Use of methanotrophic microcosms, tag sequencing and thermodynamic metabolic models to examine structure-function relationships

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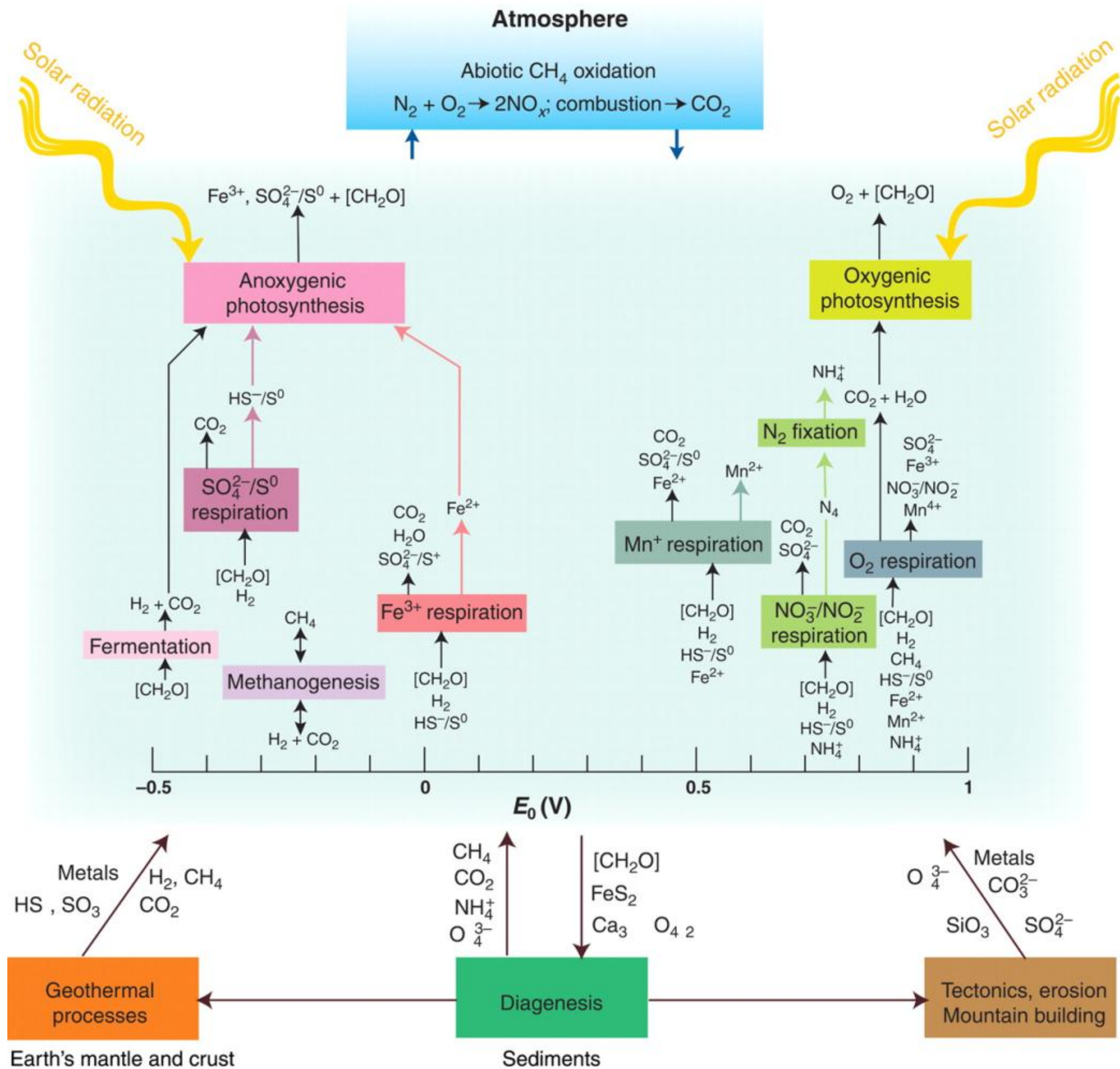
Objective: *Predictive* Understanding of Biogeochemistry

Most Reactions catalyzed by bacteria, the **molecular machines**

Falkowski et al., *Science* (2008)



Biogeochemistry: Which M.M. get built?

Organisms Define Biogeochem.

- Different communities results in different biogeochemistry.
- Need to know who’s there and their growth characteristics.
- Each ecosystem is a case study.
- Generalization very difficult.

Thermodynamics Driven

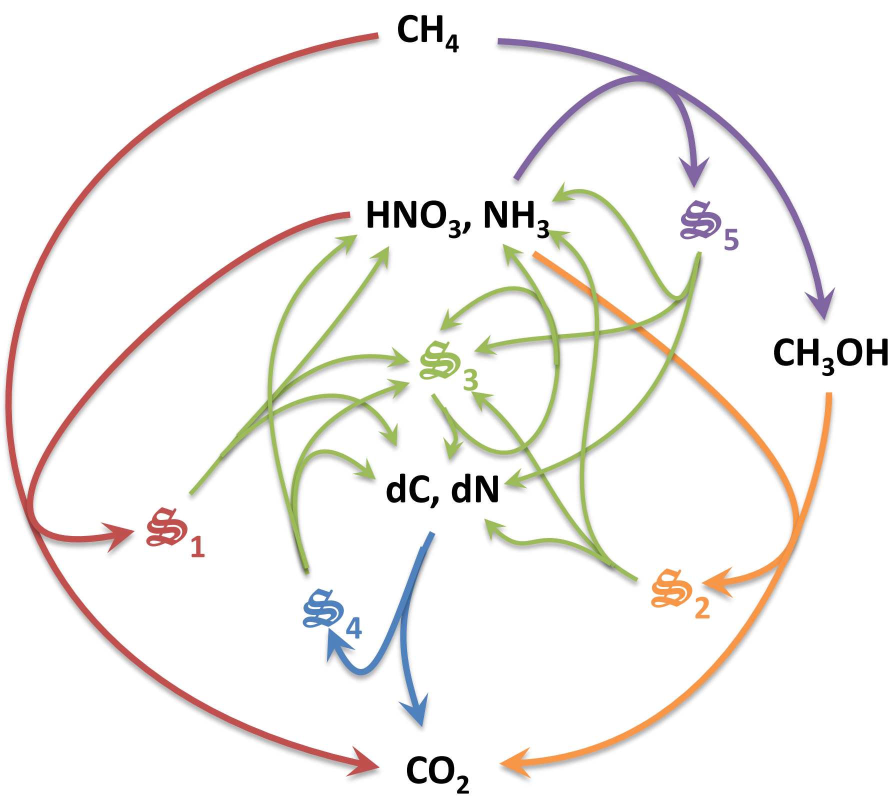
- Energy, nutrients and physical conditions define biogeochemistry.
- Species interchangeable, only function matters.
- Ecosystems operate at or towards an extremum.
- Generalization possible.

Use ***Maximum Entropy Production Theory*** as the basis for predicting microbial biogeochemistry

- Complex systems will organize to utilize (dissipate) all available free energy.
- See Paltridge (1975) (Experimental), Dewar (2003, 2005) (Theory)
- “This represents a paradigm shift from ‘we eat food’ to ‘food has produced us to eat it’” (Lineweaver and Egan, 2008)

MEP Modeling: Methanotrophic Sys.

