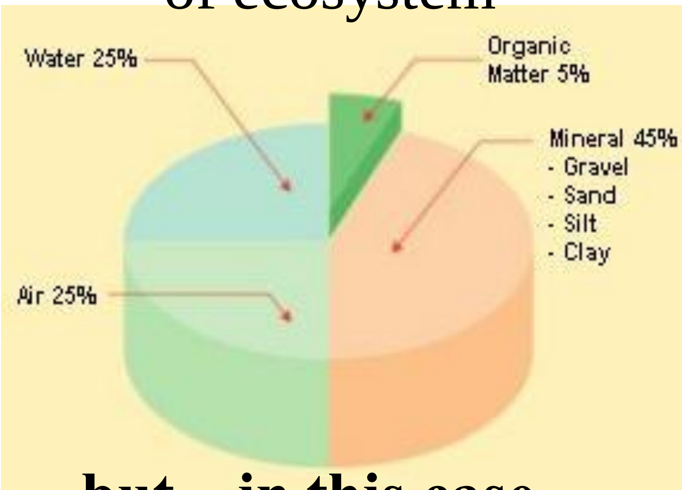
Judith Ascher*,
MariaTeresa Ceccherini*
Anna Lavecchia*
Giacomo Pietramellara*

SPACE FARMING

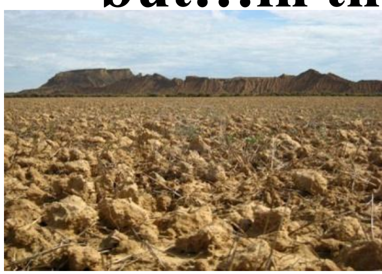
Smart soil and plant management for human extra-terrestrial base sustainability: induction of fertility in extraterrestrial soil

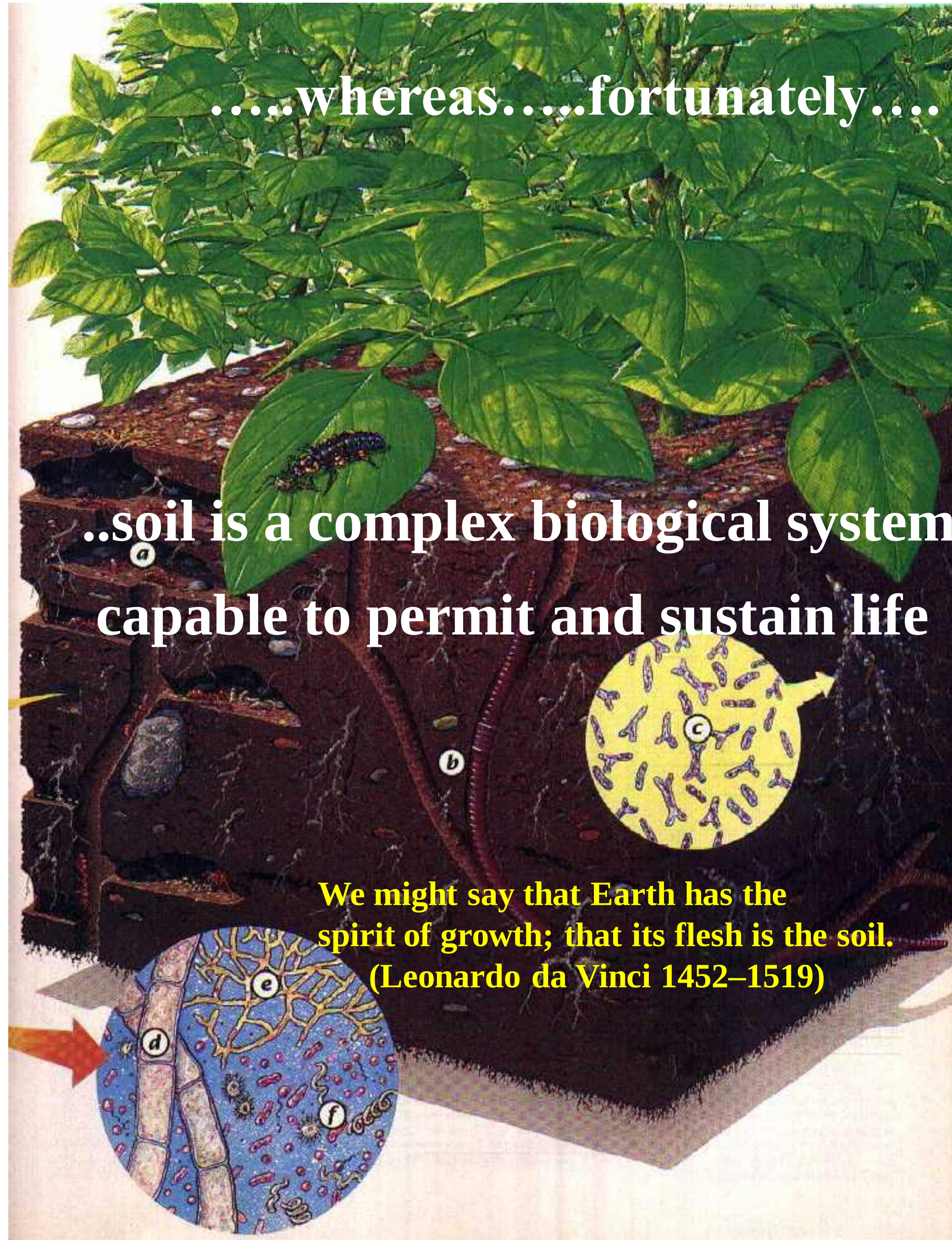
DiPSA - UNIFI *
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO *

Soil as a static component of ecosystem



but…in this case…





The capacity of soil to sustain life is defined as soil fertility

The pathway by which soil sustains life is defined as soil functionality

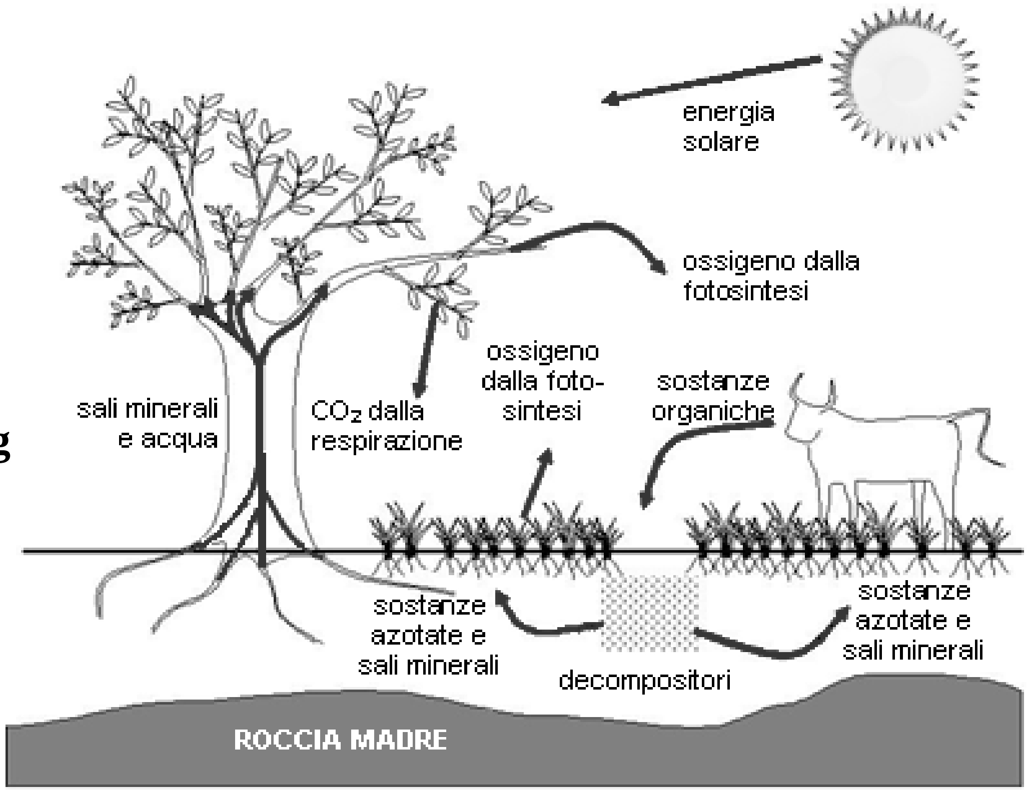
….it is possible to recognize:

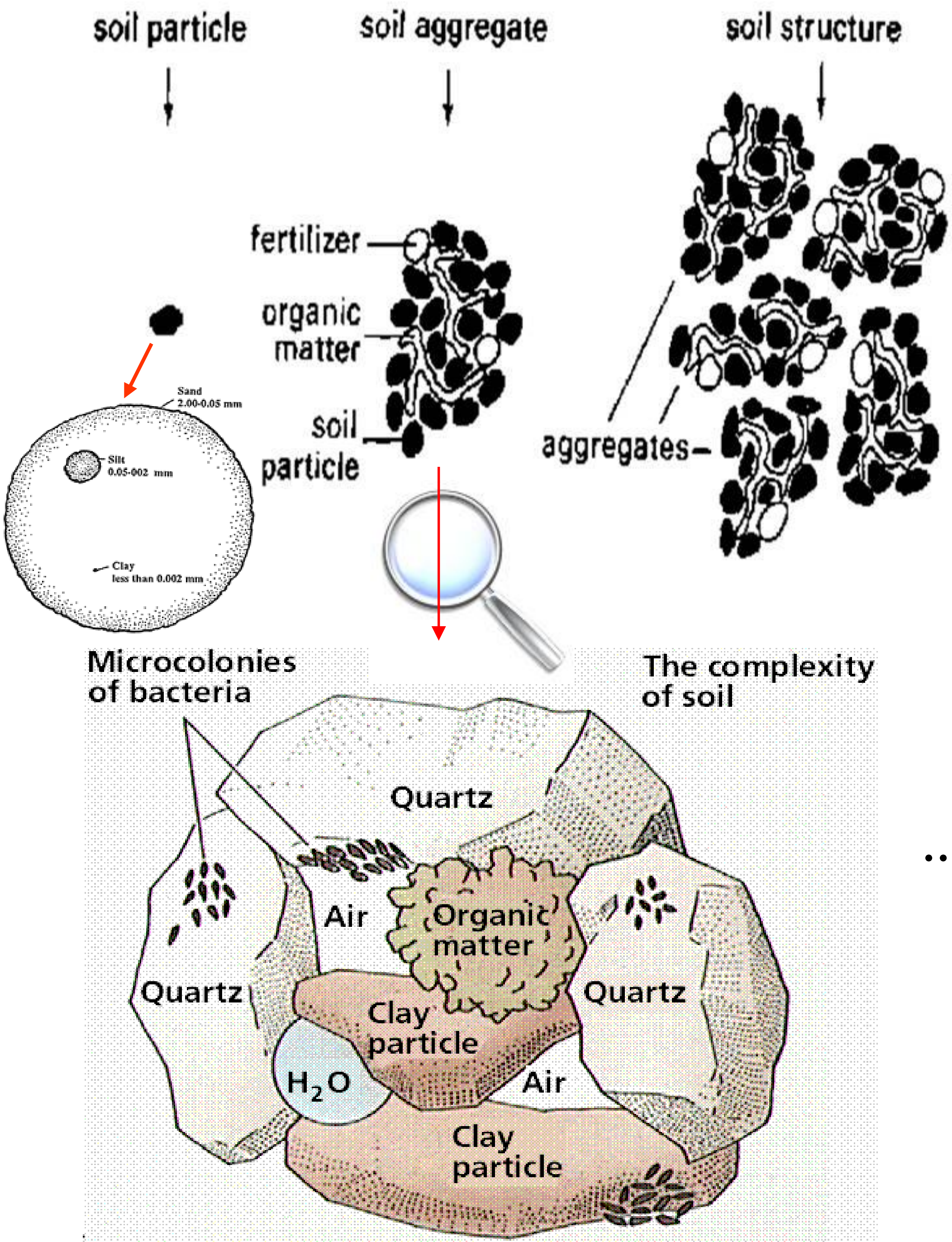
Chemical-physical fertility

Abiotic factors – moisture, pH, salinity and alcalinity, texture, **structure**, **CEC**, ESP, **soil pH buffering capacity**, O_2/CO_2 air conc., soil and air temperature.

Biological fertility

Biotic factors – soil **microbial community**
soil **organic matter** forms
extracellular enzymes





Chemical-physical fertility

...due to interaction between :

