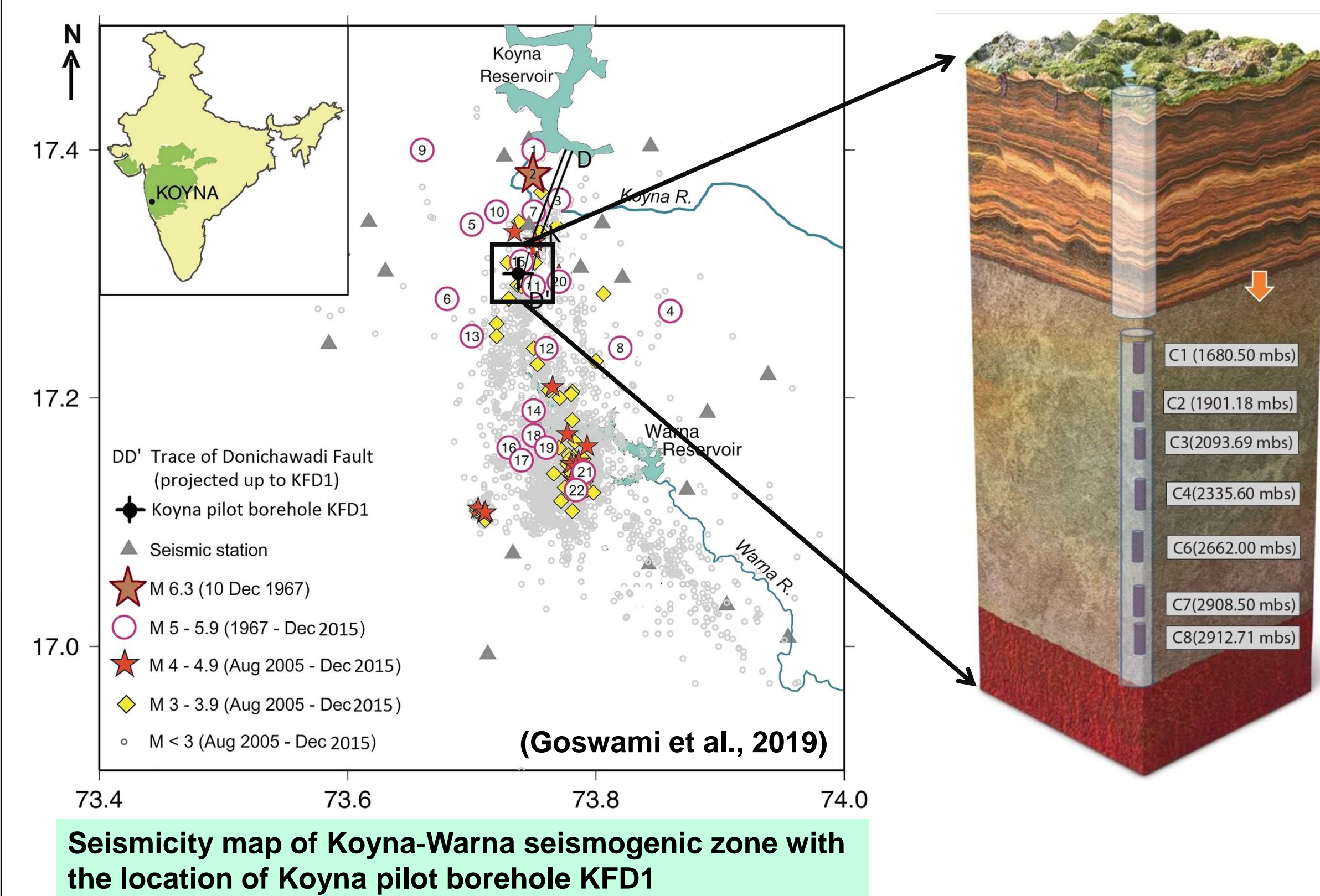


# Introduction

- Deep biosphere is a huge reservoir of diverse, novel and mostly uncultured and unidentified microorganisms
- This accounts one fifth of earth's biomass
- These microorganisms have the potential for answering the most fundamental questions of origin, adaptation and evolution of life on our planet, their significance in global elemental cycles, and providing valuable resources to humans
- Our understanding on the nature and distribution of microorganisms within the deep continental subsurface as well as their metabolic functions, especially mechanisms of carbon and energy metabolism, remained limited
- The deep microbial life within the granitic bedrock and the mechanisms of carbon and energy metabolism remained inadequately explored

