

Supplementary information

Microbial biomining from asteroidal material onboard the International Space Station

Author

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Supplementary Note 1

Microbial final cell concentration and pH

Final cell concentration was measured immediately after sample recovery, as an indication of microbial growth, by analysing the liquid fraction of each sample by spectrometric analysis (optical density, $\lambda = 600$ nm), flow cytometry and colony forming unit (CFU) assay.

Optical density data at $\lambda=600$ nm (Supplementary Figure 10, Supplementary Table 7) of the liquid fraction were obtained after spaceflight. All samples showed higher final cell concentration on Earth compared to space (ISS). Using a wavelength of 530 nm (Supplementary Figure 10, Supplementary Table 7), which can be used for filamentous fungi¹, we observed a similar trend.

Optical density at different wavelengths could potentially be sensitive to metabolic changes, therefore analysis of post-spaceflight final cell concentration was also obtained by flow cytometry (Supplementary Figure 10, Supplementary Table 7). The flow cytometry results showed no gravity-driven difference between events measured with the bacterial dye for *S. desiccabilis* samples $[(3.97 \pm 0.06) \times 10^9$ cell/mL on ISS, $(3.27 \pm 0.32) \times 10^9$ cell/mL on Earth, respectively], and a slight increase (<10 -fold) in Consortium samples (samples containing both the bacterium and the fungus) in Earth gravity compared to microgravity $[(6.47 \pm 1.98) \times 10^8$ cell/mL on ISS against $(3.64 \pm 0.14) \times 10^9$ cell/mL on Earth, respectively] (Supplementary Figure 10, Supplementary Table 7). Differences in the fungi-associated events were present, when comparing *P. simplicissimum* in different gravities, with Earth samples showing concentrations one order of magnitude higher than the ISS $[(3.50 \pm 1.99) \times 10^6$ cell/mL on ISS against $(6.37 \pm 2.11) \times 10^7$ cell/mL on Earth, respectively]. Similarly, Consortium samples showed a slight increase (<10 -fold) in final cell concentration on Earth samples compared to ISS $[(1.43 \pm 0.34) \times 10^6$ cell/mL on ISS against $(7.69 \pm 1.33) \times 10^6$ cell/mL on Earth, respectively]. The presence of positive events in the sterile diluent and non-biological controls, potentially caused by small non-biological particulate matter, were in the worst case one order of magnitude smaller than the biological samples ($<10\%$) and are thus considered negligible.

Additionally, CFU assay was performed to identify potential contaminations not identifiable by the previous two methods (Supplementary Figure 11). CFU analysis of *S. desiccabilis* showed reduced colony numbers in ISS compared to Earth samples, in both bacterial-only $[(7.17 \pm 2.88) \times 10^6$ CFU/mL on ISS, $(1.75 \pm 1.24) \times 10^{11}$ CFU/mL on Earth], and Consortium samples $[(4.39 \pm 2.38) \times 10^6$ CFU/mL on ISS against $(2.38 \pm 2.05) \times 10^{10}$ CFU/mL on Earth]. This is in contrast

with the flow cytometry results for *S. desiccabilis*, but not for the other samples. Fungal CFU analysis of *P. simplicissimum* and Consortium showed concentrations two orders of magnitude higher on the ISS compared to Earth [*P. simplicissimum*: $(1.37 \pm 1.01) \times 10^4$ CFU/mL on ISS, $(4.58 \pm 3.08) \times 10^2$ CFU/mL on Earth; Consortium: $(8.89 \pm 6.59) \times 10^3$ CFU/mL on ISS, $(8.33 \pm 4.17) \times 10^1$ CFU/mL on Earth]. This is in contrast with both the OD₆₀₀ and the flow cytometry measurements. The discrepancies observed might be explained by the fact that CFU assay measures actively dividing cells, potentially reflecting different survival rates on Earth and ISS populations.

Overall, these results on final cell concentration provide different answers depending on the technique used, bearing on the widely inconsistent reports of the effects of space on microorganisms^{2,3}. The interplay of viability, metabolic states and other factors may influence how the effects of microgravity are manifested in cell numbers depending on the method used to measure them. Thus, experiments investigating the effects of microgravity should choose the method of analysis carefully and preferably more than one method should be used.

We observed contaminations in some of our CFU assays from endogenous (*S. desiccabilis*) and exogenous (Contaminant 1 and 2, Supplementary Figure 11) species. 16S rDNA sequencing of the contaminated ISS samples could not identify any species, suggesting the contamination was not present in the original samples, but just on the plates used for CFU, as corroborated by flow cytometry. Taken together, these results confirmed that all ISS samples were clean of contamination.

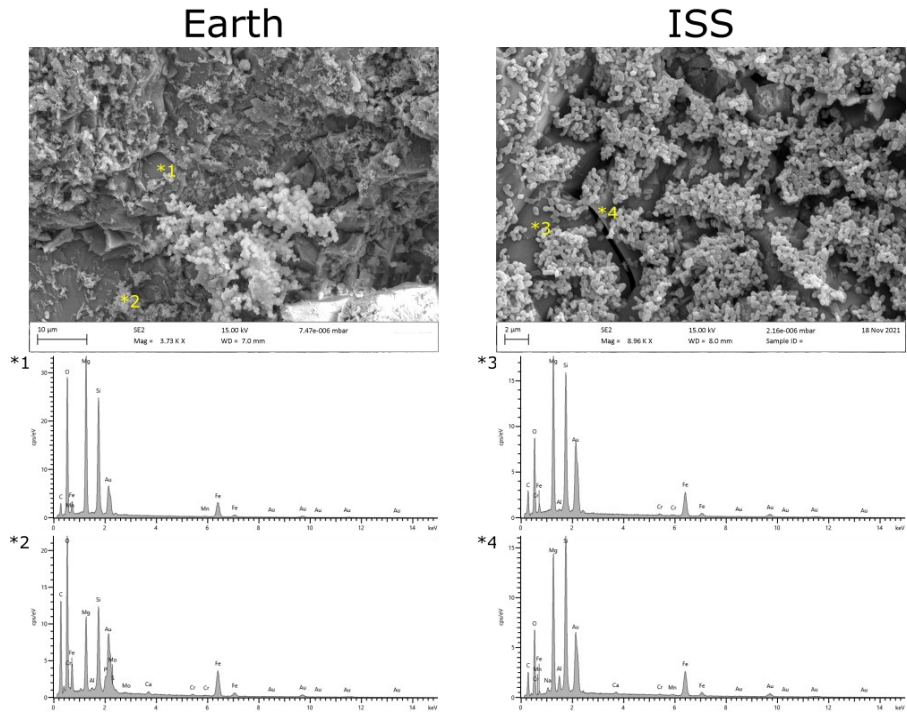
For Earth samples, 16S rDNA sequencing identified a species of the *Sphingomonas* genus for one of the two bacterial samples affected, and for two of the three Consortium samples affected. Identification failed for the remaining affected samples (Supplementary Table 8, see Methods for details). Taken together, this suggests original *S. desiccabilis* and Consortium Earth samples were not contaminated (Supplementary Figure 11). For the *P. simplicissimum* Earth samples, alignment for three of the four affected sample sequences suggested contamination from the order Bacillales, probably from the genera *Bacillus* and/or *Planococcus*. Identification failed for the fourth sample (Supplementary Table 8). Sequences of the isolated species (Contaminant 1 and 2) aligned with the genera *Bacillus* and *Paenibacillus*, suggesting these as the most probable genera for the contaminants (Supplementary Table 8). We can hypothesise that any relevant effect of the contamination would have likely influenced all the affected samples equally. From the

metabolomics analysis we note this is not the case, as suggested by the absence of contaminant-related clusters in the heatmap results and the PCA analysis (Fig. 6, S6). This might indicate that, if any effect was present, this does not have a relevant impairing role in the results. We were not able to repeat the experiment to test these hypotheses, due to the unavailability of identical material from the same L-chondrite meteorite. However, even when the Earth fungal results require these caveats, the other results are completely valid. Spare samples prepared for the Earth control experiment did not show Contaminant 1 or 2 colonies formation (data not shown), suggesting the contamination did not occur during sample preparation but probably during sample post-flight processing or hardware integration.

The pH is one of the potential influencing factors in leaching. We measured the pH of the liquid fractions after spaceflight (Supplementary Table 9), reporting values between 7.21 and 7.43 among all samples. It must be noted that the values were likely affected by the presence of the fixative (RNAlater, $\text{pH}=4.87\pm0.01$), which was necessary to halt the experiment at its end and prevented us from deriving conclusions on the changing pH during the experiment.

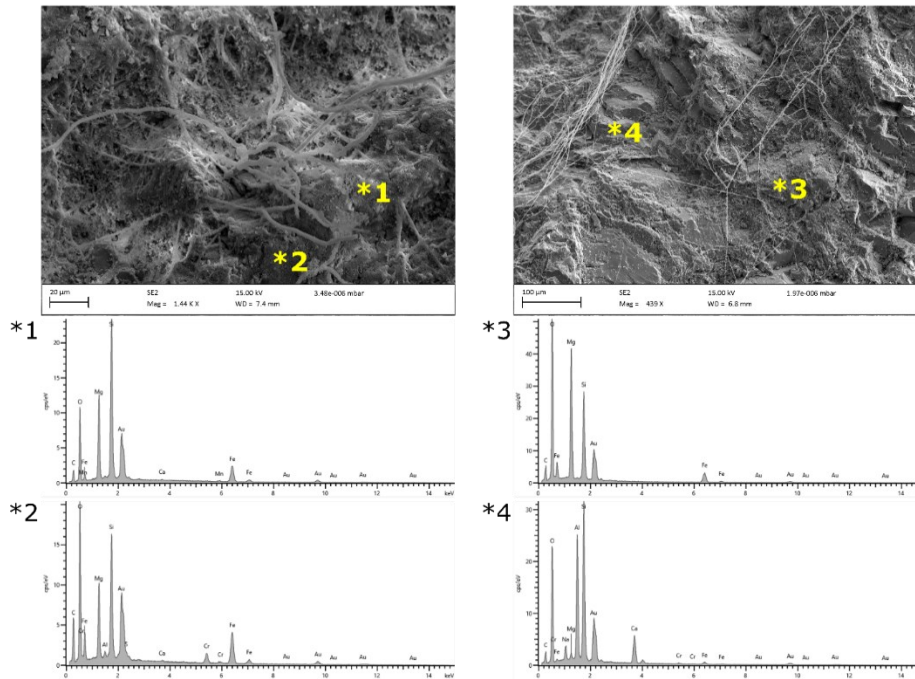
Supplementary Figures

S. desiccabilis



Supplementary Figure 1. Scanning electron microscopy images of *S. desiccabilis* biofilm formed on top of the meteorite rock fragments for ground (Earth) and space (ISS) samples. Asterisks indicate the points where energy-dispersive X-ray spectroscopy (EDS) spectra were measured. Gold peaks are from the gold sample coating.