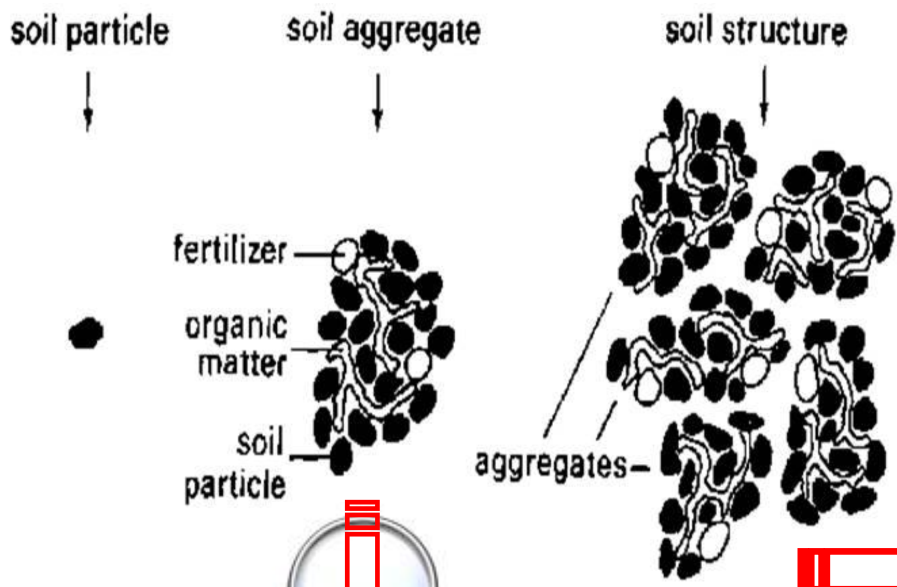
minerals + organic substances

↓

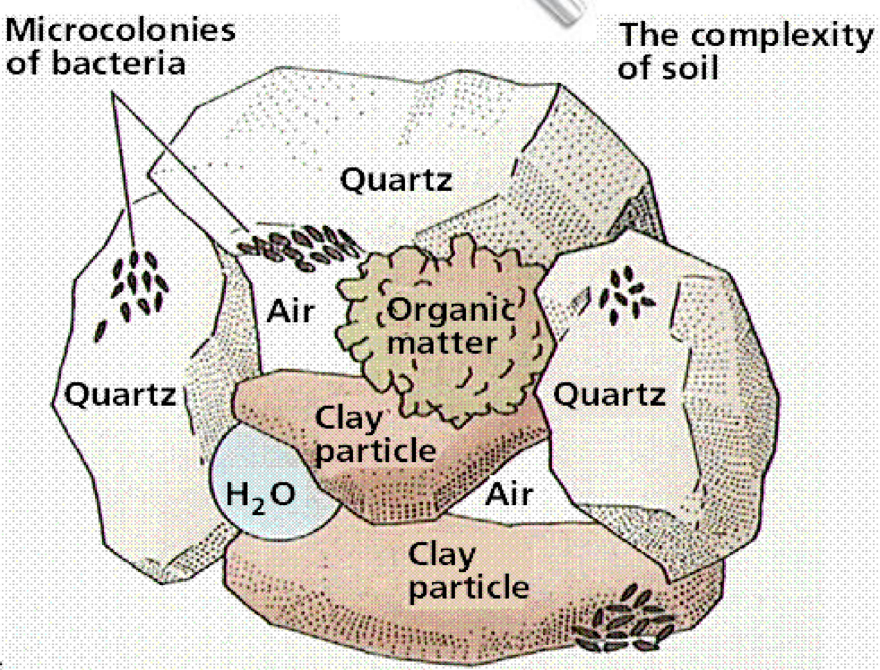
SOIL STRUCTURE

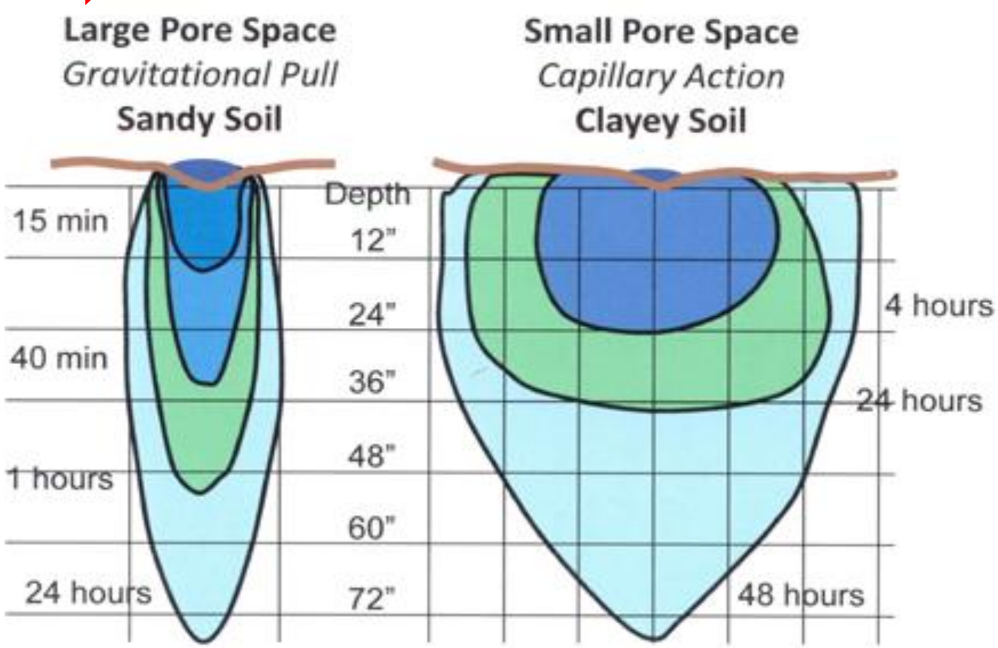
↓

... in which **AGGREGATE** is the base component...


soil particle
soil aggregate
soil structure
fertilizer
organic matter
soil particle
aggregates


Structured soil
Unstructured soil


Microcolonies of bacteria
The complexity of soil
Quartz
Air
Organic matter
Quartz
Quartz
Clay particle
H_2O
Air
Clay particle


Large Pore Space
Gravitational Pull
Sandy Soil
Small Pore Space
Capillary Action
Clayey Soil
Depth
15 min
12"
24"
40 min
36"
48"
1 hours
60"
24 hours
72"
4 hours
24 hours
48 hours

... stabilizing the soil structure is essential to reduce:

.. drying …

…and flooding

...to give stability to the soil structure…

...avoiding…

...relevance of colloids:

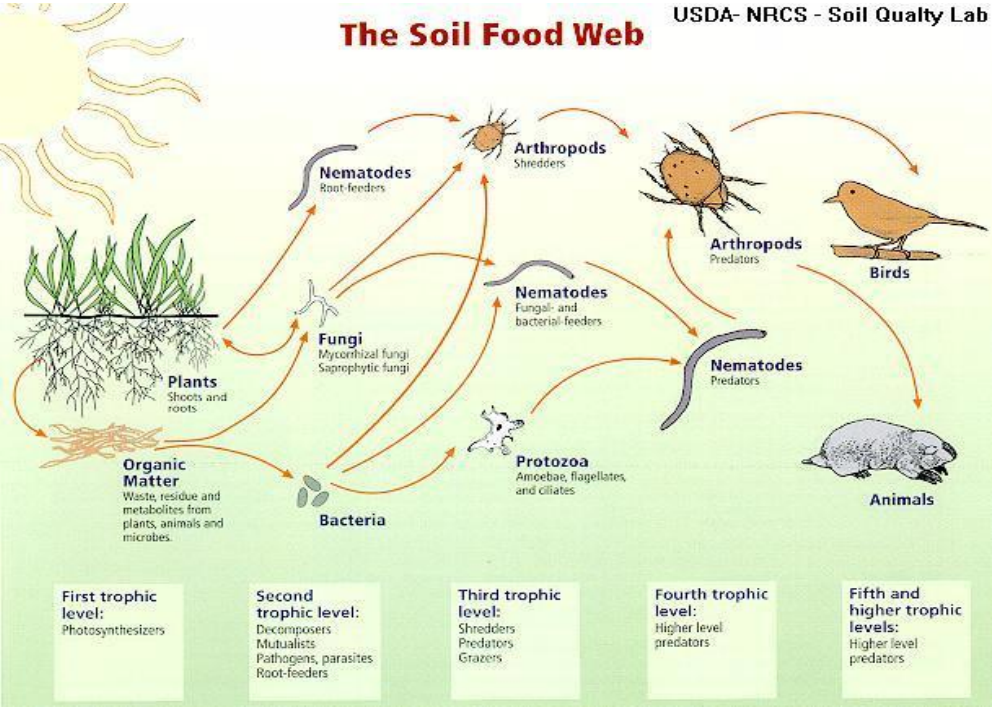
- Humic substances
- Clay minerals
- Fe and Al oxy/hydroxy

★ degradation (mineralization)

★ saturating cation

★ soil solution pH value

Biological fertility…soil inhabiting organisms…

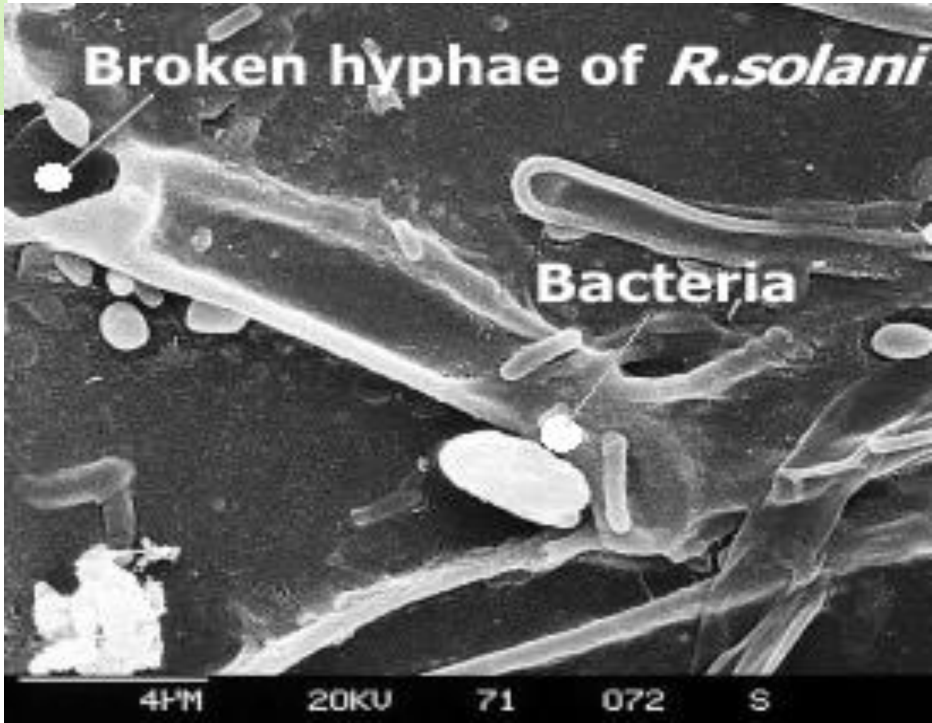


.. Classified as:

- microfauna
- mesofauna
- macrofauna

Microfauna:

- bacteria 1 – 2 t/ha
- fungi 2 – 5 t/ha

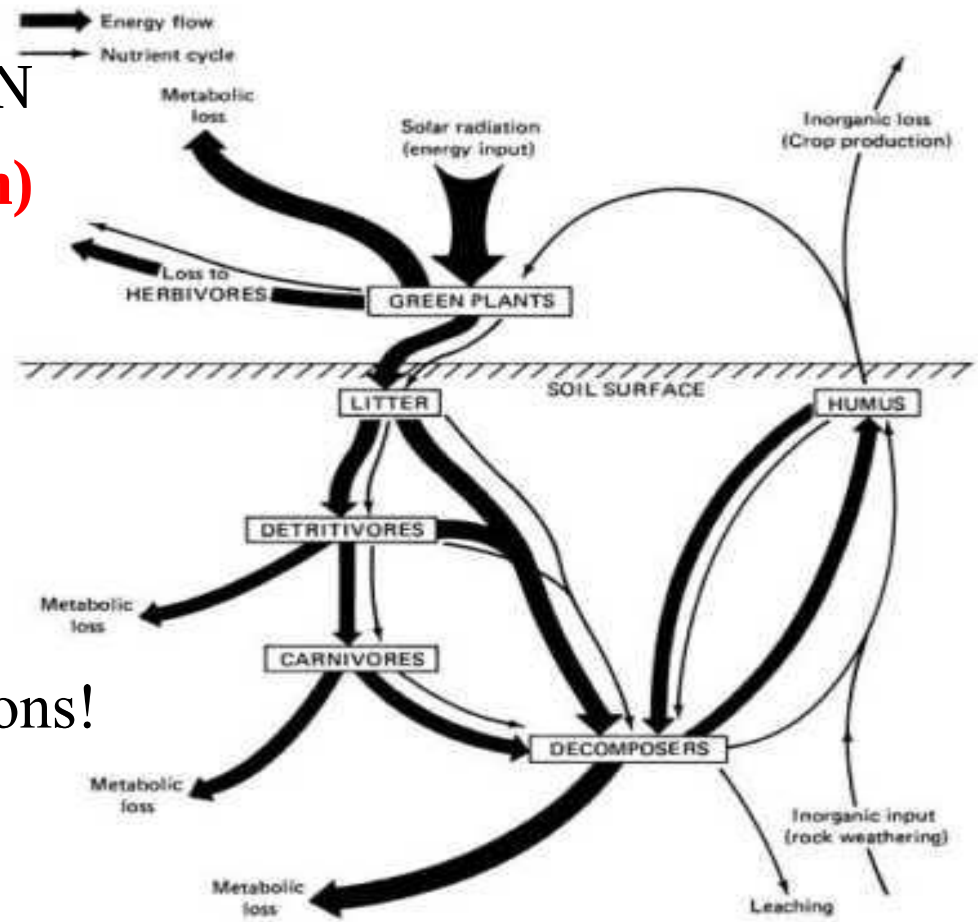


… **Microfauna relevance** → **BIOLOGICAL FERTILITY**

DEGRADATION (MINERALIZATION)

NUTRIENT CYCLE ACTIVATION

(nitrogen, phosphorus, potassium)



...consider also...

Extracellular enzymes

… active even in extreme conditions!

Biological soil functionality

Mainly depends on soil microbial community structure and activity.

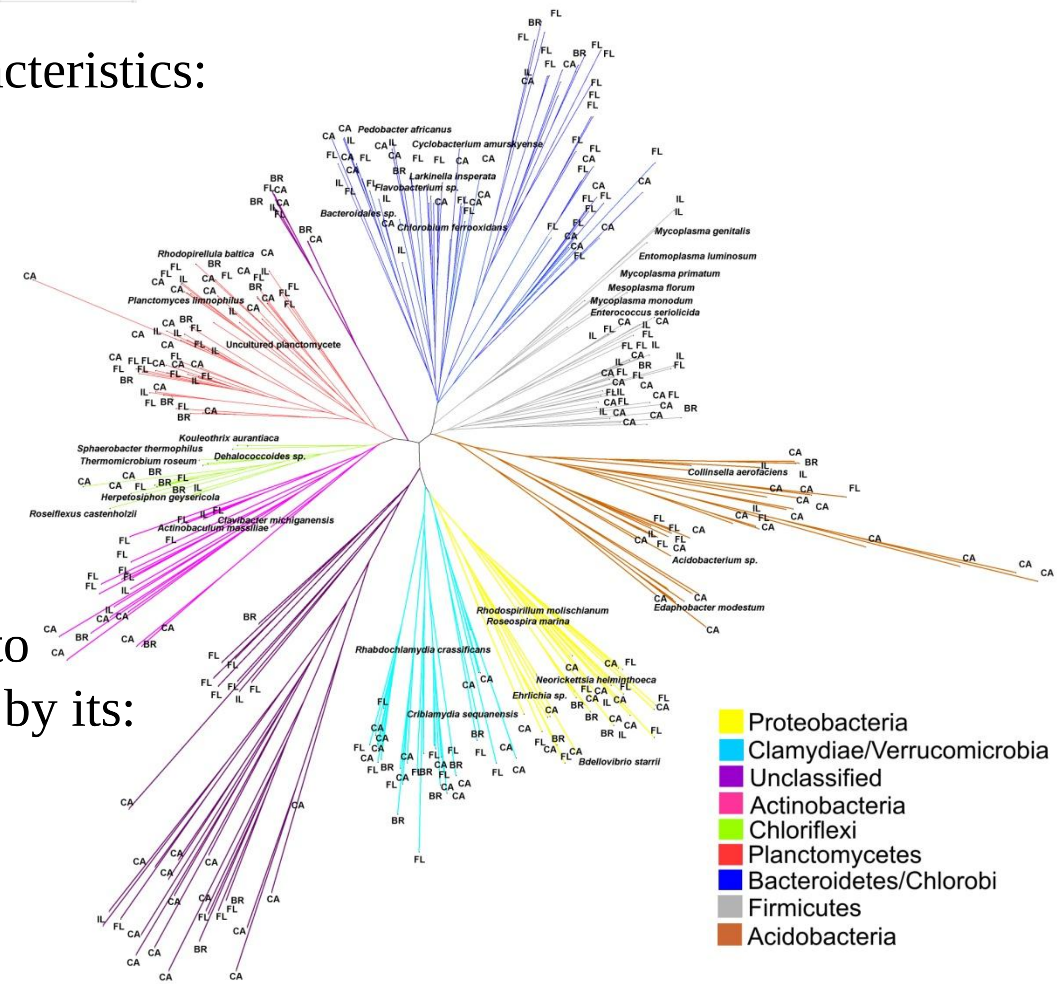
Soil microbial community characteristics:

- richness
- evenness
- r/k strategists ratio
- microbial consortia
- functional redundancy

The capacity of soil to respond to environmental stress is defined by its:

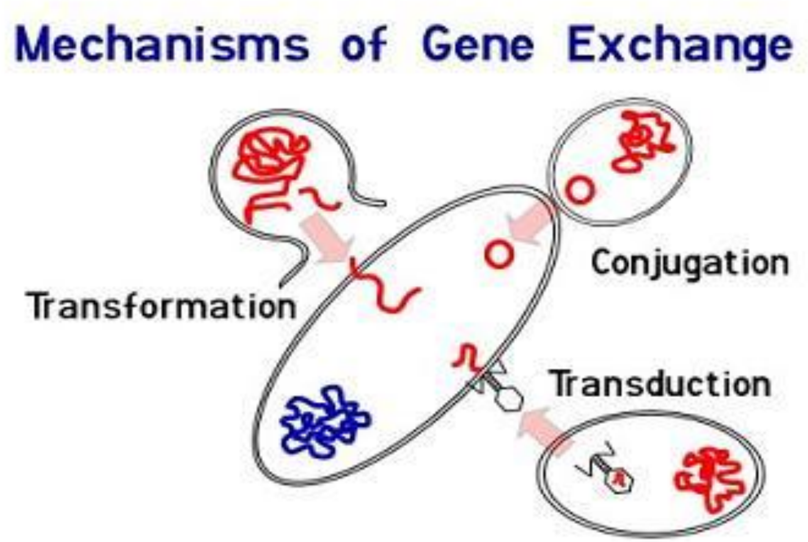
- Resistence
- Resilience

} Soil functional robustness



(Pyrosequencing enumerates and contrasts soil **microbial diversity)**

The main adaptative strategy of bacteria is based on:

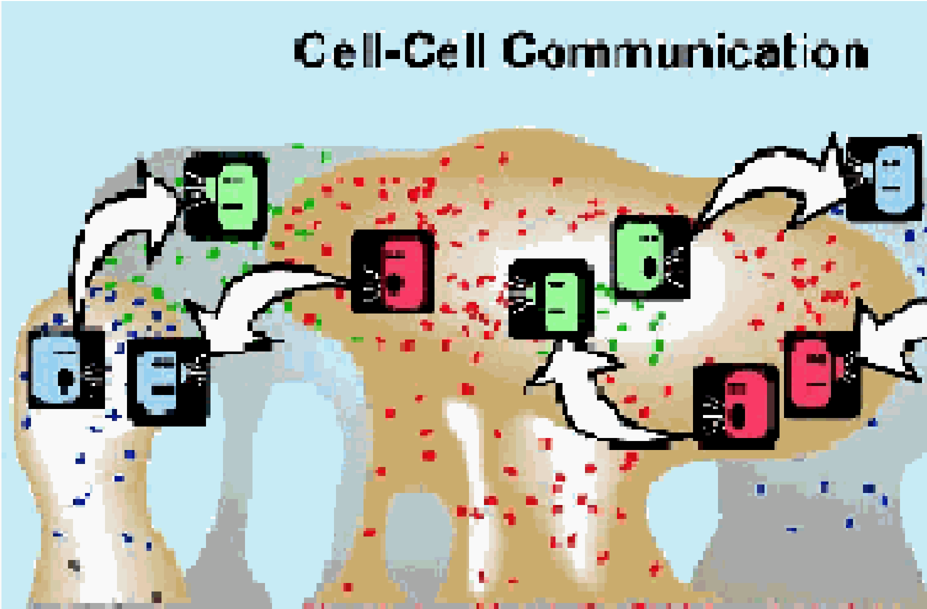


exchange of genetic information

(new metabolic functions)

situational awareness

(to adopt the appropriate strategy)



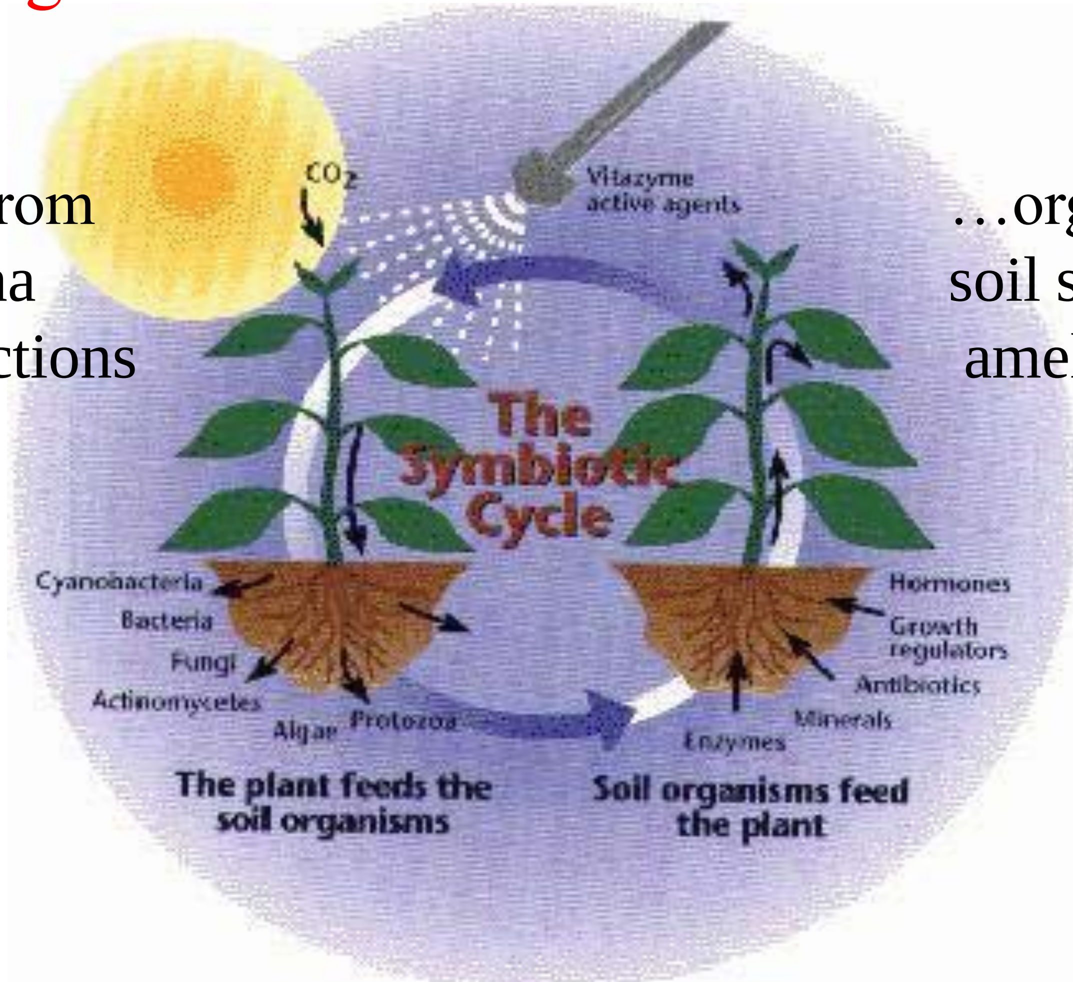
...plants relevance in soil functionality…

….receiving…

...giving…

…nutrients from
microfauna
degradative actions

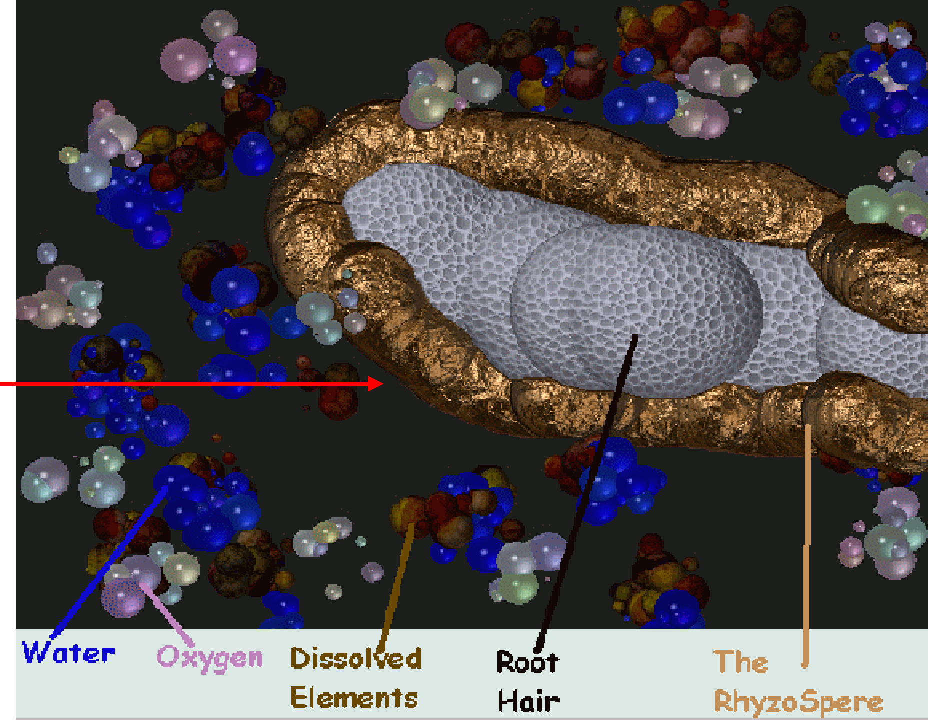
…organic matter
soil structure
amelioration



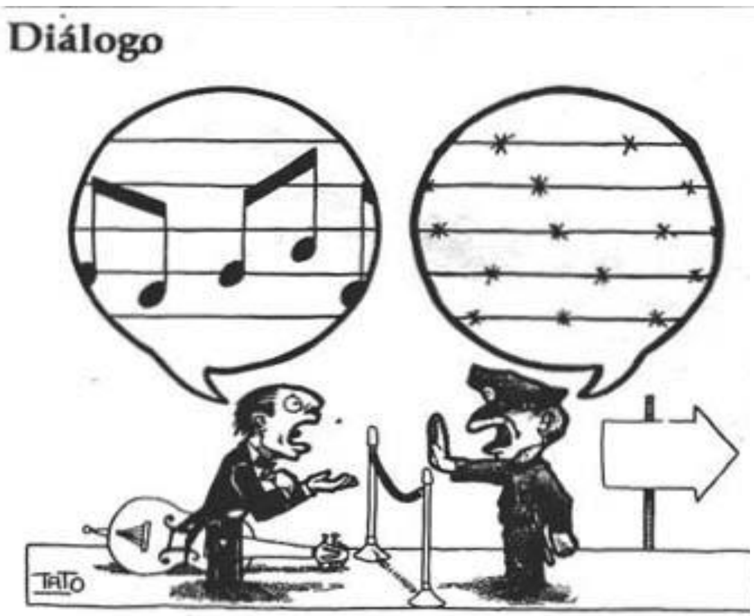
Plants

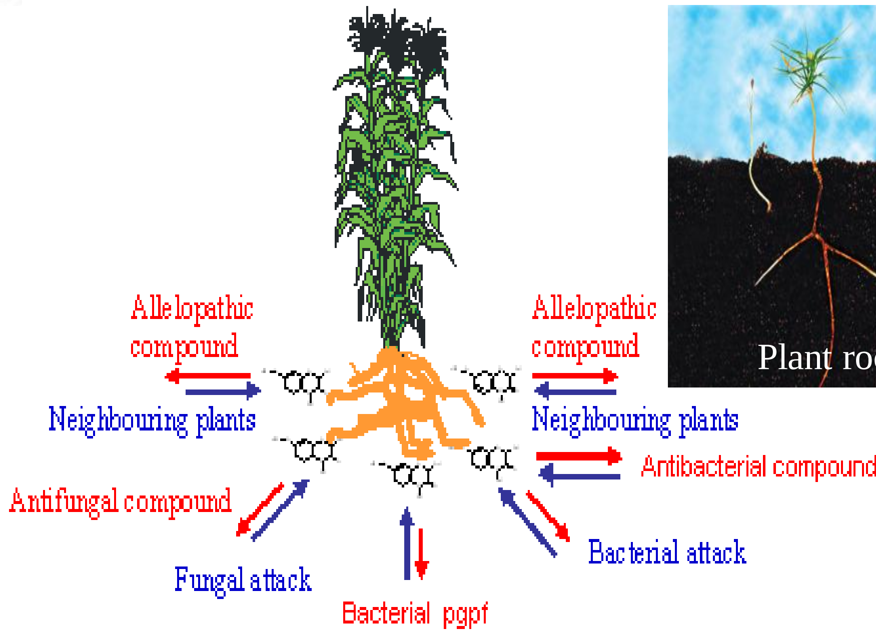
relevant to consider:

rhizosphere



Molecular talking
(*quorum sensing*)