Distributed Metabolic Network (Only "Extracellular" Metabolites Needed)



Entropy Production, $\dot{\sigma}$:

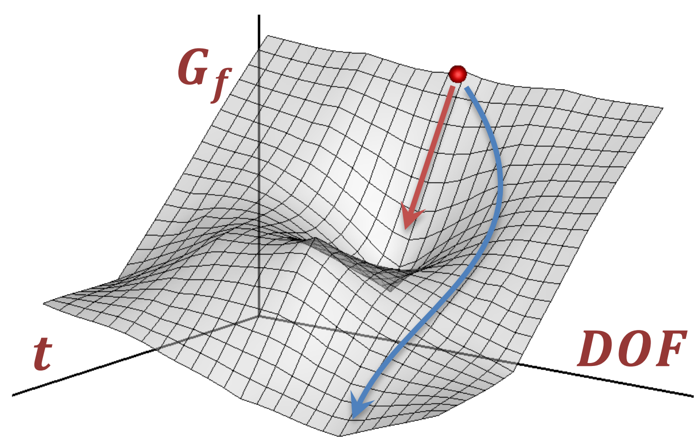
$$\dot{\sigma} = \frac{1}{T}\sum_i r_i \Delta_r G_{r_i}$$

Construct Optimal Control Problem:

$$\underset{\mathfrak{S}_i}{maximize}\ \dot{\sigma}$$

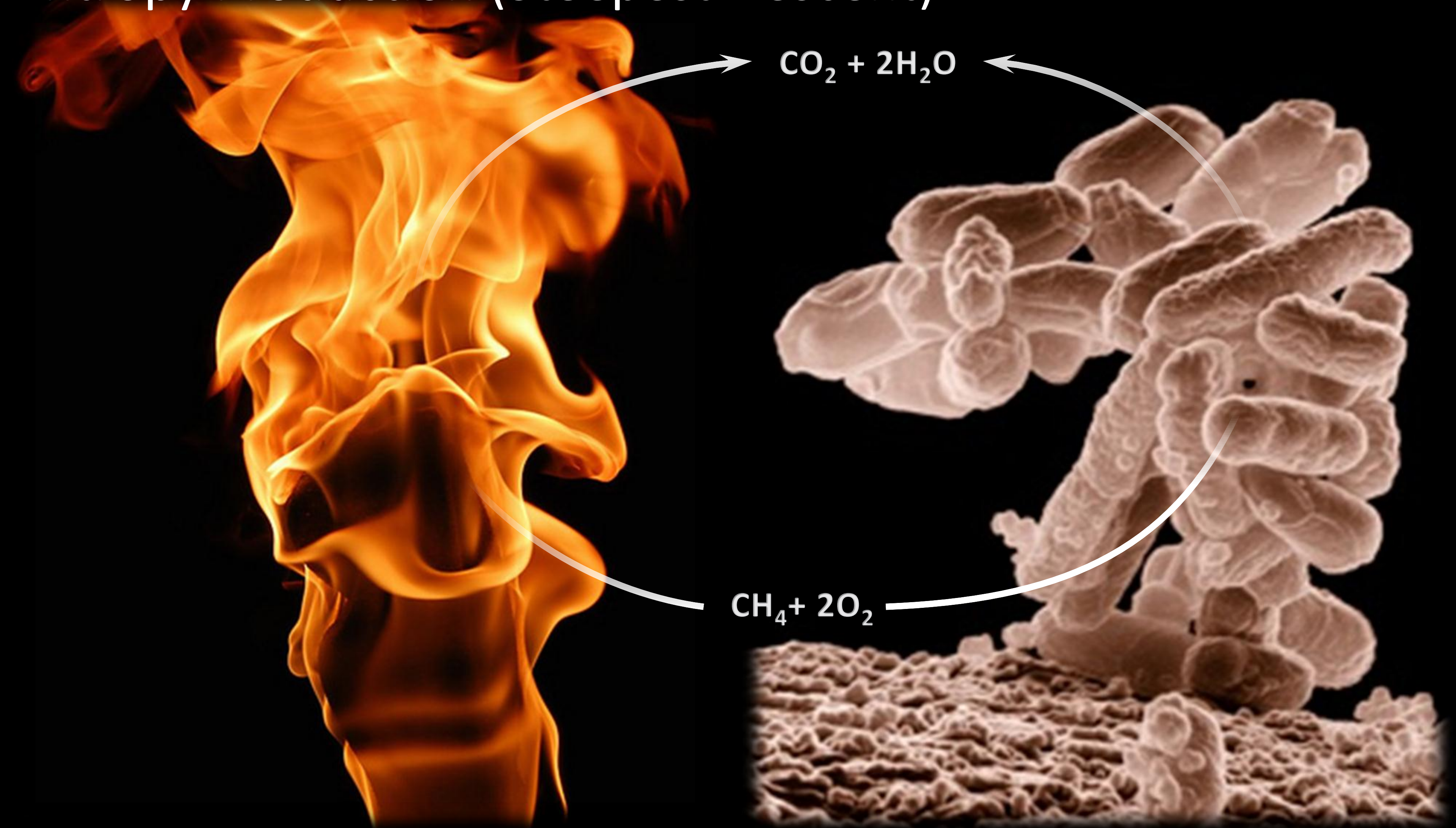
But $\dot{\sigma}$ can be maximized either:

- Instantaneously
- Over time interval



See: Vallino (2010) *Phil. Trans. R. Soc. B*
Vallino (2011) *Earth Syst. Dynam.*

Abiotic system maximize instantaneous Entropy Production (Steepest Descent)



Living systems use information stored in the metagenome to maximize EP over time

NSF ATB Project: Microbial Microcosms to Test MEP conjectures

Experiment 1: Integration of Entropy Production over time.
Examine Community Adaptation to Cyclic Energy Inputs.

Two 18 L chemostats with continuous CH_4+O_2 input

MC 2

MC 3

Two 18 L chemostats with periodic CH_4+O_2 / O_2 input (20 day period)

