Microbial Survival and Dispersal from Spacecraft on Mars – Lessons from Earth

Committee on Planetary Protection Meeting No 2 on Mars Mission Bioburden requirements

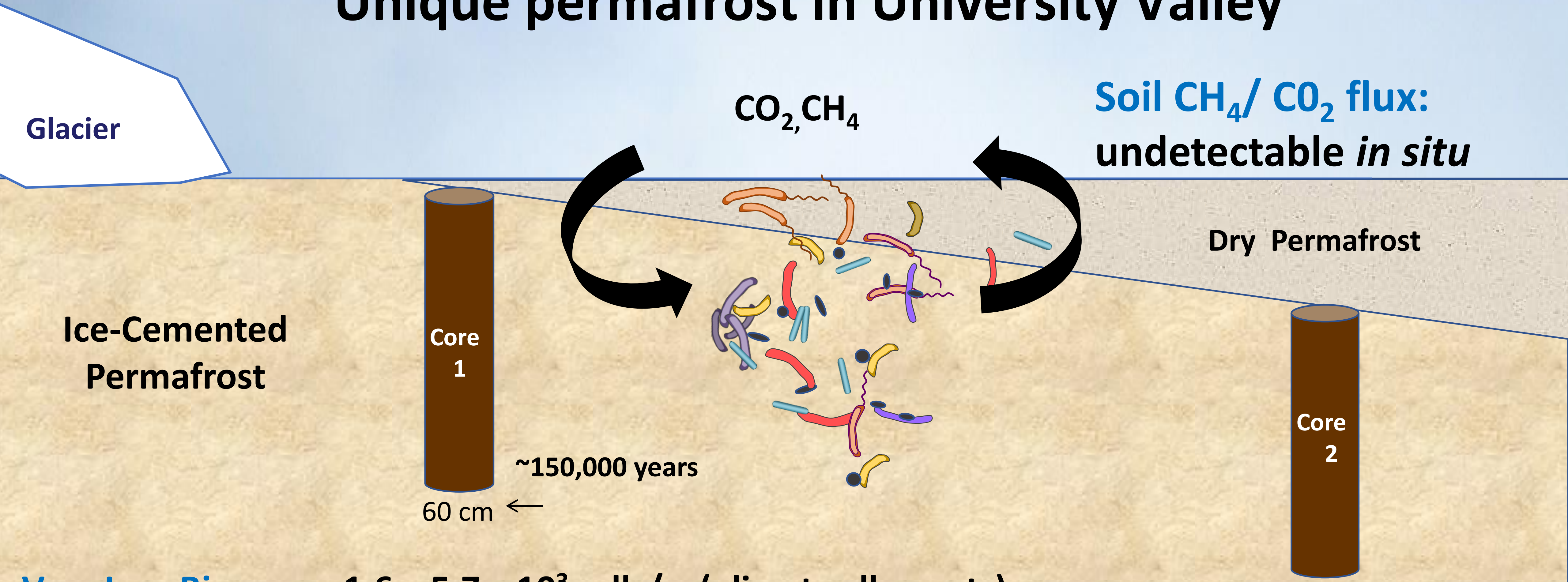
April 5, 2021

Dr. Jackie Goordial
Assistant Professor of Environmental Microbiology
University of Guelph, Canada

University Valley

- Extremely cold and dry environment
- Mean air temp: -27°C (max -3 °C)
- Nutrient Poor: 0.01-0.05 % C; undetectable- 0.09% N
- Due to a combination of low salinity and temperature: unfrozen water only present 74h/ year
- Presence of Dry permafrost

Unique permafrost in University Valley



Very Low Biomass: **1.6 – 5.7 x 10^3 cells/ g (direct cell counts)**

6 culturable isolates (2 with sub-zero growth)

Transcriptional activity: **RNA undetectable**

Soil populations are diverse! ~1600 unique "species" (OTU)

(Goordial et al., ISME 2016)

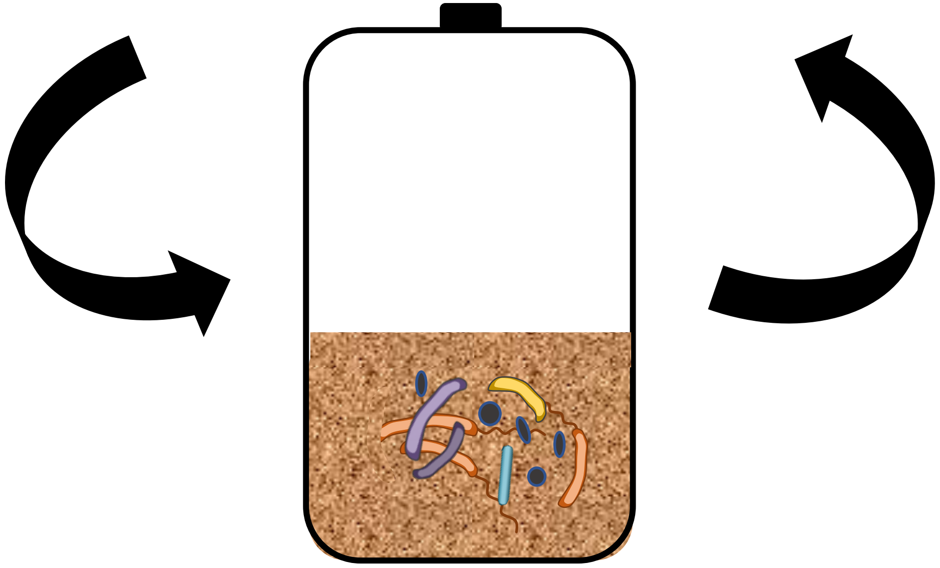
Measuring microbial activity: radiorespiration assays

Are indigenous microbial communities active at subzero temperatures relevant to *in situ* conditions?

- **Highly sensitive activity assay**
- **Activity of aerobic heterotrophs**

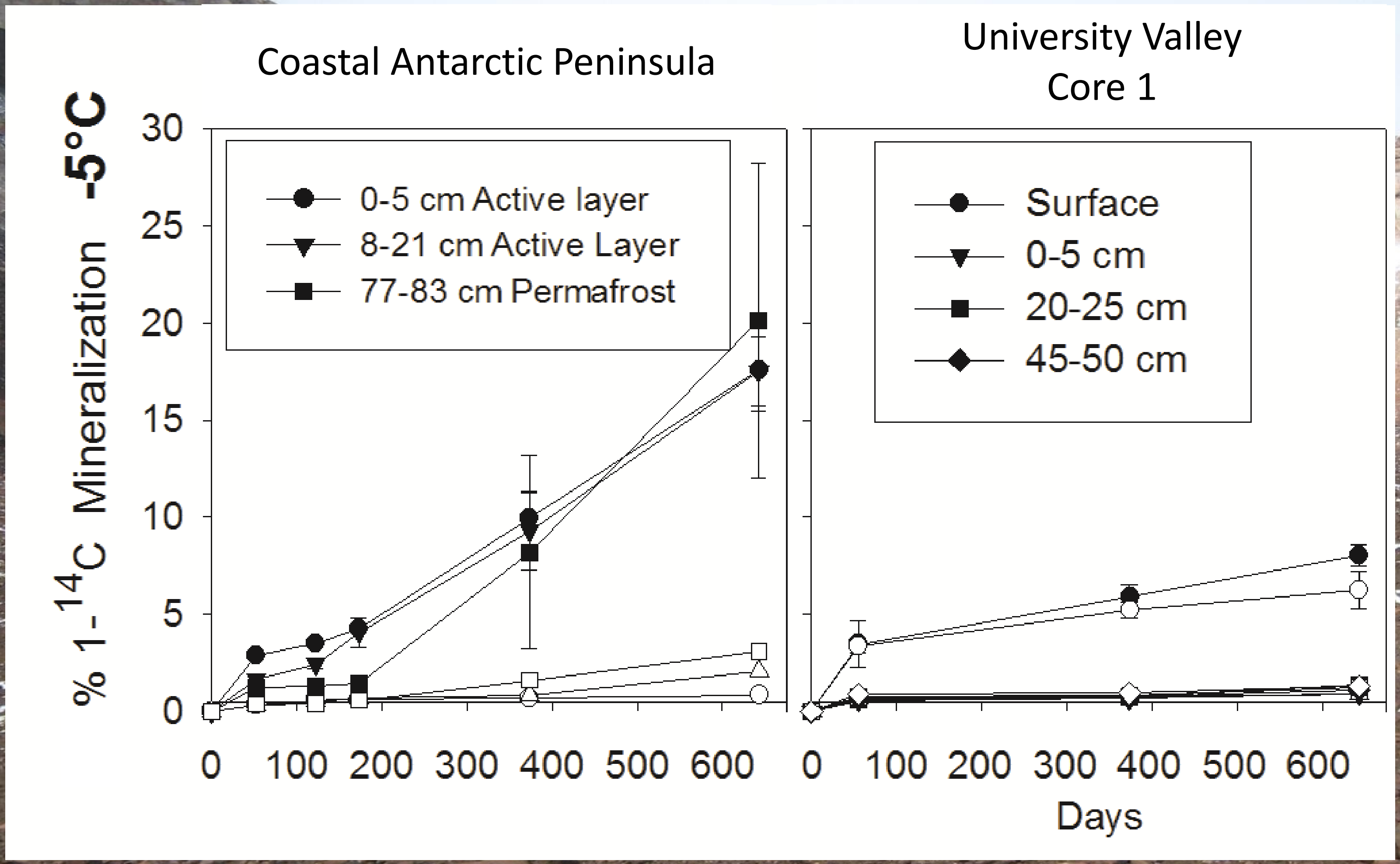
Addition of radiolabelled acetate, measure labelled CO_2 that has been mineralized.

