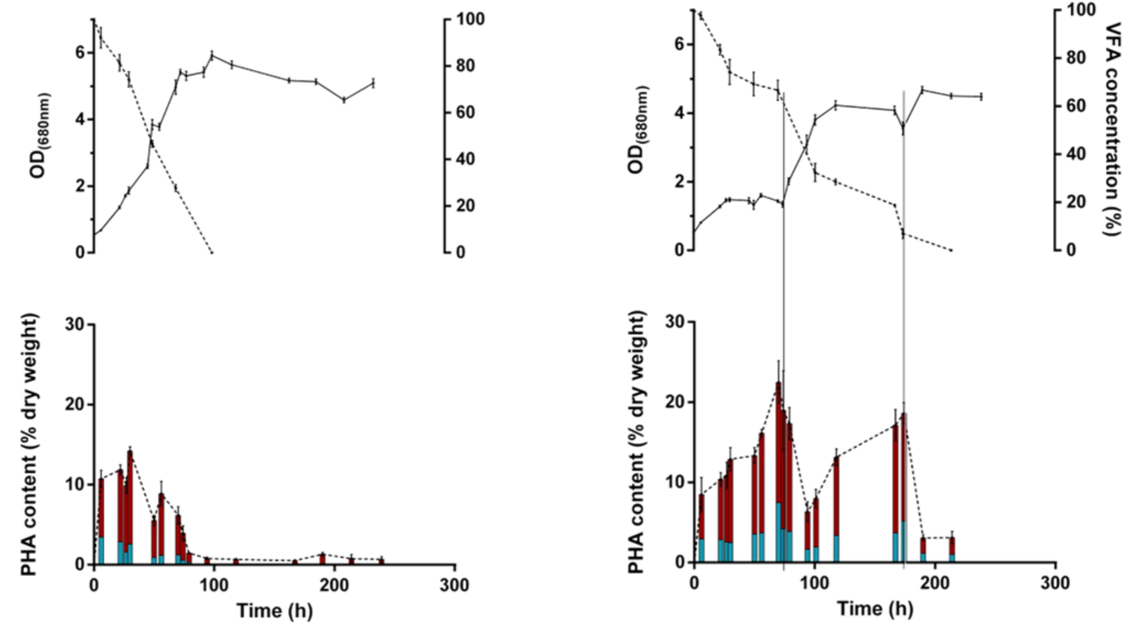


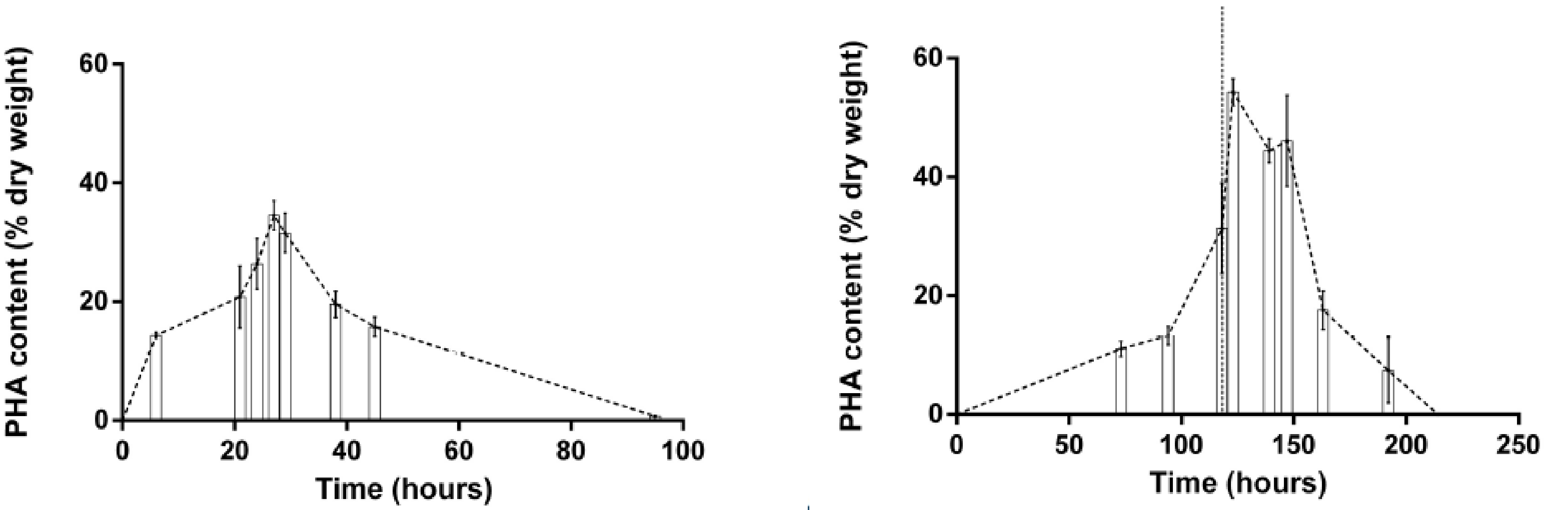
Bicarbonate non-limiting condition

Bicarbonate limiting condition

The MELiSSA metabolome …on earth

- ✓ Bioplastic production (PHA)

Light stress induced PHA accumulation



Conclusion

- Omics analysis allows detailed analysis of metabolome of MELiSSA
- Understanding the metabolome of MELiSSA is key for operational condition definitions and global monitoring, modelling and control
- Characterization of (space related) stress response is also mandatory for robust operation
- Metabolism understanding is a main source of terrestrial applications

Perspectives

- Completion of metabolic characterization of MELiSSA compartment
- Compartment exchanges of metabolite or QS molecules
- Effect of metabolites or contaminant accumulation in the loop
- Better analysis of the response to space related stresses (radiation and µgravity), adaptation to stresses
- Analysis of metabolic shift during operation in space!
- …

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IN COOPERATION WITH
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European Space Agency

MELiSSA FOUNDATION

Belgian Science Policy Office
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THANK YOU.

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