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Research Article

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Growth of Rucola on Mars soil simulant under the influence of pig slurry and earthworms

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Abstract: To feed humans on a future Mars settlement, a sustainable closed agricultural ecosystem is a necessity. On Mars, both the faeces of astronauts as well as any plant residues or other organic waste needs to be (re) used to fertilise the present regolith. The activity of earthworms may play a crucial role in this ecosystem as they break down and recycle the dead organic matter. The contribution of worms to Mars regolith forming is yet an unexplored territory. The first goal of our research was to investigate whether earthworms (*Caligonella* genus and *Dendrobaena veneta*) can survive in Mars soil simulant. The second goal was to investigate whether earthworm activity on Mars soil simulant can stimulate the growth of crops, in our case Rucola. The third goal was if earthworm activity can enhance the effect of pig slurry on the growth of Rucola. In a 75-day greenhouse experiment, we sowed Rucola in Mars soil simulant as well as in silver sand as an Earth control, amended with pig slurry, plant residues, and earthworms. During the experimental period, we observed worm activity. At the end of the experiment, the worms had propagated both in the Mars soil simulant and Earth control. However, we found no significant effect of worm activity on plant biomass production. This was probably due to the relative short duration of the experiment, being one life cycle of Rucola. Adding pig slurry stimulated plant growth significantly as expected, especially for the Mars soil simulant.

Keywords: extra-terrestrial, organic matter, regolith, crop, *Caligonella*, *Dendrobaena veneta*

1 Introduction

To feed astronauts on a future Mars settlement, a closed sustainable agricultural ecosystem will be a necessity [1,2]. Crops may be flown in, but that is costly and inefficient. Moreover, it is an uncertain factor in the food supply, since a supply ship may fail. Crop growth on Mars itself will contribute to the safe (permanent) stay of astronauts on Mars. There are basically four different ways possible to grow crops on Mars, but we are assuming that it all will be indoors or underground, given the hazardous Mars environment with a very low air pressure (6 hPa, about 0.6% of Earth pressure at sea level). Main components of Mars atmosphere are about 95% CO_2, 2.6% N_2, 1.9% Ar, 0.16% O_2, and 0.06% CO, all volume percents. The average temperature lies around −63°C with a variation from −140 to + 20°C [3]. Moreover, due to the absence of a planet wide protecting magnetic field, cosmic radiation reaches the planet surface giving a 17 times higher radiation than on Earth, which may affect plant growth [4,5].

The first possible option to grow crops is aeroponics [6]. The second option is aquaponics, as is investigated by e.g. Fu et al. [7]. A third option is underwater growth e.g. algae and fish [8]. The fourth option is to use the soil that is present on Mars, the Mars regolith, and the present water (as ice) on Mars [9,10]. The last option is further explored in this article, based on the idea to use the resources available on Mars as much as possible.

Martian regolith is not available on Earth for research. Therefore, NASA has created soil simulants for research purposes. Two variants developed under supervision of NASA are available, the JSC-1A [11], made in 1997. The second is the Mojave Mars Soil (MMS) made in 2007 and originates from the Mojave Desert near Saddleback Mountain [12]. In this experiment we applied the more recent MMS simulant (for an extensive description refer Peters et al. [12], their Table 1 and for a comparison with Mars measurements their Table 2). Important for plant growth are the absence of life, the almost absence of nitrogen and ammonium, and the absence of complicated organic

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Table 1: Overview of the experimental set-up

Worm	Manure	Soil type	Code
–	+	Earth	NME
–	+	Mars	NMM
–	–	Earth	NNE
–	–	Mars	NNM
+	+	Earth	WME
+	+	Mars	WMM
+	–	Earth	WNE
+	–	Mars	WNM

For soil type Earth indicates application of Earth soil and Mars indicates application of Mars soil simulant MMS. The code for the treatments is used in all other tables and figures.

molecules. One of the physical features of the MMS is that the minerals that make up the soil are quite sharp, as they would be on Mars. This may have consequences for all life in the soil. Edges of the minerals may be so sharp that it could damage living cells, including roots of plants or the gut of worms, leading to leaking of cell content and in the end possible death of plants and animals.

In a closed agricultural system non-eaten parts of cultivated crops must be returned into the agricultural system. A key step in the breakdown of these organic "waste products" will be the breakdown of organic matter by earthworms [13,14].