[1-^{14}C] acetate **$^{14}CO_2$**



5°C to -20°C

No microbial activity at subzero temperatures

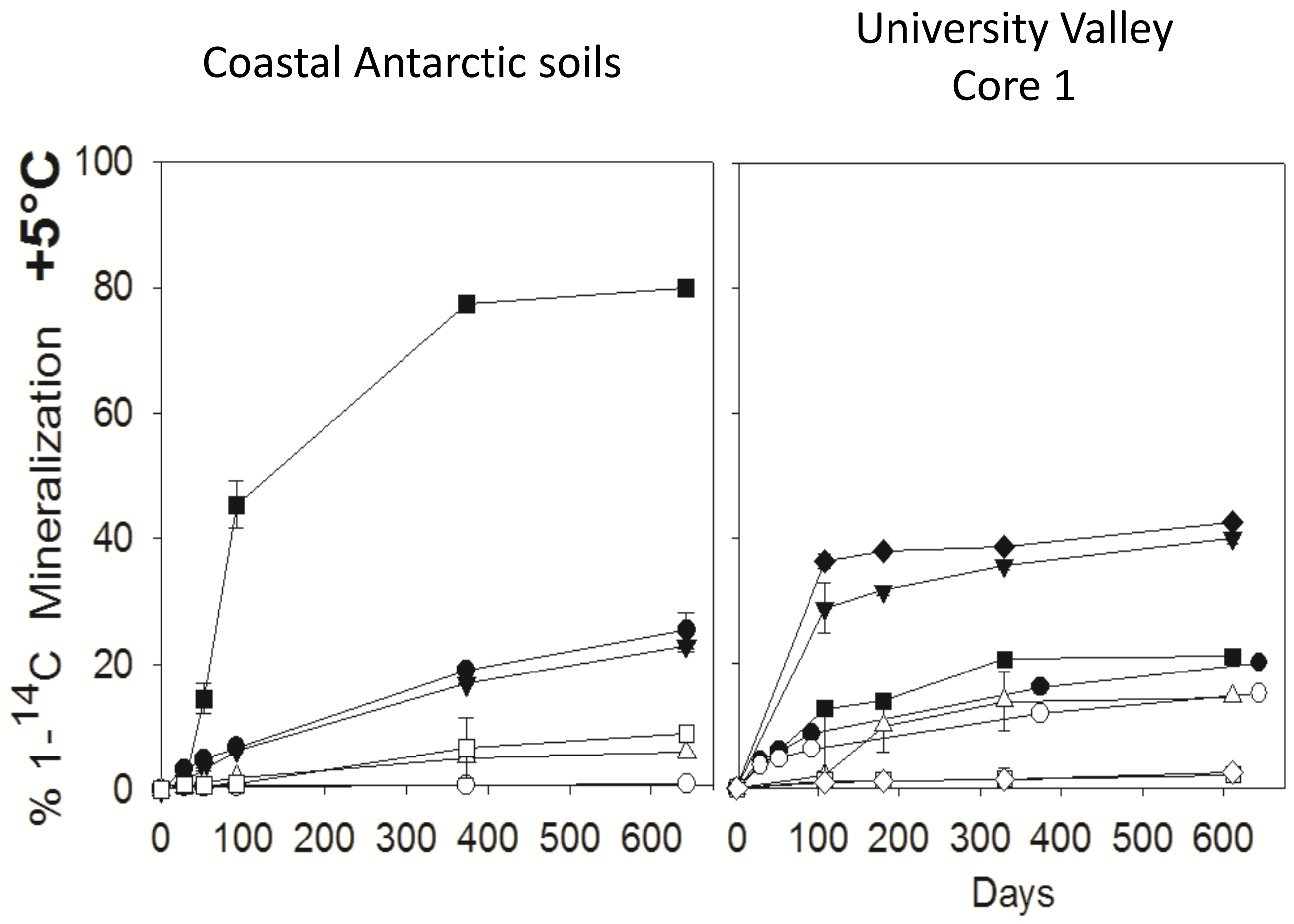


Goordial et al., ISME 2016

Microbial activity resumes above freezing (in SOME permafrost soils)

-Confirms cells remain viable in permafrost on long timescales

-genomic enrichment for dormancy traits



Nutrient Amendments do not change these results (not N/P limited)

Goordial et al., ISME 2016

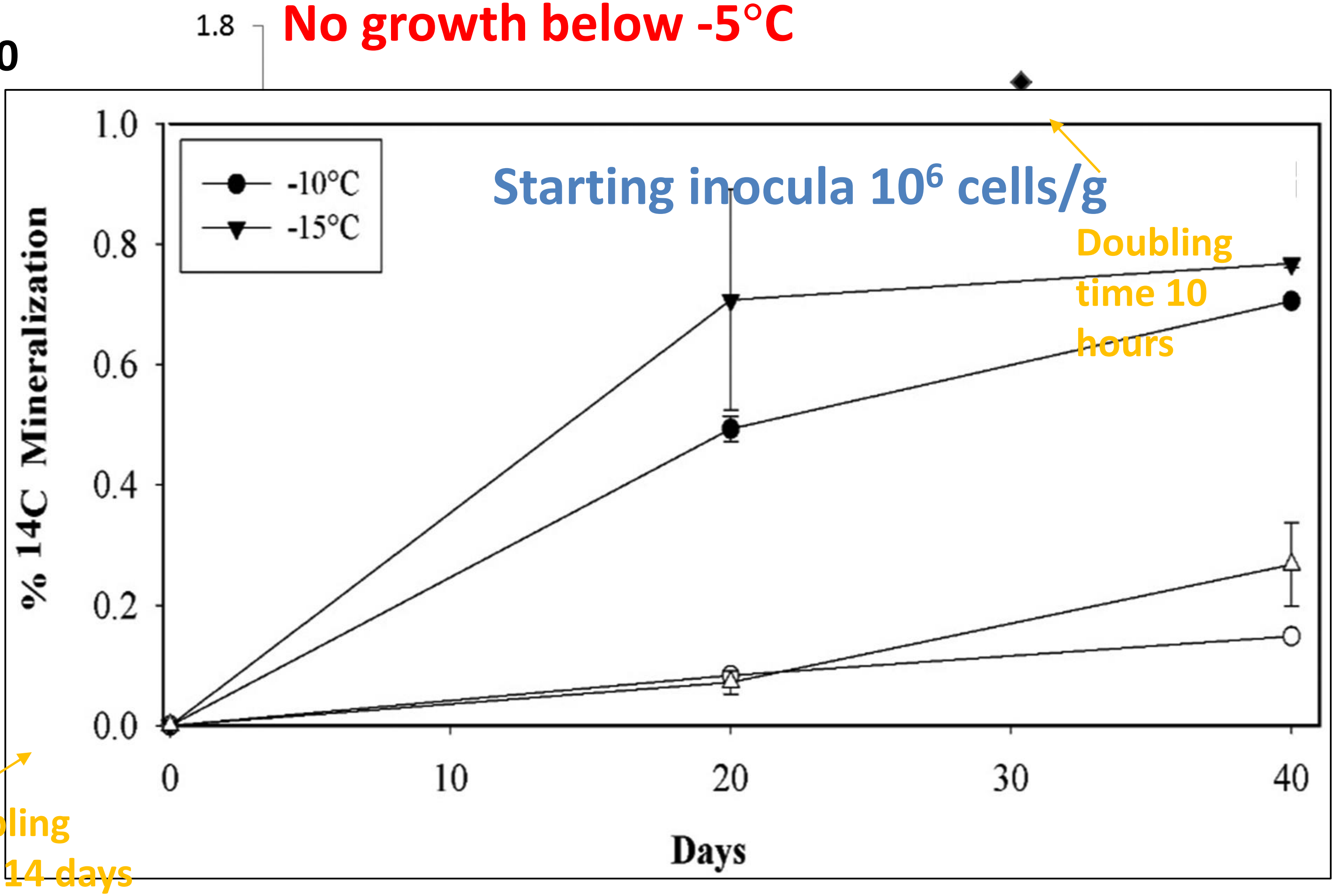
But are all cells dormant? Or is activity below our detection limits?

