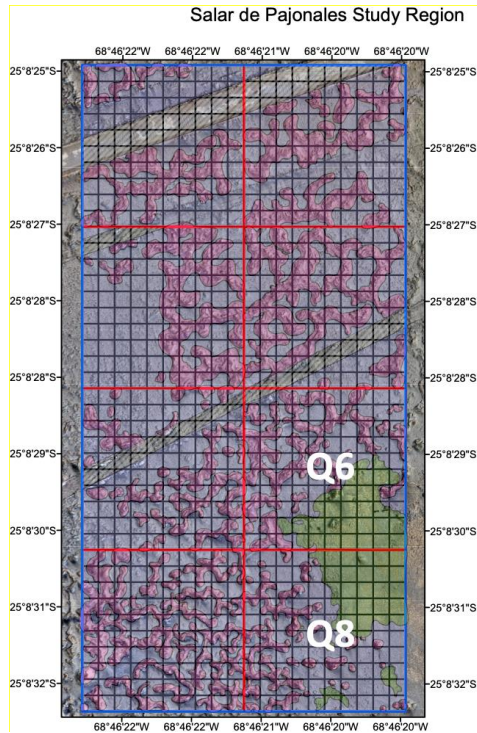


Orbit-to-ground framework to decode and predict biosignature patterns in terrestrial analogues

In the format provided by the
authors and unedited

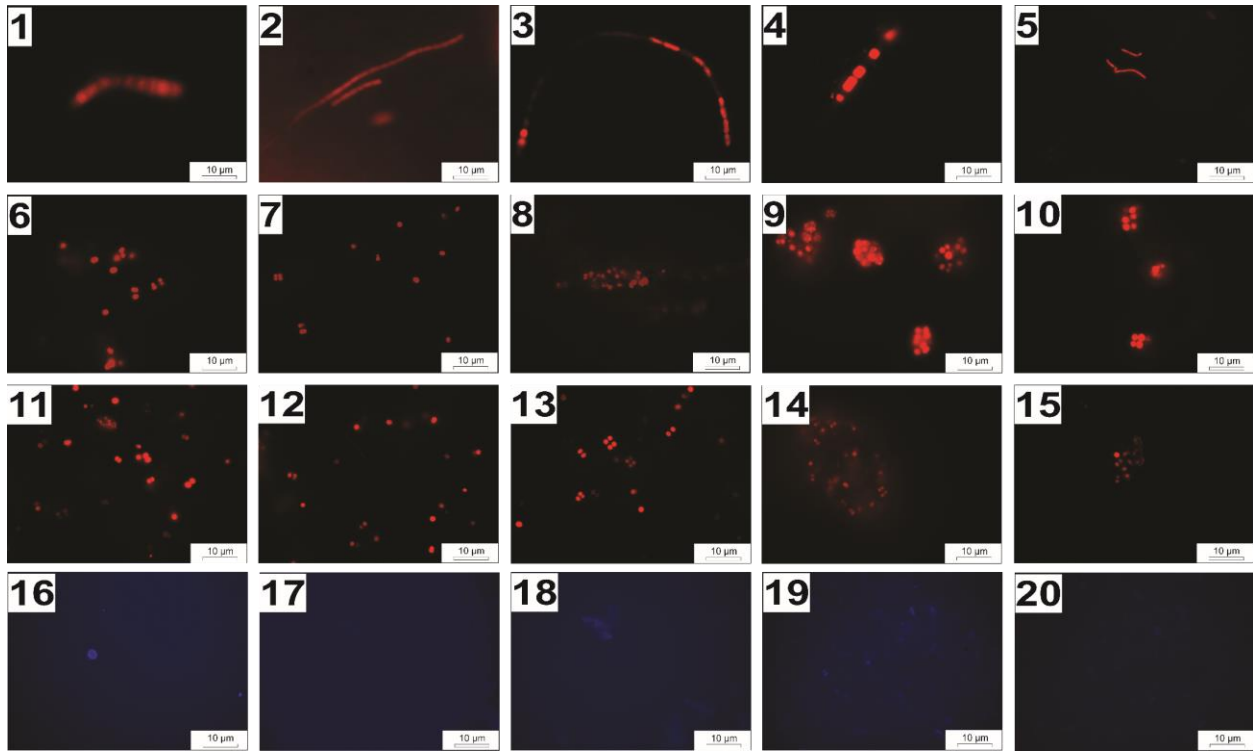
Supplementary Information

Microbial Ecology Methods Supplementary Data



Supplementary Fig. 1. UAV image of the Dome Field.

