The Microbiology of Antarctica's Lake Vida

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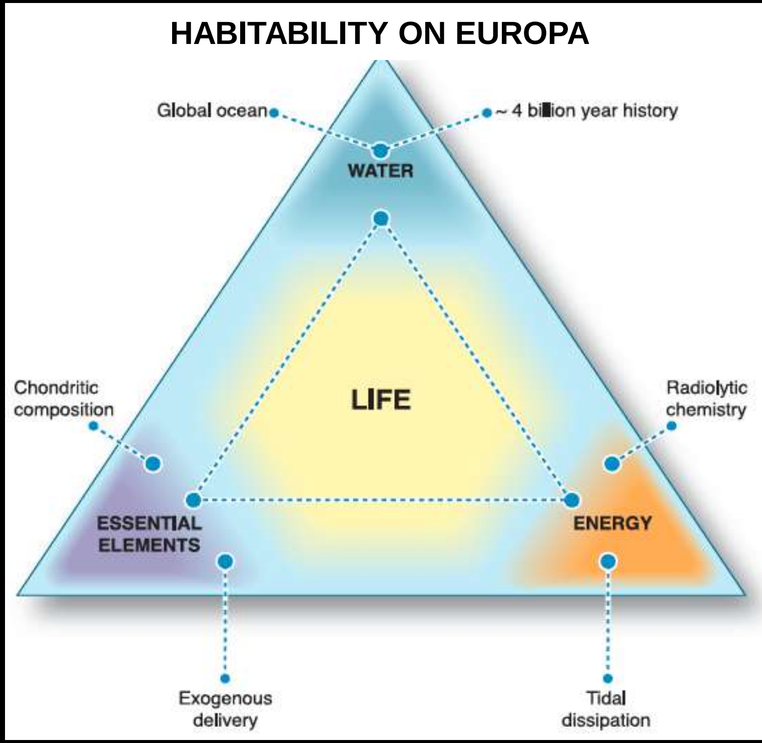
Why Lake Vida?

- Thickest lake ice on Earth
- Opportunity to study abiotic and life-associated processes in a very stable, cold, dark, and icy environment
- Improve predictive models for habitability of icy worlds (e.g. Europa, Ganamede, Enceladus)

Habitability – is Earth unique?



- Habitability relies on:
  - Water availability
  - Essential elements
  - Energy source
  - Temperature
  - Atmosphere
  - Radiation
  - pH
  - Stability & Disequilibrium

- Large habitable zone for microbes
  - Survive without oxygen
  - Boundaries – still being defined
  - Use inorganic sources of energy & minimal raw materials
  - Remain dormant for millennia

DRI

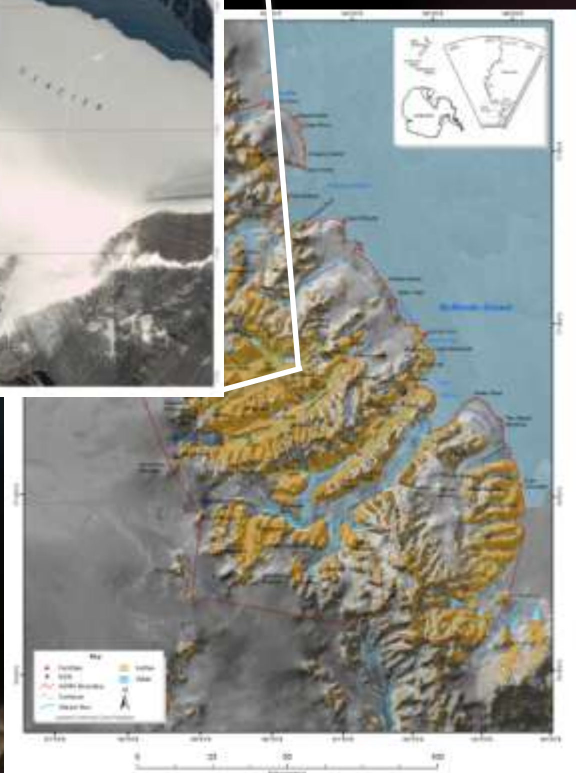
Priscu & Hand 2012, Microbe 7:167-172

Boundaries continue to expand…

Outline – Lake Vida Microbiology

- Background
- Geochemical environment
- Microbiology
  - Abundance/Activity/Diversity
- Assembling the picture
  - Geobiology of Lake Vida

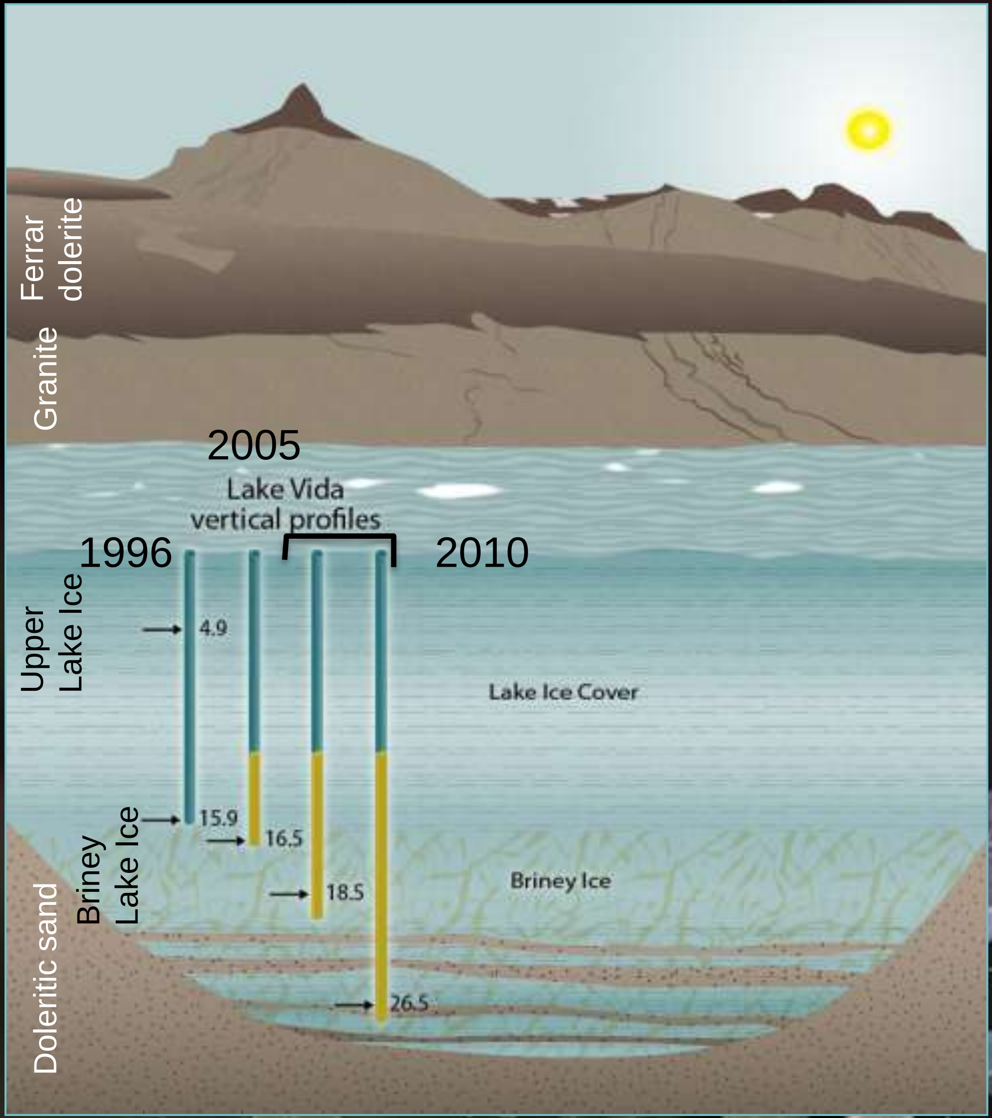
Lake Vida, Victoria Valley, McMurdo Dry Valleys



- One of the coldest and largest of the Dry Valley Lakes, 6.8 km^2
- Elevation 350 m
- Lake has been little studied (3 expeditions; ‘96, ‘05, ‘10)

Explorations into the Lake Vida Ice Cover

- 1996 Lake Vida I… the beginnings
- 2005 Lake Vida II… and then there was brine
- 2010 Lake Vida III… more brine, ice, and sediment



Vida I

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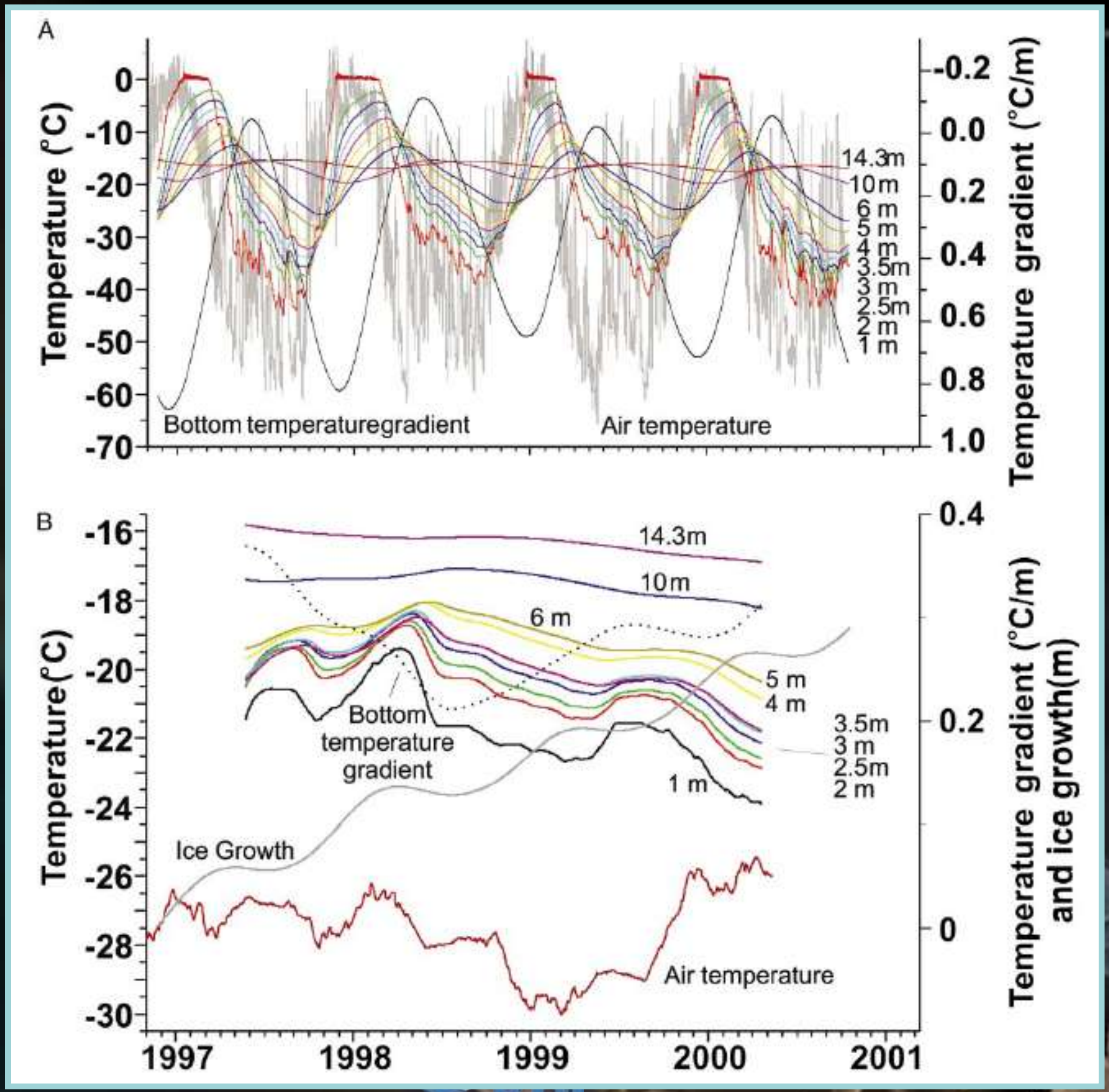
Lake Vida Ice Cover: Temperature Record