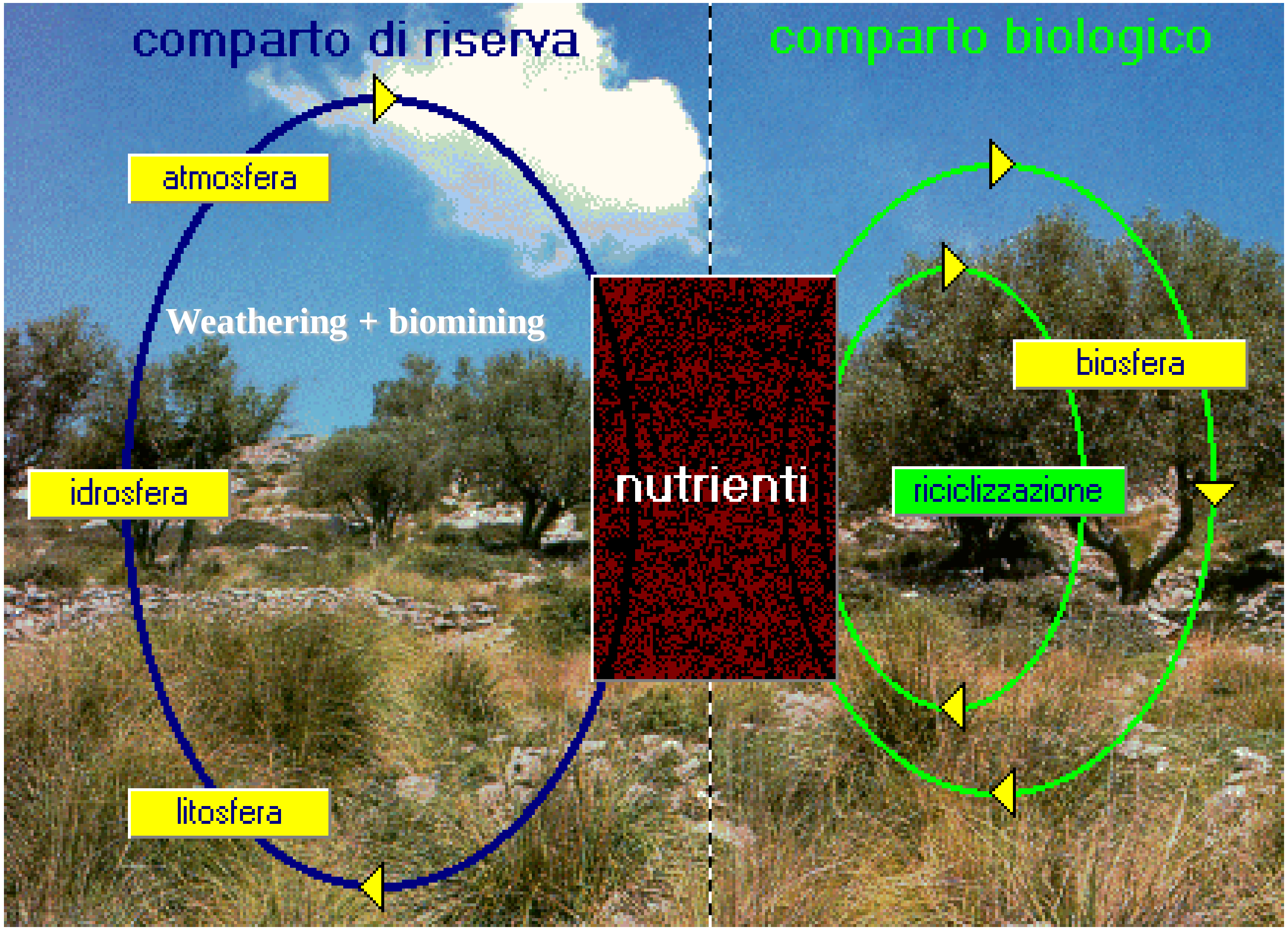




microfauna + extracellular enzymes + plants

↓

soil capacity to degrade "almost" everything…

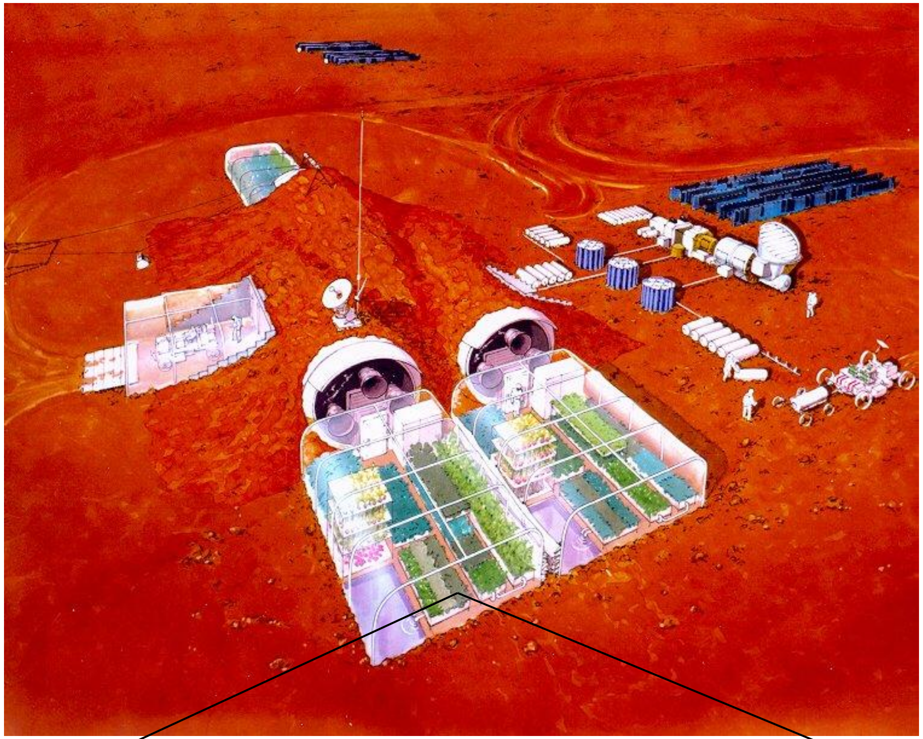


…and thus sustaining life by recycling nutrient substances

Space farming

Inducing, enhancing and maintaining fertility in extra-terrestrial soil

Need to be considered



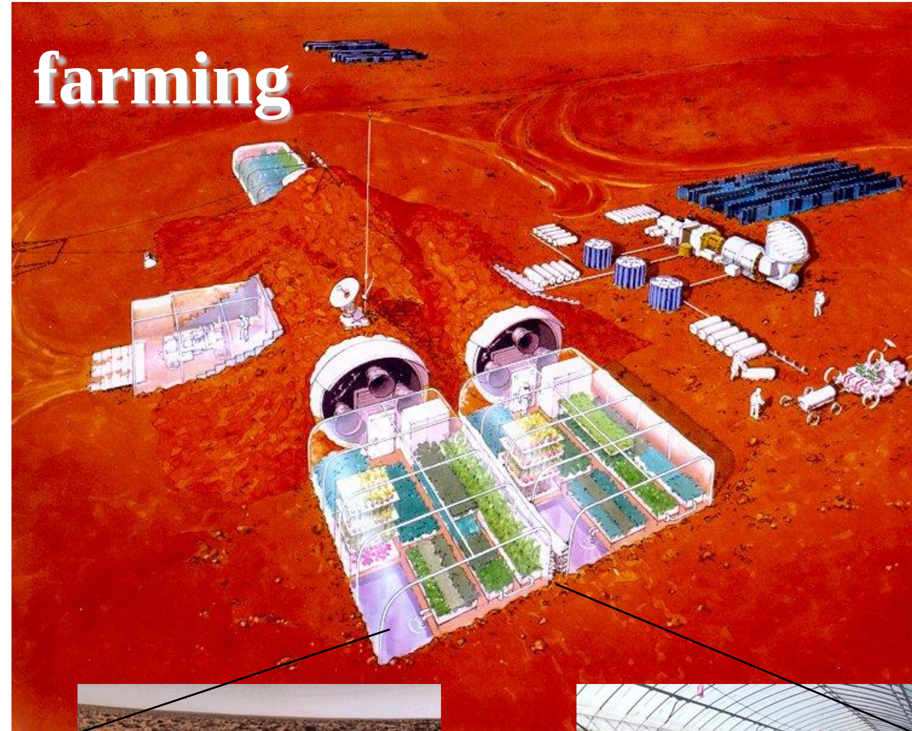
Environmental parameters:

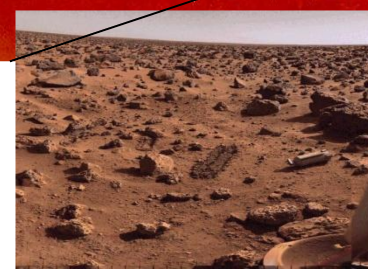
- Low gravity
- Atmosphere chemical composition
- Dangerous radiations (UV)
- Low Temperatures

Soil parameters:

- Relevance of fine particle size
- Pore size (micro/macro pores ratio)
- **Microgravity effects**
- Water content
- **Soluble salts concentration** (Na/Ca carbonates)
- micro-macro nutrient concentrations (N; K; P)
- **Toxic elements** (HM and ClO_4^-)

Space farming





1) Fertility induction:

Abiotic fertlity induction:

- **ettenuation of low gravity effects**
- dust reduction
- **pH adjustments**
- **safety (human) waste recycling**
- **soil soluble salts concentration**
- **toxic elements bioremediation**
- soil water holding capacity
- creation of terrestrial atmosphere

Biotic fertlity induction:

- **selected microorganisms inoculum preparations**
- strategy of sequencial applications
- pioneer plants

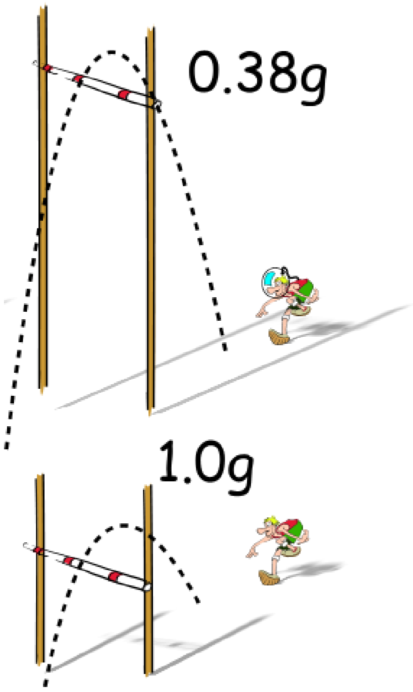
2) Fertility development:

- **monitoring** soil chemical-physical parameters
- **monitoring** soil microbial community
- **plants sequence (pioneer to cropping plants)**
- **targeted actions**

3) Fertility stabilization and maintainance:

- irrigation requests
- SOM mineralization
- DOC
- microbial community richness and activity
- root nutrient availabilty
- fertilization requests
- presence of soil born plant patgogens

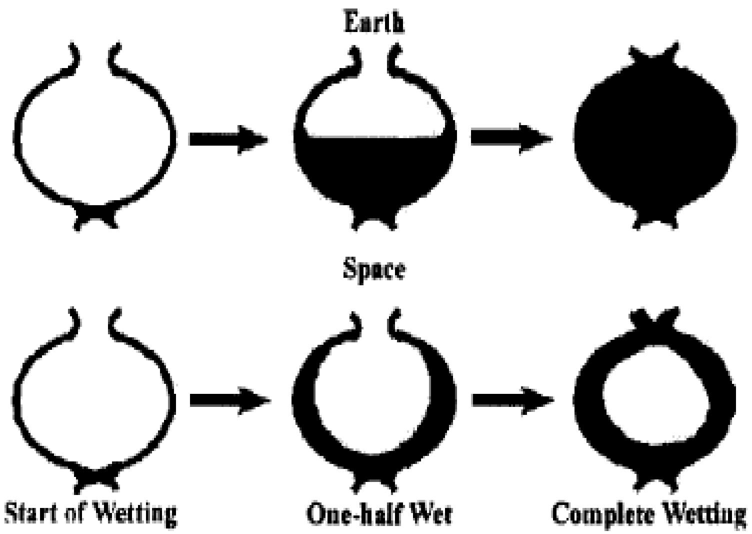
Effects of low gravity condition on terrestrial soil functionality



- **Lower water circulation by gravity**
- Higher water persistence in soil
- **Soil solution with high salts concentration**
- **Soil pH values**
- **Low gases diffusion in soil (O_2 and CO_2)** (could lead to suffocation of microorganisms and roots)
- **Boundary layer temperature**
- Emissions of toxic gases
- **Higher loss of N by denitrification** (N_2, NO, N_2O)
- **Lower N loss of nutrients by percolation** (NO_3^-)
- **No change in root zone residence time of nutrients respect to Earth gravity condition (1g)**

(Maggi and Pallud 2010. Planetary and Space Science 58: 1996-2007)

• soil water circulation

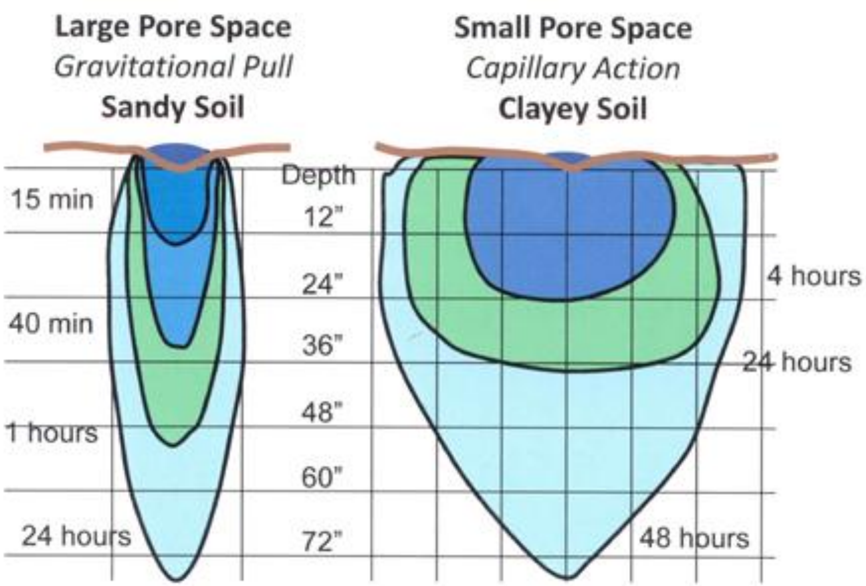


• **Micropore air entrapped cause low water circulation**

• **Less soil water holding capacity**

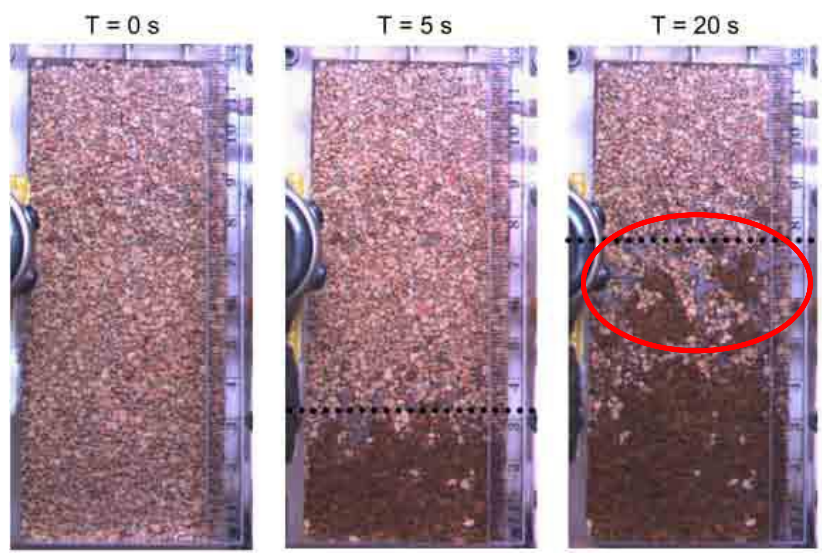
The trapped air would significantly reduce saturated hydraulic conductivity, but may also cause:

• Soil anoxic condition
• Bacterial competiton for O2 with plants
• High solutes concentration
• N losses by denitrification

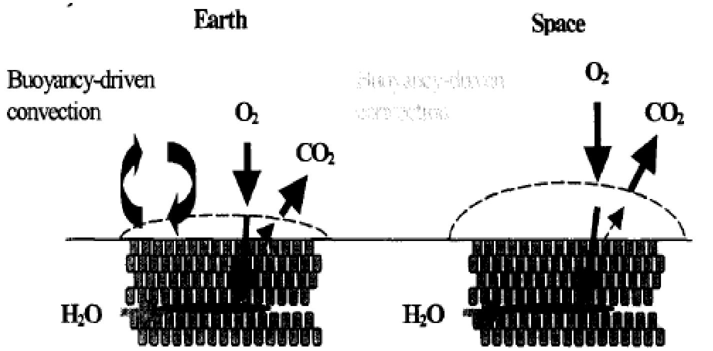


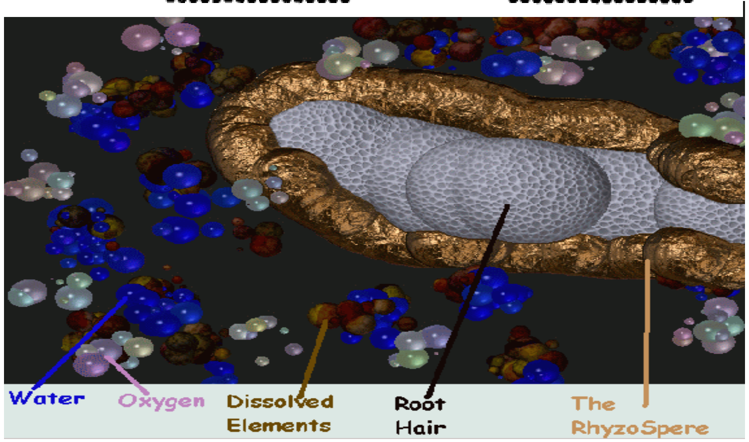
(Heinse et al. Vadose Zone Journal 2007)

Jones and Or (1999) Water Resources Research 35



Water imbibition into dry soil (1–2 mm) showing **fingering** wetting front propagation at 0g





- O_2 at boundary layers

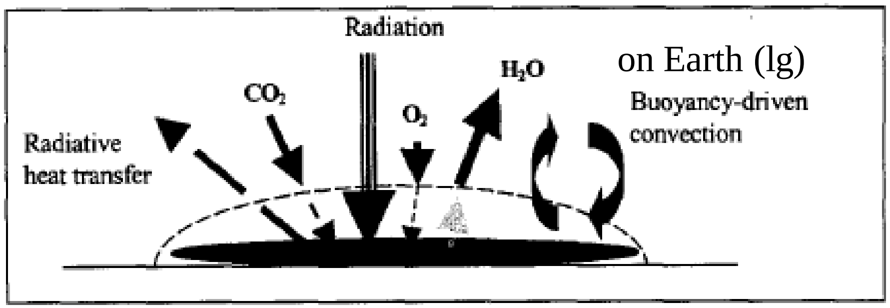
At 1 g, the boundary layers are thin enough so that metabolic processes like respiration and transpiration are rarely diffusion-limited.

