Spatially-resolved genomic, molecular organic, and stable isotopic analyses of an actively-accreting freshwater microbialite from Cuatro Ciénegas, Mexico

David Hollander, Mya Breitbart & Anthony Nitti-
College of Marine Science, University of South Florida, St. Petersburg, FL
Janet Siefert- Rice U., TX, Valeria Souza- UNAM, MX.

Outline

- Ancient & Modern Microbialites
- Introduction to Cuatro Ciénegas
  - Conceptual approach & objectives
- Research Approach
  1. Community structure
  2. Molecular signatures
  3. Carbonate accretion
  4. Isotopic composition
- Summary: Integrated Conclusions

Ancient Stromatolites
&
Modern Microbialites

Ancient Stromatolites

Fossil Evidence of Early Life

- thought to be the product of some of the earliest biological communities on Earth (>3 billion years ago)
- abundant through much of the Proterozoic
- analogous to modern microbialites

Window to the past

- geochemical signatures are retained within the $CaCO_3$
- understanding the source of these signatures could help with the understanding of life processes on early Earth

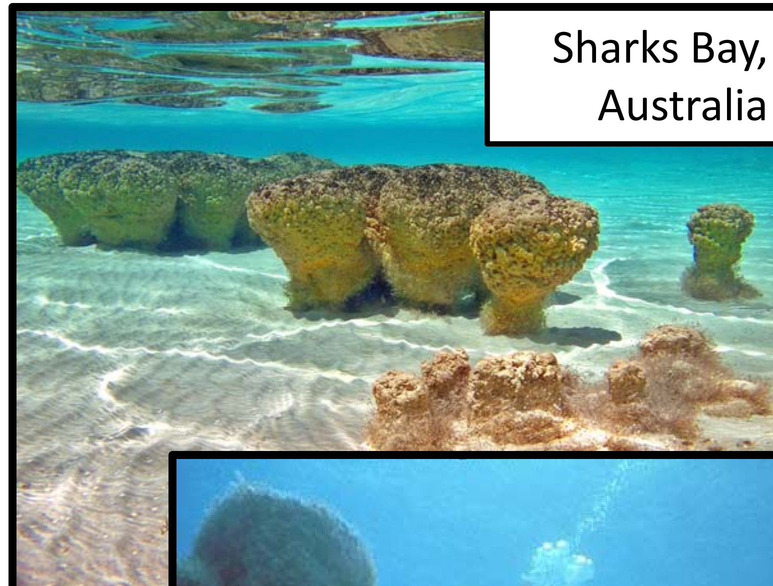
Modern Microbialites

<u>Living rocks</u>

- organosedimentary microbial mats
- microbial consortia influences $CaCO_3$ accretion (trapping and precipitation)

<u>Relatively uncommon</u>

- found in diverse environments throughout the world
  - hot/cold, marine, freshwater, hypersaline
- environmental factors (water chemistry) are often conducive to $CaCO_3$ precipitation



Sharks Bay, Australia

Highborne Cay, Bahamas

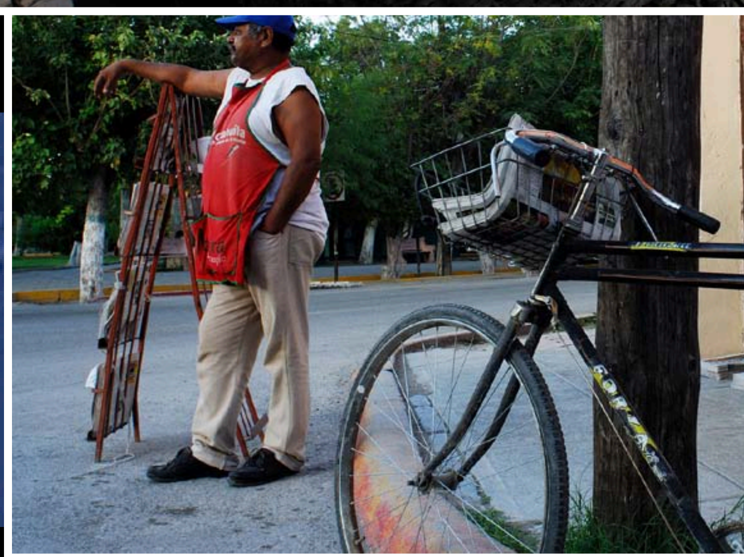
Pavilion Lake, Canada

Laguna Bacalar, Mexico

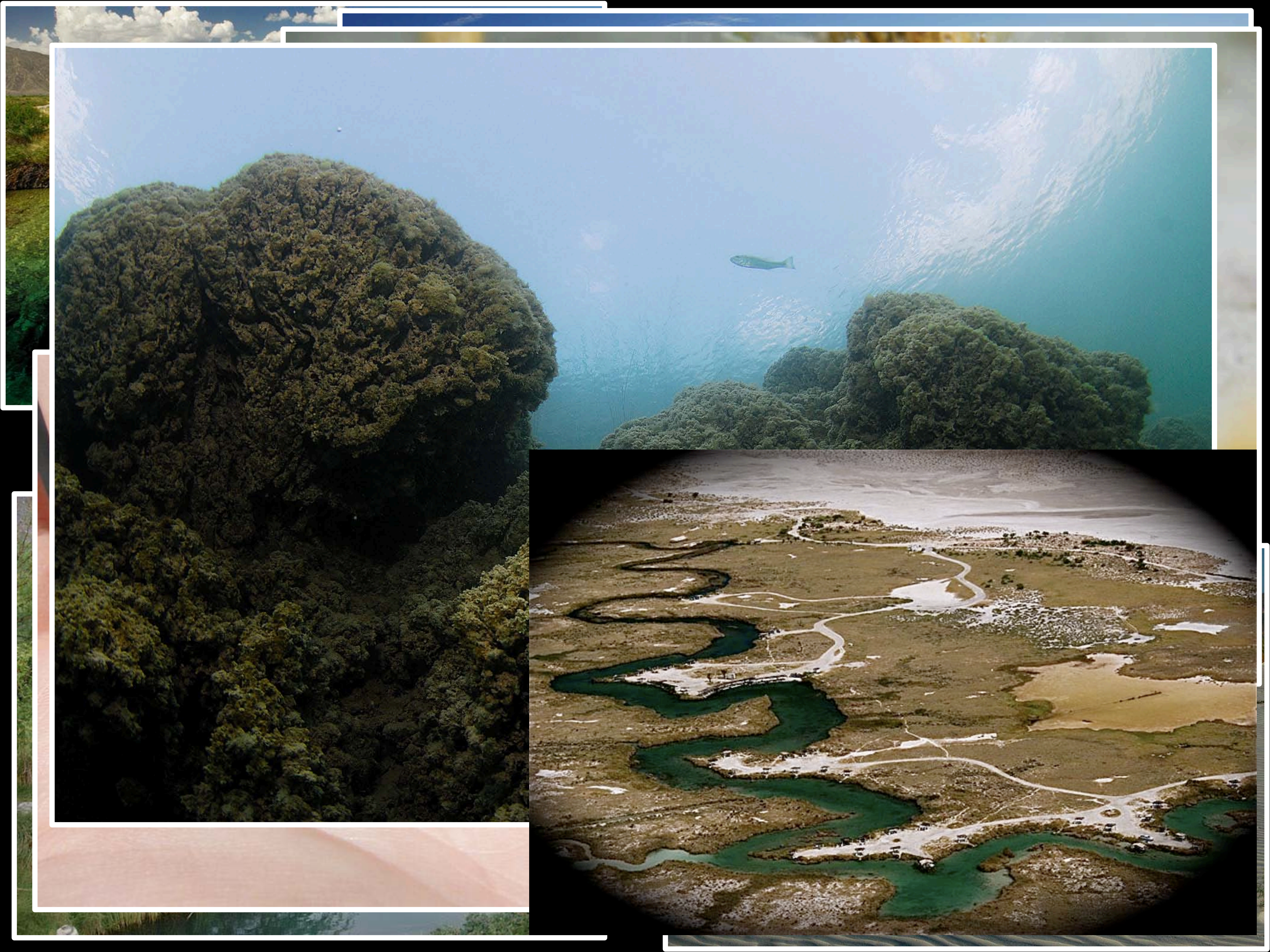
Cuatro Ciénegas


San Juan

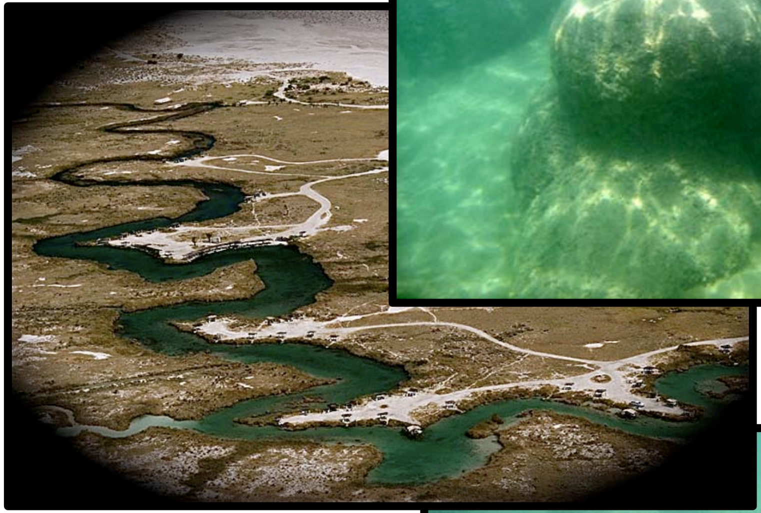

ZAPATERIA
POSADA
LAVADO Y ENGRASA
Restaurante
Restaurante




SIMAS
SISTEMA MUNICIPAL DE AGUAS Y SANEAMIENTO DE CUATRO CIENEGAS.
urban challenge
think on the run



Rio Mesquites



Spring-fed system

- Groundwater dependent habitat; karstic terrain
- Largest river in basin; 2 – 20 meters wide; 2.5 meters deep

Water Chemistry

- Rich in HCO_3^-, SO_4^{2-}
- Low Na^+, Cl^-
- Extremely high nitrate and low phosphate levels

Discharge

- Into man-made canals (flow out of basin)
- Formerly, into lagoons or “marshes” that gave the city of Cuatro Ciénegas its name

Living Microbialites

- Large amorphous and small round microbialites

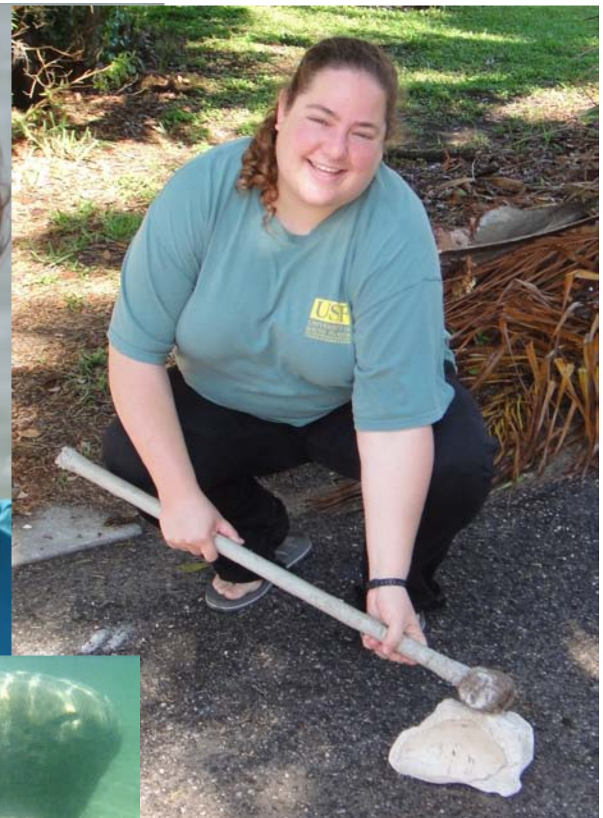
An "organosedimentary" microbial mat

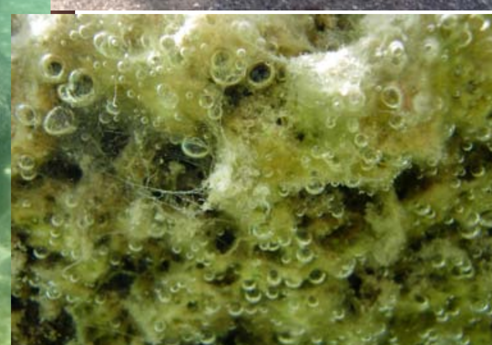
What makes a rock living?

As the microbial community traps sediment and produces excess organic material it migrates upwards and outwards

Over time the surface becomes buried, and the physical, chemical and biological characteristics of the matrix change

Previous Research "Mashed Microbialite"





(Breitbart et al., 2008, Environmental Microbiology)

"Metagenomic and stable isotope analyses of freshwater microbialites from Cuatro Cienegas, Mexcio"

Bulk results show that a complex **consortium of microorganisms, autotrophic and heterotrophic, aerobic and anaerobic**, are associated with carbonate precipitation.

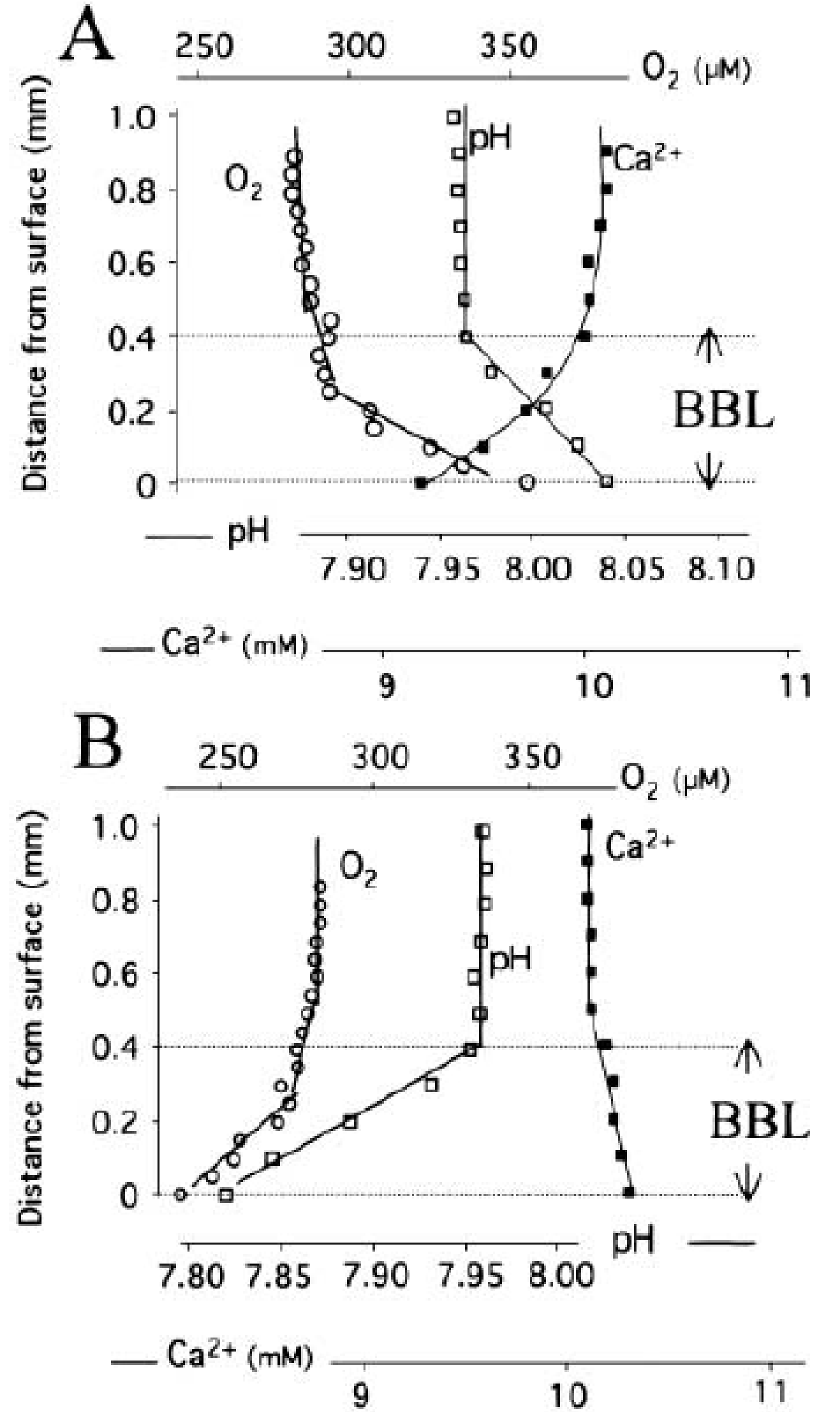
Metagenome has a high abundance of genes for:

