



2022 MELISSA CONFERENCE
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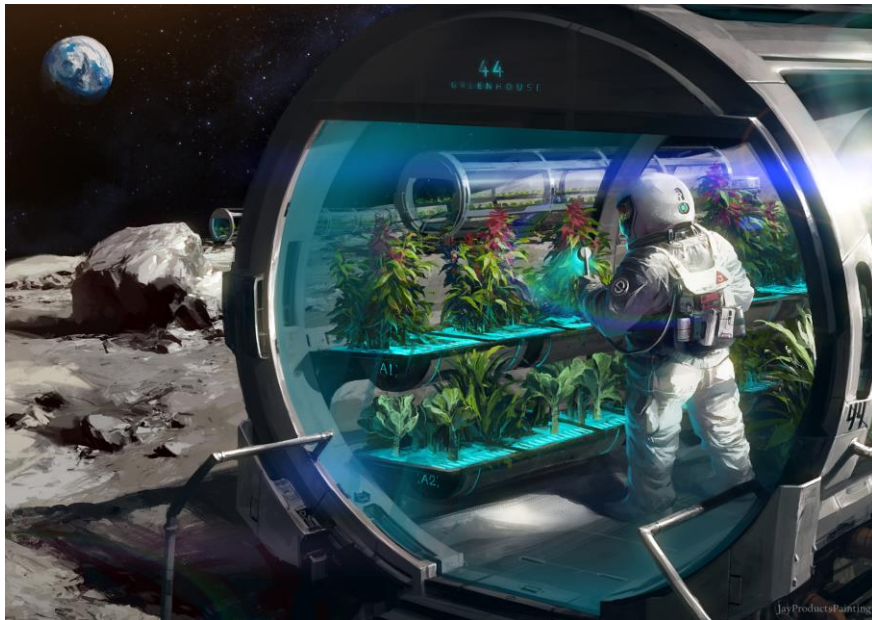
THE POTENTIAL OF LUNAR AND MARTIAN REGOLITH SIMULANTS AS PLANT GROWTH MEDIA

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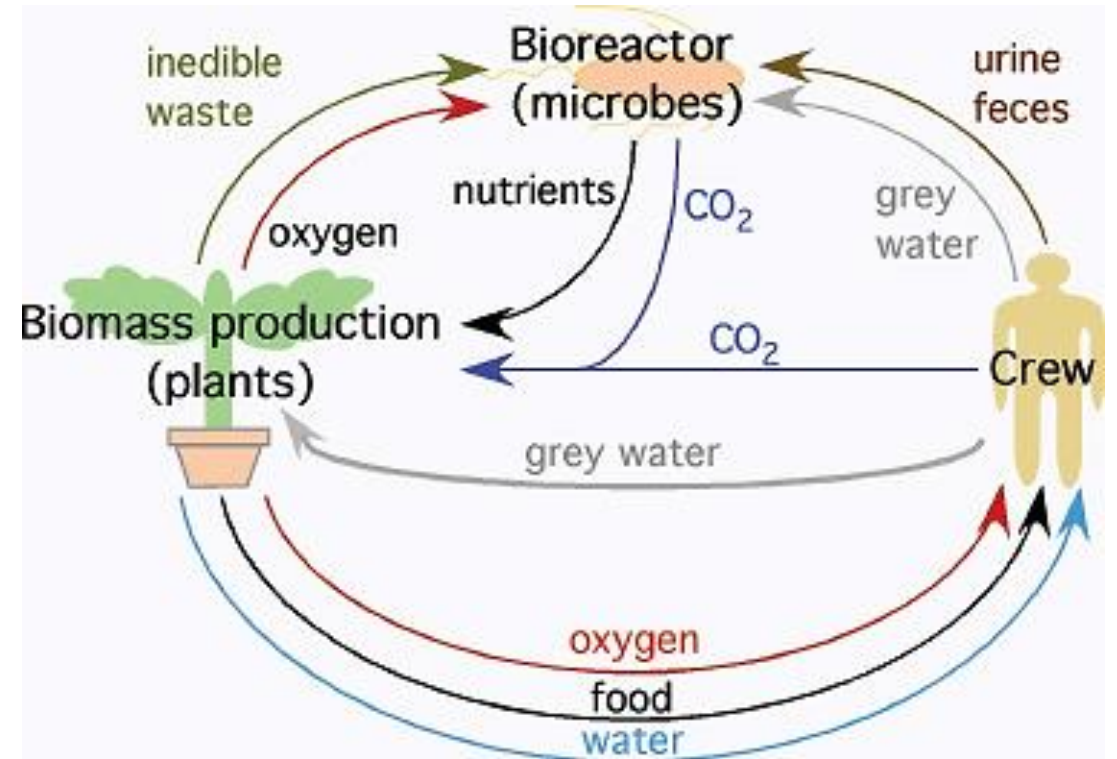


Space farming based on local resource exploitation is a promising strategy for food production on extra-terrestrial habitats (Ramírez et al., 2019)



This can also be accomplished through in situ resource utilization (ISRU), which requires the use of native materials and waste as primary resources (Karl et al., 2018)

An efficient bioregenerative life support systems (BLSS) must be capable of purifying water, revitalising the atmosphere, and producing food in a closed loop system (Llorente et al., 2018)



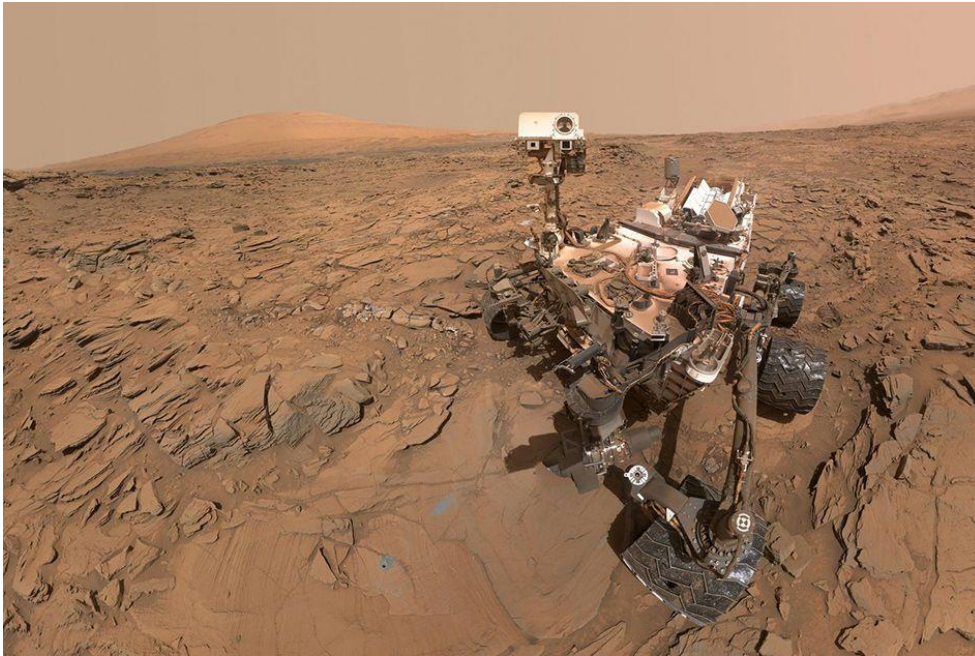


Using the local regolith as 'soil' for plant growth would be a viable way to grow food, even though 'extra-terrestrial soil' is very different from vital and fertile 'terrestrial soil'