To investigate the effect of spatial scale on the probability for photosynthetic microbial community biosignatures (as % col) the study location was subdivided into nested hierarchical scales of study, including quadrants (red grid, 50m x 50 m), areas (black grid, 5 m x 5 m), and micro-sites (see Methods). UAV imagery was initially classified as raised areas (ridges and domes) or 'flat' areas [aeolian cover (grey) and patterned ground (green)]. Initial study hypotheses (H_0) were that i) biosignatures (%Col) varied significantly by spatial scale and ii) that 'raised' geomorphic features (perhaps linked to preferential water availability or more suitable rock architecture for colonization)^{8,92,93,94} were more habitable for photosynthetic microbial communities than flat features, i.e., that geomorphic unit/ecological habitat type controlled biosignature probabilities at multiple scales. These habitat classifications were refined as an understanding of the features and associated microbial communities evolved. Furthermore, the 'abiotic' patterned ground observed in 2016 by aerial imagery (green unit) was in 2018 ground surveys ascertained to be micro-structures with living photosynthetic biological soil crust communities (BSC). To accommodate these new findings, we created micro-structure and bare salar surface micro-habitat and BSC biological morphotype categories and separated these data from the endolithic colonization mode analyses.

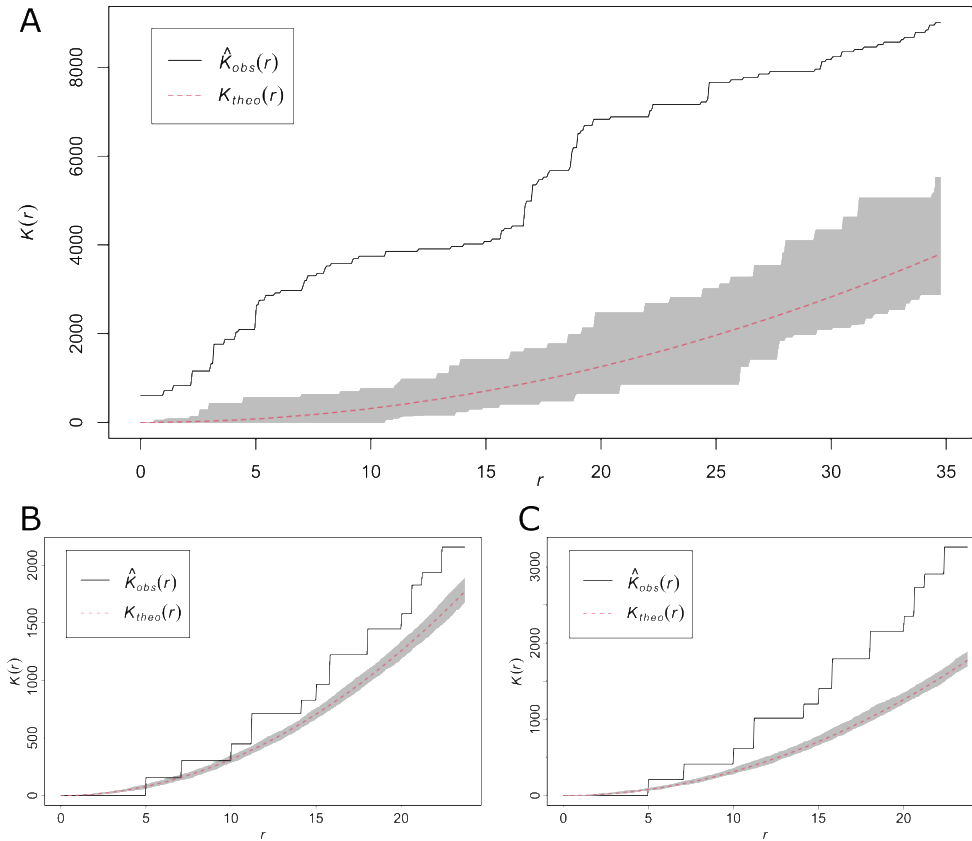


Supplementary Fig. 2. Confocal Laser Scanning Microscopy autofluorescence cell counts in green and orange pigmented biosignature layers (1-15) versus white (16-20) layers confirmed $4.49 \times 10^5 \pm 2.83 \times 10^5$ cells per mL and zero cells per mL, respectively.

Microbial Ecology Statistical Results Supplementary Data

Spatial Randomness/Aggregation Analyses (Ripley's K, Global Moran's I)

Ripley's K and Global Moran's I revealed non-random/aggregated/clustering (i.e., hotspots, islands, refugia)^{27,115,116,117} spatial distributions for endolithic and BSC communities at all scales assessed.



