COMPARATIVE PLANETOLOGY & ASTROBIOLOGY: MARS-MOON TERRESTRIAL ANALOGUE FIELD RESEARCH

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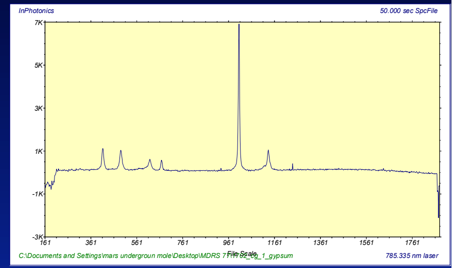
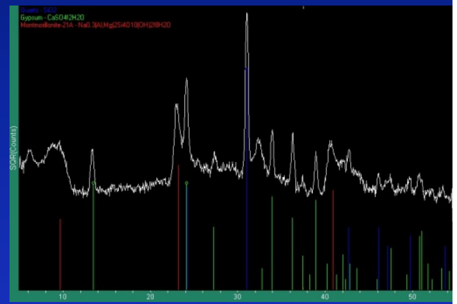
ExoGeoLabTechnology demonstration

In-situ instruments/techniques relevant to Mars habitability:
(ExoGeoLab 2007 -2013, ESTEC/ILEWG/Ames/VU Amsterdam)

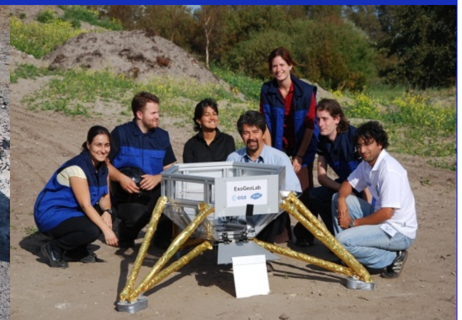




- Context imaging from remote, panoramic, closeup to microscopic
- Drilling, GPR, sample collection and handling
- X-Ray Diffractometry/Fluorescence XRD/XRF, Raman, spectro
- Polymerase Chain Reaction PCR and DNA analysis
- Soil and rock samples selected from diverse habitats & analysed

- Landers instruments
- Cooperative robotics
- Remotely controlled cameras and telescopes

- Exohab habitat
- Mobile laboratory
- Human robotic interfaces
- Extra vehicular activities



EuroMoonMars Field analogue campaigns

Goals: study habitability, analysing context & samples

Field: EuroGeoMars2009, DOMMEX/EuroMoonMars 2010-2013
(support NASA Ames, ILEWG, ESA/ESTEC, partners VU Amsterdam, NASA NAI, MEX RCL, ESA ground SocMar, ÈSTEC BIC, NWO, STFC)
6 crew participants x 2 x 2 weeks per year (MSc, PhDs, Post-docs)

- Mars Desert Research station near Hanksville in Utah
- Field test of technologies
- Comparatove plantology
- Remote sensing / in situ field truth
- Support to interpretation of data from MEX and Mars missions
- Diversity of sites relevant to Mars (cf MEX, MER & MRO)
- Simulating potential landing sites for MSL and ExoMars
- Samples sent for detailed analysis in remote laboratories

Foing et al. , Ehrenfreund et al 2011, 2012, Elsaesser et al 2013

Transient and local niches on Mars: geochemical habitats

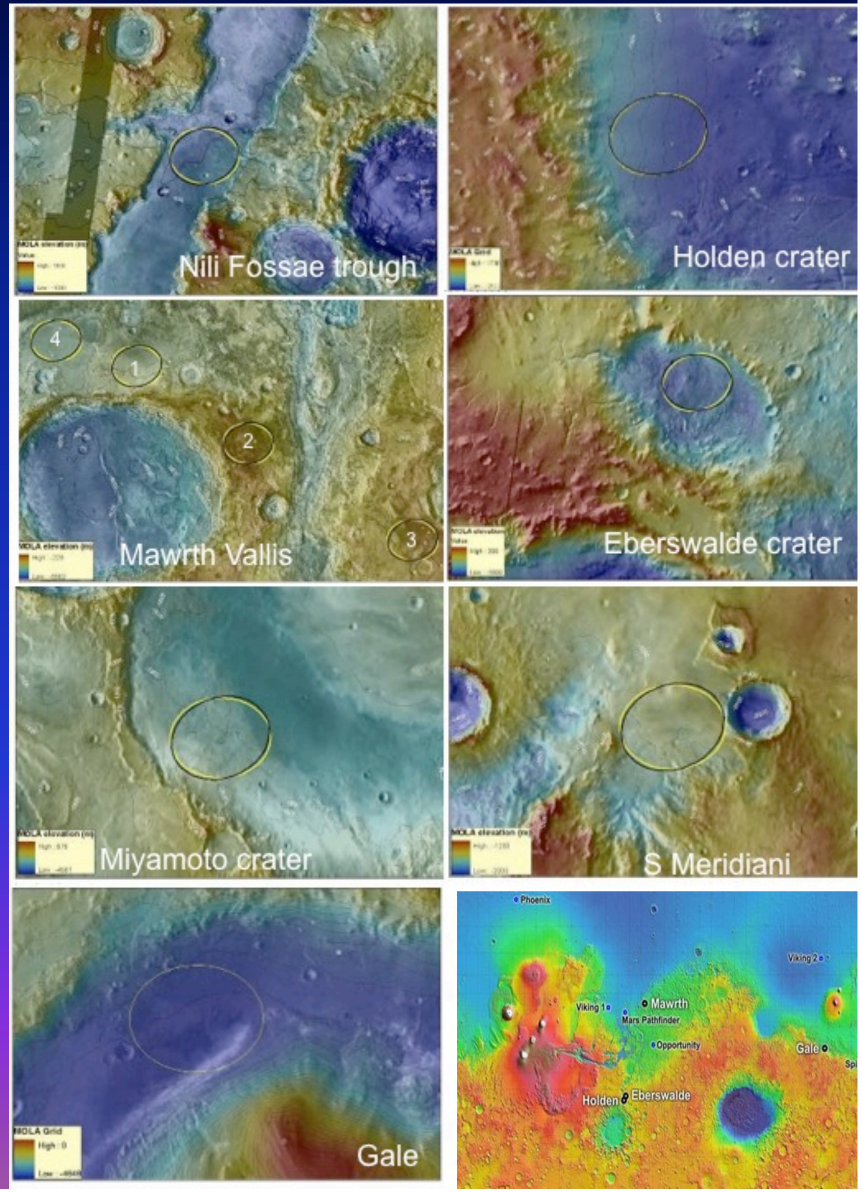
Transient environments:

- **fluvial and lacustrine deposits, gullies**
- **transient geothermal and/or hydrothermal conditions**
- **effects of large impacts or proximity with igneous activity**

Local delivery and burial of constituents:

- **volcanic ashes, spring deposits**
- **atmospheric deposits**
- **delivery of ejecta**
- **extraterrestrial material**

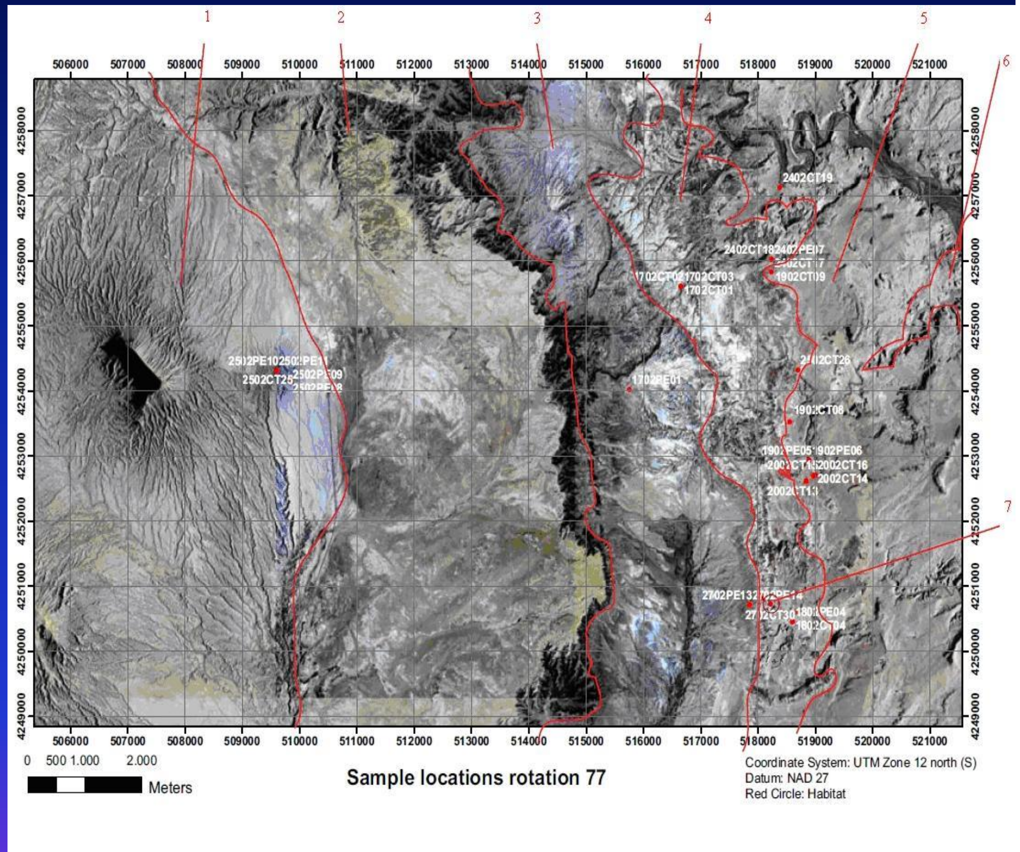
(also cometary/ meteoritic organics)



MDRS Analogue for Mars geochemical habitats

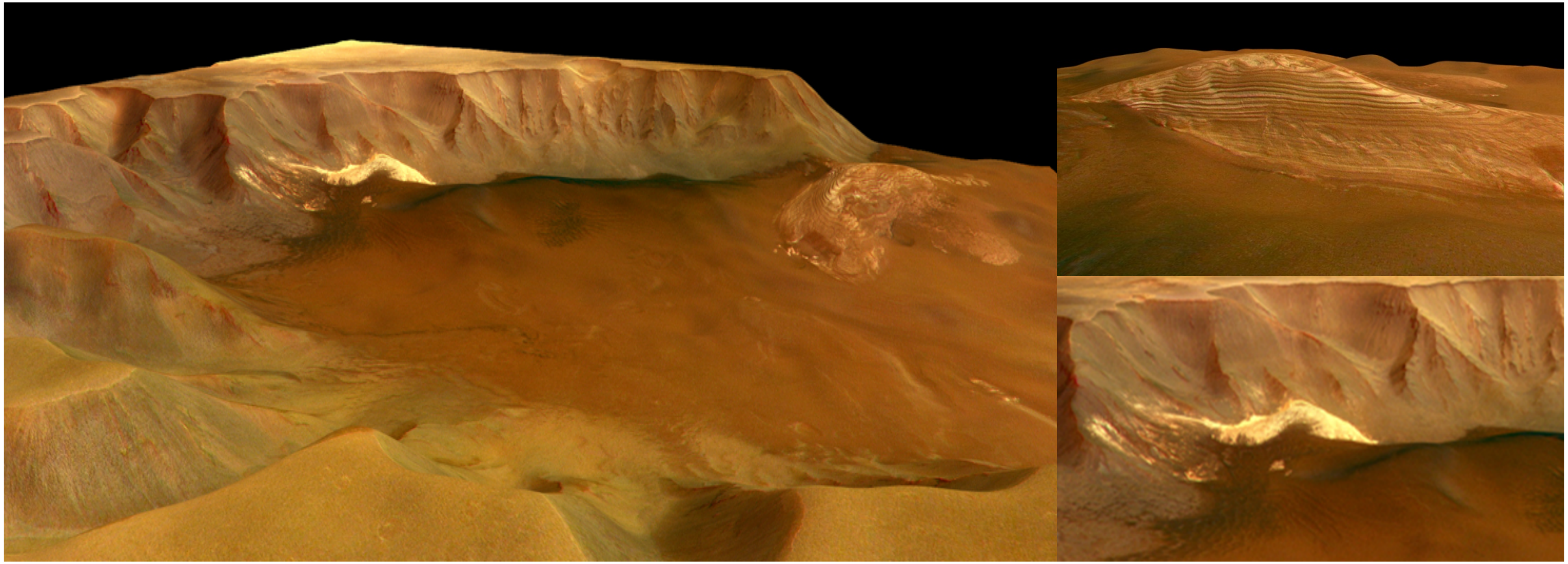
MDRS Mars Desert Research Station located in a geological formation formed 150 million years ago, with a diversity of environments:

(Foing et al. 2011, Stoker et al 2011, Zamurovic et al. 2013 a &b)

