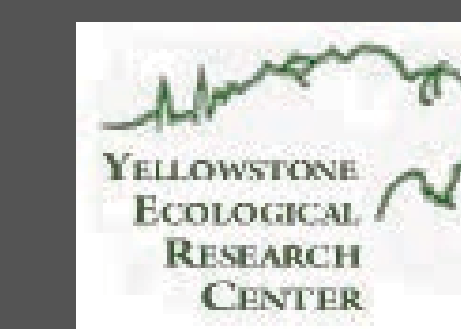


Biogeophysical interactions control the formation of iron oxide microbial biofilms in acidic geothermal outflow channels of Yellowstone National Park

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Introduction

- Mechanisms of microbial biofilm formation are widely unknown in geothermal environments.
- Fe(III)-oxide microbial mats provide an excellent opportunity to study biofilm formation *in situ*.
- Underlying biological, geochemical, and physical processes control the formation of Fe(III)-oxide microbial mats in acidic geothermal springs.

Relevant Archaeal Populations

Population Type	Relative Abundance	HCO	Fe(II)-oxidizer	Reference
OSP_B (T=75 °C, pH=3.5)				
<i>M. yellowstonensis</i> str. MK1	23	Y	Y	Kozubal et al. 2008
'Geoarchaeota'	30	Y	N	Kozubal et al., 2012
Desulfurococcales	14	N	N	Jay et al., 2011
<i>Vulcanisaeta</i>	31	N	N	Jay et al., 2011
BE_D (T=68 °C, pH=3.0)				
<i>M. yellowstonensis</i> str. MK1	12	Y	Y	Kozubal et al. 2008
Novel Archaeal Group 2	38	Y	?	Kozubal et al., 2012
Thaumarchaeota	10	Y	N	Beam et al., 2012
Novel Thermoplasmatales	12	Y	N	Kozubal et al., 2012
'Geoarchaeota'	3	Y	N	Kozubal et al., 2012
Sulfolobales_MK5	14	Y	Y	Kozubal et al., 2012

Table 1. Relative abundance of archaeal populations in BE_D and OSP_B inferred from *de novo* metagenome assemblies. HCO = heme copper oxidase. Data from Bernstein and Beam et al., 2012