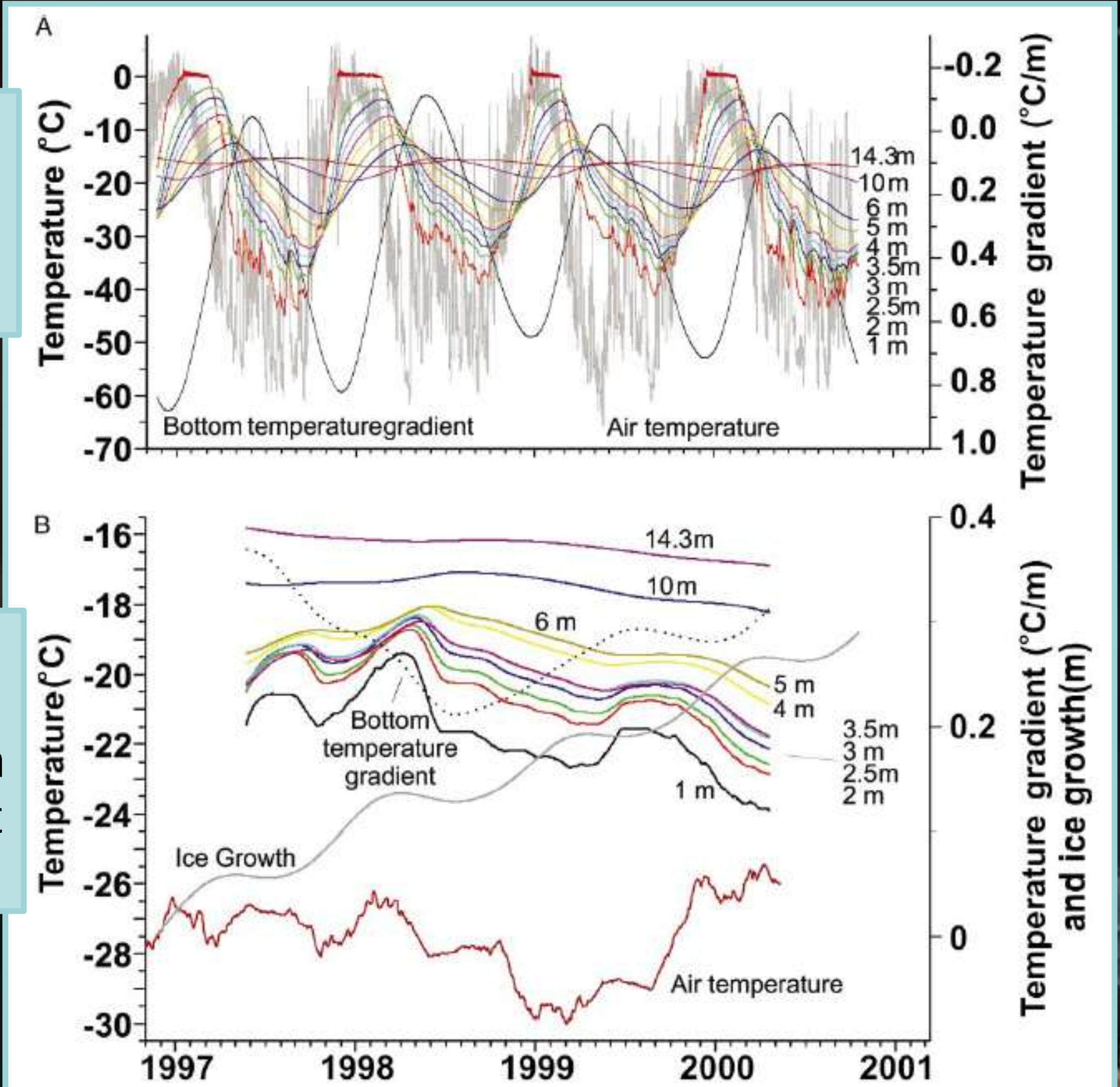


Doran et al. 2003, PNAS 100:26-31

Lake Vida Ice Cover: Temperature Record

High seasonal variation in temperature ave. -40 °C in winter

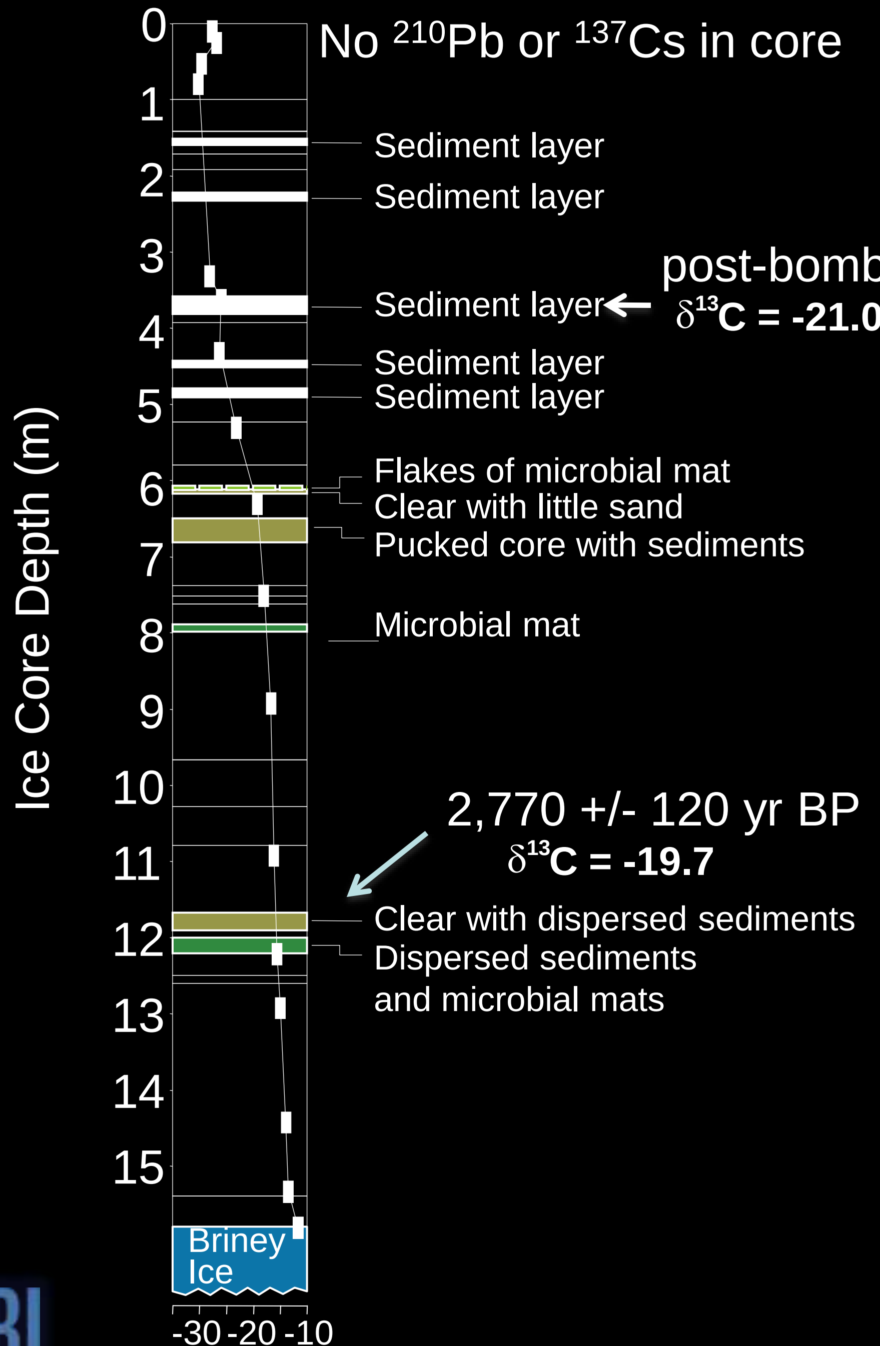
Insulation of deeper ice – low variation in temperature at 14.3 m



Doran et al. 2003, PNAS 100:26-31

Lake ice stratigraphy

- 20 m perennial sediment-laden ice cover (moat forms at edges, but no hydrologic connection).
- Upper ice cover contains microbial mats viable upon thawing
- ^{14}C-dating of mat at 13.9 m ~ 2800 yrs.
- Isolated, aphotic ecosystem



Doran et al. 2003, PNAS 100:26-31

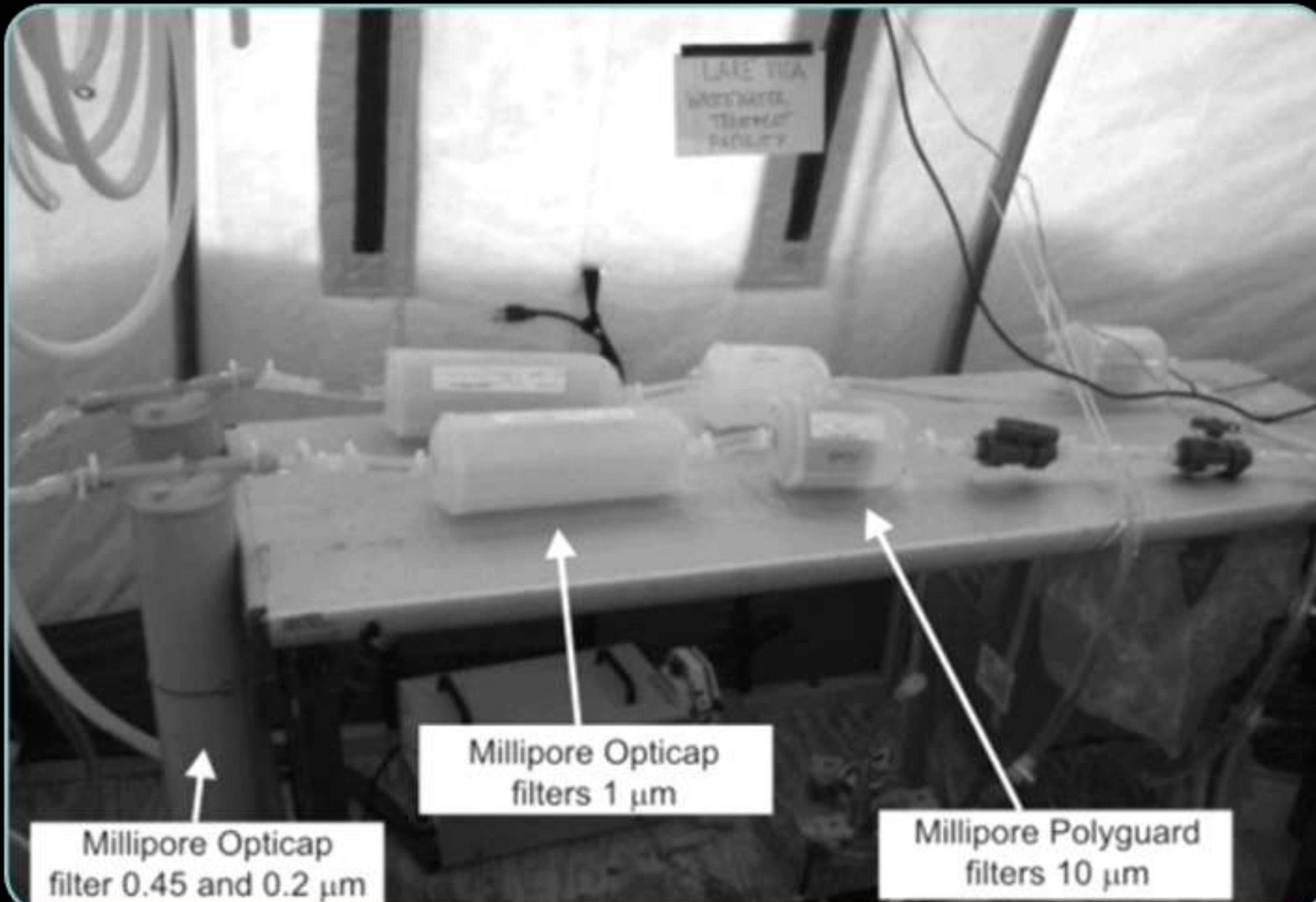
Vida II & III
Expedition Goals

- Develop clean access technology to sample an ice-sealed ecosystem
- Access the subsurface brine and describe the system – physiochemical and biological parameters
- Form and test hypotheses concerning energy and life detection signatures

Vida 2005: Camp installed….



Ice-melt, filter-sterilization system



Doran et al. 2008 L&O Methods 6: 542-547

Clean Room: Drilling, hole cleaning and sampling

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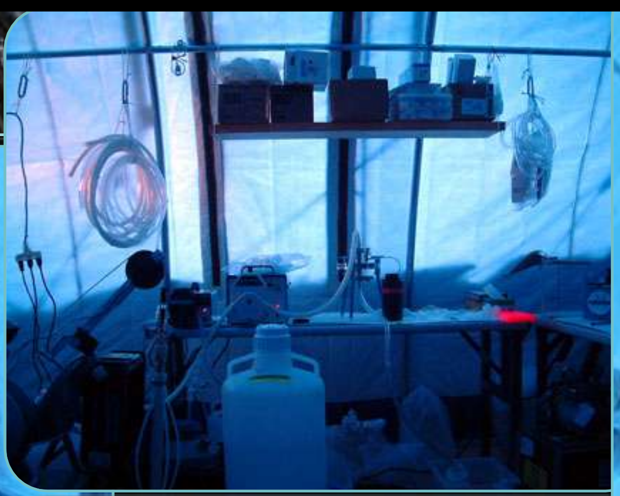
Clean Room: Drilling, hole cleaning and sampling

‘05 “Ahoy” brine in the hole at 16 m!



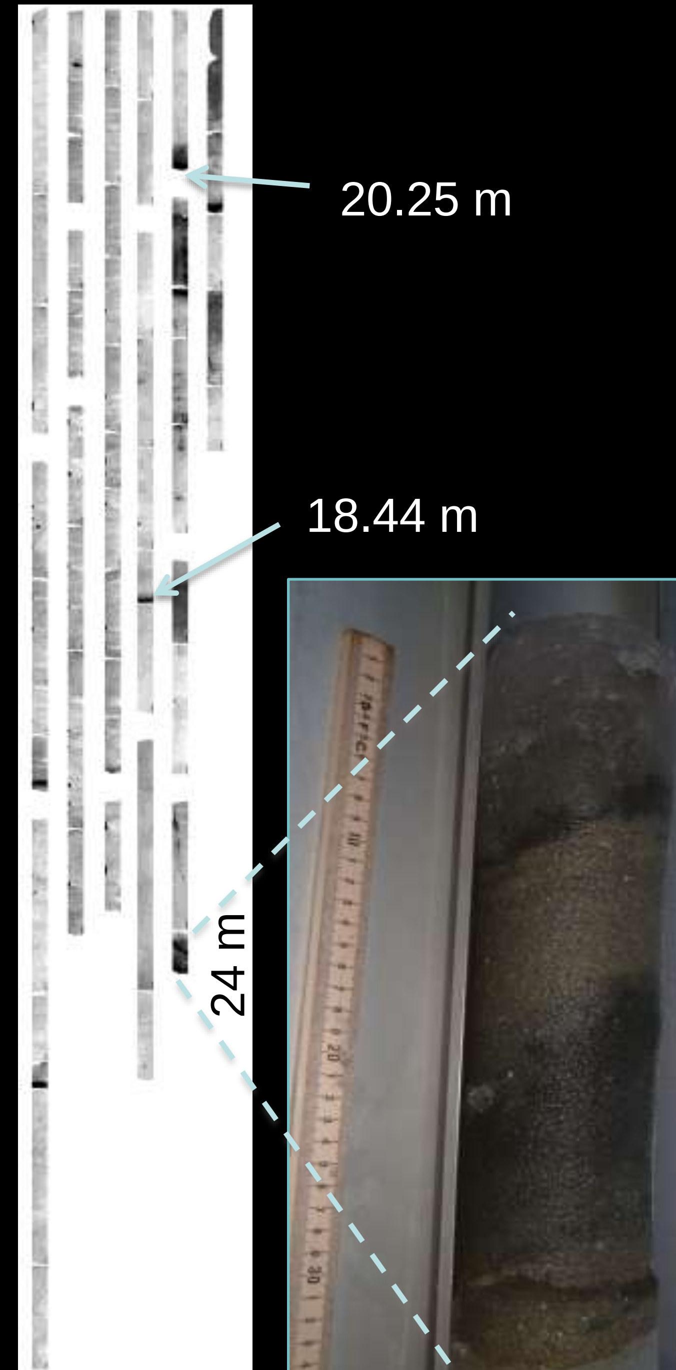
‘10 Brine sampling marathon

- Inorganic: ions, N & P, metals
- Stable isotopes (N, S, C_{org}, DIC, H, Fe)
- Biology: cells, spores, viruses, DNA, RNA, POC, DOC, PON, lipid and small molecule biomarkers

- Dissolved gasses: N_2O, CH_4, H_2, N_2, Ar + noble gasses
- Electrochemistry
- Radioisotopes: C, H

'05 & '10 Vida Core recovery/brine collection

- '05: During the drilling process brine entered the hole 16 m below the lake surface – this brine was sampled.
- '10: Following clean access protocol (< 13 m; brine initially entered hole. Drilled to 26.5 m – recovered ice/frozen sediment core.
- '10: Drilled a second hole to 20 m solely for brine collection & downstream analyses.