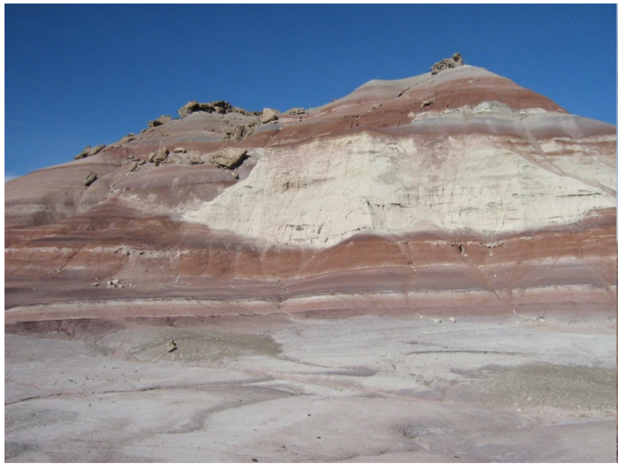


- **Middle Jurassic Summerville Formation**
- **Late Jurassic Morrison Formation**
  - **lacustrine and fluvial clays**
  - **inverted paleochannels**
- **Early Cretaceous Dakota Sandstone**
- **Middle Cretaceous Mancos Shale F.**
- **Mancos fluvial sandstone**
- **Bluegate carbonaceous pyritic units**
- **Small scale mineral & subsurface niches**
- **Concretions & endolithic environments**
- **Sedimentary deposits of sands, evaporites and clays**

Transient geological and geochemical episodes have affected local parameters (mineralogy, organics content, environment variations) and habitability



Juventae Chasmae, Mars Express HRSC

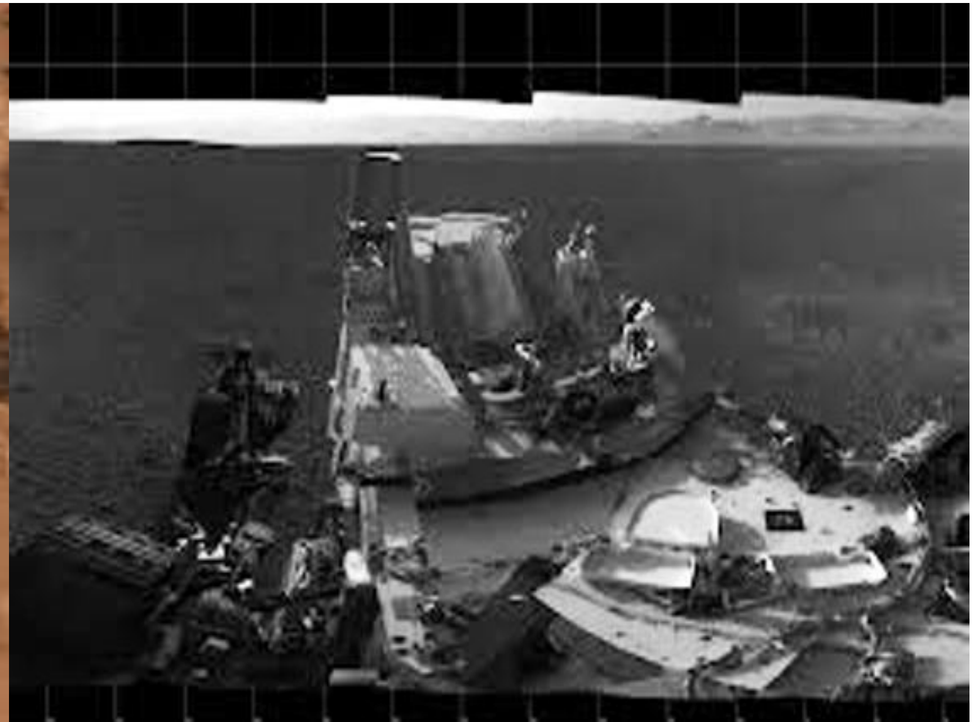


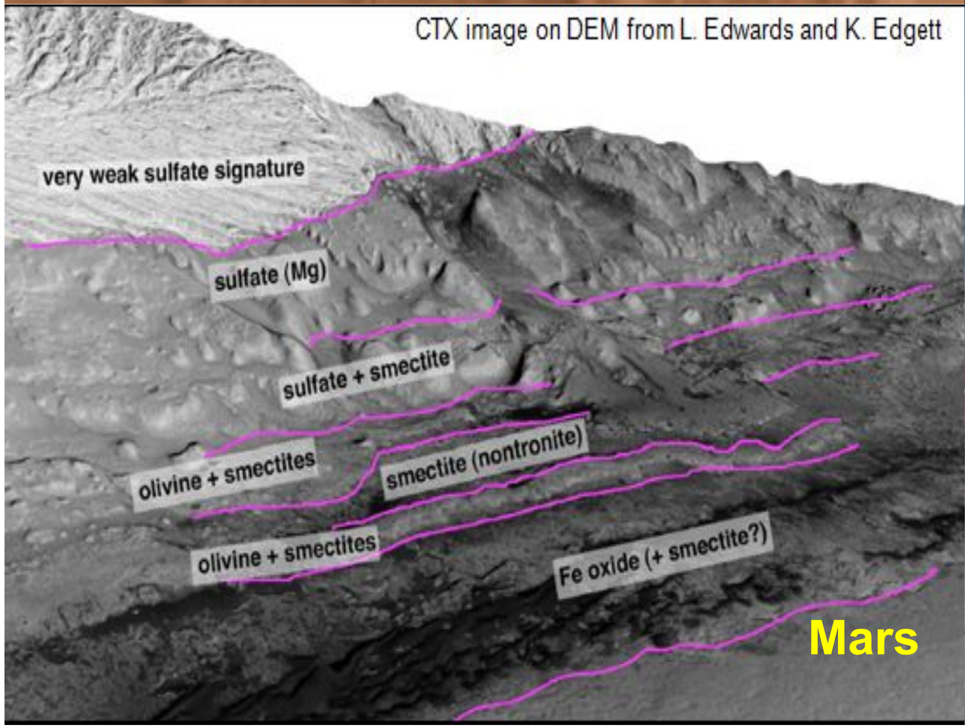
MDRS site Salt wash

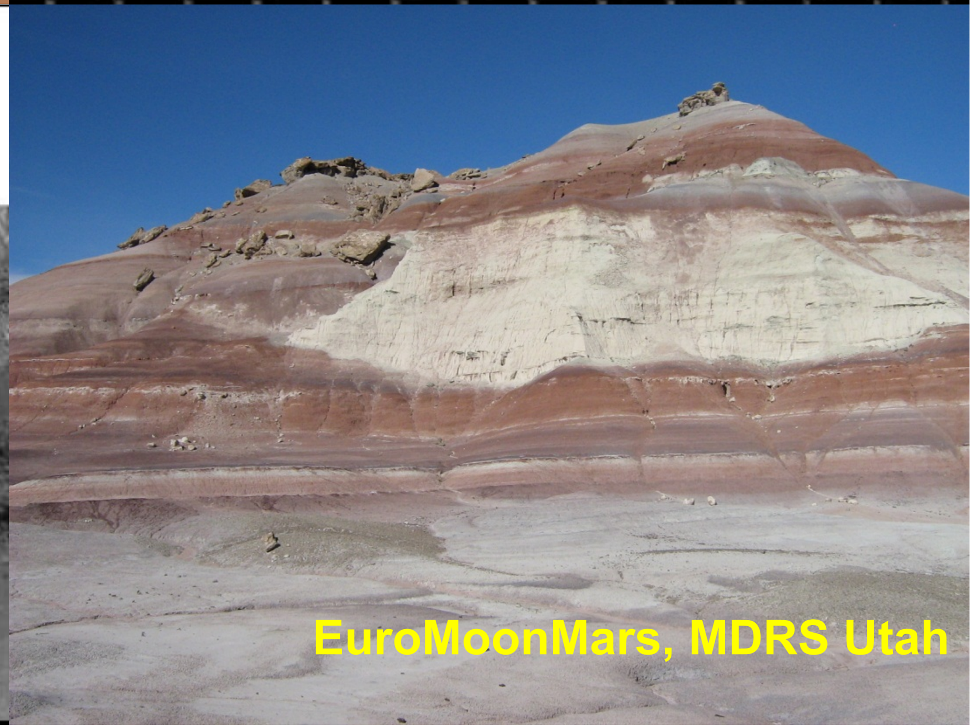
Complex channels

Thinly bedded laminated sandstone

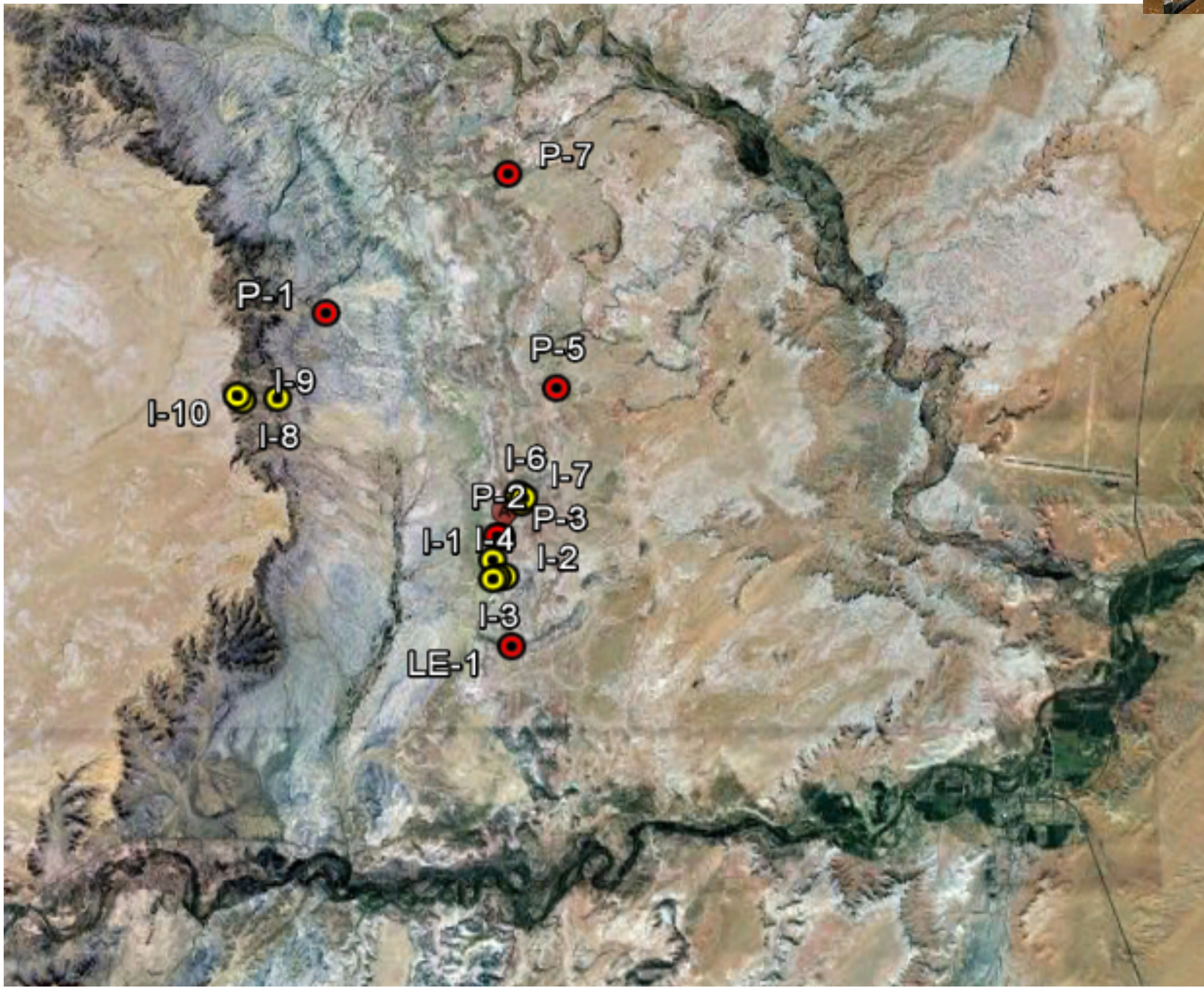

"Duststone"
(youngest)
Sulfates
Clays + Sulfates
(oldest)