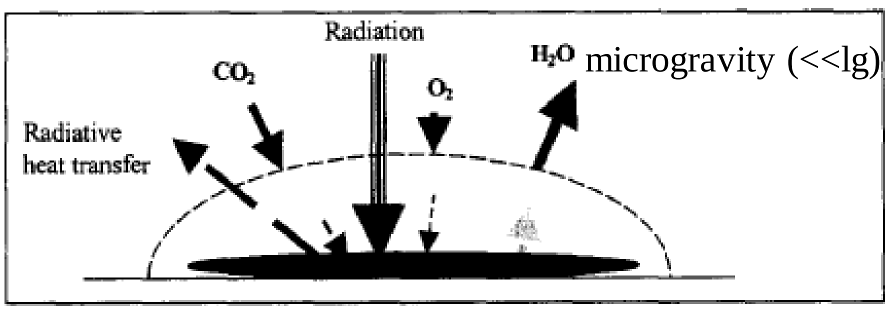
Inhibiting gravity-mediated oxygen transport may lead to biophysical limitations in O_2 availability,

- boundary layer temperature

High leaf temperatures under low gravity due to reduction of forced convection.

This result suggests that forced convection must be used to ameliorate the lack of convective mixing in microgravity.





Monje.et al. 2003. *Adv. Space Res.* 31 (1): 151-167

Dust reduction

Biological soil crusts formation

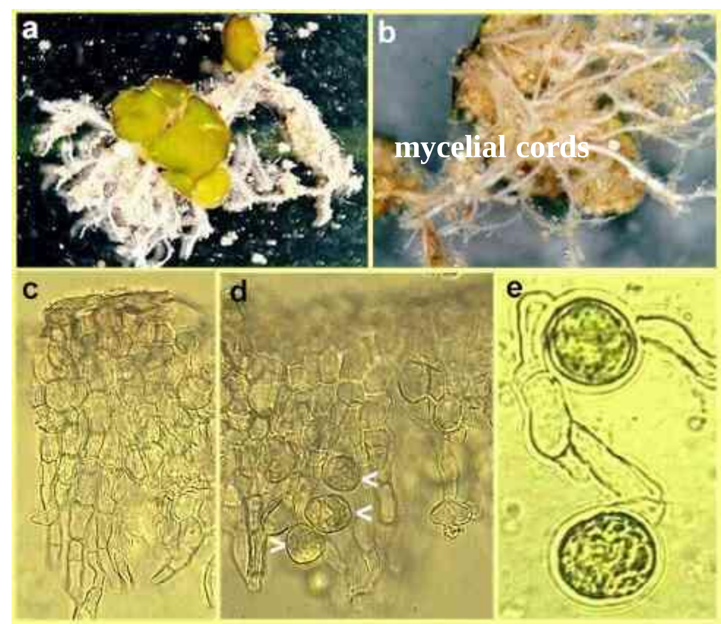
Biological soil crusts is developed by a specialized community of cyanobacteria, algae, microfungi, lichens, and bryophytes that typically cover the open spaces in arid and semiarid regions.

Biological crusts provide key ecosystem services

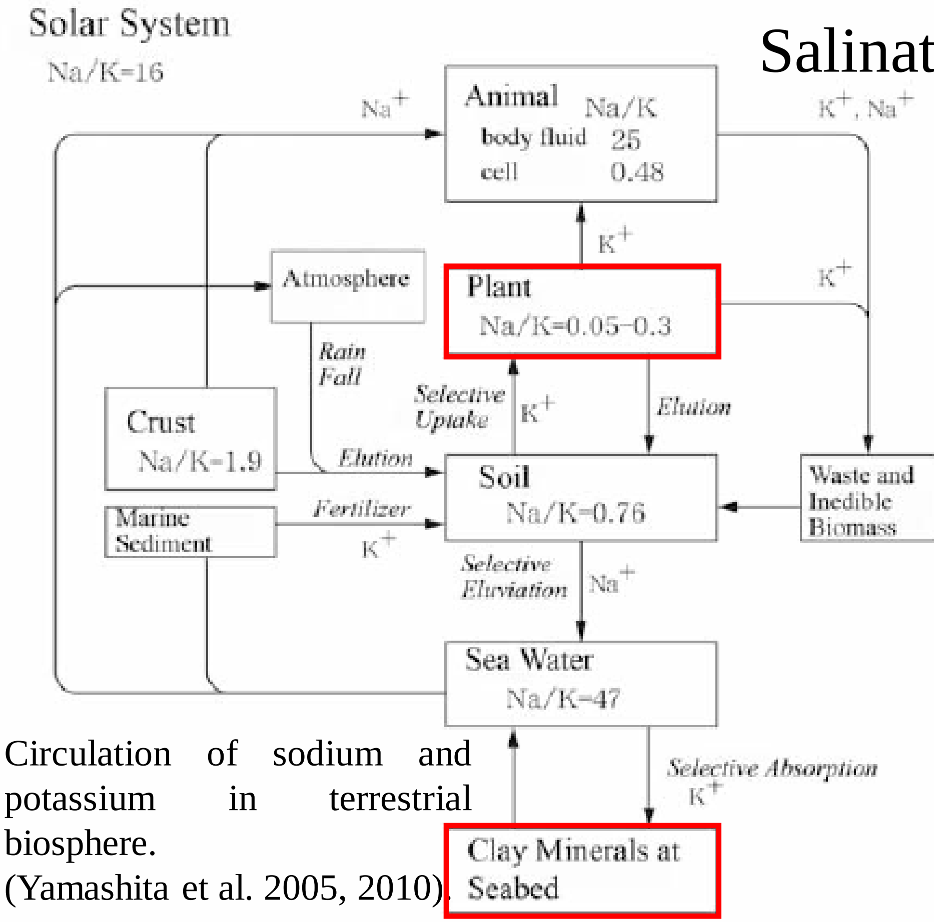
- increasing erosion resistance
- increasing infiltration
- contributing organic matter
- fixing atmospheric nitrogen.

Peltula lichen separate lobes, termed squamules, that are connected to one another by a system of fungal hyphae (A and B)

green alga *Trebouxia* (arrowheads, **d**) intimately associated with fungal hyphae (**e**) which gain carbohydrates from the algal cells.



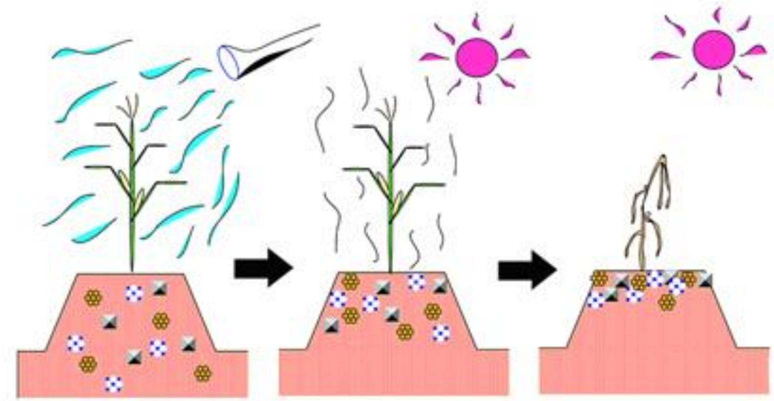
Salination a crucial problem for soil fertility

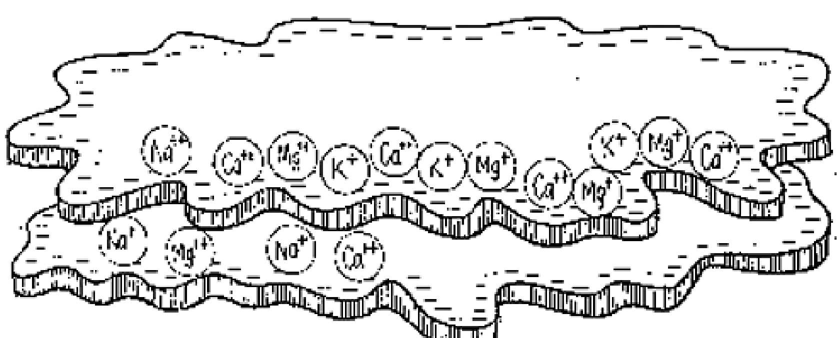


Circulation of sodium and potassium in terrestrial biosphere.
(Yamashita et al. 2005, 2010)

Extraterrestrial soil and also human waste results rich in salts.
Human feces especially urine results rich in sodium salts.

The presence of sodium salts could represent a potential risk in terms of toxicity for plants and de-structuring effect on soil structure in presence of water.





Irregated soil lost its structure

Na^{+} concentration >15% CEC

Sodium tolerant plants

Halophytes (salt-tolerant plants), which can grow in the salt-affected soil and accumulate sodium in the edible parts of the plant.

Halophytes are the ice plant (*Mesembryanthemum crystallinum*), the saltwort (*Salicornia herbacea* L.), and the New Zealand spinach (*Tetragonia tetragonoides*).

Salicornia herbacea L.

Mesembryanthemum crystallinum

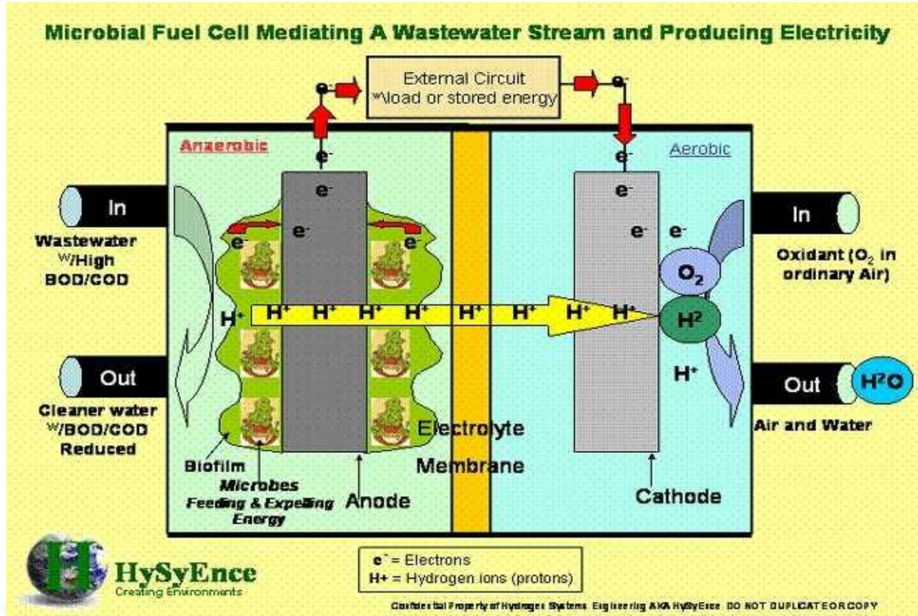
Tetragonia tetragonoides

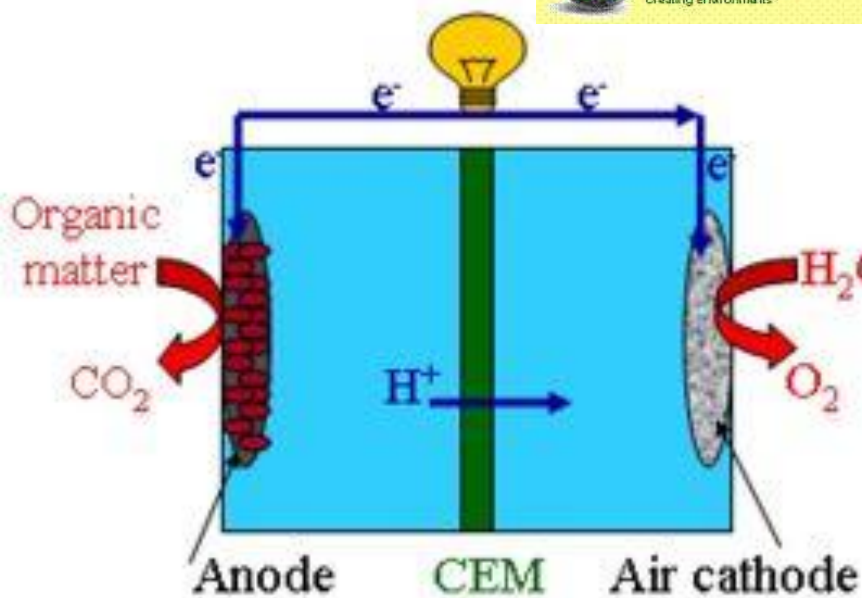
Cultivation of marine algae

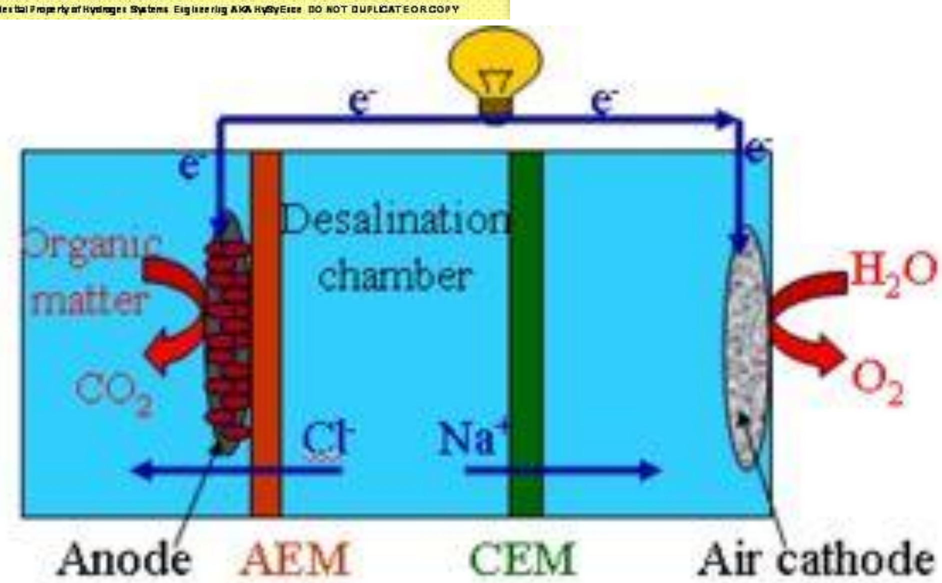
Marine algal species, *Ulva lactuca* is selected to harvest potassium from processed compost, and recycle sodium in space agriculture.