What are the challenges to living in Lake Vida?

Lake Vida Brine Geochemistry

	2005	2010
Temperature	-13.4	-13.4
pH	6.2	6.2
Salinity (NaCl) PSU	188	200
SO_4^{-2} (μM)	58.4 $\pm$ 2.3	66.35 $\pm$ 4.31
Fe (μM)	307.9 $\pm$ 22.6	256.0 $\pm$ 12.0
Mn (μM)	81. 9 $\pm$ 3. 67	83 $\pm$ 3
H_2 (g) (μM)	NA	10.47 $\pm$ 0.02
N_2O (g) (μM)	58.8	86.6$\pm$ 5.9
NH_4^+ (**mM**)	3.89 $\pm$ 0.04	3.60$\pm$ 0.19
NO_2^- (μM)	23.7 $\pm$ 1.0	27.83 $\pm$ 0.72
NO_3^- (**mM**)	0.9 $\pm$ 0.03	1.12 $\pm$ 0.13
DOC (mg L^{-1})	580	776.6

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Low temperature! One of the most stable cryobiospheres on Earth

Temperature affects:
- Protein folding
- Translation apparatus
- Membrane integrity

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6-7X the salinity of seawater.
NaCl - based brine

Water activity: 0.87

Limits:
Haloarchaea: 0.7
Fungi: 0.61

Lake Vida Brine Geochemistry

High iron concentrations – 99% in dissolved form

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Lake Vida Brine Geochemistry

Very high oxidized and reduced N species

High DOC values

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Lake Vida Brine Geochemistry

Highest values reported for a natural water

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Lake Vida Brine Geochemistry

Abundant H_2

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Temperature	-13.4	-13.4
pH	6.2	6.2
Salinity (NaCl) PSU	188	200
SO_4^{-2} (μM)	58.4 ± 2.3	66.35 ± 4.31
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What is the evidence for life in Vida brine?

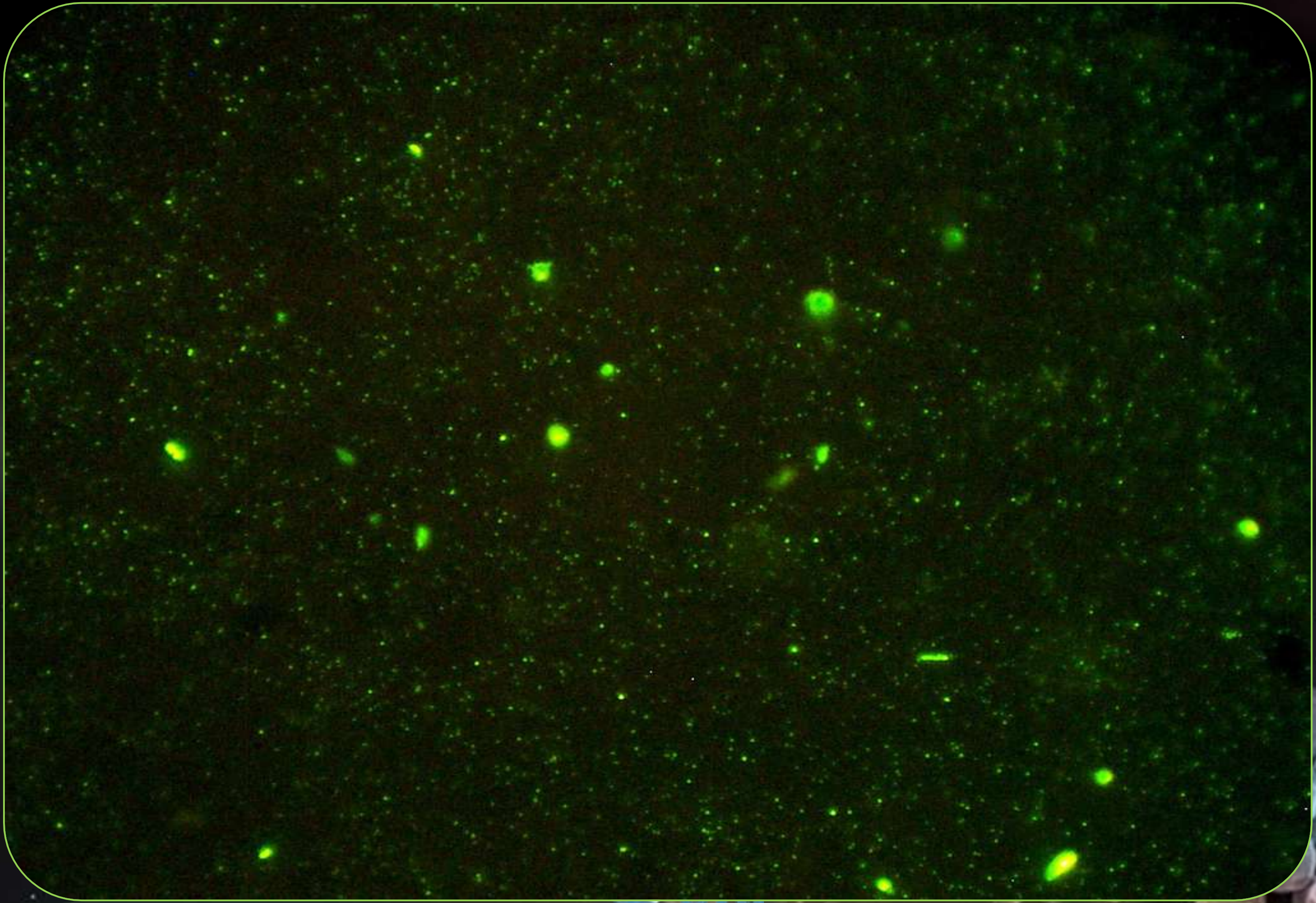
- Microscopy
- Metabolic activity determinations – protein production
- Detection of ribosomal DNA and RNA

DRI

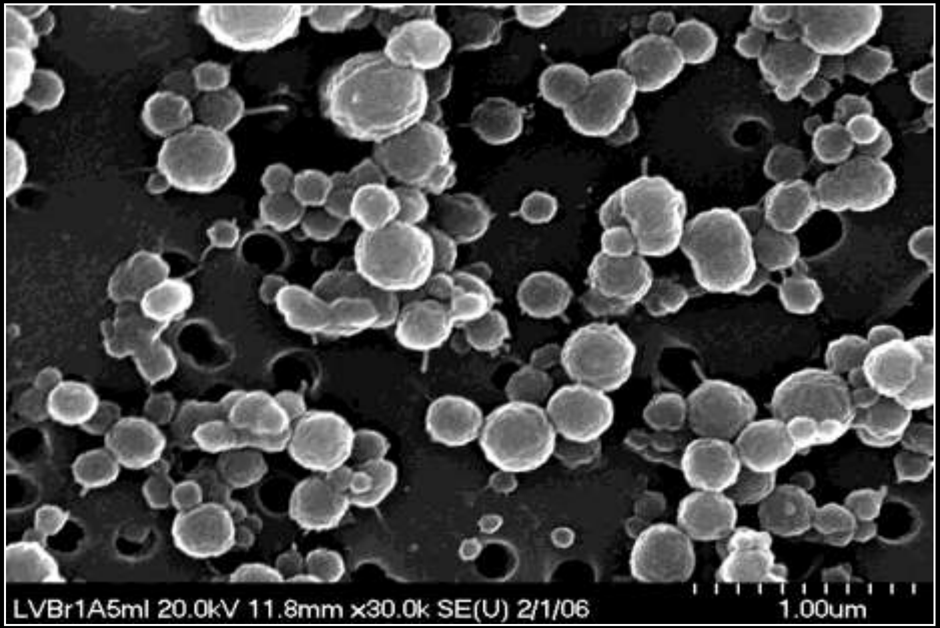
SYBR Gold-stained brine reveals two size classes

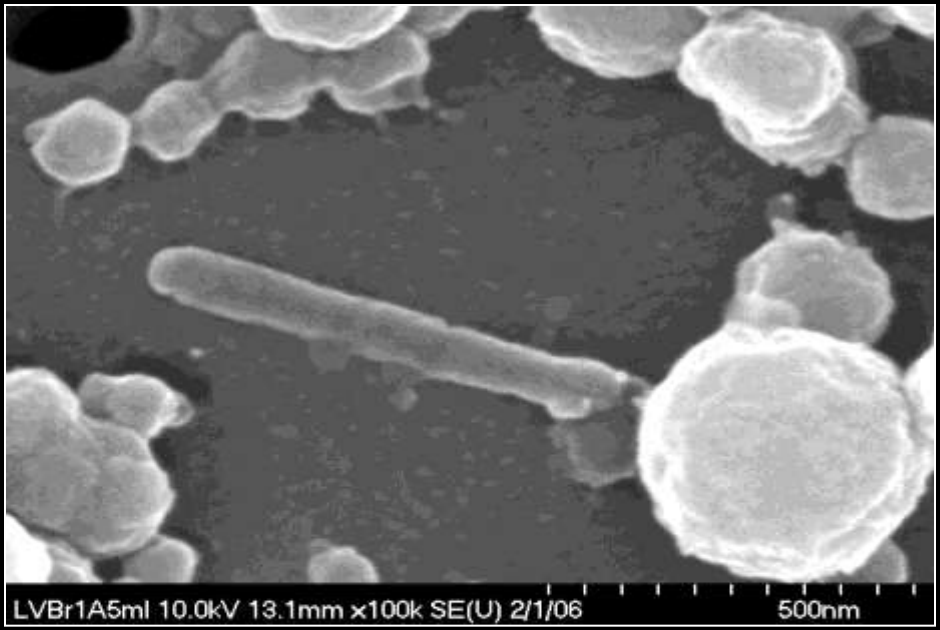
~ > 0.4 μm cells: 0.143×10^9 cells x L^{-1}

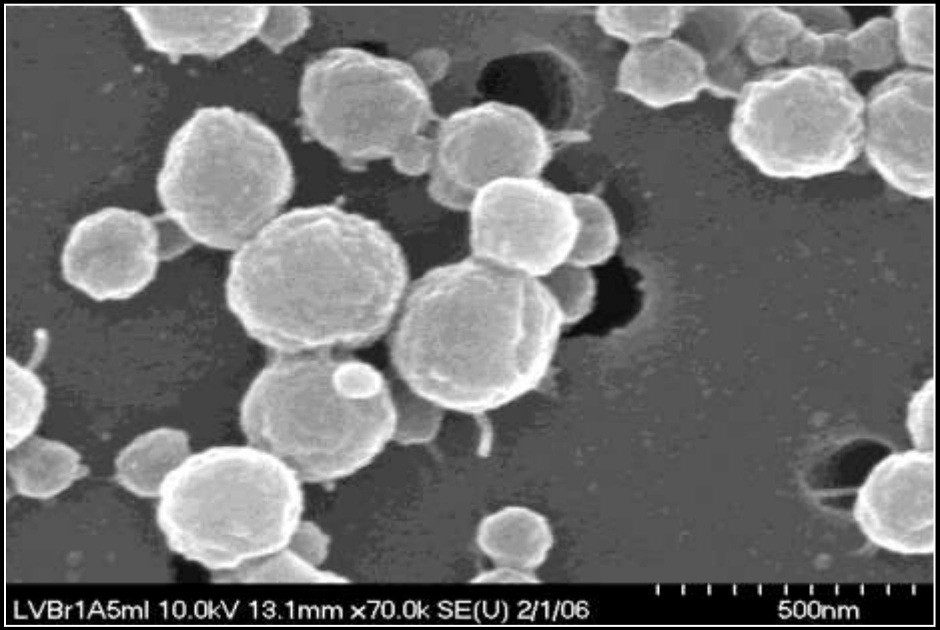
~ 0.1-0.2 μm cells: 61.4×10^9 cells x L^{-1}