Soil Taxonomy defines soil as *'a natural body that comprised solids (minerals and organic matter), liquid, and gases that occur on the land surface, occupies space, and is characterised by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment'* (Soil Survey Staff, 2014)

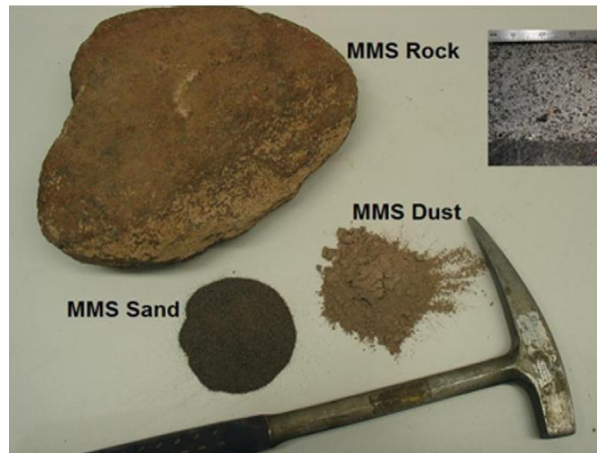




Regolith does not have associated organic matter or a microbiome. In theory, regolith can be classified as soil if it has undergone the same processes that the Earth-based parent material undergoes to become soil

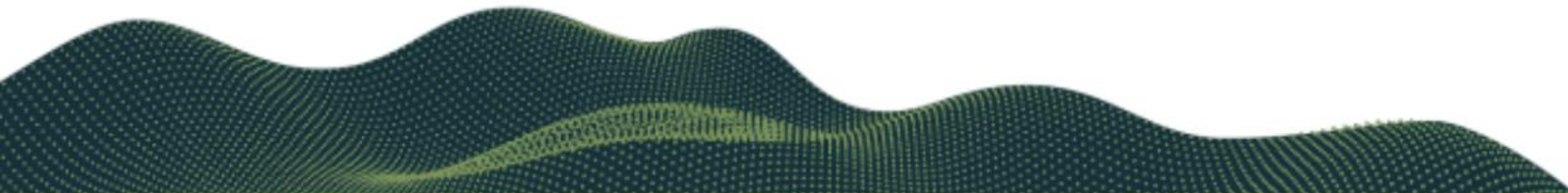
The surface deposits on planetary and other celestial bodies should be considered soils in a pedological sense (Certini et al., 2009). Lunar and Martian soils are made exclusively by inorganic minerals, are sterilised, and the Mars ones contain perchlorate salts, which can be accumulated by plants at a level considered toxic to humans

To develop a simulant, it is crucial to find terrestrial rocks (generally from desertic areas) with mineralogical and chemical compositions similar to those of Lunar and Martian regoliths. Commercial simulants contain many minerals of these regoliths, but often lack some minor phases, such as phosphates, sulfides and phyllosilicates

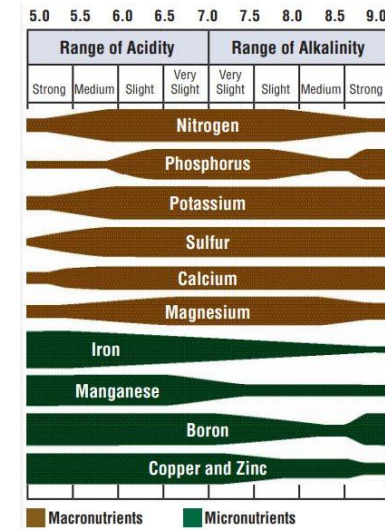
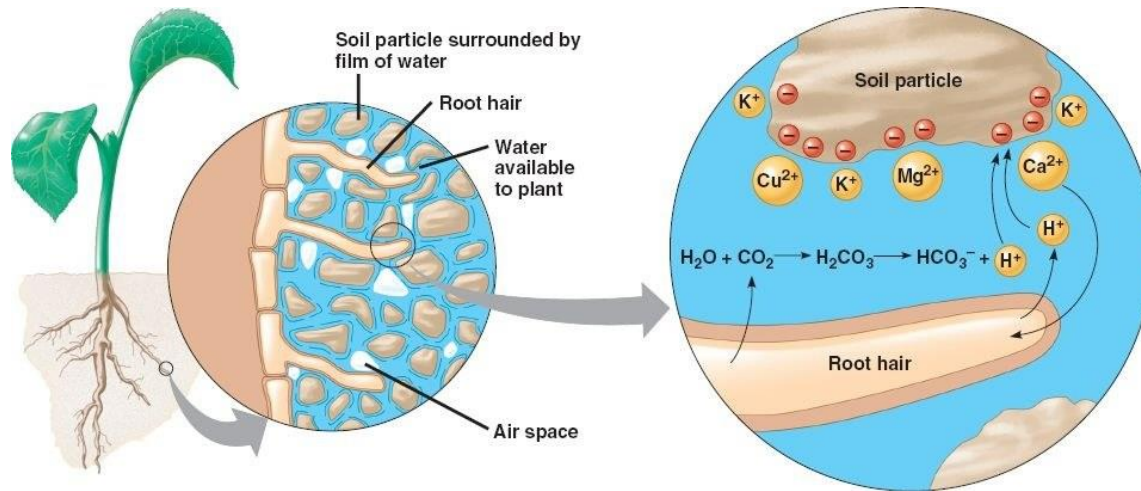


	Martian regolith								Martian simulants	
	Viking Landers		Pathfinder		MER Spirit		MER Opportunity		JSC	MMS
	VL-1	VL-2	"Soil"	SFR	"Soil"	Rocks	"Soil"	Bounce	Mars-1	
Concentration in wt%										
SiO ₂	43	43	42.0	57.7	45.8	45.6	43.8	50.8	43.48	49.4
TiO ₂	0.66	0.56	0.8	0.50	0.81	0.55	1.08	0.78	3.62	1.09
Al ₂ O ₃	7.3	(7) ^a	10.3	12.3	10.0	10.6	8.6	10.1	22.09	17.1
Cr ₂ O ₃	-	-	0.3	-	0.35	0.56	0.46	0.12	0.03	0.05
Fe ₂ O ₃	18.5	17.8	21.7	-	-	-	-	-	16.08	10.87
FeO	-	-	14.2	15.8	17.8	22.3	15.6	-	-	-
MnO	-	-	0.3	0.5	0.31	0.38	0.36	0.43	0.26	0.17
MgO	6	(6) ^a	7.3	0.8	9.3	10.7	7.1	6.4	4.22	6.08
CaO	5.9	5.7	6.1	6.7	6.10	7.50	6.67	12.5	6.05	10.45
Na ₂ O	-	-	2.8	4.2	3.3	3.0	1.6	1.3	2.34	3.28
K ₂ O	<0.15	<0.15	0.6	1.2	0.41	0.15	0.44	0.10	0.70	0.48
P ₂ O ₅	-	-	0.7	0.4	0.84	0.67	0.83	0.95	0.78	0.17
SO ₃	6.6	8.1	6.0	0.25	5.82	1.79	5.57	0.52	0.31 ^b	0.10
Cl	0.7	0.5	0.9	0.4	0.53	0.23	0.44	0.06	-	-
LOI	-	-	-	-	-	-	-	-	(17.36)	(3.39)
Total	89	89	99.8	99.2	99.4	99.6	99.3	99.7	99.7	99.4
Mean of	3	3	7	5	11	3	8	1	2 ^b	9
Method	X-ray fluorescence				Alpha Particle X-ray Spectrometer (APXS)				XRF	ICP-AES
Source	(Banin et al., 1992)		(Foley et al., 2003)		(Gellert et al., 2004)		(Rieder et al., 2004)		(Morris et al., 2000)	