ISS



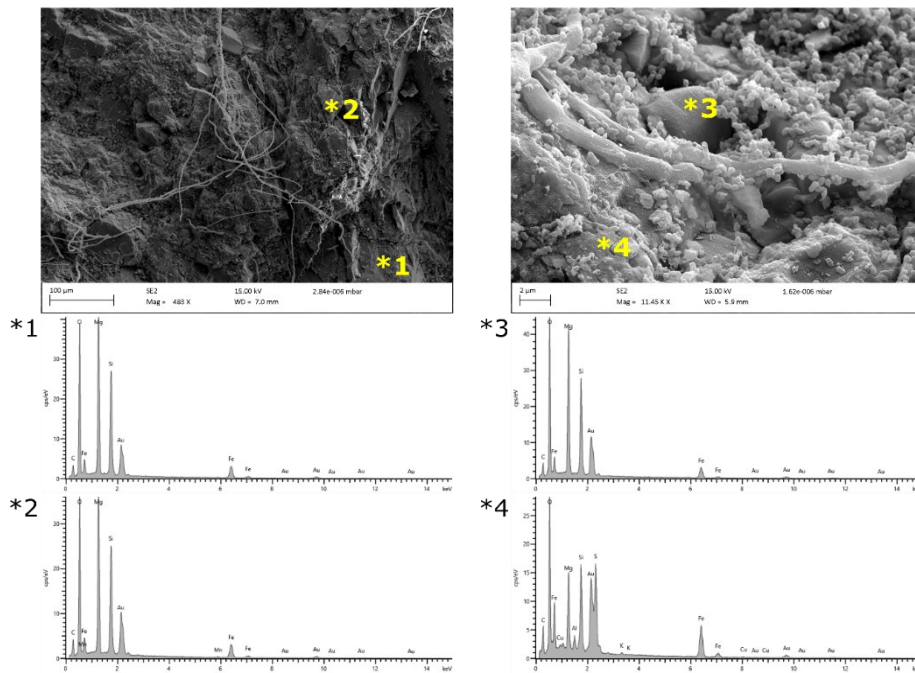
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Supplementary Figure 2. Scanning electron microscopy images of *P. simplicissimum* mycelium formed on top of the meteorite rock fragments for ground (Earth) and space (ISS) samples. Asterisks indicate the points where EDS spectra were measured. Gold peaks are from the gold sample coating.

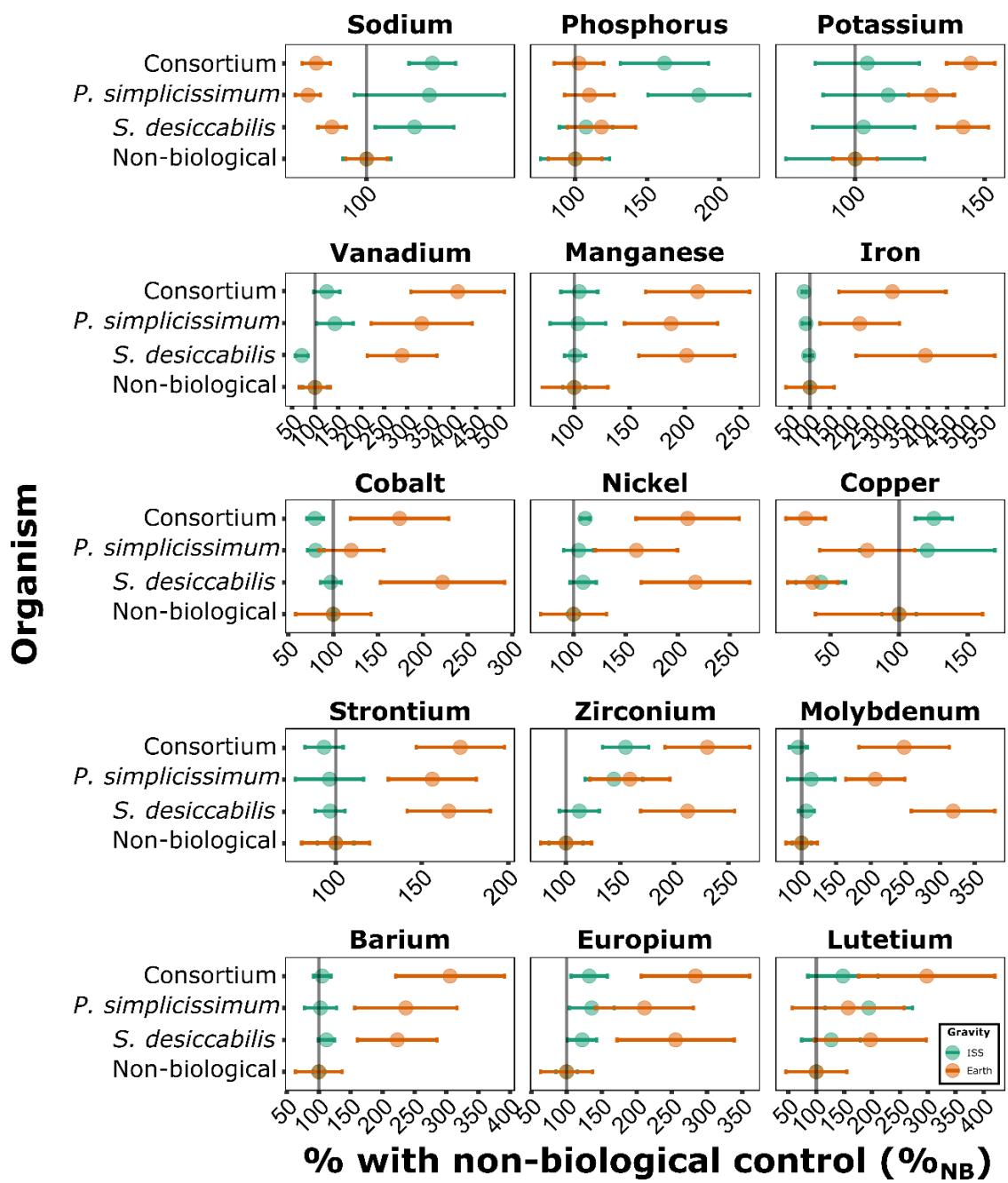
Consortium

Earth

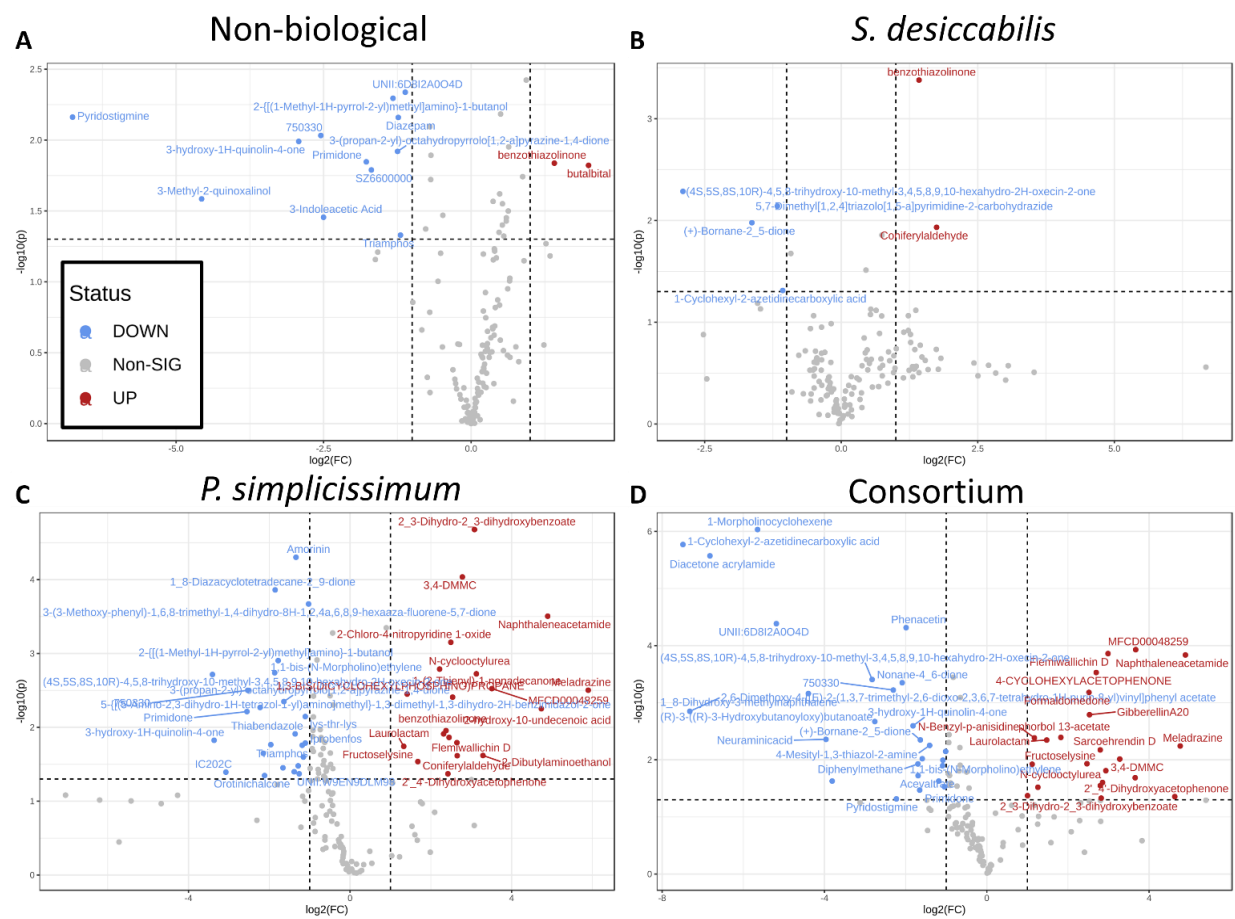
ISS



Supplementary Figure 3. Scanning electron microscopy images of bacterial biofilm and fungal mycelium (Consortium) formation on meteorite rock fragments for ground (Earth) and space (ISS) consortium samples. Asterisks indicate the points where EDS spectra were measured. Gold peaks are from the gold sample coating.