OSP_B Temporal Community Dynamics

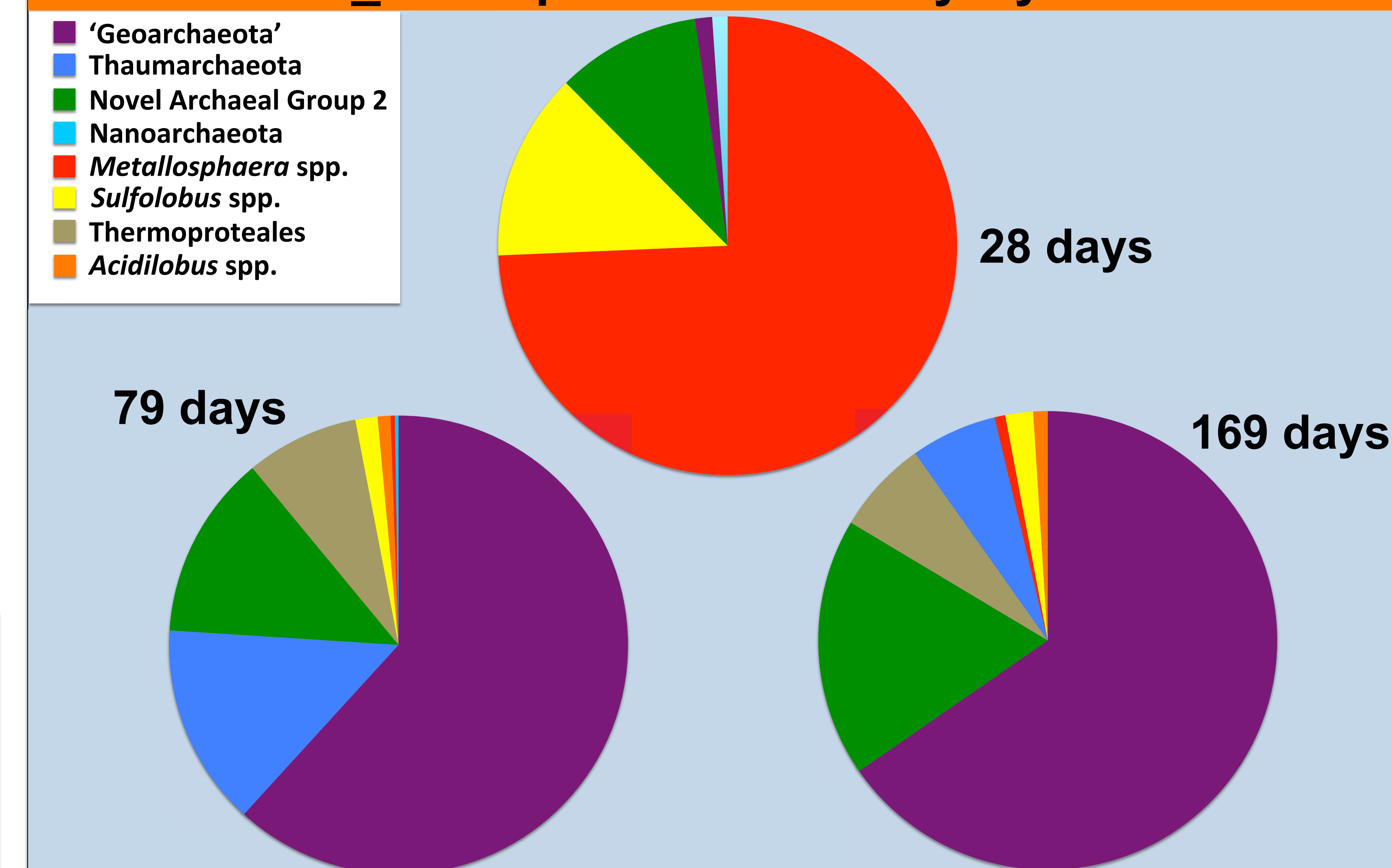


Figure 4. Community dynamics from OSP_B (2010) determined by 16S rRNA gene 454 sequencing. Phylum, order, and genus level taxon classifications based on > 97 % nucleotide identity to custom (YNP thermophiles) reference 16S rRNA gene database.

Objectives

- Quantify *in situ* Fe(III)-oxide deposition in two acidic geothermal springs.
- Determine temporal microbial community structure.
- Visualize microbial cell attachment and Fe(III)-oxide production
- Elucidate potential mechanisms controlling the formation of Fe(III)-oxide microbial biofilms.