## Rationale of the Study



## Fundamental questions

**Do life exist in this biosphere? If yes, who are they?**

## What are they doing?

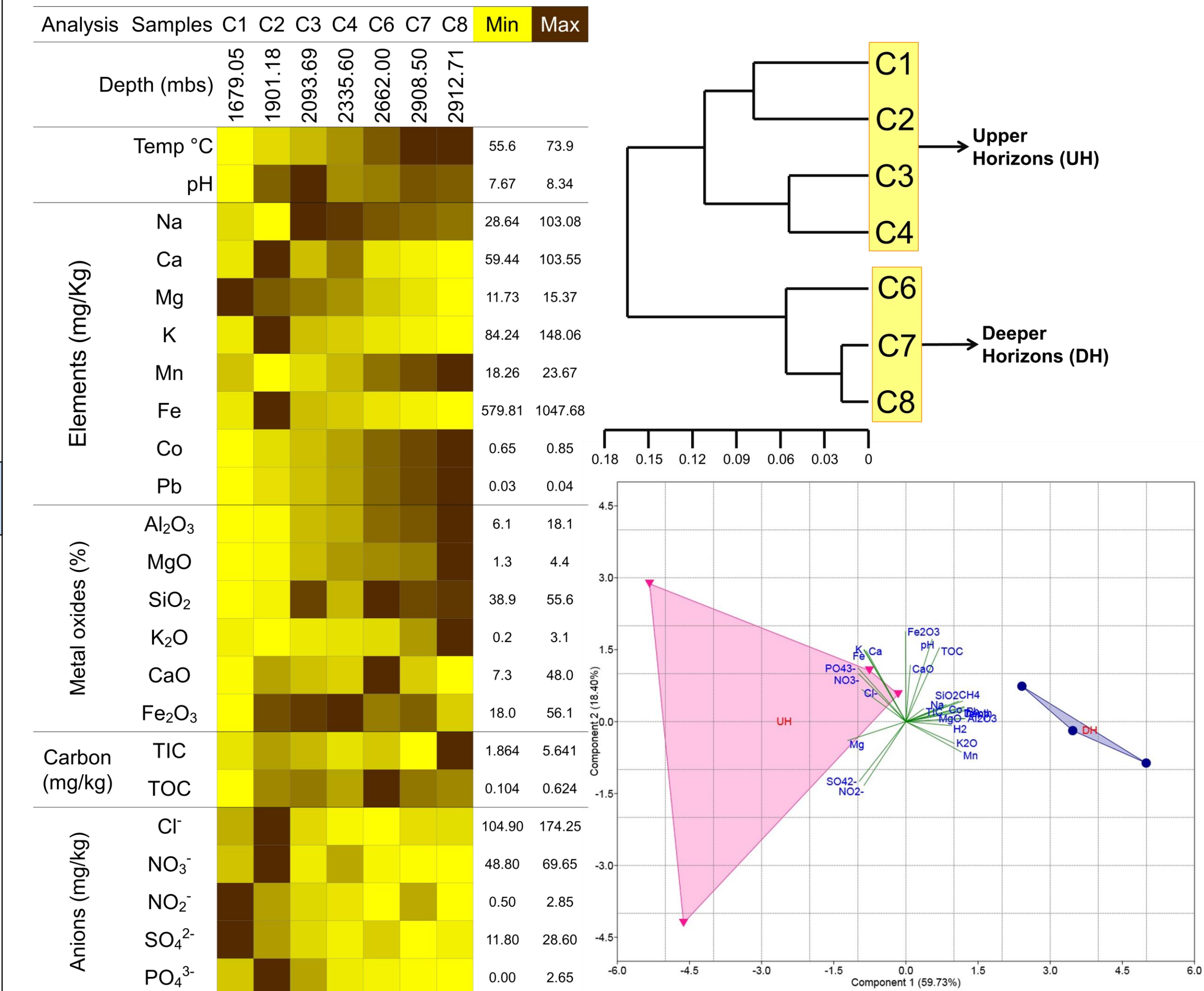
**What are the geochemical drivers of this community?**