Supplementary Fig. 3. Spatial statistical analyses reveal clustering at multiple scales. a, The clustering pattern of endolithic microbial community biosignatures at larger scales examined (ES-1, distances (r) ≤ 35 m) is indicated by the observed K(r) values (solid line) above the theoretical envelope, as indicated by the red dotted line and the grey band showing the maximum and minimum values from 99 simulations. The envelope represents the variability of the complete spatial randomness (CSR) process, as the null hypothesis. In other words, our analysis showed more clustering than a homogenous Poisson process (CSR) would predict at this scale. **b, c,** Spatial pattern at fine-scales (≤ 20 cm) for endolithic biosignatures (**b**, ES-3 dome) and endoliths + BSC biosignatures (**c**, ES-3 aeolian cover) are also more clustered than CSR. These observations are also supported by Global Moran's I, which showed significant spatial autocorrelation (Monte-Carlo simulation of Moran's I, 1000 permutations, I = 0.03924 (ES1-2); 0.12947 (ES-3 dome); 0.26812 (ES-3 aeolian cover), p-value = 0.001 for all).

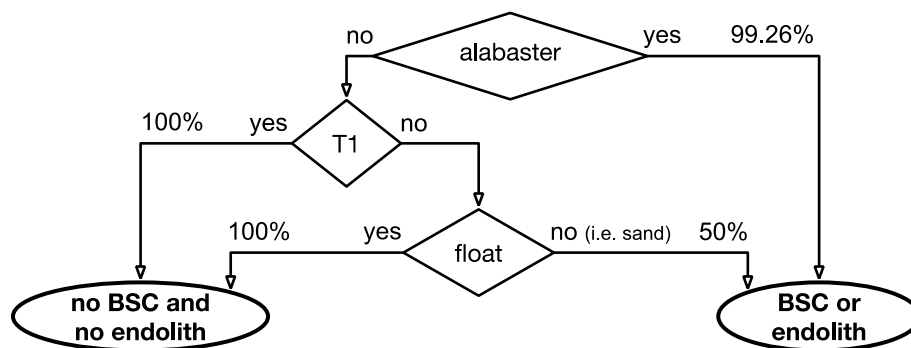
Pivot Table Statistics and Decision-Trees

Pivot-tables count the number of events, where in this case for Pajonales each event is some combination of microhabitat (e.g., alabaster, sand) and biosignature (endolith, BSC). A decision-tree is derived from the pivot-table, such that a sequence of yes/no decisions per microhabitat optimize the likelihood of detecting life/biosignatures in the fewest decisions possible. For example, based on the decision tree shown in Extended Data Fig. 3, alabaster microhabitats (in a dome macrohabitat) maximize the likelihood of observing endolithic biosignatures. Therefore, given this knowledge, at Pajonales, a rover programmed with a CNN algorithm might scan the environment with spectrometers for alabaster microhabitats, which have the highest probability for containing endolithic (or BSC) biosignatures.

Supplementary Table 1. Pivot table results for the probability of endolithic biosignatures (%Col) in the dome macrohabitat.

				<i>Endolith Present (1), Absent (0)</i>					
<i>Geologic material/microhabitat</i>				<i>0</i>		<i>1</i>		<i>Total</i>	
Alabaster	T2 crystal	T1 crystal	Sand	%	Count	%	Count	%	count
0	0	0	0	100	33			100	13
			1	100	8			100	8
		0 total		100	41			100	41
		1	0	69.54	137	30.46	60	100	197
		1 total		69.54	137	30.46	60	100	197
	0 total			74.79	178	25.21	60	100	238
	1	0	0	17.78	24	82.22	111	100	135
		0 total		17.78	24	82.22	111	100	135
	1 total			17.78	24	82.22	111	100	135
0 total				54.16	202	45.84	171	100	373
1	0	0	0			100	27	100	27
		0 total				100	27	100	27
	0 total					100	27	100	27
1 total						100	27	100	27
Total				50.50	202	49.50	198	100	400

In above table, data from the ecological survey (ES-3 above) are shown as follows: the absence of colonization, i.e., biosignatures, is registered as a “0” and the presence of endolithic community biosignatures as a “1” for each microhabitat (alabaster, Type II (T2) crystal, Type I (T1) crystal and sand) in the dome macrohabitat. As an example, of the n=400 total samples, n=8 samples were sand microhabitat, of which 100% were uncolonized, i.e., no biosignature present (n=8 with a count of “0” endolith biosignature).