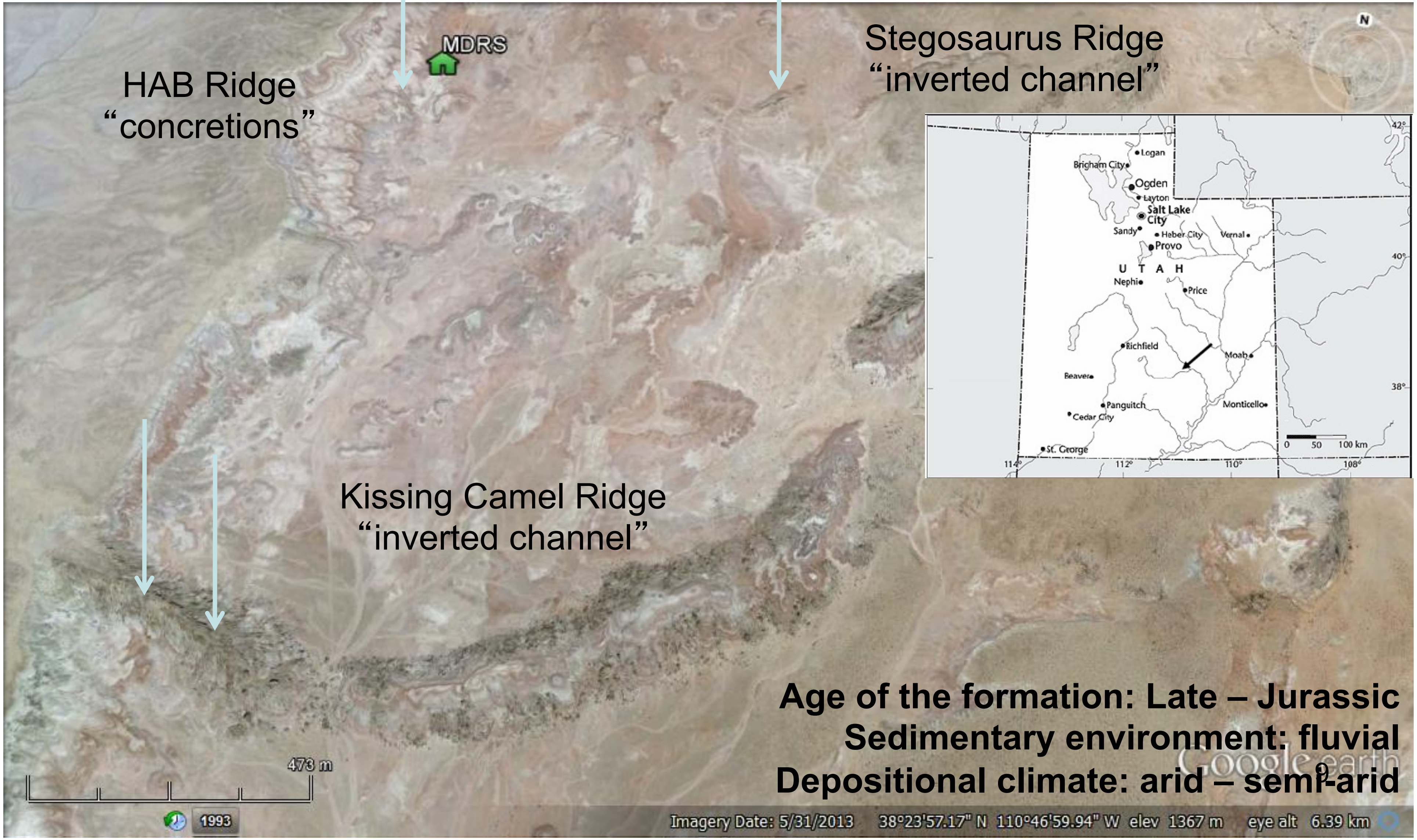

CTX image on DEM from L. Edwards and K. Edgett
very weak sulfate signature
sulfate (Mg)
sulfate + smectite
olivine + smectites
smectite (nontronite)
olivine + smectites
Fe oxide (+ smectite?)
Mars


EuroMoonMars, MDRS Utah

ILEWG EuroMoonMars 2012 & 2013 Utah in support for MSL at Gale crater

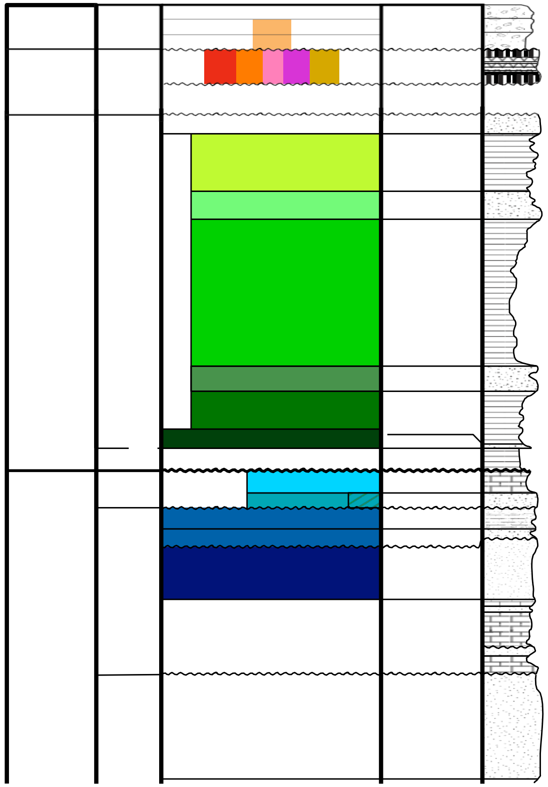


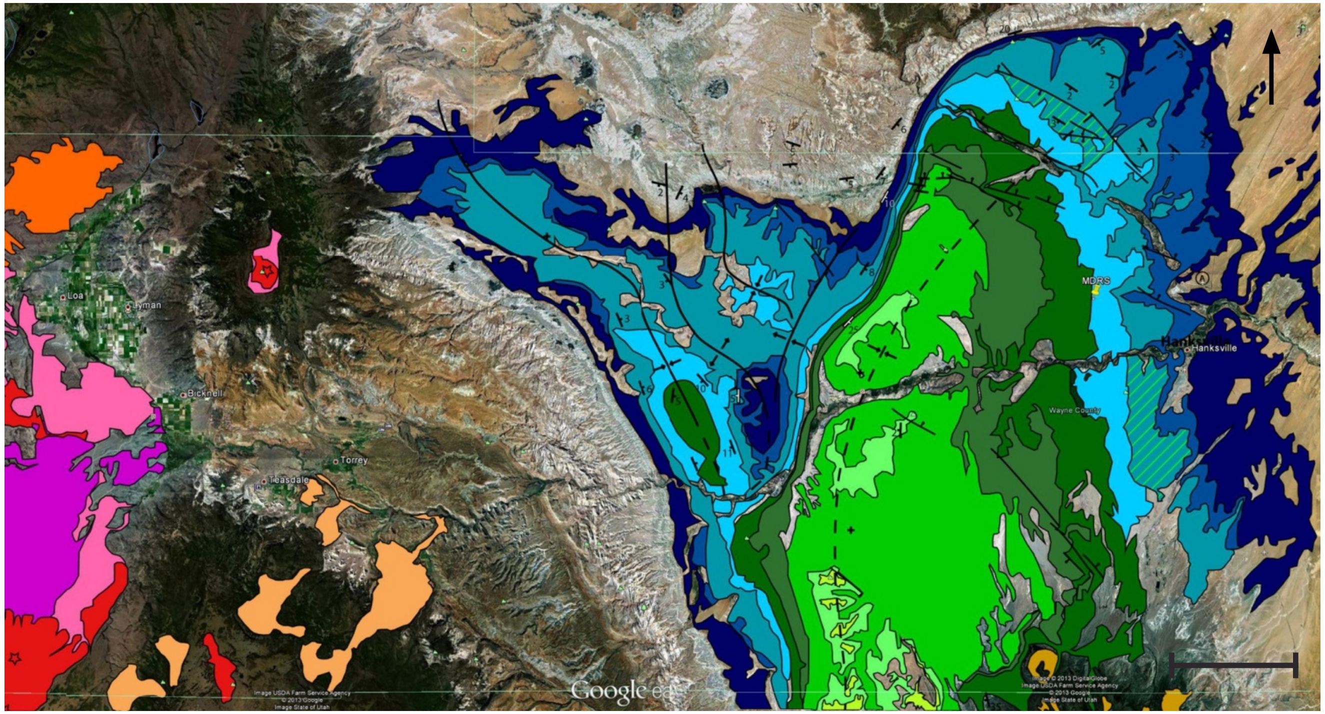
EuroMoonMars 2013, MDRS
Morrison Fm. - Brushy Basin Member

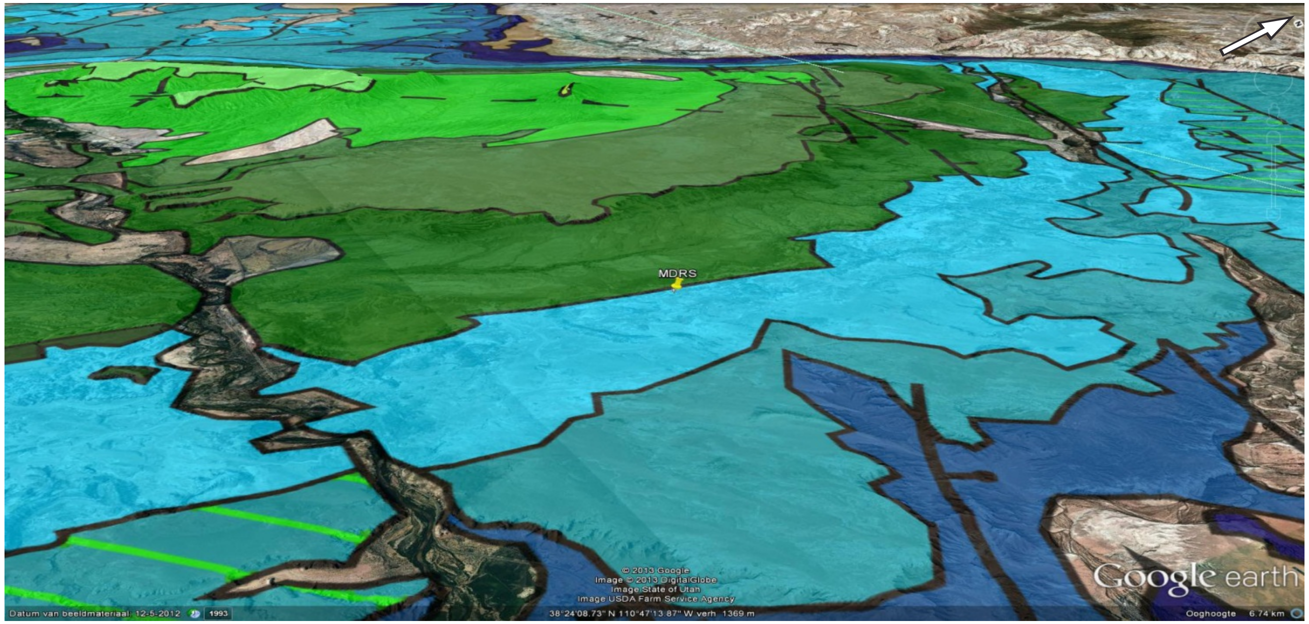


Age of the formation: Late – Jurassic
Sedimentary environment: fluvial
Depositional climate: arid – semi-arid

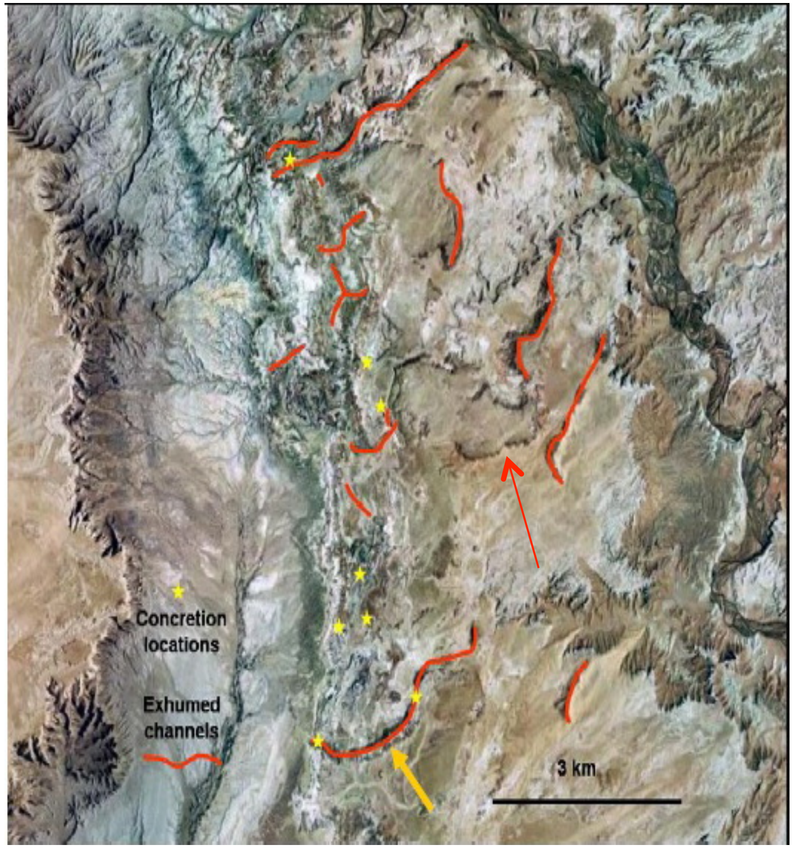
MDRS
Geology context
(Zamurovic et al 2013)