- photosynthesis, all wavelengths
- diverse phosphorous cycling
- sulfo- & nitrogen-based lipids
- biofilm formation (colonization)
- motility, quorum sensing
- chemotaxis
- temporal regulation (circadian clock)
- extracellular polymeric substances (EPS)

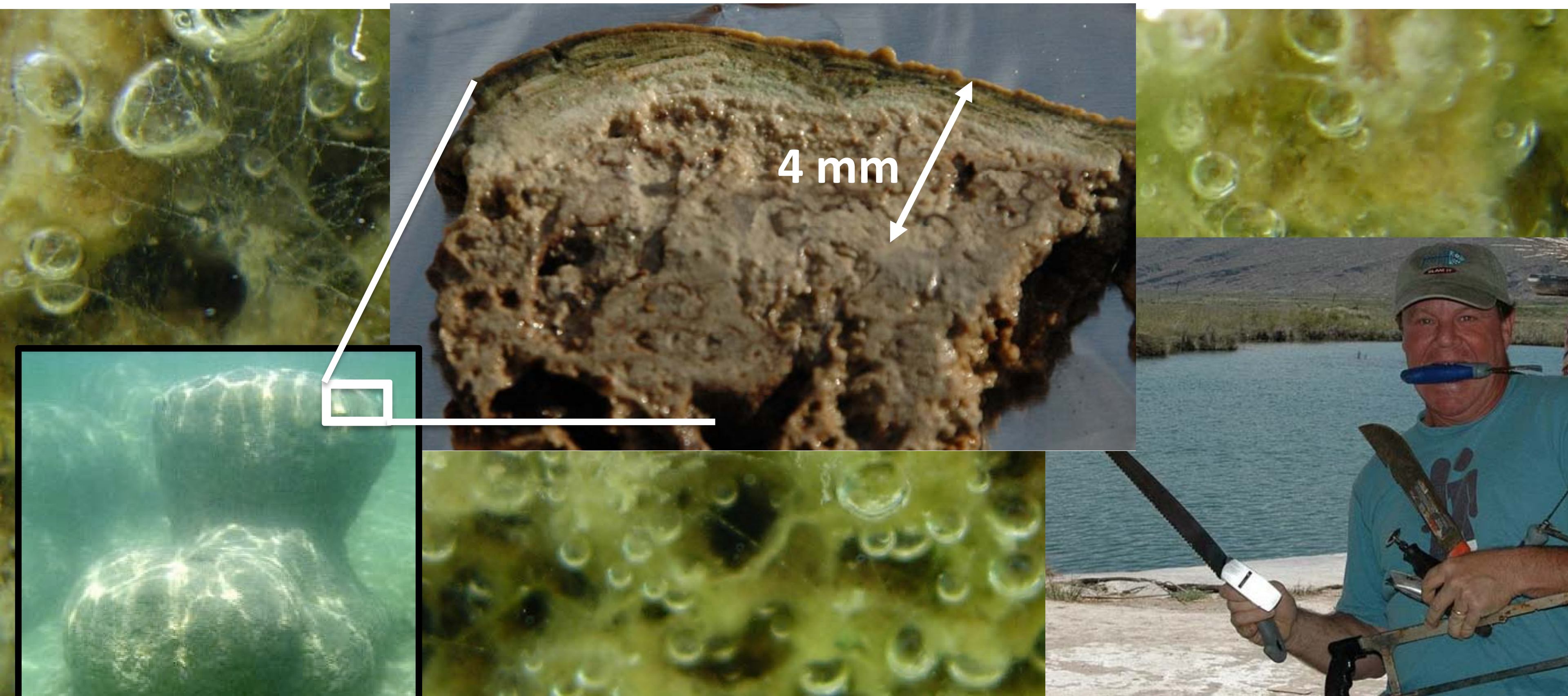
Microscale analyses…
Beauty is in the details

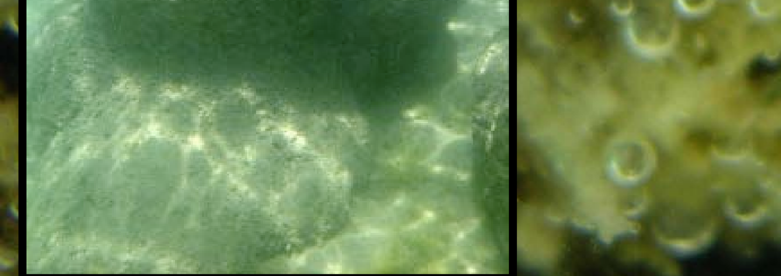
- Gene presence ≠ gene expression/activity
- Need to shift from bulk analyses to detailed spatial & temporal analyses to microbially-mediated redox processes

Garcia-Pichel et al. 2004



Spatial structure and temporal regulation are critica developing/maintaining chemical microenvironmer and coordinating microbial processes





“Detailed Dissection”

The beauty is in the detail

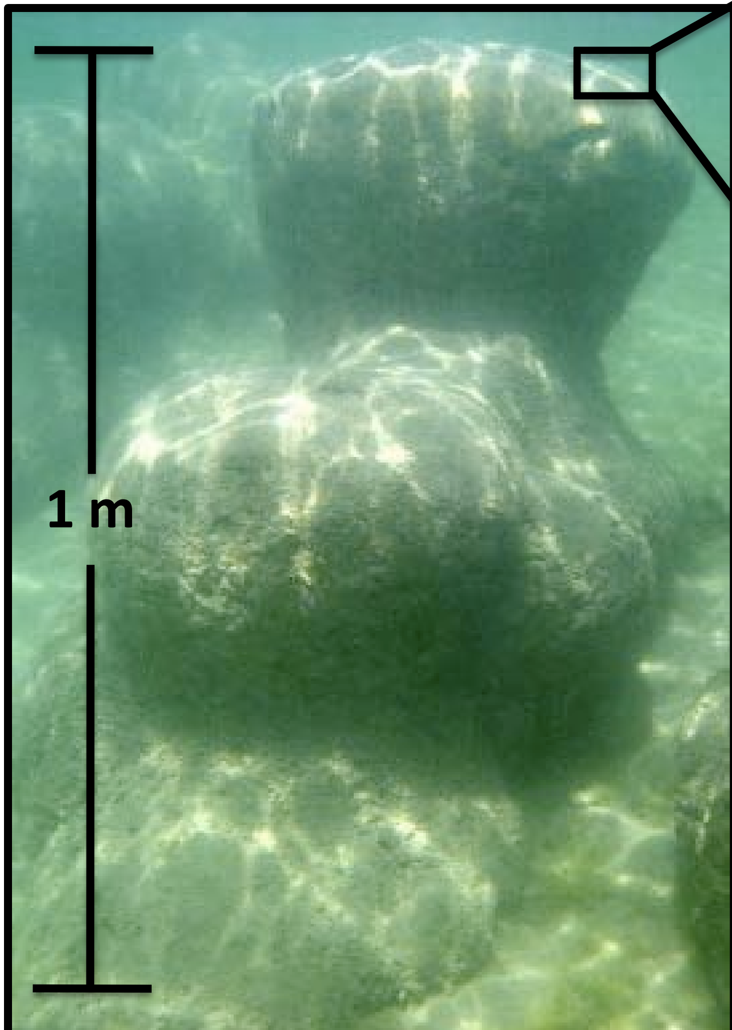
Conceptual goal

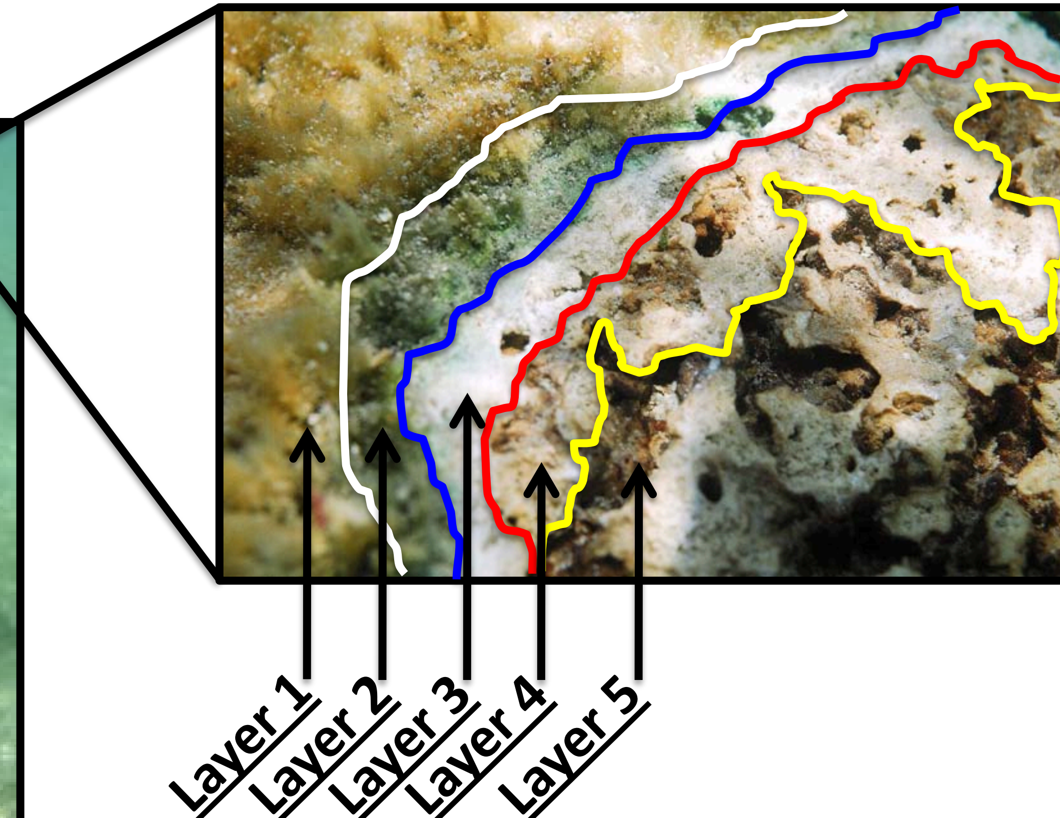
detailed spatial analysis of the bacterial community and geochemical signatures in modern, actively-accreting microbialites to understand the processes by which they form

---

- the microbial community spans the surface of the microbialite
- sharp gradients in the chemical parameters that control $CaCO_3$ precipitation occur over mm scales
- this suggests that the organisms and processes responsible for $CaCO_3$ accretion vary across similar spatial scales

Isolation of microbialite layers





- these 5 layers span the upper **<u>20 mm</u>** of the microbialite surface

Objectives

1. Characterize the bacterial communities of all 5 layers

2. Characterize the molecular composition of each layer to determine:

    a) if the lipid content directly reflects the observed microbial community

    b) how the biomass is degraded, preserved, or altered with depth

3. Determine the relative accretion of $CaCO_3$ at each layer to determine what organisms and processes contribute to microbialite formation

4. Characterize the carbon isotope composition of organic matter and $CaCO_3$ to help determine how different carbon cycling processes affect microbialite formation

1) Community Composition:

A genomic analysis

who’s there?

Methods: DNA extraction and amplification

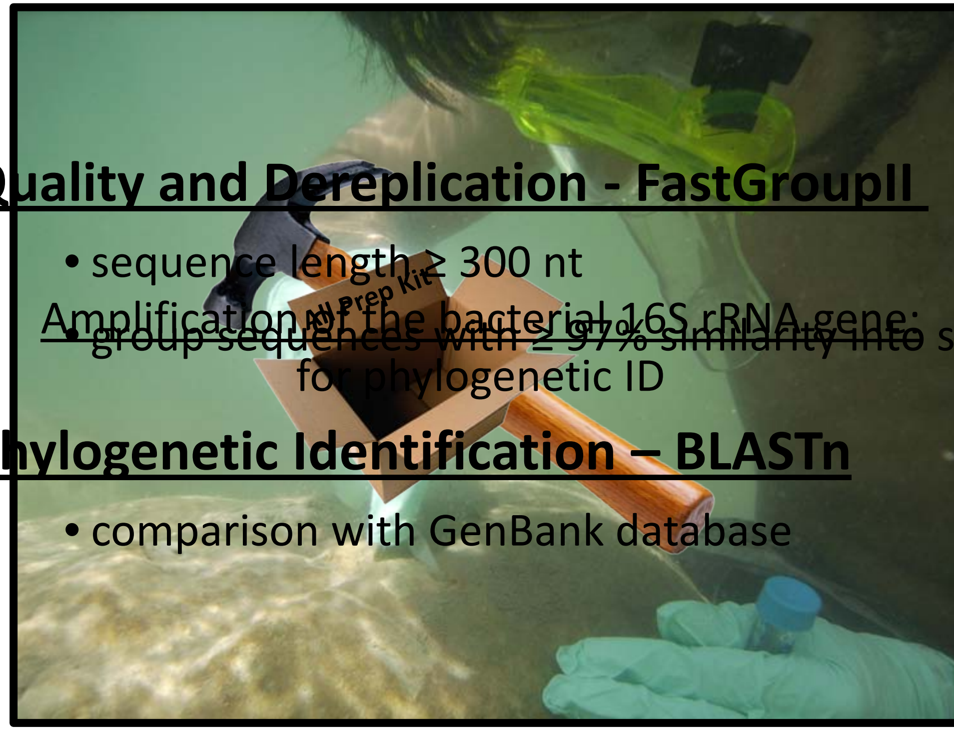
Sample collection: Isolation of microbialite layers

Sample disruption & homogenization

DNA extraction

PCR amplification

Cloning & Sequencing



Quality and Dereplication - FastGroupII

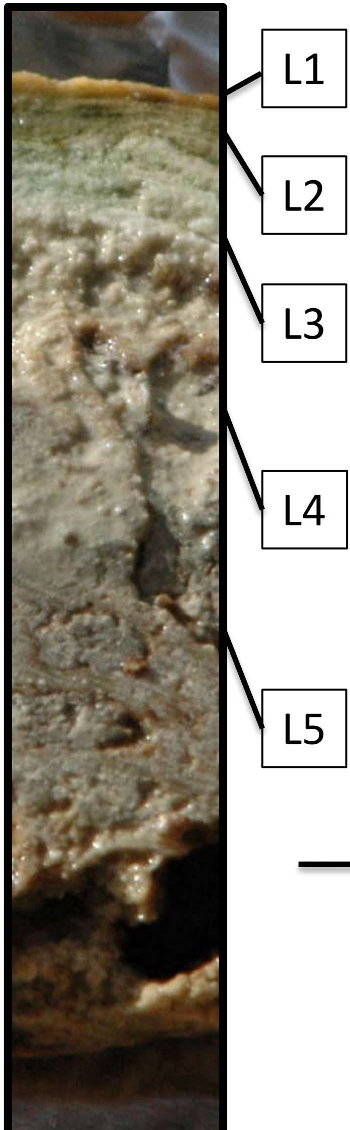
- sequence length ≥ 300 nt
- group sequences with ≥ 97% similarity into species

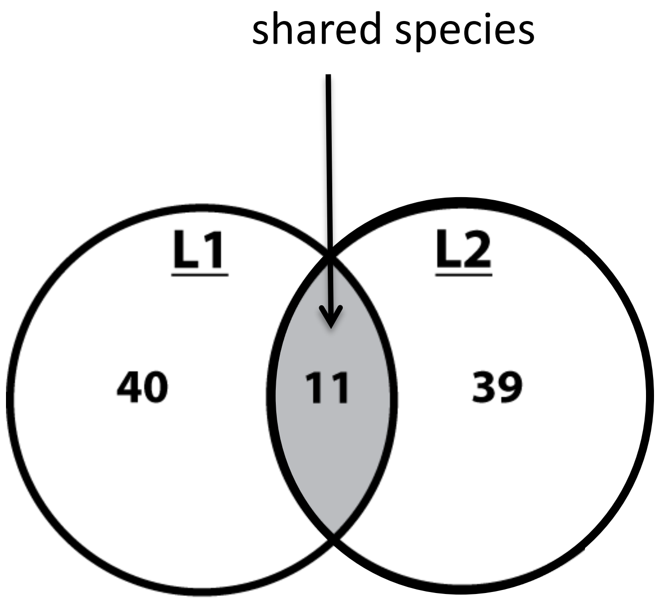
Amplification of the bacterial 16S rRNA gene: for phylogenetic ID