**What is their role In local geochemical cycle?**

**Which ecological processes are governing the community structure?**

## Geochemical characteristics of subsurface rocks

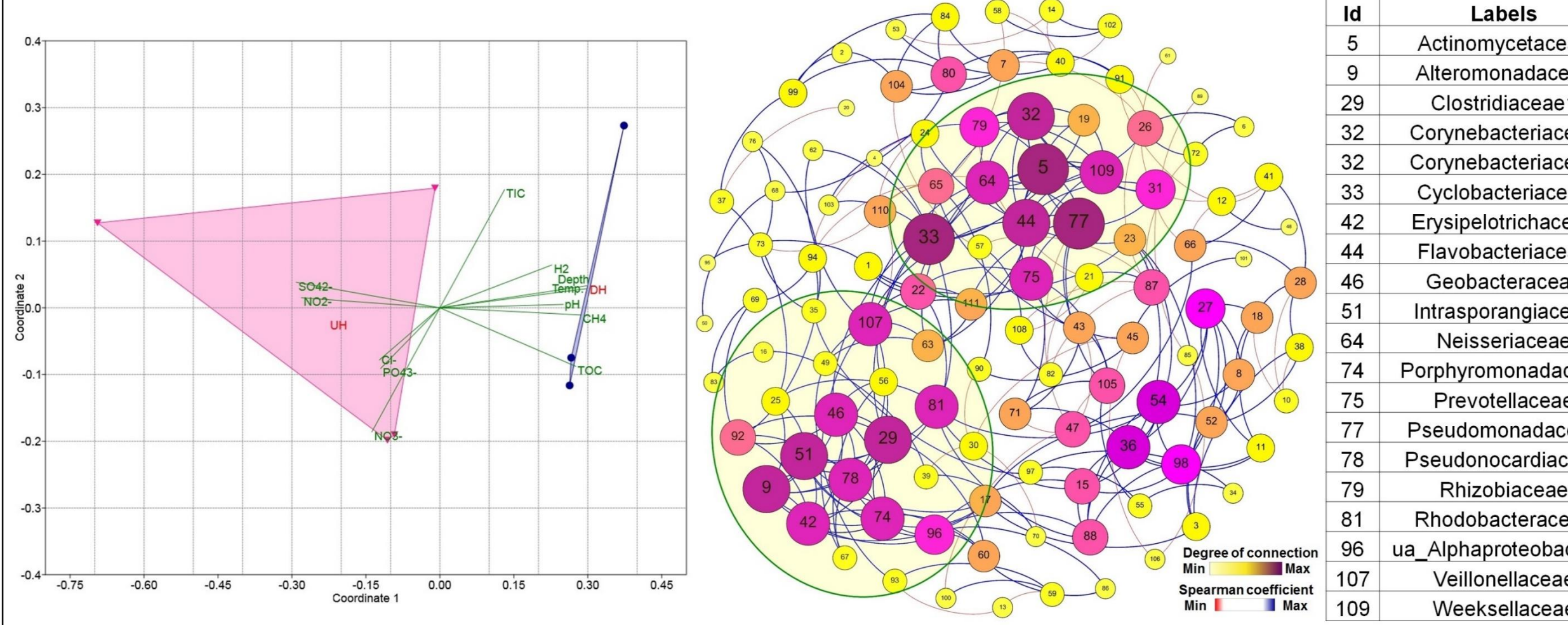
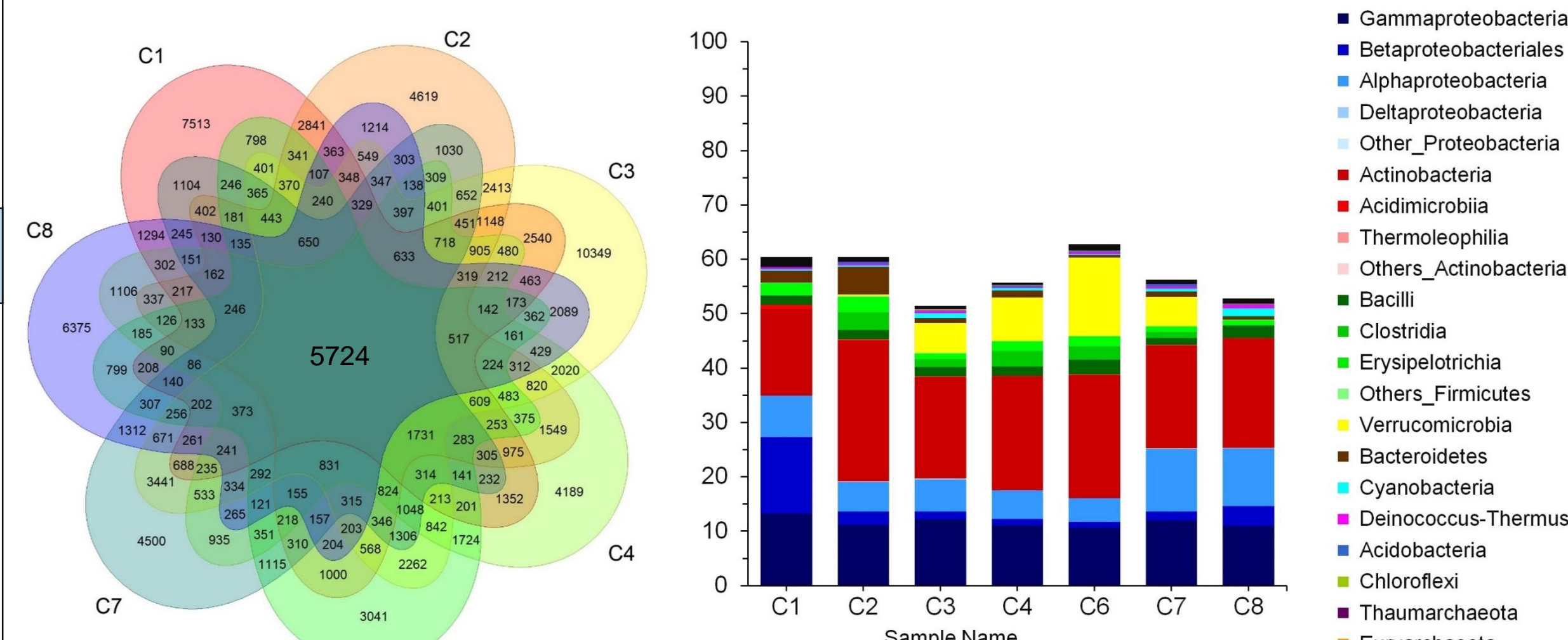
- Rock samples represented a progressively hot (50-70 °C) nutrients depleted extreme environment