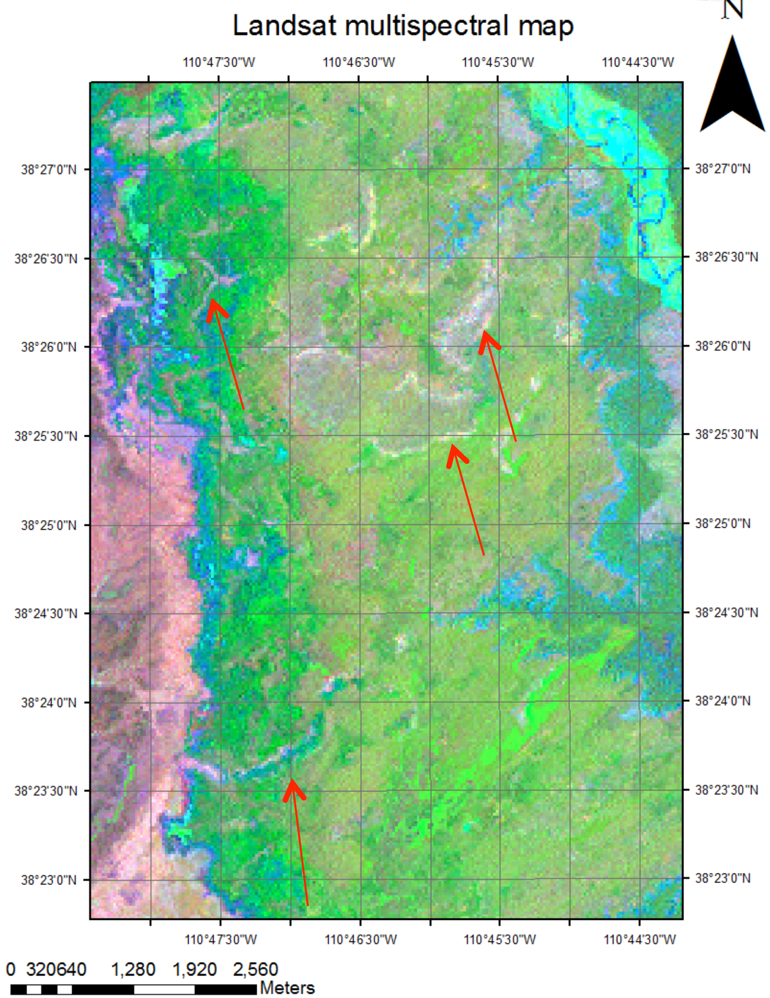






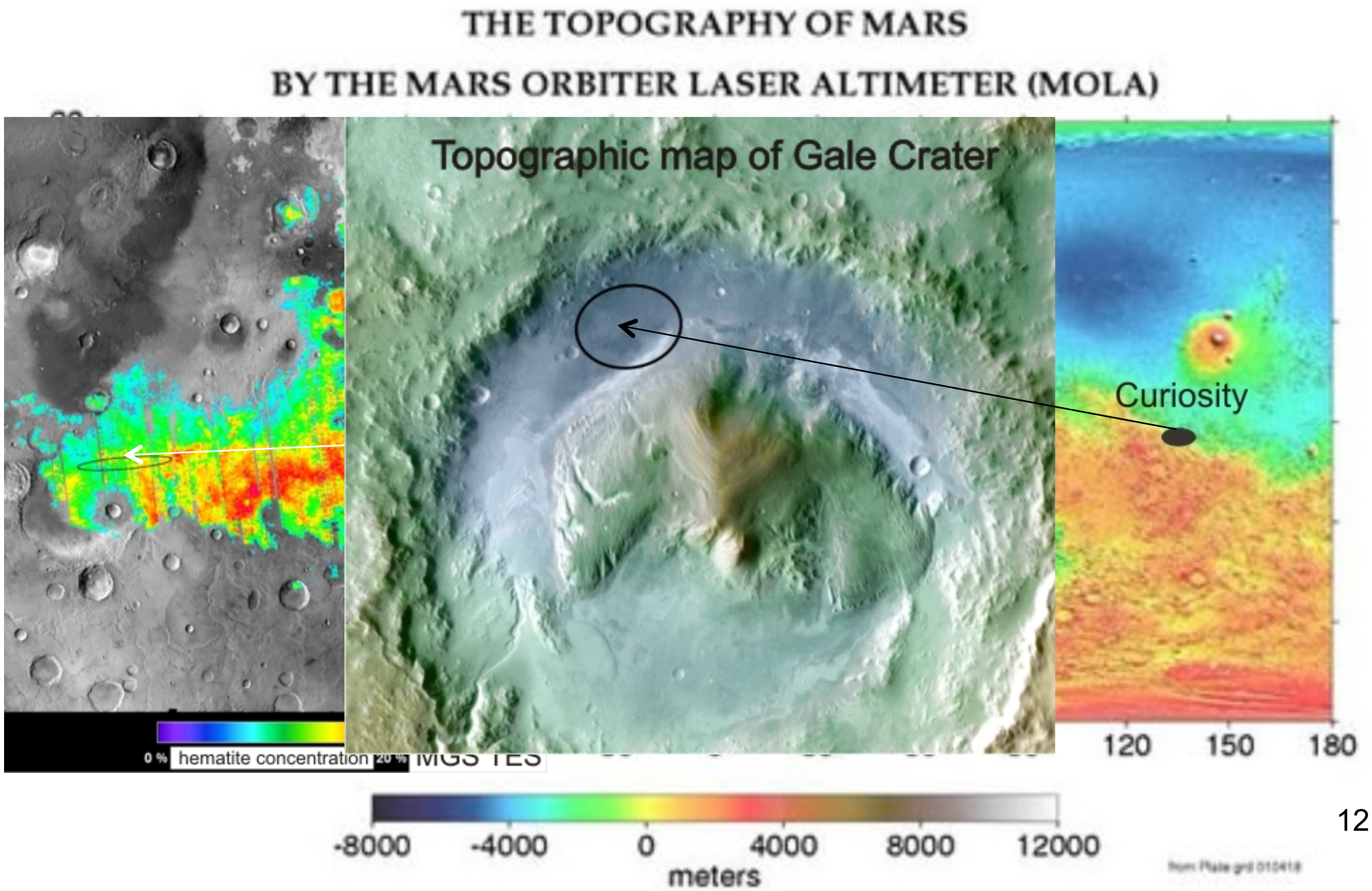
Landsat multispectral map of minerals and detection of calcite in inverted channel deposits.



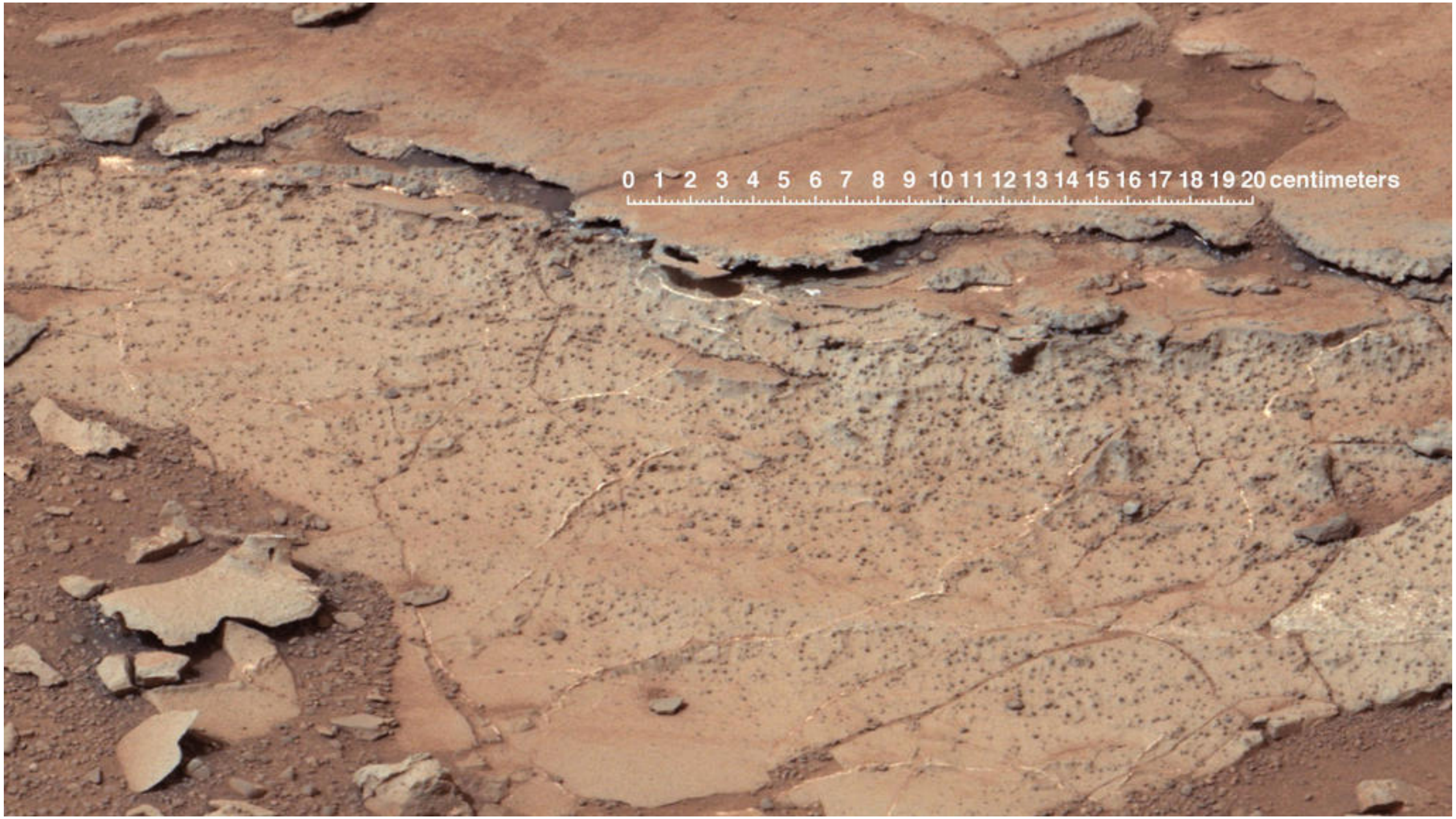


Left image: Inverted channels and location of the concretions. Inverted channels were the part of an old anastomosing river system. (Clarke and Stoker, 2011).Right image: Landsat multispectral data shows the presence of calcite (pink color) along the inverted channels

