



The effect of sand grain size on the development of cyanobacterial biocrusts



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ABSTRACT

Biocrusts are critical components of desert ecosystems, significantly modifying the surfaces they occupy. Although the presence of fine soil particles is known to be conducive to biocrust development and recovery from disturbance, their influence on the inceptive development of biocrusts has not been empirically studied. In this study, the effect of substrate granulometry on the development of biocrusts was explored, under controlled laboratory conditions of light, soil humidity, and temperature. A cyanobacterial inoculum of *Microcoleus vaginatus* was applied to five sand fractions in the range of 1–2000 μm . The results showed that the biocrusts developed more rapidly on the fine fraction (<125 μm) than on the coarser fractions. While the biocrust cover on the fine fraction was spatially homogenous, it was patchy and discontinuous on the coarse fractions. The difference in the pore size between the different fractions is suggested to be the reason for these discrepancies in biocrust development, since large pores between the particles of coarse soil restrict and regulate the filaments' spreading. It was found that the spectroscopic indices, the Normalized Difference Vegetation Index and the Brightness Index, were more sensitive to the biocrust development than the bio-physiological parameters of the biocrusts (polysaccharides, protein, and chlorophyll contents). The faster biocrust development on the fine fractions can explain various biophysical phenomena in aeolian environments.

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1. Introduction

Drylands occupy more than 40% of the earth's terrestrial surface area (Glenn et al., 1993; Lal, 2004). Throughout the world, drylands are home to biocrusts (Belnap and Lange, 2001; West, 1990). Biocrusts are dominated by cyanobacteria and may also include lichens, mosses, green algae, microfungi, and bacteria (Belnap and Lange, 2001). In natural drylands across the world, biocrusts cover vast regions and may amount to 70% of the ground cover (Buis et al., 2009; Eldridge and Greene, 1994; Karnieli et al., 2002; Qin et al., 2006).

Biocrusts under variable conditions of aridity reach various developmental stages and function in different ways (Belnap,

2006; Zaady et al., 2014). Some of these functions include carbon and nitrogen fixation (Belnap, 2002; Burgheimer et al., 2006a,b; Shields, 1957; Wu et al., 2009; Zaady et al., 1998, 2000) and profound effects on plant germination (Boeken et al., 2004; Serpe et al., 2006; Zaady et al., 1997). Biocrusts also play a crucial role in geomorphological processes, such as aeolian (Maman et al., 2011; Meir and Tsoar, 1996; Tsoar, 1990), hydrological (Akuja et al., 2003; Eldridge et al., 2002; Ram and Aaron, 2007; Zaady et al., 2014; Zaady, 2005) and pedological processes (Eldridge and Leys, 2003; Zhang et al., 2006). Biocrusts may effectively impede wind and water erosion and water flow; however due to hydrophobic properties and extracellular polysaccharides that act to clog the surface, some biocrusts may also trigger runoff resulting in nutrient and sediment translocation (Belnap, 2006; Zaady et al., 2013). The effects of biocrusts on pedological processes are due in part to their organic matter content as well as microorganism–mineral interactions (Belnap and Lange, 2001; Breckle et al., 2008). Thus, biocrusts are critical components of desert ecosystems, significantly modifying the surfaces they occupy.

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Biocrusts are essentially composed of photoautotrophic organisms and soil granules. The composition of their photoautotrophic community is dynamic and associated with a successional process. Initially, the aggregation of soil granules is performed by metabolic extracts secreted by cyanobacteria and green algae, forms the hard crust layer. This mucilage layer allows better water retention in the soil over time by reducing evaporation and desiccation (Zaady et al., 2014). Cyanobacteria, the most common element in the desert's microphytic community, are a primary producer. Their dominance as a primary colonizer in desert areas stems from their extraordinary ability to disperse through dust and runoff water, colonizing new and disturbed areas, and then binding with soil particles to produce wind resistant surfaces. As a result of their endurance for low water potential (Brock, 1975), high temperature (Buzer et al., 1985), and radiation (Levy and Steinberger, 1986), the colonization, establishment and domination of cyanobacteria on the soil surface can occur rapidly and may take only a few weeks. The soil stabilization process begins when the initial crust is generally formed by filamentous cyanobacteria of the *Microcoleus* genera (Campbell et al., 1989). Other filamentous cyanobacteria such as *Phormidium* spp., *Oscillatoria* spp., may also appear and colonize the soil surface (Belnap, 2001; Zaady et al., 2010). Following soil stabilization, nitrogen-fixating cyanobacteria, such as *Calothrix* spp. and *Nostoc* spp., are established and enrich the soil with nutrients (Rychert and Skujins, 1974). As the successional process continues, green algae appear, followed by mosses. The latter further contribute to soil stabilization since moss rhizoids penetrate into the soil and act as a skeleton, compacting the cyanobacterial crust into the soil (Zaady, 1999). Lichens appear later as they require an established biocrust in order to develop (Belnap and Lange, 2001).