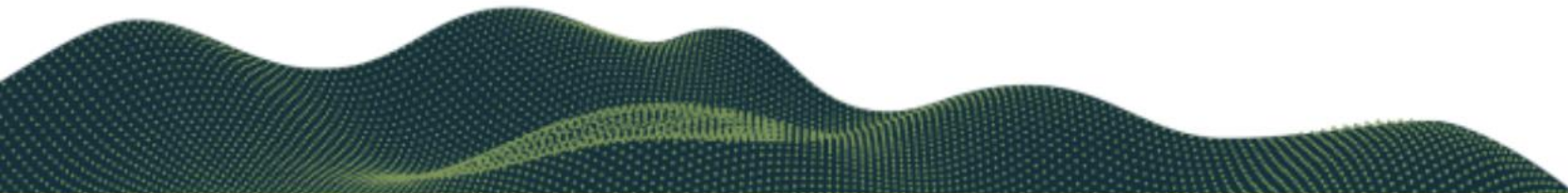
This limitation can be overcome by the exogenous addition of minerals. However, it is difficult to replicate all regolith physicochemical and hydraulic properties in a single simulant



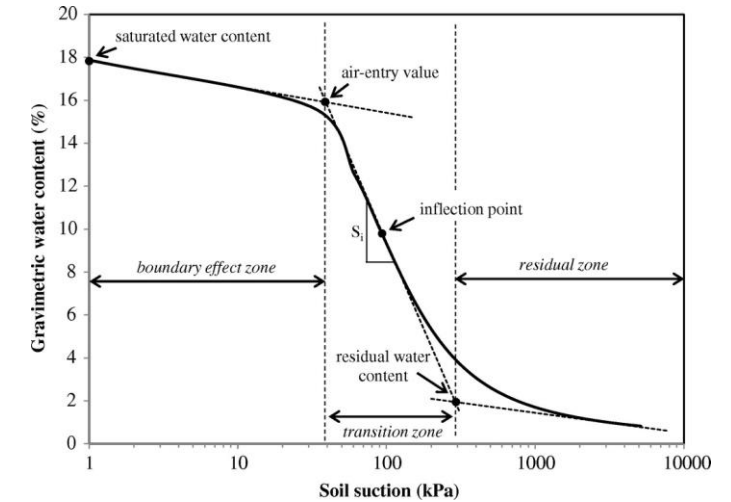
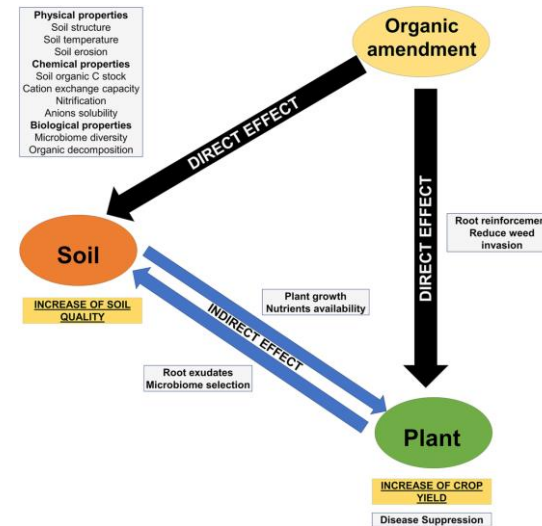
The total amount of many essential elements, such as Ca, K and Mg, may be more than adequate to satisfy the requirements for plant growth in simulants. However, plants generally take up only the bioavailable forms of elements (such as the readily soluble and exchangeable forms), and not the elements occluded in mineral structures that are released only after mineral weathering



Nutrient bioavailability in soil is governed by the pseudo-equilibrium between aqueous and solid phases. Factors such as pH, redox potential, electrical conductivity (EC), texture, type and relative abundance of fine solid particles play a key role



Simulants lack/are poor of key nutrients for plants deriving exclusively (N), mostly (P and S) or partly (Ca, K, Mg, Fe, Zn, Cu, Mn, B and Cl) from the degradation of organic matter. Micronutrients are generally occluded in accessory minerals in trace concentrations



The addition of organic amendments (e.g., compost or manure) to Lunar and Martian simulants may provide missing nutrients and enhance the bioavailability of essential nutrients. This practice can also aid in pH adjustment and have positive effects on microbial rhizosphere activity and nutrient biogeochemical cycles, as well as on physico-hydraulic properties



REBUS PROJECT - WP1200



WP1200 aims at defining possible strategies capable of making the Lunar and Martian regolith simulants suitable substrates for the growth of food crops

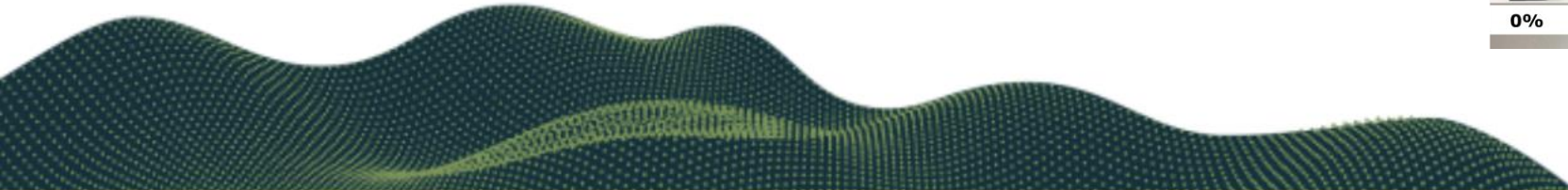
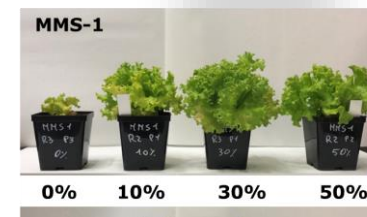
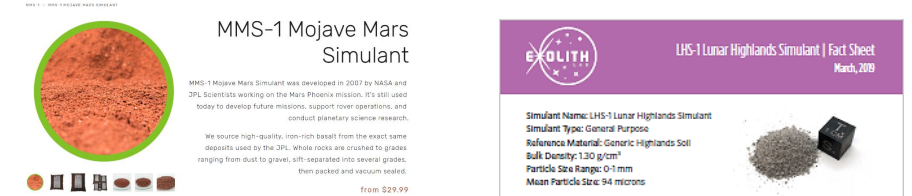
Team: Paola Adamo, Antonio G. Caporale, Mario Palladino, Roberta Paradiso

Task 1: assessment of mineralogical, chemical and physico-hydraulic properties of commercial simulants

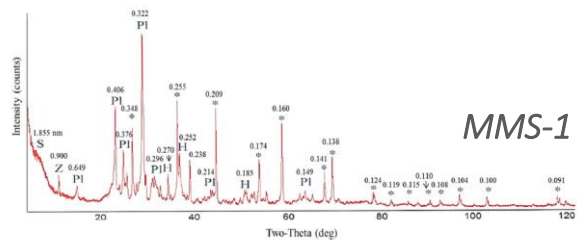
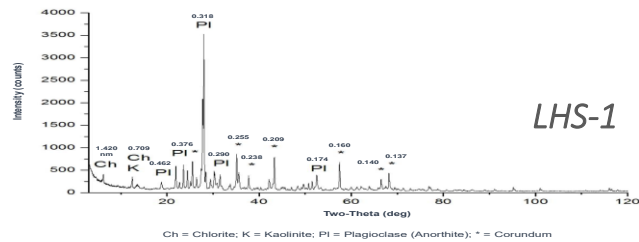
Task 2: chemical characterisation of a possible space organic-waste amendment (biodegraded as well by fungi, bacteria and larvae) and commercial analogous amendments such as monogastric-based manure and green compost