- o Geochemically the basement environment can be partitioned. The deeper horizons are distinct from the upper horizons

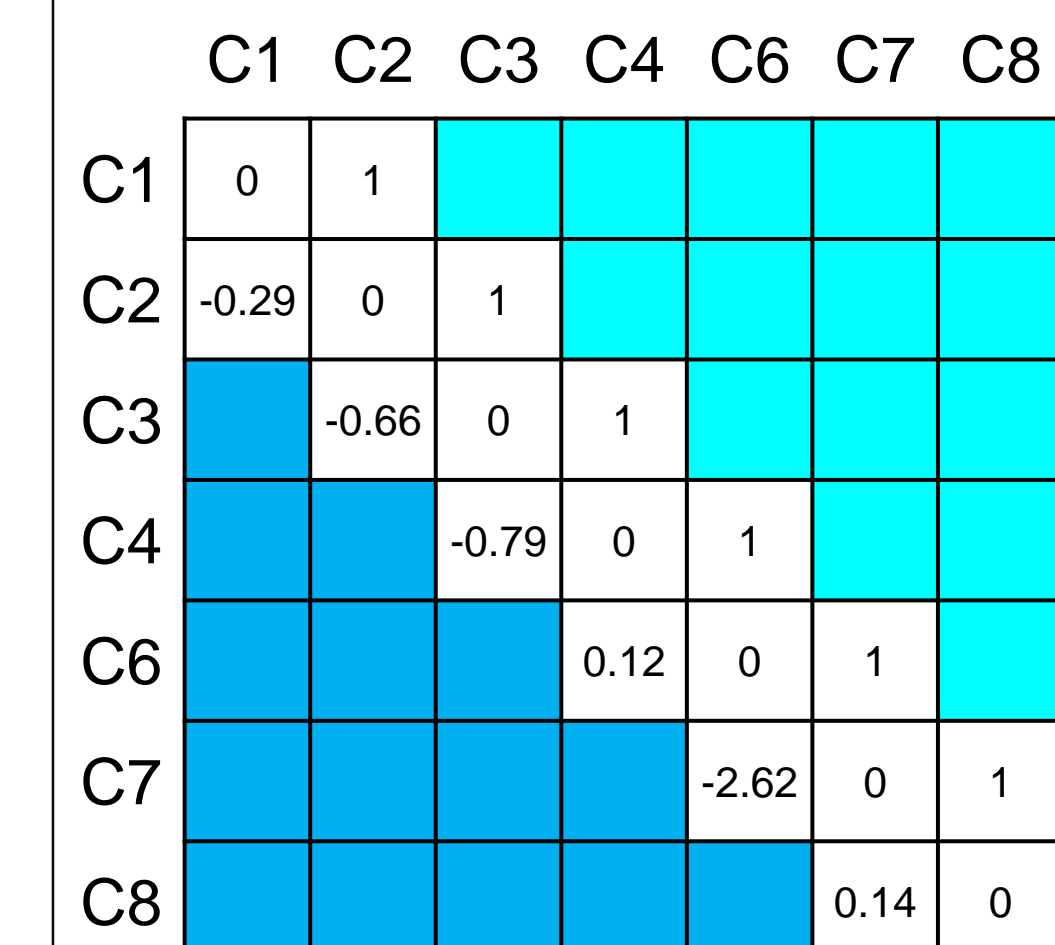
## Microbial community structure, geochemical control and co-occurrence networks

- 16S rRNA gene amplicon sequencing with multiple replicates yielded a total of **7.37 × 10<sup>6</sup> reads**. These were grouped into **118,064 OTUs**
- OTUs affiliated to **bacteria predominated (average 96.9%)**, archaea represented minor fractions (**0.1 - 0.74%**), while **0.91 - 5.07%** of reads remained **unassigned** across the samples
- **10 - 16% of the OTUs** (5724 OTUs out of the total 118,064 OTUs) from the samples were **shared across seven samples** and represented **51 - 63%** of total community