Previous studies have found links between soil texture and biocrusts (Danin, 1996; Williams et al., 2013). Topsoil texture changes with biocrust succession; as the biocrusts become more mature, they contain higher proportions of fine grained particles (Kidron, 2014; Ram and Aaron, 2007). A significant positive correlation was found between organic matter in biocrusts and the proportion of silt and clay (Danin, 1996). Further, Danin (1996) suggested a positive feedback between the biocrusts and the amount of fine particles. In addition, biocrusts are known to trap airborne dust (Danin and Ganor, 1991; Pietrasiak et al., 2014; Williams et al., 2012; Zaady and Offer, 2010). Indeed, it is this trait that was suggested to be an essential component in the formation of loess soil since biocrusts aggregate deposited dust particles into their structure and keep these particles from being eroded by water and wind (Svirčev et al., 2013). The process of enriching the topsoil with fine particles reinforces the development of biocrusts by increasing their water-holding capacity and by providing necessary mineral nutrients. Dust incorporation into the biocrusts increases the growth of cyanobacteria and strengthens the cohesion of biocrusts (Hu et al., 2002). Moreover, biocrusts cover a larger portion of the surface when the soil contains finer particles, and it was observed that at least 4–5% of clay and silt is required to support a measurable microphytic crust (West, 1990). Accordingly, cyanobacterial biocrusts are abundant on soils with fine sand and negligible rocks (Williams et al., 2013). With the succession of biocrust underway and later the establishment of mosses and lichens, biocrusts increase dust capture to form biologically mediated vesicular horizons that are finer textured than the underlying fine sands (Williams et al., 2012, 2013). Thus throughout the biocrust succession, texture, microstructures, and the potential to capture dust change with developments in biocrust composition and morphology (Felde et al., 2014; Williams et al., 2012, 2013). Following a disturbance, biocrust recovery is more rapid in fine-textured soils that form physical crusts than in coarse-textured soils that do not form physical crusts, when climate and disturbance characteristics are otherwise similar (Belnap and Eldridge, 2001). As a result, clay, silt,

and fine sand particles are recognized for their importance for biocrust establishment, development, and recovery. Yet, little research has experimentally examined the impact of the soil grain size on the establishment of initial biocrusts on bare soil. Filling this knowledge gap is crucial to understanding the process of biocrust formation. Hence, the primary aim of the current investigation is to explore how the sand-grain-size distribution affects the ability of biocrusts to establish themselves. Specifically, the objective is to compare the rate and dispersal patterns of inceptive cyanobacterial biocrusts on different grain-size fractions.

2. Materials and methods

2.1. Characterization of the sand substrate

Sand was collected from Wadi Kasuy sand dunes in the southern Negev Desert in Israel (29° 59' 14" N; 34° 59' 25" E). The sand composition in this area was previously described as 60% calcite and 35% quartz (Yizhaq et al., 2012). Its grain-size distribution is polymodal with many coarse grains. The collected material was sterilized in an autoclave and sieved into five grain-size fractions following the classification of sand sizes from the U.S. Department of Agriculture (USDA): <125 µm (very fine sand), 125–250 µm (fine sand), 250–500 µm (medium sand), 500–1000 µm (coarse sand), and 1000–2000 µm (very coarse sand) (Buol et al., 2011). A grain-size distribution analysis was performed using the laser diffraction technique (ANALYSETTE 22 MicroTec Plus) that measures particles in the range of 0.08–2000 µm. The mineral composition of the sand in each fraction was determined by phase analysis using the X-ray Powder Diffraction (XRPD) method. The data were collected on a Philips 1050/70 powder diffractometer, with a graphite monochromator on a diffracted beam providing CuK α radiation (=1.541 Å) and operating at $v = 40$ kV, $I = 30$ mA. Phase identification was performed by using the Bede ZDS computer search/match program coupled with the International Centre for Diffraction Data (ICDD) Powder Diffraction File database. The amount of definite phase concentrations of the crystalline components was estimated by using the Relative Intensities Ratio (RIR) method following Hubbard and Snyder (1988) with a determination accuracy of 10%.