Task 3: preparation, chemical and physico-hydraulic characterisation of simulant/amendment mixtures at different rates

Task 4: plant growth experiments with lettuce (source of fibers), potato (carbohydrates), soia (proteins) and microgreens (antioxidants), and nutritional quality assessment



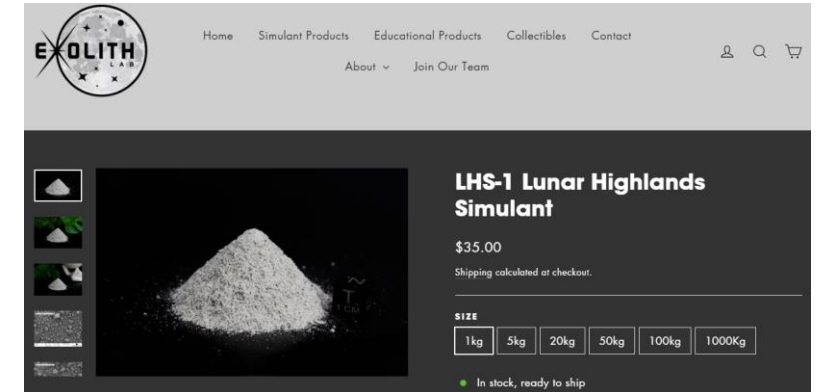
	LHS-1	MMS-1
Plagioclase (Anorthite, %)	71 ± 1.0	57 ± 0.8
Kaolinite (%)	1.6 ± 0.1	---
Chlorite (%)	3.5 ± 0.1	---
Zeolite (Clinoptilolite) (%)	---	12 ± 0.2
Hematite (%)	---	3.0 ± 0.1
Smectite (%)	---	<1.0
Amorphous Residue (%)	24 ± 0.3	28 ± 0.4



	LHS-1	MMS-1
SiO ₂ (%)	42 ± 1.4	57.3 ± 1.3
Al ₂ O ₃ (%)	23 ± 0.3	12.9 ± 0.1
Fe ₂ O ₃ (%)	3.7 ± 0.9	9.1 ± 1.0
CaO (%)	11 ± 1.0	4.9 ± 0.9
K ₂ O (%)	0.4 ± 0.1	2.1 ± 0.4
MgO (%)	2.8 ± 0.2	4.1 ± 0.3
MnO (%)	0.1 ± 0.05	0.1 ± 0.04
Na ₂ O (%)	3.6 ± 0.5	4.2 ± 0.8
P ₂ O ₅ (%)	0.1 ± 0.04	0.2 ± 0.06
TiO ₂ (%)	0.6 ± 0.1	1.1 ± 0.2
SO ₃ (%)	0.1 ± 0.02	---

Loss of ignition (%) 1.4 ± 0.2 4.0 ± 0.2

Ba (mg kg ⁻¹)	297 ± 25	566 ± 16
Cr (mg kg ⁻¹)	66 ± 11	---
Cu (mg kg ⁻¹)	25 ± 7.1	31 ± 4
Ni (mg kg ⁻¹)	79 ± 6.8	116 ± 14
Rb (mg kg ⁻¹)	4.0 ± 1.2	45 ± 2
Sr (mg kg ⁻¹)	219 ± 9.3	283 ± 2
Zn (mg kg ⁻¹)	18 ± 1.0	52 ± 3
Zr (mg kg ⁻¹)	41 ± 1.2	292 ± 2



MMS-1 - MMS-1 MOJAVE MARS SIMULANT



MMS-1 Mojave Mars Simulant

MMS-1 Mojave Mars Simulant was developed in 2007 by NASA and JPL Scientists working on the Mars Phoenix mission. It's still used today to develop future missions, support rover operations, and conduct planetary science research.

We source high-quality, iron-rich basalt from the exact same deposits used by the JPL. Whole rocks are crushed to grades ranging from dust to gravel, sift-separated into several grades, then packed and vacuum sealed.

from \$29.99

Source LHS-1 → Caporale et al., 2023 - *J Environ Manage* 325: 116455, 10.1016/j.jenvman.2022.116455

Source MMS-1 → Caporale et al., 2020 - *Sci Total Environ* 720: 137543, 10.1016/j.scitotenv.2020.137543

Horse/swine Manure



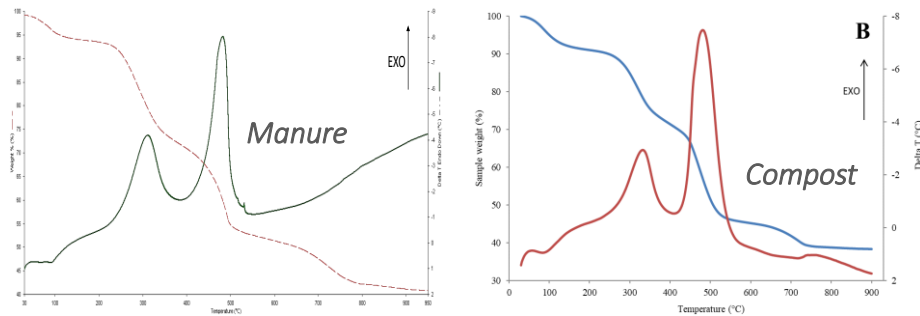
C	H	N	S	H/C	C/N	Al	Ca	Fe	K	Mg	Mn	Na	P	As	Cd	Cr	Cu	Mo	Ni	Pb	Sn	V	Zn
%				g kg ⁻¹										mg kg ⁻¹									
23.5 ± 1.1	3.0 ± 0.1	2.5 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	11 ± 0.4	4.8 ± 0.5	73 ± 4.1	7.0 ± 0.4	18 ± 1.0	6.4 ± 0.5	0.5 ± 0.1	5.3 ± 0.2	7.8 ± 0.3	2.2 ± 0.3	0.5 ± 0.1	36 ± 2.4	85 ± 5.2	2.4 ± 0.1	16 ± 0.7	32 ± 1.6	4.3 ± 0.2	23 ± 0.4	172 ± 7.1

Green compost



C	H	N	S	H/C	C/N	Al	Ca	Fe	K	Mg	Na	P	As	Cd	Co	Cr	Cu	Ni	Pb	V	Zn
%				(g kg ⁻¹)										(mg kg ⁻¹)							
29.3 ± 3.0	3.1 ± 0.3	2.3 ± 0.2	0.1 ± 0.03	1.3 ± 0.1	14.8 ± 0.6	4.7 ± 0.3	12.5 ± 3.6	4.7 ± 0.5	17.2 ± 1.7	3.9 ± 1.1	3.5 ± 0.1	2.6 ± 0.2	2.9 ± 0.6	0.2 ± 0.1	2.3 ± 0.3	7.8 ± 1.1	97 ± 5	9.0 ± 0.6	24.0 ± 1.2	6.5 ± 0.4	91.0 ± 10.3