Rhodococcus sp. JG3

- Isolated permafrost-soil aged ~150,000 years
- Does not form spores
- Genomic traits for cold adaptation

Observed Growth: -5°C to 30°C

No growth below -5°C



Activity observed down to -15°C

Goordial et al., *FEMS Microbial Ecology* 2016

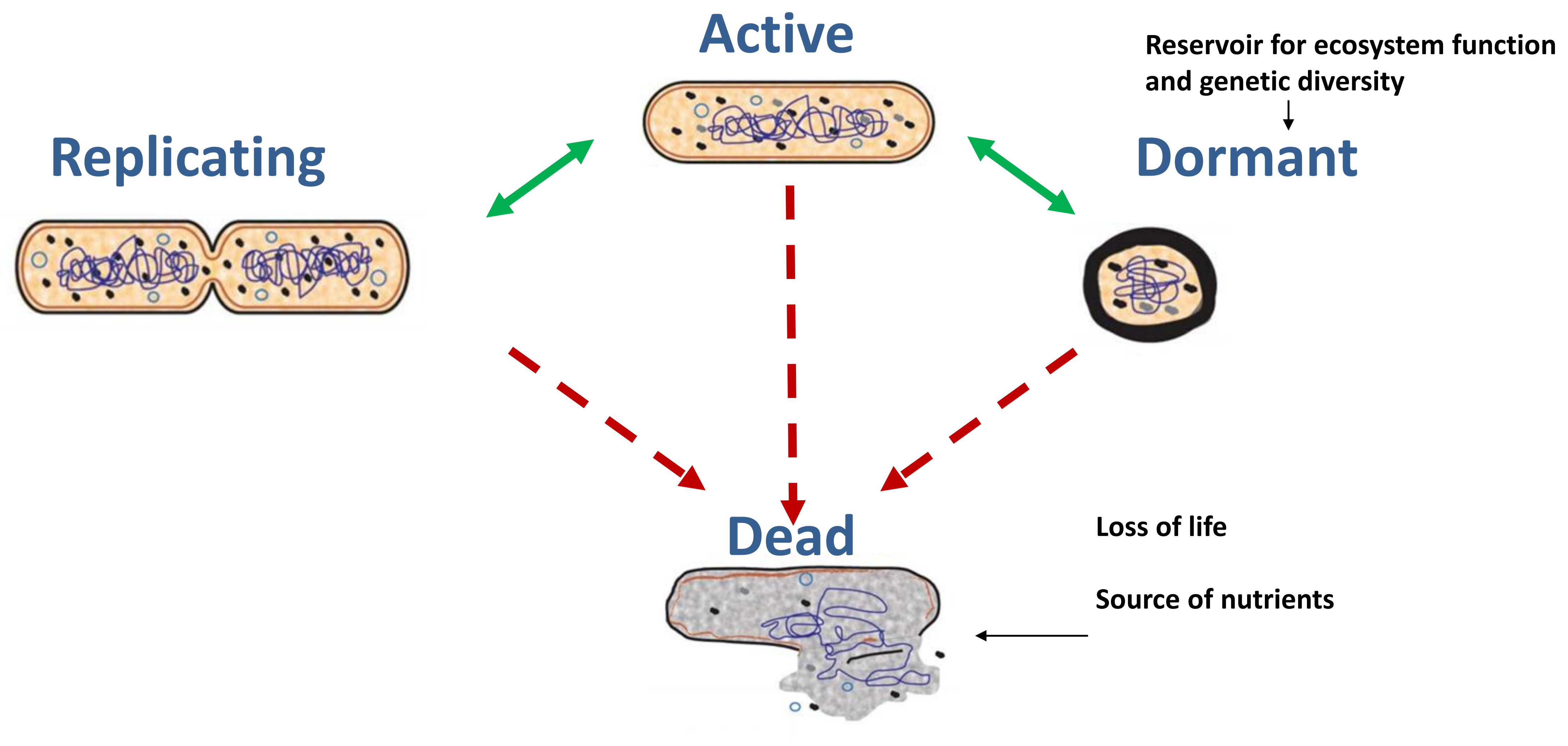
Current "limits to life" haven't changed much in recent years → But typically refer to **GROWTH**

Table 3.
Ecological limits to life

Parameter	Limit	Note
Lower temperature	∼ −15 °C	Limited by liquid water associated with thin films or saline solutions
Upper temperature	122 °C	Solubility of lipids in water, protein stability
Maximum pressure	1,100 atm	Ref. 10
Low light	∼0.01 µmol $m^{-2} \cdot s^{-1}$ = 5×10^{-6} direct sunlight	Algae under ice and deep sea
pH	0–12.5	
Salinity	Saturated NaCl	Depends on the salt and temperature
Water activity	0.6	Yeasts and molds
	0.8	Bacteria
UV	≥1,000 J m^{-2}	*D. radiodurans*