(Yamashita et al. 2009. Adv. Space res. 43: 1220-1223).

Water desalinization and depuration by microbial fuel cell (MFC)

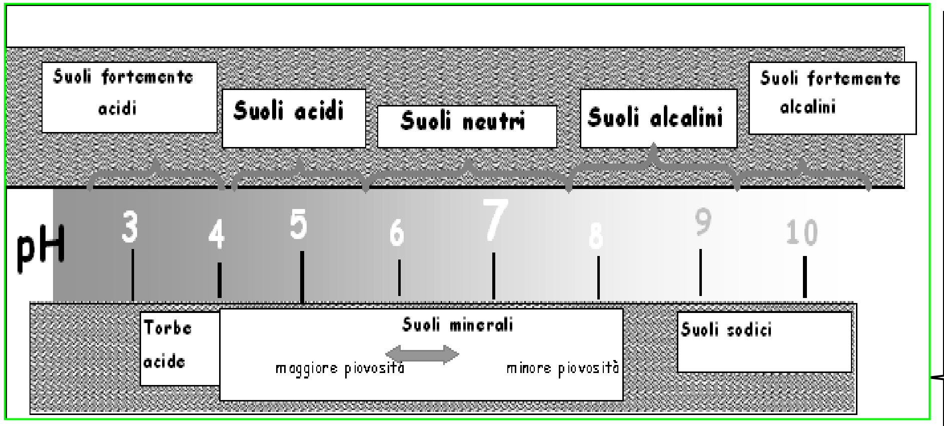






(Cao et al. 2009. Environ. Sci. Technol. 43:7148-7152; Mehanna et al. 2010.Energy Environ. Sci. 3(: 1114-1120)

Soil pH correction

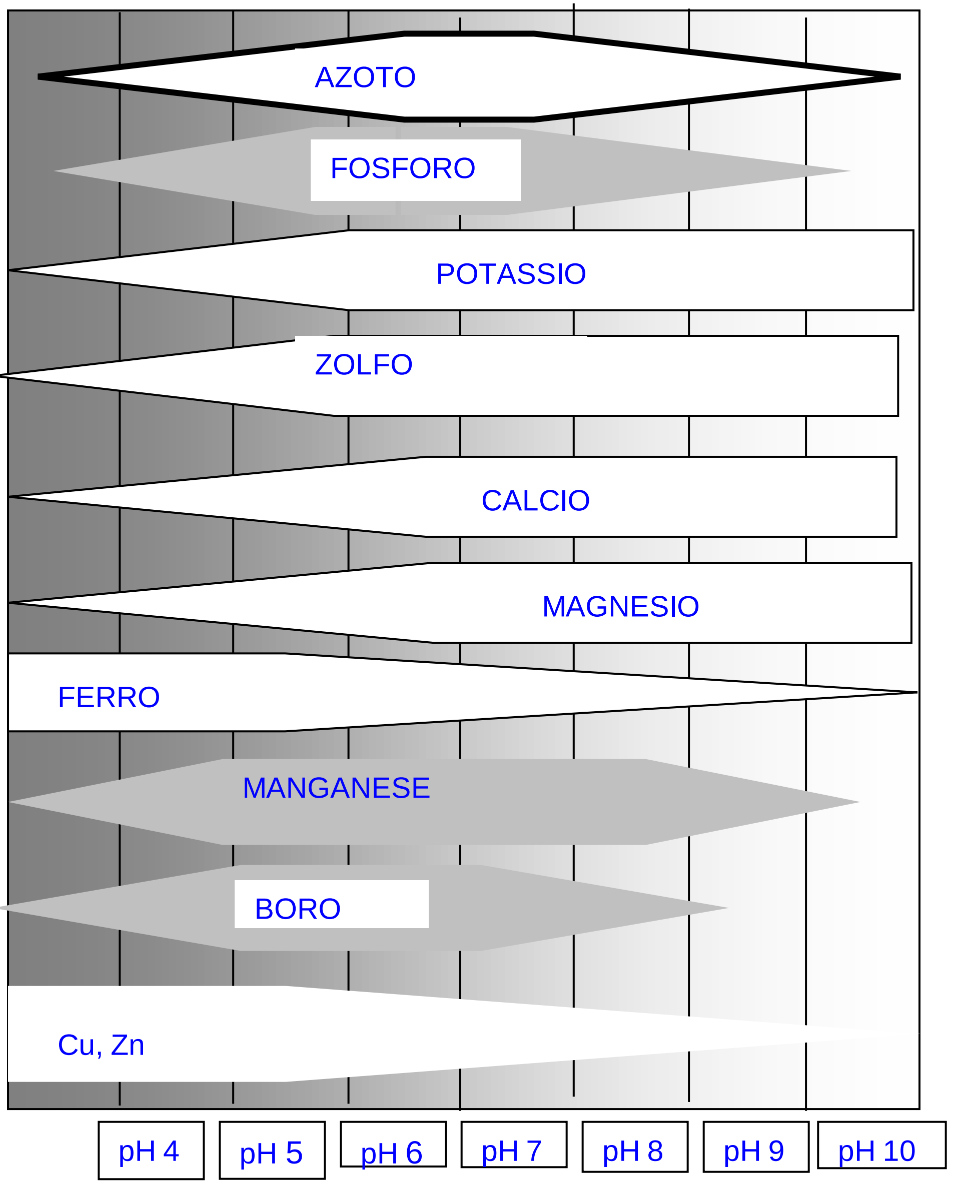


Alkaline soil correction

- Peat moss - application ratio to soil 1:4
- Green sand – application ratio to soil 1:50
- Humates – application ratio to soil 1:50
- Pumice – application ratio to soil 2.50

…**also improving soil structure**

(Silverstone et al. 2003, 2005. Adv. Space Res.)

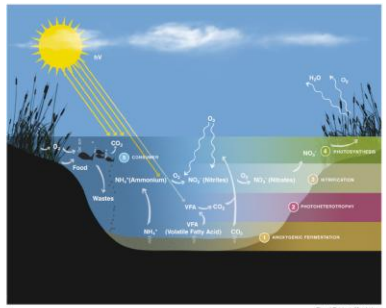


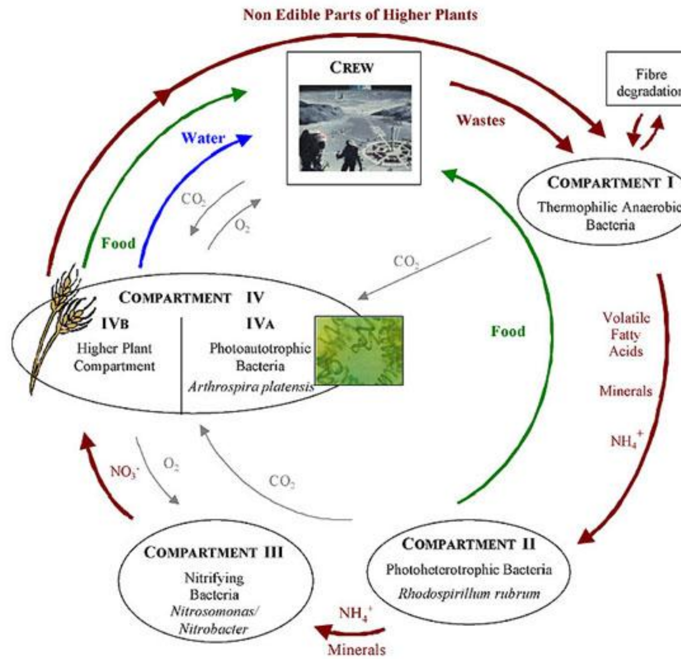
Soil nutrient elements availability at different pH values

Safety (human) waste recycling

MELiSSA (Micro-Ecological Life Support System Alternative)

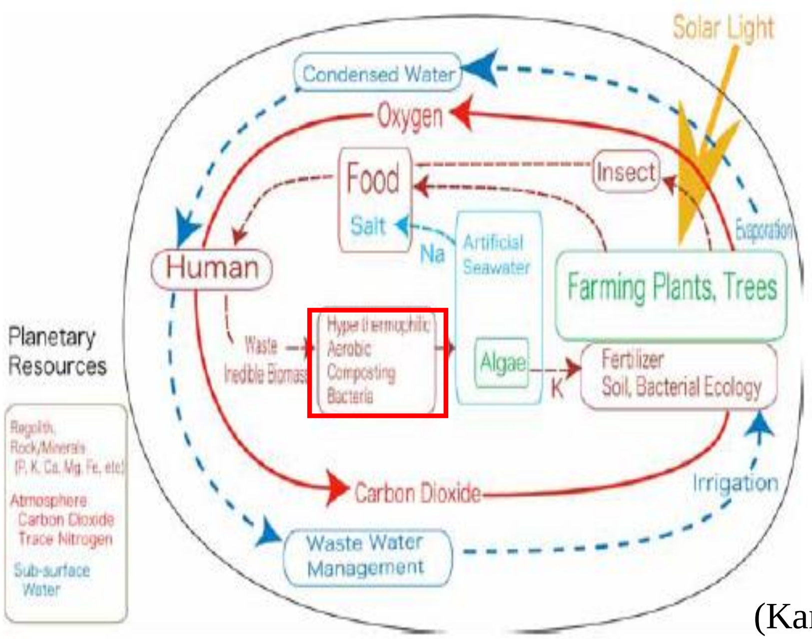
The driving element of MELISSA is the recovery of food, water and oxygen from waste (faeces, urea), carbon dioxide and minerals.





(Hendrickx et al. 2006. Research in Microbiology 157: 77–86)

Hyper-thermophilic aerobic composting bacterial



Concept of space agriculture for habitation on Mars. On site resources are employed to make the system for more than 100% recycle possible.

(Kanazawa e al. 2008. Advances in Space Research 4: 696–700)

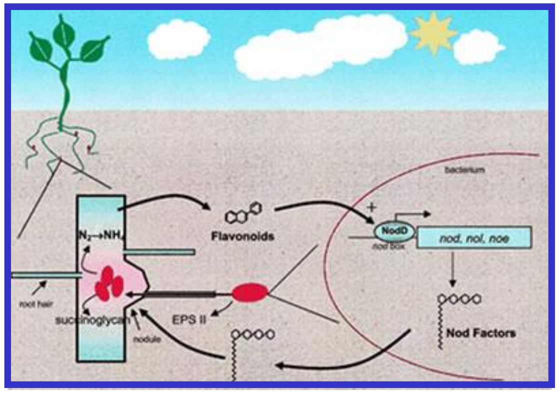
Inoculum: strategy for the selection of organisms

Characteristics of organisms

Transportability - implies characteristics such as longevity of cells during periods of inactivity, ability to be easily desiccated (e.g. freeze-dried) and stored.
Robusteness – capacity to survive against the shutdown or failure of equipment an under the expected operating environment (resistance to ionizing radiation and UV radiation)

Selection strategy

- **Richness**
- **Evennes**
- **Redundancy**
- **Key stone species**
- **Rare species**
- **Plant-endophytic mic. symbiosis**



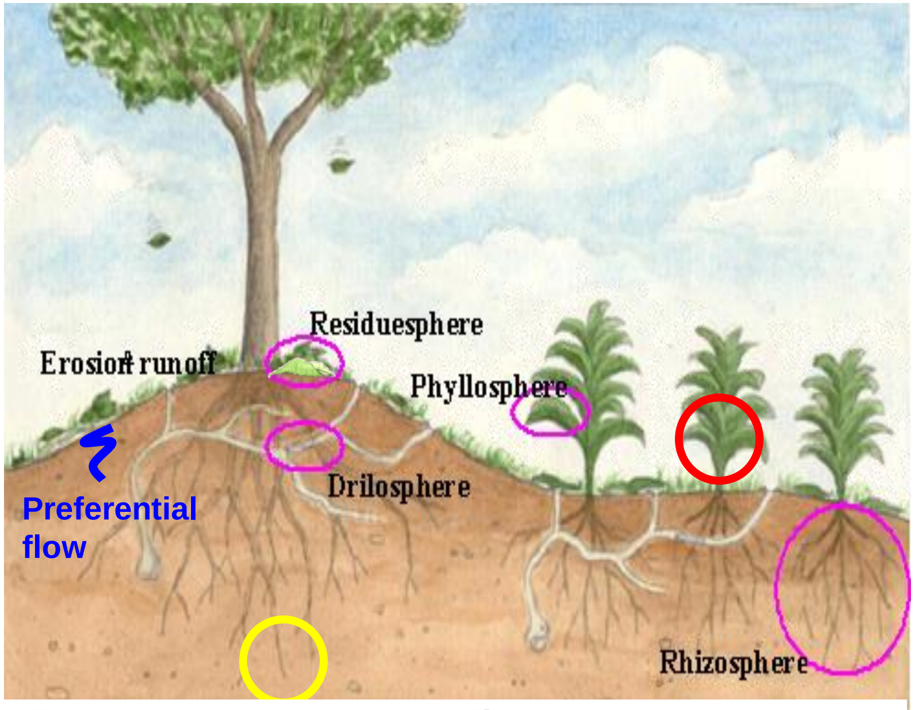
To guarantee the efficiency of the soil functionality in the case of environmental stress