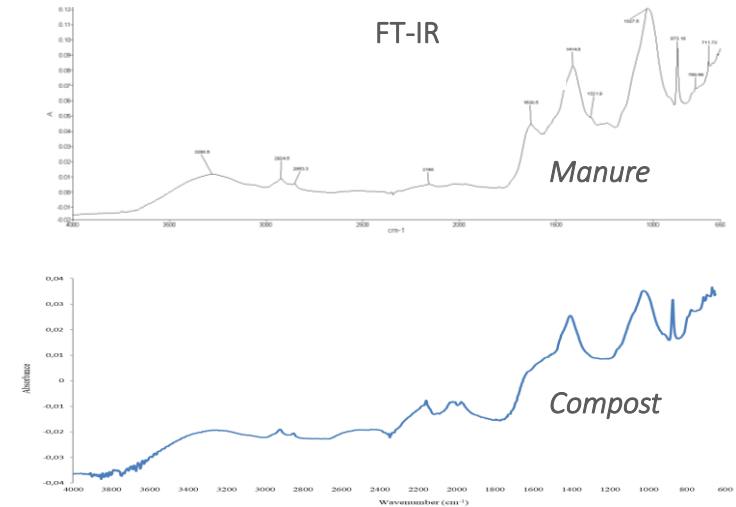
TGA-DSC



Source Manure → Caporale et al., 2023 - *J Environ Manage* 325: 116455, 10.1016/j.jenvman.2022.116455

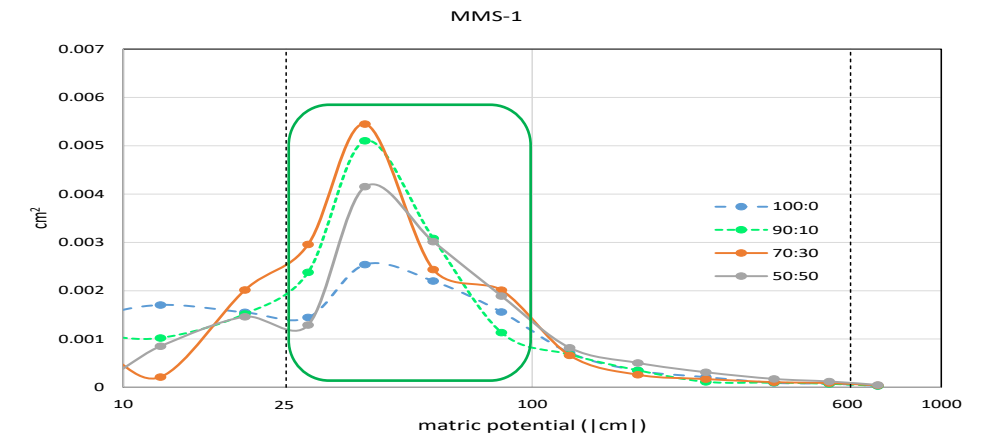
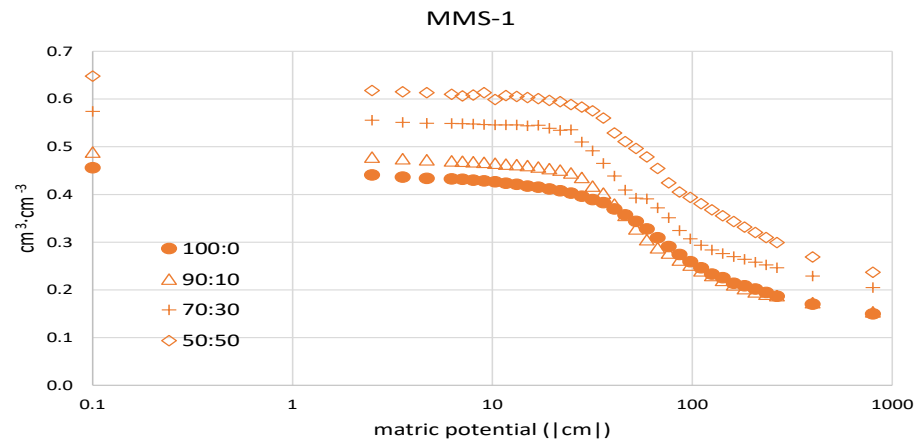
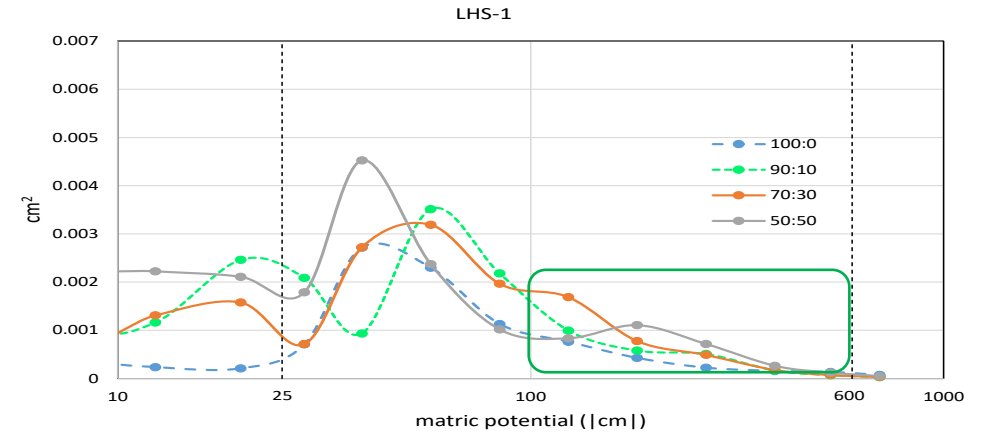
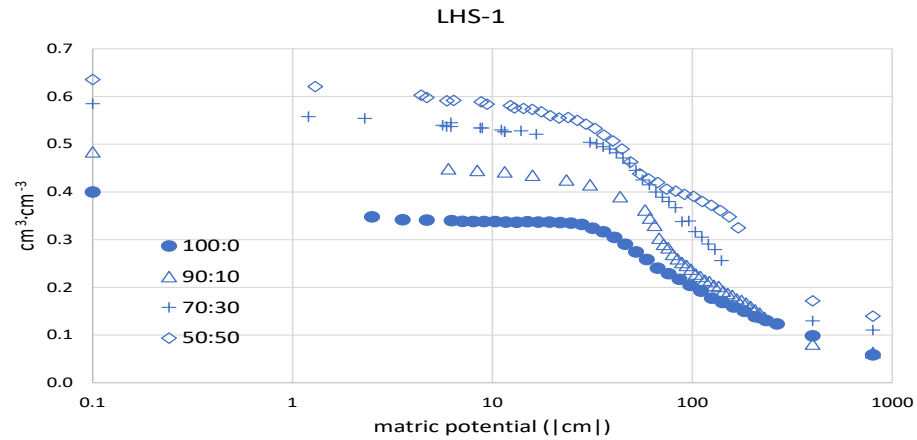
Source Compost → Caporale et al., 2020 - *Sci Total Environ* 720: 137543, 10.1016/j.scitotenv.2020.137543