2.2. Controlled experiment

The experiment and all measurements described below were conducted at laboratory of the Agricultural Research Organization (ARO), Gilat Research Center, Israel. A total of 32 samples were prepared for each size fraction of the sand. Each sample consisted of 80 g of sieved sand on filter paper in a sterile Petri-dish (90 mm in diameter) with five 1-mm drainage holes at the bottom. The inoculant of the filamentous cyanobacteria (*Microcoleus vaginatus*) was isolated from the sand dunes of the northern Negev Desert, and the Accession number of the 16S rRNA gene sequence was EF667962 (Zaady et al., 2010). The cyanobacteria were grown in a synthetic growth medium up to a certain biomass in a bioreactor (Lan et al., 2014). The medium was filtered and dried at 40 °C for 48 h. The dry powder of the cyanobacterial fragments was stored at 4 °C before the experiment took place. One gram of the cyanobacterial powder was suspended in 100 ml of double-distilled water (DDW), and 1 ml of the suspension was sprayed into each Petri-dish. The growth and the incubations began by adding DDW to the soils up to a moisture content equivalent to field capacity (approximately 22%, –30 kPa) that was maintained during the experiment. The samples were kept in a growth chamber (21 °C) with continuous illumination. The samples were covered by punctured lids to prevent rapid desiccation while

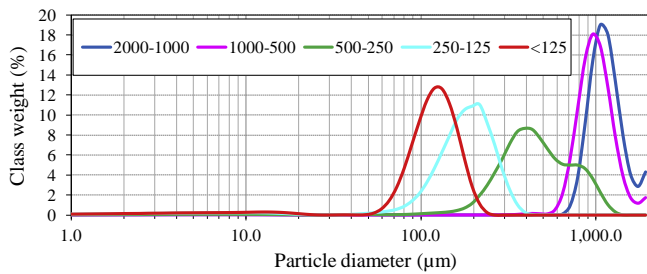


Fig. 1. Grain-size distribution of the sand samples used in the experiment measured with a High-Resolution Laser Diffractometer. USDA classification of sand sizes: <125 µm (very fine sand), 125–250 µm (fine sand), 250–500 µm (medium sand), 500–1000 µm (coarse sand), and 1000–2000 µm (very coarse sand).

simultaneously allowing for gas exchange between the samples and the room environment. The experiment continued for 81 days. Throughout this period, the specific time points when the measurements were performed were referred to by the number of days from the onset of the experiment, with day 0 referring to measurements performed prior to the onset of the experiment.

2.3. Measuring the level of biocrust development

Based on previous studies (Johansen, 1993; West, 1990), the biocrust development was evaluated via several bio-physiological variables that were previously used to measure biocrust development: chlorophyll, polysaccharides, and protein content (Zaady and Bouskila, 2002; Zaady et al., 2014). Since these measurement techniques are destructive to the biocrusts, they were combined with non-destructive spectroscopic measurements. As described below, five dishes from each fraction group were removed for destructive measurements after 32 days; the remaining dishes were tested only at the end of the experiment.

2.3.1. Destructive measurements

Chlorophyll (a+b) content was determined by extraction with ethanol and measurement of the extracts by a spectrophotometer (UV–VIS mini-1240 spectrophotometer, Shimadzu, Columbia, MD, USA). Chlorophyll was quantified by its characteristic absorbance wavelengths (Castle et al., 2011), while polysaccharide content was measured with the UV–VIS mini-1240 spectrophotometer, using an Anthron reagent and sulfuric acid (Dische, 1955). Protein content was determined by extraction from the soil using the Lowry method with 0.1 N NaOH (Lowry et al., 1951). Upon completion of the experiment, biocrust samples from the different fractions were examined for morphology with a scanning electron microscope (SEM, Quanta 200, FEI). Three magnification levels were used to compare between the different fractions (e.g. 300×, 600×, 3000×).