Another option to manure the soil is to bind N_2 from the air by nitrogen fixing bacteria that live in symbiosis with plants [15] or by cyanobacteria [16], thus enriching the soil with ammonia. Human faeces can also be a source of nutrients and should therefore also be returned into the agricultural system as manure for the plants. Instead of the application of human faeces, for experiments it is also an option to use pig slurry, which is easier and safer to handle, especially given the pathogen content in human faeces [17]. We added the pig slurry to review the effect on plant growth compared to the expected manuring effect of the worm activity.

Earthworms eat the organic matter, mixing it in the process with soil in their gut, while extracting nourishing elements and then excreting a mixture of broken-down organic matter and soil. Bacteria can then further break-down the organic matter and thus release nutrients for the next generation of plant growth [13]. The earthworms are also an important factor in the forming of soil by bringing organic matter into the soil [14]. They also dig burrows, which promotes draining of the soil and they make water supply easier. In earlier experiments with Mars soil simulant, water supply proved to be problematic due to the hydrophobic character of the simulant [9]. Adding organic matter to the soil proved to solve this problem [10,18]. The burrows of the earthworms also help aerating the soil so that the roots of the plants can take up the oxygen they need for their maintenance respiration [19].

The first goal of this experiment was to investigate whether the earthworms can survive in Mars soil simulant and whether they show normal activity as digging burrows and decomposing organic matter. The second goal was to investigate if the worm activity stimulates plant growth also in combination with the addition of pig slurry. To this end, a greenhouse experiment was set up with MMS and Earth soil control and the addition of pig slurry and earthworms. The effects of the addition were monitored using Rucola (*Eruca sativa*) as a bio-indicator.

2 Material and methods

2.1 Experimental design and greenhouse settings

The experiment lasted from 1-9-2017 to 15-11-2017 and was carried out in a greenhouse with a minimum temperature of 20°C and 65% humidity. Daytime lasted 16 h. Lamps yielding 80 µmol (HS2000 from Hortilux Schréder) were used if the sunlight intensity was below 150 W/m².

Table 2: Properties of the pig slurry

Parameter	Analysis method	(kg fresh weight^{-1})
DW (%)	Oven dried and weighed	2.9
Specific weight (kg/L)	Weighing 100 mL at 20°C	1.008
pH	Electrode at 20°C	8.06
N_{tot} (mg/kg)	$H_2SO_4/Se/H_2O_2$ destruction and measured with a segmented flow analyser	3861.7
NH_4-N (mg/kg)	Extraction with 1 M KCl and measured with a segmented flow analyser	1,284
P_{tot} (mg/kg)	$H_2SO_4/Se/H_2O_2$ destruction and measured with a segmented flow analyser	840
Ortho P (mg/kg)	Centrifuged, filtrated, and measured with a segmented flow analyser	146

The experiment had a full factorial design of three factors of two levels each (2 * 2 * 2, with $n = 4$); earthworm, pig slurry, and “soil,” giving in total 2 (worm, no worm) × 2 (manure, no manure) × 2 (Mars soil simulant, Earth control) × 4 (replicas) 32 pots (also Table 1). The treatments were randomly placed in a water bath (Figure 1). Temperatures in the greenhouse are optimal for plants, but too high for the worms. To obtain more optimal conditions for the worms, the pots were placed in a streaming water bath. The water was cool groundwater pumped up at the site with a temperature of approximately 10°C.

2.2 Pots

The experiment was carried out in circular pots with a radius of 5.0 and 15.0 cm height (1.2 l, by NIPAK, The Netherlands). Velcro (5 cm) was glued to the inside top of the pots to prevent worms from escaping the pots (as suggested and tested by Lubbers and van Groenigen, [20]).

2.3 Soil and water

In contrary to our earlier experiments, the Mojave Mars Simulant (MMS) was used instead of the JSC-1A [9,10]. The MMS soil, delivered by the Martian garden (www.themartiangarden.com), was used as the next generation Mars soil simulants. Despite its more recent origin, this simulant does not contain perchlorate, which was recently found in Martian soil and believed to be widespread on Mars [21–23]. As Earth control, we used silver sand, sand that is nutrient poor and also lacking organic material. We used 800 g MMS and 700 g silver sand per pot.