Gale crater on Mars

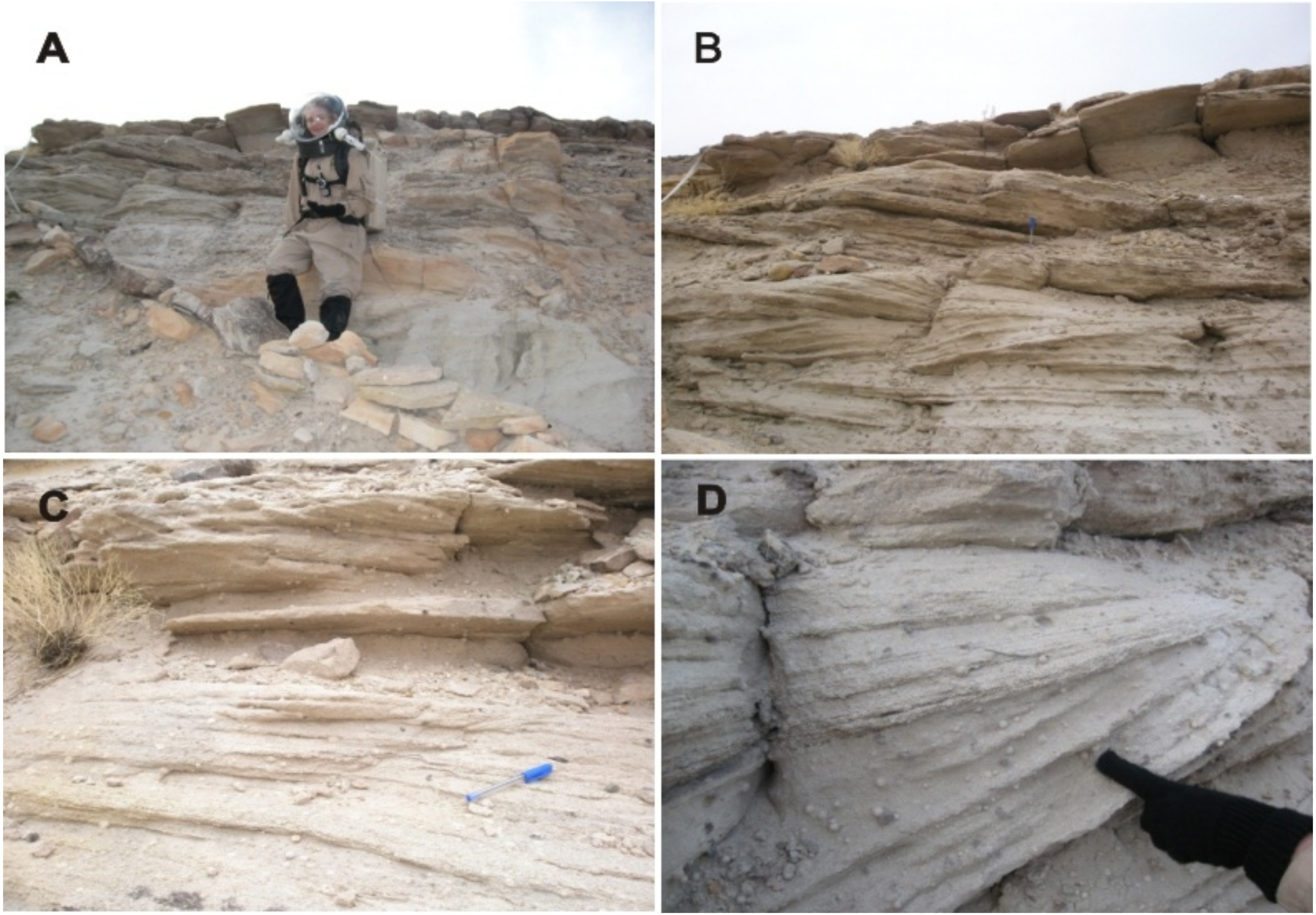


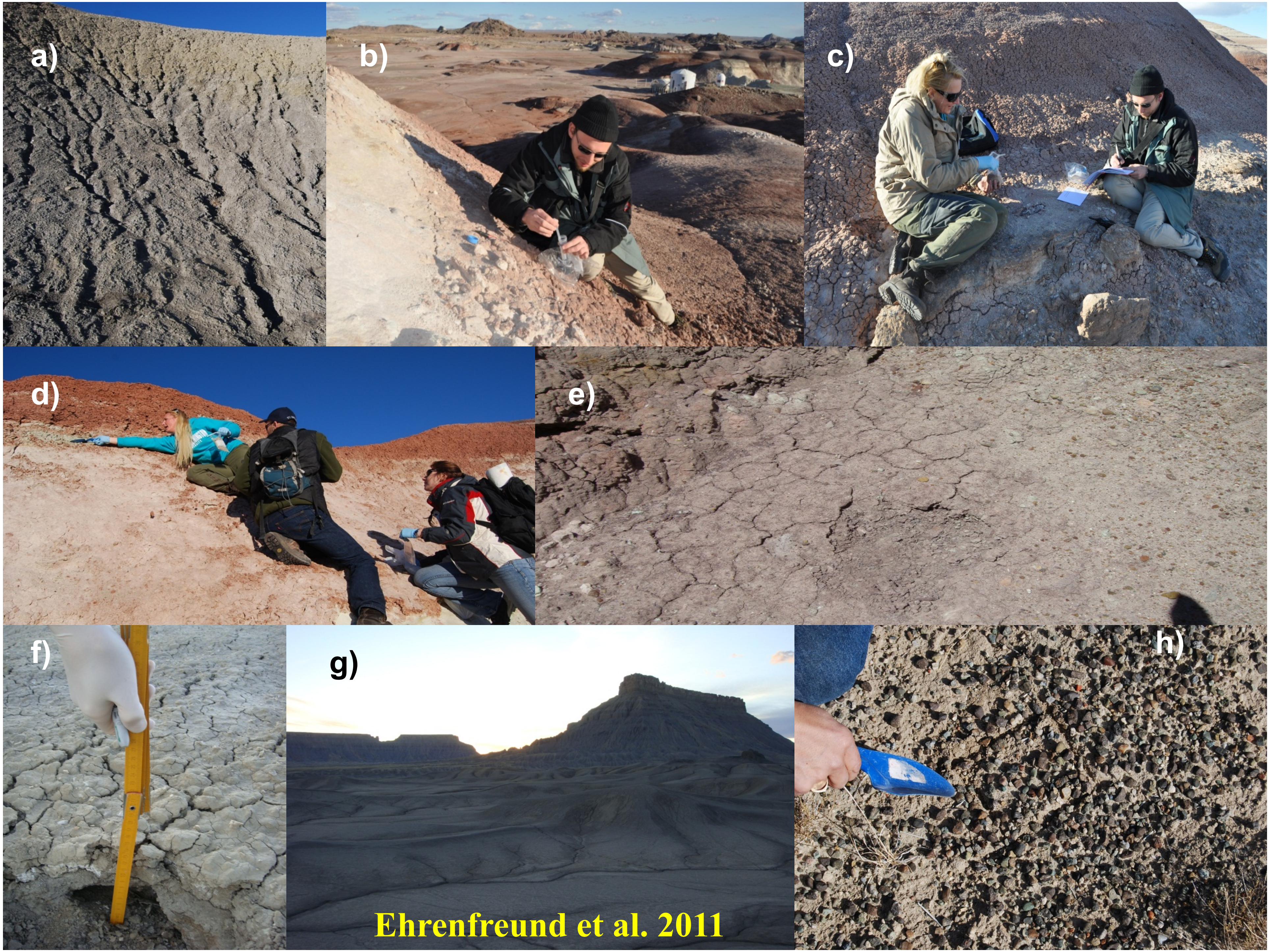
Gale Sedimentary rock strata



Spherules in Sheepbed Unit at the Yellowknife Bay with evaporites. Image taken by MastCam on 25. December 2012.

EuroMoonMars MDRS 2013 outcrop (Orgel et al)




a)
b)
c)
d)
e)
f)
g)
h)
Ehrenfreund et al. 2011

Soil sample analysis

We have characterized the mineralogy, organic compounds and microbiology of 10 selected sample sites from the Utah desert

- The samples were collected under sterile conditions
- The samples were partly analyzed in situ and later distributed to the various laboratories for post-analysis
- Soil sample properties such as pH value and elemental composition of K, P, Mg, and nitrate were measured in the MDRS habitat laboratory
- On-site Polymerase Chain Reaction (PCR) using specific primers in combination with agarose gels identified biota of several domains shortly after collection

EuroMoonMars 2012 & 2013 Methods: Sampling, Preparation, & lab analysis (Leiden/VU/ESTEC)

(Rammos et al 2012, Elsaesser et al 2013)

- Sampling
  - Brushy Basin and Bluegate Shale Member samples
  - Sterile samples taken from depths of 0, 25 and 50cm and kept cool at around 4°C
- Treatment
  - Compressed Alkali metal Halide pellet method (Kbr pellet)
  - Pellets inserted in BIO-RAD Spectrometer

-> Analysis of C, N, S total content

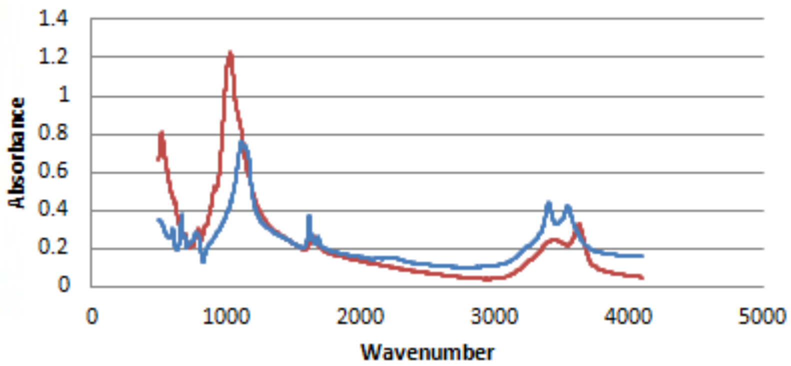
-> FTIR spectroscopy

-> X- Ray Fluorescence and Diffraction studies

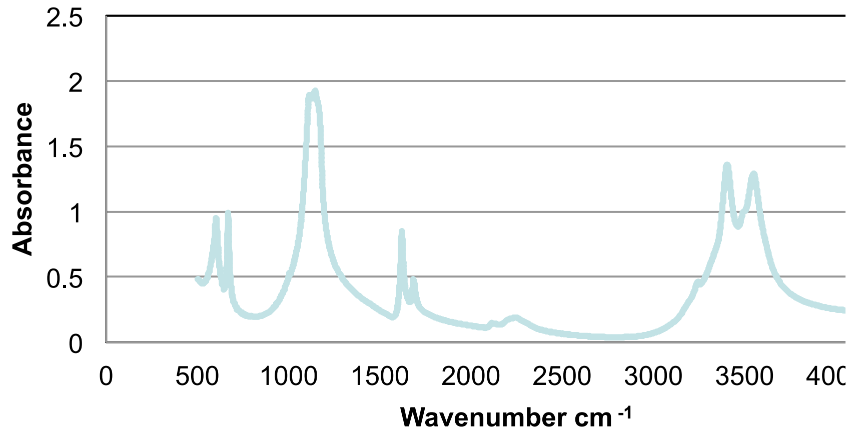
-> Microscopy, spectroscopy, Raman (ESTEC/VU)

FTIR Results Mineralogy

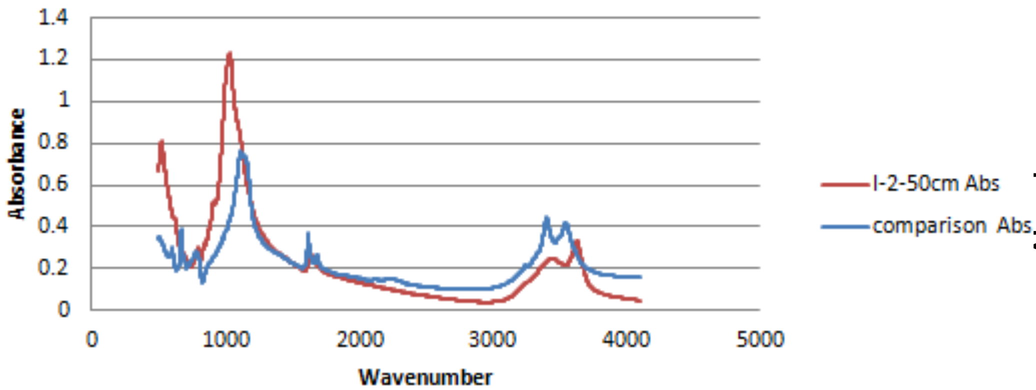
I-2-50cm:
A=0.25Gps+0.7Qz+0.01Flp+0.01Calc+0.01 hem



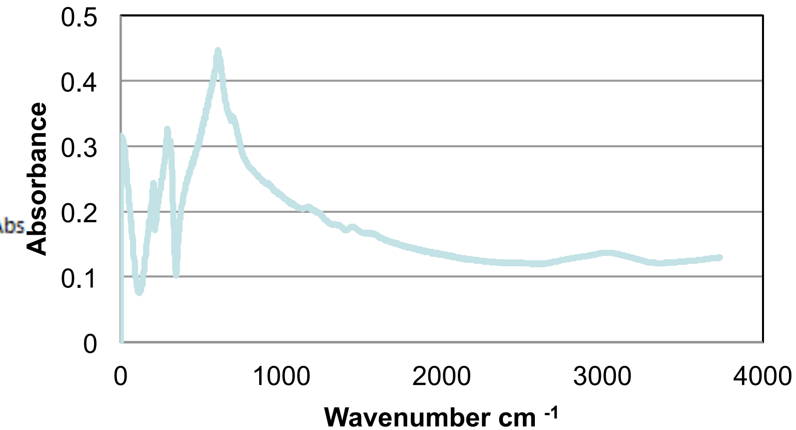
Gypsum



I-2-50cm:
A=0.5Gps+0.2Qz+0.05Flp+0.01Calc+0.05hem



Quartz



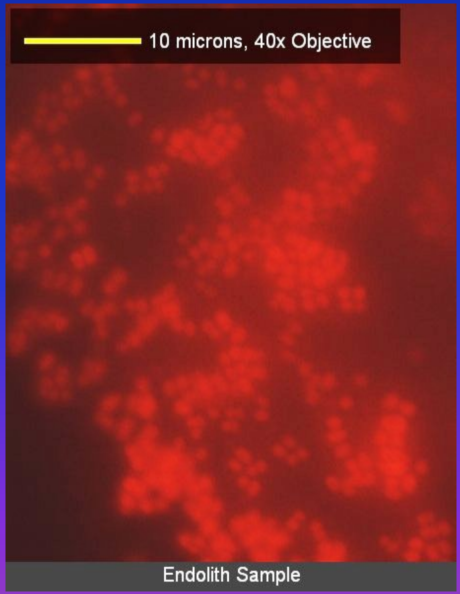
Rammos et al 2012, Elsaesser et al 2013

Endolithic microbial mats

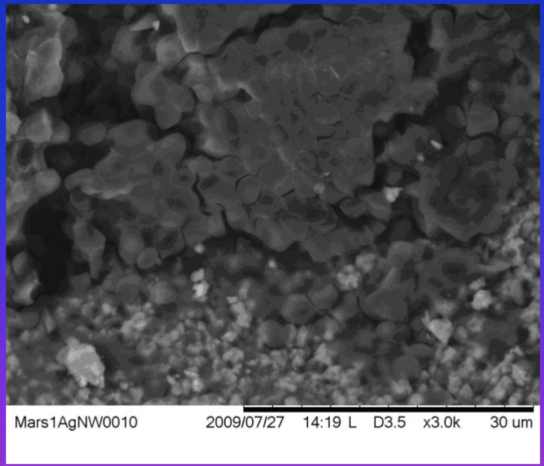
(Foing et al. 2011, 2013)

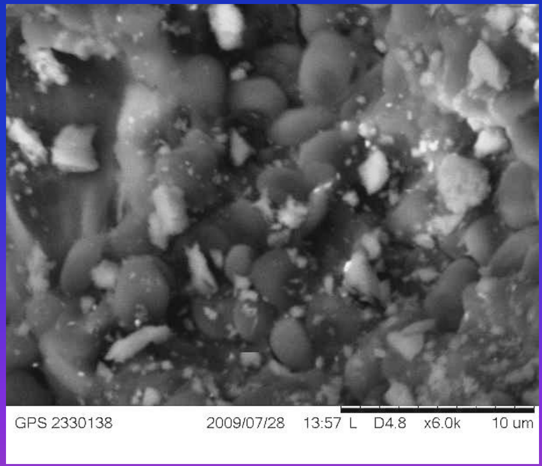
- **Phototrophs**
- **Litho-autotrophs (CO2)**
- **Litho heterotrophs (organic)**