FT-IR



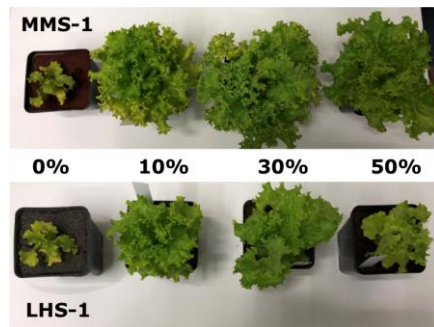
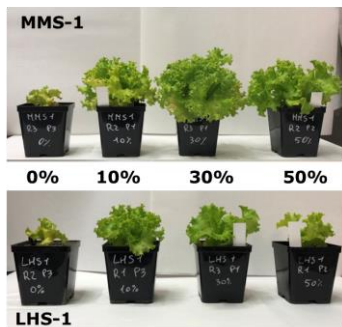
Mixture property	MMS-1				LHS-1				Simulant significance
	Simulant : Manure (w/w %)				Simulant : Manure (w/w %)				
	100:0	90:10	70:30	50:50	100:0	90:10	70:30	50:50	
Coarse sand (>200 μm - g kg ⁻¹)	650*	-	-	-	552	-	-	-	*
Fine sand (200-20 μm - g kg ⁻¹)	260*	-	-	-	346	-	-	-	**
Silt (20-2 μm - g kg ⁻¹)	65.0*	-	-	-	53.0	-	-	-	**
Clay (<2 μm - g kg ⁻¹)	25.0*	-	-	-	49.0	-	-	-	**
pH in milliQ H ₂ O	8.86* a	8.88 a	8.96 a	8.94 a	9.67 a	9.32 a	9.41 a	9.30 a	**
Electrical conductivity (dS m ⁻¹)	0.3* d	0.7 c	2.1 b	3.3 a	0.1 d	0.9 c	2.3 b	3.7 a	ns
Organic C (g kg ⁻¹ DW)	0.2* d	14 c	38 b	85 a	0.1 d	15 c	39 b	86 a	ns
Total N (g kg ⁻¹ DW)	0.1* d	1.7 c	4.2 b	10 a	<0.1 d	1.9 c	4.0 b	8.8 a	ns
C/N ratio	2.7* c	8.4 b	9.1 a	8.4 b	2.0 c	8.3 b	9.7 a	9.8 a	ns
Total S (g kg ⁻¹ DW)	<0.1 d	0.5 c	0.9 b	2.2 a	<0.1 d	0.6 c	1.0 b	2.2 a	ns
Total carbonates (g kg ⁻¹ DW)	27.0*	-	-	-	14.1	-	-	-	**
Cation exchange capacity (cmol(+) kg ⁻¹)	7.9* d	13 c	17 b	23 a	1.0 d	4.8 c	14 b	22 a	ns
Exchangeable Ca (mg kg ⁻¹ DW)	1034* b	1297 a	1307 a	1326 a	163 d	337 c	773 b	1181 a	*
Exchangeable K (mg kg ⁻¹ DW)	248* d	1027 c	1874 b	3222 a	9.0 d	574 c	2127 b	3340 a	ns
Exchangeable Mg (mg kg ⁻¹ DW)	106* d	216 c	304 b	401 a	16 d	84 c	255 b	395 a	ns
Exchangeable Na (mg kg ⁻¹ DW)	292* d	486 c	776 b	1109 a	18.3 d	214 c	682 b	1075 a	ns

Different letters indicate significant differences ($p < 0.05$) among simulant:manure rates, while ns, *, ** indicates not significant or significant (* = $p < 0.05$; ** = $p < 0.01$) differences between MMS-1 and LHS-1 simulants, according to one-way ANOVA (Duncan's multiple-range post-hoc test). DW stands for dry weight. Number of replicates = 3. * Data from Caporale et al. 2020, 10.1016/j.scitotenv.2020.137543



Element	MMS-1: Manure (w/w %)				LHS-1: Manure (w/w %)				Simulant significance
	100:0	90:10	70:30	50:50	100:0	90:10	70:30	50:50	
	mg kg ⁻¹ DW (% of total content)				mg kg ⁻¹ DW (% of total content)				
Ca	2111 a (6.0) ^o	1690 b (4.4)	1260 c (2.7)	1230 c (2.3)	518 c (0.8)	812 b (1.0)	1043 a (1.3)	1050 a (1.4)	*
K	174 d (1.0)	1140 c (6.5)	3017 b (17)	5137 a (29)	6.6 d (0.04)	1390 c (32)	4216 b (58)	6998 a (69)	ns
Mg	299 b (1.2)	353 a (1.5)	381 a (2.0)	368 a (2.4)	29 d (0.1)	126 c (0.8)	186 b (1.4)	208 a (1.8)	**
P	0.2 d (0.03)	25 c (1.6)	39 b (1.3)	53 a (1.2)	0.3 c (0.03)	58 ab (5.3)	54 b (2.1)	61 a (1.5)	ns
Fe	<0.1 d (<0.01)	19 a (0.03)	7.3 c (0.02)	16 b (0.05)	0.2 d (<0.01)	6.8 c (0.03)	23 b (0.11)	31 a (0.19)	ns
Na	1.4 d (<0.01)	4.4 c (0.02)	11 b (0.04)	18 a (0.10)	0.3 d (<0.01)	3.7 c (0.01)	11 b (0.05)	18 a (0.11)	ns
Mn	<0.1 d (<0.01)	1.2 c (0.2)	1.8 b (0.3)	2.1 a (0.3)	1.9 c (0.2)	2.0 c (0.5)	2.7 b (0.6)	3.3 a (0.7)	ns
Cu	0.04 d (0.1)	0.2 c (0.7)	1.3 b (2.7)	2.1 a (3.7)	0.2 d (0.8)	0.9 c (2.8)	1.5 b (3.5)	2.1 a (3.8)	ns
Zn	0.01 d (0.03)	0.2 c (0.3)	0.8 b (1.0)	1.8 a (1.6)	0.2 d (0.5)	0.8 c (2.3)	1.5 b (2.3)	2.2 a (2.3)	ns
Al	0.4 c (<0.01)	0.5 c (<0.01)	1.1 b (<0.01)	4.0 a (0.01)	0.9 c (<0.01)	10 b (0.01)	26 a (0.03)	26 a (0.04)	ns
Cr	0.01 d (0.01)	0.04 c (0.1)	0.15 b (0.2)	0.24 a (0.4)	<0.01 d (<0.01)	0.08 c (0.1)	0.19 b (0.3)	0.26 a (0.5)	ns
Ni	0.01 d (0.01)	0.12 c (0.1)	0.36 b (0.4)	0.54 a (0.8)	0.27 b (0.2)	0.11 c (0.2)	0.26 b (0.4)	0.36 a (0.8)	ns
Pb	0.05 c (0.4)	0.05 c (0.4)	0.07 b (0.4)	0.13 a (0.6)	0.01 d (0.1)	0.03 c (0.6)	0.11 b (0.9)	0.14 a (0.8)	ns
V	0.54 b (0.9)	0.68 a (0.9)	0.75 a (1.5)	0.72 a (1.7)	0.05 c (0.1)	0.28 b (0.5)	0.41 a (0.9)	0.45 a (1.1)	*

Different letters indicate significant differences ($p < 0.05$) among simulant:manure rates, while ns, *, ** indicates not significant or significant ($* = p < 0.05$; $** = p < 0.01$) differences between MMS-1 and LHS-1 simulants, according to one-way ANOVA (Duncan's multiple-range post-hoc test). DW stands for dry weight. ^o Values in round brackets indicate the NH_4NO_3 -extractable fractions expressed as percentages of the total element contents.