For both soils, the water holding capacity (WHC) was determined. For MMS the WHC was 21% and for silver sand 23% (soil weight). Water was added to both the soils till the saturation point. During the experiment, on 12-10-2017, water content was raised to 26% for MMS and 30% for Earth control soil to keep the pots moist to compensate for increased evaporation due to plant growth. Water was supplied twice a week bringing the pots back to their original weight.

2.4 Organic matter and manure

The soils were mixed with the harvested above ground organic matter from a previous growth experiment on Mars soil simulant and Earth control [10]. 20 g per pot was added as rough material and mixed through the

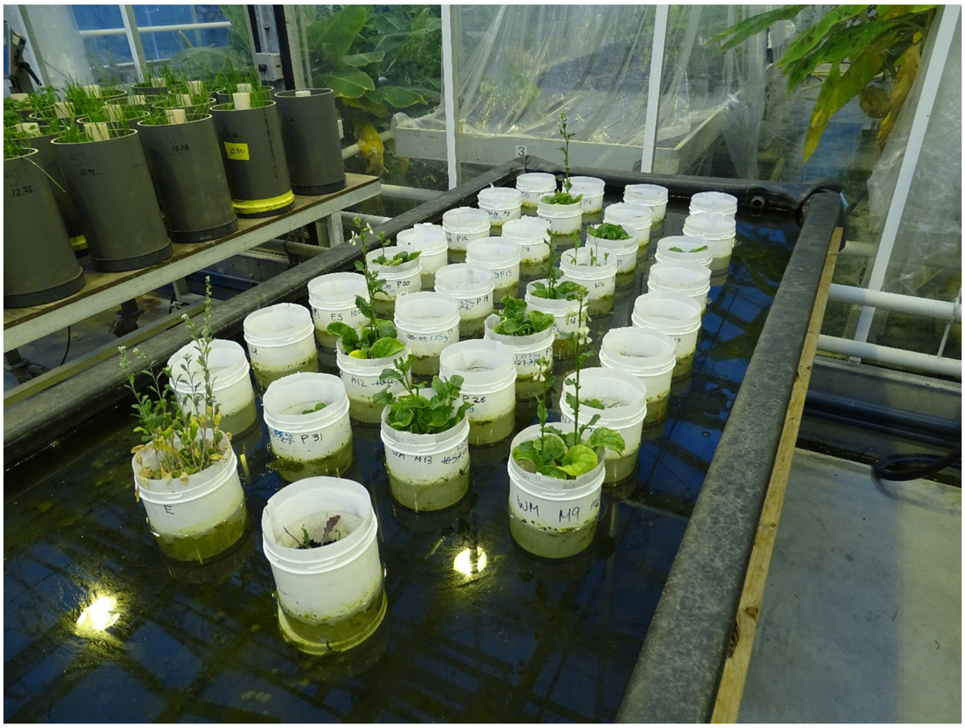

Figure 1: Set up of the experiment. Pots and treatments were placed randomly in the water bath. The photo shows the pots placed in a cold-water bath for optimal temperature conditions of the soil for the worms. A schematic diagram of the pot set up can be found in Appendix Figure 1.

upper 10 cm of the soil. 10 g ball milled organic matter was added per pot on top of the soil as a litter layer. Both the organic matter fractions were added after water was added to the soils. This gives roughly 3.8% organic matter in MMS and 4.3% organic matter in silver sand (dry weight, DW). The organic matter mainly contained above ground non-eaten parts of rye, cress, green bean, pea, and carrot. The organic matter contained on average 18.8 g/kg DW (±7.0) potassium, 12.4 g/kg DW (±3.6) nitrogen, and 2.16 g/kg DW (±1.00) phosphorous. The variation between the samples taken from the organic matter was quite large, hence the large standard errors. The N content is rather low, but not outside the range what is found for N-content in organic matter.

12.5 mL of Pig slurry was added as manure (Table 2 shows its content), after water and organic matter were added. It was added on top of the soil. The slurry did sink in the silver sand immediately after adding to the soil, in the MMS soil it took minutes to sink in. This shows the hydrophobic character of the MMS, which was also found for the JSC-1A we used in an earlier experiment [9].