Supplementary Fig. 4. Rules for the probability a sample contains endolithic or BSC biosignatures in microhabitats of aeolian cover macrohabitat. The above is a visual representation of the main results of Supplementary Data Table 2. For instance, in the first decision in the figure, if the microhabitat is alabaster, then the probability of endolithic and/or BSC biosignatures present in a sample is 99.26% (%Col is: 135 colonized samples/136 total alabaster samples x100). If the microhabitat is not alabaster, this leads to the next decision choice of whether the microhabitat is a Type I (T1) crystal or not, and so forth.

Supplementary Table 2. Pivot table results for the probabilities of endolithic and/or BSC biosignatures (%Col) in microhabitats of the aeolian cover macrohabitat.

Geologic material/microhabitat				BSC or Endolith Present (1), Absent (0)					
				0		1		Total	
Alabaster	T1 crystal	Float	Sand	%	Count	%	Count	%	count
0	0	0	1	50	113	50	113	100	226
		0 total		50	113	50	113	100	226
		1	0	100	86			100	86
		1 total		100	86			100	86
	0 total			63.78	199	36.22	113	100	312
	1	0	0	100	3			100	3
		0 total		100	3			100	3
		1	0	100	25			100	25
		1 total		100	25			100	25
	1 total			100	28			100	28
0 total				66.76	227	33.24	113	100	340
1	0	0	0	0.74	1	99.26	135	100	136
		0 total		0.74	1	99.26	135	100	136
	0 total			0.74	1	99.26	135	100	136
1 total				0.74	1	99.26	135	100	136
Total				47.90	228	51.10	248	100	476

In the above table, data from the ecological survey (ES-3 above) are shown: counts for endolithic or BSC biosignatures (presence = "1", absence = "0") and counts (presence = "1", absence = "0") for each of the microhabitats (alabaster, T1 crystal, float and sand) in the aeolian cover macrohabitat.

GAM Models and Supplementary Data

Predictive model results for endolithic biosignatures in dome macro-habitat

Supplementary Table 3. Parameter estimates for the spatial logistic GAM. The response (dependent) variable is the absence/presence of endolithic colonization in 1 m x 1 m quadrat of dome face macro-habitat, with x,y spatial resolution at 5 cm x 5 cm. The predictor (independent) variables are the absence/presence of Type I (T1) crystal and Type II (T2) crystal micro-habitats, and the x-y coordinates of the 5 cm x 5 cm samples that form the 1 m x 1 m quadrat.

Coefficient	Estimate, β_i	Standard error	z-value	Odds ratio, $\exp(\beta_i)$	95% Confidence interval for odds ratio
Intercept	-3.4965	0.6606	-5.2931	0.030	(0.008; 0.110)
T1_1	3.5877	0.7517	4.7727	36.152	(8.284; 157.763)
T2_1	4.7733	0.7927	6.0217	118.315	(25.020; 559.484)

Interpretation of the model results: The model shows that the likelihood of endolithic biosignatures was 36 times greater in the T1 micro-habitats and 118 times greater in the T2 micro-habitat when compared to areas without T1 and T2 micro-habitats, respectively. In terms of probability, it is 97.3% more likely to observe endolithic biosignatures if T1 is present when compared to areas that do not have T1. Likewise, it is 99.16% more likely to observe endolithic biosignatures if T2 is present when compared to areas that do not have T2. If the odds ratio is greater than one, then the micro-habitat shows a greater association (or higher

likelihood of having endolithic biosignatures). If the odds ratio is less than one, the micro-habitat has a negative relationship with biosignatures (or a lower likelihood of having endolithic biosignatures present when compared to areas that do not have that micro-habitat).

Predictive models for endolithic and BSC biosignatures of aeolian cover

Endolithic Biosignatures in Aeolian Cover Macro-Habitat (Supplementary Table 4a): In the dome macro-habitat model above, sand micro-habitat was always negatively correlated with endolithic biosignatures (close to -1, i.e., sand was predominantly in areas where there was no endolithic biosignatures or the alabaster was bare) and was therefore not included in Methods eq. (1) above. However, in the aeolian cover macro-habitat, sand was observed in some areas where alabaster was also present and colonized by endoliths. This is illustrated in the probability contour map for endolithic biosignatures in the aeolian cover macro-habitat (Fig. 5d, right box).