Source → Caporale-Amato et al. - Plants (under review)

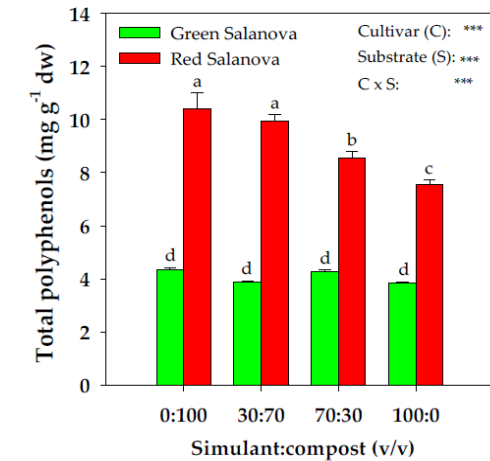
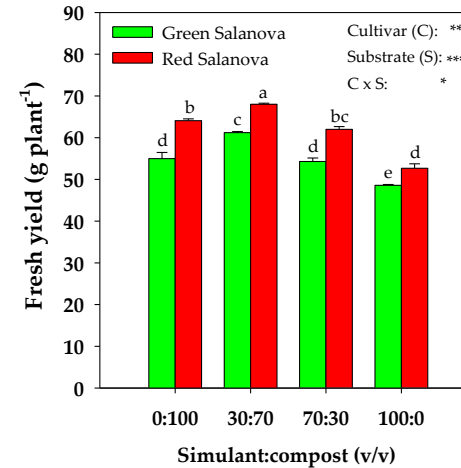
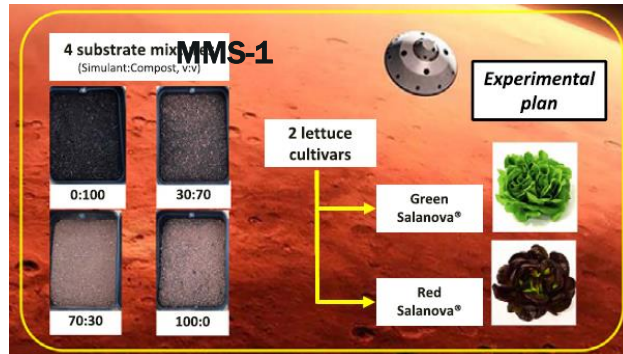


Source → Caporale-Paradiso et al. - Plant & Soil (under review)



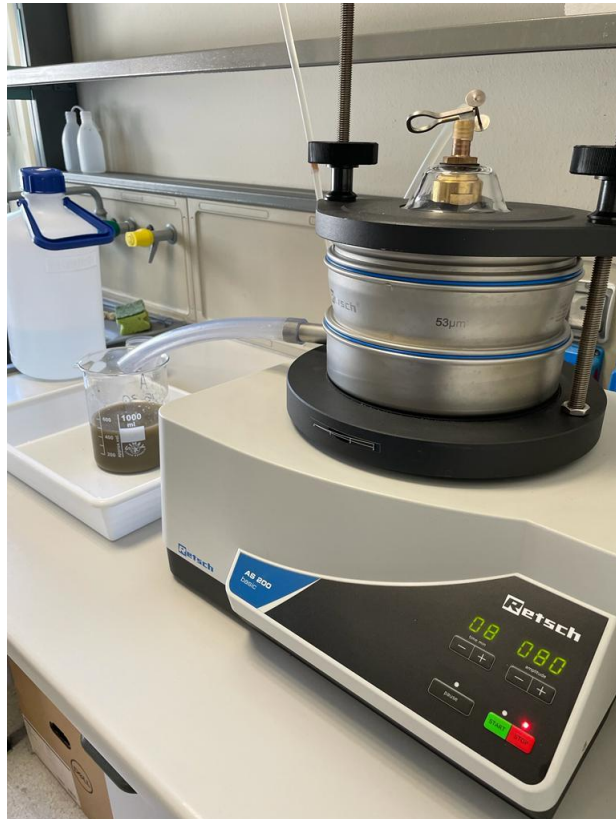
Source → Caporale-Paradiso et al. - Plants (under review)





Source of Variance	NO ₃ (g kg ⁻¹ dw)		PO ₄ (g kg ⁻¹ dw)		K (g kg ⁻¹ dw)		Ca (g kg ⁻¹ dw)		Mg (g kg ⁻¹ dw)		Na (g kg ⁻¹ dw)		Cl (g kg ⁻¹ dw)		SO ₄ (g kg ⁻¹ dw)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Cultivar (C)																
Green Salanova	27.6	42.9 a	9.0	5.7	64.7	50.6	7.1	6.2	2.5	2.8	1.0	5.9	3.3	2.2	1.5 b	8.9
Red Salanova	30.4	28.8 b	10.4	7.5	71.7	44.6	6.2	6.0	2.5	2.9	1.0	5.2	3.1	1.9	2.5 a	9.4
Simulant:compost (v:v) (S)																
0:100	29.4 a	33.3 ab	11.2 a	8.7 a	82.7 a	69.2 a	4.8 c	5.6 ab	2.2 b	2.2 c	0.8 b	2.0 c	6.9 a	2.8 a	2.2 a	9.6 ab
30:70	32.5 a	24.9 b	11.9 a	7.3 a	75.2 b	48.4 b	6.4 b	6.7 a	2.4 b	2.5 bc	0.8 b	2.7 bc	2.0 b	1.7 b	2.3 a	9.3 b
70:30	32.4 a	43.6 a	9.5 b	7.3 a	69.5 c	57.4 b	6.9 b	6.6 a	2.3 b	3.0 b	0.9 b	4.8 b	2.0 b	2.0 b	2.0 a	11.1 a
100:0	21.7 b	41.6 a	6.2 c	2.9 b	45.2 d	15.5 c	8.5 a	5.3 b	3.2 a	3.9 a	1.7 a	12.8 a	1.7 b	1.9 b	1.4 b	6.6 c

Non-significant (ns). *, **, *** Significant at P 0.05, 0.01, and 0.001, respectively. Cultivar means were compared by t-test. Substrate mixture means and interaction were compared by Duncan's multiple-range test (P = 0.05). Different lowercase letters within each column indicate significant differences (P 0.05).



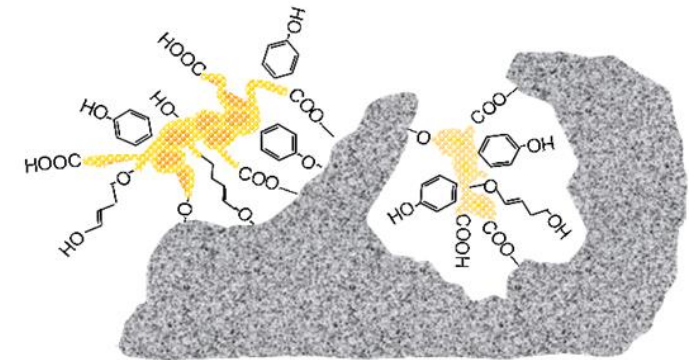
Stabilisation of exogeneous OM by minerals, including iron oxides, over time is of paramount importance for the survival of crops

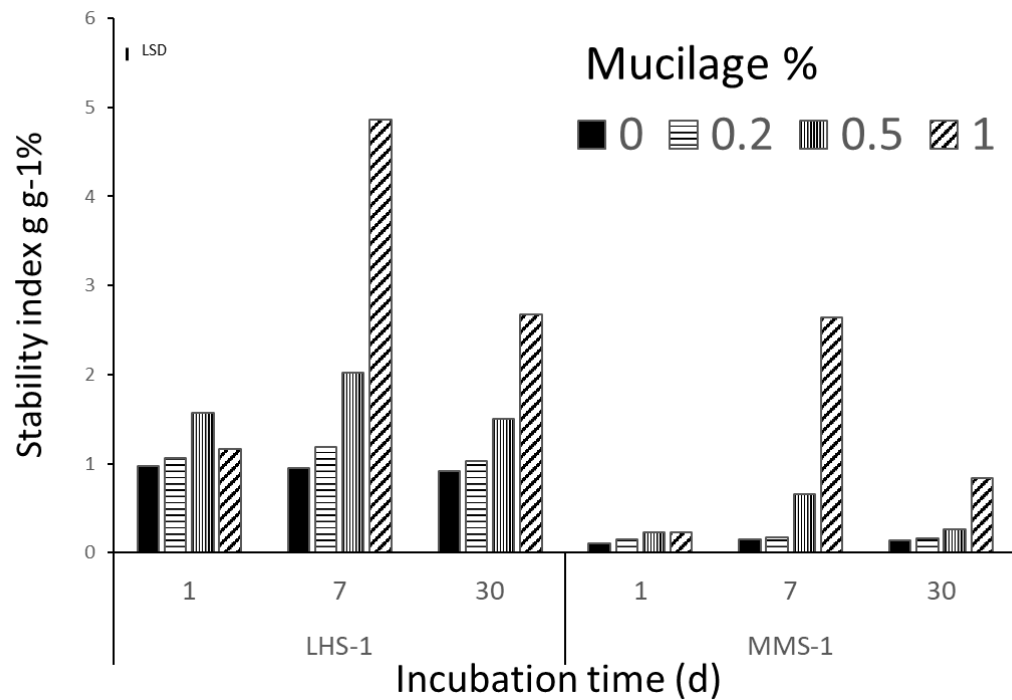
Source → Giannetta-Zaccone et al. - SISS conference 2022