2.5 Worms

Two species of worms were added to the soils. The first were from the *Caligonella* genus, the most common endogenic species found in The Netherlands. They were caught in the grass field next to the institute. The second worm species was *Dendrobaena veneta*, a compost worm. These worms were supplied by "De Polderworm" in Rutten, The Netherlands. Adult worms were put on tissue paper and water for two days to empty their guts, to prevent interference of gut material with the experiment. Each worm treatment in the experiment received two *Caligonella* and two *Dendrobaena* worms. The worms were added after germination and establishment of the seedlings of the Rucola on 22-9-2017, 3 weeks after the start of the experiment.

2.6 Plant growth

To investigate the effect of the treatments on plant aboveground biomass growth, we used a round leaved Rucola cultivar (argula or rocket, *Eruca sativa* Mill. cv Sparkle RZ, delivered by Rijk Zwaan) as bio-indication. A teaspoon full of seeds (50 ± 5) were sown randomly in each pot. After germination, the young plants were not thinned. At the end of the experiment, aboveground biomass was harvested and fresh and dry weights were measured. The biomass was dried in an oven for 2 days at 70°C.

2.7 Statistics

A full factorial 3-way-ANOVA was carried out for both fresh weight (FW) and dry weight (DW) of the Rucola for all treatments and interactions in SPSS (IBM, [24]). Statistically significant differences between DWs was tested with a student *t*-test ($p = 0.05$).

3 Results

3.1 Earthworms

As long as the experiment lasted, worms did escape the pots despite the Velcro that should prevent this [20]. They were removed from the water bath but not put back in the pots, since they had not been labelled per pot. During the experiment all pots were infested with fungi, some formed even mushrooms. The mushrooms were removed, the fungi were not treated.

At the end of the experiment worms were retrieved from the soil. Most of the worms had by then escaped from the pots (Appendix Table 2). However, two young worms were found in two different pots in the Mars soil simulant and one in the Earth control. Recovered worms from the pots were all alive and lively. The effect of the worms on the aboveground biomass growth (DW) of Rucola was not significant (Figure 2; for the statistics see Appendix Table 3). Comparing the treatments NNE and WNE, NME and WME, NNM and WNM, and NMM and WMM in Figure 2 clearly shows that there are no differences between the paired treatments with and without worms on the DW.

3.2 Biomass growth

All pots produced growing plants. However, the difference between the treatments were huge (Figure 2; Appendix Tables 3 and 4). The pig slurry treatment yielded the highest biomass (DW) of Rucola and differed statistically significant from the non-treated pots. The MMS soil simulant gave a significant higher biomass production than the Earth control ($p = 0.010$). This was mainly due to the relative low biomass production of the pig slurry addition to the Earth control. The two-way interaction between planet and manure and the three-way interaction between planet, manure and earthworm were (just) significant. No significant interactions were found for the harvested DW of the aboveground biomass.

4 Discussion

The worms did survive in the MMS soil simulant, indicating that uptake of sharp soil particles, present in the MMS, is not a major problem for their survival. The fact that they were healthy is also supported by the young worms that were born during the experiment. Many worms escaped from the pots, but there was no difference between the Earth control and the MMS soil simulant. Another indicator of good health of the worms were the burrows dug and the poop heaps found on the surface of some of the pots. Soil forming processes were observed in the pots with the worms. The effect of the worms on the biomass growth, however, was absent despite our expectation that the worms would positively influence biomass production. The absence of a positive stimulus may also be due to the time the experiment lasted and the time it takes for worms to process the organic matter and, subsequently, for bacteria to mineralise the worm excrement and release the nutrients for the plants [13,14,25].

The growth of Rucola was clearly stimulated by the addition of pig slurry. The fact that adding manure stimulates the growth is not very surprising and the effect is well known [25,26]. Pig Slurry was chosen because it mimics the addition of human faeces well [27]. In a closed agricultural system, the human faeces will have to be brought back in the system, otherwise there will be a loss of nutrients from the system, especially nitrate, which is not easily replaced. The human faeces will have to be sterilised before application, to prevent unwanted bacteria from the human gut to enter the agricultural system. Worms can also play a role in bringing the faeces back into the soil when it will be applied to the soil. However, in this experiment, the interaction between worm and manure was not significant (p = 0.685).