Varnish, crust & 3 colour units of endoliths



Coccoid autofluorescence





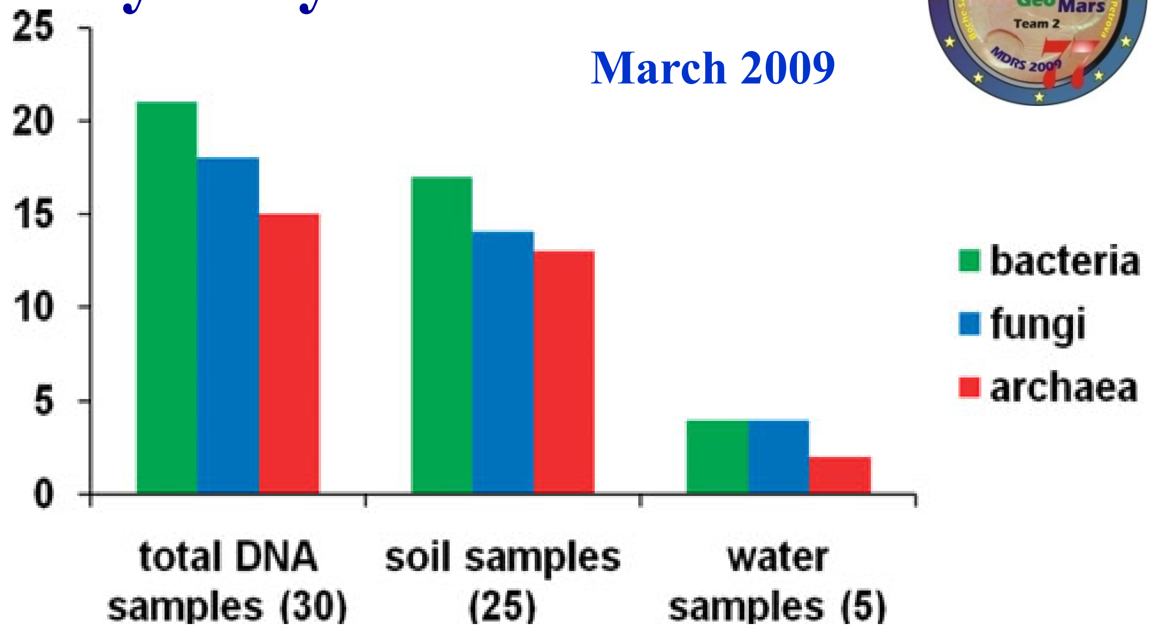
SEM of coccoid cells, abiotic particles in biofilm

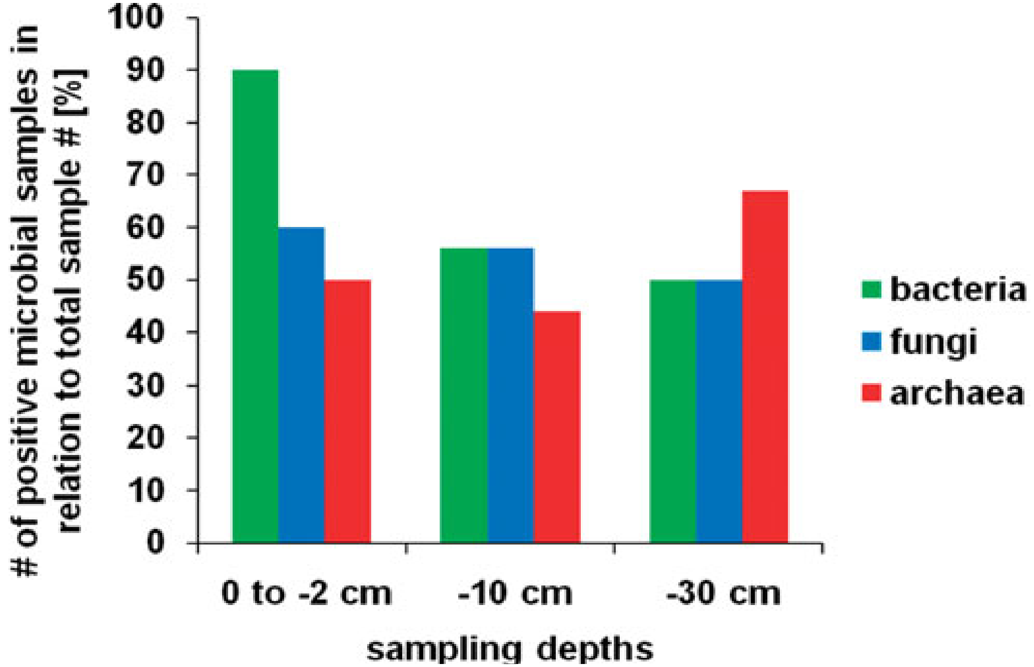
First time on-site DNA analysis by PCR at MDRS

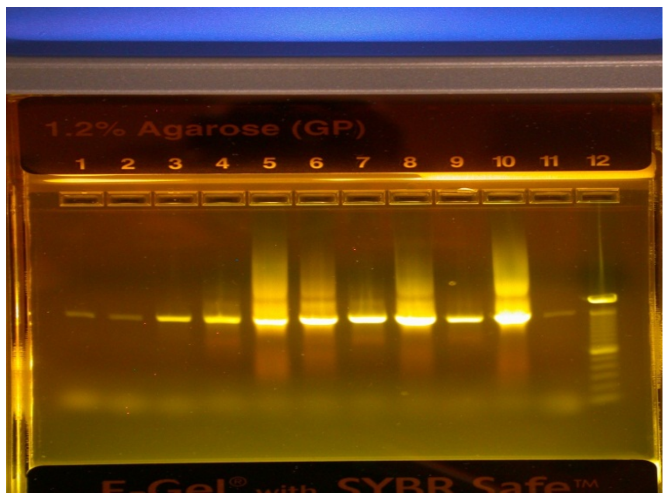
March 2009

Total number of samples positive for bacterial, fungal and/or archaeal DNA

Thiel et al. 2011





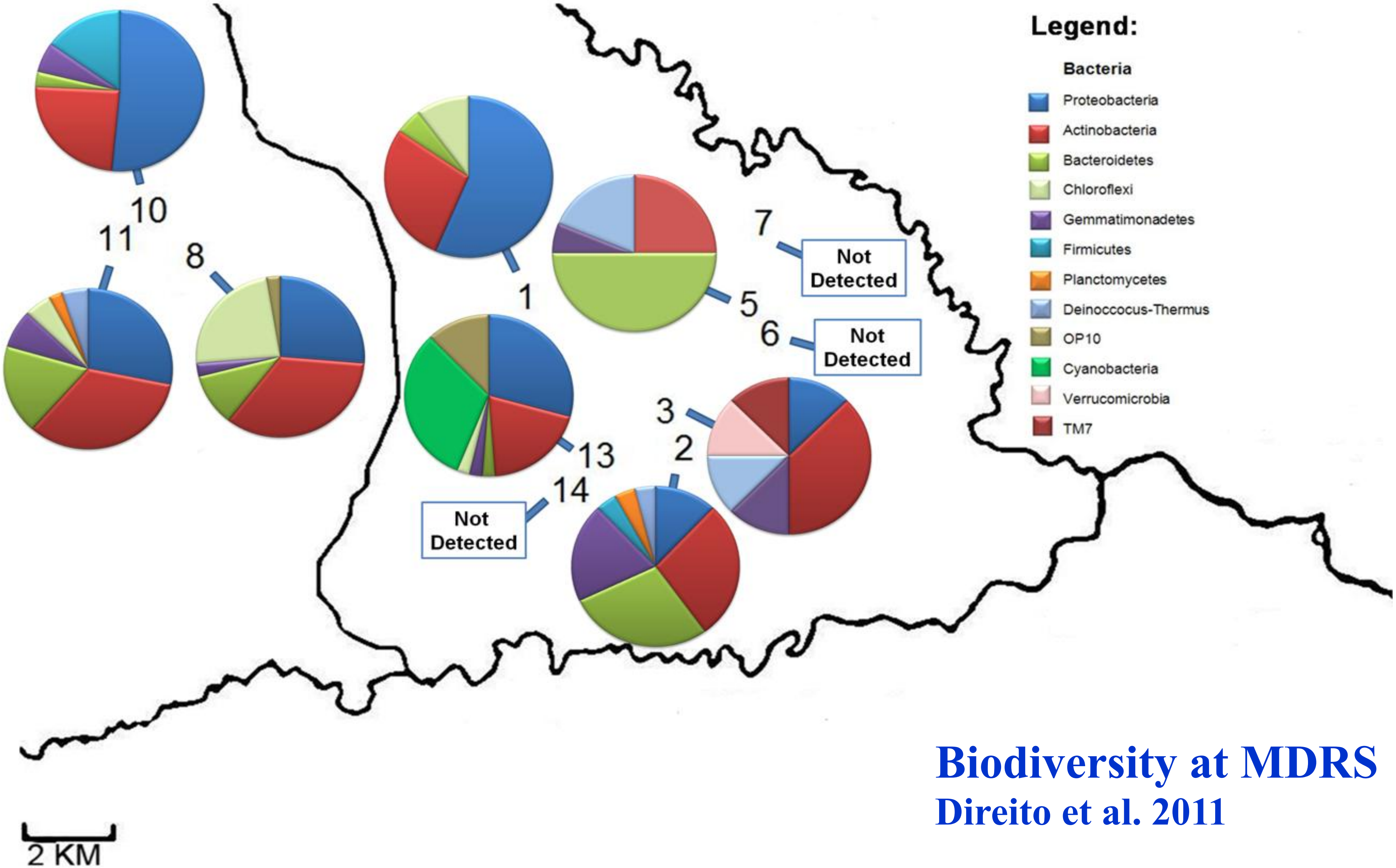


Cultivation-independent rRNA gene-based characterization and phylogenetic analysis

- All domains of life were detected at MDRS; the environment proved to be quite diverse (e.g., for Bacteria); large differences in occurrence and diversity over short distances
- Spiking results indicate low DNA recovery from clay-rich samples!
- Extraordinary variety of putative extremophiles - mainly Bacteria but also Archaea and Eukarya: radioresistant, endolithic, chasmolithic, xerophilic, hypolithic, thermophilic, thermoacidophilic, psychrophilic, halophilic, haloalkaliphilic/alkaliphilic microorganisms

Direito et al. 2011

Relative contributions of Bacteria phyla to clone libraries derived from samples taken at various locations at MDRS

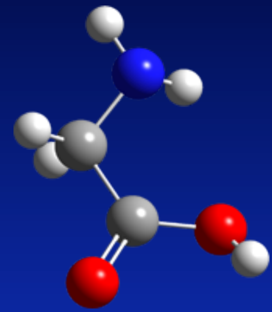


Biodiversity at MDRS

Direito et al. 2011

Life and Amino Acids in dry deserts….

Atacama: Biomass $8.5x10^6 – 6x10^7$ cells/g (PLFA)
$6.3x10^2$-$5.2x10^3$ CFU/g
600 ppm TOC (Lester et al. 2007)



Amino Acid (ppb)	Atacama Peru	Atacama Yungai	Utah Morrison	Utah Mancos	Utah Dakota
Glycine	91	102	92	1324	17390
L-Alanine	283	92	76	1114	32934
L-Glutamic Acid	62	116	194	600	10892
L-Aspartic Acid	35	57	102	220	7775