Table 1

Statistical parameters and soil fractions calculated from the particle size analysis by the laser diffractometer. Skewness is a measure of the asymmetry of the grain size distribution curve while kurtosis is a measure of whether the curve is peaked or flat relative to a normal distribution.

Fraction (µm)	1000–2000	500–1000	250–500	125–250	<125
USDA classification	Very coarse sand	Coarse sand	Medium sand	Fine sand	Very fine sand
Sample description	Unimodal very well sorted	Unimodal very well sorted	Unimodal moderately sorted	Unimodal moderately sorted	Unimodal moderately sorted
Mean grain diameter (µm)	1134.3	1038.2	521.4	189.3	122.0
Skewness	–1.331	–0.623	0.726	–0.214	–0.571
Kurtosis	6.433	6.201	3.203	3.364	3.791

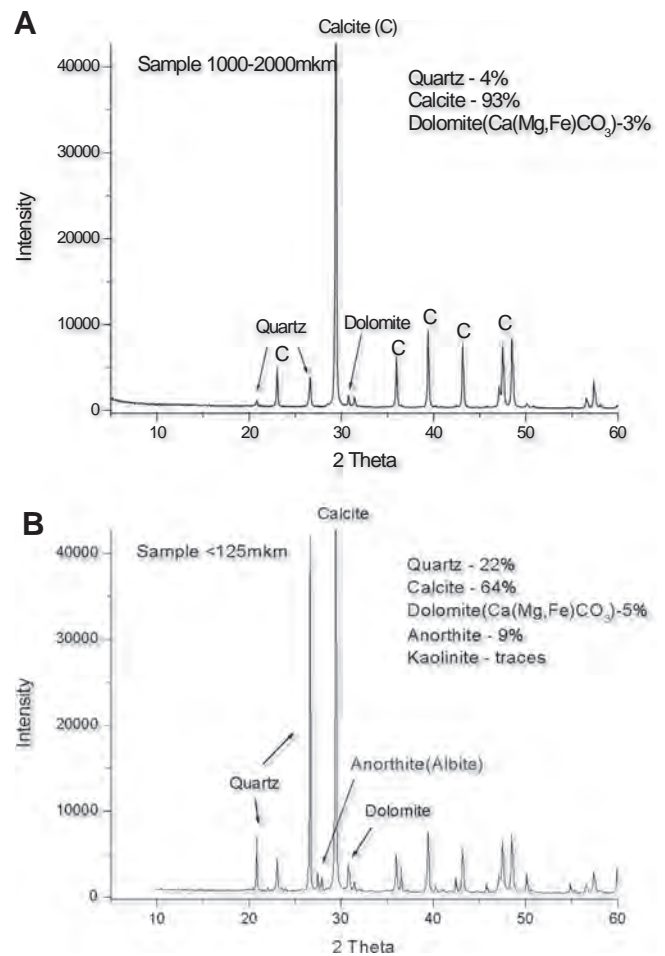


Fig. 2. X-ray Powder Diffraction (XRPD) phase patterns and mineral composition analysis of sediment fractions (A) 1000–2000 µm and (B) <125 µm.

2.3.2. Spectral measurements

Samples were measured in controlled laboratory settings using an Analytical Spectral Devices' (ASD) FieldSpec[®]Pro FR spectrometer (Analytical Spectral Devices Inc., Boulder, CO, USA) with a spectral range of 350–2500 nm in 1-nm intervals. The spectral measurements of the samples took place on days 0, 14, 32, 46, and 81 of the experiment. The samples were irrigated up to saturation about an hour before taking the measurements in order to allow excess water to drain, so that the moisture content of the samples would be at about field capacity during the measurements. This was done in order to standardize the moisture content during the spectral measurements, and to minimize the moisture content changes between measurements. During these measurements, each sample was placed under the spectrometer's 25° bare fiber aperture at a distance of 18 cm to permit the a circular