BSC Biosignatures of Aeolian Cover Macro-Habitat (Supplementary Table 4b-d). In the aeolian cover macro-habitat, only alabaster or sand can be included in the model, not both. When both variables are included, the model will not converge because some combinations do not exist in the original dataset (see Supplementary Table 2). Indeed, as indicated below, the presence of sand was positively associated with, and increased the chances for, the presence of BSC biosignatures (Supplementary Table 4c). More specifically, in cases where both sand and alabaster are absent ("sand=0, alabaster=0"), there were no cases where BSC biosignatures was present. Conversely, BSC biosignatures will always be present if both sand and alabaster are present ("sand=1, alabaster=1"). Thus, both these conditions were removed from the models to ensure convergence. Below, we first examined sand and alabaster combinations in separate models (Supplementary Table 4b-c) and then created a model to consider situations with alabaster *only* and sand *only* (Supplementary Table 4d). The model (Supplementary Table 4d) that included alabaster *only* (without any sand) and sand *only* (without any alabaster) showed that the direction of the relationships to BSC biosignatures is consistent with those observed for Supplementary Table 4b-c. However, the magnitude of the association was much stronger when *both* micro-habitats were present in the same sample (Supplementary Table 4d).

Supplementary Table 4. GAM predictive models for endolithic (a) and BSC biosignatures in aeolian cover macro-habitat.

a). Estimated parameters for predicting likelihood of endolithic biosignatures in aeolian cover.					
Parametric Coefficients	Estimate, β_j	Standard error	z-value	Odds ratio, $\exp(\beta_j)$	95% Confidence interval for odds ratio
Intercept	-92.7851	28.9458	-3.2055	5.06 e-41	(1.161 e-65; 2.204 e-16)
sand	-3.4207	1.0694	-3.1988	0.033	(0.004; 0.266)
Smooth term					
Term	edf	Ref,edf	χ^2 -value	p-value	
f(x_coord,y_coord)	10.5	11.96	31.99	0.0014	
Model information					
Adjusted R ² = 0.88	Deviance explained = 86.7%			n = 400	
b). Estimated parameters for predicting likelihood of BSC given presence of alabaster.					
A. Parametric Coefficients	Estimate, b_j	Standard error	z-value	Odds ratio, $\exp(b_j)$	95% Confidence interval for odds ratio
Intercept	-11.8796	2.9134	-4.078	6.93 e-06	(2.295 e-08; 2.092 e-03)
Alabaster	-1.5913	0.6008	-2.649	0.2037	(0.063;0.661)
A. Smooth term					
Term	edf	Ref,edf	χ^2 -value	p-value	