Scanning electron micrographs







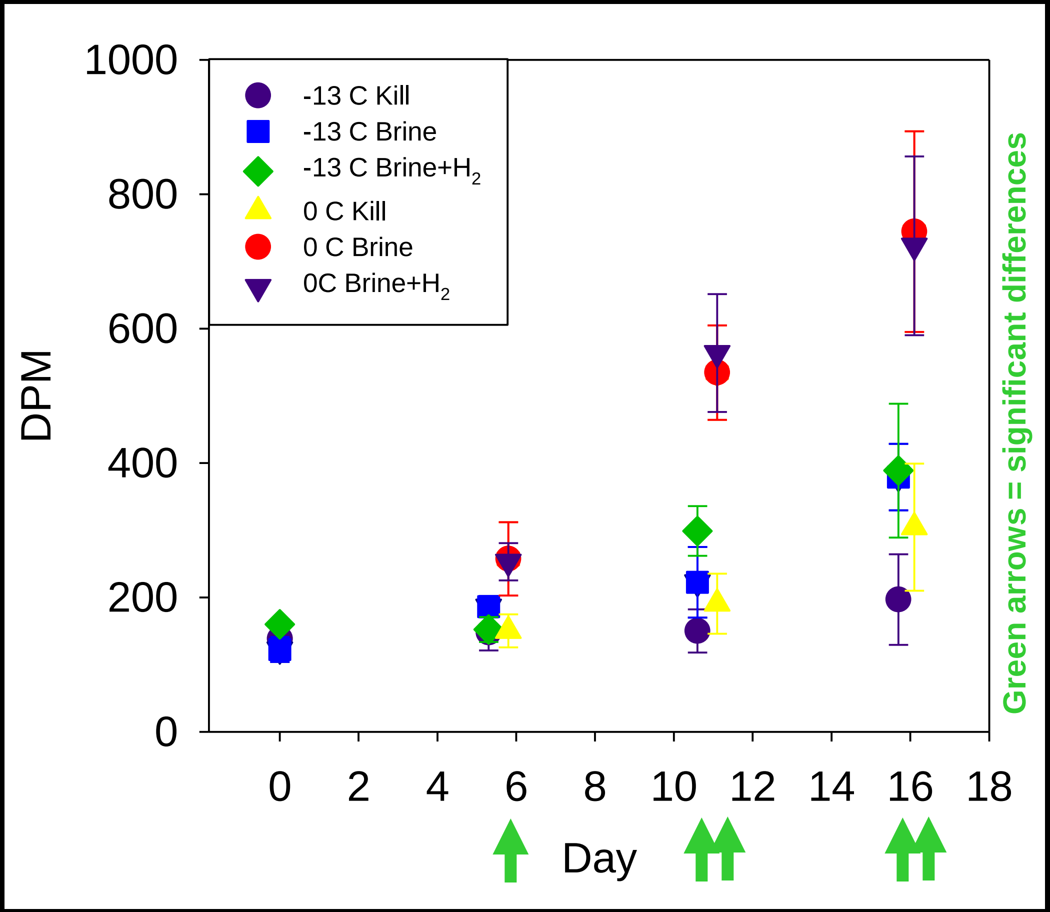
Life Detection

Testing both human and microbial activity at -13˚C

- Protein biosynthesis
- DNA biosynthesis
- C-fixation into organic matter

Evidence for protein biosynthesis: Microbial uptake of ^3H-leucine into protein

Hungate tubes with N_2 and H_2:N_2 headspace, n=5

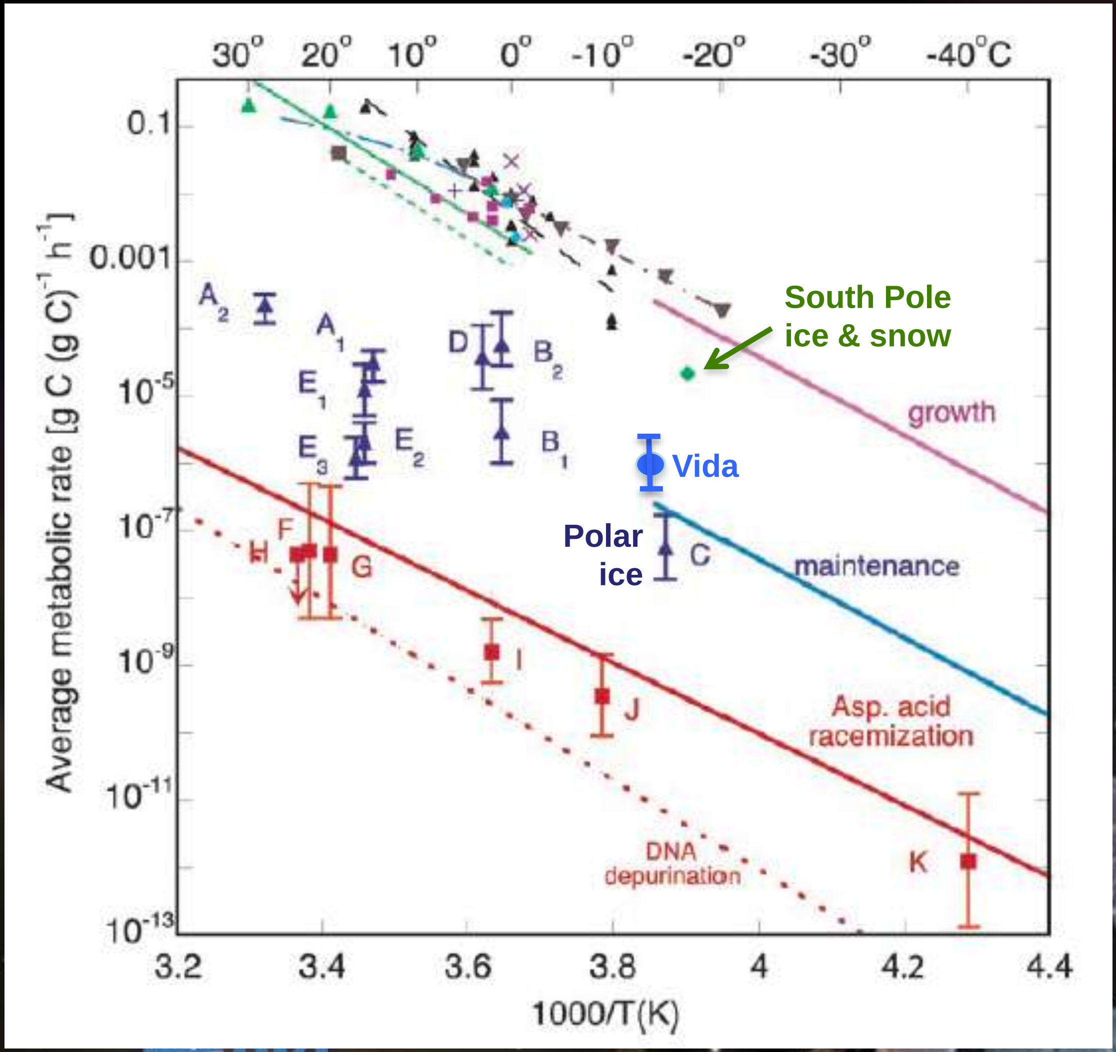


- Rates at 0 °C are higher than at -13.5 °C
- Significant levels of protein biosynthesis at 0 °C after 6 days; and at -13 °C after 11 days.
- H_2 did not have a significant effect on protein biosynthesis rates.

** results were not significant for ^3H-thymidine or ^{14}C-bicarbonate uptake

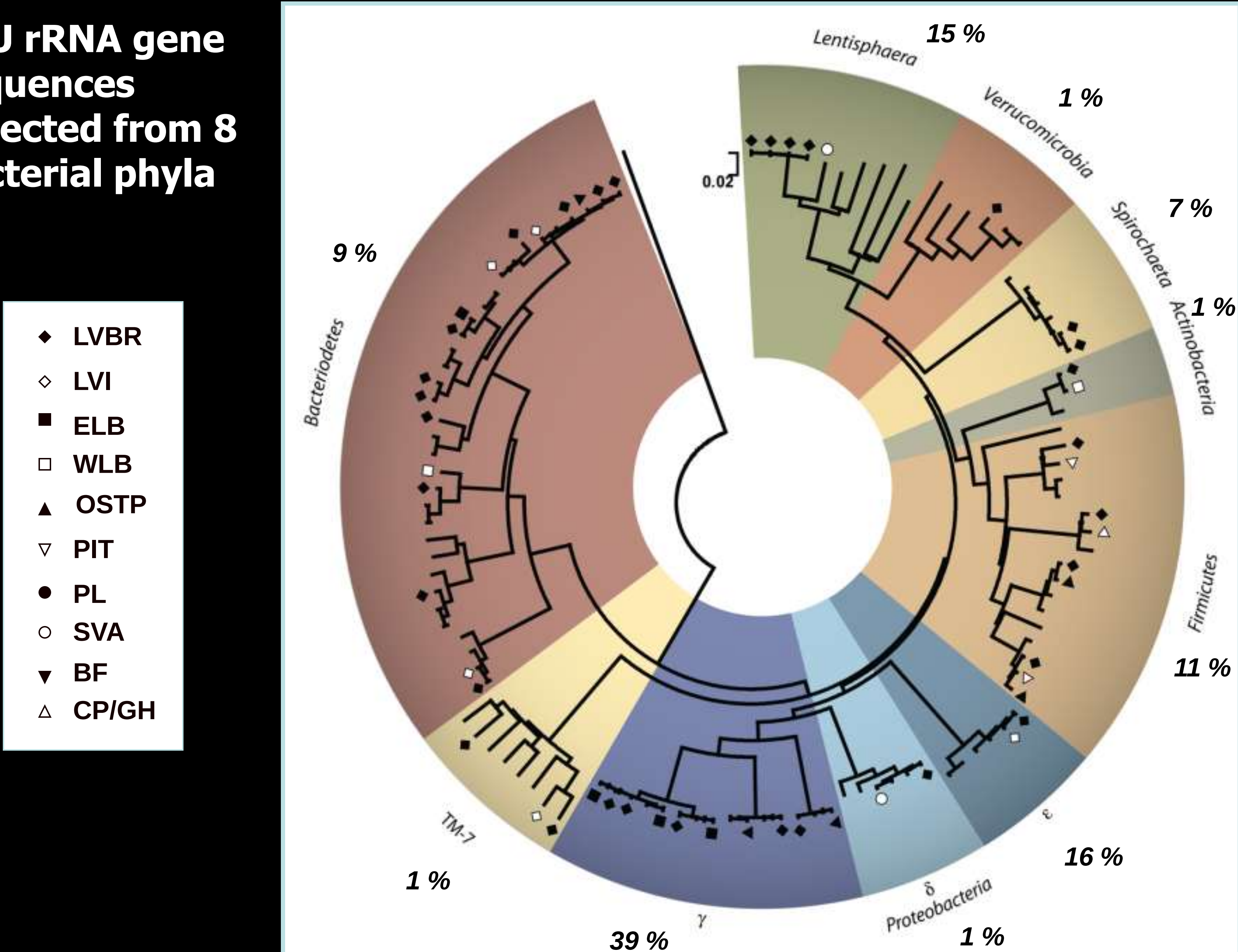
Metabolic rate as a function of temperature

- Considering only the larger cells…
- Metabolic rate falls between maintenance and growth metabolism
- Lake Vida microbiota may double only every 120 yrs
- Rates are averaged across all cells