Conceptual Model of Biofilm Development

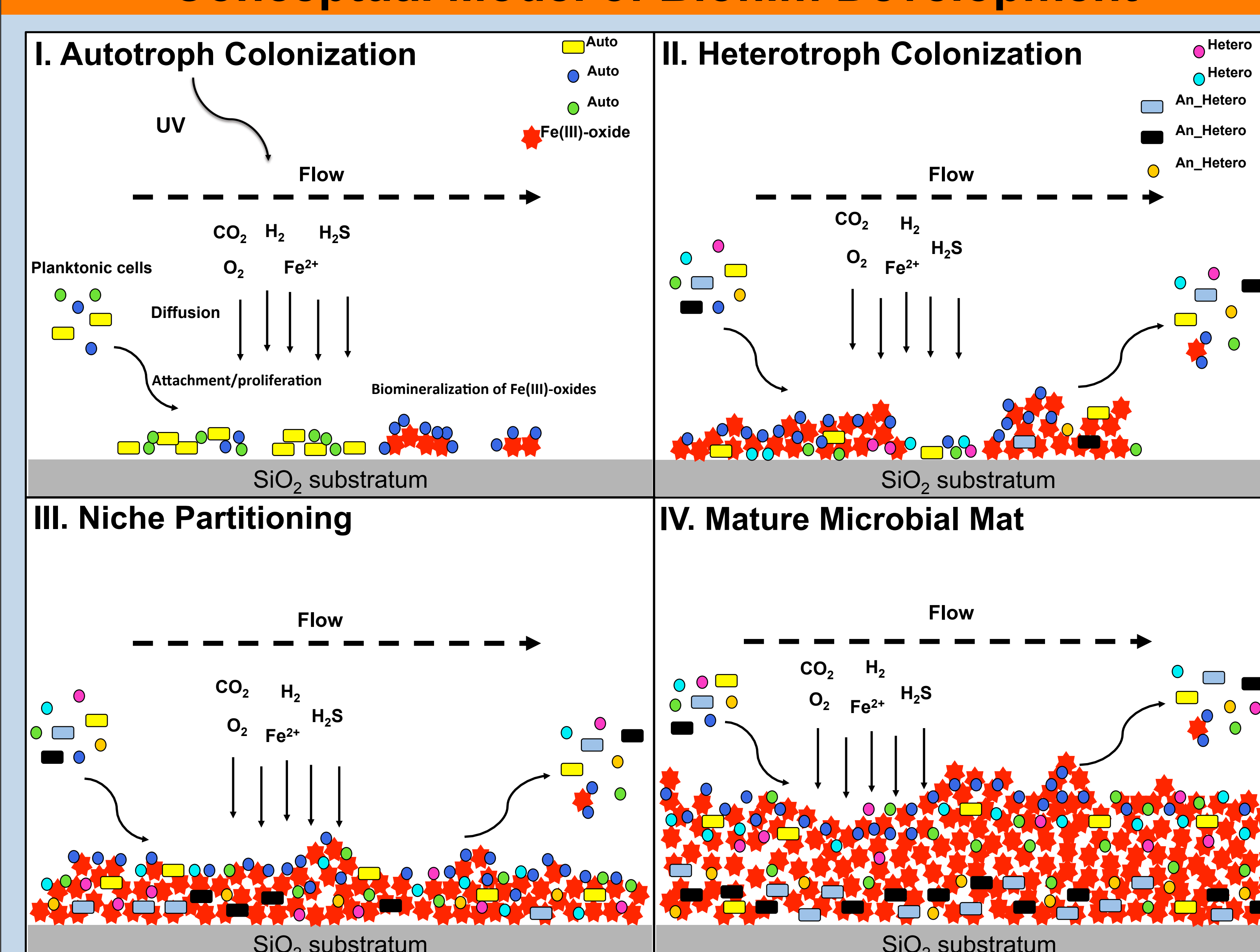


Figure 2. Conceptual model of Fe(III)-oxide microbial biofilm development

Methods

Glass (SiO₂) microscope slides were placed in the primary outflow channel of two acidic geothermal springs in YNP.

Analyzed for total Fe accumulated (oxalate Fe extraction, ferrozine) and DNA extracted to determine microbial community structure (e.g., 16S rRNA gene).

Scanning electron microscopy (SEM) used to visualize cell attachment and biomineralization.