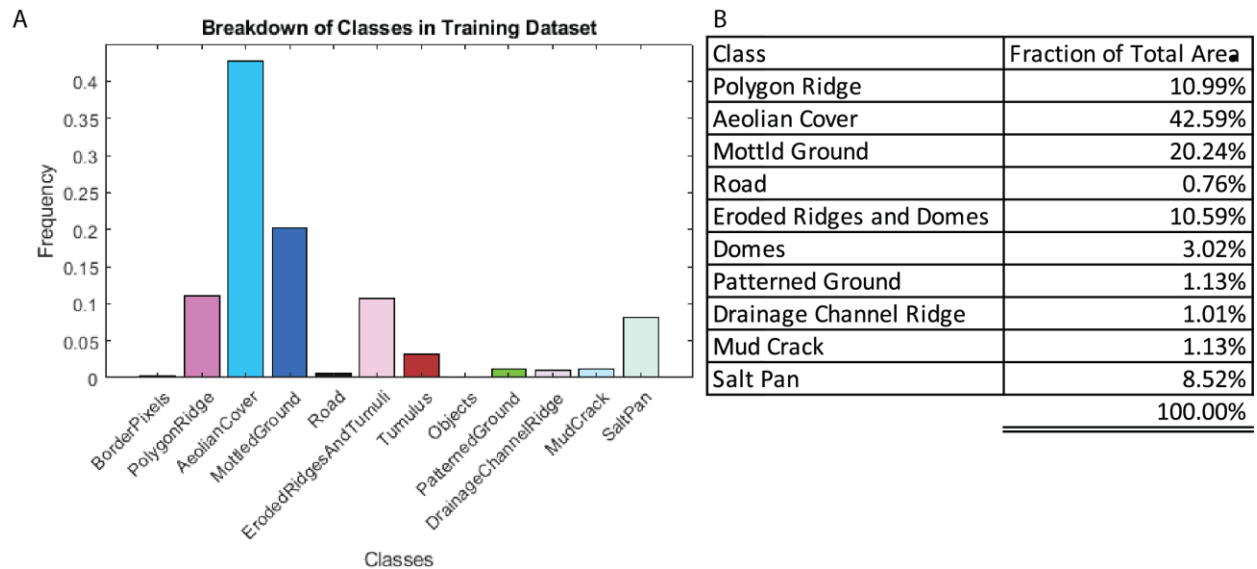
f(x_coord,y_coord)	12.69	14.58	46.6	< 0.0001	
B. Model information					
Adjusted R ² = 0.56	Deviance explained = 56.5%		n = 400		
c). Estimated parameters for predicting likelihood of BSC given presence of sand.					
Parametric Coefficients	Estimate, b _i	Standard error	z-value	Odds ratio, exp(b _i)	95% Confidence interval for odds ratio
Intercept	-39.4748	9.6868	-4.0751	7.183 e-18	(4.080 e-26; 1.265 e-09)
sand	9.3319	1.6123	5.7811	11292.567	(479.087; 266175.40)
Smooth term					
Term	edf	Ref,edf	χ ² -value	p-value	
f(x_coord,y_coord)	12.5	14.18	30.33	0.0087	
Model information					
Adjusted R ² = 0.92	Deviance explained = 90.4%		n = 400		
d). Estimated parameters for predicting the likelihood of BSC for sand <i>only</i> and alabaster <i>only</i> .					
Parametric Coefficients	Estimate, β _i	Standard error	z-value	Odds ratio, exp(β _i)	95% Confidence interval for odds ratio
Intercept	-21.4441	5.2621	-4.075	4.86 e-10	(1.614e-14; 1.466 e-05)
Alabaster only (no sand)	-5.0169	0.7144	-7.023	0.0066	(0.002; 0.027)
Sand only (no alabaster)	2.5901	1.0267	2.523	13.3305	(1.782; 99.727)
Smooth term					
Term	edf	Ref,edf	χ ² -value	p-value	
f(x_coord,y_coord)	11.45	13.12	43.43	< 0.0001	
Model information					
Adjusted R ² = 0.83	Deviance explained = 80.5%		n = 400		

a, Results for GAM for endolithic biosignatures in aeolian cover macro-habitat. Estimated parameters for predicting the likelihood of endolithic biosignatures (absent, present) in the 1 m x 1 m quadrat of aeolian cover, with x,y spatial resolution at 5 cm x 5 cm. The predictors are the presence of sand (1=yes, 0=no) and the x-y coordinates of the 5 cm x 5 cm samples in the 1 m x 1 m quadrat. Interpretation of the model results: Supplementary Table 4a shows that sand is negatively associated with the presence of endolithic biosignatures. When sand was observed where alabaster was also present, there was a 30-fold (1/OR_{sand}=1/0.033) decrease in observing endolithic biosignatures. We hypothesize this finding is related to light effects, where a layer of alabaster that is covered by sand has significantly lower light penetration, making it a less suitable habitable micro-environment for endolithic colonization; **b, Estimated parameters for predicting the likelihood of BSC biosignatures given the presence of alabaster.** In this GAM model, alabaster is denoted as present if the 5 cm x 5 cm sample area contains *alabaster micro-habitat and alabaster/sand mix* (i.e., the latter is when *both* micro-habitats are present in a sample) in a 1 m x 1 m quadrat of aeolian cover, with x,y spatial resolution at 5 cm x 5 cm. In this model, alabaster micro-habitat present = yes, alabaster and sand micro-habitat both present = yes; all other conditions = no; **c, Estimated parameters for predicting the likelihood of BSC biosignatures given the presence of sand.** In this GAM model, sand micro-habitat is denoted as present if the 5 cm x 5 cm sample area contains *sand or sand/alabaster mix* (i.e., the latter is when *both* micro-habitats are present in the sample) in a 1 m x 1 m quadrat of aeolian cover, with x,y spatial resolution at 5 cm x 5 cm. In this model, sand micro-habitat only = yes; sand and alabaster micro-habitats both present = yes; all other conditions = no; **d) Estimated parameters for predicting the likelihood of BSC biosignatures.** This GAM model considers the presence of *sand only* (sand only = yes; all other conditions = no) and *alabaster only* (alabaster only = yes; all other conditions = no) in a 1 m x 1 m quadrat of aeolian cover, with x,y spatial resolution at 5 cm x 5 cm.