MC 1

MC 4

Media: mineral salts, with HNO_3 limiting (50 μM)
Dilution rate: 0.1 d^{-1}

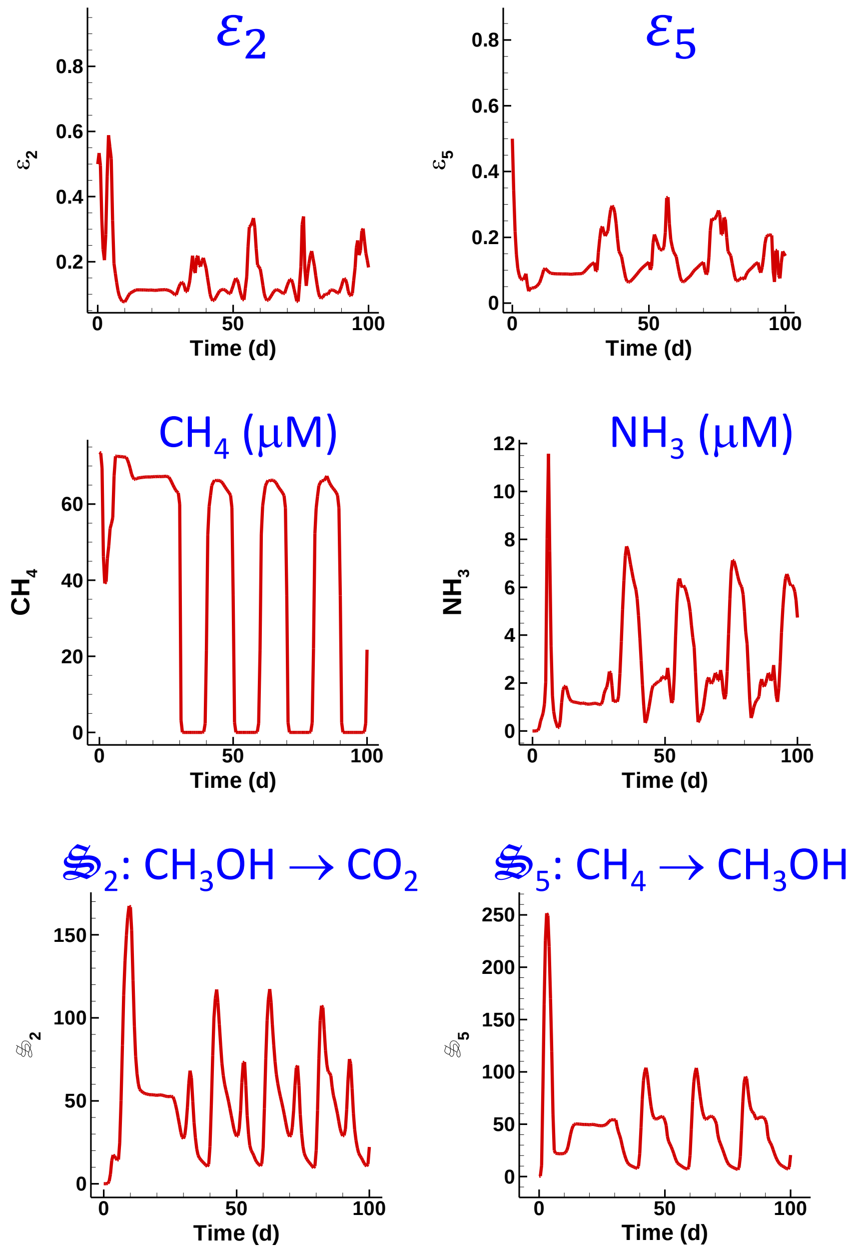
(slide *not* presented at ASLO 2012)

Example of MEP model output

Reaction Efficiencies, ε_i:

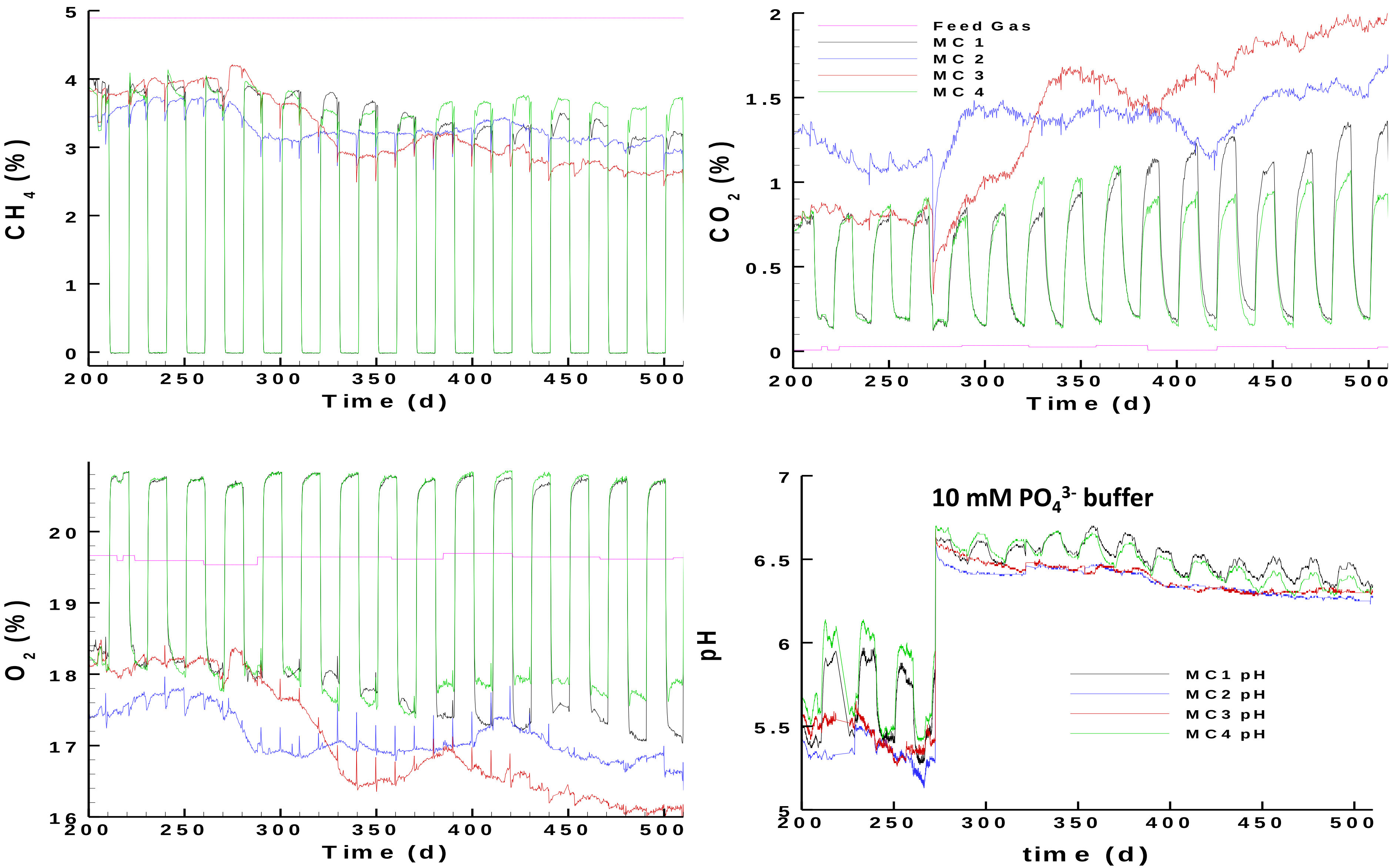
State Variables:

Biological Structures, $\mathfrak{S}_i$:



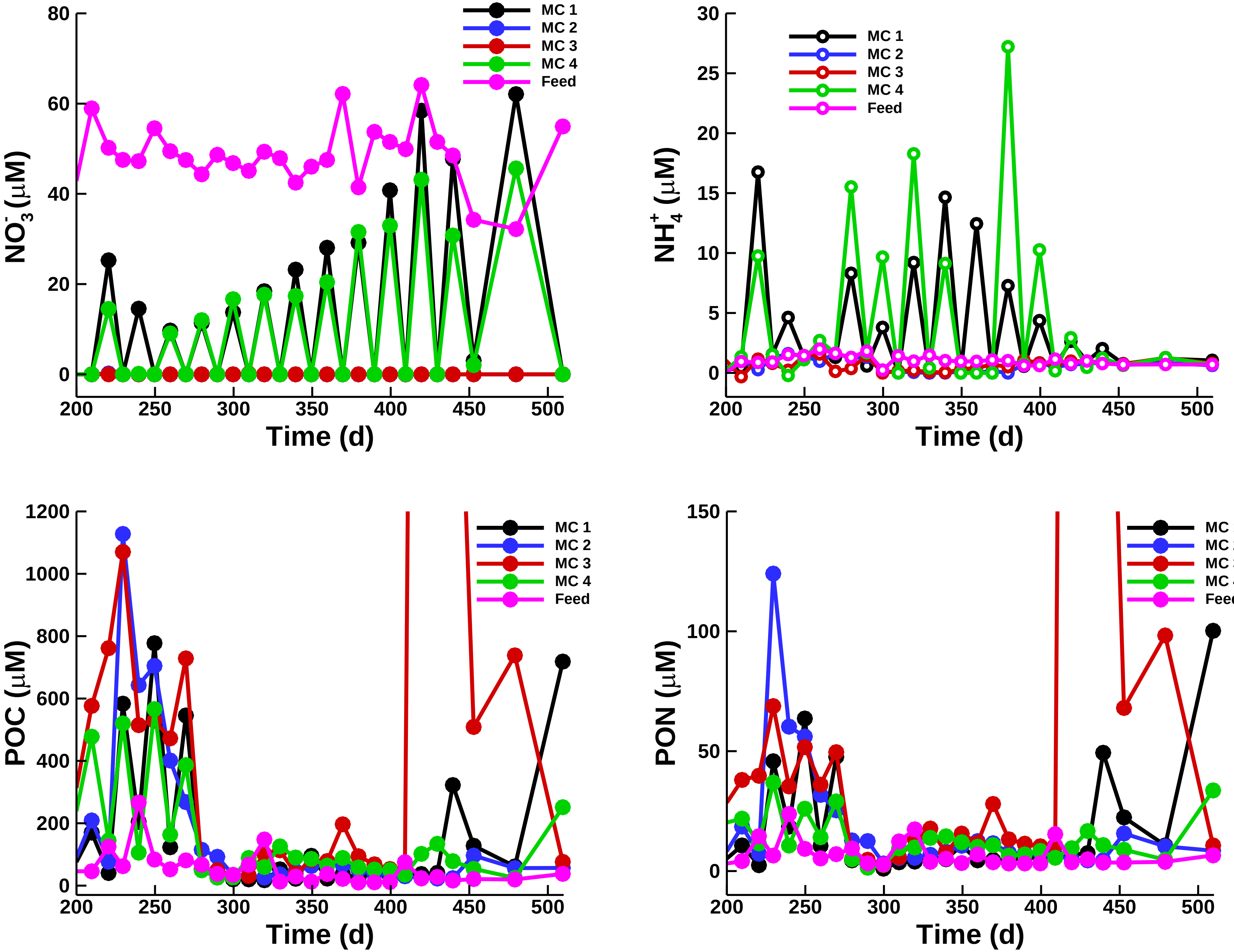
Experimental Gas and pH Data

MC 2 and MC 3 Controls
MC 1 and MC 4 Cycled



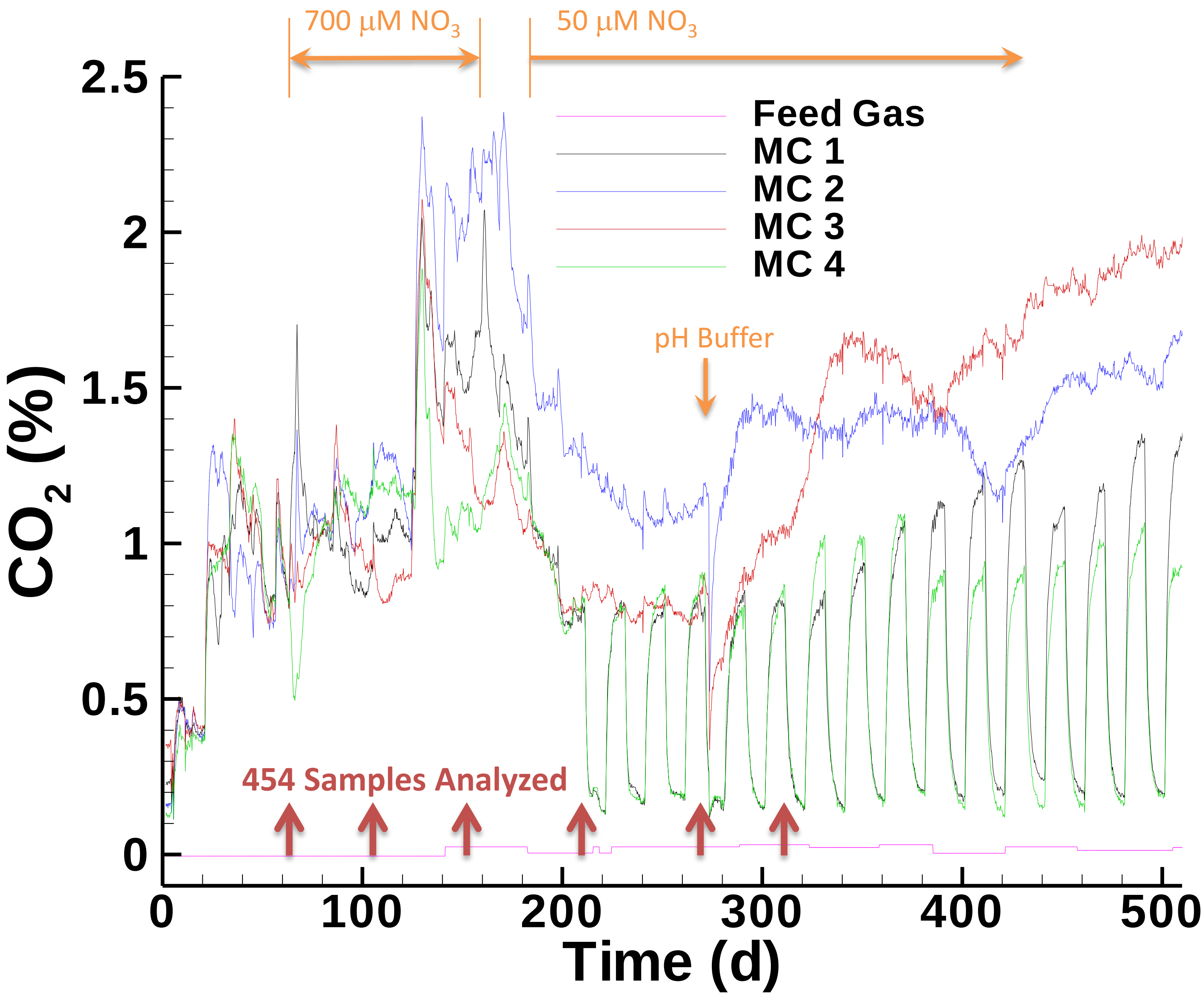
Real time data posted at: http://ecosystems.mbl.edu/MEP