Microbial Attachment and Biomineralization

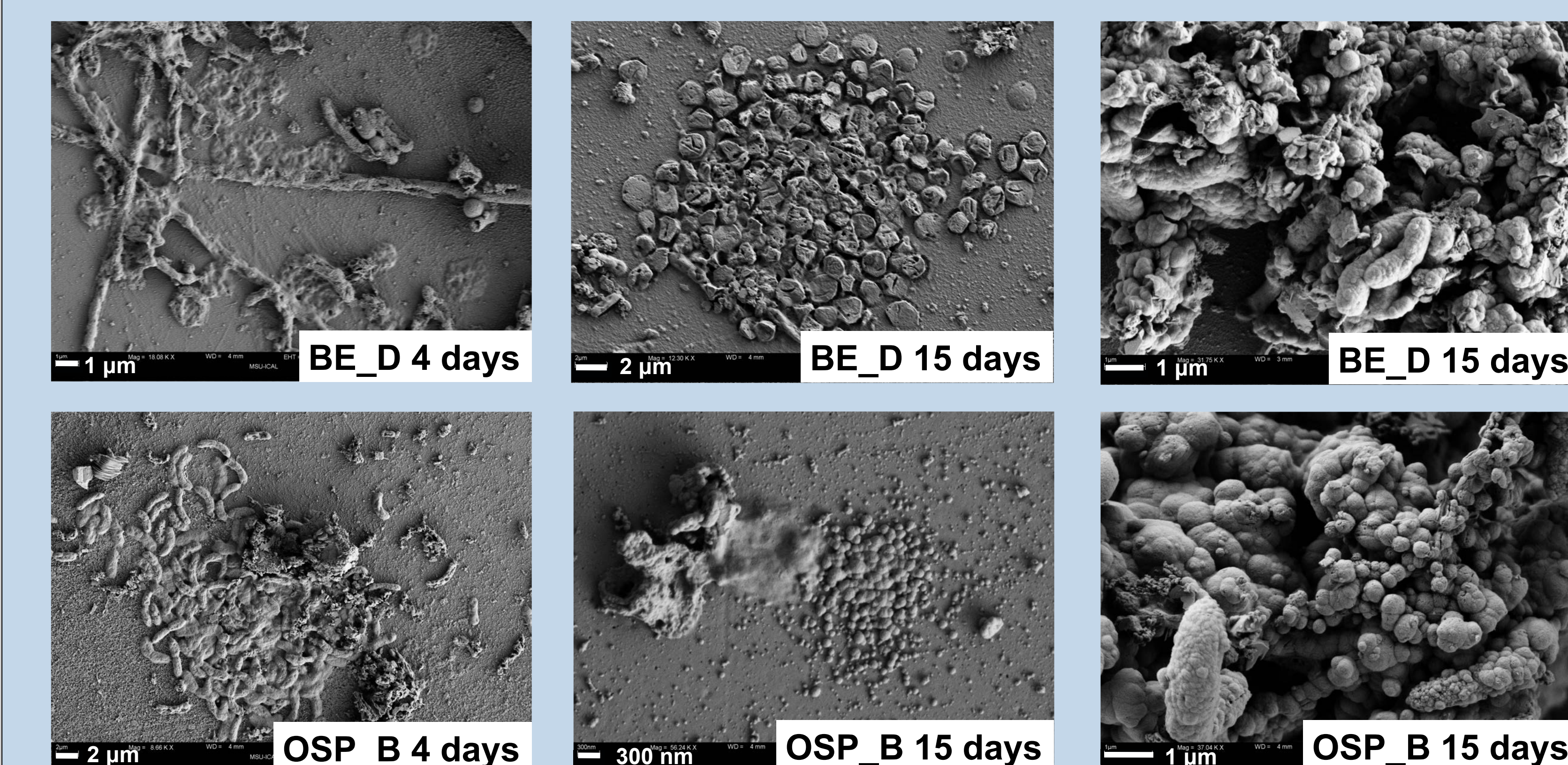
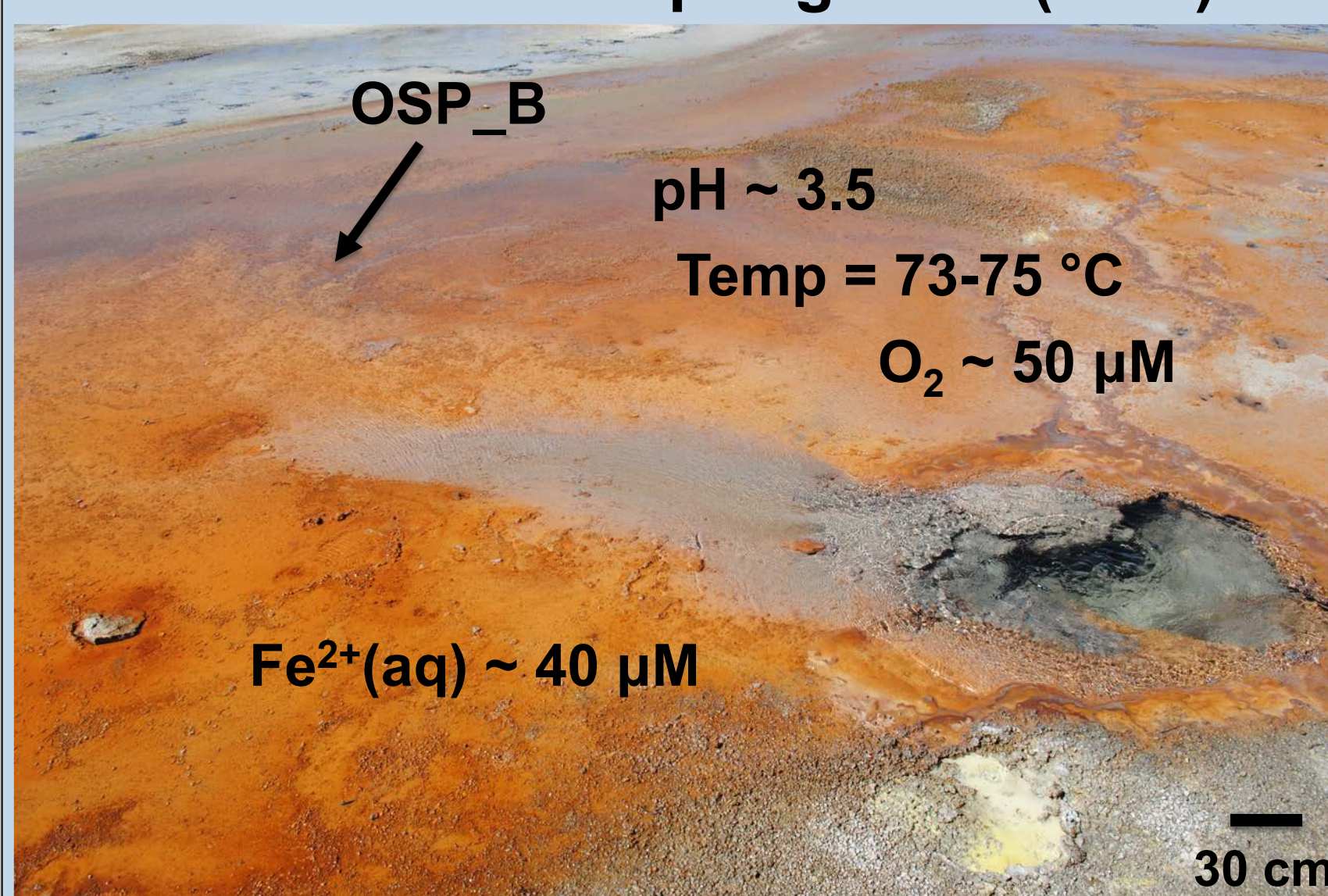