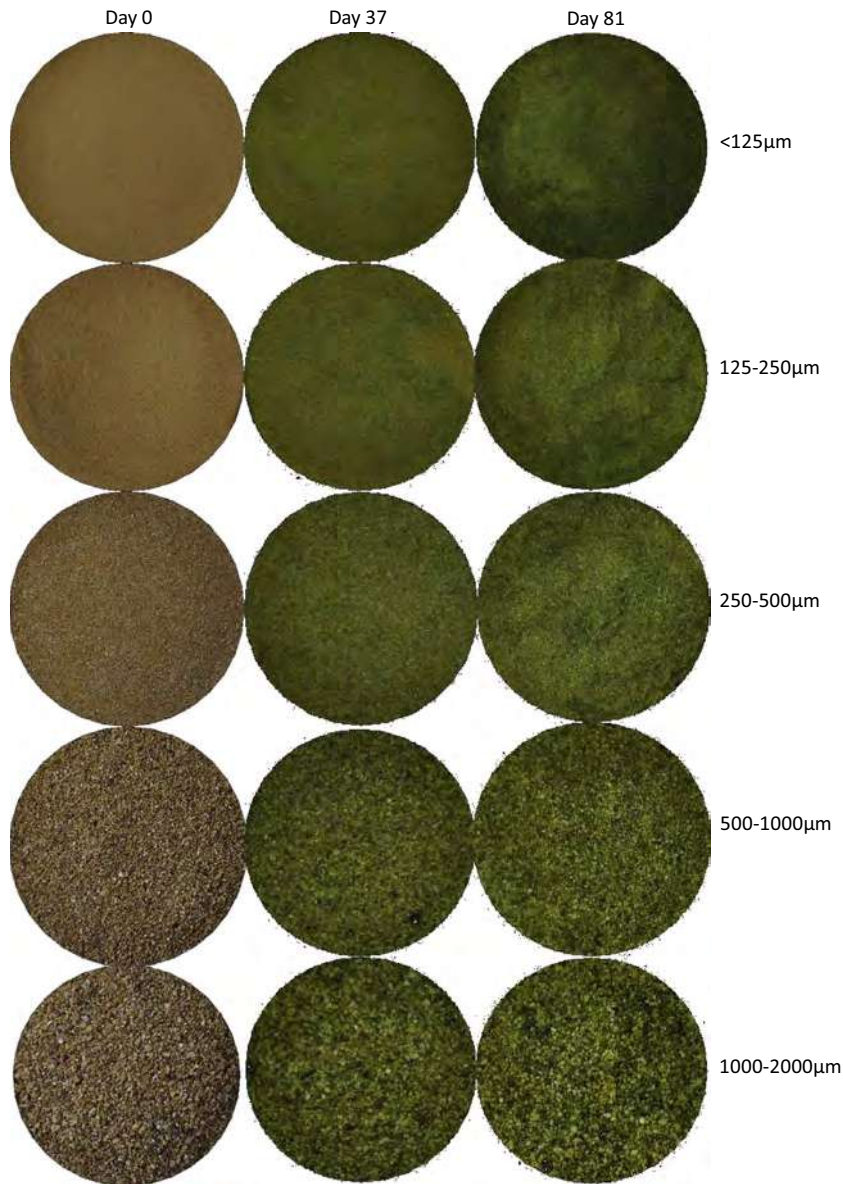


Fig. 3. Representing samples of each fraction on day 0 (prior to inoculation), day 37, and day 81 (at the end of the experiment).

instantaneous field of view of 8 cm in diameter. The surfaces around the sample were opaque and black, thus absorbing all incident radiance in the relevant spectral range. Samples were illuminated from two opposite directions using dedicated illuminator reflectance lamps. The lamps were designed specifically for accurate indoor diffuse reflectance measurements taken with the ASD spectrometer by producing stable illumination over the 350–2500 nm spectral range. The bidirectional illumination reduced the effects of micro-topography shadowing. To further eliminate bidirectional reflectance distribution function (BRDF) effects¹, each sample was measured four times, while rotating 90° between each reading (Karnieli et al., 1996; Roskin et al., 2012). A white Spectralon panel was measured for reference between measurements. Preprocessing of the spectral data included the correction of a bias caused by offsets between the three internal

detectors of the spectrometer (Hatchell, 1999), and the average of the four repeated readings for each sample.