Phylogenetic Identification – BLASTn

- comparison with GenBank database

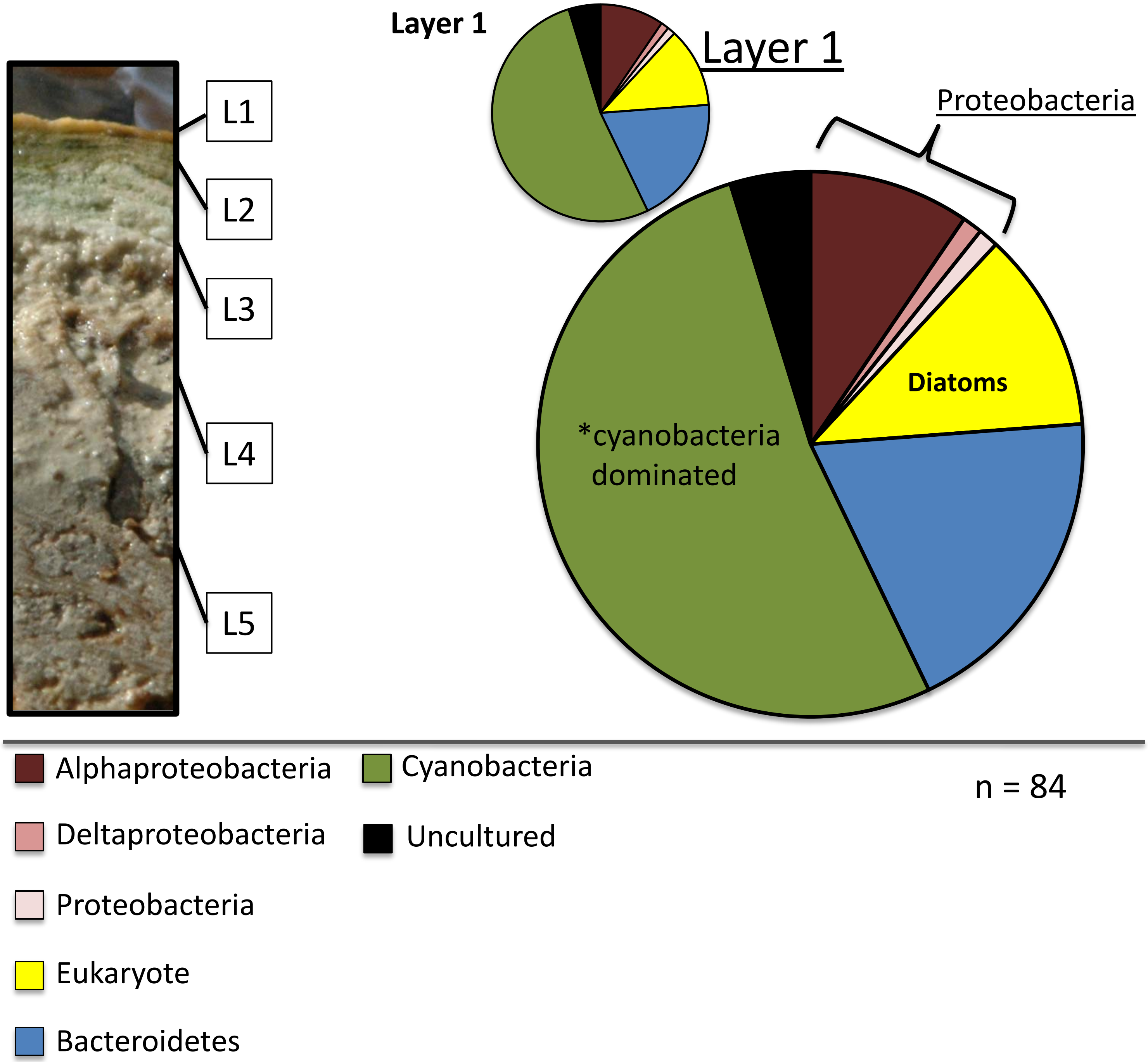
Results: discrete bacterial communities

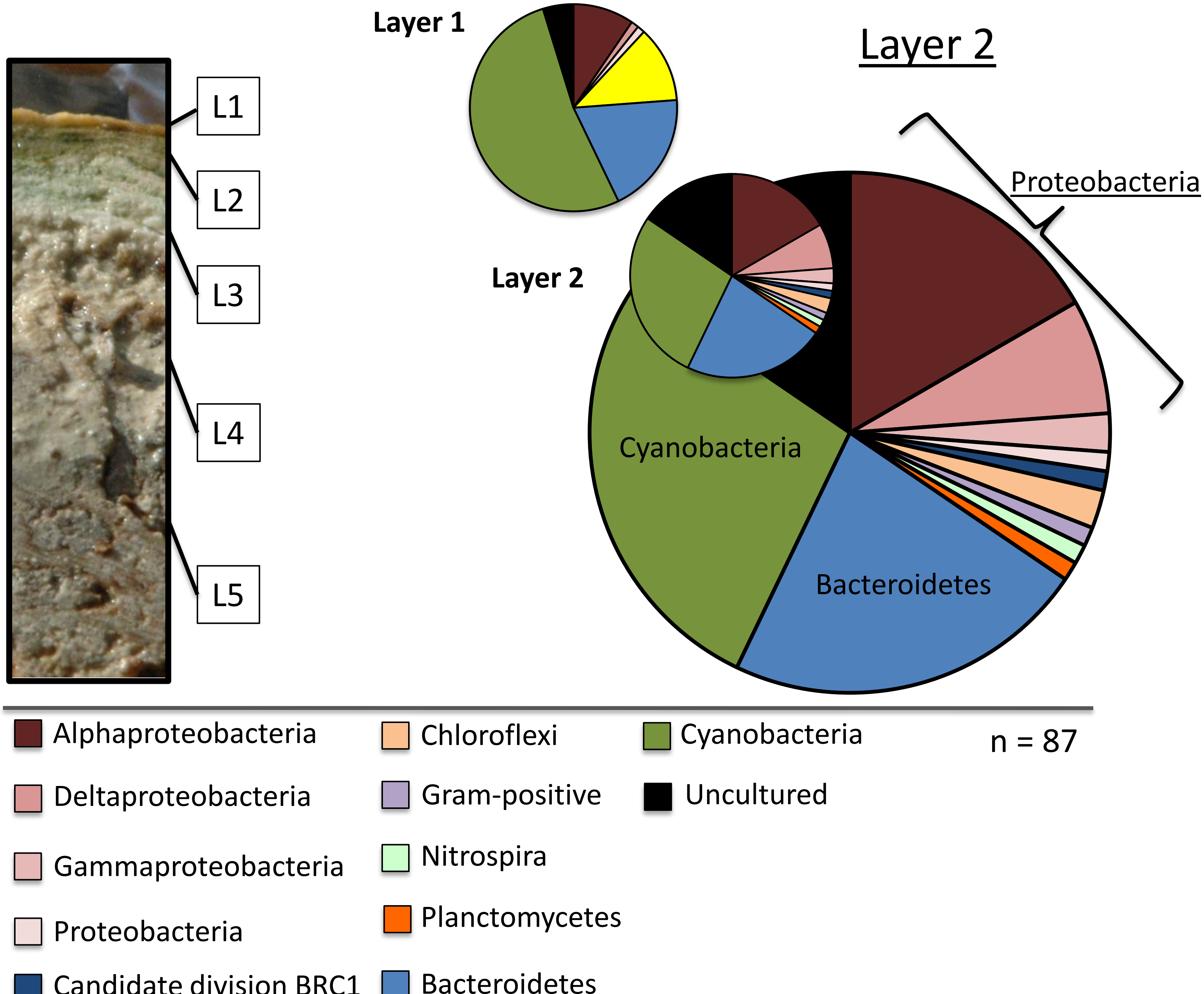


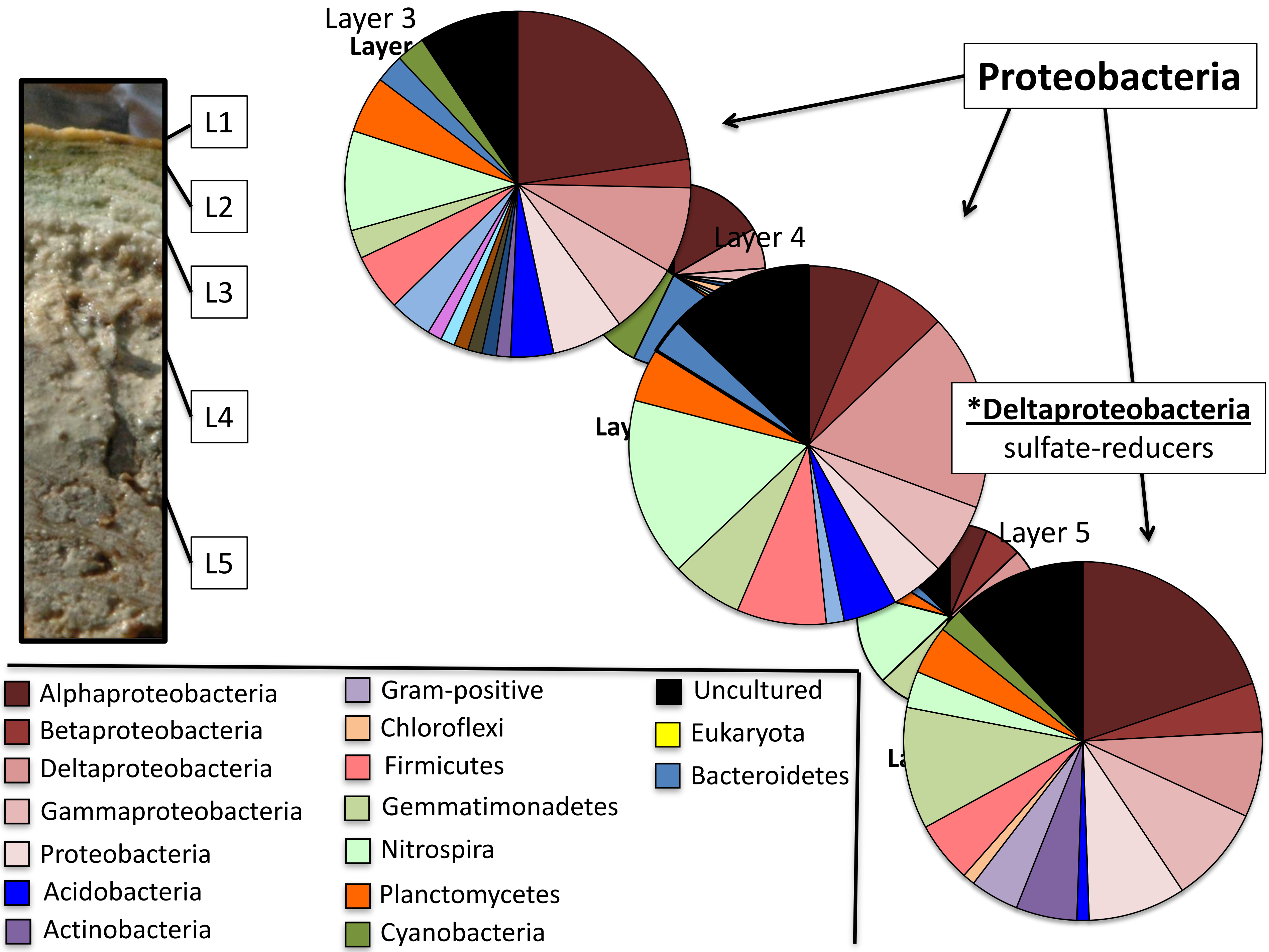


- 261 different species (399 total sequences)
- Little overlap in bacterial community of individual layers
- Validates layer-specific approach

Results: community composition

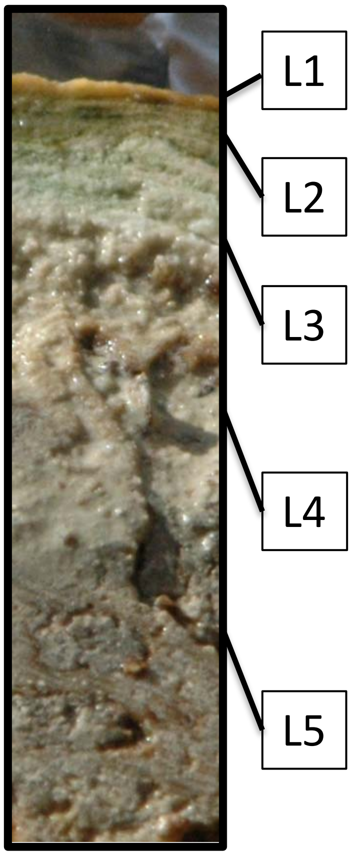

L1
L2
L3
L4
L5
Layer 1
Layer 1
Proteobacteria
Diatoms
*cyanobacteria dominated
Alphaproteobacteria
Deltaproteobacteria
Proteobacteria
Eukaryote
Bacteroidetes
Cyanobacteria
Uncultured
n = 84

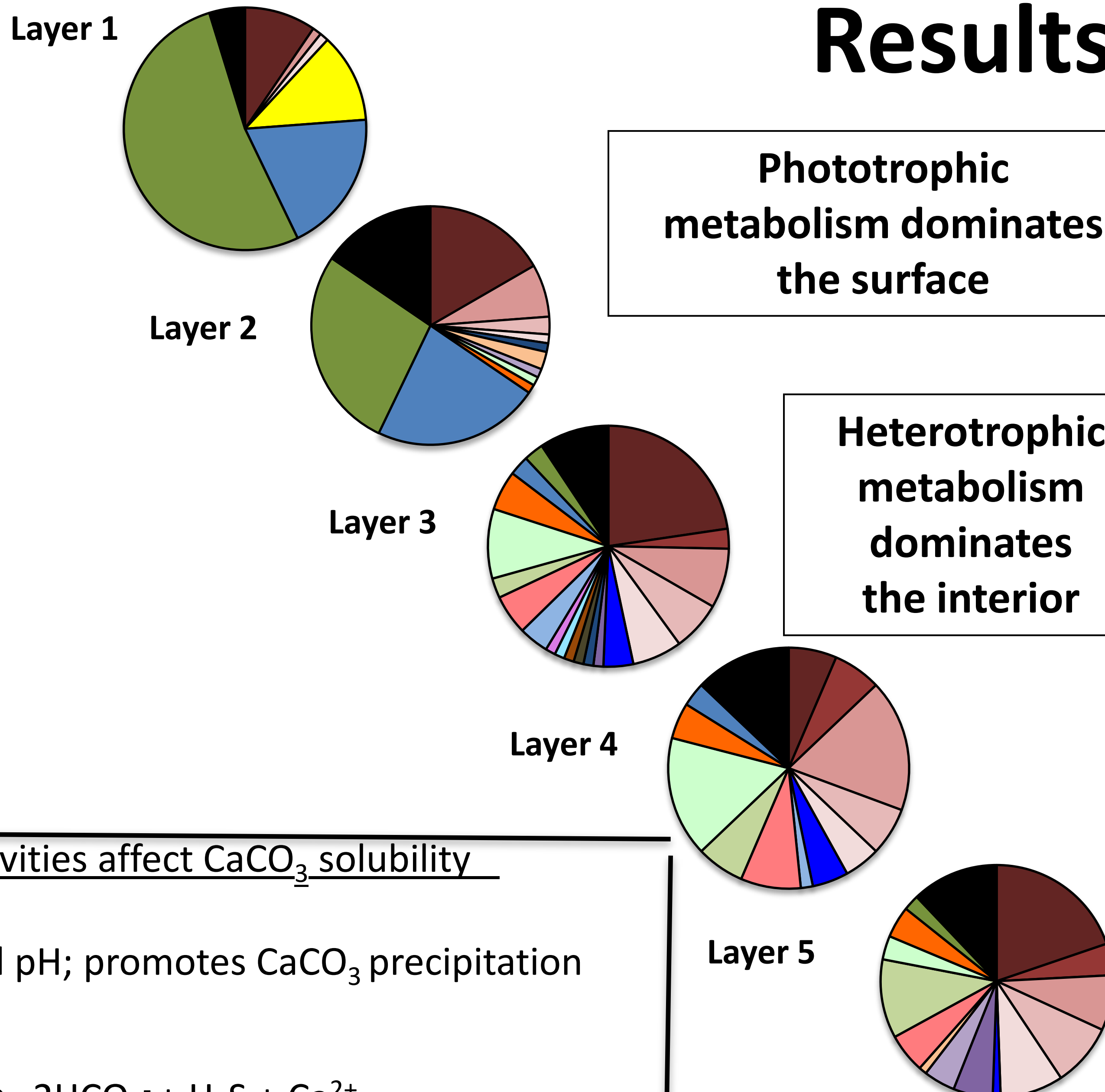

L1
L2
L3
L4
L5
Layer 1
Layer 2
Layer 2
Proteobacteria
Cyanobacteria
Bacteroidetes
Alphaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Proteobacteria
Candidate division BRC1
Chloroflexi
Gram-positive
Nitrospira
Planctomycetes
Bacteroidetes
Cyanobacteria
Uncultured
n = 87



Layer 3
Layer 4
Layer 5
L1
L2
L3
L4
L5
Proteobacteria
*Deltaproteobacteria
sulfate-reducers
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Proteobacteria
Acidobacteria
Actinobacteria
Gram-positive
Chloroflexi
Firmicutes
Gemmatimonadetes
Nitrospira
Planctomycetes
Cyanobacteria
Uncultured
Eukaryota
Bacteroidetes

Results





Phototrophic metabolism dominates the surface

Heterotrophic metabolism dominates the interior

metabolic activities affect $CaCO_3$ solubility

photosynthesis

CO_2 uptake raises local pH; promotes $CaCO_3$ precipitation

sulfate-reduction

$$Ca^{2+} + 2CH_2O + SO_4^{2-} \Rightarrow 2HCO_3^{-} + H_2S + Ca^{2+}$$

$$\Rightarrow \mathbf{CaCO_3} + H_2S + H_2O + CO_2$$

Conclusions: community composition

1) Discrete populations of bacteria in each layer

2) Photoautotrophic organisms dominate the surface
   - not observed at depth

3) Sulfate reducing δ-proteobacteria are abundant at the interior
   - primarily layer 4

Question:

Does the lipid signature change in the same way as the genomic signature?