Figure 5. Scanning electron microscopy of *in situ* incubated slides revealing abundant microbial attachment and biomineralization.

Acidic Geothermal Springs

One Hundred Spring Plain (OSP)



Beowulf Spring East (BE)

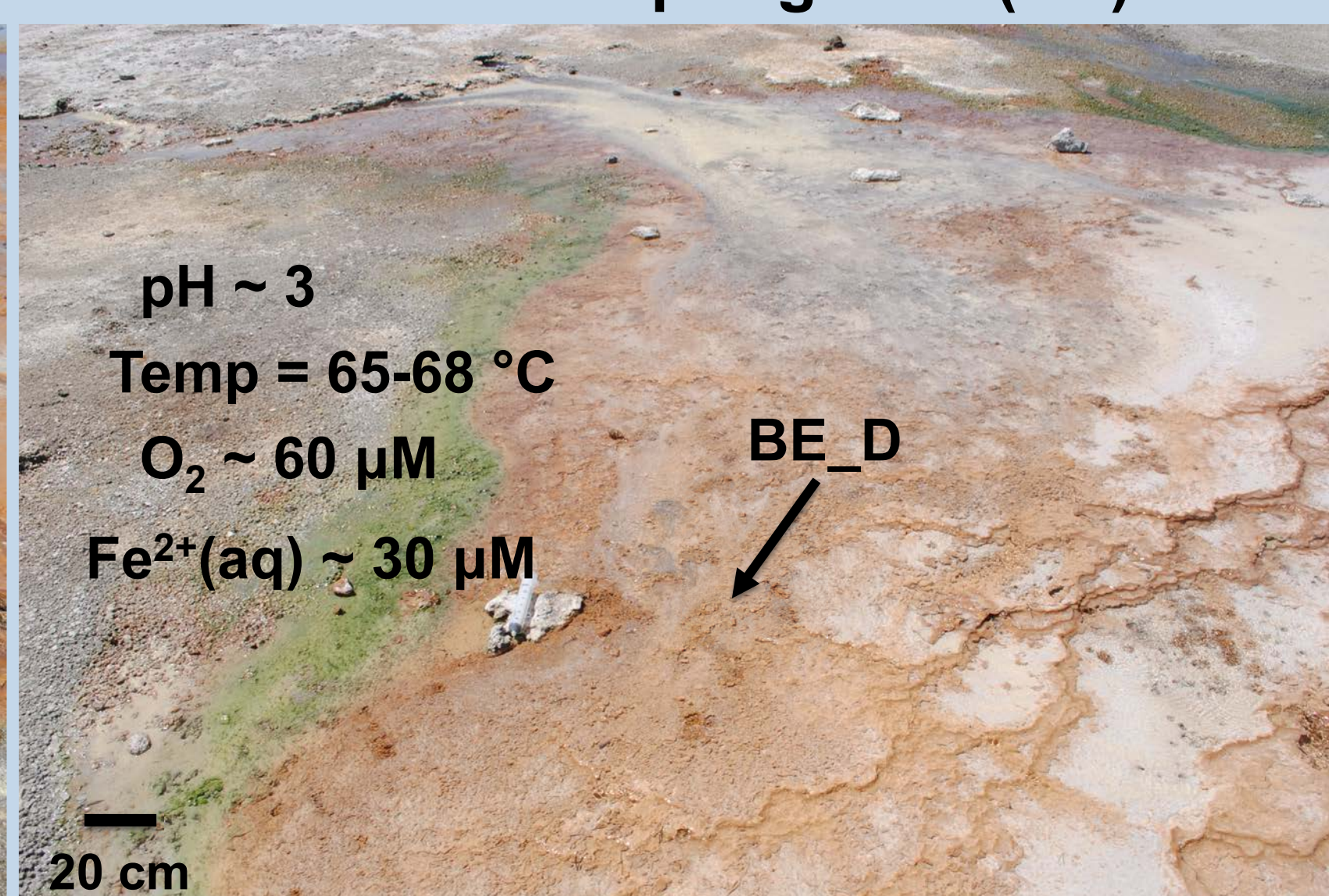


Figure 1. Photographs of One Hundred Spring Plain (OSP) and Beowulf Spring East located in Norris Geyser Basin, Yellowstone National Park, WY, USA. Flow channel velocities are approximately 0.1 – 0.2 m sec⁻¹ at each site.