After potato plant growth, samples of each substrate were fractionated, obtaining particulate OM (POM) and mineral associated OM (MAOM), and characterised for total and organic carbon (OC) and for Fe K-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS)

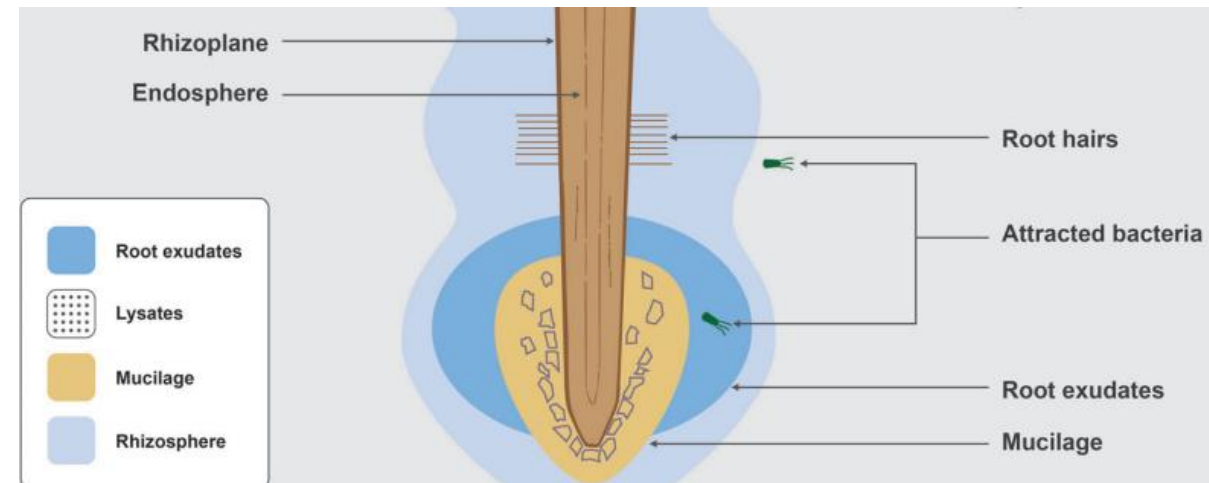


Mineral-associated organic matter (MAOM)

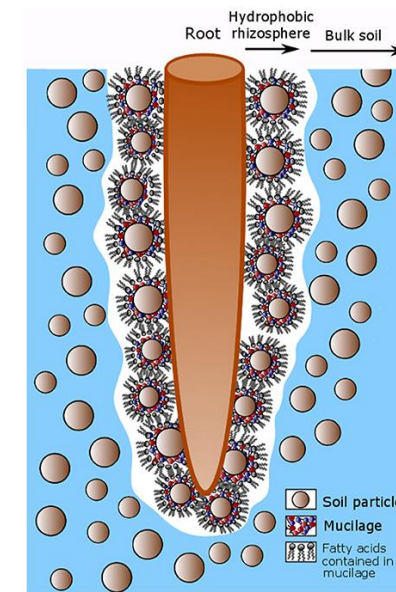




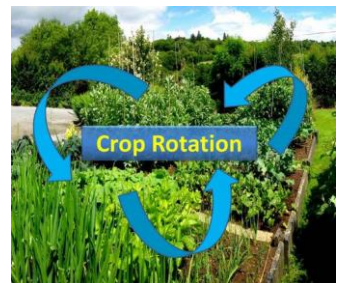
Aggregate stability index as affected by different percentages of root exudate analogue at increasing incubation times



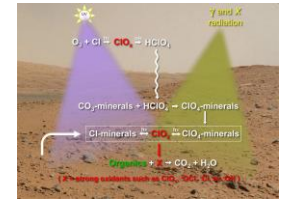
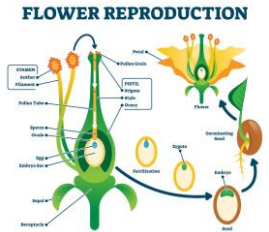
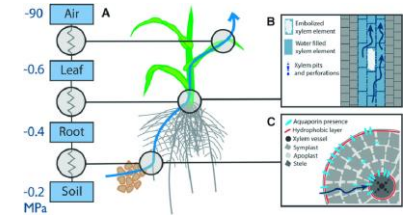
Source → Amato-Bochicchio et al. - SIA conference 2021



- ✓ Pure regolith simulants might be suitable media for plant growth (at least for limited periods) and function as a source of essential nutrients such as Ca, Mg, K. They lack OM and key macronutrients like N, P and S
- ✓ Simulants exhibit critical features for plant health, such as alkaline pH, high availability of Na, scarce cohesion of mineral components, predominance of large vs. micro pores, and scant water holding capacity
- ✓ A promising strategy is the adding of in situ recycled organic matter to enrich regolith simulants. This is a sustainable and effective technique to enhance the chemical and biological fertility and physico-hydraulic properties of regolith-based substrates
- ✓ Consecutive cycles of plant cultivation on the same regolith-based substrate can allow prolonged root exudation and the release of organic acid molecules and CO₂. This can lower the substrate pH, increase mineral weathering rates, enhance nutrient release/availability, promote particle aggregation (to form a more efficient porous system), and overall contribute to soil improvement



- ✓ Water movement and fluxes in regolith-based substrate/plant systems under partial- and micro-gravity. Water dynamics would regulate the extent of mineral weathering and the rate of organic matter decomposition, thus greatly affecting the biogeochemistry and bioavailability of nutrients and plant growth
- ✓ Effect of the space environment on plant physiology (e.g., the biophysical limitations on gas exchange and transpiration), and how this affects plant growth and productivity and substrate properties
- ✓ In sustainable space farming scenarios, regolith-based substrates are required to sustain plant growth throughout the plant life cycle, including the complete seed maturation needed for reproduction
- ✓ The presence of microorganisms and addition of biofertilisers/biostimulants will add further complexity to extraterrestrial BLSS
- ✓ The occurrence and remediation of perchlorates in the Mars regolith is a challenge to its use as growth medium





The Potential for Lunar and Martian Regolith Simulants to Sustain Plant Growth: A Multidisciplinary Overview

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Duri-Caporale et al., 2022 - Front Astron Space Sci 8: 747821, 10.3389/fspas.2021.747821



- ✓ Melissa conference scientific and organising committees, and session chairs
- ✓ ReBUS project, WP1200 leader (Prof. Adamo) and research team





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