Nutrients



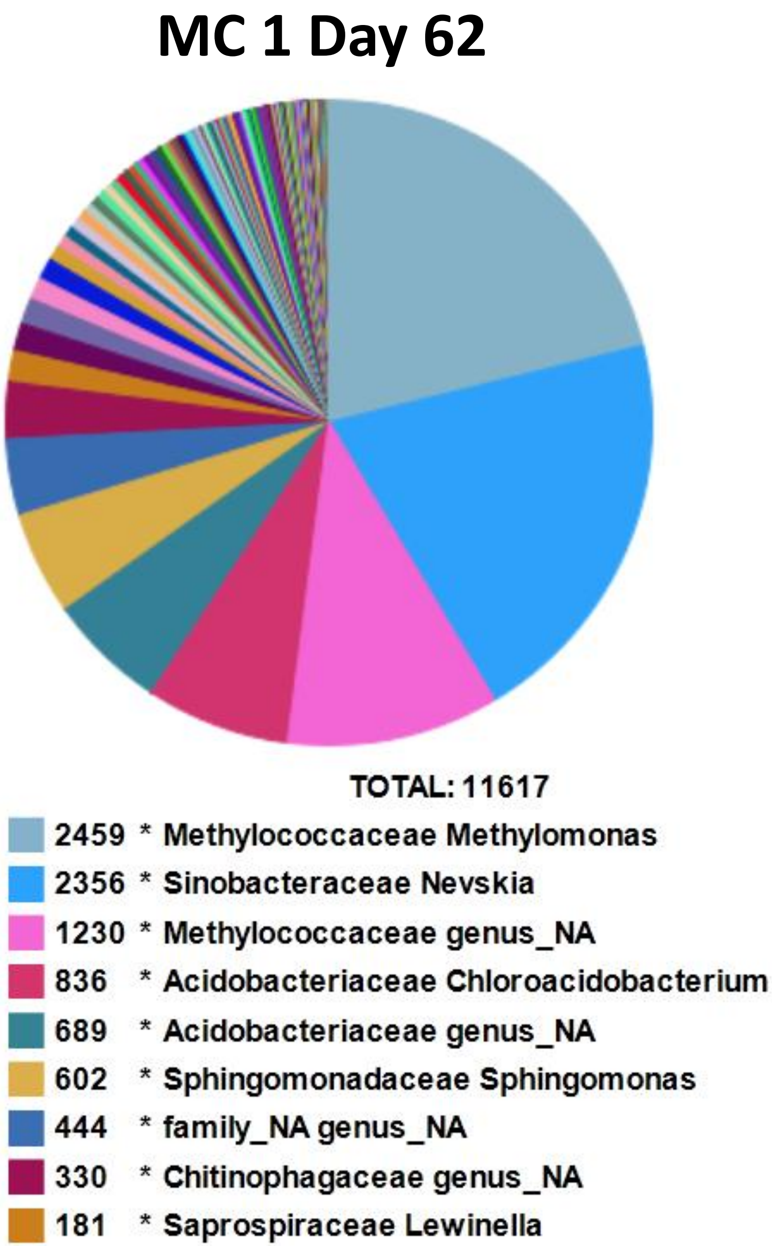
MC 1
MC 2
MC 3
MC 4
Feed
NO3- (μM)
Time (d)
NH4+ (μM)
Time (d)
POC (μM)
Time (d)
PON (μM)
Time (d)