123 **Supplementary Figure 4.** Percentage ratios with the non-biological control (%_{NB}) for further 15
124 elements whose bioextraction showed at least one p-value < 0.05 (ANOVA). Values are separated
125 per gravity condition (ISS = microgravity, green circles, Earth = 1 x g, orange circles). Circles
126 indicate mean values; error bars indicate standard error; grey vertical lines indicate 100% values
127 (i.e., same bioleaching efficiency as non-biological samples).



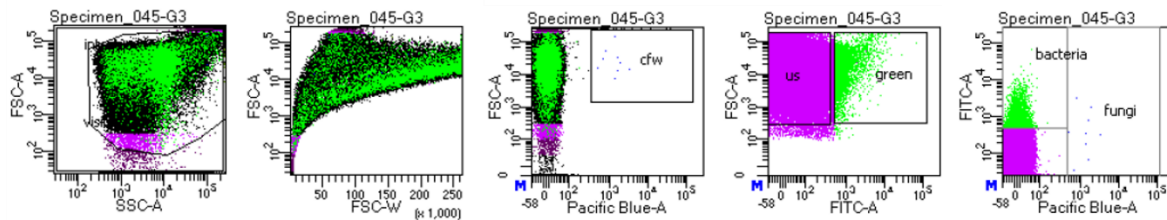
Supplementary Figure 5. Volcano plots showing up- or downregulations of features in space compared to Earth. Comparisons are shown for A) non-biological, B) *S. desiccabilis*; C) *P. simplicissimum* and D) Consortium samples.



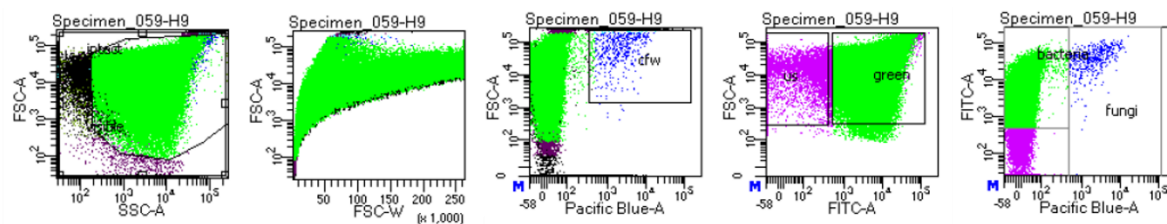
Supplementary Figure 6. Heatmap showing the top 70 features measured in the metabolomics analysis, clustered per gravity condition and organism. Each column represents a single sample.

Numbers on the left represent 6 visual clusters suggesting metabolomic patterns dependent on the organism and/or the gravity condition. Cluster 1 represents features expressed almost solely in the presence of *P. simplicissimum* in space. Cluster 2 shows features present in all samples except those containing the fungus (alone or in Consortium) on the ISS, potentially indicating features consumed by the fungi when in microgravity conditions. Cluster 3 shows features present in the non-biological control samples regardless of gravity condition, while cluster 4 shows features mostly present in the non-biological control samples in Earth gravity. Cluster 5 shows features highly associated with the presence of *S. desiccabilis*. Complementary to Cluster 1, Cluster 6 represents features solely produced in the presence of the fungus in Earth gravity. Interestingly, the observation of these clear patterns, together with the separate clusters observed in Fig. 6D, suggests that the contaminant species (Supplementary Figure 11), if present in the original samples, had no or minor effects on the metabolomics results. Features with long names were indicated with their chemical formula, with asterisks showing the complete names at the bottom of the figure.

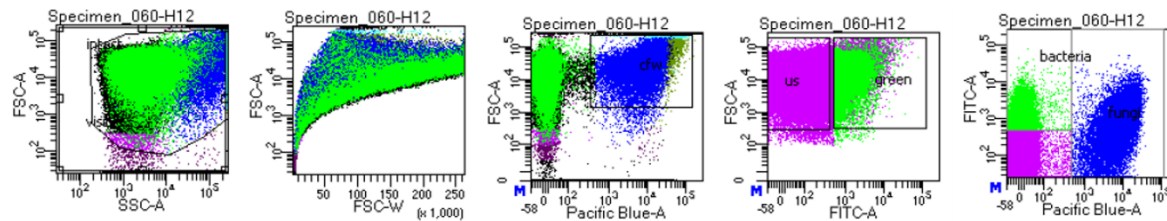
Non-biological sample



S. desiccabilis-containing sample

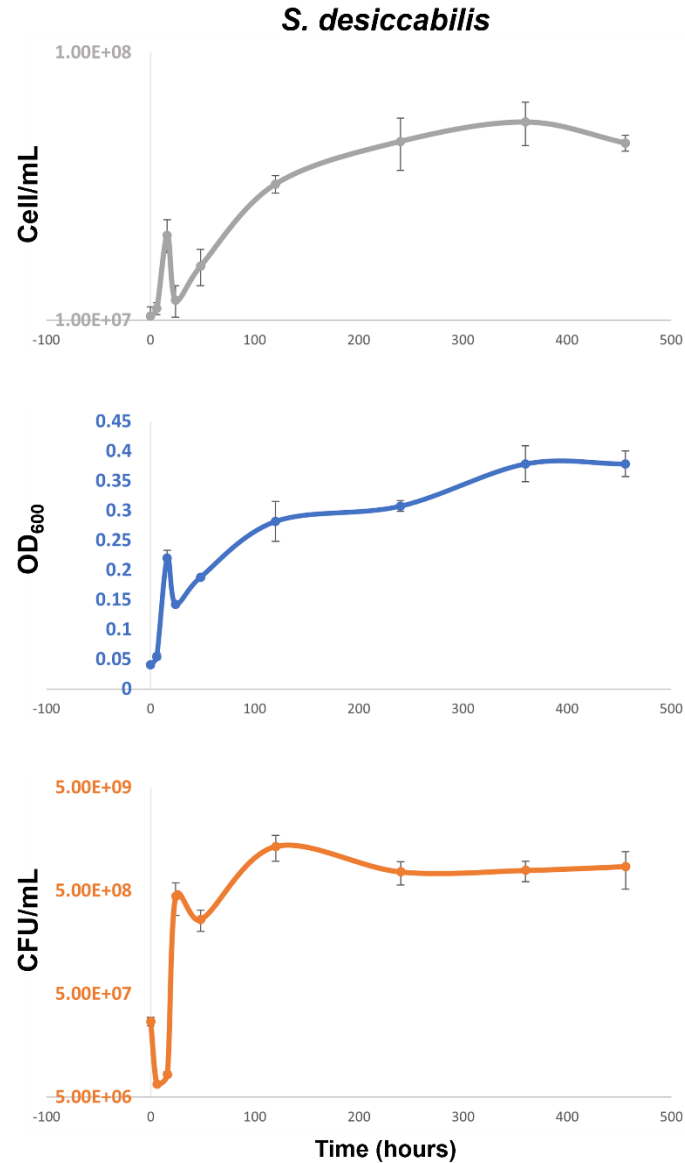


P. simplicissimum-containing sample



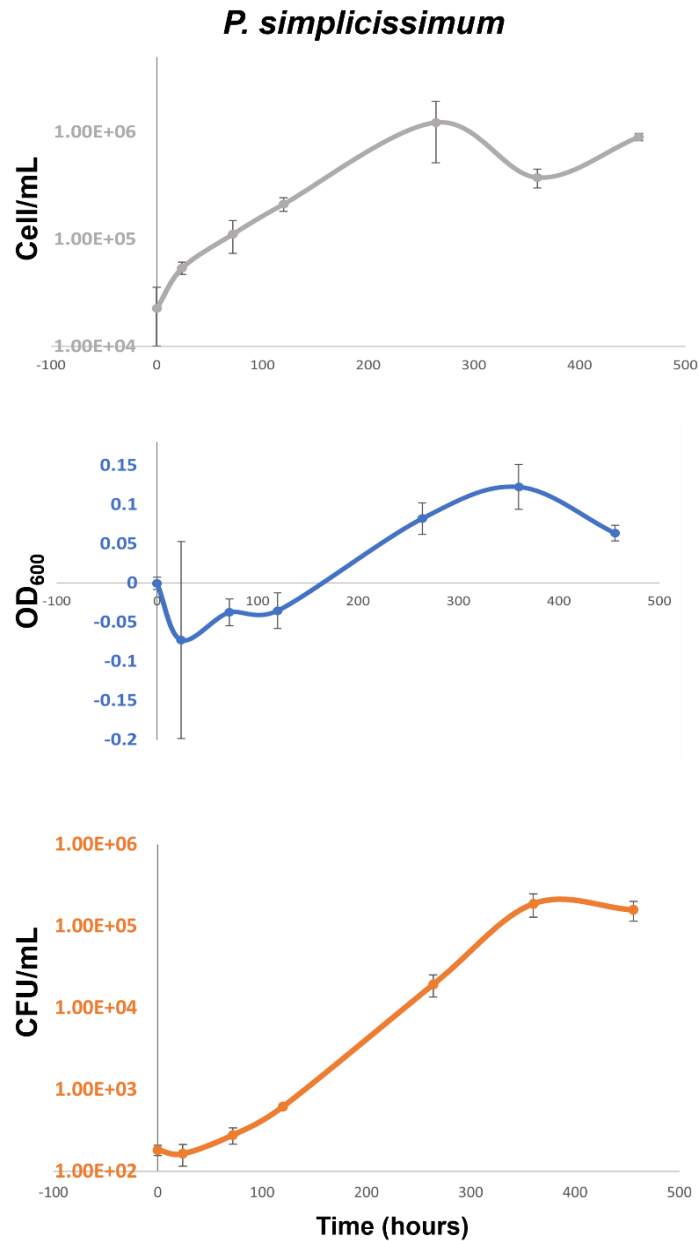
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152 **Supplementary Figure 7.** Gating strategy for the flow cytometry analysis, constructed using the
 153 software BD FACS Diva 8.0.1, to distinguish bacterial from fungal cells. Three representative
 154 samples are shown here (top: a non-biological space control; middle: a *S. desiccabilis* containing
 155 sample; bottom: a *P. simplicissimum* containing sample). The events reported in this manuscript
 156 were obtained from the fungi and bacteria gating. The Pacific Blue-A filter was used to visualise
 157 calcofluor white (cfw, fungi-specific dye), while FITC-A filter was used for backlight green
 158 (green, bacteria-specific stain). us gating indicates unstained particles.