## Results and Discussions

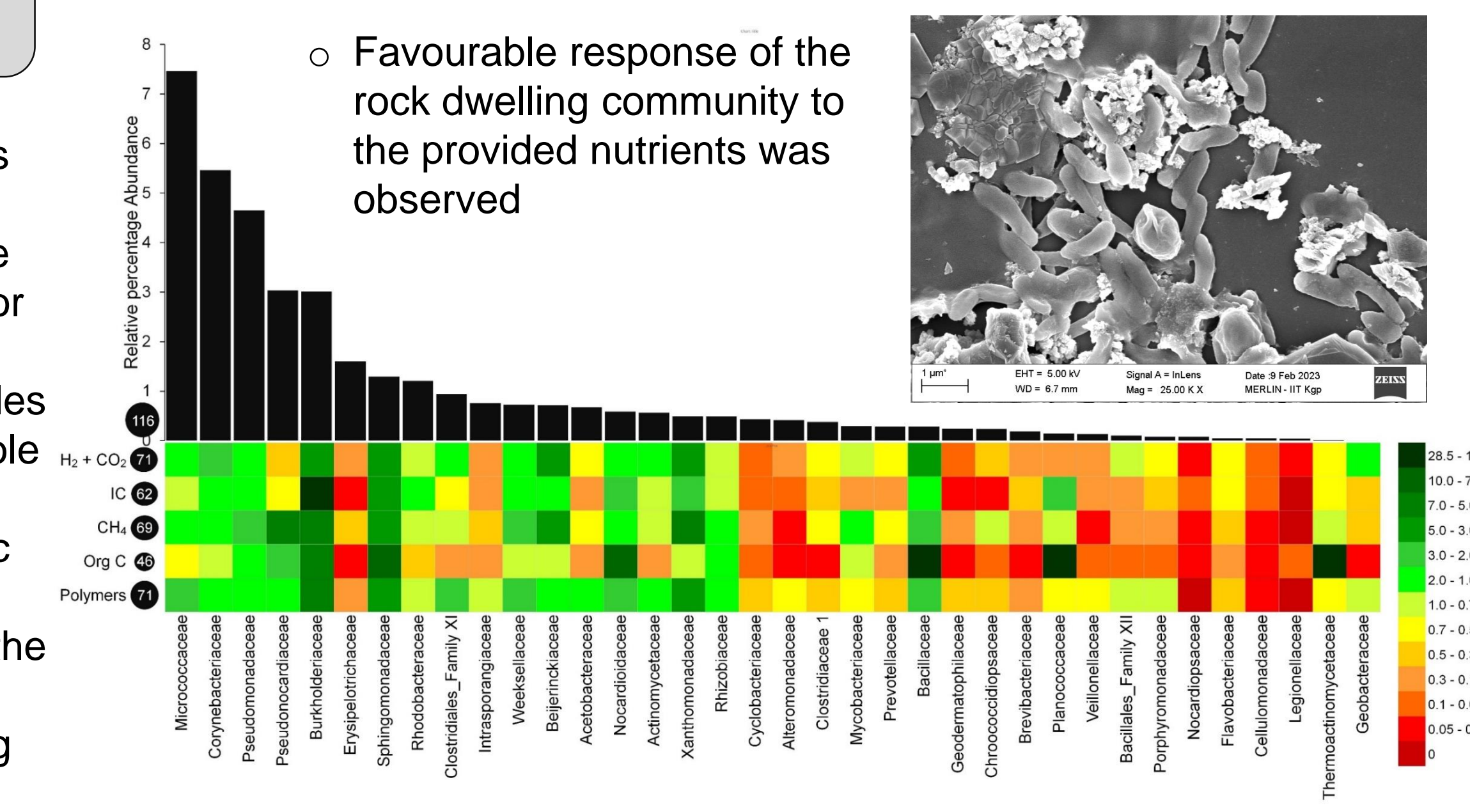
## Ecological processes driving the community



- $\beta$ NTI and  $RC_{BC}$  models were utilized to determine the selective pressures explaining for community variation
- $|\beta NTI| < 2$  of the samples suggested the major role of **stochastic factors** controlling the endemic community assembly
- $RC_{BC} > 0.95$  depicted the role of **dispersal limitation** in controlling the diversity