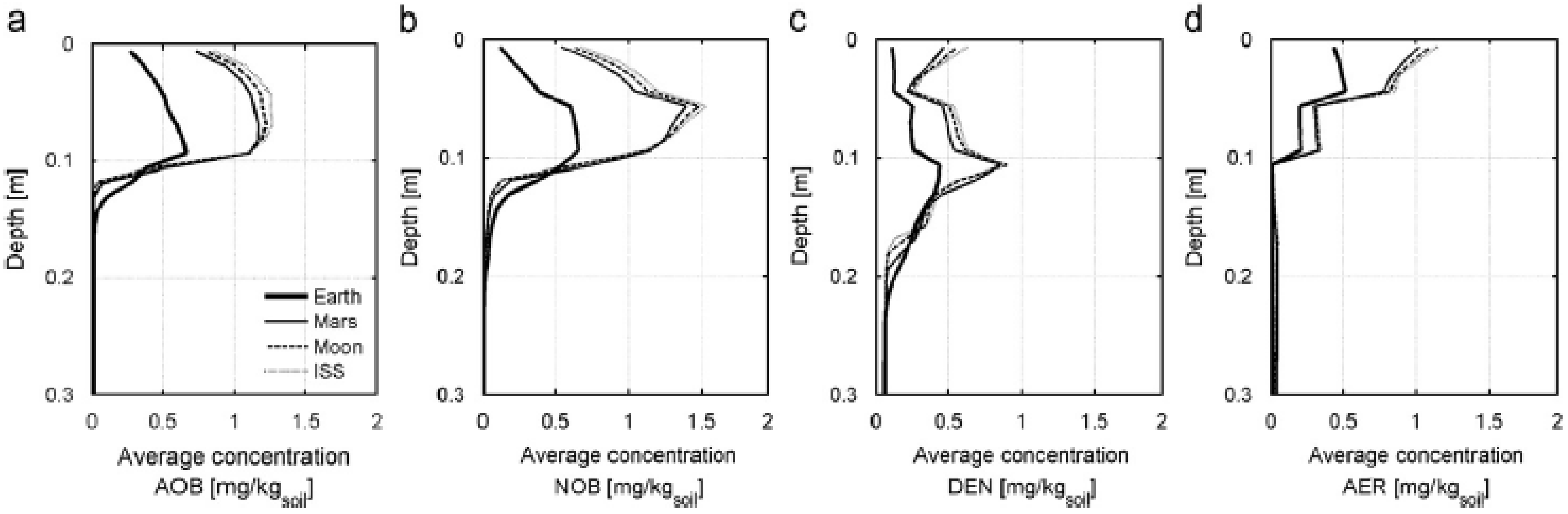
Relevance of the studies on:

biogeography, geomicrobiology and space adaptability of organisms

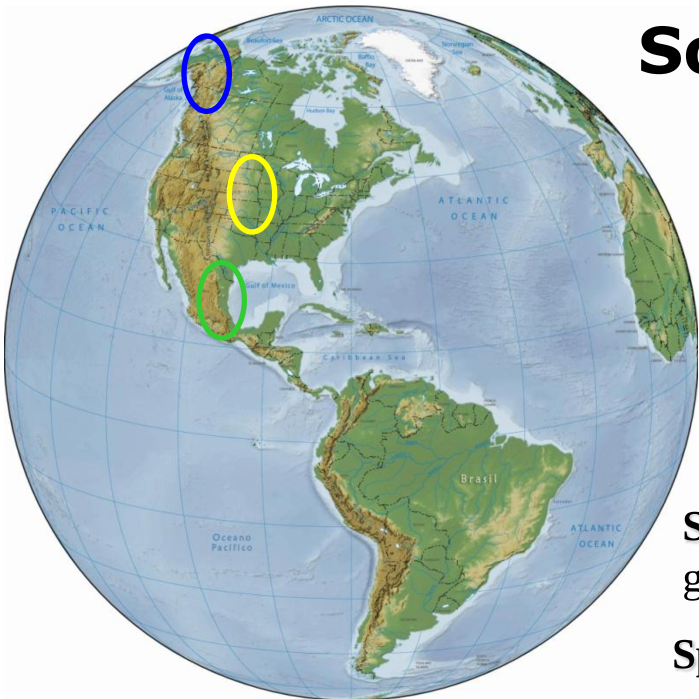
Practical aspect to consider preparing soil inoculum

- **Microrganism distribution in soil**
  - **Bulk soil**
  - **Hot spots** - residuesphere, drillosphere, rhizosphere, preferential pathflow
  - **depth**





(Maggi and Pallud 2010. Planetary and Space Science 58: 1996-2007)



Soil metagenomics: biogeography

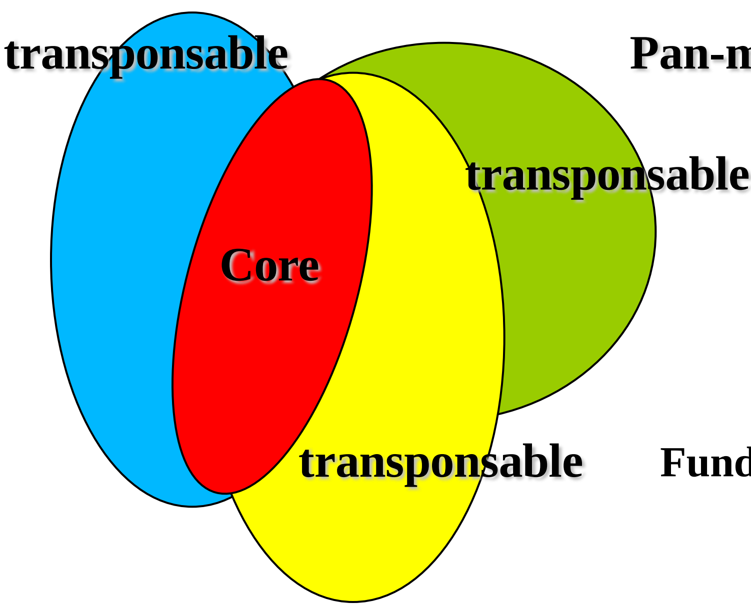
To characterize soil microbial community and to detect the activated functions and the involved microorganisms

Core-metagenome : genes existing in all soils

Core-metapopulation : species found in all soils

Specific-metagenome (transponsable genome)
genes present in one or more soils and genes unique to single soils

Specific-metapopulation
species present in one or more soils and species unique to single soils



Pan-metagenome or -metapopulation : core + transponsable

«everything is everywhere*, but, *the environment selects»

Beijerinck M.W. (1913) De infusies en de ontdekking der backterien, in: Jaarboek van de Knoniklijke Akademie van Wetenschappen, Muller, Amsterdam.

Fundamental questions: Pan/Core the actual size of specific

(Fierer and Jackson.2006.PNAS 103: 626-631)

Example of soil extreme microbial communities investigated in extraterrestrial simulation environments

Sample	MSE[a]	LEO[b]/ simulation	Reference
Environmental			
Soils	x		Green et al. (1971), Fulton (1958), Hansen et al. (2005)
Colonised sandstone, Antarctica	x	x	Onofri et al. (2008)
Permafrost; Arctic, Siberia and Antarctica	x	x	Novotoskaya-Vlassova et al. (2002), Morozova et al. (2007)
Halite rock, Atacama Desert	x	x	Wierzchos et al. (2006), de la Torre et al. (unpublished)
Coastal limestone cliff, Beer, UK	x	x	Olsson-Francis (unpublished)
Lichens			
Rhizocarpon geographicum	x	x	de la Torre Noetzel et al. (2007)
Xanthoria elegans	x	x	Sancho et al. (2007), de Vera et al. (2004)
Aspicilia fruticulosa	x	x	de la Torre et al. (unpublished)
Fulgensia bracteata	x	x	de la Torre (unpublished), de Vera et al. (2004)
Xanthoria parietina	x	x	de la Torre (unpublished), de Vera et al. (2004)

[a] Mars Simulated Environment.
[b] Low Earth Orbit.

(Olsson-Francis and Cockell 2010. J. Microbiol. Methods 80: 1-13)

Geomicrobiology

It investigates on the interactions of microorganisms with geological substrates evidencing enormous potential in the exploration and settlement of space.

- Microorganisms can be used to extract useful elements from extraterrestrial materials for industrial processes or for use as nutrients in life support systems, and energy production by MFC.

- Microorganisms able to degrade and remediate soil from pollutants by biomining and bioleaching

- Microorganisms could be used to create soil from lunar and Martian rocks.

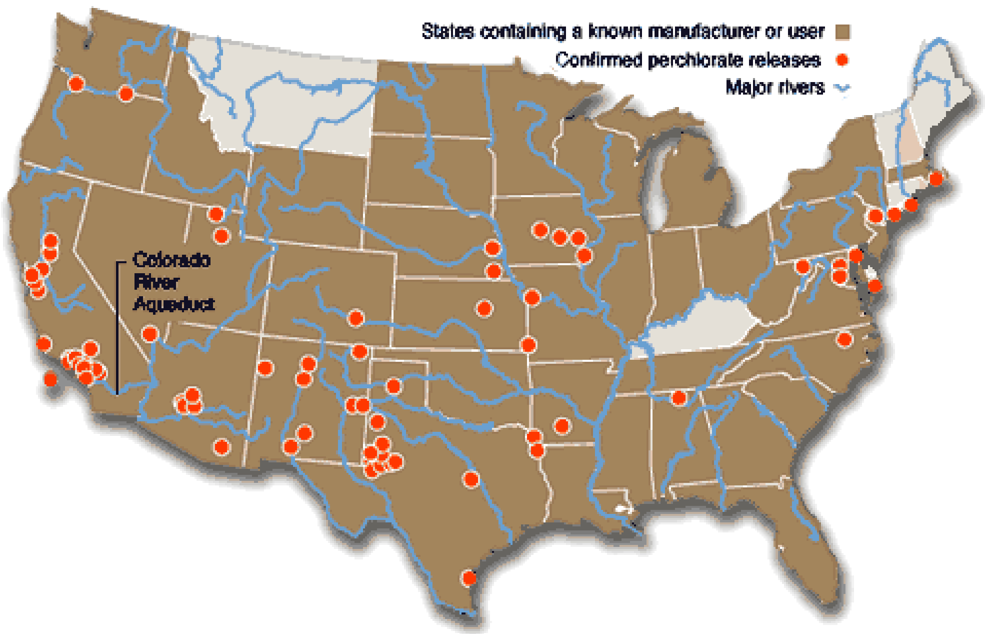
- Understanding the interactions of microorganisms with rocks is essential for identifying mineral biomarkers to be used in the search for life on other planetary bodies.

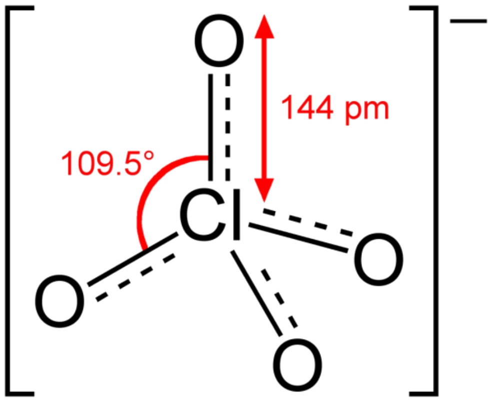
Organism	Mineral substrate	Reference
Halophilic bacteria	salts	(Mancinelli et al. 1998. Adv. Space Res. 22; 327-334)
Bacillus	interior many rock types	(Horneck G. 1993. Orig. Life Ecol. Biosph. 23: 37-52)
Fungi	surface and interior of a variety of rocks	(Onofri et al. 2008. Stud. Mycol. 61: 99-109)
Lichens	surface of a variety of rocks	(Sancho et al. 2007. Astrobiology 7: 443-454)
Cyanobacteria	carbonate rocks	(Olosson-Francis et al. 2009. Orig. Life Evol.Biosph. 39: 565-579)

(Cockell 2010. Trends in Microbiology 18: 308-314)

Water and soil perchlorate and chlorate contamination

Perchlorates are the salts derived from perchloric acid ($HClO_4$)





Under Martian environmenatl condition perchlorate:
- do not oxidise organics
- exert a strongly disseccating activity (highly hygroscopic salt)

Geomicrobiology relevance

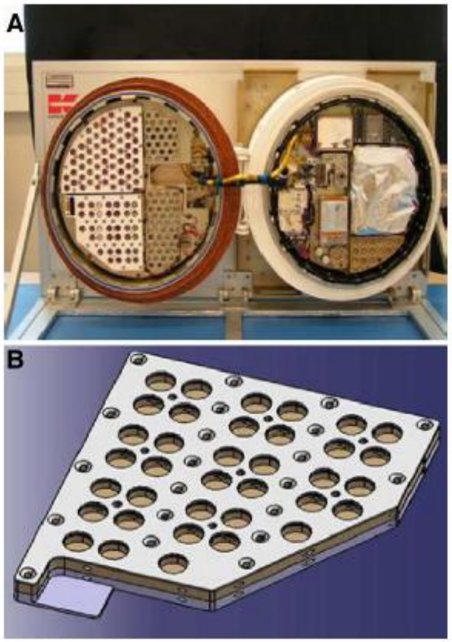
studies on extreme soil like Atacama and polar deserts detected:

- **presence of perchlorate**
- **perchlorate anaerobic degrading bacteria**

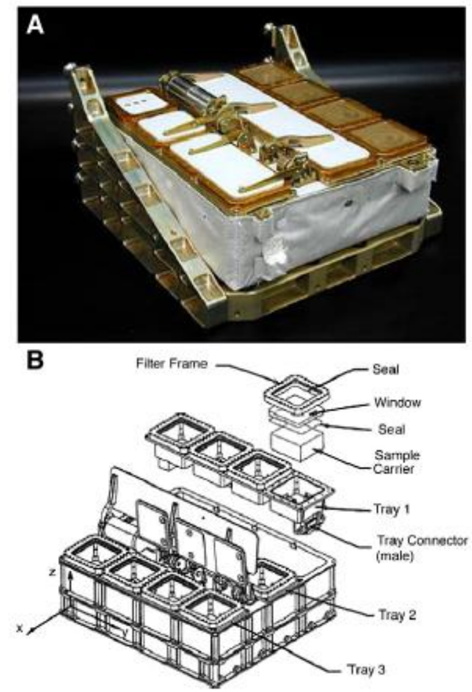
(Hecht et al. 2009 Science 325; Thrash et al. 2010 Appl. Environ. Microbiol.76; Wu et al. 2001 Bioremediation J. 5)

Resistence of organisms to space conditions

BIOPAN facility, used for short-term exposure (1994-2007)



(A) The inside lid of the BIOPAN module to expose the biological samples to LEO conditions.
Two plates; a top (level-1) and a bottom (level-2) plate where the samples were located inside the BIOPAN. (38x23 cm).
(B) The cells of the top plate were covered by optical long-pass filters.



EXPOSE facility, used for long-term exposure

(A) A photograph of EXPOSE.
(B) A schematic drawing of the EXPOSE facility (images courtesy of ESA). The experiments are accommodated in three sample trays (77×77 mm inner width and 36 mm inner depth).