The biomass production (DW) of Rucola was higher on the Mars soil simulant compared to the Earth control (p = 0.010). These results are in line with the earlier research of Wamelink et al. [9]. In their experiment, the Earth control was nutrient poor soil as well. In later research, Wamelink et al. [10] used organic soil as a control. Our expectation is that when this would have been applied in this experiment as well, the Earth control would have outperformed the MMS. However, the idea was to build a soil from Earth sand as well as from the MMS and then the approach followed here is more appropriate. Effects can be better compared and studied and the *Caligonella* genus fits these circumstances better.

The fresh weight analyses were in line with the DW; however, here also two interactions were found to be just significant, for the two-way interaction between planet and manure (p = 0.033) and the three-way interaction between planet, manure and earthworm (p = 0.042; Appendix Table 4). The significant effect of the three-way interaction is most likely a result of the two-way interaction between planet and manure. We cannot explain this effect.

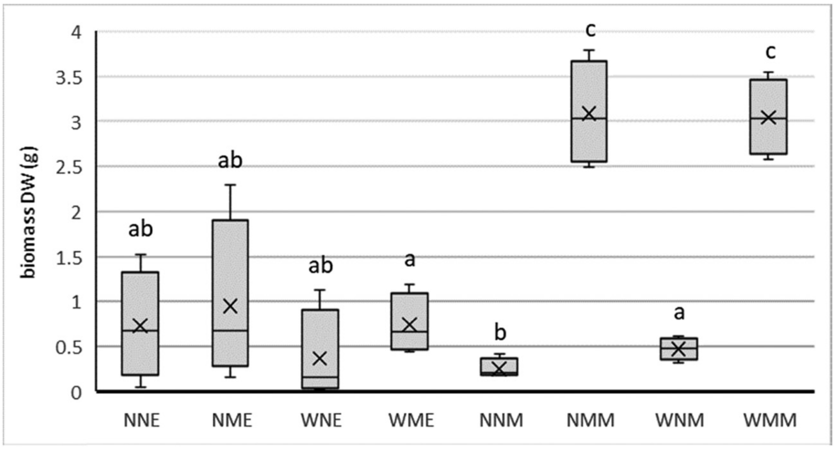

Figure 2: Box plot of the harvested biomass (dry weight) per treatment. Treatment code indicates for first letter for worm (W) or no worm (N) added; for the second letter for manure (M) or no manure (N) added; for the third letter for Earth control (E) and Mars soil simulant (M). Different letters indicate significant differences at p = 0.05.

We used *Dendrobaena veneta* and worms from the *Caligonella* genus. *D. veneta* is a mulching species and is used to high temperatures (The supplier Polderworm breeds them at around 20°C). Worms of the *Caligonella* genus, however, like it colder. To accommodate the *Caligonella* genus worms, we put the pots in a cold-water bath. However, this is suboptimal for *D. veneta* and plant growth. For this first trial experiment this is acceptable, but it is less optimal, and the water bath complicates the experimental set up. Therefore, in the next experiments, we recommend using worms that thrive at 20°C and can mix organic matter with soil.

One of the most disputed issues is the presence of perchlorate in the Mars soil, at least in the upper layers [21–23,28]. There was no perchlorate present in the JSC-1A or the MMS soil simulant used here, nor was it added to its successors [11,12,29]. Perchlorate is poisonous for plants and humans and most likely for earthworms as well. To test the effect, it could be added to the soil simulant, as was done by Oze et al. [30]. They found a significant negative effect of perchlorate on both the germination and growth, if any, on a Mars soil simulant. This result was confirmed by Eichler et al. [31]. However, it remains disputable if the perchlorate is present everywhere on Mars including deeper soil layers and in caves.

5 Conclusion

The added worms were clearly active during the experiment and showed to be able to propagate. However, the worms did not significantly affect the plant biomass production, probably due to the short experimental period; a longer experiment is needed to assess whether or not there is a long-term effect.

The addition of pig slurry stimulated plant growth significantly as expected, especially in the Mars soil simulant. The biomass production on Mars soil simulant was higher than on the nutrient poor Earth soil.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analysed during this study are included in this published article.

References

[1] Horneck G, Facius R, Reichert M, Rettberg P, Seboldt W, Manzey D, et al. HUMEX, a study on the survivability and adaptation of humans to long-duration exploratory missions, part II: Missions to Mars. Adv Space Res. 2006;38:752–9. doi: 10.1016/j.asr.2005.06.072

[2] Cousins CR, Cockell CS. An ESA roadmap for geobiology in space exploration. Acta Astron. 2016;118:286–95.