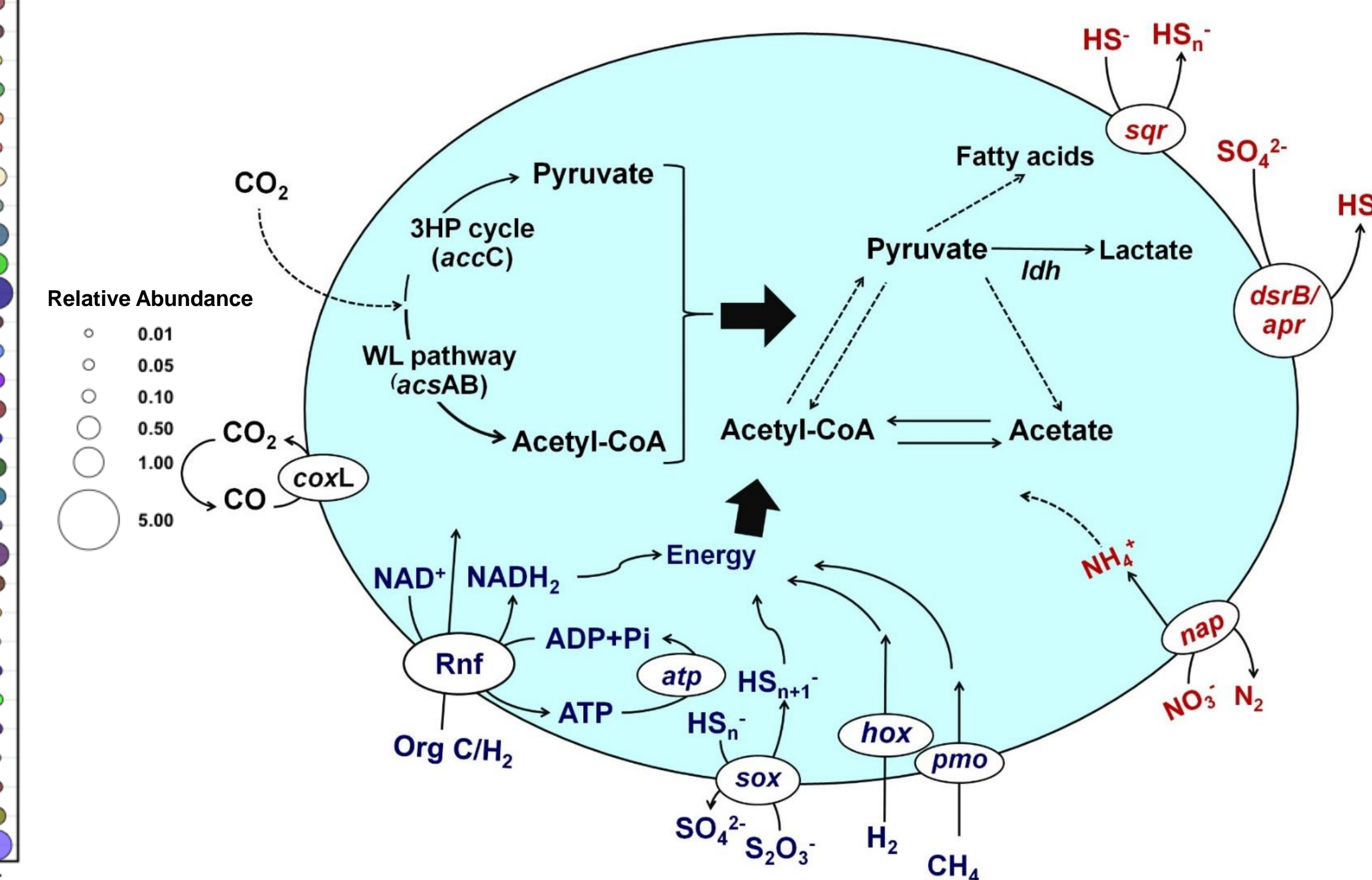
### Enrichment-based biomass reactivation

- Favourable response of the rock dwelling community to the provided nutrients was observed