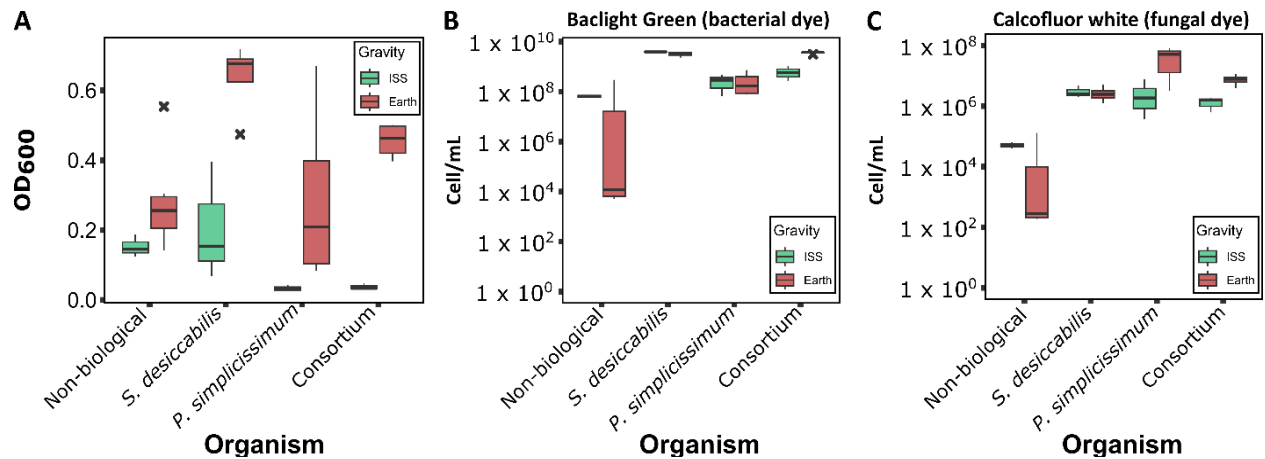


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160 **Supplementary Figure 8.** Growth curve for *S. desiccabilis* showing comparison between the three
 161 methods for final cell concentration used in this work. *S. desiccabilis* growth curve was determined
 162 in 19 days (456 hours), which is the timeline used for the BioAsteroid experiment. Flow cytometry
 163 (cell/mL, in grey), OD₆₀₀ (in blue) and CFU/mL (in orange), are shown. Points represent average
 164 values, error bars represent standard errors.

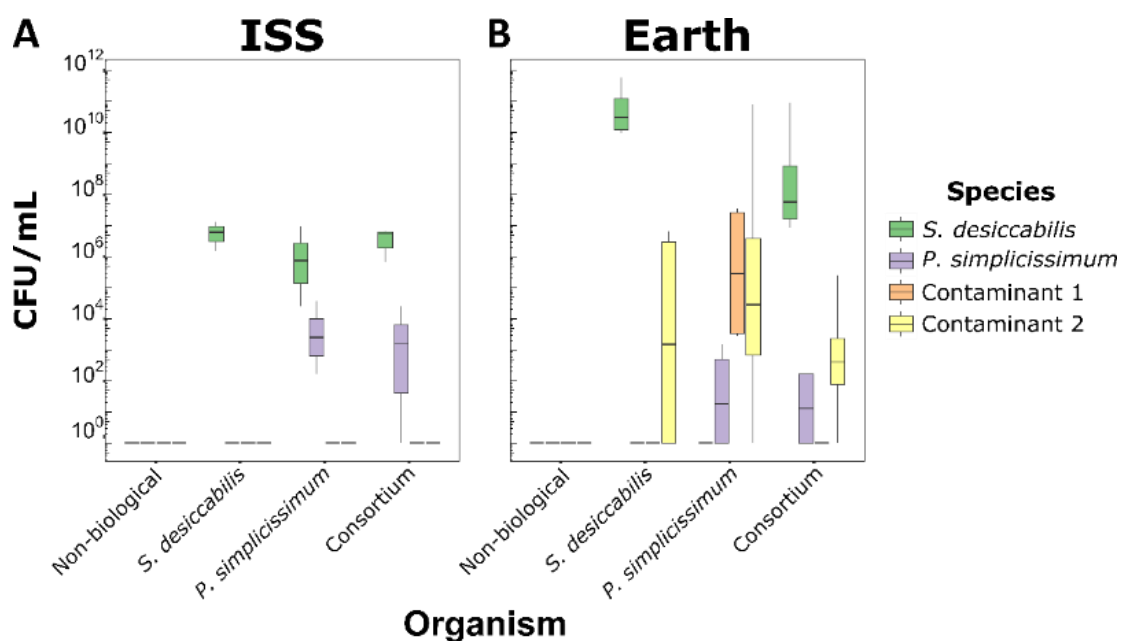


Supplementary Figure 9. Growth curve for *P. simplicissimum* showing comparison between the three methods for final cell concentration used in this work. *P. simplicissimum* growth curve was determined in 19 days (456 hours), which is the timeline used for the BioAsteroid experiment. Flow cytometry (cell/mL, in grey), OD₆₀₀ (in blue) and CFU/mL (in orange), are shown. Points represent average values, error bars represent standard errors.



Supplementary Figure 10. Final cell concentration in the liquid fraction of the BioAsteroid experiment. Optical density ($\lambda = 600\text{nm}$, A) and flow cytometry analysis measured (B-C) from the liquid fraction of the ISS and Earth samples. For flow cytometry, values represent the average events counted in 100 μL of sample stained with B) Baclight Green (specific for bacterial cells) and C) Calcofluor White (specific for the fungal cells). Black X represent outliers; $n=3$ for ISS samples, $4 \leq n \leq 6$ for Earth samples.

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182 **Supplementary Figure 11.** Final cell concentrations measured in the liquid fraction of the space
 183 (ISS, A) and ground control (Earth, B) samples in terms of CFU/mL. CFU concentration of *S*
 184 *desiccabilis*, *P. simplicissimum*, Contaminant 1 and Contaminant 2 species are indicated. A
 185 horizontal line indicates a value of CFU/mL = 0.

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Supplementary Tables

Supplementary Table 1.

Statistical values calculated with ANOVA for the raw concentrations (ICP-MS data) of the 22 elements whose p-value was ≤ 0.05 for at least one of the variables analysed (*Organism*, *Gravity* or their interaction), and for at least one biologically relevant pairwise comparison. In pale yellow the PGEs analysed in this work; in bold p-values ≤ 0.05 .

#	Element	<i>Organism</i>		<i>Gravity</i>		<i>Organism x Gravity</i>	
		F	p value	F	p value	F	p value
1	Sodium	0.830	0.492	15.282	0.001	3.384	0.036
2	Aluminium	0.254	0.858	4.730	0.041	5.054	0.008
3	Silicon	10.449	0.0002	18.389	0.0003	3.172	0.044
4	Phosphorus	2.467	0.089	0.166	0.688	3.344	0.038
5	Potassium	5.778	0.005	7.354	0.013	2.720	0.069
6	Scandium	2.028	0.139	29.871	0.00002	1.354	0.283
7	Vanadium	10.941	0.0001	3.548	0.073	3.488	0.033
8	Manganese	6.042	0.004	1.18910	0.287	2.745	0.067
9	Iron	9.908	0.00025	29.340	0.00002	6.908	0.002
10	Cobalt	4.731	0.011	15.279	0.001	3.138	0.046
11	Nickel	8.519	0.001	29.196	0.00002	2.060	0.135
12	Copper	1.536	0.233	6.473	0.019	0.695	0.565
13	Strontium	4.118	0.018	7.106	0.014	3.057	0.050
14	Zirconium	11.172	0.0001	5.442	0.029	2.187	0.118
15	Molybdenum	5.745	0.005	10.495	0.004	2.668	0.073
16	Ruthenium	6.744	0.002	15.859	0.001	1.506	0.241
17	Palladium	12.148	0.0001	10.405	0.004	6.709	0.002
18	Barium	6.041	0.004	0.167	0.687	2.862	0.060
19	Europium	6.051	0.004	12.202	0.002	0.764	0.526
20	Erbium	2.010	0.142	21.970	0.0001	1.530	0.235
21	Lutetium	3.783	0.025	23.987	0.0001	1.690	0.198
22	Platinum	9.992	0.00024	30.137	0.00002	7.478	0.001

Supplementary Table 2.

P-values from the pairwise comparison of the raw concentrations with Tukey post-hoc test for the PGEs (ruthenium, palladium and platinum). Green shadowed cells indicate p-values < 0.05 from biologically relevant comparison. NB = non-biological; SD = *S. desiccabilis*; PS = *P. simplicissimum*; CN = Consortium.