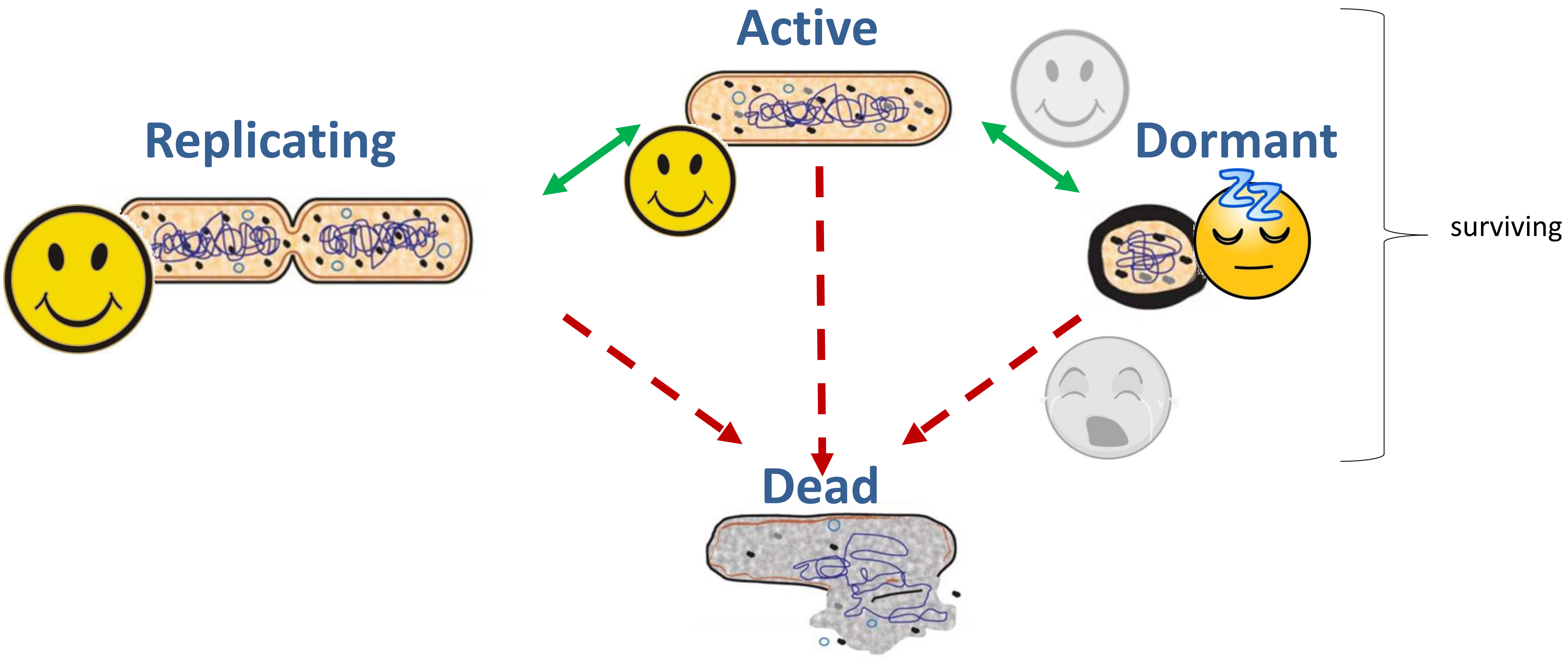
McKay, PNAS, 2014

Microbial physiological state matters when we consider ‘survivability’

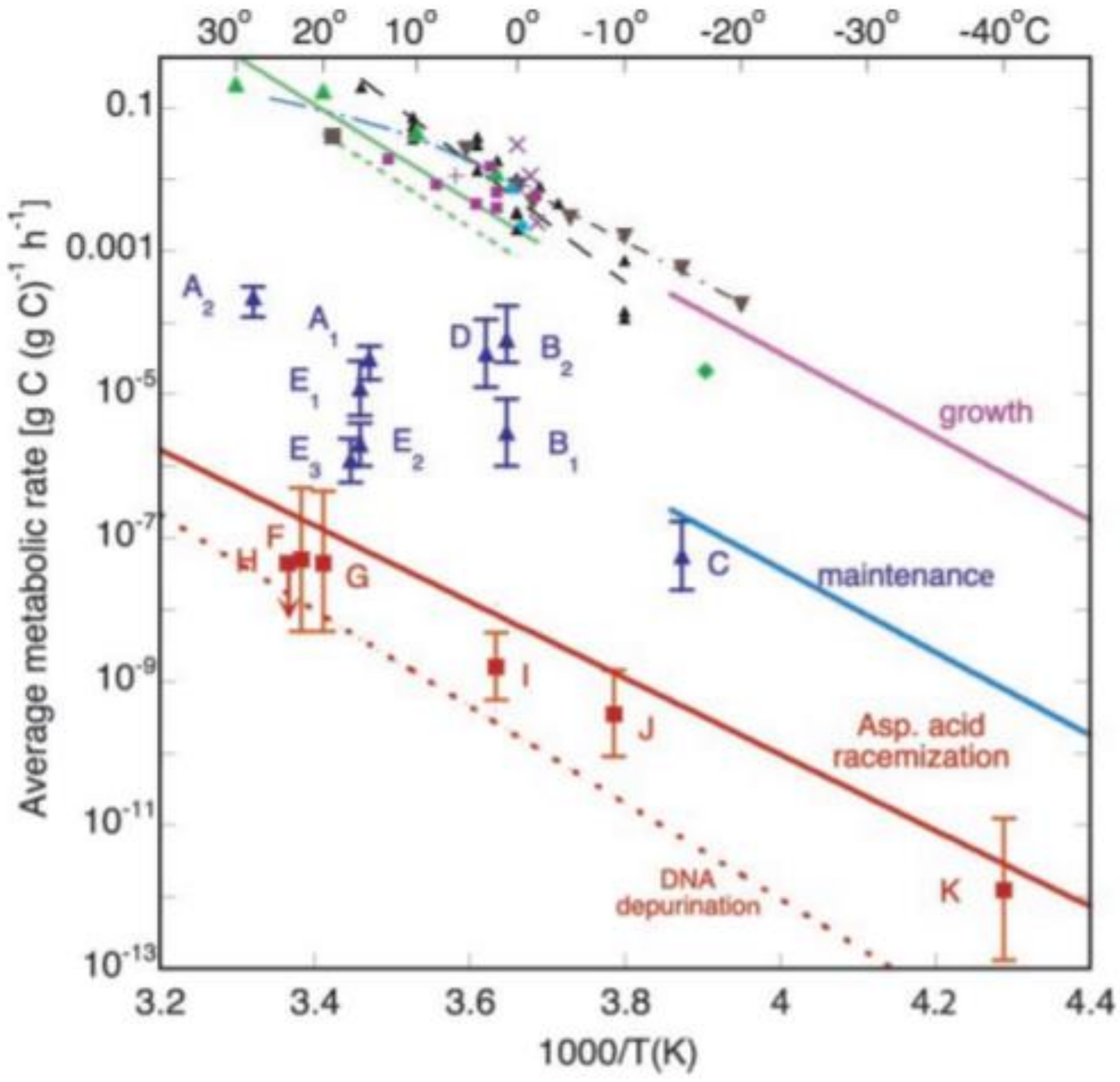


Adapted from Blazewicz et al., 2013.

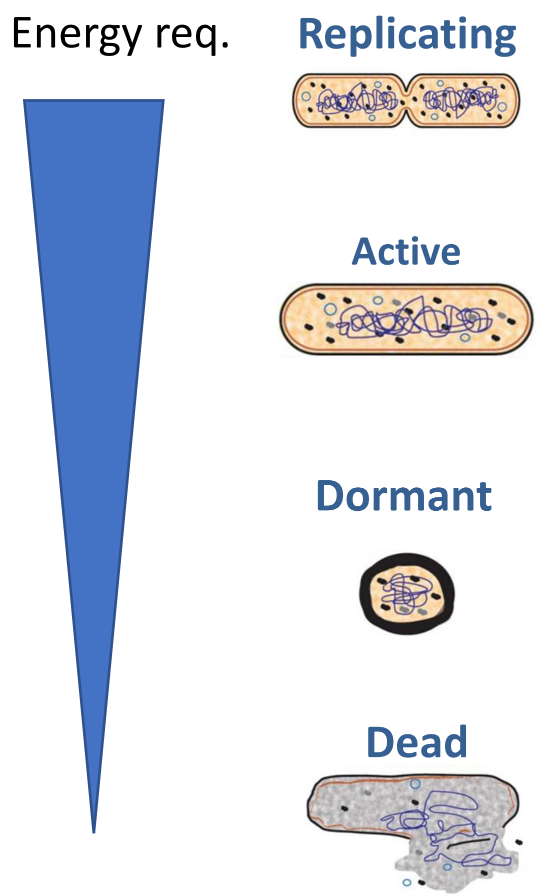
Microbial cell state *reflects habitability*