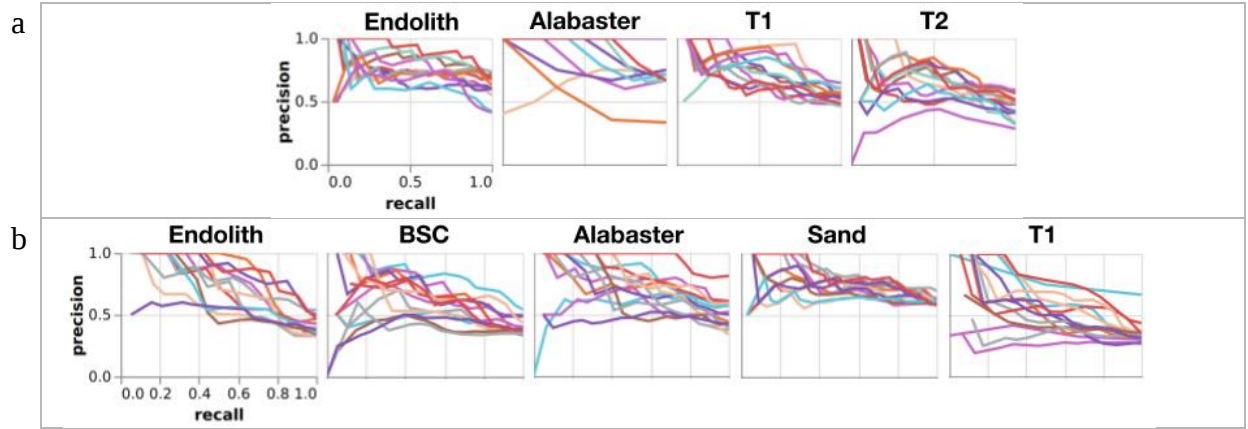
Together, the above models confirm the critical importance of the spatial correlation of sand and alabaster micro-habitats both being present as providing the set of conditions with the highest likelihood for BSC biosignatures (i.e., ‘habitability’). As Supplementary Table 4d shows, when *only* sand is present, BSC biosignatures is 13 times more likely to be present versus 11,292 times higher likelihood when the sand/alabaster ‘mix’ (i.e., both sand and alabaster are present in same sample area) is included in the model (Supplementary Table 4c). When alabaster *only* is considered (Supplementary Table 4d), there is a 151 times lower likelihood of BSC biosignatures being present, compared to 5 times less likely odds in the alabaster plus alabaster/sand mix.

These results confirm that while the presence of a sand micro-habitat is important for BSC colonization, it is when a sand micro-habitat is co-located with or adjacent to alabaster that the likelihood of observing BSC biosignatures is greatest. The findings in Supplementary Table 4 above are perhaps more easily understood when mapped visually in a BSC probability contour map (Fig. 5d, center box). When sand is co-located with alabaster (bottom half of Fig. 5d, center box), an 11,292 times greater likelihood (‘biotic hotspot’) of observing BSC biosignatures exists. When only sand is present but in proximity to (but not co-located with) alabaster there is still a 13 times higher likelihood to observe BSC biosignatures than when no sand was present. Alternatively, when the sand sample was *not* co-located with or adjacent to alabaster, then sand remained overwhelmingly uncolonized by BSC (i.e., top half of Fig. 5d, center box). Thus, a positive correlation between the co-occurrence of sand (or Type I crystals) and alabaster and BSC biosignatures existed [(Fig. 5 d, center box, right-tailed t-test, $t(61)=10.697$, $p<0.001$); Fig. 5 d, center box, two-tailed t-test, $t(87)=43.254$, $p<0.001$, Supplementary Table 4 above)].