			ISS				Earth			
			NB	SD	PS	CN	NB	SD	PS	CN
Ruthenium	ISS	NB								
		SD	1							
		PS	0.76	0.83						
		CN	0.83	0.89	1					
	Earth	NB	0.54	0.04	0.0009	0.0012				
		SD	1	1	0.59	0.68	0.042			
		PS	0.96	0.94	0.22	0.28	0.212	0.99		
		CN	1	1	0.62	0.71	0.038	1	0.99	
Palladium	ISS	NB								
		SD	0.99							
		PS	0.73	0.58						
		CN	0.99	0.99	0.86					
	Earth	NB	0.0006	0.0003	0.05	0.0013				
		SD	0.99	1	0.44	0.99	7.02 x 10 ⁻⁵			
		PS	0.62	0.47	1	0.79	0.03	0.31		
		CN	0.92	0.81	0.99	0.98	0.006	0.67	0.99	
Platinum	ISS	NB								
		SD	0.44							
		PS	0.65	0.016						
		CN	0.66	0.016	1.00					
	Earth	NB	0.001	0.20	0.00001	0.00001				
		SD	0.97	0.91	0.13	0.13	0.005			
		PS	0.32	1.00	0.007	0.01	0.15	0.83		
		CN	0.98	0.88	0.14	0.15	0.004	1.00	0.79	

202 **Supplementary Table 3.**

203 **Platinum group element (PGEs) biomining.** Total concentration (in ng/mL, or ppb) extracted from the meteorite rock, percentage of
 204 element extraction compared to total concentration in the meteoritic material (%_M), and percentage ratios with the non-biological
 205 controls (%_{NB}), for each gravity condition (ISS = microgravity; Earth = Earth gravity) of ruthenium, palladium and platinum. NB = non-
 206 biological; SD = *S. desiccabilis*; PS = *P. simplicissimum*; CN = Consortium.

	Ruthenium						Palladium						Platinum					
	ng/mL (ppb)		%_M		%_{NB}		ng/mL (ppb)		%_M		%_{NB}		ng/mL (ppb)		%_M		%_{NB}	
	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth	ISS	Earth
N	0.22	0.10	14.78	6.59	100.00	100.00	0.05	0.69	2.17	29.47	100.00	100.00	0.22	0.12	0.24	0.13	100.00	100.00
B	±0.02	±0.03	±2.19	±2.13	±13.23	±42.73	±0.01	±0.10	±0.58	±6.47	±29.82	±20.65	±0.02	±0.01	±0.04	±0.02	±10.07	±12.25
S	0.23	0.21	15.20	14.32	102.87	217.12	0.02	0.00	0.73	0.05	33.55	0.18	0.17	0.20	0.19	0.22	78.03	166.09
D	±0.02	±0.02	±2.40	±2.30	±14.67	±69.93	±0.01	±0.00	±0.43	±0.05	±20.11	±0.18	±0.00	±0.01	±0.02	±0.03	±5.91	±12.25
P	0.29	0.19	19.29	12.44	130.56	188.64	0.29	0.29	11.91	12.25	549.34	41.58	0.26	0.17	0.29	0.19	118.39	142.16
S	±0.03	±0.03	±3.22	±2.37	±19.94	±63.81	±0.10	±12.3	±4.83	±6.70	±234.4	±22.53	±0.03	±0.02	±0.05	±0.03	±17.63	±20.14
C	0.28	0.22	18.89	14.44	127.84	218.90	0.09	0.21	3.74	9.03	172.37	30.65	0.26	0.20	0.29	0.22	118.24	167.33
N	±0.02	±0.01	±2.46	±1.88	±14.24	±67.45	±0.01	±0.03	±0.72	±1.91	±40.23	±6.05	±0.01	±0.2	±0.04	±0.03	±9.72	±14.86

207

208

209 **Supplementary Table 4.**

210 Percentage ratios with the non-biological controls (%_{NB}) and with the meteorite (%_M), for each gravity condition (ISS = microgravity;
 211 Earth = Earth gravity) of the further 15 elements whose at least one biologically relevant pairwise comparison provided a p-value <0.05
 212 (mean±standard error). Elements are listed in order of atomic number.

		% _{NB}				% _M			
		Non-biological	<i>S. desiccabilis</i>	<i>P. simpliciss.</i>	Consortium	Non-biological	<i>S. desiccabilis</i>	<i>P. simpliciss.</i>	Consortium
Na	ISS	100.00±6.41	112.83±10.51	116.79±20.09	117.57±6.24	0.57±0.07	0.64±0.09	0.66±0.14	0.67±0.08
	Earth	100.00±5.61	90.72±3.77	84.31±3.36	86.53±3.78	0.82±0.11	0.74±0.09	0.69±0.08	0.71±0.09
P	ISS	100.00±23.98	107.58±18.54	185.86±35.28	162.02±30.60	0.12±0.03	0.15±0.02	0.26±0.04	0.23±0.04
	Earth	100.00±18.59	118.30±23.72	109.84±17.17	102.63±17.22	0.18±0.03	0.21±0.04	0.19±0.03	0.18±0.03
K	ISS	100.00±26.79	103.22±19.65	112.77±25.14	104.67±20.06	1.13±0.23	1.16±0.09	1.27±0.18	1.18±0.10
	Earth	100.00±8.55	141.65±9.85	129.44±8.85	144.62±9.30	1.06±0.10	1.50±0.13	1.37±0.11	1.53±0.12
V	ISS	100.00±27.00	71.40±13.97	143.47±39.69	125.54±28.67	NA	NA	NA	NA
	Earth	100.00±33.98	289.49±75.64	332.18±109.81	410.51±101.8	NA	NA	NA	NA
Mn	ISS	100.00±10.21	100.76±9.45	103.37±25.16	104.55±16.58	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Earth	100.00±30.30	201.47±43.39	187.34±41.98	211.31±46.98	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Fe	ISS	100.00±2.84	96.67±10.93	89.83±10.15	85.12±5.26	0.11±0.01	0.10±0.02	0.10±0.02	0.09±0.01
	Earth	100.00±61.61	394.35±176.82	227.03±101.55	309.94±136.26	0.02±0.01	0.10±0.02	0.06±0.01	0.08±0.01
Co	ISS	100.00±2.40	97.34±11.64	80.24±9.23	79.53±9.28	0.05±0.10	0.49±0.11	0.40±0.10	0.40±0.10
	Earth	100.00±42.01	221.93±69.40	120.03±36.20	174.04±54.83	0.21±0.10	0.46±0.10	0.25±0.05	0.36±0.10
Ni	ISS	100.00±5.89	109.18±12.58	104.75±14.25	111.02±4.68	0.15±0.01	0.16±0.02	0.16±0.02	0.17±0.01
	Earth	100.00±31.77	217.12±52.12	160.30±39.46	209.64±49.57	0.06±0.01	0.14±0.01	0.10±0.01	0.13±0.01
Cu	ISS	100.00±12.48	43.01±18.10	120.60±49.22	125.38±13.51	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Earth	100.00±60.88	37.07±18.33	76.83±34.74	31.92±14.42	0.01±0.01	0.01±0.00	0.010±0.00	0.00±0.00

Sr	ISS	100.00±10.55	96.73±8.75	96.41±19.79	93.22±11.09	0.39±0.06	0.37±0.05	0.37±0.09	0.36±0.06
	Earth	100.00±19.73	165.53±24.11	156.02±25.57	172.28±25.62	0.21±0.04	0.35±0.05	0.33±0.05	0.37±0.05
Zr	ISS	100.00±15.67	112.00±12.63	144.23±26.72	155.06±21.38	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Earth	100.00±23.54	212.33±43.33	159.03±37.02	230.62±39.13	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Mo	ISS	100.00±13.90	106.77±11.63	113.83±34.27	95.05±12.86	0.03±0.01	0.03±0.01	0.03±0.02	0.03±0.01
	Earth	100.00±22.93	319.16±60.41	206.61±42.90	248.24±65.63	0.01±0.01	0.03±0.01	0.02±0.01	0.02±0.01
Ba	ISS	100.00±9.13	112.00±12.63	102.73±24.92	105.31±13.78	0.11±0.02	0.13±0.02	0.12±0.03	0.12±0.02
	Earth	100.00±36.61	223.05±62.37	236.39±80.09	305.66±85.39	0.05±0.02	0.12±0.02	0.13±0.03	0.16±0.03
Eu	ISS	100.00±15.20	122.22±20.78	135.81±31.78	132.31±26.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Earth	100.00±36.97	255.23±83.41	210.97±69.65	283.56±77.18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lu	ISS	100.00±54.73	126.63±52.78	194.13±78.51	147.77±63.01	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.00
	Earth	100.00±54.70	197.23±100.03	157.16±100.53	294.13±122.21	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Supplementary Table 5.

Fold-change (ratio) of the mean raw concentration values for the 18 elements (including 3 PGEs, in pale yellow) whose at least one biologically relevant pairwise comparison provided a p-value <0.05. Fold-changes values between organisms within the same gravity condition, and between gravities (ISS = microgravity; Earth = Earth gravity) within the same organisms (NB = non-biology, SD = *S. desiccabilis*, PS = *P. simplicissimum*, CN = Consortium), are indicated. Ratios ≥ 1.5 have been highlighted in green. Elements are listed in order of atomic number.