[3] Sagan C, Mullen G. Evolution of atmospheres and surface temperatures. Science. 1972;177(4043):52–6.

[4] Guo J, Slaba TC, Zeitlin C, Wimmer-Schweingruber RF, Badavi FF, Böhm E, et al. Dependence of the Martian radiation environment on atmospheric depth: Modeling and measurement. J Geophys Res Planets. 2017;122:329–41. doi: 10.1002/2016JE005206.

[5] Tack N, Wamelink GWW, Denkova AG, Schouwenburg M, Hilhorst H, Wolterbeek HT, et al. Influence of Martian radiation-like conditions on the growth of Secale cereale and Lepidium sativum. Front Astron Space Sci. 2021;8:665649. doi: 10.3389/fspas.2021.665649.

[6] Maggi F, Pallud C. Space agriculture in micro- and hypo-gravity: A comparative study of soil hydraulics and biogeochemistry in a cropping unit on Earth, Mars, the Moon and the space station. Planet Space Sci. 2010;58:1996–2007.

[7] Fu Y, Li L, Xie B, Dong C, Wang M, Jia B, et al. How to establish a bioregenerative life support system for long-term crewed missions to the moon or mars. Astrobiology. 2016;16:925–36.

[8] Bluem V, Paris F. Possible applications of aquatic bioregenerative life support modules for food production in a Martian base. Adv Space Res. 2003;31:77–86. doi: 10.1016/S0273-1177(02)00659-2.

[9] Wamelink GWW, Frissel JY, Krijnen WHJ, Verwoert MR, Goedhart PW. Can plants grow on Mars and the Moon: a growth experiment on mars and moon soil simulants. PLoS ONE. 2014;9(8):e103138. doi: 10.1371/journal.pone.0103138.

[10] Wamelink GWW, Frissel JY, Krijnen WHJ, Verwoert MR. Crop growth and viability of seeds on Mars and Moon soil simulants. Open Agriculture. 2019;4:509–16. doi: 10.1515/opag-2019-0051.

[11] Rickman D, McLemore CA, Fikes J. Characterization summary of JSC-1A bulk lunar mare regolith simulant; 2007. http://www.orbitec.com/store/JSC-1AF_Characterization.pdf.

[12] Peters GH, Abbey W, Bearman GH, Mungas GS, Smith JA, Anderson RC, et al. Mojave Mars simulant-characterization of a new geologic Mars analog. Icarus. 2008;197:470–9.

[13] Edwards CA, Fletcher KE. Interactions between earthworms and microorganisms in organic-matter breakdown. Agric Ecosyst Environ. 1988;24:235–47.

[14] Edwards CA, Hendrix PF, Arancon NQ. Biology and ecology of Earthworms. 3rd edn. US: Springer; 1996.

[15] Harris F, Dobbs J, Atkins D, Ippolito JA, Stewart JE. Soil fertility interactions with Sinorhizobium-legume symbiosis in a simulated Martian regolith; effects on nitrogen content and plant health. PLoS ONE. 2021;16(9):e0257053. doi: 10.1371/journal.pone.0257053.

[16] Verseux C, Heinicke C, Ramalho TP, Determann J, Duckhorn M, Smagin M, et al. A low-pressure, N_2/CO_2 atmosphere is suitable for Cyanobacterium-based life-support systems on Mars. Front Microbiol. 2021;12:611798. doi: 10.3389/fmicb.2021.611798.

[17] Tyrrel SF, Quinton JN. Overland flow transport of pathogens from agricultural land receiving faecal wastes. J Appl Microbiol Symp Suppl Vol. 2003;949(32):87S–93S.

[18] Caporale AG, Vingiani S, Palladino M, El-Nakhel C, Duri LG, Pannico A, et al. Geo-mineralogical characterisation of Mars simulant MMS-1 and appraisal of substrate physico-chemical properties and crop performance obtained with variable green compost amendment rates. Sci Total Environ. 2020;720:137543. doi: 10.1016/j.scitotenv.2020.137543.

[19] Raich JW, Tufekcioglu A. Vegetation and soil respiration: Correlations and controls. Biogeochemistry. 2000;48:71–90.

[20] Lubbers IM, van Groenigen JW. A simple and effective method to keep earthworms confined to open-top mesocosms. Appl Soil Ecol. 2013;64:190–3.