2) Biomarker Distribution:
A molecular organic analysis

Why use lipid biomarkers?

- Like genetic signatures, certain lipid compounds are specific to a single group of organisms, making them "biological markers"

- Unlike genetic material, lipid compounds are often resistant towards decomposition processes, allowing them to be used to identify organisms long after they die

Methods: lipid extraction, separation, & ID

Sample disruption & homogenization

Total Lipid Extraction

Compound Class Separation

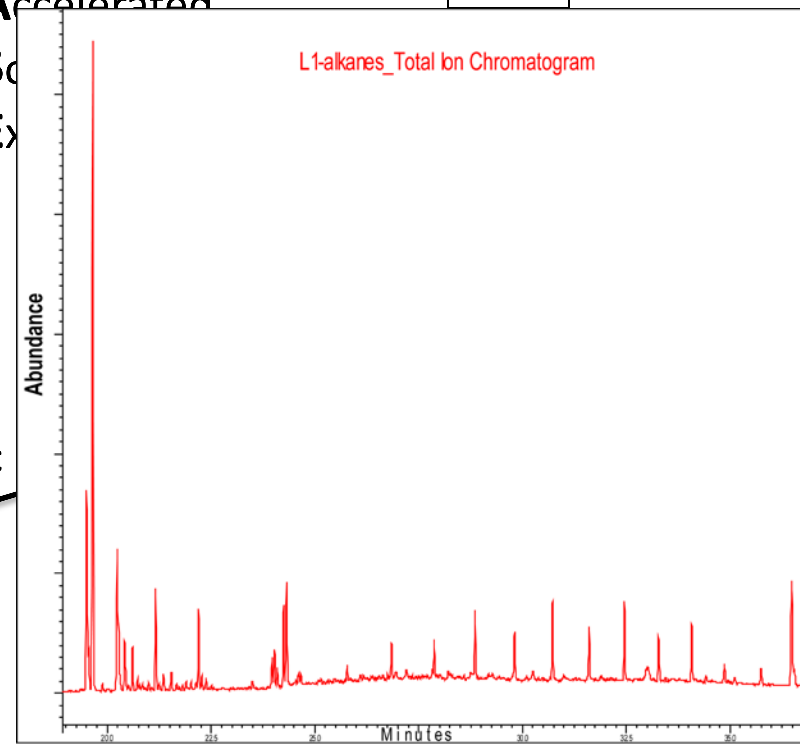
Derivatization

Compound ID and Quantification

Gas Chromatography
Mass spectrometry

TLE

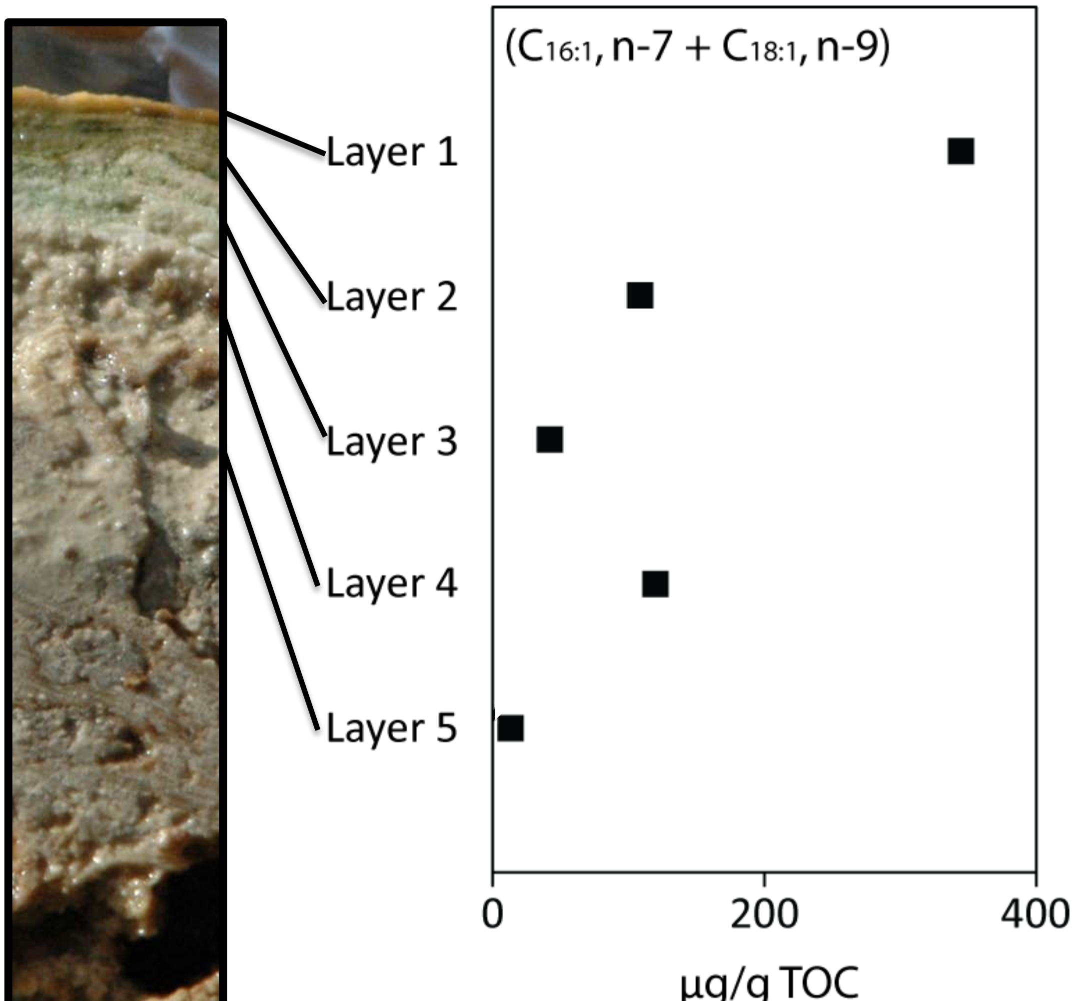
Accelerated
Sc
Ex



- trying to detect compounds that match the genomic data

fatty acids

Results: changing signatures with depth



Cyanobacterial biomarkers

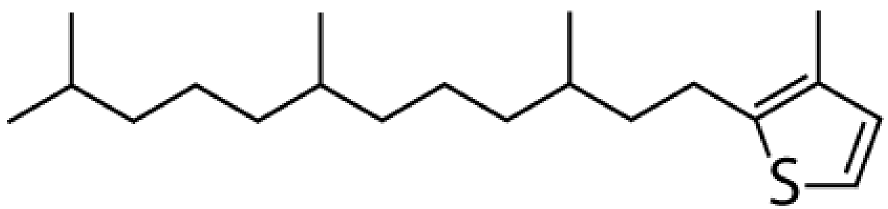
Diatom biomarkers

Results: changing signatures with depth

layer 5: two thiophene isomers

- sulfurized derivatives of phytol

phytol + $H_2S \Rightarrow$



- indicative of both the phototrophic community and sulfate reducing organisms

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with

Conclusions: lipid composition

1) Lipid composition reflects community as determined by genomics:
    - abundant phototrophic biomarkers
    - Sulfate-reducing bacteria biomarkers

2) Photoautotrophic biomass is efficiently degraded by the heterotrophic community at depth

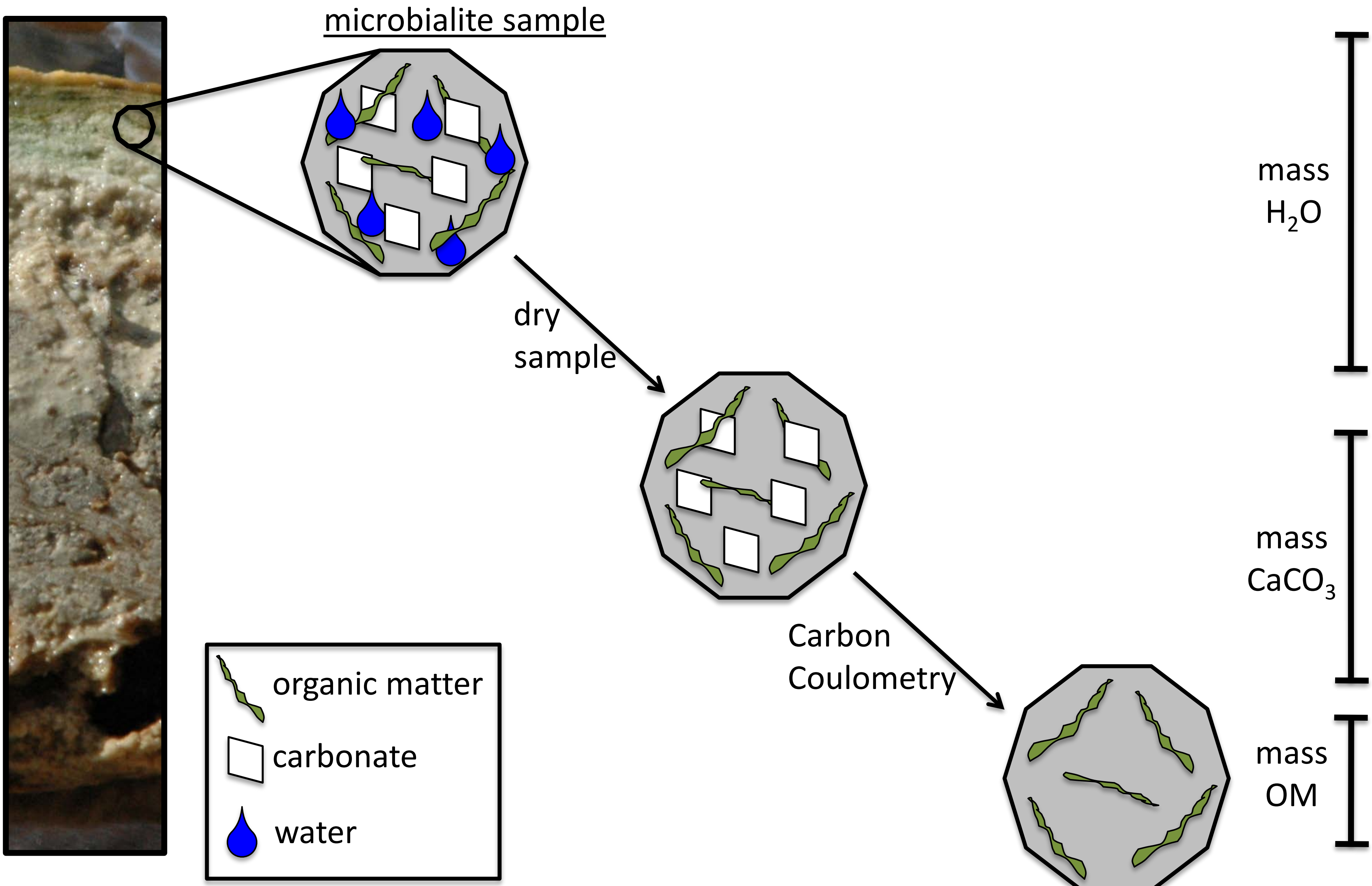
Question:

How does the distribution of organisms and changes in molecular composition relate to carbonate accretion?

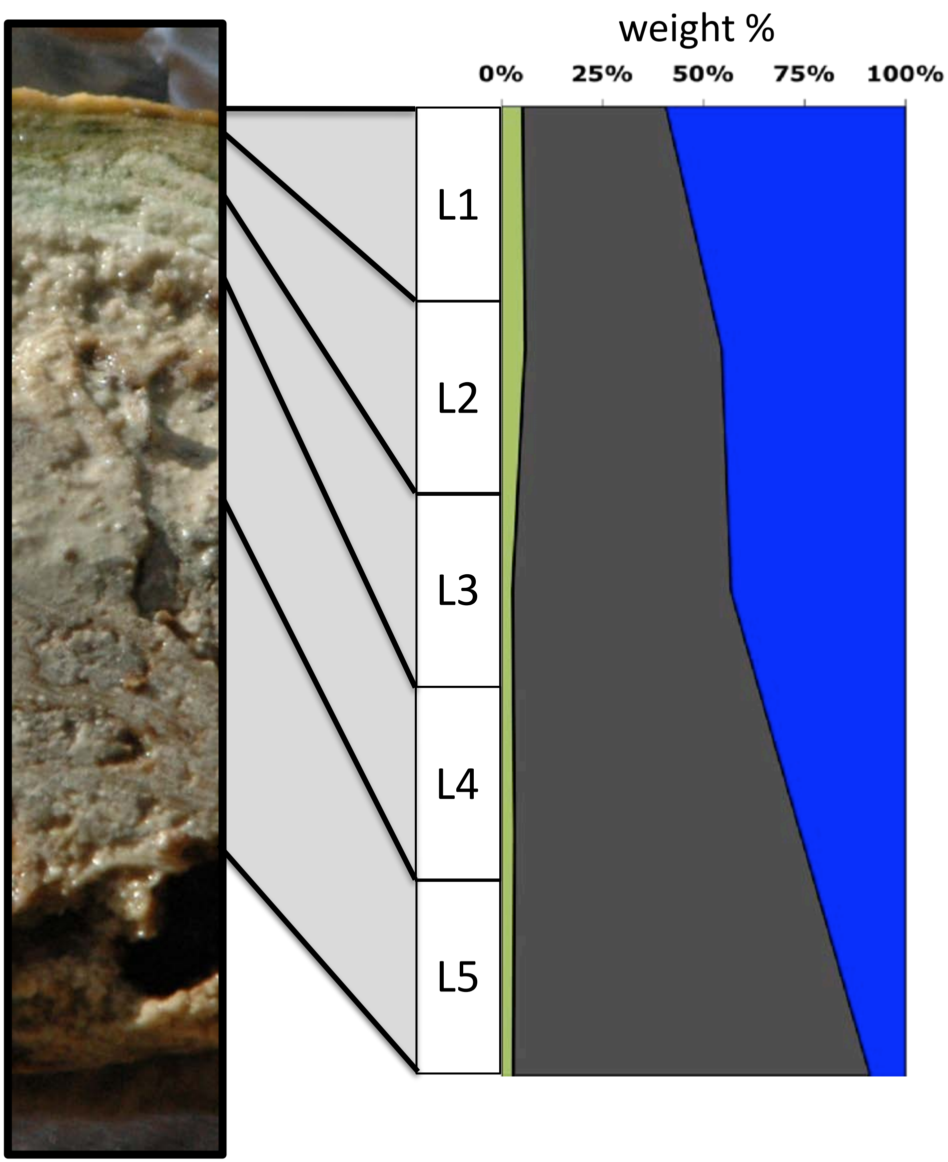
3) Carbonate Accretion:

A mass balance analysis

Methods: microbialite composition



Results: mass balance



<u>low organic matter content</u>

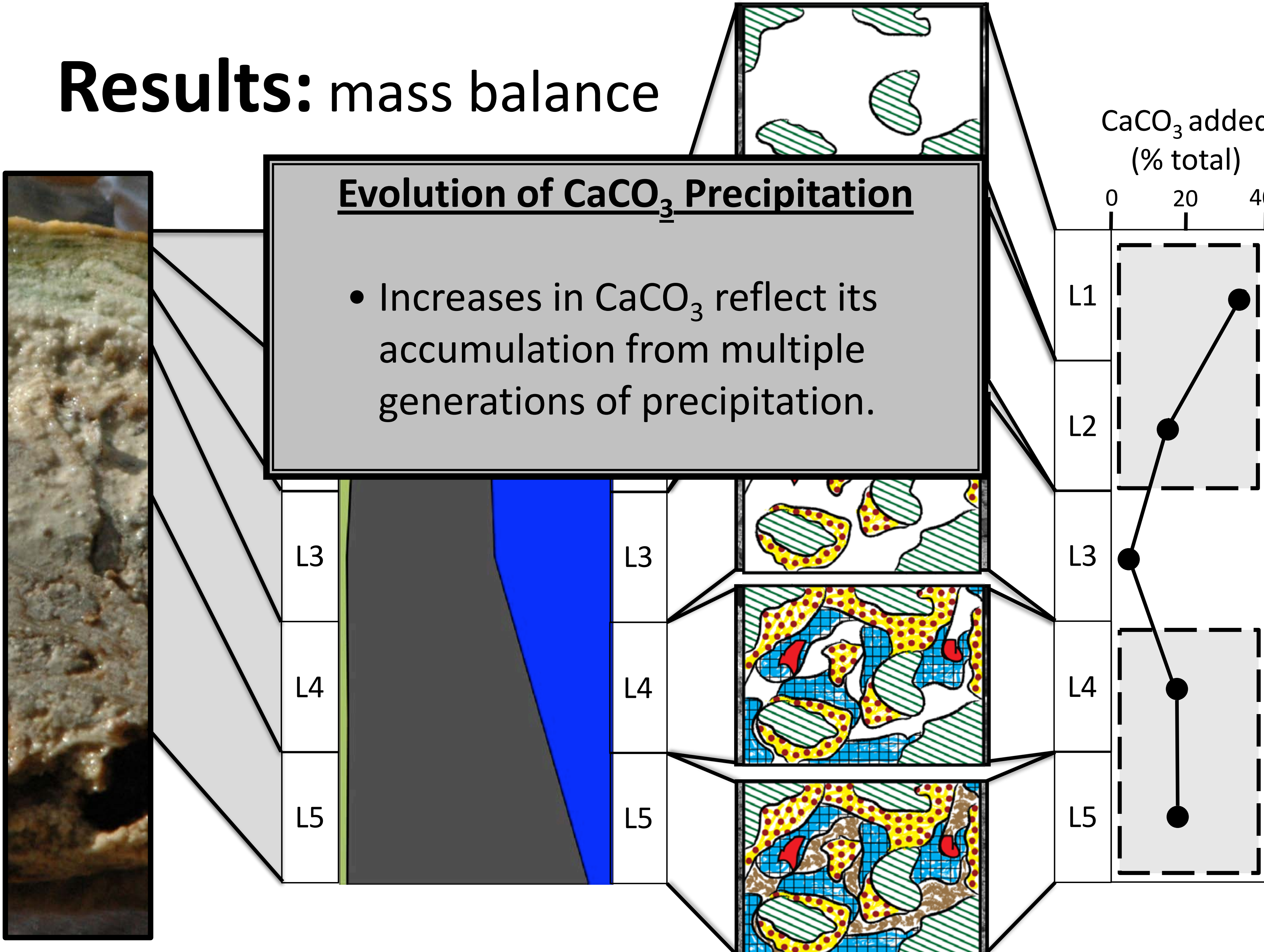
- less than 6% (by wt)
- decreases with depth

<u>$CaCO_3$ increases w/ depth</u>

- 35 – 90%
- precipitation not only at surface

*Where does $CaCO_3$ precipitation occur?

Results: mass balance



Evolution of $CaCO_3$ Precipitation

- Increases in $CaCO_3$ reflect its accumulation from multiple generations of precipitation.

Conclusions: carbonate accretion

1) Carbonate accounts for the majority of the microbialite matrix
   - 90% in layer 5
   - low OM content throughout

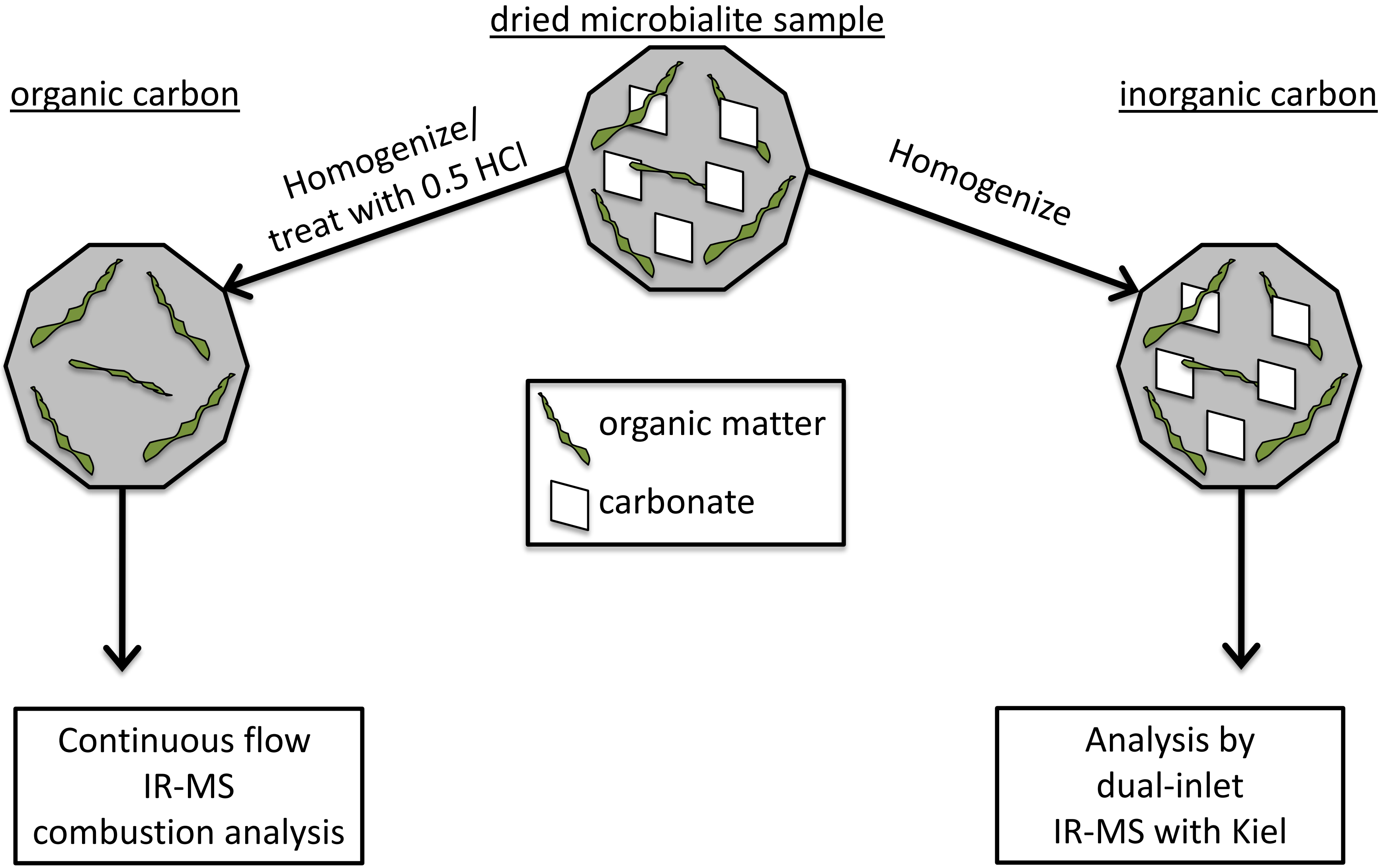
1) Multiple generations of $CaCO_3$ precipitation
   - 2 distinct zones
   - directly adjacent to areas of high phototroph and sulfate-reducer abundance

Question:

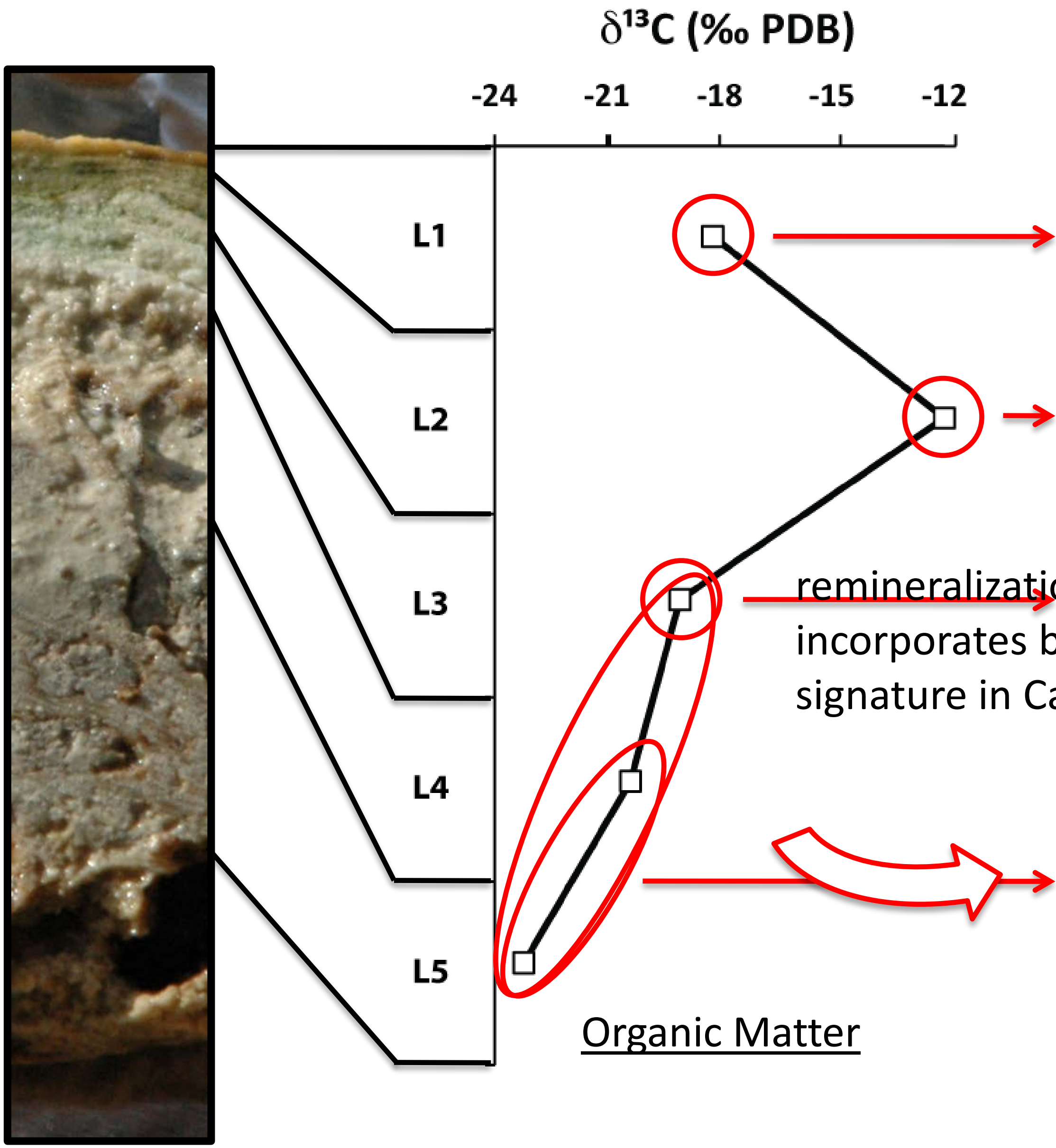
Can the $\delta^{13}C$ of $CaCO_3$ in the layers provide insight into what metabolic processes result in the precipitation of $CaCO_3$ in the different layers?

4) Carbon Cycling Processes:
An isotopic analysis

Methods: carbon isotope analyses



Results: carbon isotope profile



remineralization of OM incorporates biogenic signature in $CaCO_3$

<u>Inorganic $CaCO_3$</u>

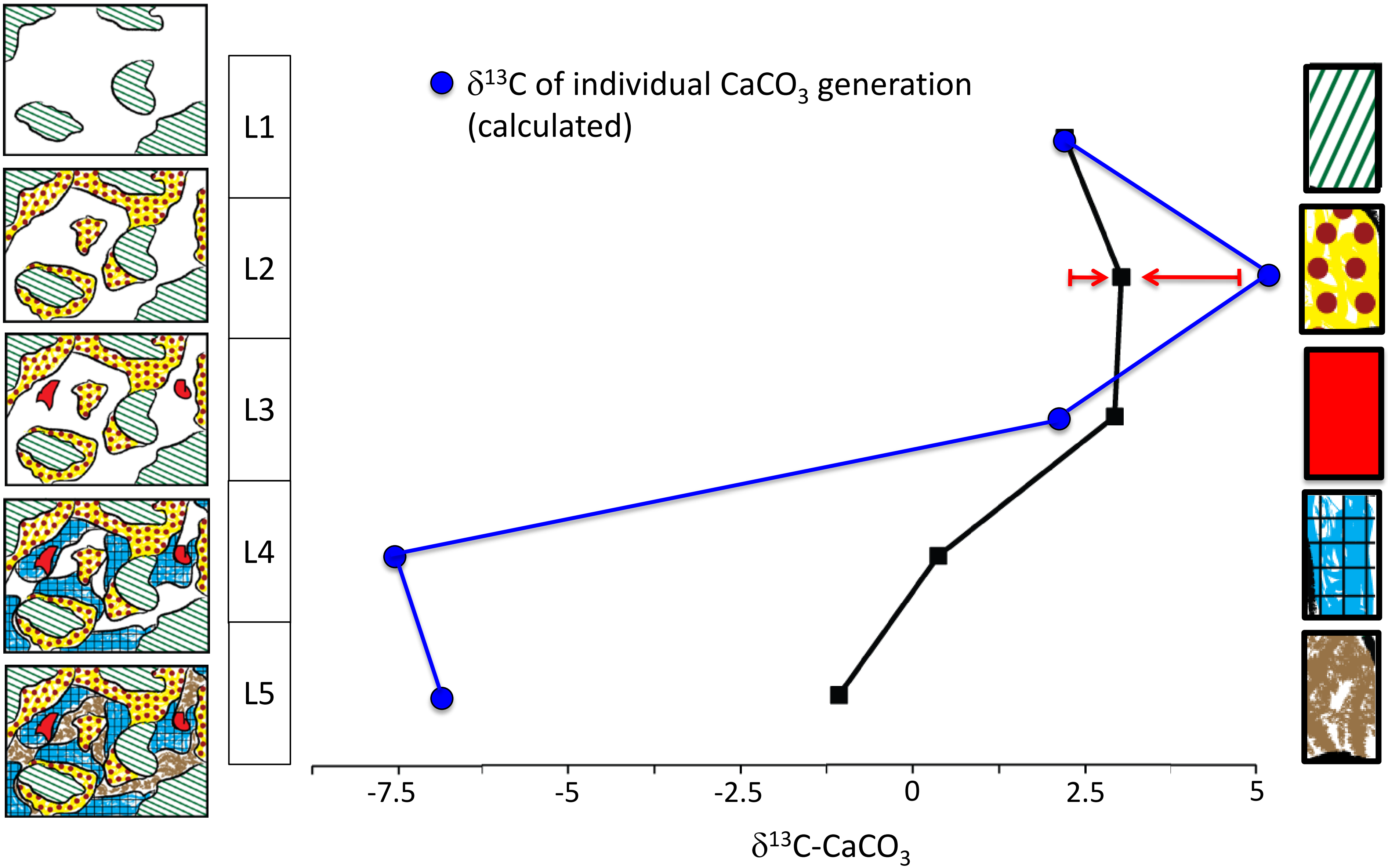
<u>$\delta^{13}C$-DIC</u>
+4 ‰

- $\delta^{13}C$ typical of cyanobacterial fractionation
- relatively ^{13}C enriched
  - carbohydrate rich EPS
  - HCO_3^- utilization
- remineralization of ^{13}C rich biomass (EPS?)