## Metabolic potential of the community

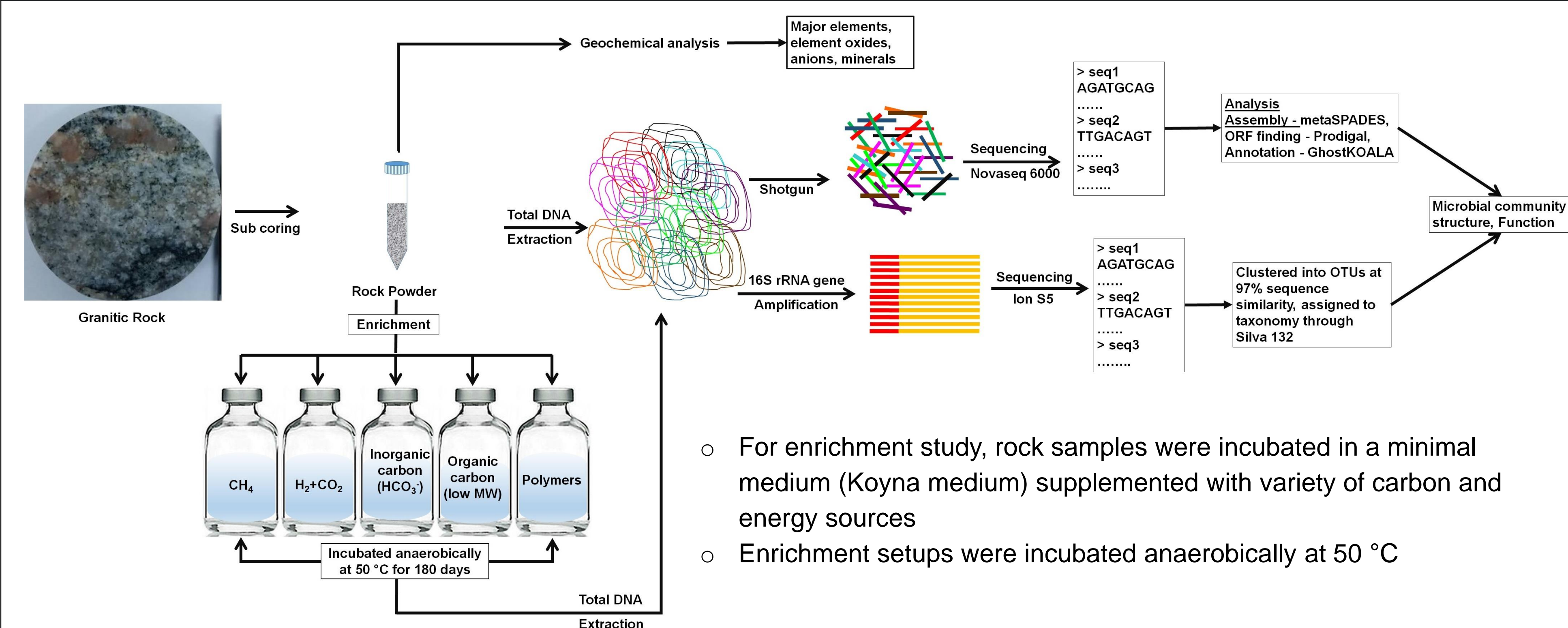
- Metabolic potential of the communities was deciphered by analysing specific marker genes associated with carbon, energy and biomass production
- Diverse genetic potential of the rock dwelling community to utilise locally available electron donor, acceptor and carbon source was noted
- Wood-Ljungdahl pathway was detected as the major carbon assimilation pathway
- Significant role of Rnf complex for energy conservation and ATP generation was observed
- Important role of acetate and acetate switch in maintaining homeostasis via controlling cellular biosyntheses vis-à-vis stress response in this biosphere was perceived



## Aim

- Investigate the nature of life that evolved and exists within the deep crystalline Archaean granitic crust underneath the Deccan Traps, India
- Elucidate the geological/environmental and metabolic drivers of the microbial community harbouring this biosphere

## Materials and Methods



- For enrichment study, rock samples were incubated in a minimal medium (Koyna medium) supplemented with variety of carbon and energy sources
- Enrichment setups were incubated anaerobically at 50 °C

## Summary

- In spite of energy and nutrient-limited extreme condition, this biosphere harbours high bacterial diversity and an endemic community whose composition is highly constrained by depth and geochemical factors
- Close interactions among autotrophic and organotrophic bacteria facilitate the community sustenance
- Dispersal limitation is playing a major role in community assembly
- Wood–Ljungdahl pathway coupled with Rnf-based energy conservation metabolism is the major chemoautotrophic carbon assimilation process
- A significant role of acetate metabolism, acetate switch is noted
- Overall, this study provides a better understanding on organization and functioning of microbial life within this energy-starved biosphere, highlighting the species interaction and biogeochemical role

## References

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## Acknowledgement

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## Abbreviation

mbs - meters below surface, OTUs - Operational Taxonomic Units, PCA - Principal Component Analysis, NMDS - Non-Metric Multi Dimensional Scaling,  $\beta$ NTI -  $\beta$ -nearest taxon index,  $RC_{RC}$  - Raup-Crick (Bray-Curtis)