Two spectral indices were calculated for each sample at each point of the measurement series. The Normalized Difference Vegetation Index (NDVI) (Tucker, 1979) and the Brightness Index (BI) (Escadafal and Bacha, 1996) were calculated as:

$$\text{NDVI} = \frac{\text{NIR} - \text{RED}}{\text{NIR} + \text{RED}} \quad (1)$$

$$\text{BI} = \sqrt{\text{GREEN}^2 + \text{RED}^2 + \text{NIR}^2} \quad (2)$$

where GREEN is the integrated reflectance for 500–600 nm, RED is the integrated reflectance for 650–700 nm for NDVI and 600–700 nm for BI, and NIR is the integrated reflectance for 800–900 nm for NDVI and 700–1100 nm for BI.

BI and NDVI were chosen following the findings of Zaady et al. (2007). They illustrated that BI can serve as a good indicator for biocrust development during the early stages following a disturbance. They also recognized that NDVI can be a useful indicator only after crusts have become established. The difference between

¹ The reflectance of a target is a function of illumination geometry and viewing geometry. The BRDF is wavelength dependent and determined by the structural and optical properties of the surface, such as shadowing, multiple scattering, facet orientation distribution and facet density.

the value of each spectral index on day 0 and on every other measurement day was calculated for every sample. This neutralized the influence of mineralogical and grain-size differences between samples from different fractions, since changes to the reflectance spectrum of each sample occurring as a result of possible biocrust development are inferred in relation to the reflectance spectrum at the beginning of the experiment.

2.3.3. Statistical analysis

The statistical analysis was performed with STATISTICA software (StatSoft Inc., Version 12, 2013). The average and the standard deviations for each type of measurement were calculated for each of the five fractions. Differences between the average values were assessed using a one-way analysis of variation (ANOVA) test, followed by Tukey's honest significance difference (HSD) post-hoc test. Results were considered significant at $p \leq 0.05$.

3. Results and Discussion

Fig. 1 and Table 1 show that the difference between the two coarse fractions is not large ($D_{50} = 1134 \mu\text{m}$ and $D_{50} = 1038 \mu\text{m}$), indicating that most of the coarse grains in Wadi Kasuy are around $1000 \mu\text{m}$ in diameter. The other fractions are better separated ($D_{50} = 521 \mu\text{m}$, $D_{50} = 189 \mu\text{m}$, and $D_{50} = 122 \mu\text{m}$). Note that the dominant range in the finest fraction ($<125 \mu\text{m}$) is $50\text{--}125 \mu\text{m}$. Thus, the finest-grained substrates are primarily fine sand grains, and do not represent a significant amount of silt and clay. Fig. 2 presents the XRPD phase patterns and mineral composition analysis of the coarsest (A) and finest (B) fractions. The sand was found to be highly carbonatic, with 93% calcite and 3% dolomite in the coarsest fraction and 64% calcite and 5% dolomite in the finest fraction. Furthermore, the sand was relatively poor in silicates, with 4% quartz in the coarsest fraction and 22% quartz and 9% anorthite in the finest fraction.

Fig. 3 depicts representative samples of each fraction prior to inoculation (day 0), at day 37 of the experiment, and at the end of the experiment (day 81). During the initial stages of the experiment, the surfaces of the fine sand samples were swiftly and homogeneously covered by inceptive biocrusts. However, on coarse sand, the initial biocrusts presented patchy patterns. In these coarse sand samples, cyanobacteria were first established on single grains and appeared to create a dense green coating on these grains before spreading from them to the nearby grains. This dispersal pattern appeared as patchy green spots that grew throughout the experiment. Fig. 4 demonstrates this state in images of representative samples of the extreme grain-size fractions by the end of the experiment. These patches were still visible after 81 days, under growth conditions, despite the thin biocrust cover of the entire surface, including the spaces between these initial points of origin.

Fig. 5 presents the images of the SEM obtained at the end of the experiment, illustrating changes in the cyanobacterial filament distribution and their mode of growth according to the grain-size fraction. While the filaments are relatively thin with a density appearing to be relatively low in the two finer fractions where they form a thin layer, they become dense and create web-like patterns of thick filaments in the two coarsest fractions. The middle fraction shows a transition pattern, containing both thin, low-density filaments, and thicker, denser, web-like patterns likely due to the relatively wider particulate size distribution. The middle fraction may also represent a threshold between the two different patterns of the filaments' spatial distribution, namely, a layer and a web-like pattern.