* $CaCO_3$ $\delta^{13}C$ follows a similar trend to OM

- continued remineralization of ^{13}C rich biomass by heterotrophic community

Results: $CaCO_3$ ^{13}C depletion



Conclusions: carbon isotope profile

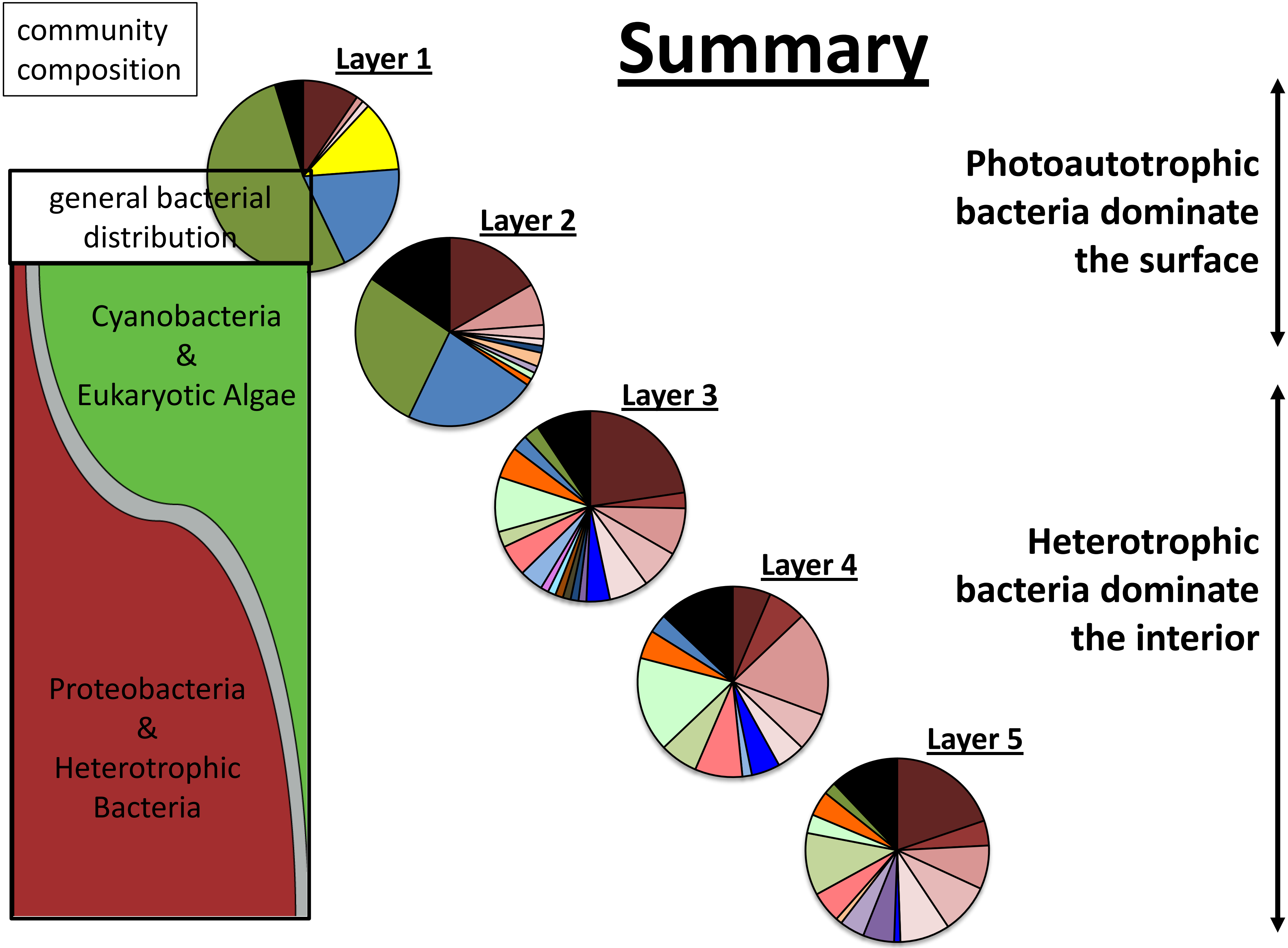
1) Organic matter $\delta^{13}C$ reflects the community composition
   - photoautotrophs at surface
   - heterotrophic degradation at depth

1) The remineralization of OM by heterotrophic bacteria significantly affects the $\delta^{13}C$ of $CaCO_3$ at depth
   - incorporates a biological isotopic signature in the inorganic matrix

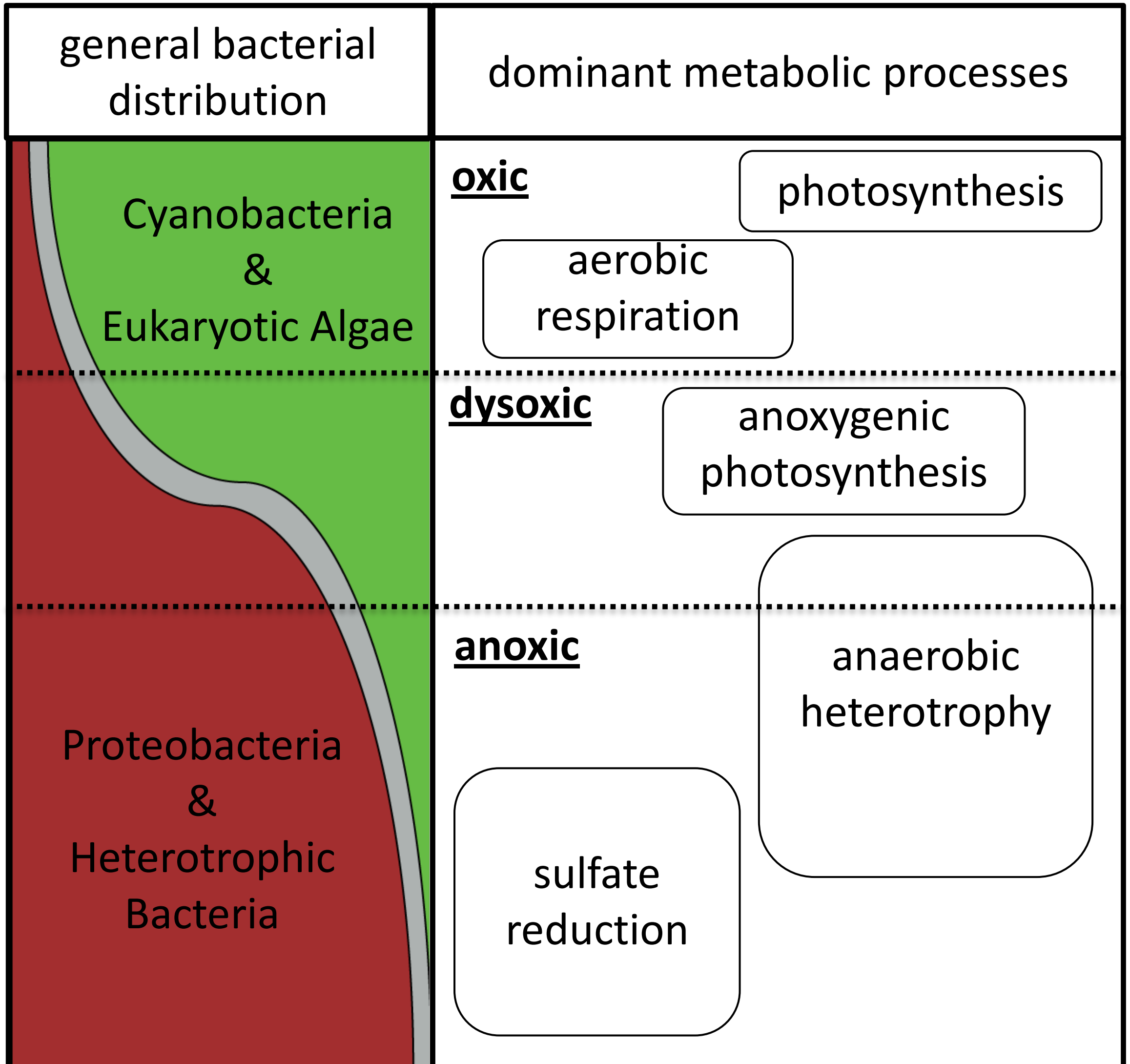
Summary:

integrated conclusions

Summary


community composition
general bacterial distribution
Cyanobacteria & Eukaryotic Algae
Proteobacteria & Heterotrophic Bacteria
Layer 1
Layer 2
Layer 3
Layer 4
Layer 5
Photoautotrophic bacteria dominate the surface
Heterotrophic bacteria dominate the interior

Summary



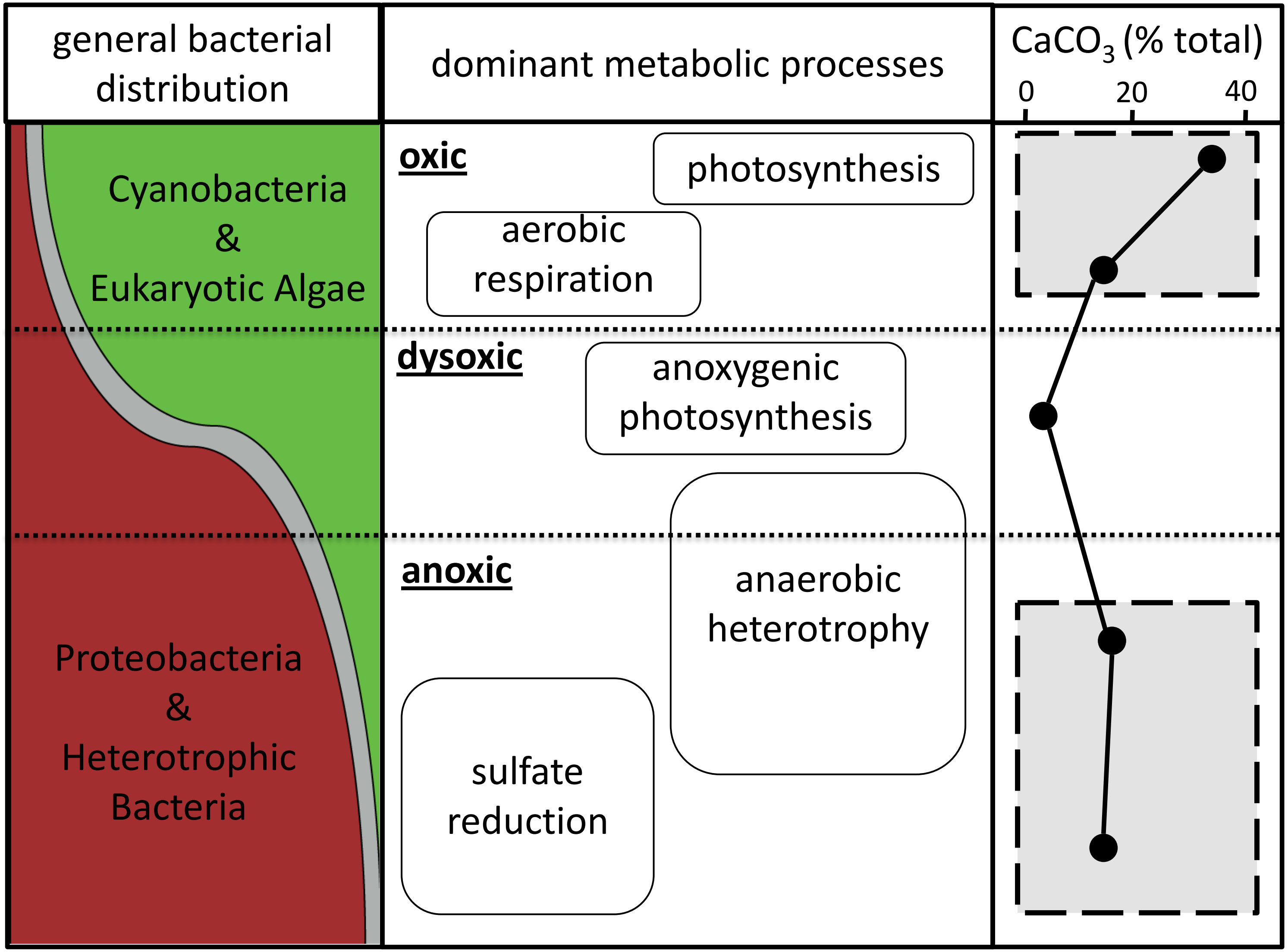
Biomarkers support 16S

- numerous cyano & diatom lipids
- SRB lipids abundant
- $\delta^{13}C$ of organic matter

Signature changes w/ depth

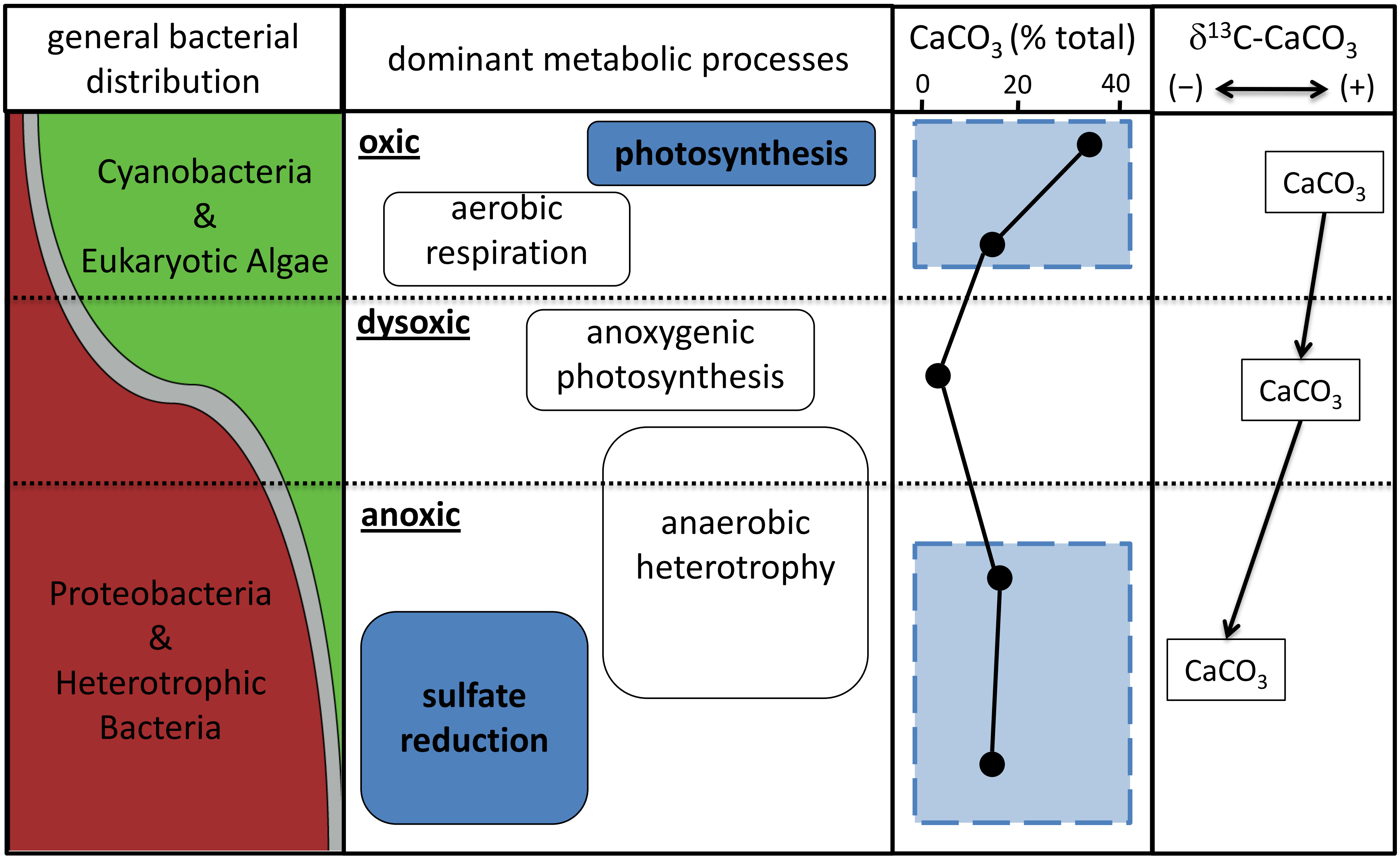
- phototrophic markers lost
- sulfate reduction active at depth (if not dominant)
- shift from aerobic phototrophic to anaerobic heterotrophic system