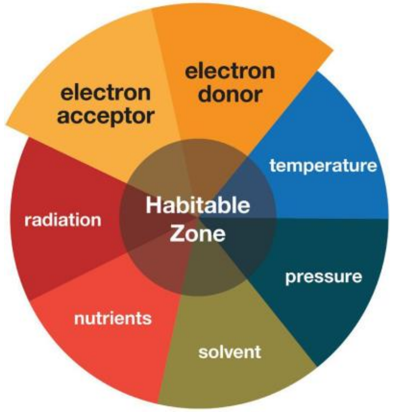


“Follow the energy” approach

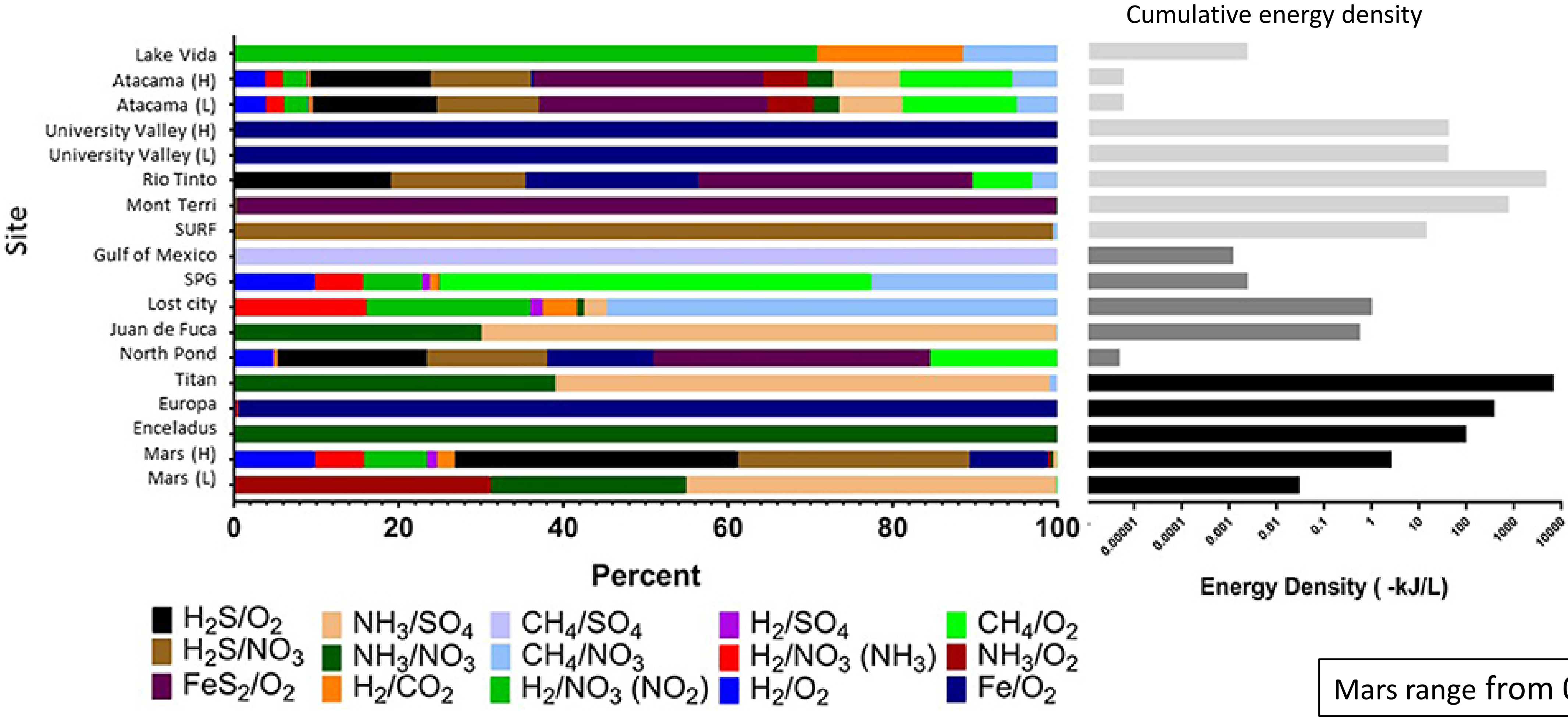


Price and Sowers, PNAS, 2004





Mars can support the energetic requirements for microbial life

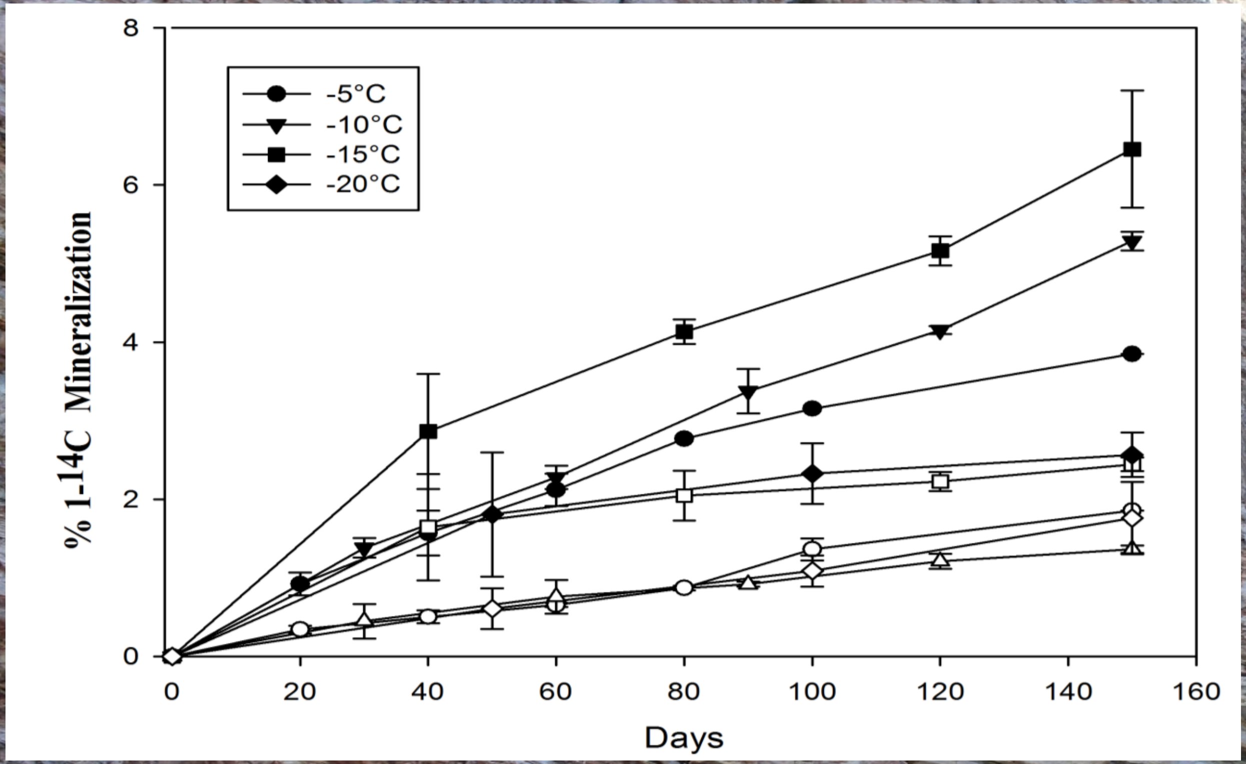


Mars range from 0.03 to 3 kJ L−1

Jones et al., Front. Microbiol., 2019

Dispersal lessons come from natural environments too

Cryptoendoliths are thriving in University Valley

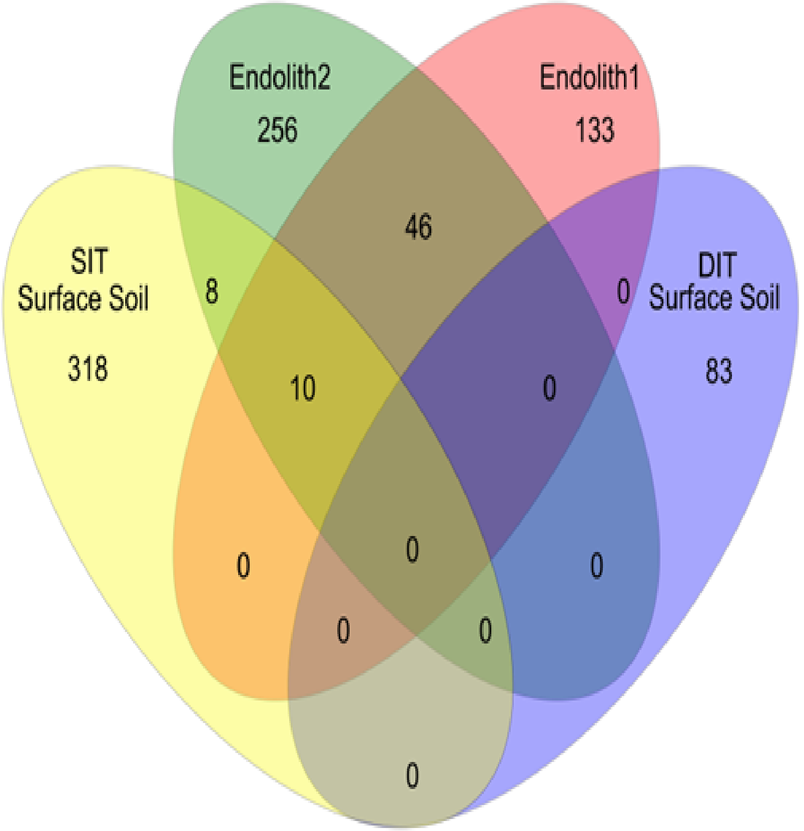


- Heterotrophic and photosystem activity detected down to -20°C
- 13 aerobic heterotroph isolates capable of growth at -5°C
- Photoautotrophic algae (*Stichococcus, Desmococcus*) , in culture. Growth and photosystem activity at -5°C