Element	Gravity	Organism	Within same gravity Fold-change to NB	Fold changes to SD	Fold changes to PS	Fold changes to CN		Within same organism
Na	ISS	Non-bio	1.0	1.1	0.9	0.9	ISS vs Earth	0.7
		<i>S. desicc</i>	1.1	1.0	1.0	1.0		0.9
		<i>P. simplic</i>	1.2	1.0	1.0	1.0		1.0
		Consortium	1.2	1.0	1.0	1.0		0.9
	Earth	Non-bio	1.0	0.9	1.2	1.2	Earth vs ISS	1.4
		<i>S. desicc</i>	0.9	1.0	1.1	1.0		1.2
		<i>P. simplic</i>	0.8	0.9	1.0	1.0		1.0
		Consortium	0.9	1.0	1.0	1.0		1.1
P	ISS	Non-bio	1.0	1.1	0.5	0.6	ISS vs Earth	0.8
		<i>S. desicc</i>	1.1	1.0	0.6	0.7		0.7
		<i>P. simplic</i>	1.9	1.7	1.0	1.1		1.4
		Consortium	1.6	1.5	0.9	1.0		1.3
	Earth	Non-bio	1.0	1.2	0.9	1.0	Earth vs ISS	1.2
		<i>S. desicc</i>	1.2	1.0	1.1	1.2		1.4
		<i>P. simplic</i>	1.1	0.9	1.0	1.1		0.7
		Consortium	1.0	0.9	0.9	1.0		0.8
K	ISS	Non-bio	1.0	1.0	0.9	1.0	ISS vs Earth	1.1
		<i>S. desicc</i>	1.0	1.0	0.9	1.0		0.8
		<i>P. simplic</i>	1.1	1.1	1.0	1.1		0.9
		Consortium	1.0	1.0	0.9	1.0		0.8
	Earth	Non-bio	1.0	1.4	0.8	0.7	Earth vs ISS	0.9
		<i>S. desicc</i>	1.4	1.0	1.1	1.0		1.3
		<i>P. simplic</i>	1.3	0.9	1.0	0.9		1.1
		Consortium	1.4	1.0	1.1	1.0		1.3
V	ISS	Non-bio	1.0	0.7	0.7	0.8	ISS vs Earth	3.1
		<i>S. desicc</i>	0.7	1.0	0.5	0.6		0.8
		<i>P. simplic</i>	1.4	2.0	1.0	1.1		1.3
		Consortium	1.3	1.8	0.9	1.0		1.0
	Earth	Non-bio	1.0	2.9	0.3	0.2	Earth vs ISS	0.3
		<i>S. desicc</i>	2.9	1.0	0.9	0.7		1.3
		<i>P. simplic</i>	3.3	1.1	1.0	0.8		0.7
		Consortium	4.1	1.4	1.2	1.0		1.1
Mn	ISS	Non-bio	1.0	1.0	1.0	1.0	ISS vs Earth	1.8
		<i>S. desicc</i>	1.0	1.0	1.0	1.0		0.9
		<i>P. simplic</i>	1.0	1.0	1.0	1.0		1.0
		Consortium	1.0	1.0	1.0	1.0		0.9
	Earth	Non-bio	1.0	2.0	0.5	0.5	Earth vs ISS	0.5
		<i>S. desicc</i>	2.0	1.0	1.1	1.0		1.1
		<i>P. simplic</i>	1.9	0.9	1.0	0.9		1.0
		Consortium	2.1	1.0	1.1	1.0		1.1

Fe	ISS	Non-bio	1.0	1.0	1.1	1.2	ISS vs Earth	4.3
		<i>S. desicc</i>	1.0	1.0	1.1	1.1		1.0
		<i>P. simplic</i>	0.9	0.9	1.0	1.1		1.7
		Consortium	0.9	0.9	0.9	1.0		1.2
	Earth	Non-bio	1.0	3.9	0.4	0.3	Earth vs ISS	0.2
		<i>S. desicc</i>	3.9	1.0	1.7	1.3		1.0
		<i>P. simplic</i>	2.3	0.6	1.0	0.7		0.6
		Consortium	3.1	0.8	1.4	1.0		0.9
Co	ISS	Non-bio	1.0	1.0	1.2	1.3	ISS vs Earth	2.5
		<i>S. desicc</i>	1.0	1.0	1.2	1.2		1.1
		<i>P. simplic</i>	0.8	0.8	1.0	1.0		1.6
		Consortium	0.8	0.8	1.0	1.0		1.1
	Earth	Non-bio	1.0	2.2	0.8	0.6	Earth vs ISS	0.4
		<i>S. desicc</i>	2.2	1.0	1.8	1.3		0.9
		<i>P. simplic</i>	1.2	0.5	1.0	0.7		0.6
		Consortium	1.7	0.8	1.4	1.0		0.9
Ni	ISS	Non-bio	1.0	1.1	1.0	0.9	ISS vs Earth	2.4
		<i>S. desicc</i>	1.1	1.0	1.0	1.0		1.2
		<i>P. simplic</i>	1.0	1.0	1.0	0.9		1.5
		Consortium	1.1	1.0	1.1	1.0		1.3
	Earth	Non-bio	1.0	2.2	0.6	0.5	Earth vs ISS	0.4
		<i>S. desicc</i>	2.2	1.0	1.4	1.0		0.8
		<i>P. simplic</i>	1.6	0.7	1.0	0.8		0.6
		Consortium	2.1	1.0	1.3	1.0		0.8
Cu	ISS	Non-bio	1.0	0.4	0.8	0.8	ISS vs Earth	0.1
		<i>S. desicc</i>	0.4	1.0	0.4	0.3		0.2
		<i>P. simplic</i>	1.2	2.8	1.0	1.0		0.2
		Consortium	1.3	2.9	1.0	1.0		0.6
	Earth	Non-bio	1.0	0.4	1.3	3.1	Earth vs ISS	7.0
		<i>S. desicc</i>	0.4	1.0	0.5	1.2		6.0
		<i>P. simplic</i>	0.8	2.1	1.0	2.4		4.5
		Consortium	0.3	0.9	0.4	1.0		1.8
Sr	ISS	Non-bio	1.0	1.0	1.0	1.1	ISS vs Earth	1.8
		<i>S. desicc</i>	1.0	1.0	1.0	1.0		1.1
		<i>P. simplic</i>	1.0	1.0	1.0	1.0		1.1
		Consortium	0.9	1.0	1.0	1.0		1.0
	Earth	Non-bio	1.0	1.7	0.6	0.6	Earth vs ISS	0.5
		<i>S. desicc</i>	1.7	1.0	1.1	1.0		0.9
		<i>P. simplic</i>	1.6	0.9	1.0	0.9		0.9
		Consortium	1.7	1.0	1.1	1.0		1.0
Zr	ISS	Non-bio	1.0	1.1	0.7	0.7	ISS vs Earth	1.7
		<i>S. desicc</i>	1.1	1.0	0.7	0.7		0.9
		<i>P. simplic</i>	1.4	1.4	1.0	1.0		1.6
		Consortium	1.5	1.4	1.0	1.0		1.2
	Earth	Non-bio	1.0	2.0	0.7	0.5	Earth vs ISS	0.6
		<i>S. desicc</i>	2.0	1.0	1.4	0.9		1.1
		<i>P. simplic</i>	1.5	0.7	1.0	0.7		0.6
		Consortium	2.1	1.1	1.4	1.0		0.9
Mo	ISS	Non-bio	1.0	1.1	0.9	1.0	ISS vs Earth	2.9
		<i>S. desicc</i>	1.1	1.0	0.9	1.1		1.0
		<i>P. simplic</i>	1.1	1.1	1.0	1.2		1.7
		Consortium	1.0	0.9	0.8	1.0		1.2
	Earth	Non-bio	1.0	3.1	0.5	0.4	Earth vs ISS	0.3
		<i>S. desicc</i>	3.1	1.0	1.6	1.3		1.0
		<i>P. simplic</i>	2.0	0.6	1.0	0.9		0.6
		Consortium	2.3	0.7	1.2	1.0		0.8
Ru	ISS	Non-bio	1.0	1.0	0.8	0.8	ISS vs Earth	2.2
		<i>S. desicc</i>	1.0	1.0	0.8	0.8		1.1
		<i>P. simplic</i>	1.3	1.3	1.0	1.0		1.6

	Earth	Consortium	1.3	1.2	1.0	1.0	Earth vs ISS	1.3
		Non-bio	1.0	2.2	0.5	0.5		0.4
		<i>S. desicc</i>	2.2	1.0	1.2	1.0		0.9
		<i>P. simplic</i>	1.9	0.9	1.0	0.9		0.6
		Consortium	2.2	1.0	1.2	1.0		0.8
Pd	ISS	Non-bio	1.0	0.3	0.2	0.6	ISS vs Earth	0.1
		<i>S. desicc</i>	0.3	1.0	0.1	0.2		13.6
		<i>P. simplic</i>	5.5	16.4	1.0	3.2		1.0
		Consortium	1.7	5.1	0.3	1.0		0.4
	Earth	Non-bio	1.0	0.0	2.4	3.3	Earth vs ISS	13.6
		<i>S. desicc</i>	0.0	1.0	0.0	0.0		0.1
		<i>P. simplic</i>	0.4	229.0	1.0	1.4		1.0
		Consortium	0.3	168.8	0.7	1.0		2.4
	Ba	Non-bio	1.0	1.1	1.0	0.9	ISS vs Earth	2.1
		<i>S. desicc</i>	1.1	1.0	1.1	1.1		1.1
		<i>P. simplic</i>	1.0	0.9	1.0	1.0		0.9
		Consortium	1.1	0.9	1.0	1.0		0.7
Eu	ISS	Non-bio	1.0	1.2	0.7	0.8	ISS vs Earth	2.6
		<i>S. desicc</i>	1.2	1.0	0.9	0.9		1.2
		<i>P. simplic</i>	1.4	1.1	1.0	1.0		1.7
		Consortium	1.3	1.1	1.0	1.0		1.2
	Earth	Non-bio	1.0	2.6	0.5	0.4	Earth vs ISS	0.4
		<i>S. desicc</i>	2.6	1.0	1.2	0.9		0.8
		<i>P. simplic</i>	2.1	0.8	1.0	0.7		0.6
		Consortium	2.8	1.1	1.3	1.0		0.8
Lu	ISS	Non-bio	1.0	1.0	0.7	0.7	ISS vs Earth	2.3
		<i>S. desicc</i>	1.0	1.0	0.7	0.7		1.6
		<i>P. simplic</i>	1.5	1.5	1.0	1.0		2.0
		Consortium	1.4	1.4	1.0	1.0		1.7
	Earth	Non-bio	1.0	1.4	0.6	0.5	Earth vs ISS	0.4
		<i>S. desicc</i>	1.4	1.0	0.8	0.7		0.6
		<i>P. simplic</i>	1.7	1.2	1.0	0.9		0.5
		Consortium	1.9	1.4	1.1	1.0		0.6
Pt	ISS	Non-bio	1.0	0.8	0.8	0.8	ISS vs Earth	1.8
		<i>S. desicc</i>	0.8	1.0	0.7	0.7		0.9
		<i>P. simplic</i>	1.2	1.5	1.0	1.0		1.5
		Consortium	1.2	1.5	1.0	1.0		1.3
	Earth	Non-bio	1.0	1.7	0.7	0.6	Earth vs ISS	0.5
		<i>S. desicc</i>	1.7	1.0	1.2	1.0		1.2
		<i>P. simplic</i>	1.4	0.9	1.0	0.8		0.7
		Consortium	1.7	1.0	1.2	1.0		0.8

Supplementary Table 6.