Experiments have involved both culture-dependent and independent methods

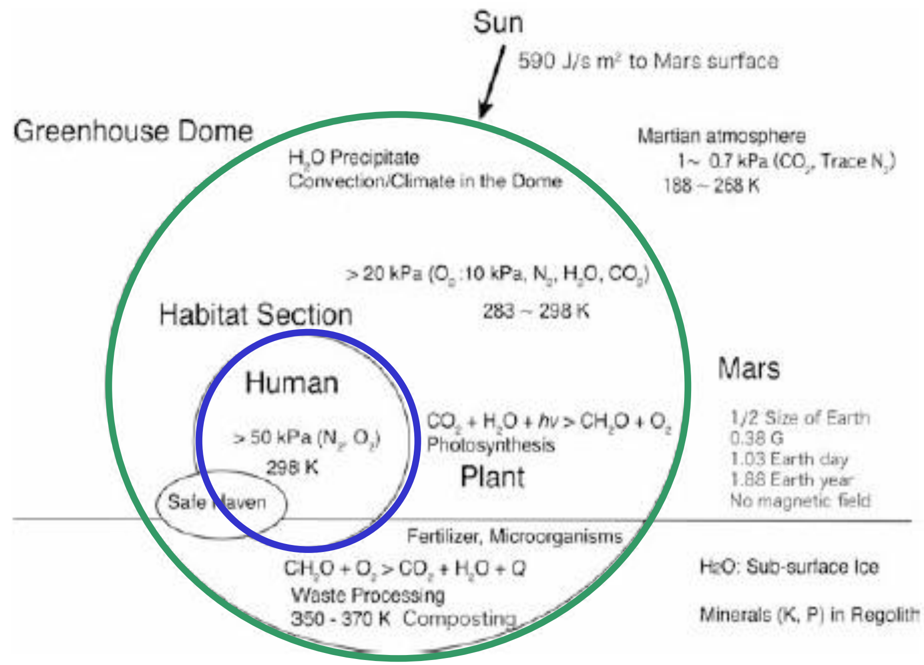
(Olsson-Francis and Cockell.2010. J. Microbiol. Meth. 80: 1-13)

Resistence of organisms to space conditions
extraterrestrial base building environmental parameters

Greenhouse section
are determined by the physiological requirements of plants:
lower limit at 10 kPa of oxygen partial pressure for plant cultivation (Goto et al. 2003;. Hinokuchi et al. 2005; Levine et al. 2008)

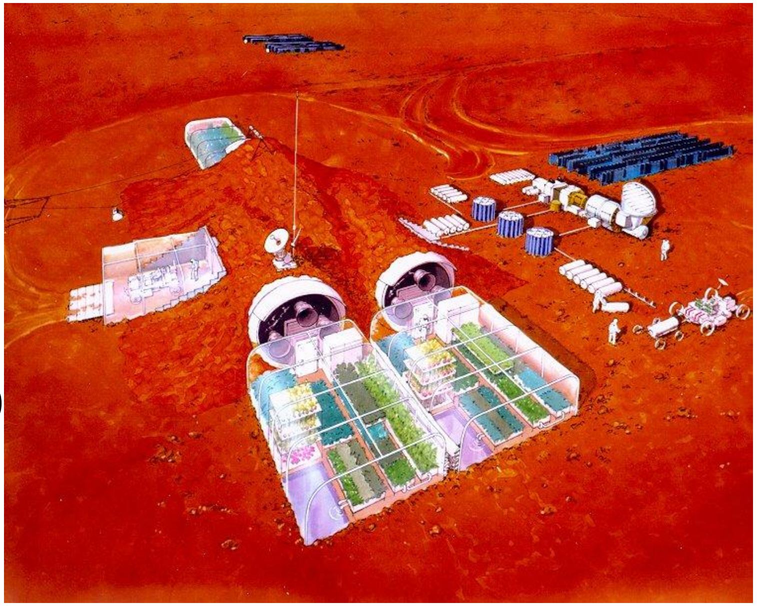
Higher plants tolerate hypobaria at least down to 0.1 bars with physiological effects:
- reduced plant growth
- increased respiration
- induces stomata closure
- induces multiple genes expression mostly related to drought and/or anoxia stress

Fire safety required 20 kPa of total pressure, balanced by inert nitrogen, together with carbon dioxide and water vapor at minor level (Yamashita et al. 2007).



human living section
are designed to meet the physiological and medical requirements for humans.
The highest altitude of ordinary living on Earth is around 4,000 m, where atmospheric pressure indicates 60 kPa.

Fertility development, stabilization and maintenance



Space farming soil

Isolated system – characterized by limited species pool

Relevant to consider

Population evolution defined by its **mutation rate**
(**Mutation rate** = adaptive mutation and gene transfer mutation)

Closed system in extreme conditions

attended relevant mutation rate mainly due to gene transfer

High mutation rate → high rate of speciation → new species

New species affermation depends on its **soil competence**

Successional dynamic

Initial prevalence of **opportunistics** and successive dominance of **specializers**

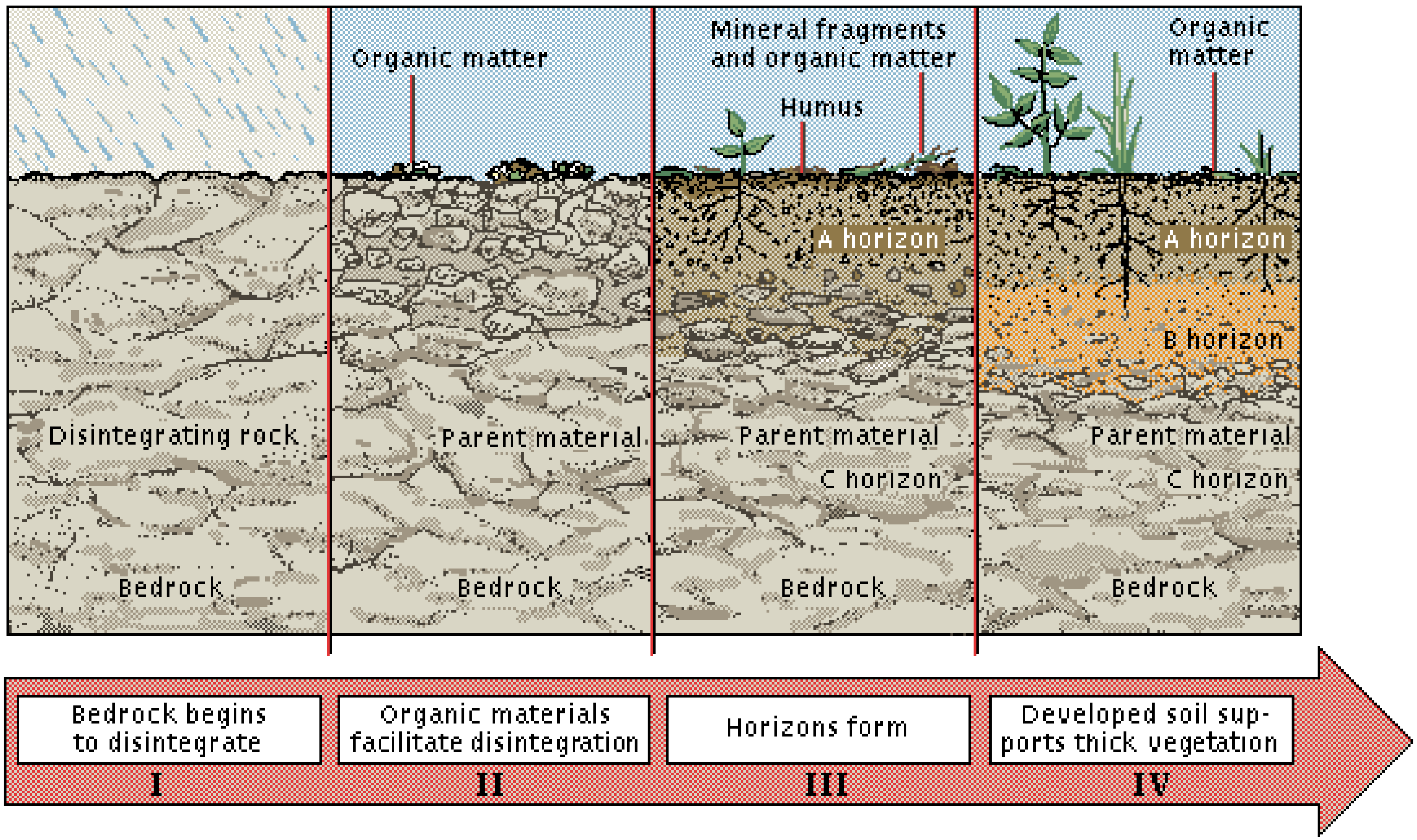
Biosystem stability

Relevance of **richness**, **redundancy** and **resistence-resilience**

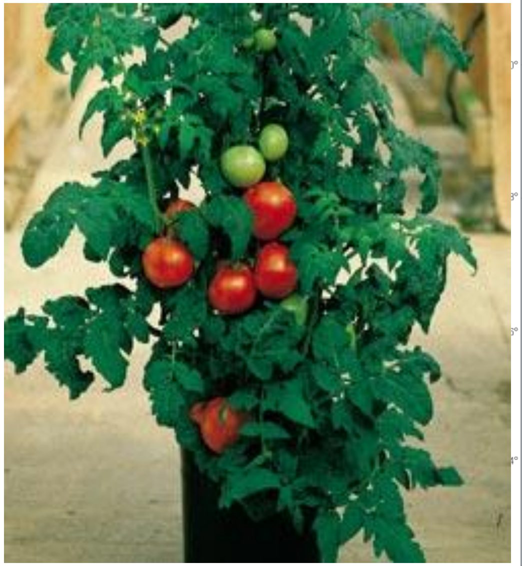
Important to avoid contamination of the biosystem and surrounding environment

Relevant to consider also the soil evolution

Relevant to consider also the soil evolution



Fertility development , stabilization and maintainance



TOUGHREACT-N model applications

(Maggi and Pallud 2010. Planetary and Space Science 58: 1996-2007)

Main results

- **Reducing gravity force decrease the soil leaching**
- **Increase the soil solute concentrations (nutrients)**
- **Increase the microbial biomass (60-100%) and activity**
- **Increase the concentration of NO3$^-$ and N gasses**
- **NO_3^- roots residence time still remains similar to earth** due to its high conversion rate to N gasses and lost whereas on earth the main NO_3^- depletion causea its leaching

Agronomical effects

- Low request of irrigation - 40-70% less than on earth
- Lower N fertilization request – 30-50% less than on earth
- Lower lost of N due to less N gasses emission
- Improve modern techniques of precision fertilization/ irrigation

Future needs

- Study the biogeochemical cycles of other macronutrients
- Study the microbial adaptation to low gravity

Maggi and Pallud (2010) Planetary and Space Science 58: 1996–2007

PCR-DGGE and BIOLOG EcoPlates to determine the survivability of soil communities in Mars simulation conditions

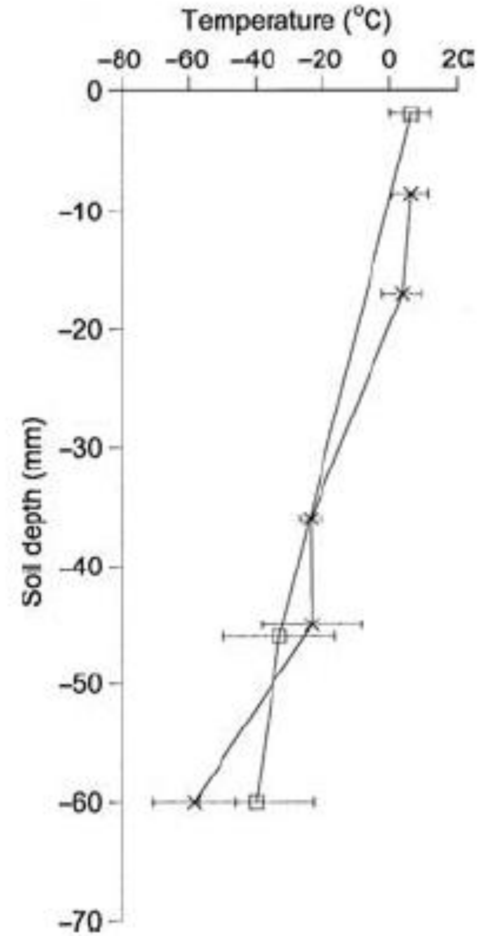
Mars simulant soil

Salten Skov, Denmark, was used as a Mars-analogue because of its high content of the iron oxides haematite, maghaemite and goethite (Nørnberg et al. 2004)

Physical conditions in the Mars simulation chamber and on the surface of present-day Mars

Parameter	Simulated conditions	Martian conditions[a]
Temperature (°C)	−95.4 to +12[b]	−123 to +25
Mean pressure (mbar)	9 and 13[c]	5.6
UV radiation (nm)	>200	>200
UV intensity at 239 nm ($W\ m^{-2}\ nm^{-1}$)	0.207	0.006[d]
Gas composition (%)	CO_2: 77.5; N_2: 8.7; O_2: 1.3[e]	CO_2: 95.3; N_2: 2.7; O_2: 0.13

[a] From Horneck (2000).
[b] See Fig. 1.
[c] Without and with UV radiation, respectively.
[d] Annual average intensity at 11.6° N (M. Patel, personal communication).
[e] Composition when flushed with CO_2 during the experimentation.



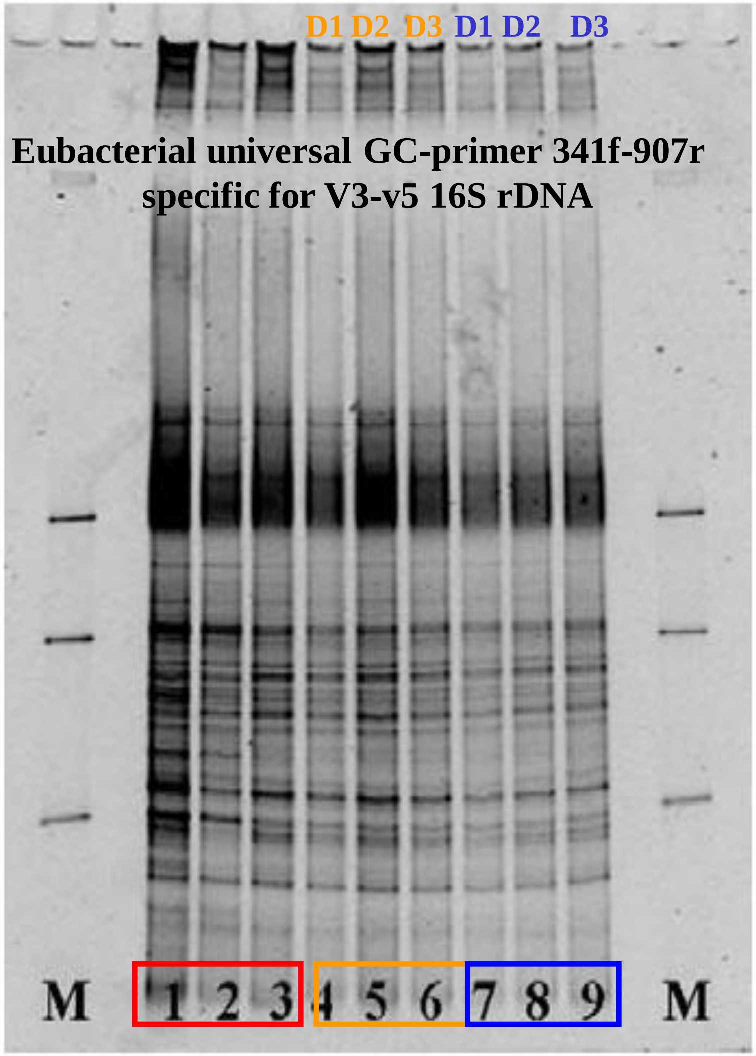
Temperature profiles in soil in the Mars simulation chamber, with (x) and without (□) UV radiation

Samples:

control soils - fresh, air-dried and freeze-dried **samples under Martian conditions** without (**Cha**) and with UV radiation (**UV**) D1(0–1cm); D2 (2–3cm); D3 (4–5 cm)

(Hansen et al. 2005. Int. J. Astobiol. 4: 135–144)

PCR-DGGE



Lanes 1–3: **control soils**
Lanes 4–6: **soil under Martian conditions no UV**
from D1 (0–1 cm), D2 (2–3 cm) and D3 (4–5 cm)
Lanes 5–7: **soil under Martian conditions with UV**
from D1(0–1 cm), D2 (2–3 cm) and D3 (4–5 cm).
M: **marker**.

Main results

PCR-DGGE

- **great similarity between all samples**
- **variations in the intensity of the bands**
- **Dissecation and UV exposure cause dominance of endospore forming bacteria and Gram positive bacteria**

BIOLOG EcoPlates

dissecation and UV exposure do not change soil microbial community fingerprinting
but dratsically reduced its:

- **functionality**
- **substrate utilization potential**

UV exposure effects were

- **confined in the first 0-3 cm of soil**
- **soil particles protect bacteria and spore from UV exposure damages**

(Hansen et al. 2005. Int. J. Astobiol. 4: 135–144)

Avoiding risks of contamination