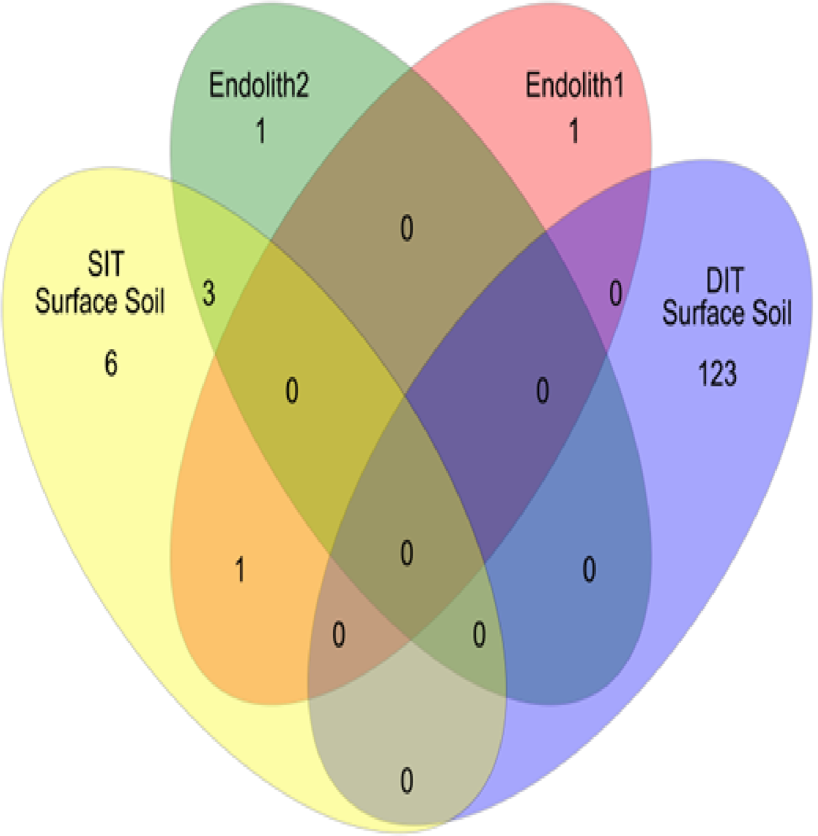
Goordial et al., Environmental Microbiology 2017

Cryptoendoliths are not the primary source of permafrost diversity

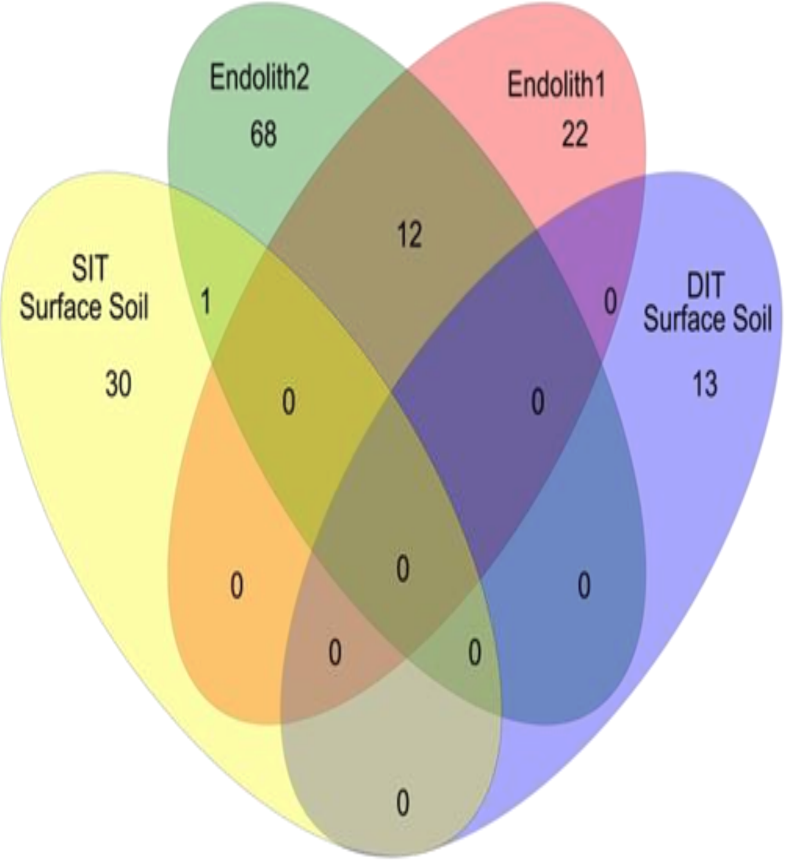
Possibly deposited via aeolian processes?



Bacteria



Archaea



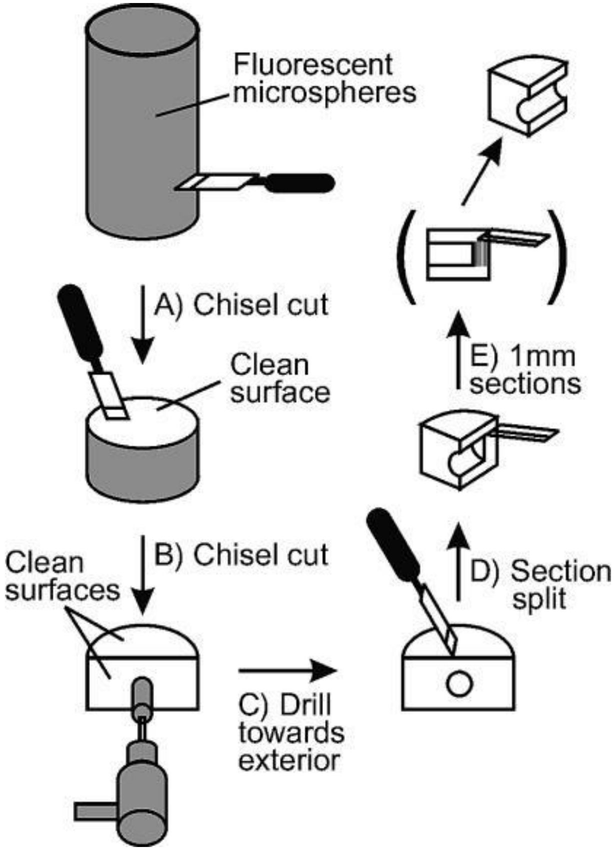
Fungi

Shared species (OTUs) -97% cutoff

Goordial et al., ISME 2016

Another point source: Drilling

- Typically remove outside of a core for analysis of earth samples → contamination of sample