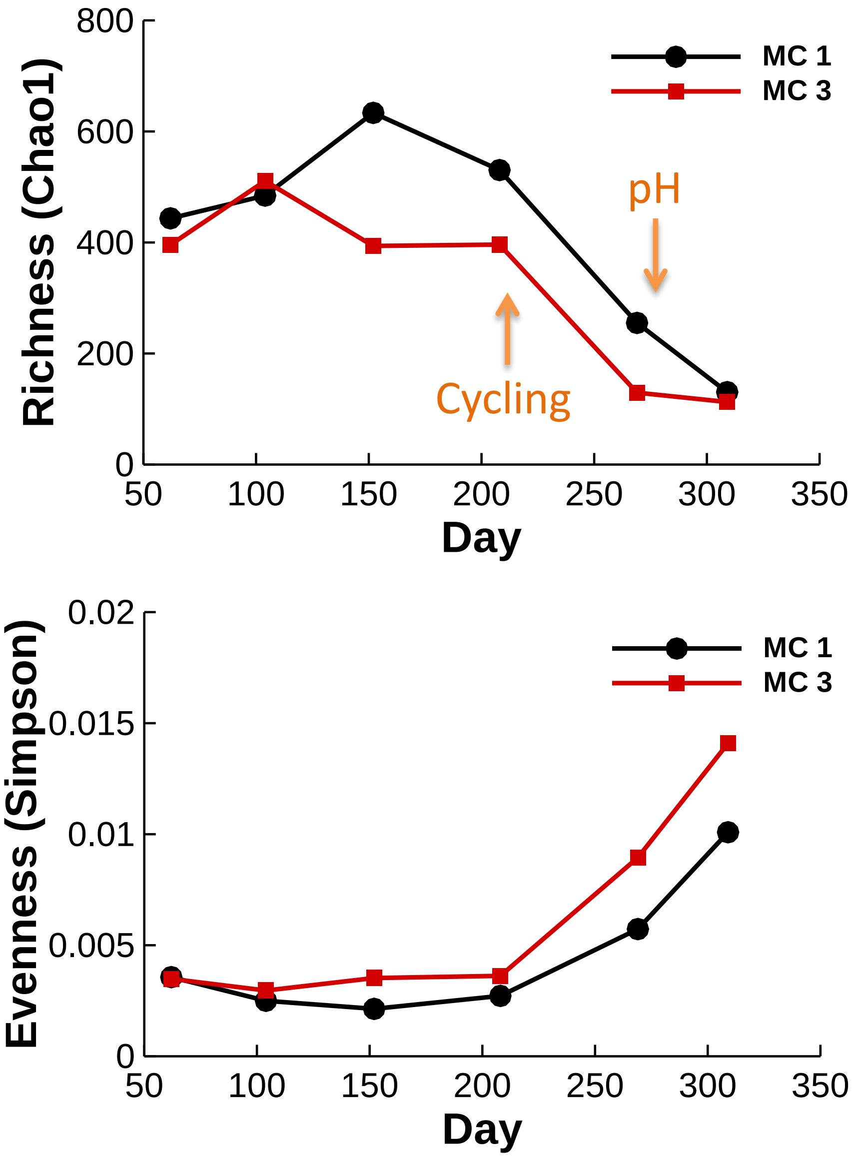
454 V6+V4 Tag Analyses to date



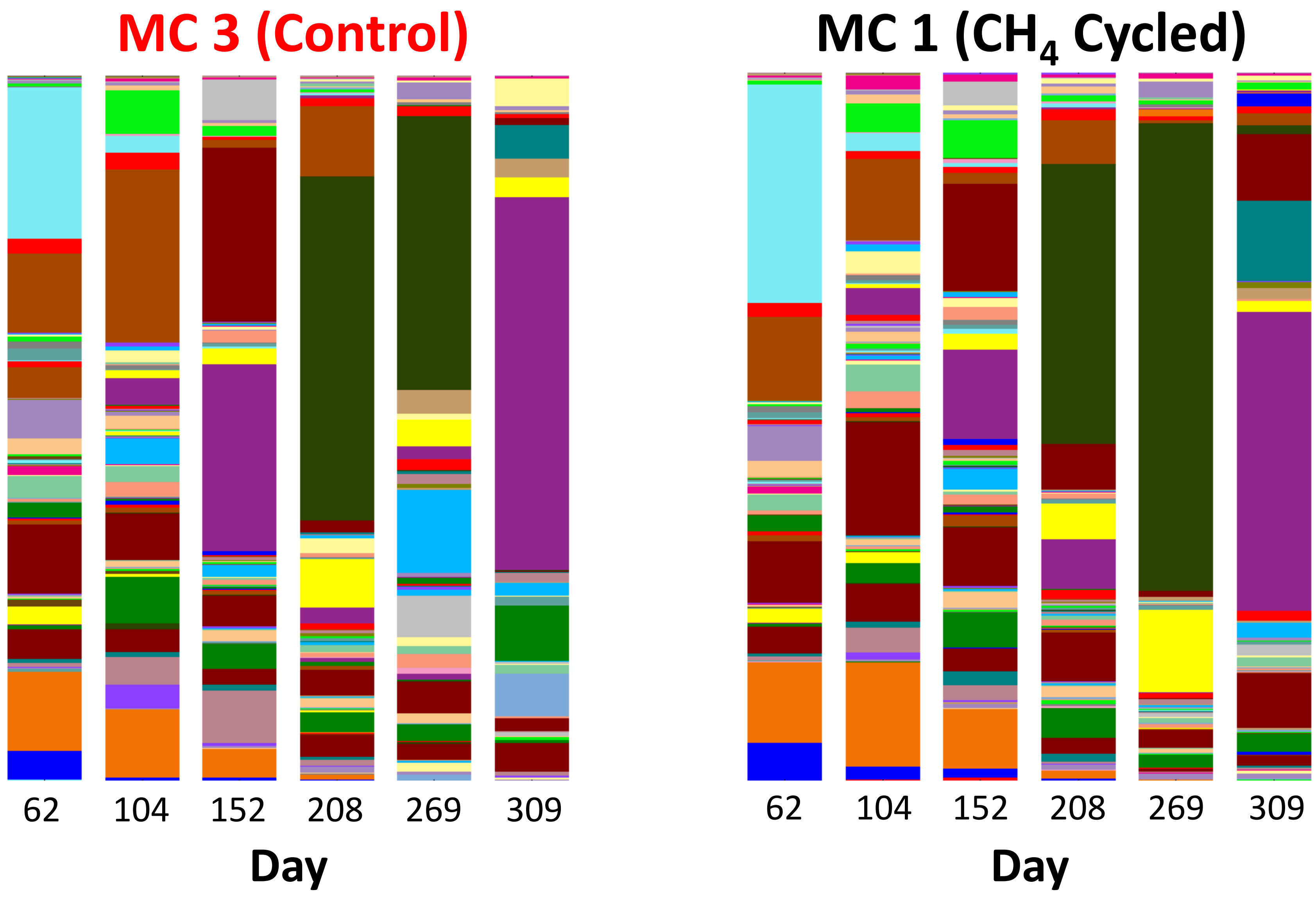
10k-18k tag reads per sample, ~300k total for 22 samples