[21] Hecht MH, Kounaves SP, Quinn RC, West SJ, Young SM, Ming DW, et al. Detection of perchlorate and the soluble chemistry of martian soil at the phoenix lander. Science. 2009;325:64–7.

[22] Chevrier VF, Hanley J, Altheide TS. Stability of perchlorate hydrates and their liquid solutions at the phoenix landing site, Mars. Geophys Res Lett. 2009;36:L1020.

[23] Clark BC, Kounaves SP. Evidence for the distribution of perchlorates on Mars. Int J Astrobiol. 2016;15:311–5.

[24] Nie NH, Bent DH, Hull CH. SPSS: Statistical package for the social sciences. New York: McGraw-Hill; 1970.

[25] Atiyeh RM, Arancon N, Edwards CA, Metzger JD. Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. Bioresour Technol. 2000;75:175–80.

[26] Herrero M, Thornton PK, Notenbaert AM, Wood S, Msangi S, Freeman HA, et al. Smart investments in sustainable food production: revisiting mixed crop-livestock systems. Science. 2010;327:822–5.

[27] Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Disposition. 1995;16:351–80.

[28] Navarro-González R, Vargas E, De La Rosa J, Raga AC, McKay CP. Reanalysis of the Viking results suggests perchlorate and organics at midlatitudes on Mars. J Geophys Res E: Planets. 2010;115:E12010.

[29] Zeng X, Li X, Wang S, Li S, Spring N, Tang H, et al. JMSS-1: a new Martian soil simulant. Earth, Planets Space. 2015;67:72. doi: 10.1186/s40623-015-0248-5.

[30] Oze C, Beisel J, Dabsys E, Dall J, North G, Scott A, et al. Perchlorate and agriculture on Mars. Soil Syst. 2021;5(37). doi: 10.3390/soilsystems5030037.

[31] Eichler A, Hadland N, Pickett D, Masaitis D, Handy D, Perez A, et al. Challenging the agricultural viability of martian regolith simulants. Icarus. 2021;354:114022. doi: 10.1016/j.icarus.2020.114022.

Appendix

Table 1: Fresh and dry weights of the Rucola.

Code	Treatment	Fresh weight (g)	Dry weight (g)
E12	NME	4.4	0.63
E16	NME	1.13	0.16
E4	NME	9.301	2.29
E8	NME	3.89	0.71
M12	NMM	16.79	3.33
M16	NMM	15.95	3.79
M4	NMM	14.7	2.74
M8	NMM	15.65	2.49
E10	NNE	4.68	0.59
E14	NNE	0.7	0.05
E2	NNE	9.29	1.52
E6	NNE	4.65	0.75
M10	NNM	2.73	0.41
M14	NNM	1.53	0.19
M2	NNM	1.05	0.22
M6	NNM	1.52	0.18
E1	WME	7.57	0.8
E13	WME	4.23	1.19
E5	WME	3.7	0.53
E9	WME	2.28	0.44
M1	WMM	13.39	3.21
M13	WMM	17.74	3.54
M5	WMM	15.28	2.57
M9	WMM	13.37	2.86
E11	WNE	0.48	0.02
E15	WNE	0.49	0.23
E3	WNE	0.62	0.07
E7	WNE	6.73	1.13
M11	WNM	3.45	0.32
M15	WNM	3.1	0.46
M3	WNM	4.2	0.61
M7	WNM	3.14	0.49

The code gives the soil type (E for Earth control and M for Mars soil simulant) and pot number. For treatment W/N first letter for worm or no worm added; for the second letter M/N for manure or no manure added; for the third letter E/M for Earth control and Mars soil simulant.

Table 2: Number of worms added and retrieved.