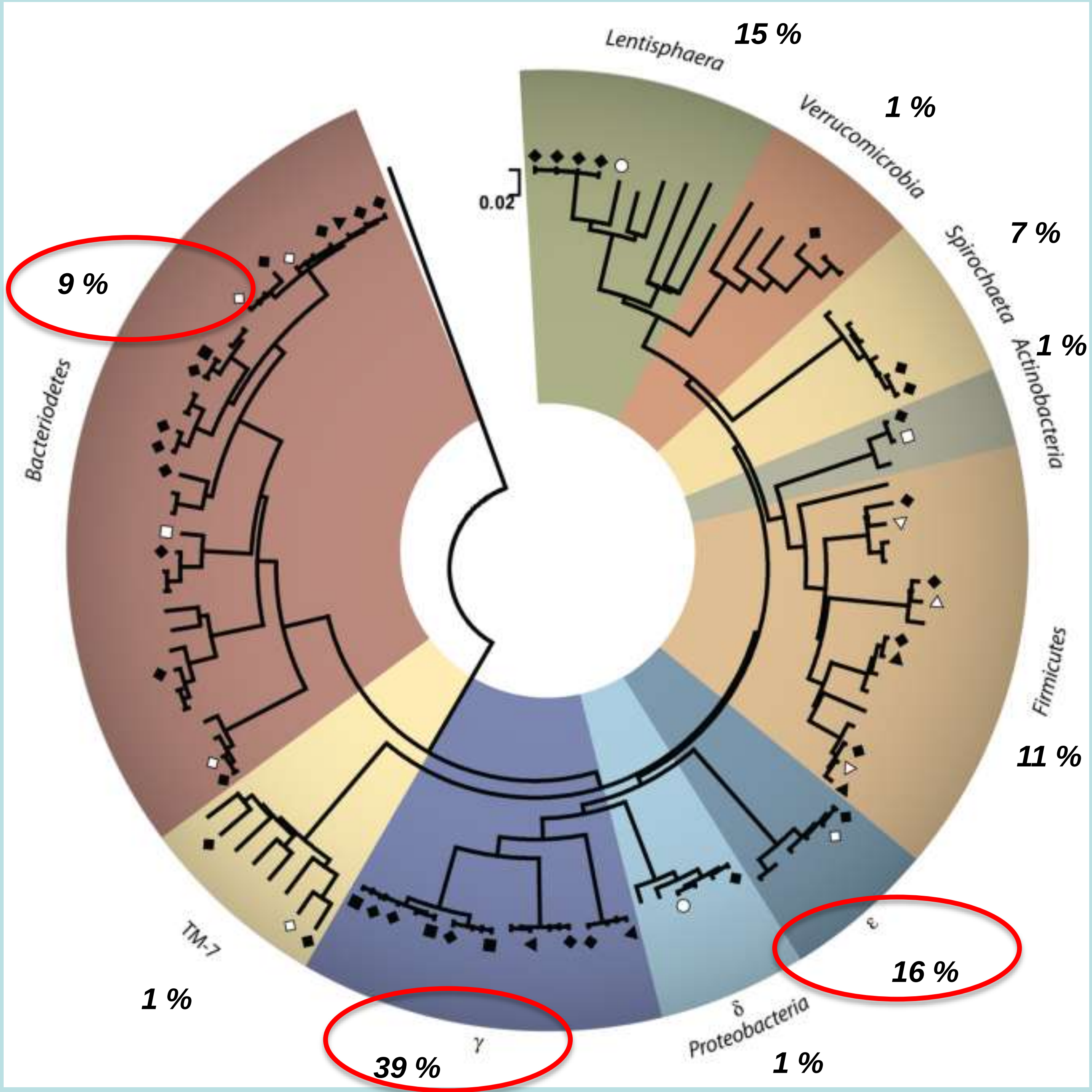
Price & Sowers, PNAS 2004 101:4631-4636

SSU rRNA gene sequences detected from 8 bacterial phyla

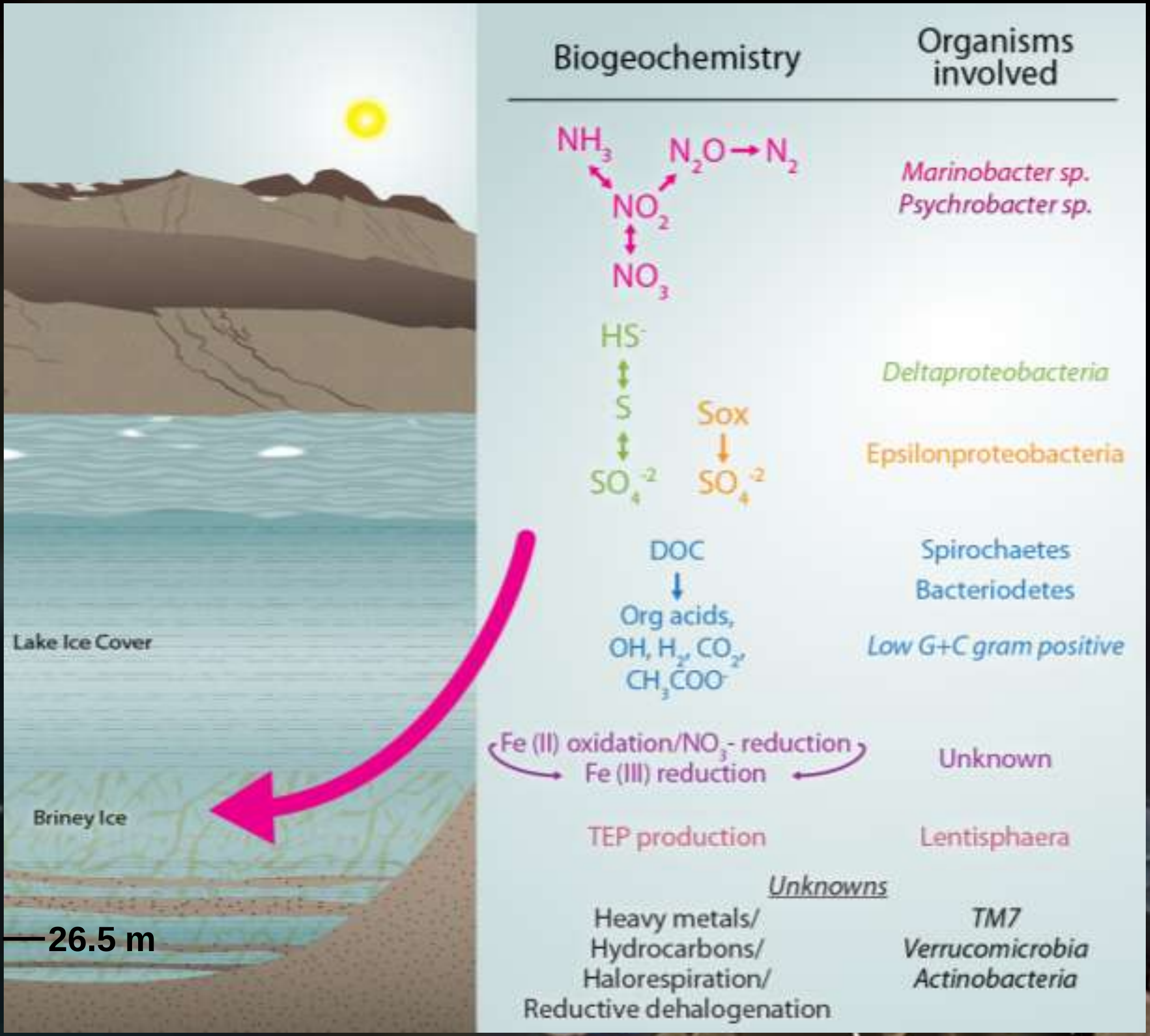


SSU rRNA sequences detected from 2 bacterial phyla

- ◆ LVBR
- ◇ LVI
- ■ ELB
- □ WLB
- ▲ OSTP
- ▽ PIT
- ● PL
- ○ SVA
- ▼ BF
- △ CP/GH



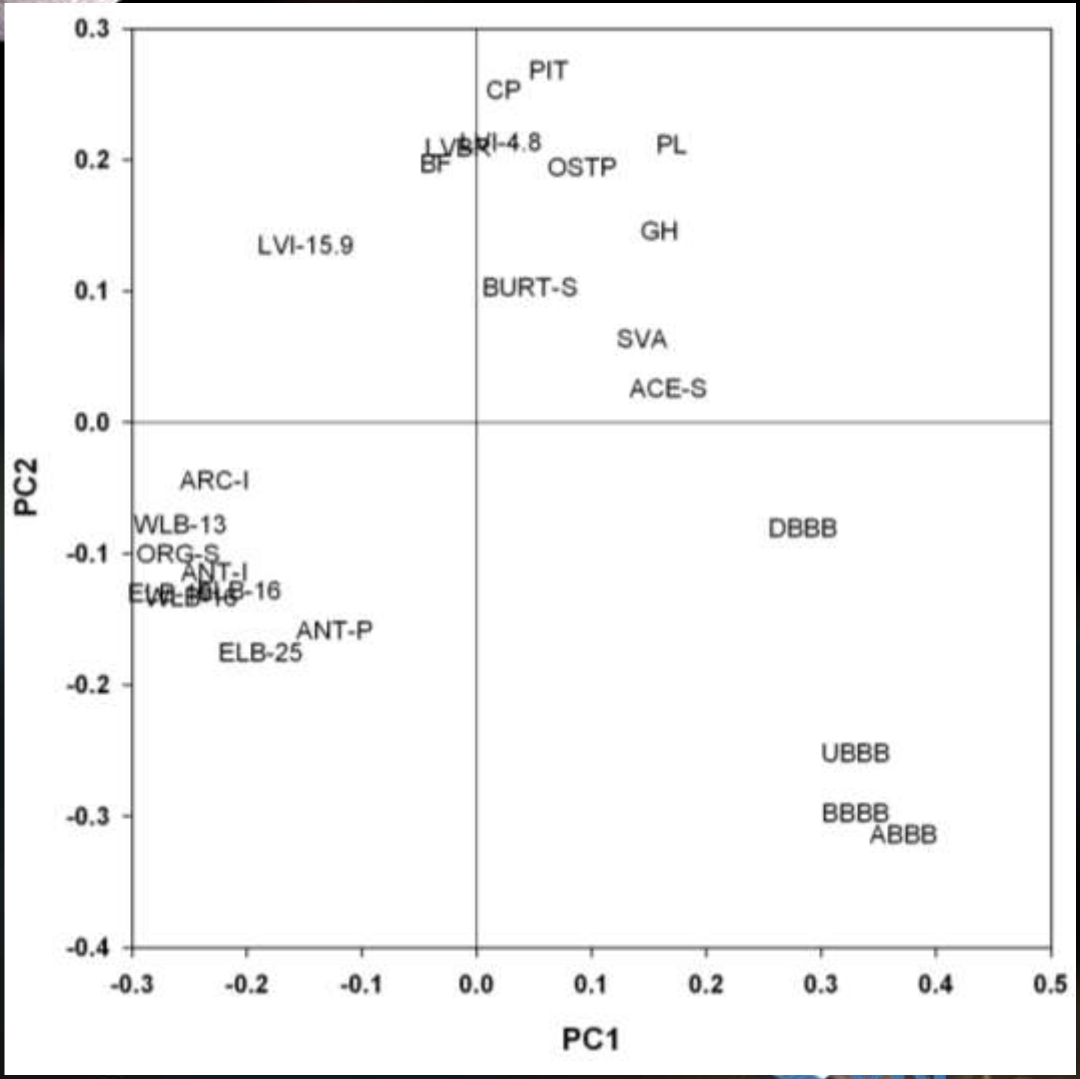
Putative Biogeochemical Capabilities



Are there similar microbial communities found in other habitats on earth?

- Statistical comparisons between Lake Vida brine SSU rRNA gene diversity and other habitats based on phylogenetic distance
  - Antarctic Lakes, Blood Falls
  - Antarctic marine and sea ice
  - Brine systems
  - Sulfidic springs
  - Deep Mediterranean brine basins
  - Natural and contaminated systems – **sequences with close relatives in LVBr** (i.e. Firmicutes sequences were related to sequences from oil sands tailings pond, oil-contaminated soils)

Relationship between microbial community structure and diversity between sites



LVBr- Lake Vida brine
LVI- Lake Vida ice
BF- Blood Falls
ELB- East Lobe Lake Bonney
WLB- West Lobe Lake Bonney
ACE-S- Ace Lake sediments
BUR-S- Burton Lake sediments
ORG-S- Organic Lake sediments
ANT-P- Antarctic bacteriroplankton
ANT-I- Antarctic sea ice
ARK-I-Arctic sea ice
CP- Colour Peak, Arctic spring
GH- Gypsum Hill, Arctc spring
SVA- Svalbard Sediments
ABBB- L'Atalante Basin brine
BBBB- Bannok Basin brine
DBBB- Urania Basin brine
UBBB- Urania Basin briine
OSTP- Oil sands tailings pond
PL- Canadian biodegraded oil resevoir
PIT- oil-contaminated soil

Hypotheses regarding sustainability of life in Lake Vida:

1. Given time scales of exponential decay, the organic resources at *-13.4 C* would be degraded within 100’s-few 1000’s yrs; expect the system would be dominated by methanogenesis.

DRI

Hypotheses regarding sustainability of life in Lake Vida:

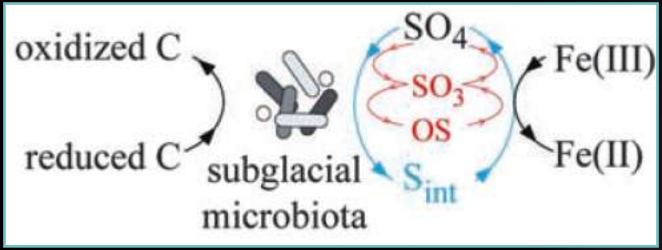
1. Given time scales of exponential decay, the organic resources at *-13.4 C* would be degraded within 100’s-few 1000’s yrs; expect the system would be dominated by methanogenesis.
2. The system is severely limited (resource, toxin, or temperature) and low metabolic rates are sustained by autochthonous resources; the phylogenetic and metabolic diversity suggest otherwise.

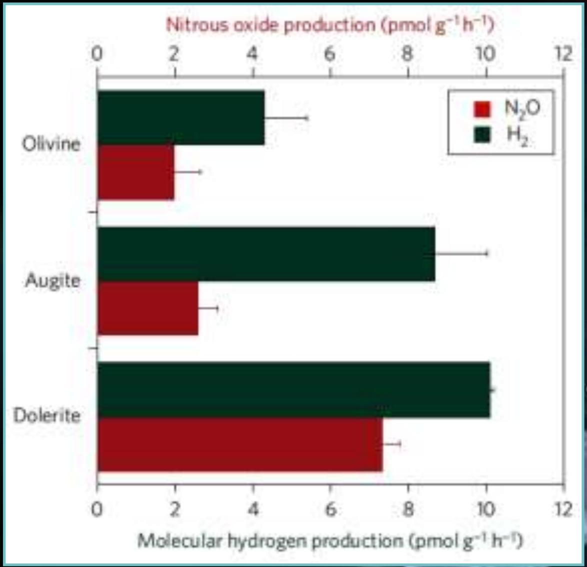
Hypotheses regarding sustainability of life in Lake Vida:

1. Given time scales of exponential decay, the organic resources at *-13.4 C* would be degraded within 100's-few 1000 yrs.
2. The system is severely limited (resource, toxin, or temperature) and low metabolic rates are sustained by autochthonous resources.
3. There is an external supplemental energy source, or catalytic reaction that fuels productivity.

Sustainability of life – coupled to biogeochemistry (I):

- Catalytic sulfur cycle: non-sulfidic SO_4 - Fe (III) cycle – Blood Falls (Mikucki et al. 2009, Science 324:397-400)
- Abiotic N_2O and H_2 formation from chemodenitrification/serpentenization-like reactions - Don Juan Pond (Samarkin et al. 2010, Nature Geosci 3:341-344)





Sustainability of life in Lake Vida?