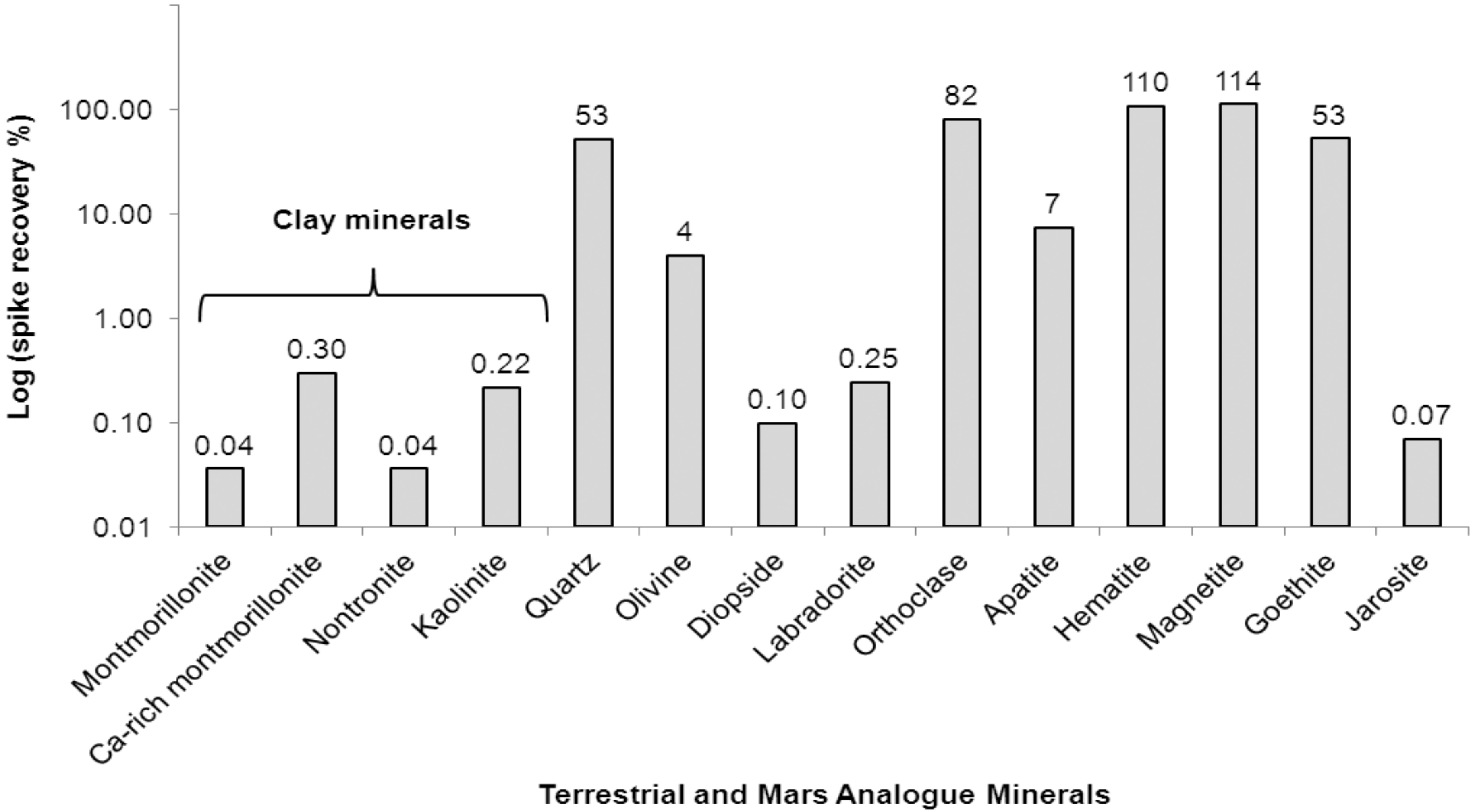
Peeters et al. 2009, Martins et al. 2011

Results from the analyses of mineralogy, organic compounds and biodiversity

Sample	Geo context	Sulfates %	Carbonates %	Clays %	Organic Matter %	PAHs/LE sum (ng/g)	Amino acids (ppb)	Post Bacteria PS /FDNA
P-1	Mancos	7	11	15	2	46	280	+ +
P-2	Morrison		<1	16	1	38	n.d.	+ -
P-3	Morrison	7	3	18	1	57	730	+ +
P-5	Morrison		2	23	2	44	n.d.	+ -
P-6	Morrison	14	<1	33	2	55	n.d.	- -
P-7	Morrison		22	6	2	48	n.d.	- -
P-8	Mancos	59	8	11	4	51	4,600	- +
P-10	Mancos	18	20	20	5	116	2,300	+ +
P-13	Dakota		16	9	2	51	100,000	+ +
P-14	Dakota	73	11	1	3	45	17,000	- -

Ehrenfreund et al. 2011

Recovery of DNA spike from a range of minerals



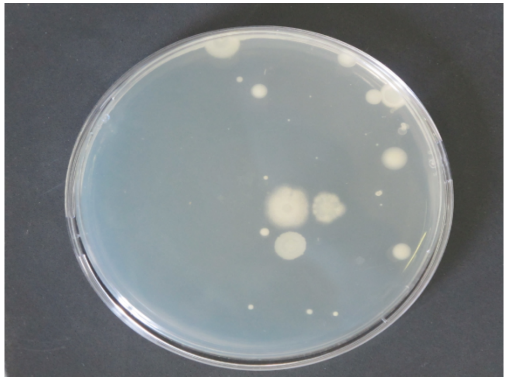
Direito et al. 2012, FEMS

Percentage of DNA spike recovered was calculated relative to a spike-only control after qPCR, and expressed on a logarithmic scale. The percentages of spike recovery are indicated above each column.

ON-SITE QUALITY ASSURANCE PROCEDURES

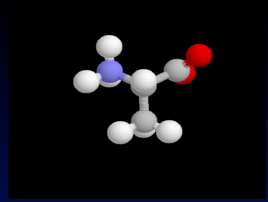
Luisa Rodrigues (PhD student VU Amsterdam/Aveiro U.)

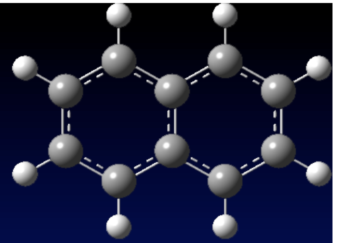
Contamination control is VITAL to assess microbial contamination



- ON-SITE AIRBORNE MICROBIAL CONTAMINATION ASSESSMENT
- BLANKS (e.g., sterile soil)
- CHEMICAL TRACERS in FLUIDS (e.g., sodium chloride, bromide, potassium sulphate, fluorescent dyes, and persulfates)
- PHYSICAL TRACERS in FLUIDS (fluorescent microspheres - size and surface charge similar to bacteria)
- PASSIVE TRACERS (natural isotopes e.g., C, Sr, S, tritium)
- BIOLOGICAL TRACERS

Post-analysis techniques





- The concentrations of polycyclic aromatic hydrocarbons (PAHs) have been determined by using the Solid Phase Microextraction (SPME) method that provides good recoveries for small PAHs that are usually targeted by planetary missions
- Amino acids were extracted from soil samples and analyzed on a Gas Chromatograph Mass Spectrometer (GC-MS)
- Culture-independent molecular analysis directed at ribosomal RNA, was used to investigate the detailed microbiology of desert samples, including a phylogenetic analysis
- Mineralogy investigations were performed using Infrared spectroscopy, XPS and X-ray diffraction analysis

EXTREME ENVIRONMENTS

- **RIO TINTO (SPAIN)**
- **UTAH DESERT, MDRS (U.S.A.)**
- **ATACAMA DESERT (CHILE/PERU)**
- **SVALBARD (NORWAY)**
- **ICELAND**
- **ICE CAVES, OCEAN CRUST,...**

EXTREMOPHILES

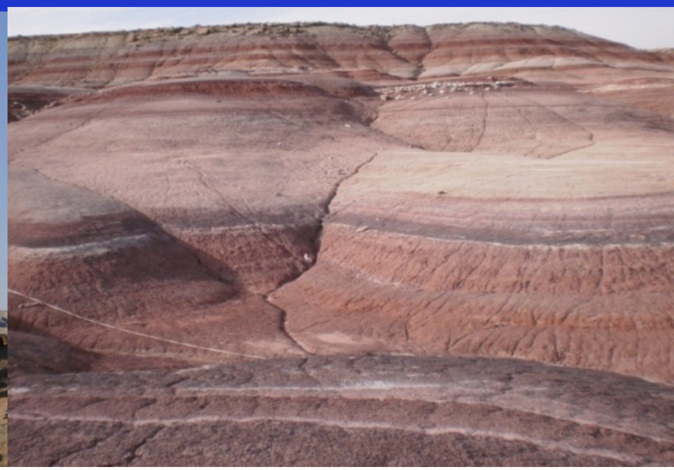
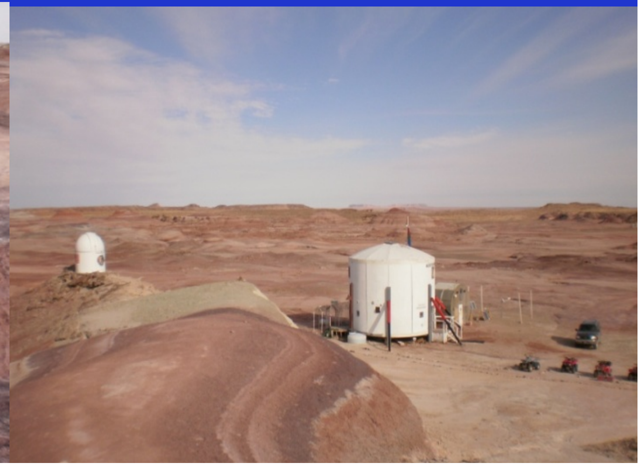
THRIVE IN PHYSICALLY OR GEOCHEMICALLY EXTREME CONDITIONS

temperature, humidity, pressure, radiation, salinity, pH

Earth Deserts as Mars astrobiology analogue

Sampling methodology optimization:

- Large difference in occurrence and diversity over short distances, indicating the need for high-sampling frequency at similar sites
- The phylogenetic analysis showed a diversity of life in contrast to the low recoverable content of amino acids (ppb) and PAHs (ng/g)
- The dominant factor in bacterial number may be soil porosity and lower small (clay-sized) particle content
- Composition of clay fractions (smectite/illite) determines environment for organics and microbes

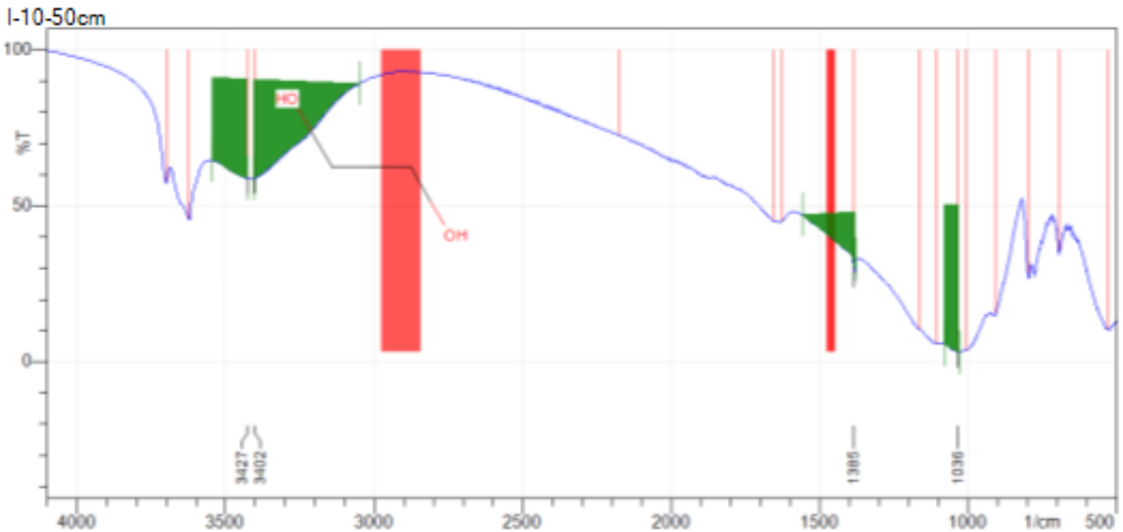
EuroGeoMars EuroMoonMars lessons learned



- Remote sensing, geology context and in-situ measurements
- Comparison between analysis techniques (spectro, samples)
- Contamination issues
- Geochemical evolution and habitats
- The extraction efficiency of amino acids and DNA material from the mineral host matrix will strongly depend on
  - the depositional environment of the minerals
  - the degree of illitization of the clay
  - the instrument performance
- Optimized protocols are needed for the extraction of fragmented and degraded organics and ancient DNA
- *References: Foing Stoker Ehrenfreund Editors, IJ astrobiology 2011 (& 12 articles), EuroMoonMars special issue : articles in preparation 2013*


MDRS NASA/ESA
EuroGeoMars 2009
6
5
1.2% Agarose (GP)

Very low concentrations of Organics
C content < 0.1 %



Estimation based on database of organic materials and peak density

Matching Functional Groups

Quality	Functional Group	Chemical Sub Class	Chemical Class
100	**Hydroxy, Possibly 1,2-Diol**		**Aliphatic Alcohol**
44	Unsaturated Hydrocarbon, Cyclic, > C5		Alkene