Biodiversity



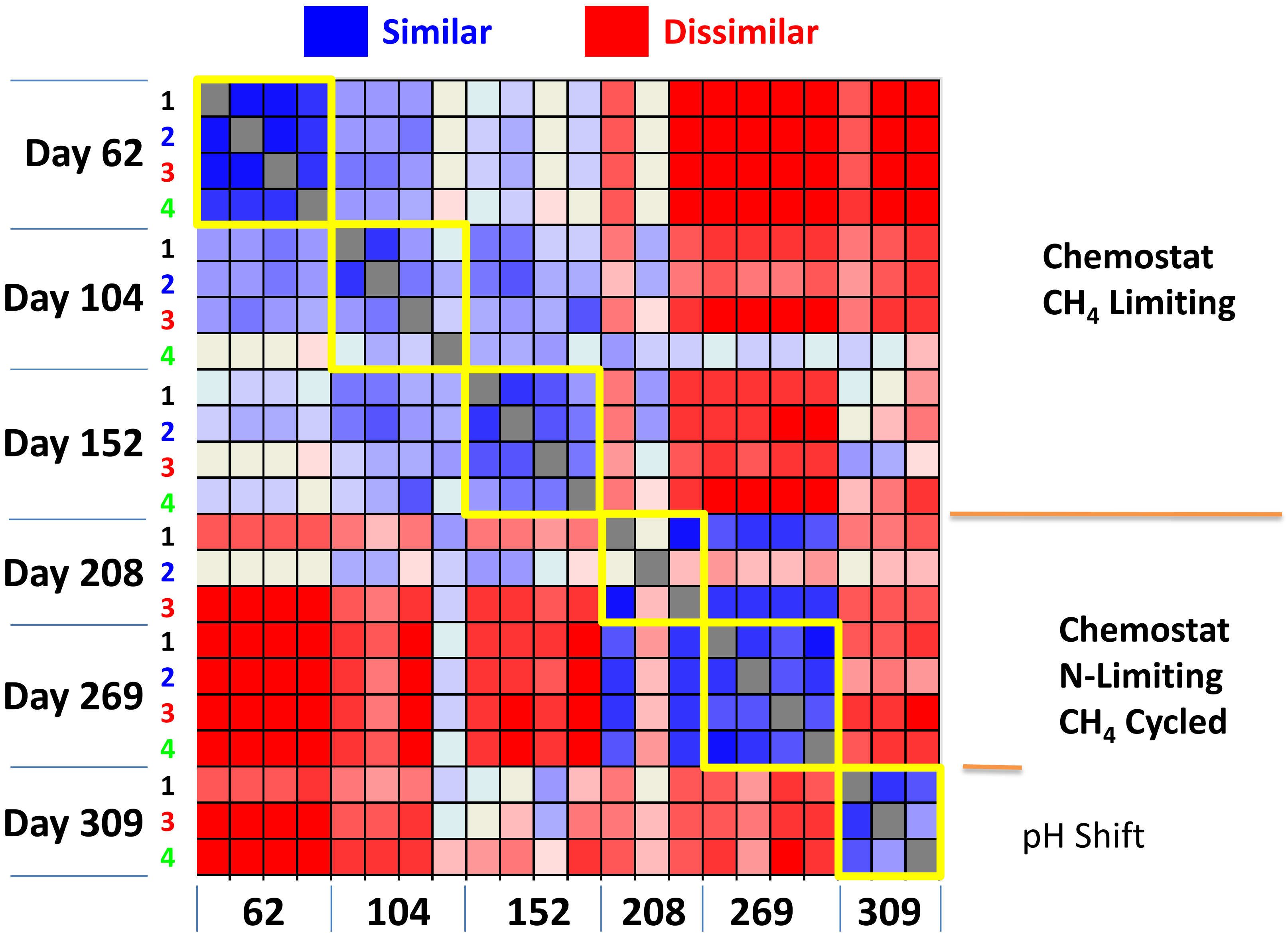


OTU Community Changes

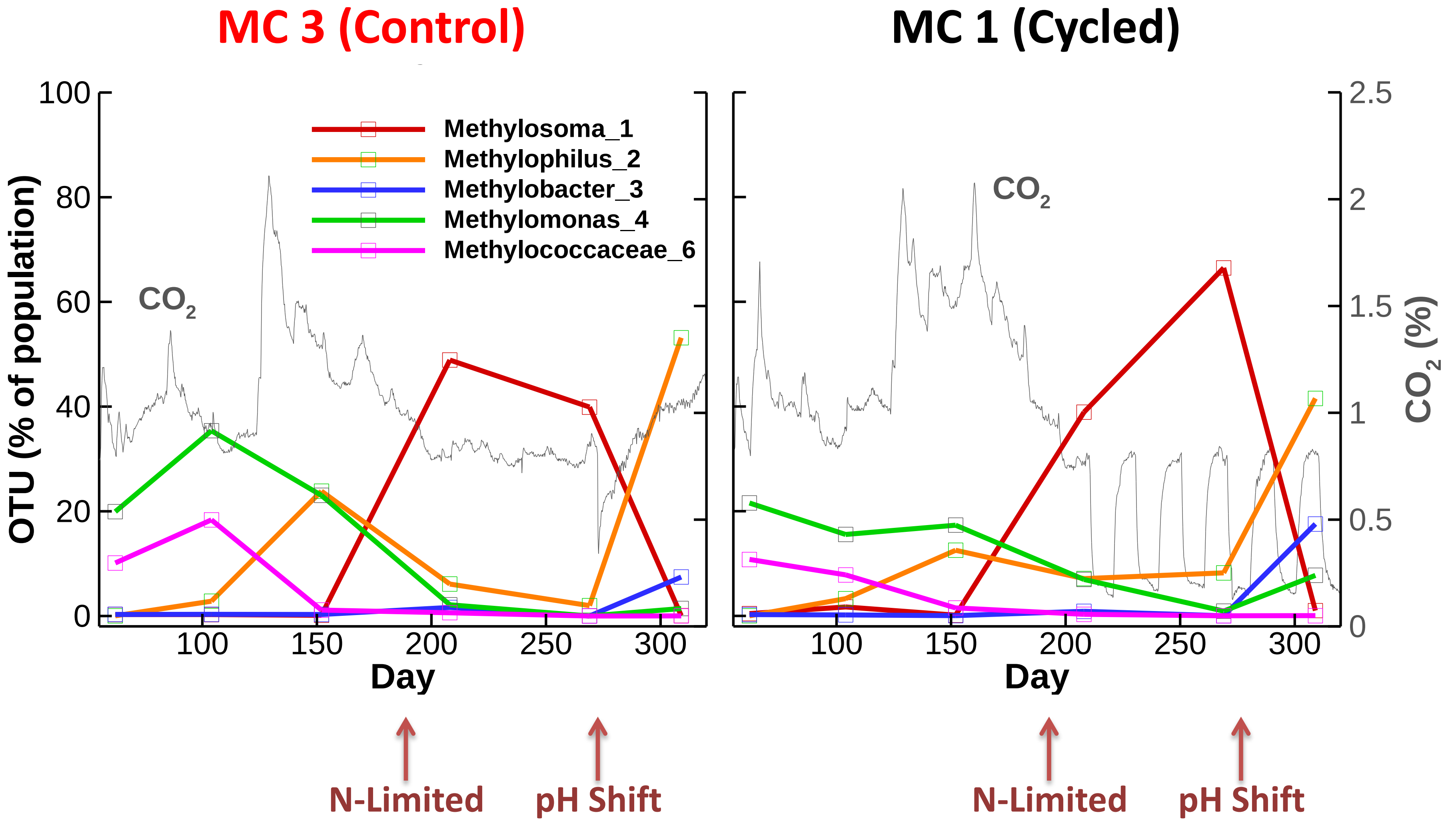


Heat Map (454 V4+V6, genus level)

OTU community similarity between microcosms over time



Methanotroph OTU Time Series



Other OTU's

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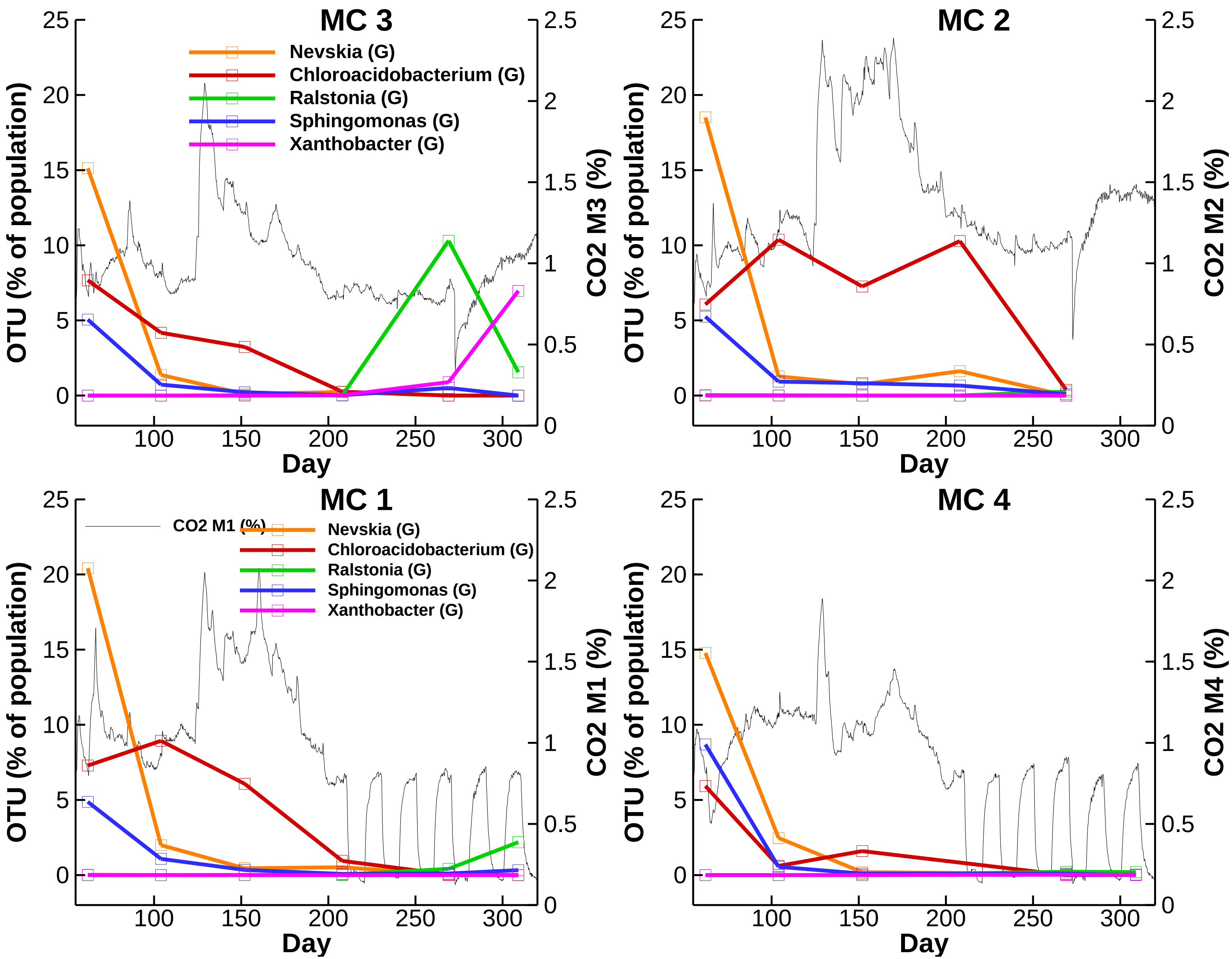
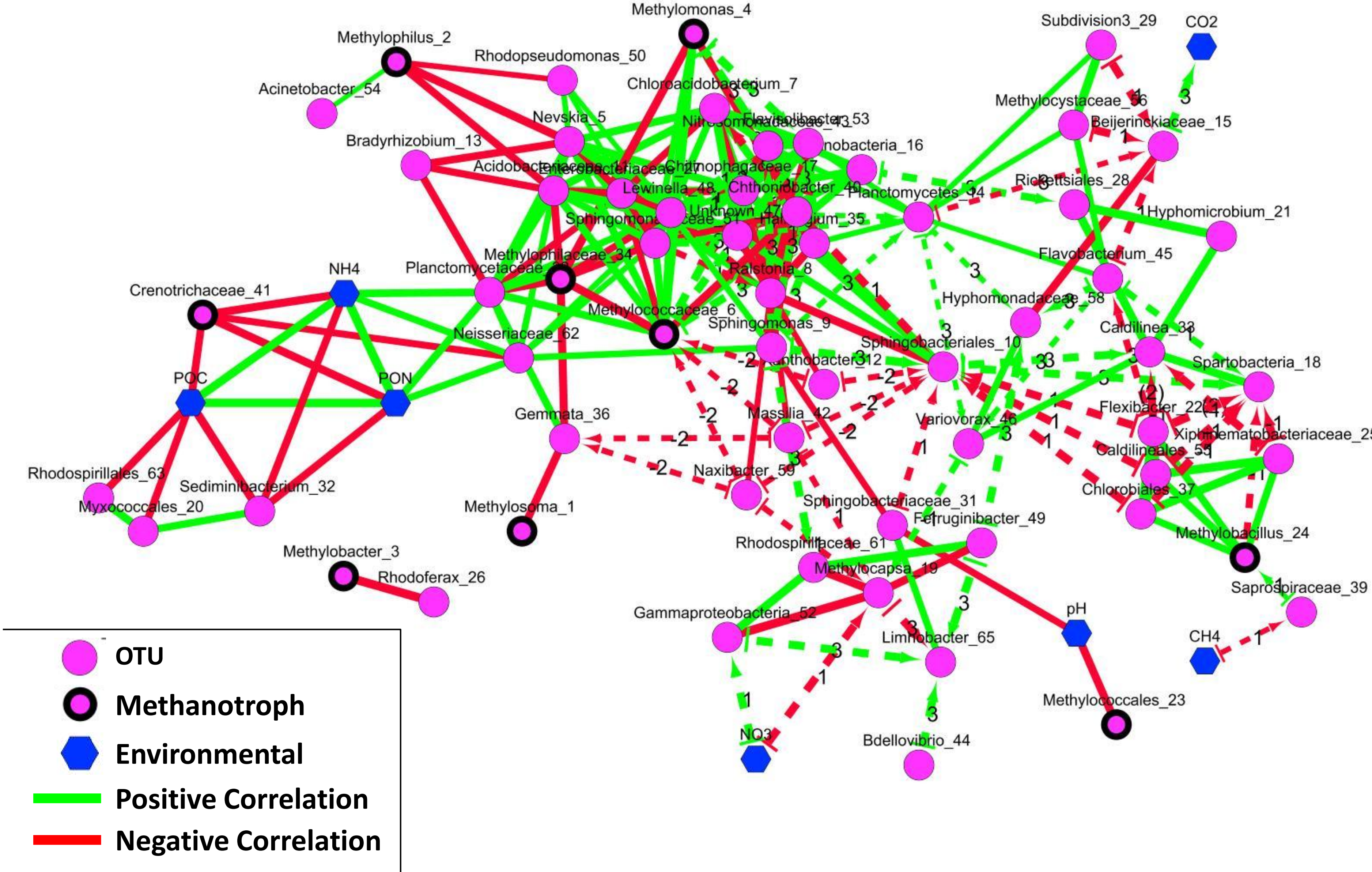