List of features of potential biotechnological/industrial interest, identified in the metabolomic analysis of this work.

Associated organism	Associated gravity	Feature	Type	Potential biotech interest
<i>S. desiccabilis</i> (alone and in Consortium)	ISS, Earth	(R)-3-((R)-3-Hydroxybutanoyloxy)butanoate	Butanoic acid derivate	Biomining
		2'-deoxymugineic acid	Carboxylic acid	Biomining
		Quinoline-3,4-diol	Associated with biofilm formation	Biomining
<i>P. simplicissimum</i>	ISS	Phosphine, 1,3-propanediylbis[dicyclohexyl-	Associated with chemical palladium-catalysed carbonylation (unknown biological activity) ⁴	Biomining
		Undeca-2,5-dienal	Fatty acid associated with bacterial siderophore biosynthesis	Biomining
		2,3-Dihydro-2,3-dihydroxybenzoic	Fatty acid associated with bacterial siderophore biosynthesis	Biomining
		Lastar A	⁵	Pharmaceutical
		Flemiwallichin D	Chalcone flavonoid ⁶	Pharmaceutical
		Sarcoehrendin D	Prostaglandine derivative	Pharmaceutical
		Lauro lactam	Used for the plastic production, i.e. Polyamide 12 (nylon 12) and copolyamides ⁷ . Natural bioproduction not reported.	Pharmaceutical, bioplastics and biomaterials
	Earth	1-morpholinocyclohexene	Belongs to the morpholino-compounds class, known for abiotic metal dissolution ⁸	Biomining
		1-Cyclohexyl-2-azetidinecarboxylic acid	Carboxylic acid	Biomining
		Hexanoic acid	Carboxylic acid	Biomining

Supplementary Table 7.

Spectrometric analysis (optical density, $\lambda = 600\text{nm}$, 530 nm) and flow cytometry analysis measured from the liquid fraction of the space (ISS) and ground control (Earth) samples of the BioAsteroid samples. A wavelength of 530 nm , instead of the classical 600 nm , was used as an additional measure for measuring filamentous fungi growth¹. For flow cytometry, values represent the average events counted in $100\text{ }\mu\text{L}$ of sample stained with Baclight green (specific for bacterial cells) and calcofluor white (specific for the fungal cells). Values are indicated as mean $\pm$ standard error.

		OD ₆₀₀		OD ₅₃₀		Flow cytometry (Cell/mL)			
		ISS	Earth	ISS	Earth	Baclight Green (bacterial dye)		Calcofluor White (fungal dye)	
						ISS	Earth	ISS	Earth
Sample	Non-biological	0.15 $\pm$ 0.01	0.28 $\pm$ 0.05	0.21 $\pm$ 0.01	0.47 $\pm$ 0.15	(6.66 $\pm$ 0.37) $\times 10^7$	(7.77 $\pm$ 4.75) $\times 10^7$	(6.33 $\pm$ 1.19) $\times 10^4$	(2.68 $\pm$ 1.94) $\times 10^4$
	<i>S. desiccabilis</i>	0.21 $\pm$ 0.08	0.64 $\pm$ 0.05	0.35 $\pm$ 0.10	1.18 $\pm$ 0.15	(3.97 $\pm$ 0.06) $\times 10^9$	(3.27 $\pm$ 0.32) $\times 10^9$	(3.15 $\pm$ 0.75) $\times 10^6$	(2.80 $\pm$ 0.70) $\times 10^6$
	<i>P. simplicissimum</i>	0.03 $\pm$ 0.00	0.29 $\pm$ 0.12	0.06 $\pm$ 0.01	0.44 $\pm$ 0.14	(2.80 $\pm$ 0.98) $\times 10^8$	(3.07 $\pm$ 1.22) $\times 10^8$	(3.50 $\pm$ 1.99) $\times 10^6$	(6.37 $\pm$ 2.11) $\times 10^7$
	Consortium	0.04 $\pm$ 0.00	0.46 $\pm$ 0.02	0.06 $\pm$ 0.01	0.74 $\pm$ 0.06	(6.47 $\pm$ 1.98) $\times 10^8$	(3.64 $\pm$ 0.14) $\times 10^9$	(1.43 $\pm$ 0.34) $\times 10^6$	(7.69 $\pm$ 1.33) $\times 10^6$
	Diluent (PBS)						(4.90 $\pm$ 2.40) $\times 10^4$		(4.50 $\pm$ 2.50) $\times 10^2$

237 **Supplementary Table 8.**

238 Sanger sequences of the V3-V4 region of the 16S rDNA of the samples affected by
 239 contamination and isolated contaminants. Sequences obtained with 805R primer have been
 240 reversed and complemented.

Primer	Original sample name	Type of sample	Organism	Sequence
241F	175-1	ISS	<i>P. simpliciss.</i>	TTTTGGGATAAAAGACGATAATCCGTTCTTTTATCTGGCTACATTTTCATGATAG GCTTGGTTGTCTGTAACCTCGTATAGCGTACATTGTACGA
805R		ISS	<i>P. simpliciss.</i>	TGGTTCCAAGGGATCTCAAGTCTCCAACACACCATCACTTTACCTTAGGGTGA GTCTCCTCTTGCGCAGTTCATAACTTAGTACACAGATGCTATACGAATAATAT CGATCCTATCCTGTAAATGCGGTAGTCTTCAAATACAGATAATGGTCTACC CACTCCGAAGGGGTCACTG
241F	175-2	ISS	<i>P. simpliciss.</i>	TCCTAGTAATATAGGGGGTTAATGGCGAGAAAGCAGAAACCTGTTCTTTTAGGA CTAGCCACTTTTACATGATAGGTTGCATTTCAATAACTTCGAATGCATAAATTATA CGAAGT
805R		ISS	<i>P. simpliciss.</i>	NNNN
241F	176-1	ISS	<i>P. simpliciss.</i>	GCGTGGATTATGCTTTTGGCGAAAAAGACAGAAAATCTGTTCTTTTAACTA GCTACATTTTACATGGGGGGCTTGGATTCTATAACTTCGAAGCATACATTATA CGAGTTATAAAC
805R		ISS	<i>P. simpliciss.</i>	TAGCTCTTAAACGGGTGGTGCCATCCTGCTCCAACAAGGTGGTTCTCCAAGGG ATACTTATAGTCTCAAAACACACAATTACTTTACAGTTAGGGTGAGTTTCTTCTG TGATGTCTATAACTTCGTATAATGTATGCTATACGAAGTTATAGAAATCCAAGCC TATCATGTAAATGTAGCTAGTATTAAGAAAGACAGATTATCTGTCTTTATCGC ACATAAGCCTCCACAGTAC
241F	7-1	Earth	<i>S. desiccabilis</i>	GCCGCGGATGAGGGGTTTGGGGGAAGTCCGTGTCGTTCTTTAGACAGCTCATT TTTTGTTAGGTTGGATTCTACTTCGTTATCATACTTTGTCGATTTTT
805R		Earth	<i>S. desiccabilis</i>	ATATTAAACAGGAACGCTGCTTTCCTTTTCAAATGTATTACGTCGCCGCTGGTTC AAACTGTGAAACAAATGTAGATACCAAGGTAGTGATNCCTGGAGATTTGGGA NAAATCCGAGTCTACGTTGTGCCATTCTAGATATTTGAAGAATCACCAGTGG GCAACGGCCGCCATTGGACC
241F	8-1	Earth	<i>S. desiccabilis</i>	CGNAAGCCTGAATCCAGCAATGCCGCTGAGTGATGAAGGCCTTAGGGTTGTA AAGCTCTTTACCCGGGATGATAATGACAGTACCGGGATAATAAGCCCCGGCTA ACTCCGTGCCACCAGCCGCTGGCATAATTATACGAAGATCTAAACACCA
805R		Earth	<i>S. desiccabilis</i>	GAAGCCAATGCGCTGTTGGTGAGGAAGCCGAANTAACTAGAATACCTTTAA GACTCATCGCTTGATCCGGAGAGGTGAGTGGAANTCCGAGTGTAGAGGTGAAA TTCGTAGATATTCGGAAGAACACCAAGTGGCGAAGGCGGCTCACTGGACCTGGC ATTGACGCTGAGGTGCGAAG
241F	9-1	Earth	<i>P. simpliciss.</i>	TGACGGAAGCAACGCCGCTGAGTGATGAAGGTTTTCGGATCGTAAACTCTGT TGTCAGGGAAGAACAAGTGCCAGGAGCATCTGCACAGCAGTGCACGGTACC TCTATAACTTCGCATAGCATACATTATACGAAGTTATATACAGCACAAGGAAA CTACCCCTAACTGTAACTGTTGTTT
805R		Earth	<i>P. simpliciss.</i>	GGGATAATTATAGTCTCGAAAAACACGAATCACTTGACAGTTAAGGTCAAGTTCC TAATGTGCTGTCTACCTTGGTGGGACGTAGGAACTGGAAGACATAAGTGCC GAAGAGGAAAGTGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA GGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACGCTGAG
241F	9-2	Earth	<i>P. simpliciss.</i>	NNNN
805R		Earth	<i>P. simpliciss.</i>	NNNN
241F	10-1	Earth	<i>P. simpliciss.</i>	NNNN
805R		Earth	<i>P. simpliciss.</i>	CACACCGCCCTCTACTATTCTCAAGGGAAAAACAGACTTCGTTGTTAAAGTGAG TCTCGTCAAGACCGGTTTAAAGTGGTATAACATATAAGATTCGGGCTGTTGAC ATAGAAGAGGAAAAATAGAATCCAAGTTCGTGCGTGAAATGCGTAGAGATGTG GAGGAACACCAGTGGCGAAGGCGACTTCTGGTCTGTAAGTACACTAG
241F	10-2	Earth	<i>P. simpliciss.</i>	GACACGCCGCTGAGTGAGAAGGTTTCGGAGATAAAGACGGTAATCTGTTCTTT TTAATACTAGATACATTTTACATGATAGGCTTGATTGTATAACTTCGTATAGCA TACATTATACGAAGTTATGAACAGCACAAGGAATCTCAACCTAACTGTAAG TAGTTGTGTGTTGAGACTATCAGTATCCCTTGAA