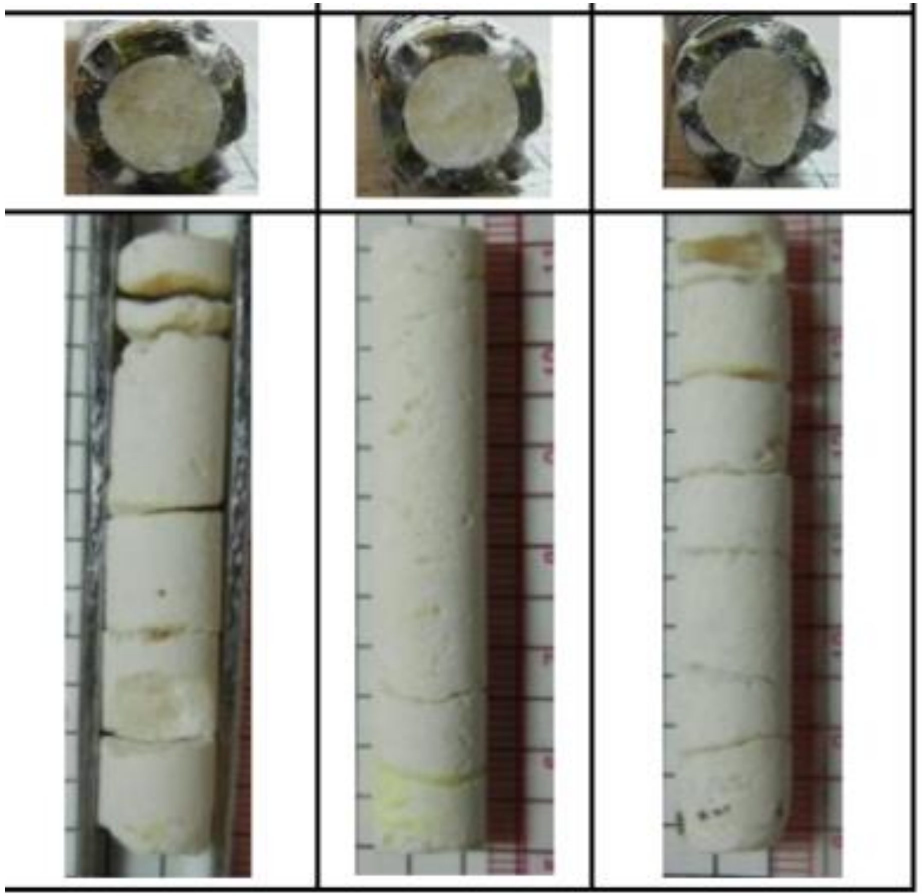


Juck et al., Appl. Env. Micro, 2005

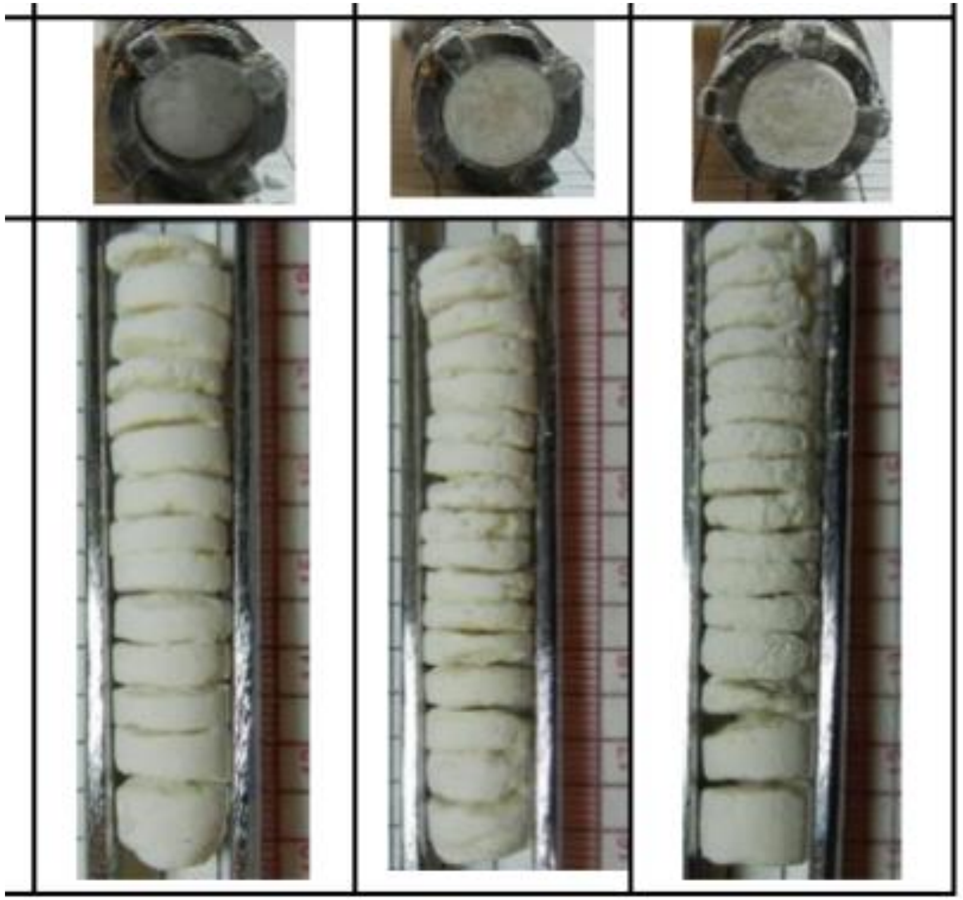
Quantified microsphere deposition on cores and cuttings

1. Painted core barrel with microspheres (0.5 and 0.05 um) ~10^{10} cells/cm^2
2. Drilled under atmospheric (Earth) and vacuum (Mars) pressures
3. Examined sphere deposition

Atmospheric pressure



Mars like pressure (7 torr)



Goordial et al., LPSC, 2017

Fluorescent microsphere distribution

Top

Bottom

Earth (760 torr)

Mars (7 torr)

Goordial et al., LPSC, 2017

Table 3. Microsphere distribution in travertine

Depth along length of core	Depth within rock	Earth conditions*	Mars Pressure*
0 cm (top of core)	4-6 mm (center)	1.2×10^3	5.7×10^1
	2-4 mm	1.4×10^3	1.5×10^3
	0-2 mm (outer core)	2.1×10^3	1.4×10^3
2.5 cm	4-6 mm	1.8×10^2	2.5×10^1
	2-4 mm	1.3×10^2	1.9×10^1
	0-2 mm	3.9×10^2	1.8×10^1
5 cm	4-6 mm	5.8×10^1	0
	2-4 mm	1.6×10^2	0.3
	0-2 mm	2.9×10^2	2.5×10^1
Inner Cuttings		2.7×10^3	2.0×10^2
Outer Cuttings		1.4×10^3	3.0×10^2

*microspheres per ul volume of crushed rock.

- Lower deposition under Mars-like conditions
- Highest deposition outside of core
- Inside penetration decreased with depth
- Core break faces similar deposition as outside of core
- Inner and outer cuttings highly contaminated.
- Cuttings which are left behind would be a concern for planetary protection of sampling sites in the Martian environment.

Goordial et al., LPSC, 2017

Conclusions

- Predictions of survival of microbial cells on Mars must go beyond **growth** of isolates in a lab on Earth. Natural analog environments on Earth are key.
- Must consider constraining microbial life in a state of dormancy/maintenance
- Redox pairs and temperature on Mars can support microbial survival (how long?)
- Potential for dispersal from drilling processes – onto samples, and onto surface

Thanks! Questions

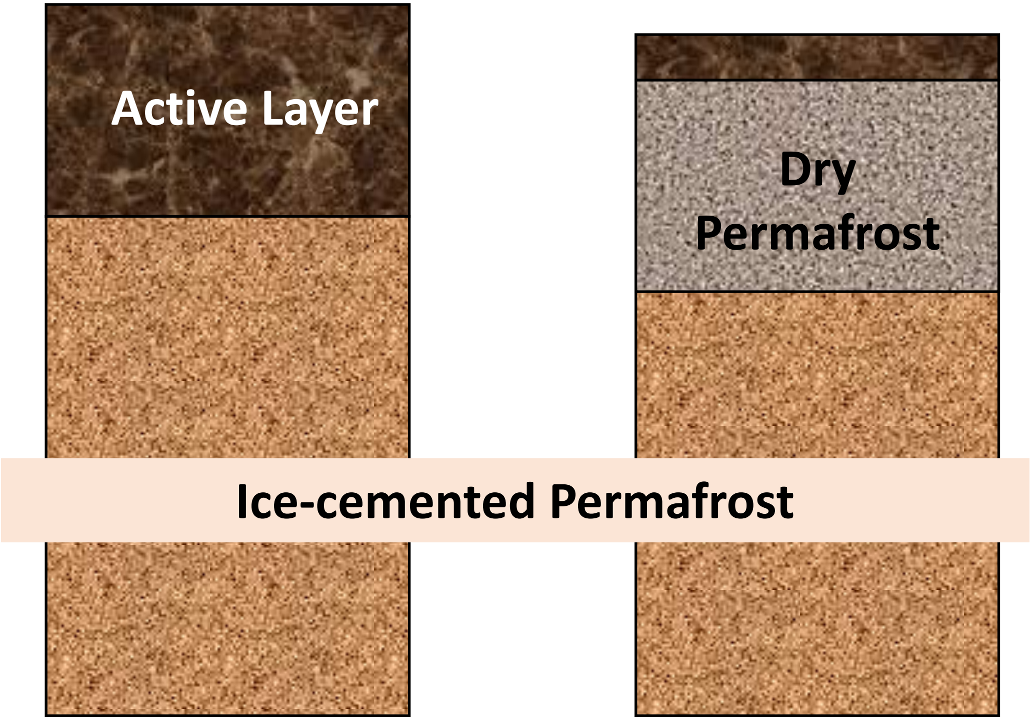
Outline

- Lessons from a Mars analog environment on Earth
    - Natural systems to look at limits of microbial life
- Microbial physiological state matters when we consider ‘survivability’
- Microbial Dispersal
    - Contamination considerations from drilling