- Excessive inventories of organic and inorganic carbon, both oxidized and reduced nitrogen, and high biomass are discerning characters compared to Blood Falls
- Has high Fe (Ferrar dolerite-(pyroxene) rock, sands, sediments), N_2O, and H_2
- Molecular (16S rRNA gene) support for H_2, NO_3^{2-}, and SO_x chemolithoautotrophic pathways (Epsilonproteobacteria)
- Stable isotopic signatures …

2010 Lake Vida Brine Geochemistry: H_2 & O Stable Isotopes

<u>Hydrogen and Oxygen</u>

$\delta^2H\text{-}H_2O = -240 \pm 20.2$ ‰

$\delta^{18}O\text{-}H_2O = -36.7 \pm 2.3$ ‰

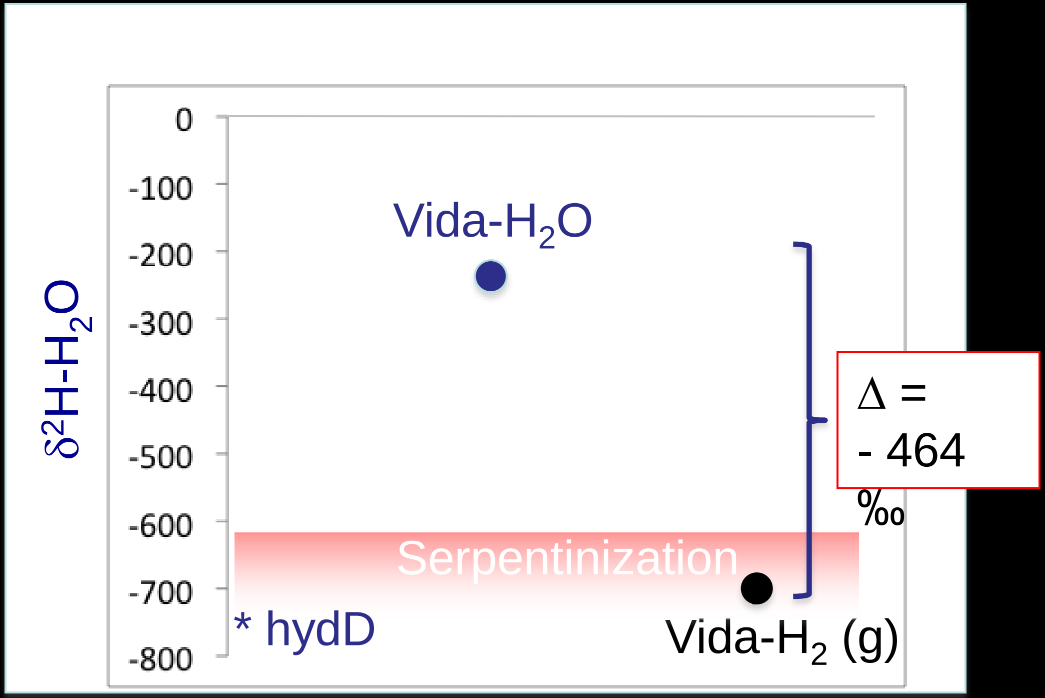
(Lake Bonney (Priscu et al. 2008):

$d^{18}O\text{-}H_2O \sim -40$ ‰)

$[H_2] = 10.47 \pm 0.02$ mM

$\delta^2H\text{-}H_2 = -704 \pm 12.2$ ‰

$\alpha_{H2\text{-}H2O} = 0.389$ ($\Delta = 464$ ‰)



Biological H_2 Production (Yang et al 2011 RCM 26: 61-68)

NiFe-hydrogenase: $\delta^2H\text{-}H_2 = -742 \pm 12.6$ ‰; $\alpha_{H2\text{-}H2O} = 0.273$

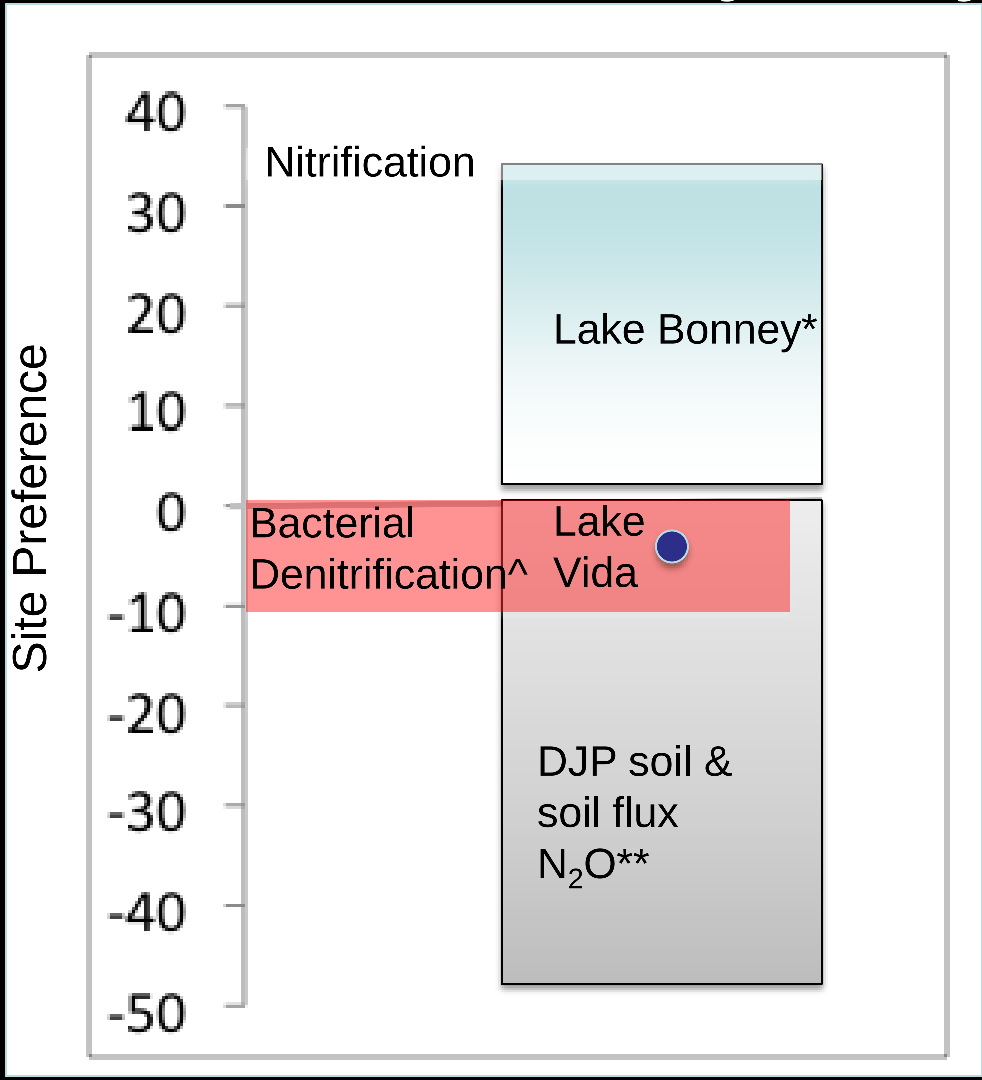
Other hydrogenases: $\Delta = -640$ to -340 ‰ (Yang, Hegg & Ostrom unpublished)

Abiological H_2 Production (Sherwood-Lollar et al., 2007 Astrobiology 7: 971-986)

Serpentinization or radiolysis

$[H_2]$ up to 7.4 mM, δ^2H -738 to -619 ‰

Nitrous oxide site preference in McMurdo Dry Valley Lakes & brines



Nitrous oxide:

Lake Vida 2010
$[N_2O]$ = 86.6 ± 5.9 μM
$d^{15}N$ = -22.17 ± 0.03
$d^{18}O$ = 2.97 ± 0.11

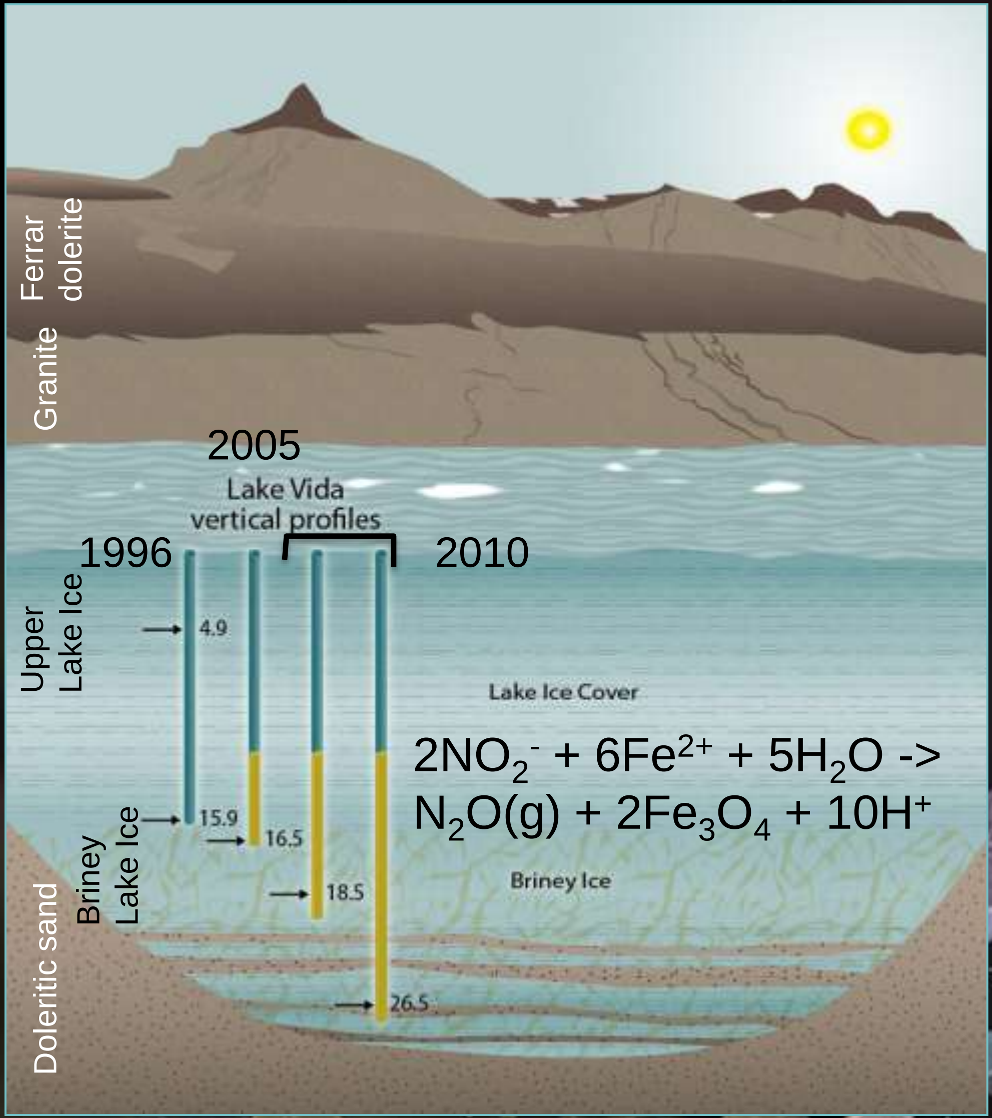
Lake Bonney
$[N_2O]$ = <1 to 43.3 μM
$d^{15}N$ = -79.6 to -14.9 ‰
$d^{18}O$ = -4.7 to 24.8 ‰

Don Juan Pond Soils
$[N_2O]$ = 3.9-6.8 μM
$d^{15}N$ = -43 to -35 ‰
$d^{18}O$ = 50.6 to 76.7‰

* Priscu et al. 2008 L&O 53:2439-2450; ^ Ostrom & Ostrom, 2011 Handbook Env Iso. Geochem. 453-476; Frame & Casciotti 2010 Biogeosci Disc. 7:3019-3059; # Samarkin et al. 2010, Nature Geosci. 3:341-344

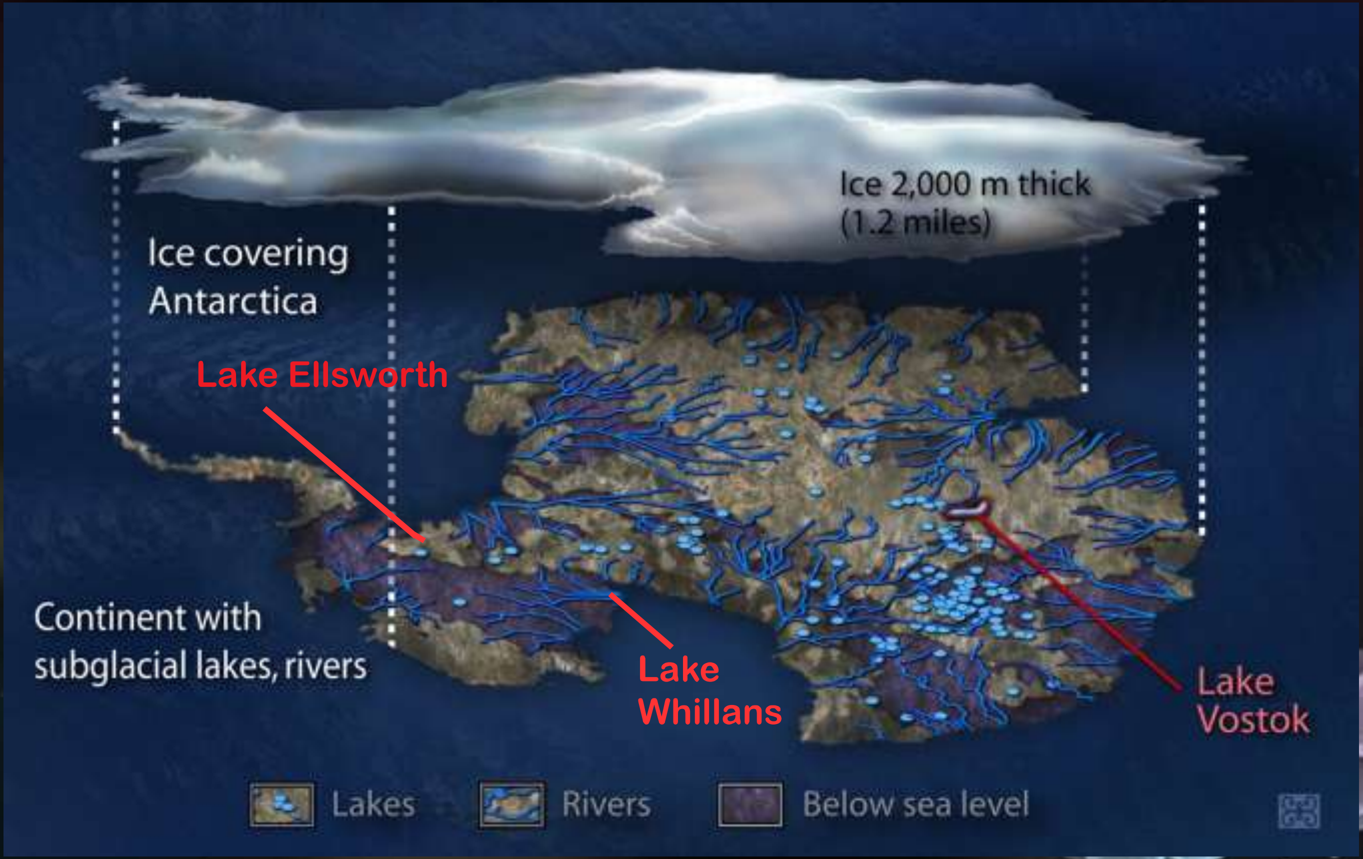
Ice-Water-Rock Systems as icy world analogs:

- Lake Vida abiotic and biotic processes could be coupled



Ice-Water-Rock Systems as icy world analogs:

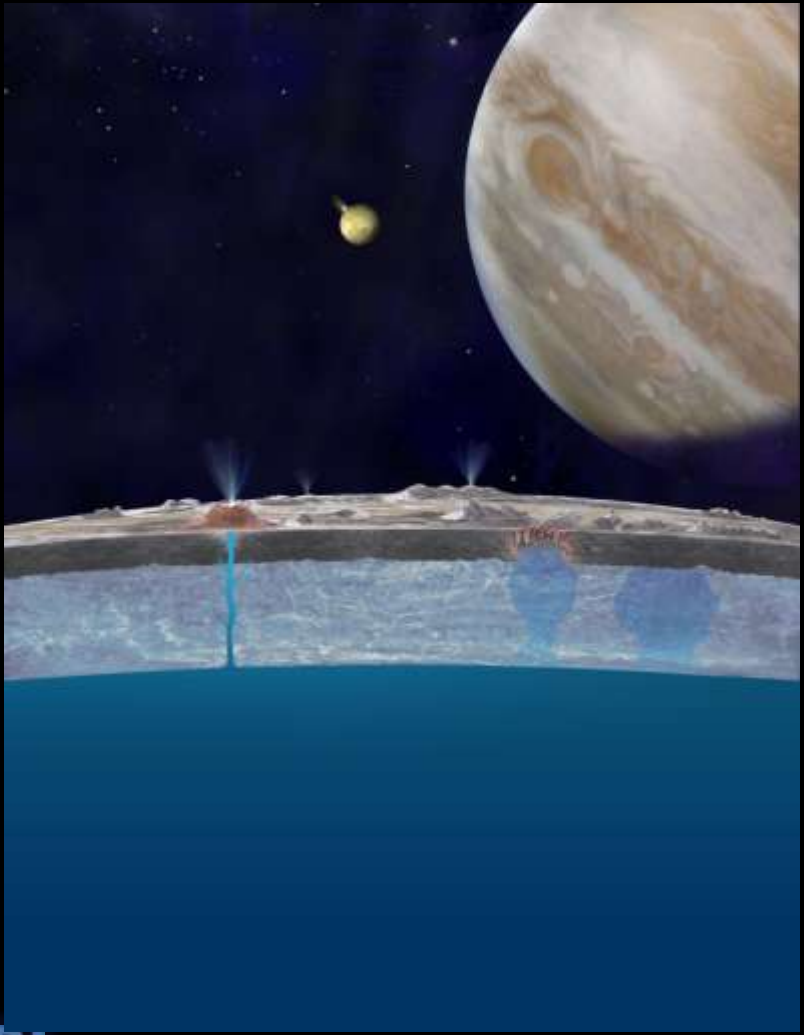
- Antarctic subglacial lakes provide new windows into ancient systems



Zina Deretsky / NSF

Potential for ice-covered saline oceans on icy worlds (Europa, Enceladus, *Titan*)

- $MgSO_4 \cdot 7H_2O$ detected at Eurpoa's surface suggests exchange with underlying ocean and surface ice



NASA/JPL-CalTech

Brown and Hand, Astronom. J. *in press*

Current state of understanding

- Lake Vida ice cover contains a cold, dark, anoxic and stable subsurface brine.
- The brine is a habitat for diverse microbial life forms that are metabolically active, albeit at rates perhaps just beyond maintenance metabolism levels.
- The energetics of the system remain under investigation – use of autochthonous resources supplemented with abiotic energy is one possibility.
- Future studies of geobiological and abiotic processes in sub-ice ecosystems are key to icy world investigations.

Vida Team

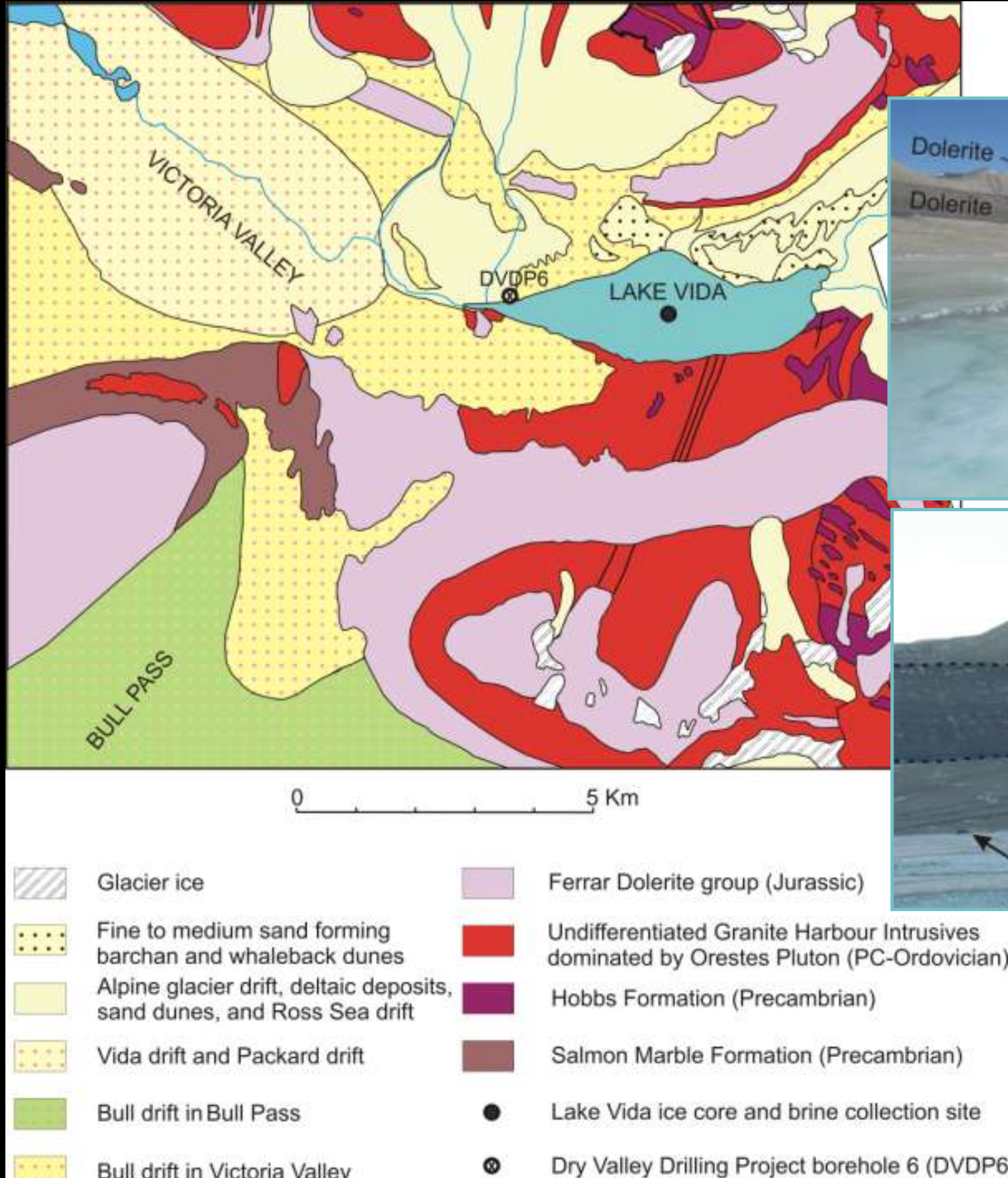
Life Detection

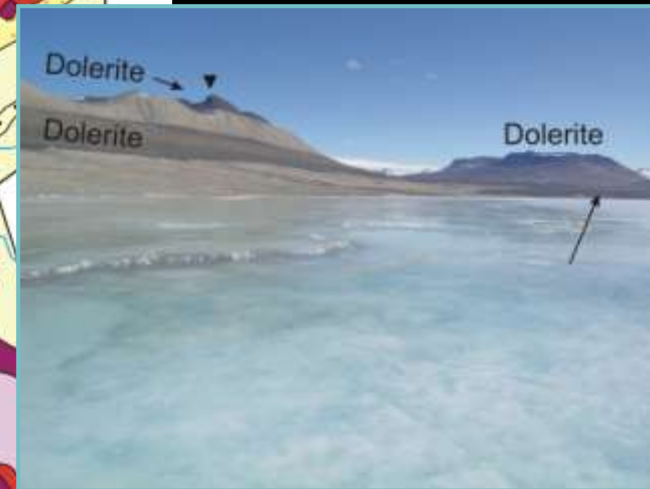
- DRI: **Chris Fritsen, Giles Marion,** Clint Davis, *Ema Kuhn*, *Protima Wagh*, Vivian Peng
- U. Ill – Chicago: **Peter Doran, Fabien Kenig,** *Hilary Dugan*, Peter Glenday
- NASA-AMES: Chris McKay
- JPL: Adrian Ponce
- UH: Brian Glazer
- MSU-Bozeman: John Priscu
- U. S. Ill: Mike Madigan

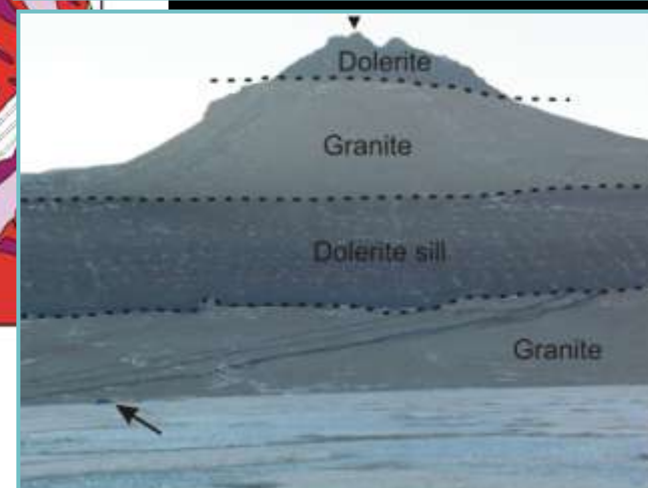
Geo/Chemistry Characterization

- UC Boulder: Diane McNight, *Kaelin Cawley, Mike Sanclements*
- SFSU, Andrew Ichimura
- UGA - Athens: Vladamir Samarkin
- IU – Bloomington: Lisa Pratt, *Seth Young*
- Curtain Univ. - Perth, Aus: Ross Edwards
- McMurdo LTER: Kathy Welsh
- Univ. Cologne: Bernd Wagner

Federal Support: NSF-OPP, NASA-ASTEP, NAI (Icy Worlds/JPL)
Logistics Support: ICDS (Jay Kyne), RPSC, PHS





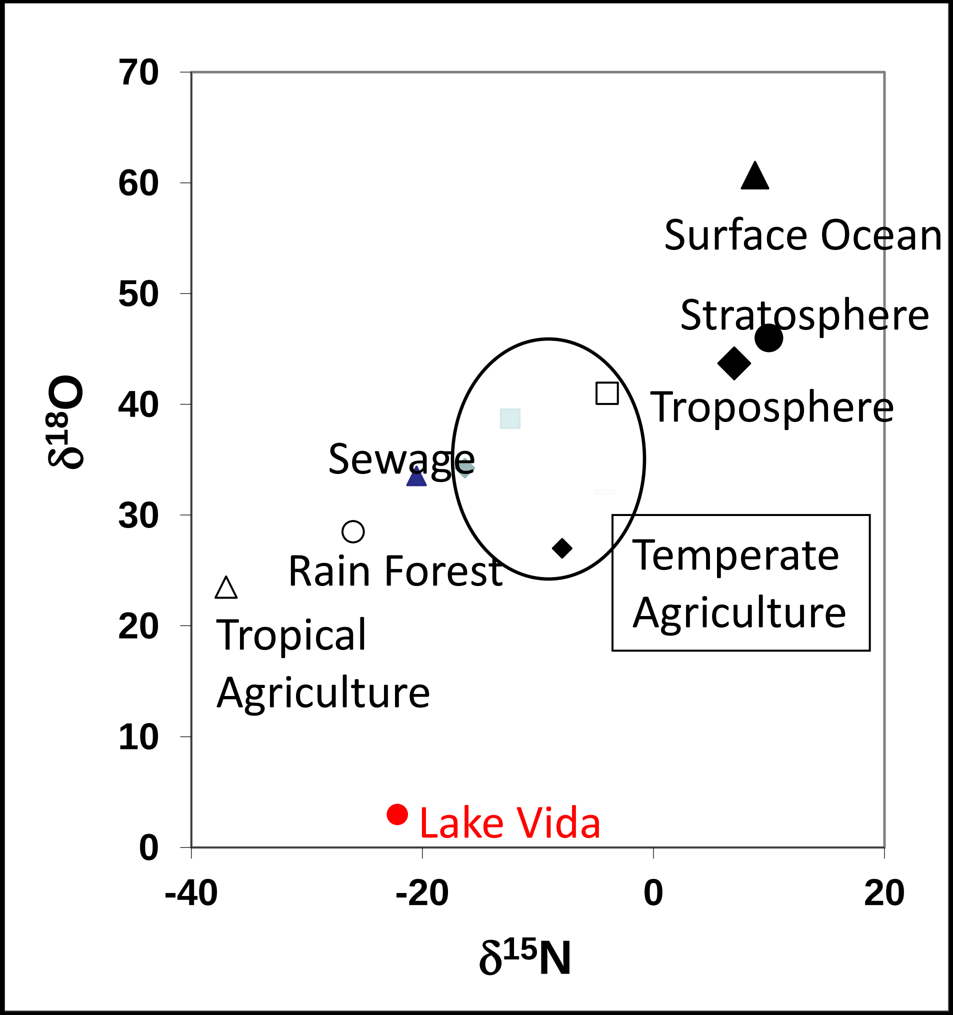


adapted from Turnbull et al. 1994

2010 Lake Vida Brine Geochemistry: Stable Isotopes

Nitrous oxide:
Lake Vida 2010
$[N_2O] = 86.6 \pm 5.9$ mM
$\delta^{15}N = -22.17 \pm 0.03$
$\delta^{18}O = 2.97 \pm 0.11$
$SP = -3.64 \pm 0.28$