Local Similarity Analysis (LSA): MC 1

(Ruan et al. 2006; Xia et al. 2011) **Only included OTU's ≥ 1% of population**



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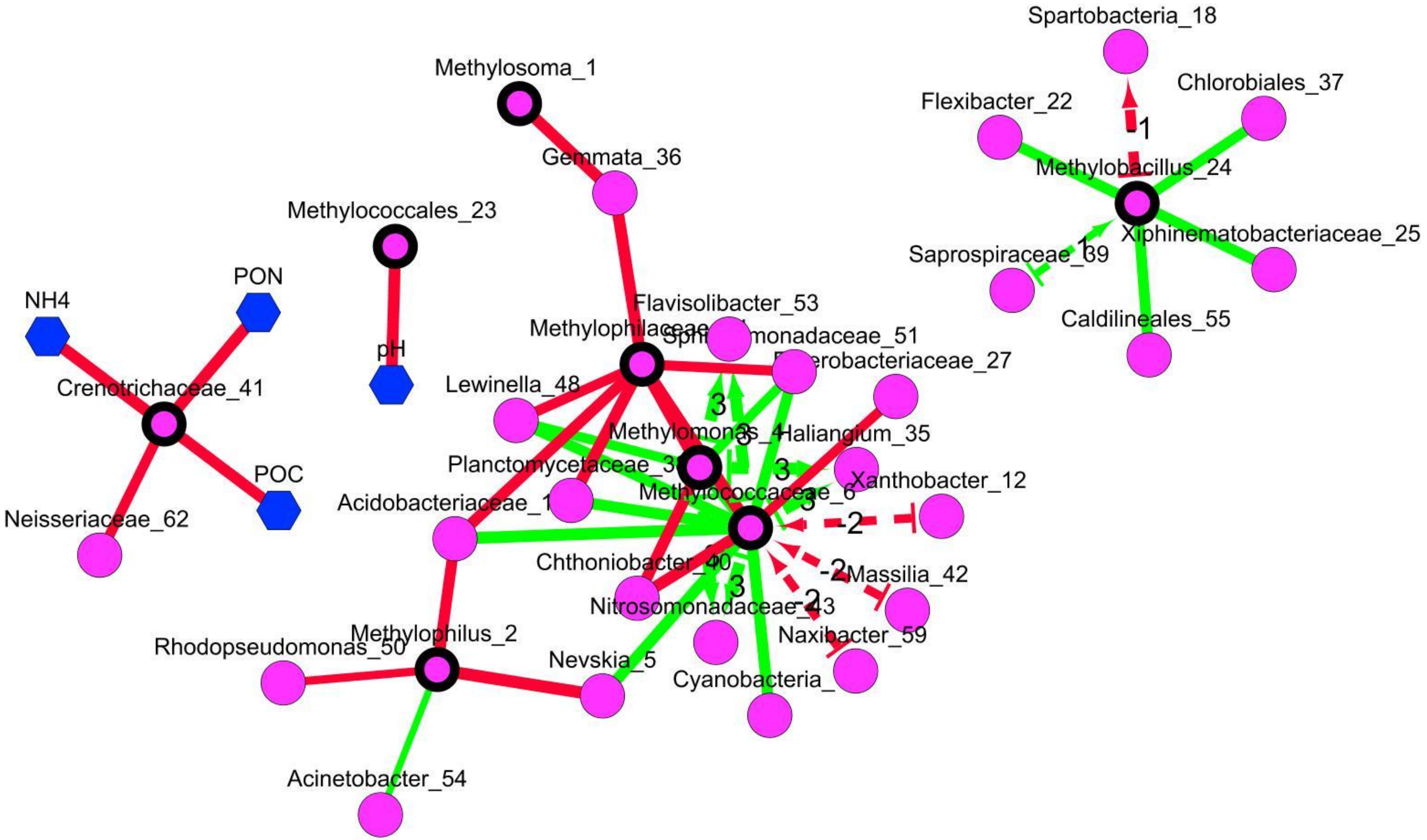
Methanotroph Network



Summary

- Microcosms maintain high diversity even after several years.
- Diversity between microcosms is very similar, but changes significantly over time.
- Ecosystem function (methane oxidation) is stable, while community is dynamic.
- Cross feeding is important ($CH_4 \rightarrow CH_3OH \rightarrow CO_2$).
- Some members of rare biosphere become dominate.
- Microcosms develop dynamic spatial heterogeneity, which likely facilitates maintenance of diversity.
- We still need much more functional information for OTU's
- Have not seen significant adaptation to energy cycling yet.

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WHERE DISCOVERIES BEGIN