Summary



general bacterial distribution
dominant metabolic processes
CaCO3 (% total)
0
20
40
Cyanobacteria & Eukaryotic Algae
Proteobacteria & Heterotrophic Bacteria
oxic
photosynthesis
aerobic respiration
dysoxic
anoxygenic photosynthesis
anoxic
anaerobic heterotrophy
sulfate reduction

Summary



general bacterial distribution
dominant metabolic processes
CaCO3 (% total)
0
20
40
δ13C-CaCO3
(–)
(+)
Cyanobacteria & Eukaryotic Algae
Proteobacteria & Heterotrophic Bacteria
oxic
photosynthesis
aerobic respiration
dysoxic
anoxygenic photosynthesis
anoxic
anaerobic heterotrophy
sulfate reduction
CaCO3
CaCO3
CaCO3

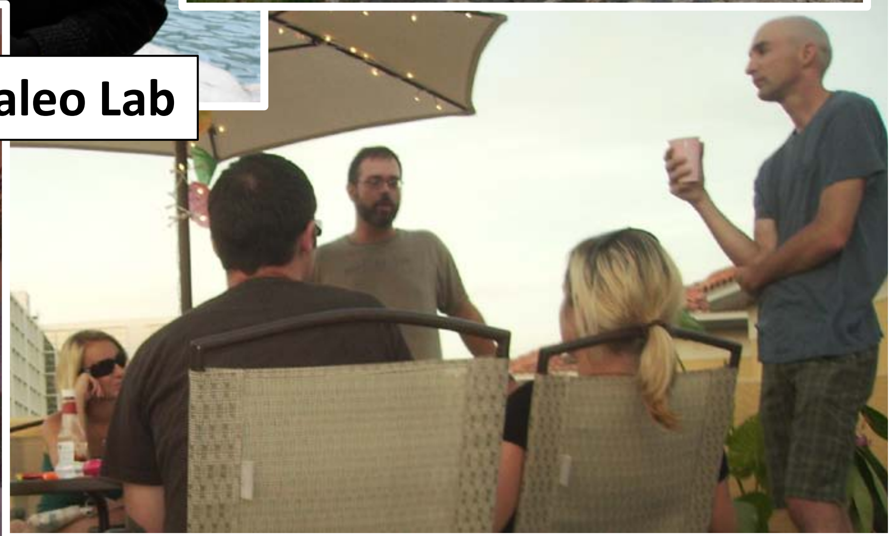
Conclusions

1. The visually distinct layers of the microbialite correspond to diverse consortiums of bacteria that are both taxonomically and physiologically different

2. Various autotrophic and heterotrophic processes are associated with microbialite formation and carbonate precipitation, occurring at both the surface and interior.
   - photoautotrophic organisms dominate surface
   - heterotrophic organisms are more abundant at the interior

3. The observed alteration of geochemical signatures (both molecular organic and isotopic) from surface to the interior results in a the preservation of a chemical "fingerprint" that can be used to interpret the total microbial community and the processes occurring within.
   - this fingerprint can also be applied to the interpretation of ancient systems

Acknowledgements



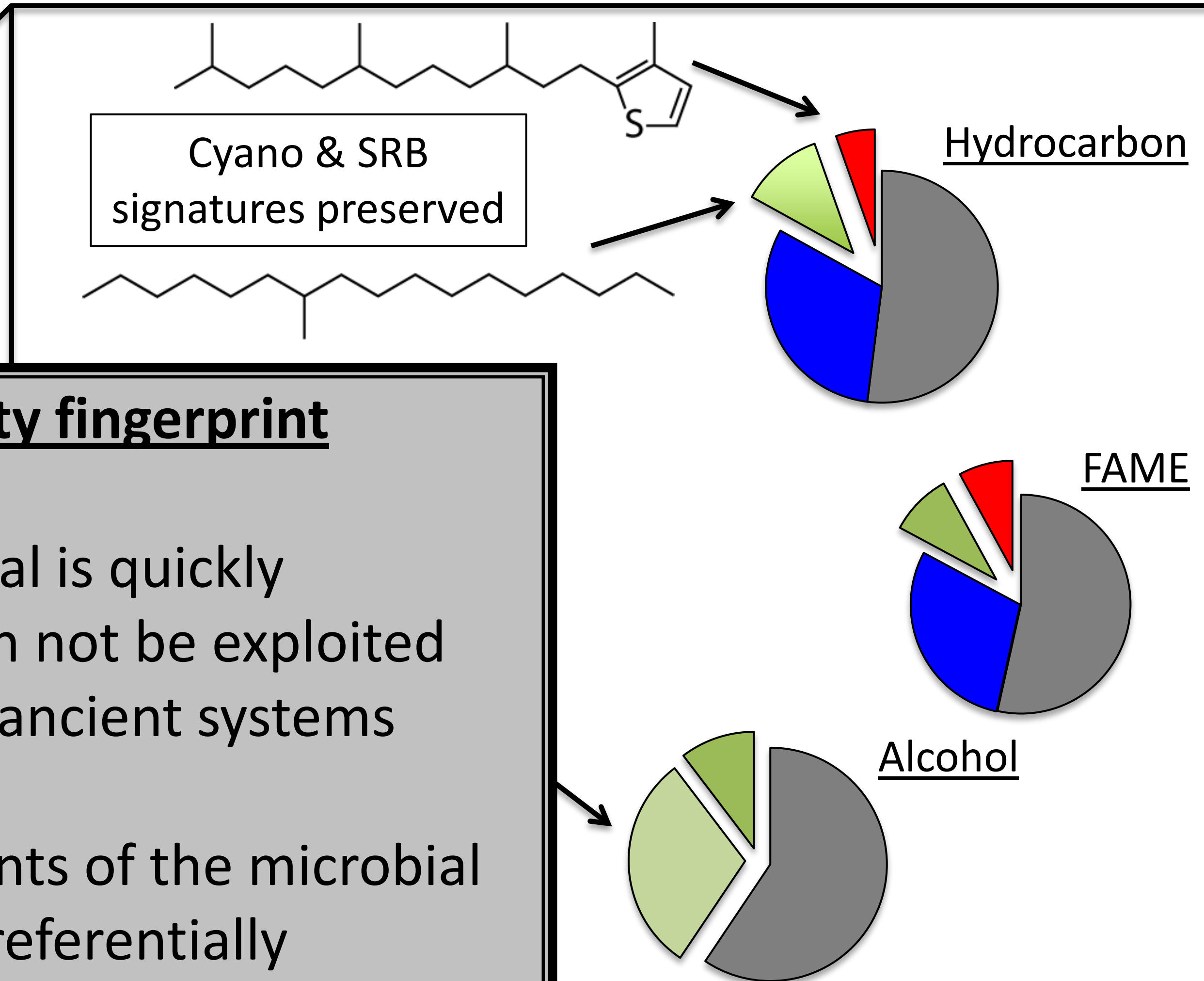
Funding

NATIONAL GEOGRAPHIC

USGS
science for a changing world

USF UNIVERSITY OF SOUTH FLORIDA
COLLEGE OF MARINE SCIENCE

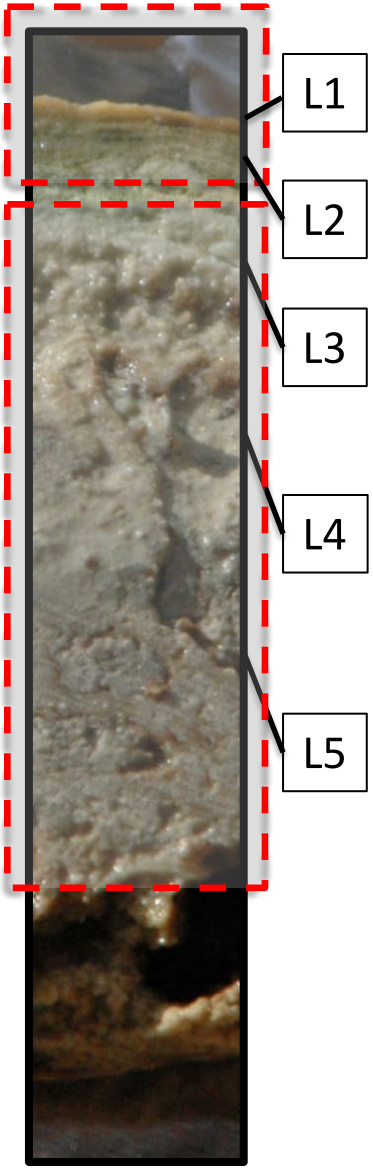
Implications: final molecular signature



A community fingerprint

- genetic material is quickly degraded – can not be exploited for analysis of ancient systems
- lipid components of the microbial biomass are preferentially preserved in the microbialite

Results: community composition



Cyanobacteria

- Supports metagenomic work – 74% Cyano sequences
  (Breitbart *et al.* 2009)
- Pleurocapsales similar to Highborne Cay, Bahamas clones

Diatoms

- Detected by 16S; confirmed by 18S and SEM

α-proteobacteria

- Group with purple non-sulfur bacteria species
- Anoxygenic photoautotrophs

Bacteroidetes

- Heterotrophic – exopolysaccharide degraders

Proteobacteria

- 40 - 50% of clones in L3, L4, and L5
- Diverse metabolic capacity
- Sulfate reducing δ-proteobacteria
  25% of L4 clone library
- Anaerobic heterotrophy

Results: notable lipid components

General bacterial lipids

- hydrocarbons *n*-C_{16} through *n*-C_{22}
- diploptene

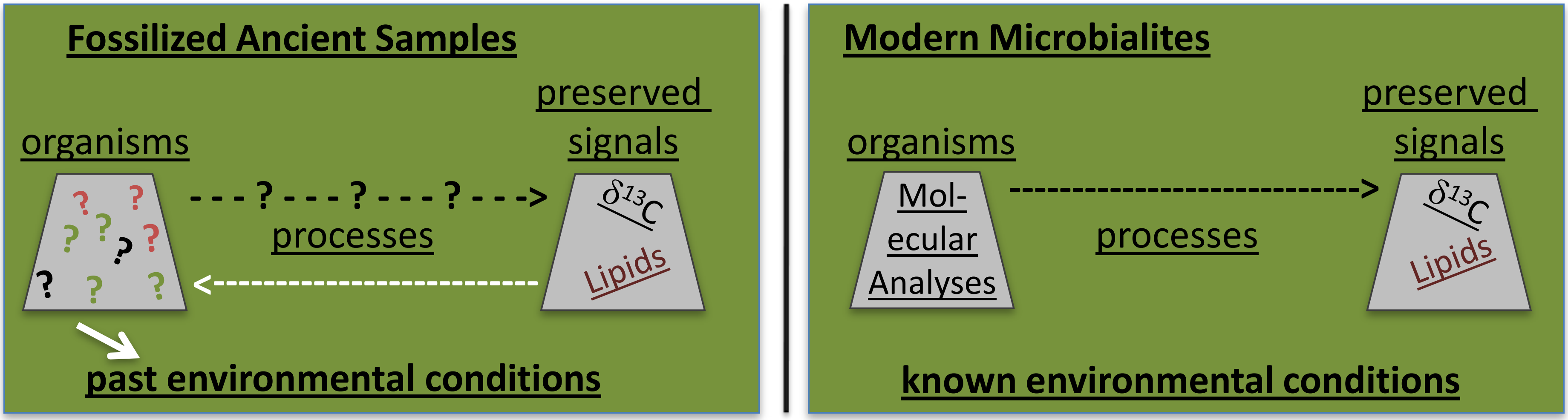
Cyanobacterial lipids

- unsaturated fatty acids
  - common in cyanobacteria and phototrophic eukaryotes
- phytol
  - side-chain of chlorophyll-a & bacteriochlorophyll-a
- mid-chain branched hydrocarbons
  - 18 – 20 carbons

Sulfate reducing bacteria

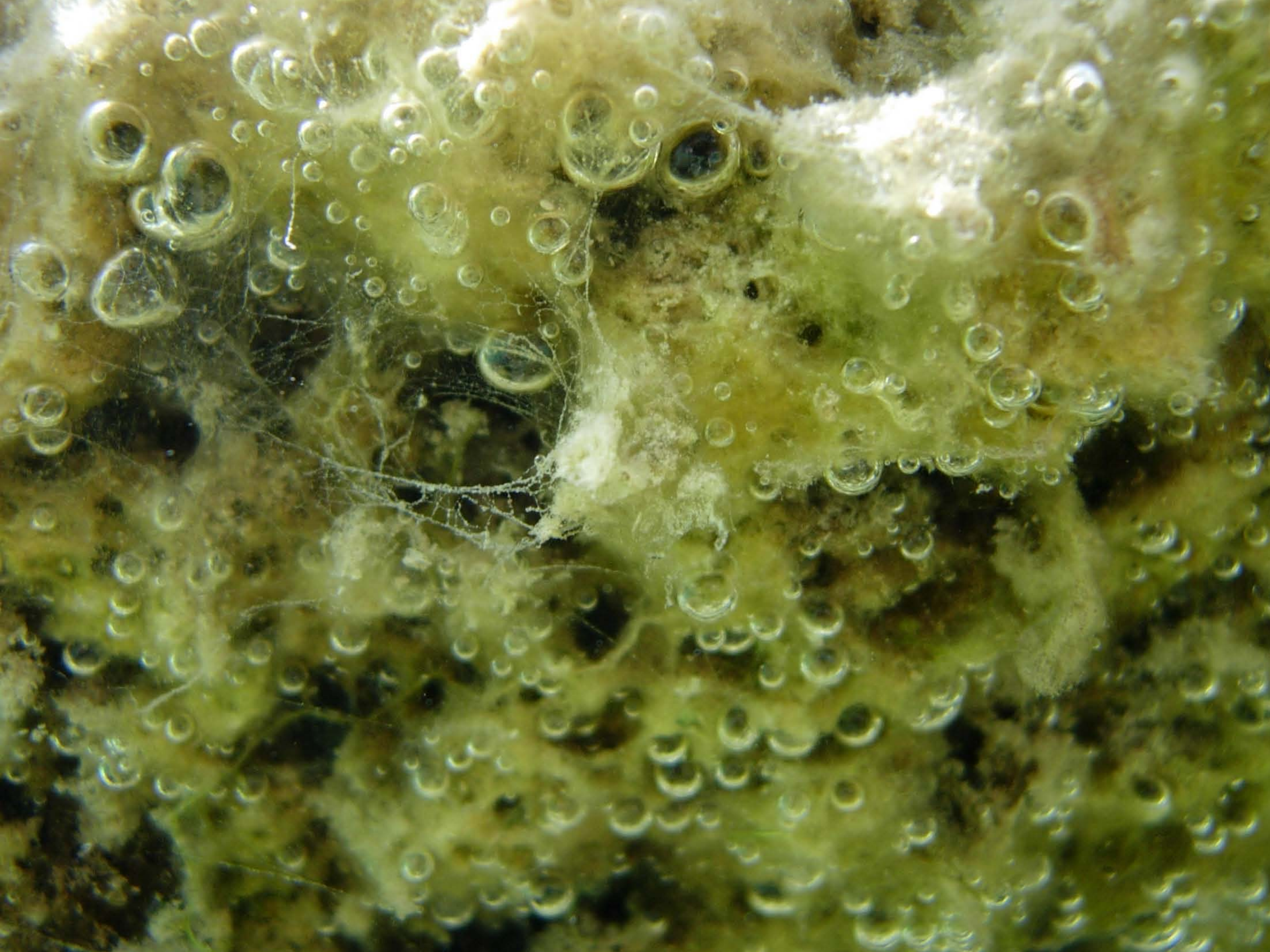
- mid-chain methyl-branched fatty acids
- sulfurized derivatives of phytol
  - indicative of both the phototrophic community and sulfate reducing organisms

Implications



The lipid content and $\delta^{13}C$-$CaCO_3$ values observed in layer 5 reveal some (not all) information about the composition and physiological function of the total community