$\delta^{18}O$ lowest reported (?)
Not consistent with a tropospheric origin
Consistent with ^{18}O depleted Lake Vida water



Yoshida and Toyoda, 2000; Toyoda et al., 2002; Toyoda et al., 2001, Yamulki et al., 2001; Perez et al., 2000; Ostrom et al., 2007; Opdyke et al., 2009; Ostrom et al., 2010

2010 Lake Vida Brine Geochemistry: Stable Isotopes

Dissolved Inorganic Carbon

[DIC] 72.4 ± 2.9 mM

S-Sulfate

[DIC] 72.4 ± 2.9 mM

δ^{13}C-DIC 1.44 ± 0.28 ‰

Conc. ~ 35 x seawater

Consistent with an inorganic origin

Nitrogen Biogeochemistry

Nitrate:

δ^{15}N-NO_3 = -7.94 ± 0.22

δ^{18}O-NO_3 = 31.68 ± 0.33

indicative of atm. or mixed atm. and biological origins

no evidence of denitrification

Dinitrogen:

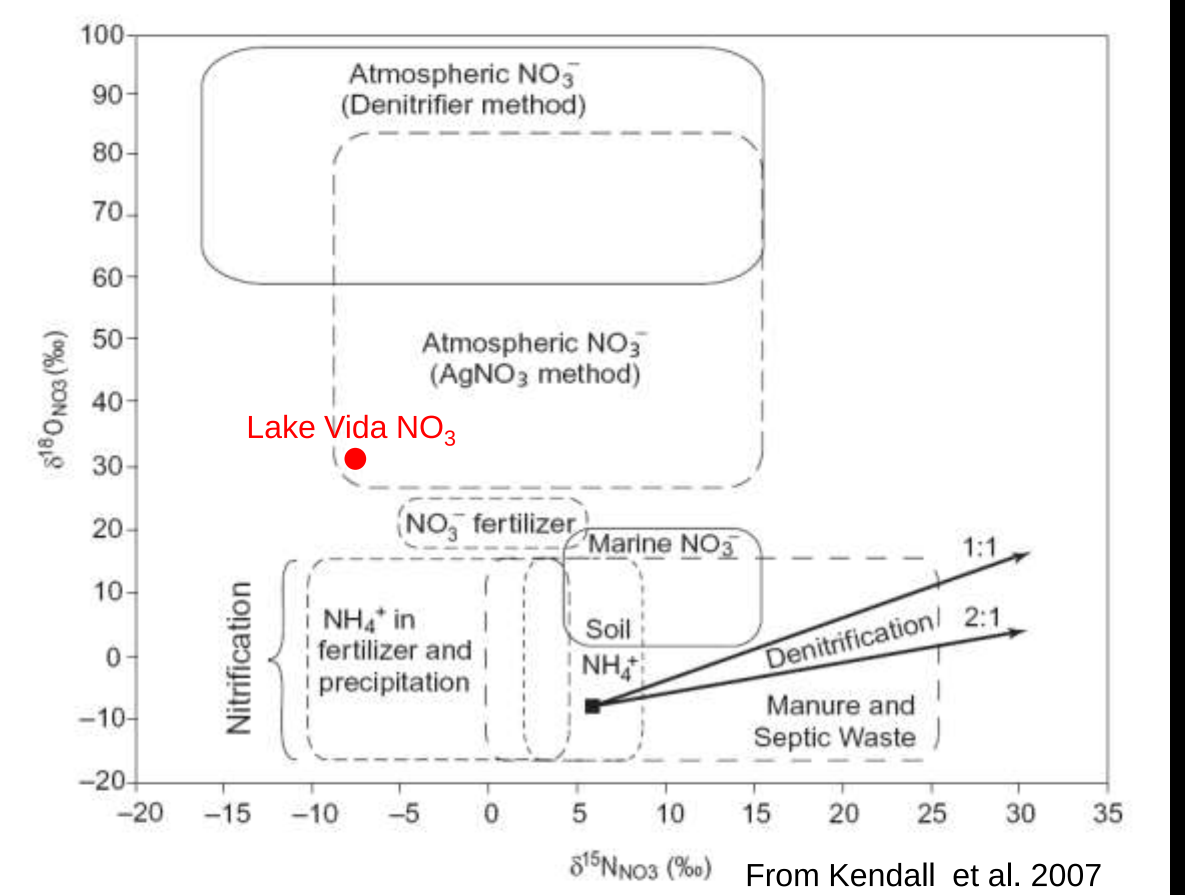
δ^{15}N-N_2 = 0.33 ± 0.27

Similar to atm. values

Ammonium:

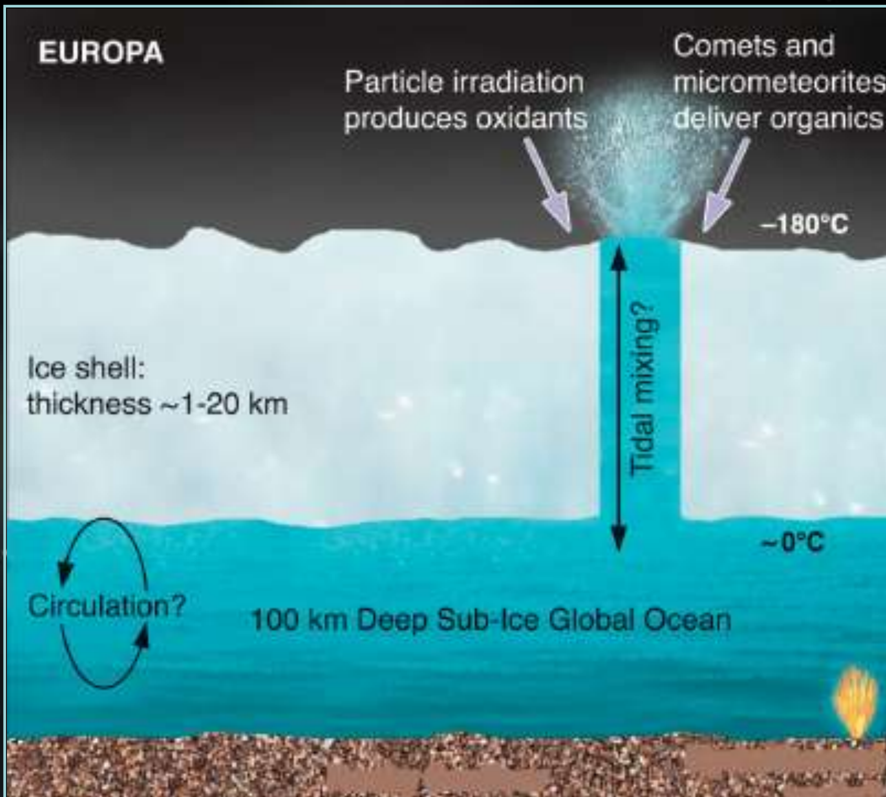
δ^{15}N-NH_4 = -4.80 ± 0.12

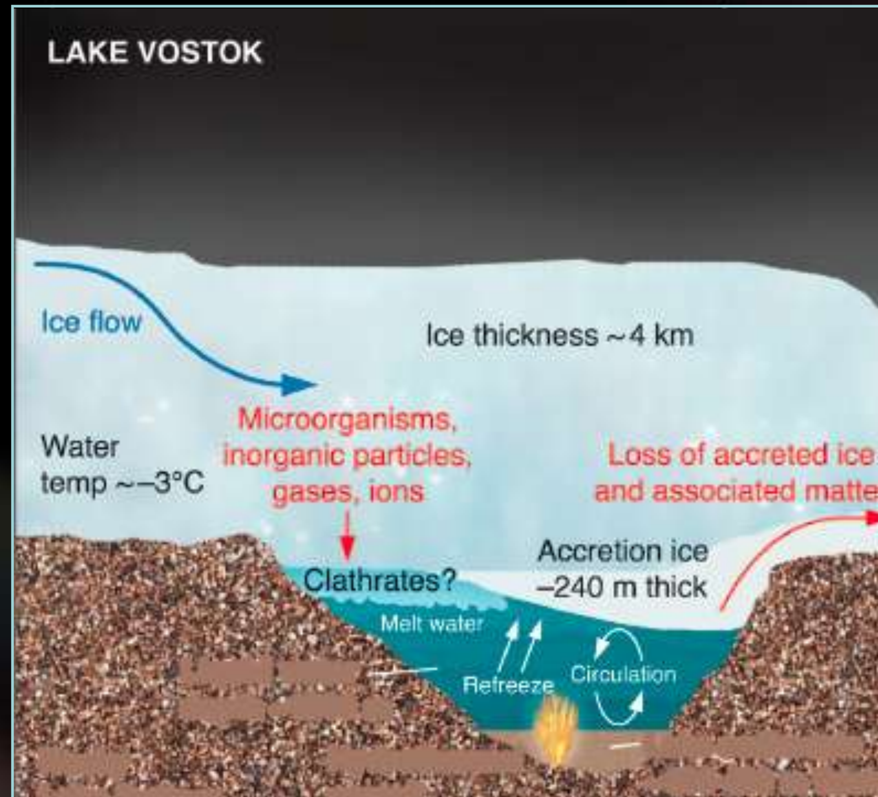
consistent with an atm origin



From Kendall et al. 2007

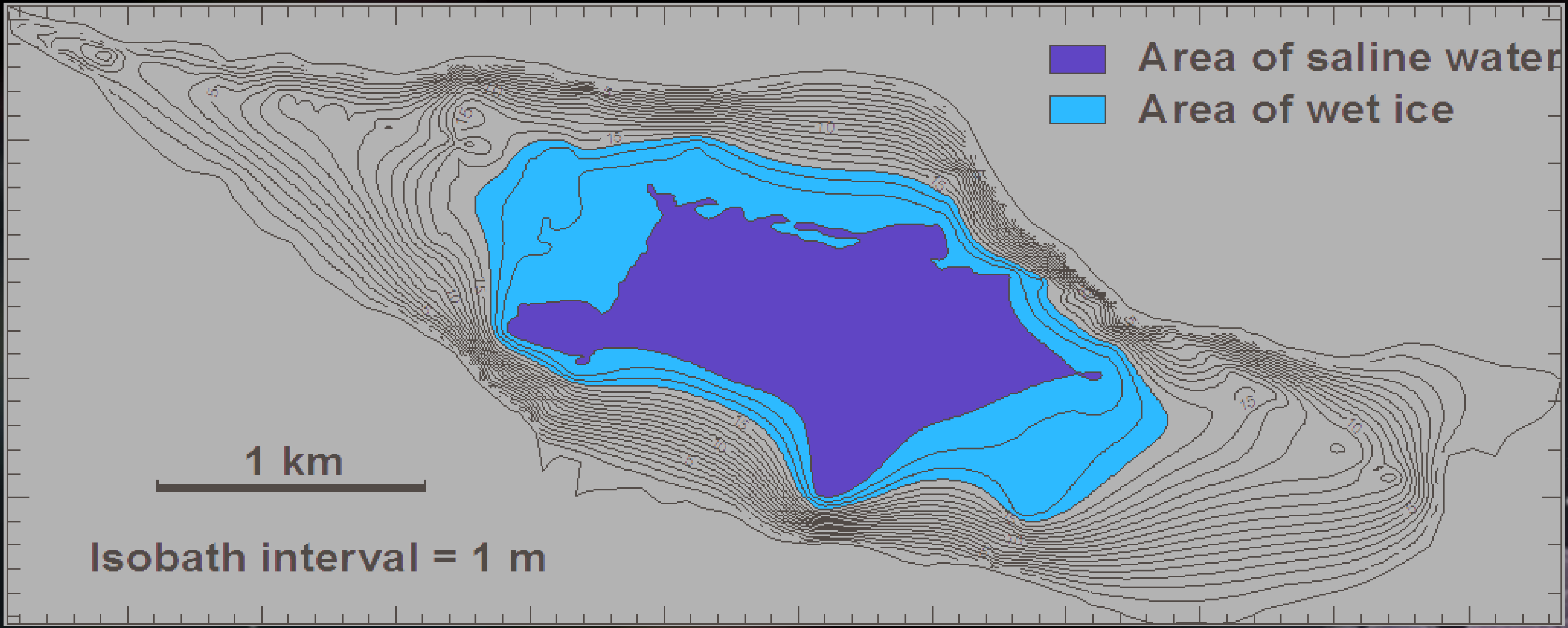
Ice-Water-Rock Processes: Europa and Sub-glacial Lake Vostok or Whillins





Priscu & Hand 2012, Microbe 7:167-172

Lake Vida Bathymetry
GPR survey (Vida I)



Doran et al. 2003,
PNAS 100:26-31

Sustainability of life – coupled to biogeochemistry (I):

- Subsurface serpentinizing systems on Earth (H_{2}-generating, CH_4-producing): Columbia River Basalt Group (Stevens & McKinley, 1995); subsurface Lidy Hot Springs Chapelle et al. 2002
- Subsurface radiolytic systems on Earth (H_2 and SO_4^{2-} produced): South African gold mine (Lin et al. 2006)
- Warm, low biomass systems dominated by methanogenic/sulfidic/chemolithoautotrophic metabolisms
- Lower temperature H_{2}-generating abiotic reactions feasible (Hellevang et al. Astrobio. 11:711-724)