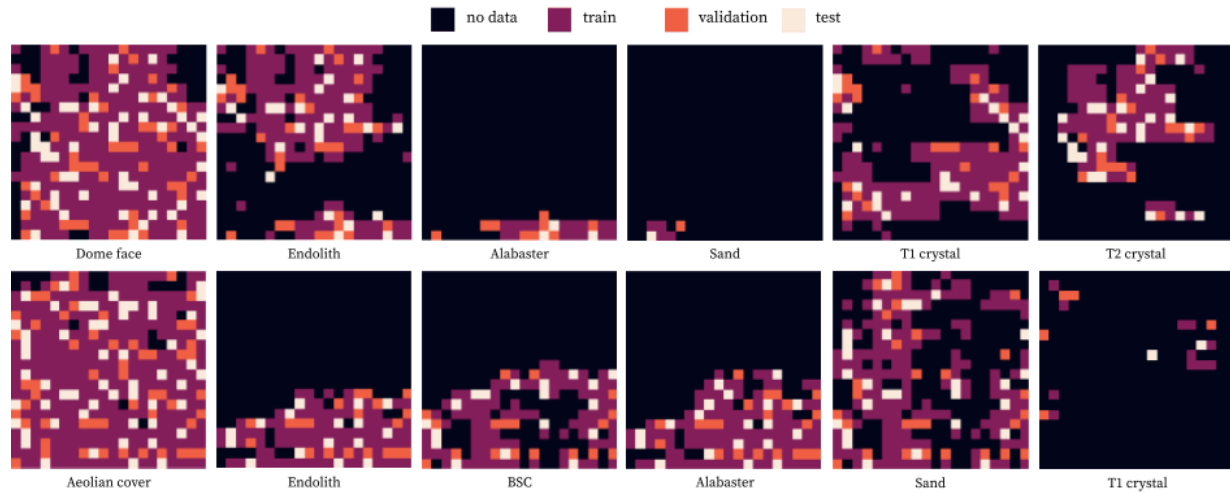
CNN Models Supplementary Data



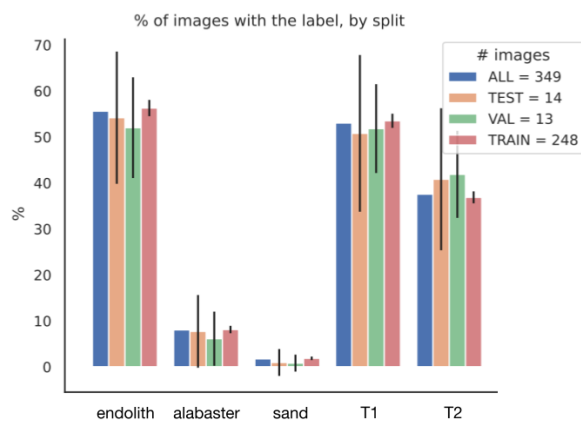
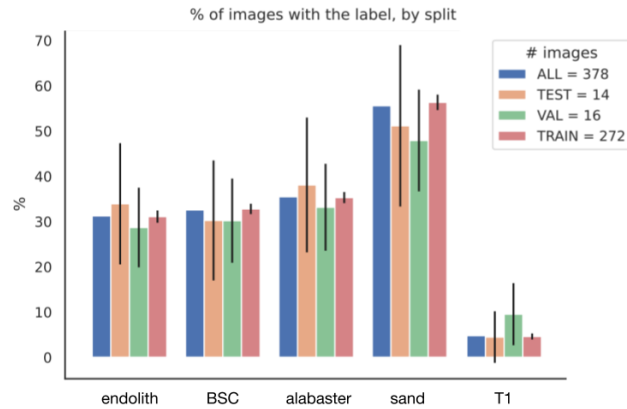
Supplementary Fig. 5. Class distribution in the training set for overhead FCN analysis. A) graphical representation of the relative frequency of each class in the training set. B) Values corresponding to the plot in part A. An inversely proportional weighting scheme was applied during training to account for the unequal distribution of classes in the training set.



Supplementary Fig. 6. Recall-Precision curves, by macrohabitat and label, show the variability across the 10 randomized runs. Each run uses a different randomized training/validation/test split (see Supplementary Fig. 7). Top: dome macrohabitat. Bottom: aeolian cover macrohabitat. Recall = True Positives / [True Positives + False Negatives]. Precision = True Positives / [True Positives + False Positives].



Supplementary Fig. 7. One example of 10 randomized partitions (also referred to as “splits”) of macrohabitats into training, validation, and test sets. Left-most column shows the split across the entire macrohabitat. Right-most columns show the split within cells containing the label. Top: training/validation/test split in the dome macrohabitat. Bottom: training/validation/test split in aeolian cover macrohabitat. Training sets are shown in purple, validation in orange, test in white.



Supplementary Fig. 8. Percentage of images by label and by split. Top: Aeolian cover macrohabitat. Bottom: dome macrohabitat. Bars shown in red, green, orange are the % of training, validation, test images, respectively, by label. Bars shown in blue are the union of train, validation, and test images (i.e. the entire macrohabitat). Each randomized split (see Supplementary Fig. 7 for examples) creates a different distribution of labels in the training, validation and test sets. The bar heights measure the average percentage across 10 randomized splits, and the black vertical bars are ± 1 std. deviation from the average percentage. The bar plots show that the label distributions (red, orange, green bars) are consistent with the macrohabitat distribution (blue bars). The set sizes do not change across all randomized splits. The absolute counts are shown in the legends.

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