		Added		Retrieved			
Type	Code	*Caliginosa*	*Dendrobaena*	Total	*Caliginosa*	*Dendrobaena*	Baby
WM	E1	2	2	0	0	0	0
NN	E2	0	0				
WN	E3	2	2	0	0	0	0
NM	E4	0	0				
WM	E5	2	2	0	0	0	0
NN	E6	0	0				
WN	E7	2	2	5	4	1	1
NM	E8	0	0				
WM	E9	2	2	0	0	0	0
NN	E10	0	0				
WN	E11	2	2	0	0	0	0
NM	E12	0	0				
WM	E13	2	2	0	0	0	0
NN	E14	0	0				
WN	E15	2	2	0	0	0	0
NM	E16	0	0				
WM	M1	2	2	0	0	0	0
NN	M2	0	0				
WN	M3	2	2	2	2	0	0
NM	M4	0	0				
WM	M5	2	2	1	0	1	1
NN	M6	0	0				
WN	M7	2	2	2	2	0	0
NM	M8	0	0				
WM	M9	2	2	3	3	0	1
NN	M10	0	0				
WN	M11	2	2	2	2	0	0
NM	M12	0	0				
WM	M13	2	2	0	0	0	0
NN	M14	0	0				
WN	M15	2	2	1	1	0	0
NM	M16	0	0				

Type gives the treatment: with W/N first letter for worm or no worm added; M/N for manure or no manure added. Code gives the soil type, with E for Earth control and M for Mars soil simulant; the number is the pot number. In total 16 worms that escaped were retrieved from the water bath. We found 16 worms in the pots, of which 3 were offspring. Thus, of the 64 worms added 29 were accounted for.

Table 3: Average FW and DW of the aboveground biomass of Rucola.

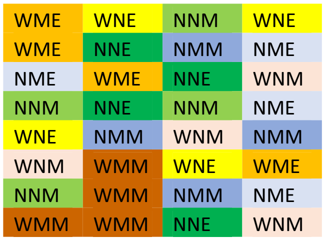
Treatment	FW		DW	
	Avg	**S.E.**	**Avg**	**S.E.**
NME	4.68	3.40	0.95	0.93
NMM	15.77	0.86	3.09	0.59
NNE	4.83	3.51	0.73	0.61
NNM	1.71	0.72	0.25	0.11
WME	4.4	2.24	0.74	0.34
WMM	14.95	2.07	3.05	0.42
WNE	2.08	3.10	0.36	0.52
WNM	3.47	0.51	0.47	0.12

Treatment codes are built up as follows: first letter for earthworm (W) or no worm (N) added; for the second letter for manure (M) or no manure (N) added; the third letter for Earth control soil (E) and Mars soil simulant (M) added. Results per pot can be found in Appendix Table 1.

Table 4: Results of the 3-way-ANOVA for FW and DW for all treatments and interactions.

	Source	Sum of squares	Degrees of freedom	Mean square	*F*	sign. (*p*-value)
FW	Model	16.455	8	2.057	21.404	**0.000**
	Planet	0.987	1	0.987	10.269	**0.004**
	Manure	2.557	1	2.557	26.612	**0.000**
	Earthworm	0.018	1	0.018	0.188	0.668
	Manure * earthworm	0.026	1	0.026	0.267	0.610
	Planet * earthworm	0.321	1	0.321	3.339	0.080
	Planet * manure	0.490	1	0.490	5.095	**0.033**
	Planet * manure * earthworm	0.443	1	0.443	4.609	**0.042**
	Error	2.306	24	0.096		
	Total	18.762	32			
DW	Model	7.656	8	0.957	5.950	**0.000**
	Planet	1.256	1	1.256	7.811	**0.010**
	Manure	3.918	1	3.918	24.359	**0.000**
	Earthworm	0.015	1	0.015	0.093	0.763
	Manure * earthworm	0.027	1	0.027	0.168	0.685
	Planet * earthworm	0.277	1	0.277	1.719	0.202
	Planet * manure	0.565	1	0.565	3.511	0.073
	Planet * manure * earthworm	0.333	1	0.333	2.071	0.163
	Error	3.860	24	0.161		
	Total	11.516	32			

Planet indicates the effect of the difference between Earth potting soil and Mars soil simulant MMS. Data were natural log transformed to gain normal distribution. In **bold**, *p*-values <0.05.



WME	WNE	NNM	WNE
WME	NNE	NMM	NME
NME	WME	NNE	WNM
NNM	NNE	NNM	NME
WNE	NMM	WNM	NMM
WNM	WMM	WNE	WME
NNM	WMM	NMM	NME
WMM	WMM	NNE	WNM

Figure 1: Schematic diagram of the pot set up of the experiment. Treatment codes are built up as follows: first letter for earthworm (W) or no worm (N) added; for the second letter for manure (M) or no manure (N) added; the third letter for Earth control soil (E) and Mars soil simulant (M) added.