Corresponding Chemical Classification Rules

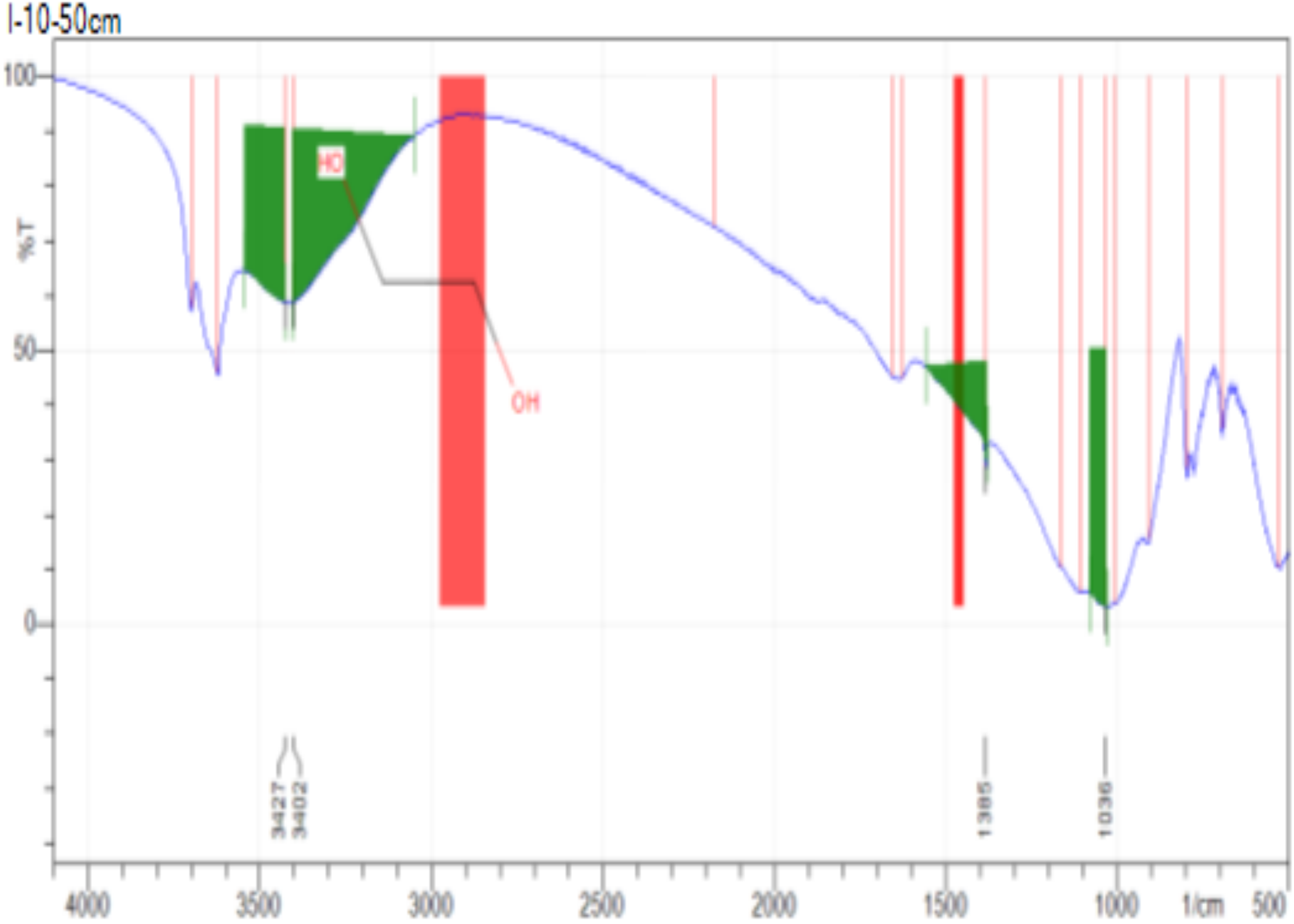
Functional Group: 'Hydroxy, Possibly 1,2-Diol', Chemical Class: 'Aliphatic Alcohol'

Vibration	Start WN	End WN	Threshold	Priority
C-O Stretch	1070	1020	0	6
O-H Def	1400	1300	0	6
O-H Stretch	3450	3300	0	6

Missing Chemical Classification Rules

Functional Group: 'Hydroxy, Possibly 1,2-Diol', Chemical Class: 'Aliphatic Alcohol'

Vibration	Start WN	End WN	Threshold	Priority
C-H Stretch, CH2	2975	2840	0	3
C-H Bend, CH2/CH3	1475	1445	0	4


I-10-50cm
100
%T
50
0
HO
OH
3427
3402
1385
1036
4000
3500
3000
2500
2000
1500
1000
1/cm
500

2 - Characterisation of rock microbial communities from Mars terrestrial analogues

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3 *ESA-ESTEC, The Netherlands*
4 *NASA Ames Research Center, USA*
5 *Mars Society Australia, Australia*
6 GWU, Leiden & FWF, Austria

EXTREME ENVIRONMENTS

- **RIO TINTO (SPAIN)**
- **UTAH DESERT, MDRS (U.S.A.)**
- **ATACAMA DESERT (CHILE/PERU)**
- **SVALBARD (NORWAY)**
- **ICELAND**
- **ICE CAVES, OCEAN CRUST,...**

EXTREMOPHILES

THRIVE IN PHYSICALLY OR GEOCHEMICALLY EXTREME CONDITIONS

temperature, humidity, pressure, radiation, salinity, pH

DRILLING ACTIVITIES

Avoid introduction **non-indigenous microorganisms** & **chemical contamination**

Lithoautotrophic microorganisms adapted to **oligotrophic conditions** (low nutrients); inhabit variety of rock-types

Extracting DNA a challenge:

- low microorganism abundance
- rock substrate

AVOID CONTAMINATION OF SAMPLES:

-sampling – handling – transport - analytical procedures

DRILLING AT MDRS (DOMEX Drilling Campaign 2011)

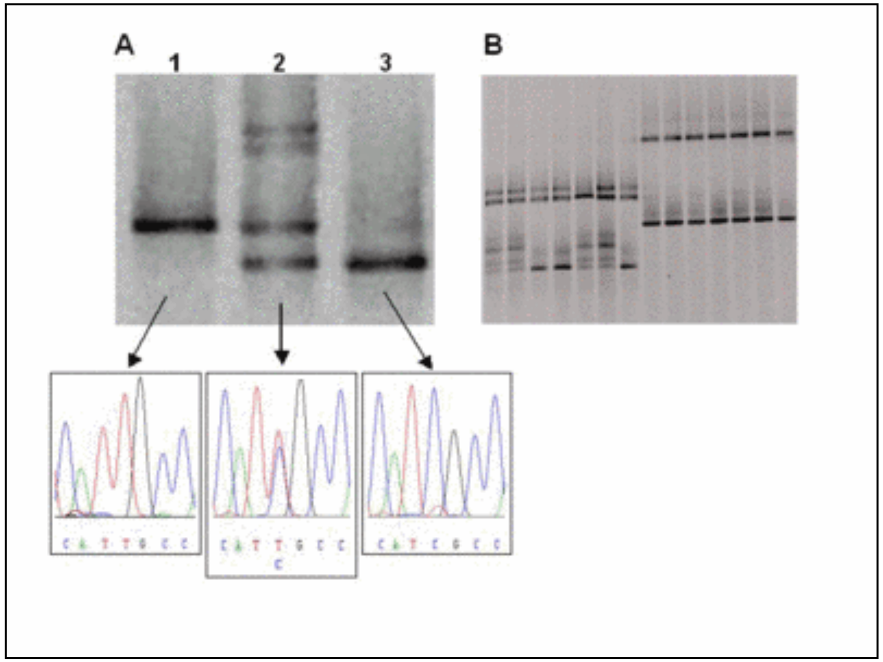
Coring drill system MARTE (Mars Astrobiology Research and Techonology Experiment Drilling System)

- **remote system**
- **compressed air coring**
- **250 mm drill lengths**
- **10 m depth, 48mm diameter**

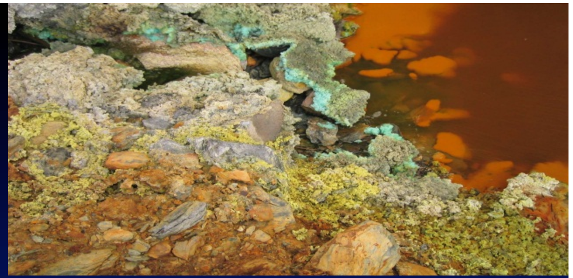
LABORATORY QUALITY ASSURANCE PROCEDURES

PCR (culture independent method) to assess microbial diversity:
- identify uncultivatable organisms

- LAMINAR-FLOW HOOD
- AEROSOL RESISTANT PIPETTE
- NEGATIVE CONTROLS
- LOW-BIOMASS CONTAMINANT DATABASE
(Compilation of negative controls)



Correlation studies



- Samples in which microorganisms could be observed after PCR amplification, had small (clay-sized) particle content of 9-21%. Outside this clay content range, microorganisms were not detected

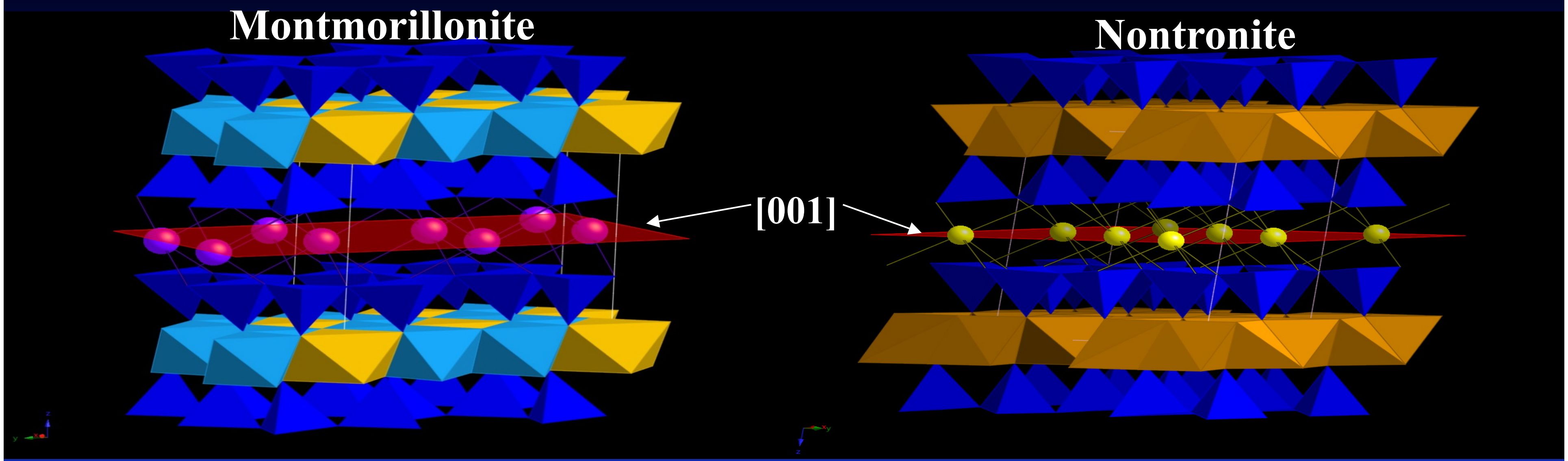
- No significant correlation was observed between amino acids and DNA yield or positive PCR signals

- Microbial numbers and diversity do not appear to be correlated with neither organic content nor mineralogy

- Both indirect and direct gradient analysis did not reveal environmental parameters that significantly explained the microbial community structure

Ehrenfreund et al. 2011, Elsaesser et al 2013

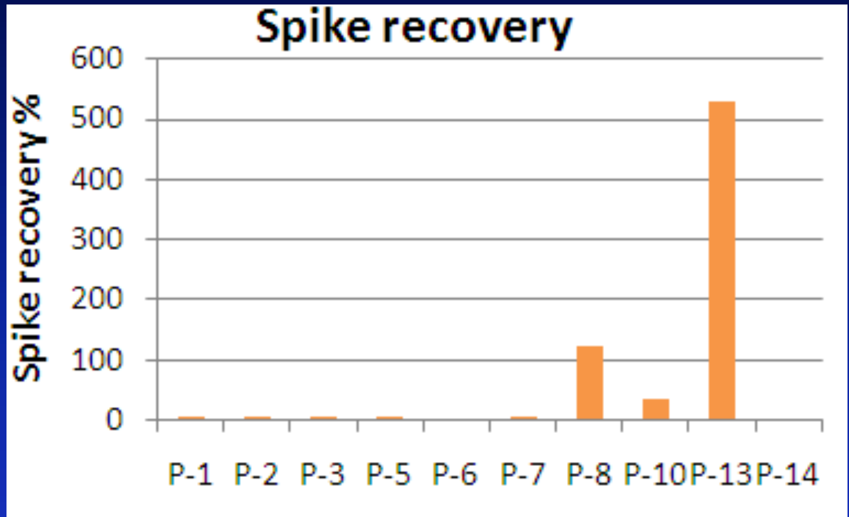
Clay binding



- The level of organic material present in clay will strongly depend on its **deposition history, diagenesis and specific composition**
- **Adsorption of biological material**, e.g., amino acids and DNA can **complicate the extraction procedure** from clays (Henneberger et al. 2006)
- RNA molecules bind efficiently to montmorillonite that can catalyze oligomers (Ferris 2005). **Most clay minerals bind organic compounds in the interlayers** and the clay expands to accommodate them

Quantification of DNA recovery from soil samples

Internal Control Experiment:

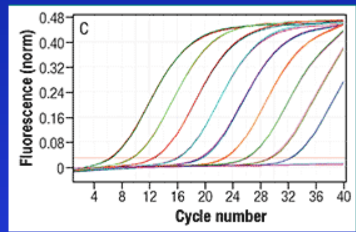


	Formation	Clays %	pH	P ppm	Amino Acids ppb	PAHs ng/g
P1	Mancos	15	7.6	5	280	46
P2	Morrison	16	8.1	12	n.d.	38
P3	Morrison	18	8.0	12	730	57
P5	Morrison	23	9.0	100	n.d.	44
P6	Morrison	33	7.6	85	n.d.	55
P7	Morrison	6	9.6	10	n.d.	48
P8	Mancos	11	8.5	5	4,600	51
P10	Mancos	20	8.5	5	2,300	116
P13	Dakota	9	8.7	100	100,000	51
P14	Dakota	1	7.0	80	17,000	45

Spiking procedure:

Spike: YHXK1 gene
fragment of yeast
(not present in samples)

DNA Extraction



qPCR (to determine spike recovery)

- **Clays interfere with extraction!**
- **Use of an external spike is necessary to account for possible losses**

Ehrenfreund et al. 2011, Direito et al. 2012,