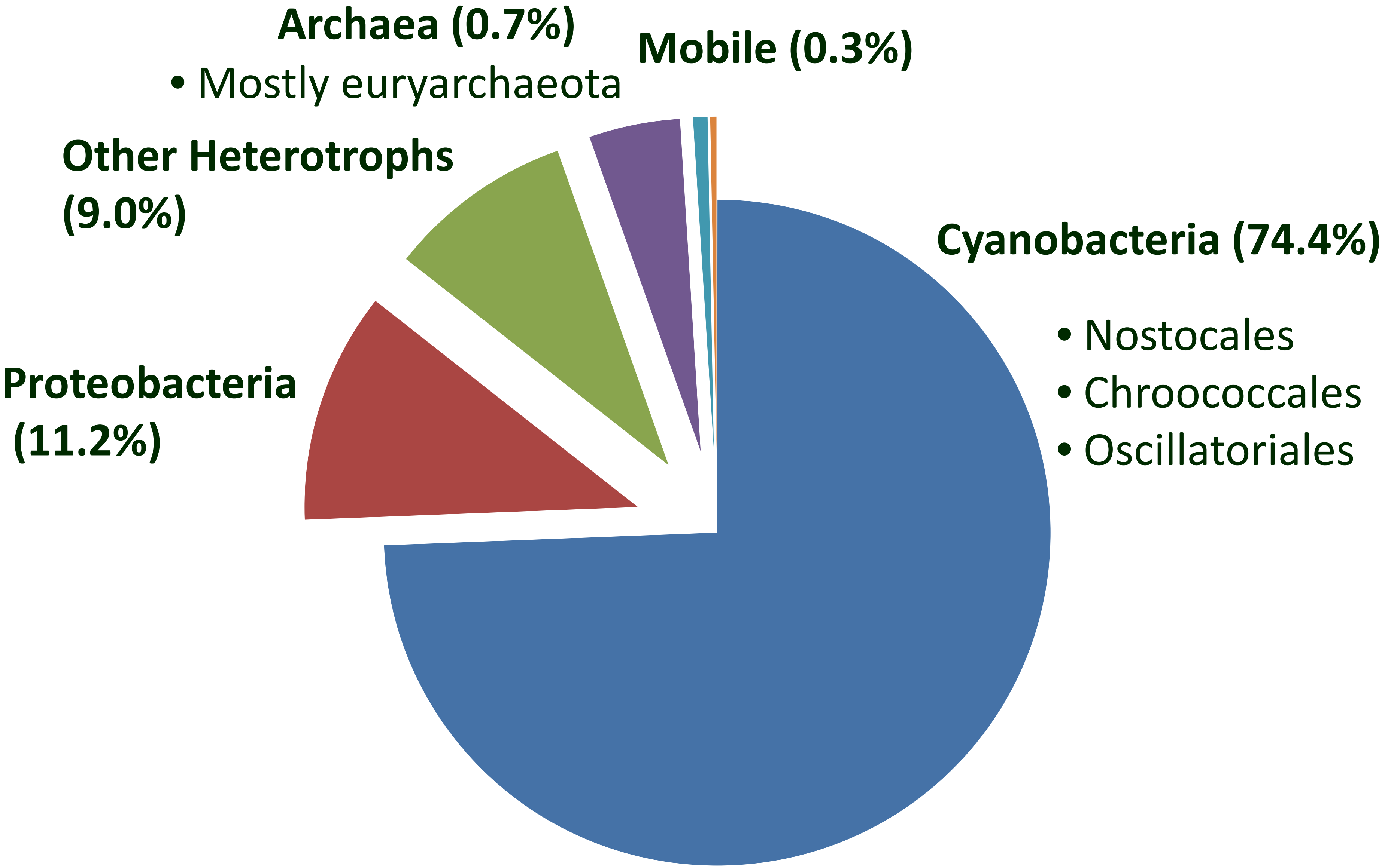
Can similar information be obtained from ancient fossilized stromatolites?



Microbialite metagenome shows a high degree of environmental adaptation

- Phosphate sensing and regulation (Pho regulon)
- Phosphate transporters (Pst genes)
  - Genes induced by P starvation (alkaline phosphatase) removes phosphate groups from nucleotides/ proteins
- Phosphonate utilization (C-P bond): general C-P lyase and specific phosphonatases
  - phosphonates may be prebiotic carriers of phosphorus
- Polyphosphate metabolism (polyphosphate kinase/
  - exopolyphosphatase)
  - polyphosphate strongly chelates metals (e.g., Ca) – may play role in carbonate precipitation
- Use of sulfolipids instead of phospholipids

Taxonomic Composition (~30,000 seqs)

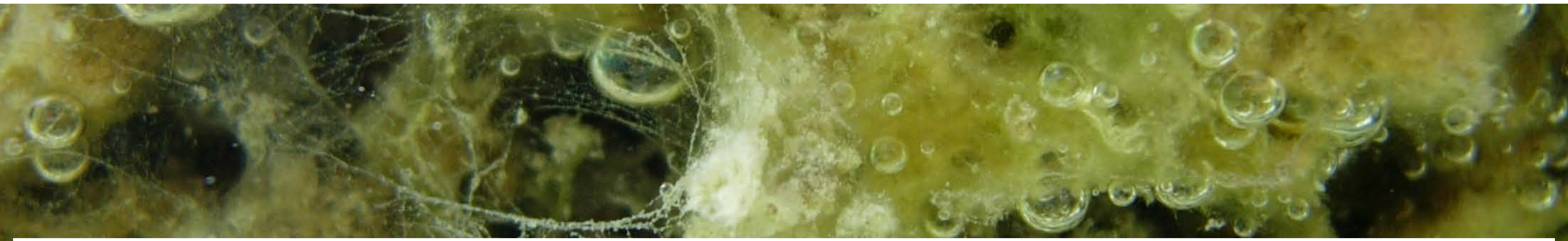


Genomic evidence for Photosynthesis

- Cyanobacterial sequences were abundant (74%)
- Genes for both light-dependent and light-independent reactions
- Photosynthetic CO_2 uptake raises pH locally, which promotes $CaCO_3$ precipitation
- Genes for photosynthetic pigments to access a wide variety of light wavelengths
    - **Chlorophyll** absorbs blue & red light (430 and 660 nm)
    - **Phycoerythrin** absorbs green light (540-570 nm)
    - **Phycoerythrocyanin** absorbs yellow light (570 nm)
    - **Phycocyanin** absorbs yellow-orange light (620-655 nm)
    - **Allophycocyanin** absorbs red light (650 nm)
    - **Phytochromes** absorb near infra-red light (650-740 nm)

The microbialites contain a diverse community of <u>autotrophic and heterotrophic microbes</u> performing <u>aerobic and anaerobic processes</u>

How are their activities coordinated?



Metagenome has a high abundance of genes for:

- biofilm formation (widespread colonization island)
- motility
- chemotaxis
- quorum sensing
- temporal regulation (circadian clock genes)
- extracellular polymeric substances (EPS)

Extracellular Polymeric Substances (EPS)

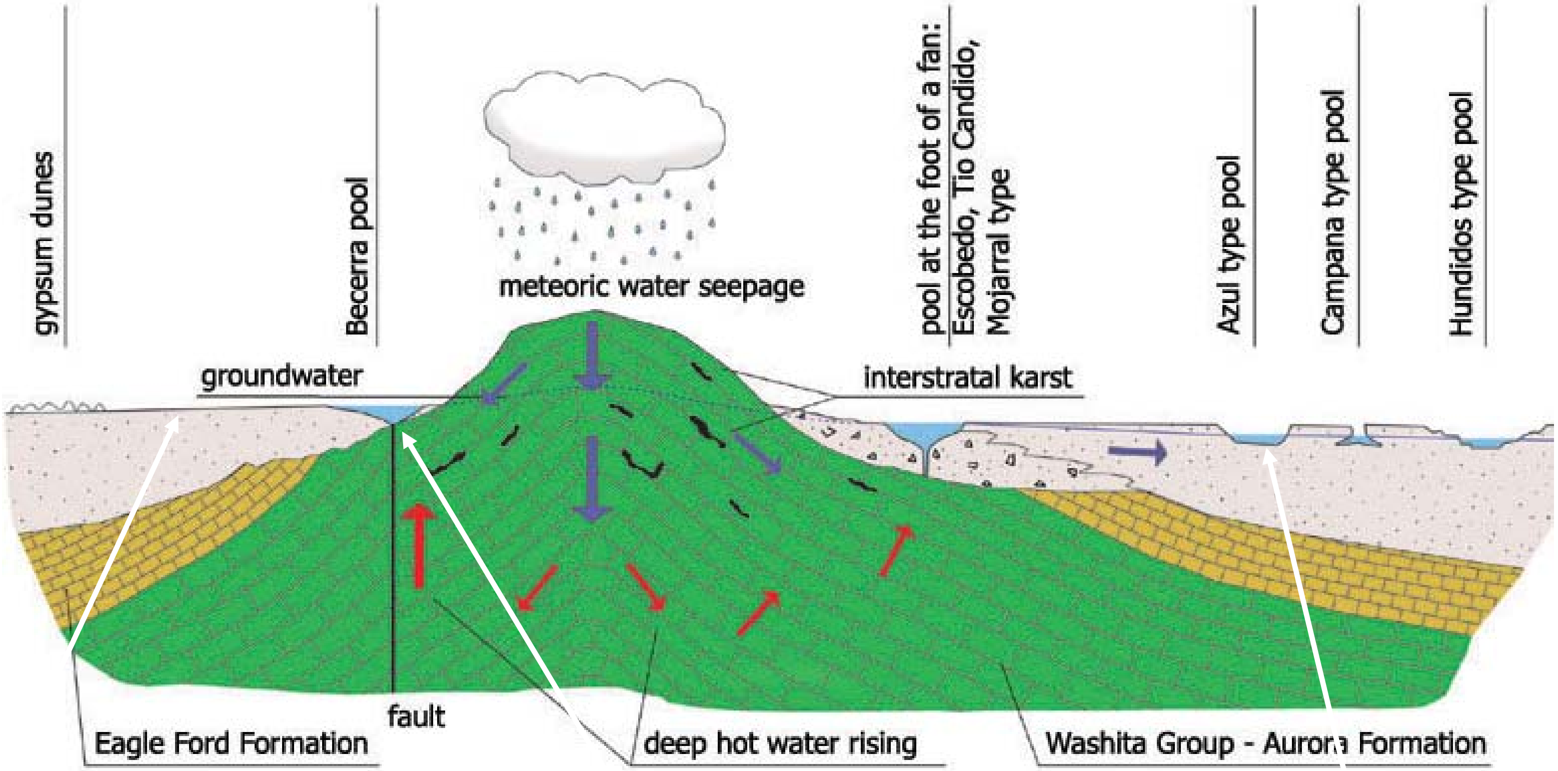
EPS Genes in Metagenome

- EPS synthesis
  alginate, colonic acid, sialic acid, rhamnose-containing glycans
- EPS degradation
  sulfatases, hydrolases, glycosidases, lyases

Roles of EPS

- Aids in the development of sharp geochemical gradients and stable microenvironments
- Binds and concentrates calcium ions, inhibiting $CaCO_3$ precipitation
- Microbial degradation of EPS releases the calcium, favoring localized precipitation

POOLS FORMATION Karst terrain in Jurassic & Cretaceous Platform Carbonates

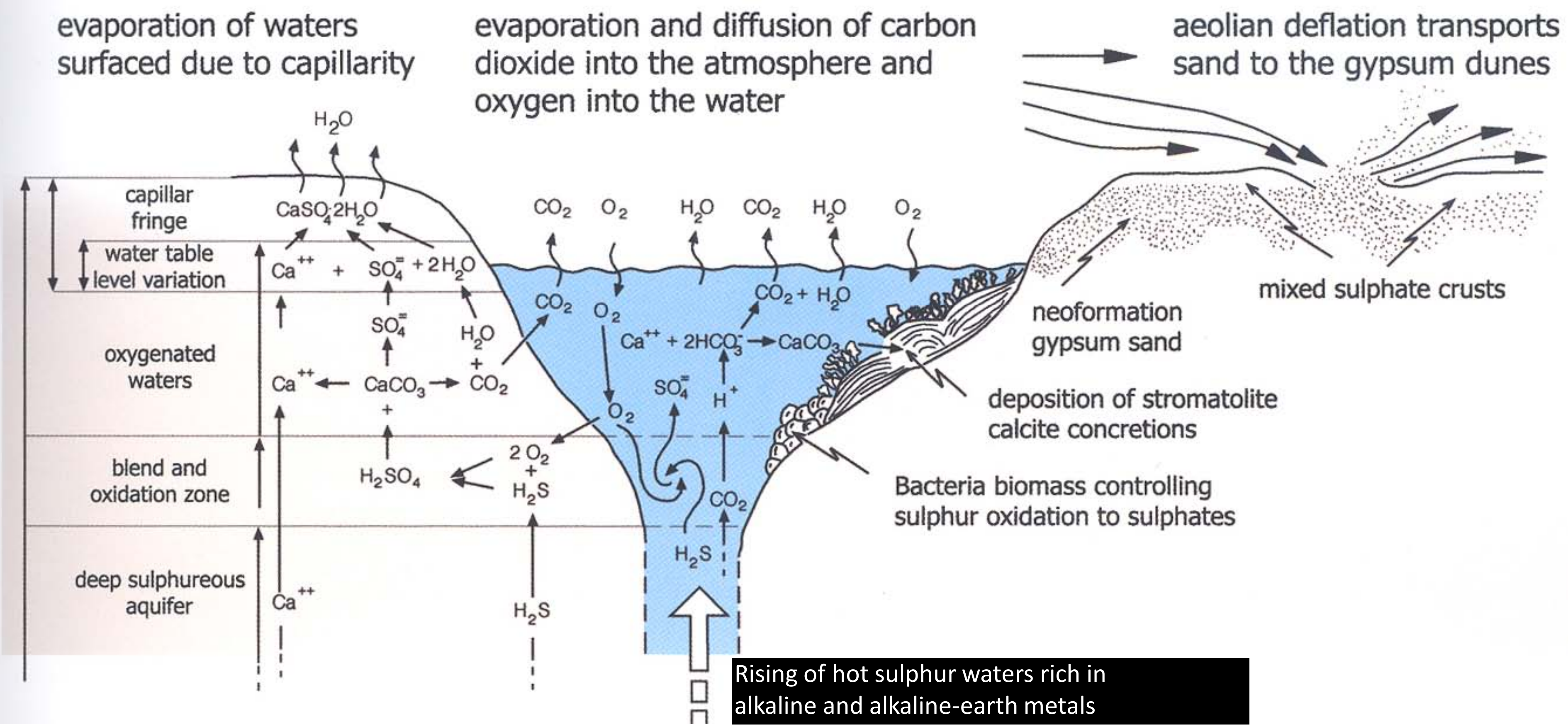


Laguna Churince

Poza Becerra

Poza Azules II

MINERAL GENETIC PHENOMENA IN THE POOLS



In this karst terrain, the ponds and microbialites are ephemeral

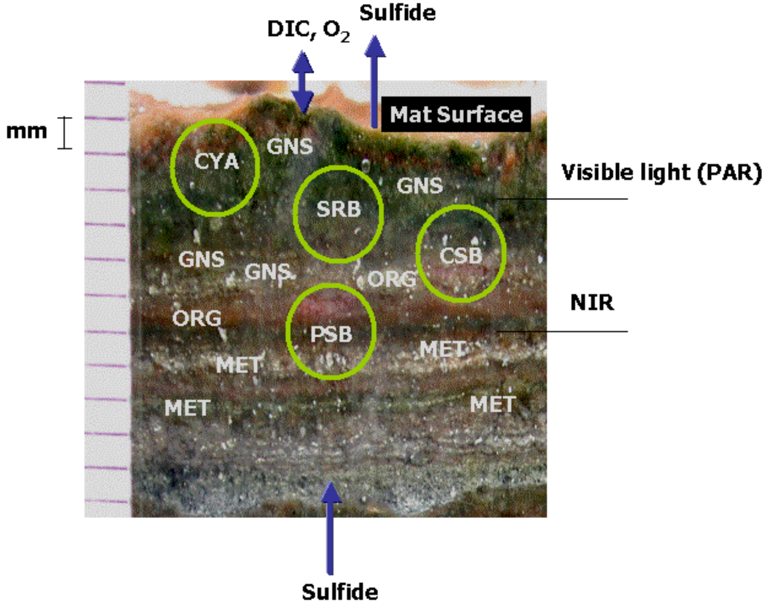
- Cuatro Cienegas = 4 Marshes
- At the time of settlement the valley was filled with water
- Climate likely controlled ground water table in past

Poza Roja – An Extreme Environment

Very hot (~65°C) and salty

High pH (~12)

Microbial mats and layering



Poza Roja Sediments
A Microbial-Climate Archive?

- Salt crust with an exotic microbial consortium likely to include
  - Purple sulfur-oxidizing bacteria
  - Iron oxidizing bacteria
  - Anaerobic photoautrophs
  - Other primitive life form

- Likely represent the influence that varying hydrologic conditions have on the water levels and chemistry in the pozas and the dominant microbial community.

- Strongly laminated with high frequency variations: A Climate Record ? Need to evaluate climate history of region