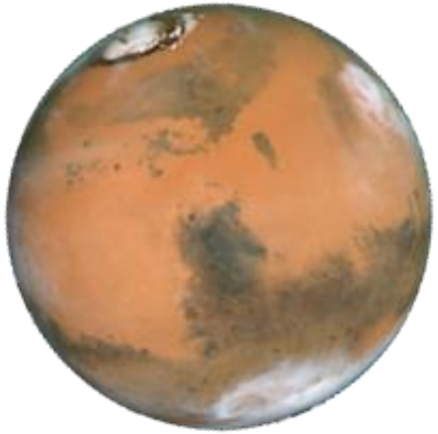
On Earth: Dry Permafrost is a rare

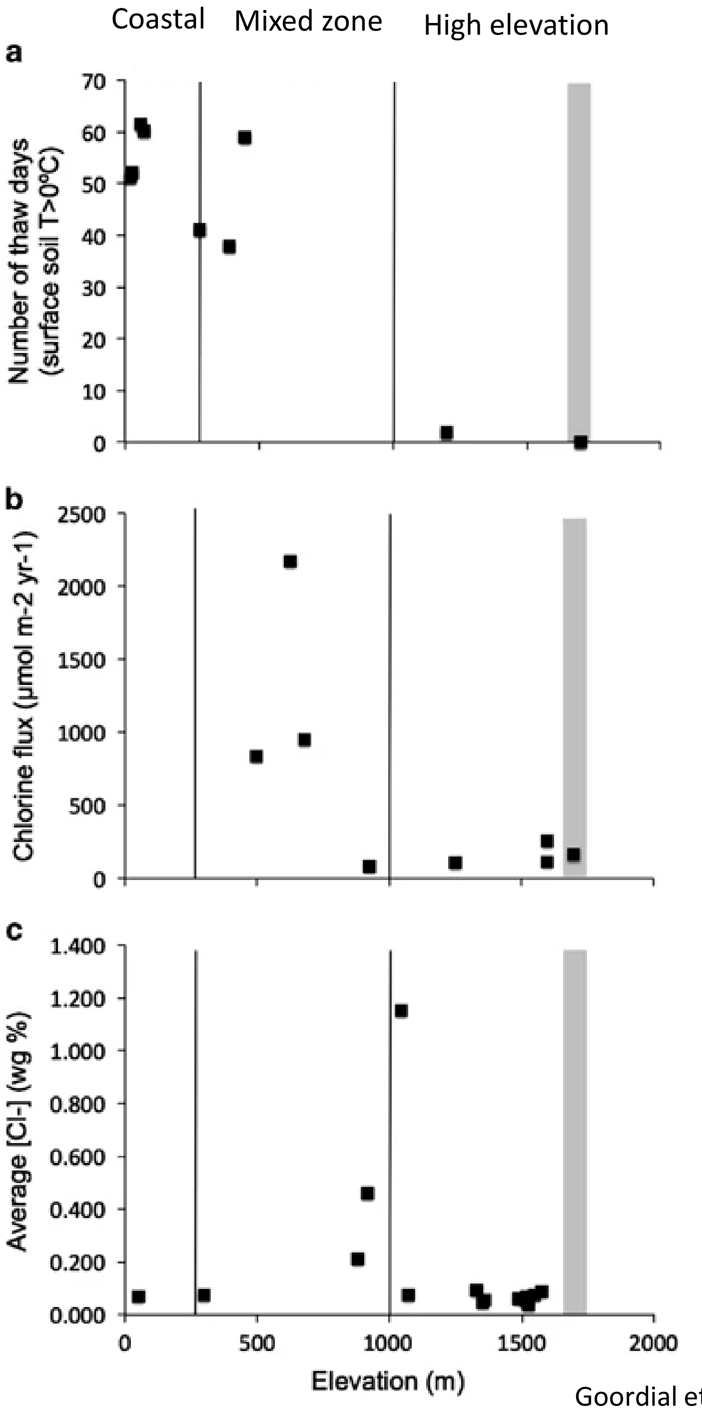
Permafrost = **Temp does not rise above freezing**
Dry permafrost = **Negligible water content**

Arctic, Coastal Antarctica, Alpine regions



High Elevation Dry Valleys,
Mars





Goordial et al., ISME, 2016

Unfrozen water in University Valley soils is only present for 74 h / year

due to:

(a) low temperature

(b) low salt influx

(c) low salt concentrations

Acknowledgements

Lyle Whyte (McGill University)

Chris McKay (NASA Ames)

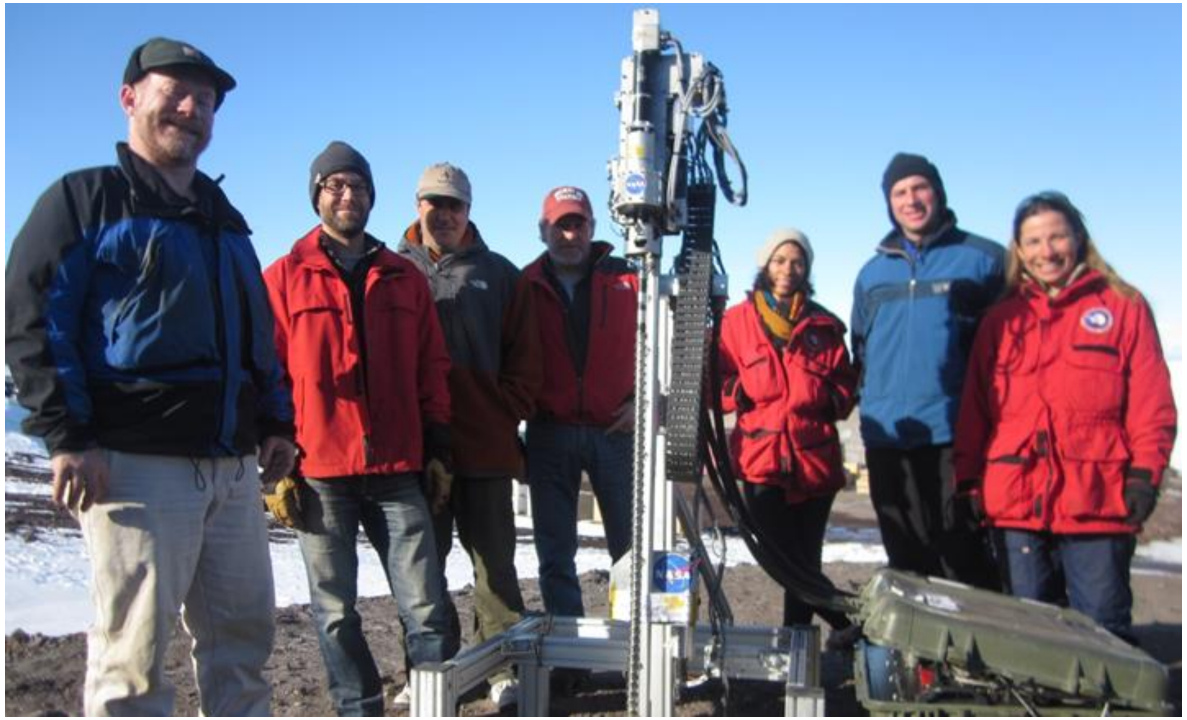
USAP project B-302

Margarita Marinova

Alfonso Davila (SETI)

Wayne Pollard (McGill)

Denis Lacelle (University of Ottawa)



NSERC
CRSNG

CATP

NASA

HONEYBEE ROBOTICS
Spacecraft Mechanisms Corporation

Institut Spatial de McGill
McGill Space Institute