Fe(III)-oxide Accretion Rates

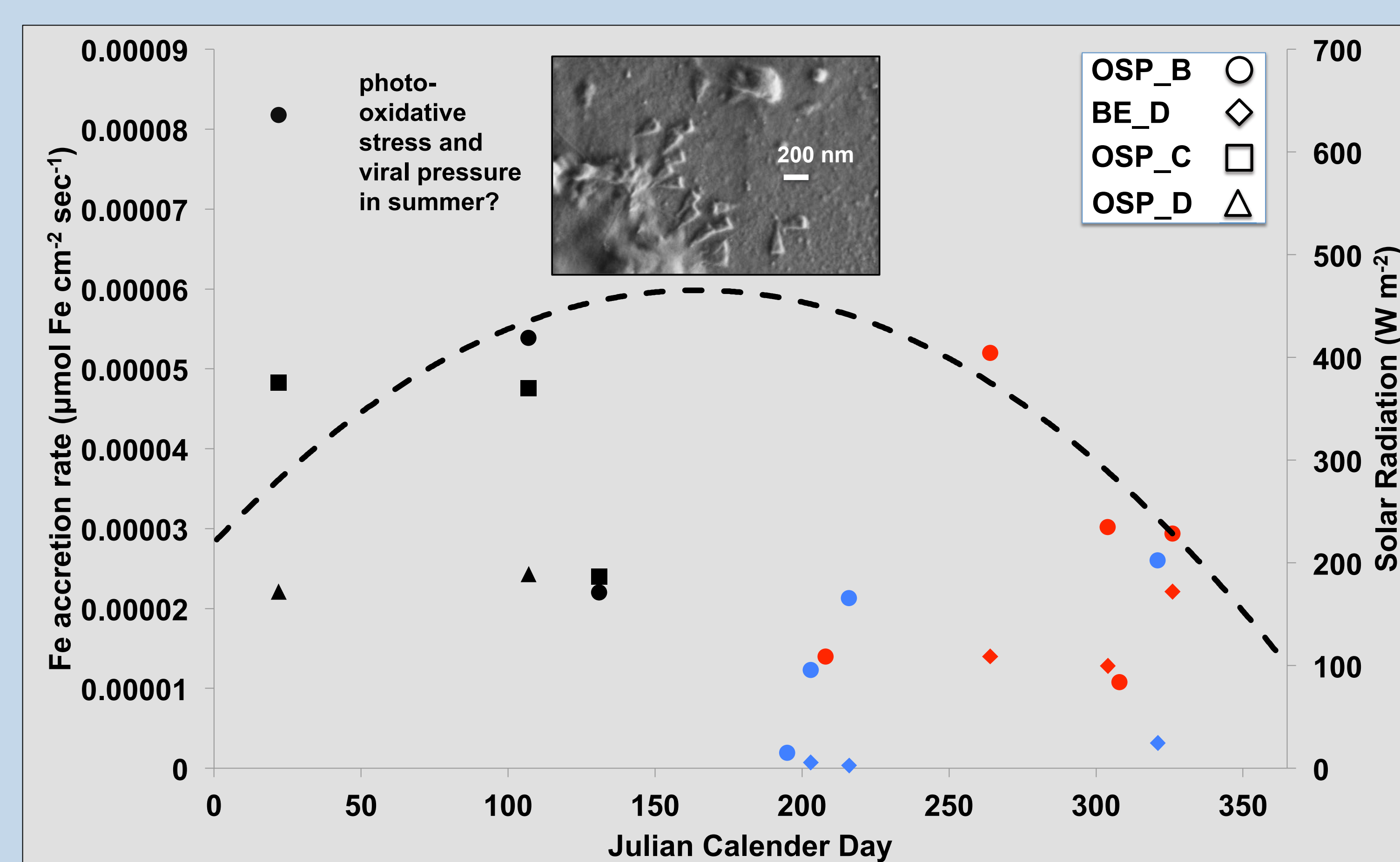


Figure 3. Yearly Fe(III)-oxide accretion rates from 2010-2012 and solar radiation data from 2009 (dotted line, <http://www.ntsg.umt.edu/project/topomet#data-product>). Black symbols = 2010, blue symbols = 2011, and red symbols = 2012. 2011 data from Bernstein et al., 2012.

Discussion

- Fe(III)-oxide biofilm formation is a **dynamic process**.
- Photo-oxidative** (e.g. UV) stress over summer months may reduce Fe(III)-oxide formation due to **viral pressure**.
- Autotrophic, Fe(II)-oxidizing *Metallosphaera* spp. are **abundant** during **early** biofilm formation.
- Heterotrophic members of a new candidate phylum 'Geoarchaeota' are more abundant in mature microbial mats.
- Microbial cells are attached and **abundant** within **4 days**.
- A combination of **biological, geochemical, and physical** processes **control** the formation of Fe(III)-oxide microbial mats.
- Future work will focus on temporal dynamics using 16S rRNA gene pyrotag sequencing, ¹³C analysis, FISH, and nanoSIMS.

Acknowledgements

J. P. B., H. C. B., and Z. J. J. were supported by NSF-IGERT in Geobiological Systems (DGE 0654336) at Montana State University. Additional thanks to C. Hendrix and S. Gunther for providing YNP research permits, D. B. Rusch (Indiana University-Bloomington), S. G. Tringe (DOE-JGI) for computational and sequencing support, and Pacific Northwest National Laboratory (Foundational Science Focus Area in Biological Interactions) for additional project support.