805R		Earth	<i>P. simpliciss.</i>	GTCTCAAAACCACAATCACTTTACAGTTAGGGAGAAATTCCTNTTGAGCCGTCT ATACCTTCGTATATACATTGGAAACACGGGGTTATANAAGNCCAAGAGGAGAAT GGAATAACTCTCGTGTAGTCGGTGAATAGAGATGTGGAGGAACACCAG TGCGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAAGGAGTGAAG
241F	11-1	Earth	Consortium	AGCTGATCAGATGCCCGTGAGTGATGAAGGCTTAGGGTTGTAAAGCTCTATTA CCTTTGATGATATGACAGTCTGGTTGAATAAGCCACGCTTGCAATTTCTATAACAT CTGATAGCATACATGATACGAATATAT
16R		Earth	Consortium	GTGGCGAGAACACACCAGAAGCGTTACAGTTNCGGTAAGTTTCGTAGCCGTG GTCTTATACTCTCAGAATTACCTTTAAGACTGCATCGTTTGAATCCGGGAGAGGT GAGTGGAATTCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCA GTGGCGAAGGCGGCTCACTGGACCGGCTGAGCTGAGGTGCGAAAGNCGTA NGGGGNNCCC
241F	11-2	Earth	Consortium	ATGCCCCGGGTGATTAGGGCGTATGGGGAGAAAGACAGATAACCTTTCTTTT TATACTAGCTACATTTTACATGATAGGCTTGGATGTCTATAACTTCGAAGGATA CATTATACGAAGTTATAGACAGCAGAAAAGGAACTCACCTAACTGTAAC
805R		Earth	Consortium	CCAGCTTAGCTCTTAAACGGGTGGTGGCCATCTTGGTCCAACAAGGTGGTCTCC AAGGGATACTTATAGTCTCAAAACACACAATTACTTTACAGTTAGGGTGAGTTTC CTTCTGTGCTGTTTATAACTTCGTATAATGTATGCTATACGAAGTTATAGAAATCC AAGCCTCTCATGTAAATGTAGCTAGTATTAAAAAGAACAGATTTTCGGTAGTAC ACCACTCACGAAGGCGGCTCACTGGACCGGCTGTCGNGCTG
241F	12-1	Earth	Consortium	GGCCGAAGCCTGAATCAGCAATGCCGCTGAGTGATGAAGGCCCTTAGGGTTGT AAAGCTCTTTACCCGGGATGATAATGACAGTACCGGAGAAATAGCCCCGGCT AACTCCGTGCCAGCAGCCGCGTAATACTAGAGGGCTAGATATACAGCGAAT AACGGGACTCACCTAACTGT
805R		Earth	Consortium	CACTCGTTGACGGAGGCGTCCGTTACCAGTGTCTATTATAAACGCCGAAAGCC ACTACGGTCAGTTAAGTGGTTTGAAGCCTGGAGCTCAACTCCGAATTGCGTTT AAGACTGCATCGCTTGAATCCGGGAGTGGTGAGTGGAATTCGAGTGTAGAGG TGAAATTCGTAGATATTCGGAAGAACCAGTGGCGAAGGCGGCTCACTGACC GGCATTGACGCTGAGGTCAAG
241F	8-1w4	Isolate	-	NNNN
805R		Isolate	-	GGTGTACTATATGCTATACGAAGTTATTTAAATCCAAGCGGATCATGAAAATGTA GCTATATGAGAAATAACAGATTTTTTTGTTCTCCGAGCTAAGGCGCTTCTGGTC TT
241F	9-1w1	Isolate	-	CGCCGCTGAGTGATGAAGGTTTTCGGATCGTAAGCTCTGTTGTCTGGGAAGAA CAGTCTAGCTACATTTTAGATACCGCTTGTATTTCTATACCTTCTATAGCCTTC ATTATAGGAAGTGATACACAGCACGAATGAACTCACTCAATTTTAAGGAAG TGTTTGTGTTGCTACTATGGGTATGCTTA
805R		Isolate	-	TCGTAAGGGCTCGCAGGCGTTTCTTGAGTCTGATTGAAAGCCCCGGCTCA ACCGGGGAGGGTCATTGGAACTGGGAACTTGAGTGCAGAAGAGGAGAGTG GAATTCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCACTGGC GAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGAA
241F	10-1w5	Isolate	-	CGTGAGCAACGCCGCTGACTGATGATGGTTTTCTGATCATAAAGCTCATGTT GCCTGTGCTTTNANACTACTAGCATACATTTACATGATATGCTTGGATTCTATA ACTTCGTATAGCATATTATACGAAGTTATACACAGCACAAAAGTAAACTCACC CTAACTGTGAAGGAATGGTGTGTTT
805R		Isolate	-	GAGCGCTCATTGCTGTTAGAGTGATAGTGTAAACGCGGGGCTCAACTTCGT ATCACGCTGGAACTGGGGGCTTGGAGAGCAGAAAGAGGAGAGTGGAATTCAC GTGTAGCGGTGAAATGCGTAGATATGTGGAGGAACACCACTGGCGAAGGCGAC TCTCTGGGCTGGAAGTACGNGCTAG
241F	10-2w8	Isolate	-	AAGTCTTGAACGGAGCAACGCCGCTGAGTGATGAAGTTTTCGGATCGTAAA GCTCTGTTGTTAGGGAAGAATTAGTACCGTTTCAATAGTACATGGGAGGCTAGG ATCTCTAACTTCGTATAGCATACATTGTANCAAGTAATAGANNCGACAGAAG GAATCTACCGCTAGCTGTAAAGTAAGGTGTGCTGTTTGCGAGA
805R		Isolate	-	GGGTCCCCGTGAGATATGCAGTGTAGACGCTCTAGACTCCGAGGCTGAGCAGT NCTGCGCTGAATTAAGTGGTGGCAGAGATCTTGTCCGGAATTTTGGCGTAA AGGGCTAGCAGGCGGTTTCTTAAGTCTGATGTGAAGCCCCGGCTCAACCGGG GAGGTCATTGGAACTGGGGAAGTGTGAGTGCAGAAGGAGAGTGAATTC ACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCACTGGCGAAGGC GACTCTCTGGTTCTGTTAACTTGACGCTGAGGAAGCGAAAG
241F	11-1w3	Isolate	-	GGGGGGGGGGCCCCCANTTGAATNAAGTCTTGACGGAGCCAACGCCGCTG AGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAGGGAAGAACAAGTACC GTTCAATAGGCGGTACCTTGACGGTACCTAACAGAAAGCCACGGCTAACTA

				CGTGCCTGCAGCCGCGCTAGGCGTAGGTGGCAAGCGTTGTCCGGAATTATTG GGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCGGCTC AACCAGGGGAGGGTCATTGGAACTGGGGAAC TTGAGTGCAGAAGAGGAGAGT GGAATTCACGTGTAGCGGTGAAAATGCTTATAGATGTGGAGTGAACATTTATT TGTCGAGTCGATTTCTGGCCTGTAATTAGA
805R		Isolate	-	ACCTACGGGTGGCAGCAGTAGGGAATCTCCGCAATGGACCAAAGTCTGACGG AGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGG GAAGAACAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAA AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT TGTCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGT GAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAACTGGGGAAC TTGAGTG CAGAAGAGGAGAGTGGAATTCACGTGTAGCGGTGAAATGCGTAGAGATGTGG AGGAACACCAAGTGGCGAAGGCGACTCTCTGGTCTTGTAACTTGACCGCTGAAG GAACNNAAGCGGGNGCC

Supplementary Table 9.

pH of the liquid fraction of the space (ISS) and ground control (Earth) samples. Values are indicated as mean±standard error. As pH is one of the potential influencing factors in leaching, pH of the liquid fractions was measured after spaceflight. ANOVA test was performed to compare pH values. The variables organism (F = 15.170, p-value = 1.45×10^{-5}), the gravity (F = 11.945, p-value = 0.002), as well as the interaction between the two (F = 5.044, p-value = 0.008) had an effect on the pH values. Pairwise comparison (post-hoc Tukey test) between non-biological and biological samples on the ISS had a p-value < 0.05 in all cases (Non-bio/*S. desiccabilis*: p-value = 0.012; Non-bio/*P. simplicissimum*: p-value = 0.026; Non-bio/Consortium: p-value = 9.36×10^{-6}), while similar comparisons on Earth reported p-values > 0.05. Comparison between non-biological samples on the ISS and on Earth had a p-value = 0.001. Comparison between *P. simplicissimum* and Consortium on the ISS had a p-value = 0.039. All other comparisons had a p-value > 0.05. The rock may sequester protons and buffer the solution, and this effect might be different under space and Earth conditions. This suggests that pH does not have a bioleaching role for *S. desiccabilis* and *P. simplicissimum*. Similar observations were made for rare earth element and vanadium extraction in space by *S. desiccabilis*, where bioleaching occurred under circumneutral pH^{9,10}. However, the pH values reported were likely buffered by the presence of the fixative (RNAlater), thus impairing our capacity to drive definitive conclusion on the effects of pH in our system.

Organism	pH	
	ISS	Earth
Non-biological	7.21±0.03	7.35±0.02
<i>S. desiccabilis</i>	7.34±0.01	7.38±0.01
<i>P. simplicissimum</i>	7.33±0.03	7.35±0.02
Consortium	7.43±0.01	7.43±0.01
R2A 50%	-	7.2
RNAlater	-	4.87±0.01
R2A 50% + RNAlater (1:5 v/v)	-	5.16±0.04

262 **Supplementary Data**

263 **Supplementary Data 1. (separate file)**

264 Supplementary excel file.

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