On day 32, no significant differences in the protein levels were found between any of the fractions. However, on day 81, the

protein level in the finest fraction was significantly higher than in the other fractions, suggesting that biocrusts developed better on the fine fraction (Fig. 6A). The chlorophyll measurements draw a more complex picture. On day 32, the chlorophyll level in the finest fraction was significantly higher than in the other fractions (Fig. 6B). On day 81, no significant differences in the chlorophyll levels were found between any of the fractions, except for the $125\text{--}250 \mu\text{m}$ fraction that had a significantly lower chlorophyll level than the finest fraction. This suggests that biocrust development occurred more rapidly on the finer fraction, but that over time, biocrusts were established on all fractions and chlorophyll levels caught up with those of the finest fraction. Polysaccharide levels did not differ between fractions and did not change

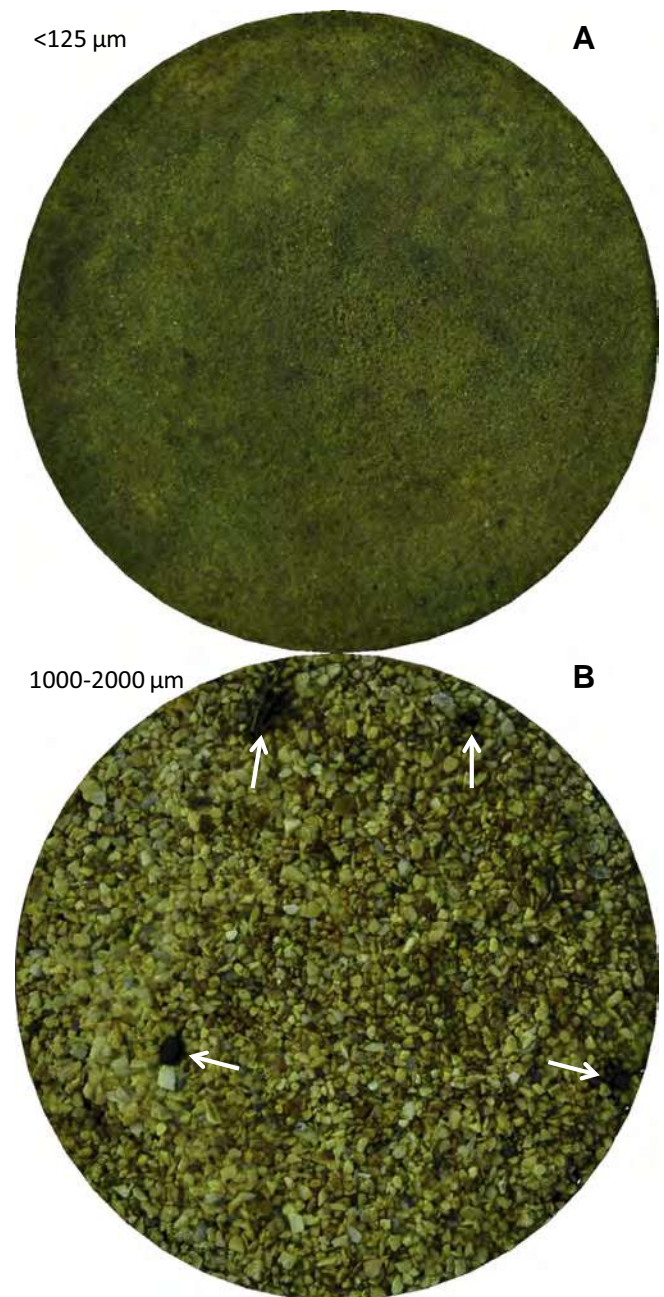


Fig. 4. Close-up on representative samples from the finest fraction (A) and the coarsest fraction (B) at the end of the experiment. Note the relatively homogenous biocrust cover of the finest fraction, compared to the dark patches (marked by white arrows) appearing in the coarsest fraction, where the initial biocrust colonization occurred, and from which it spread to the entire surface of the sample.

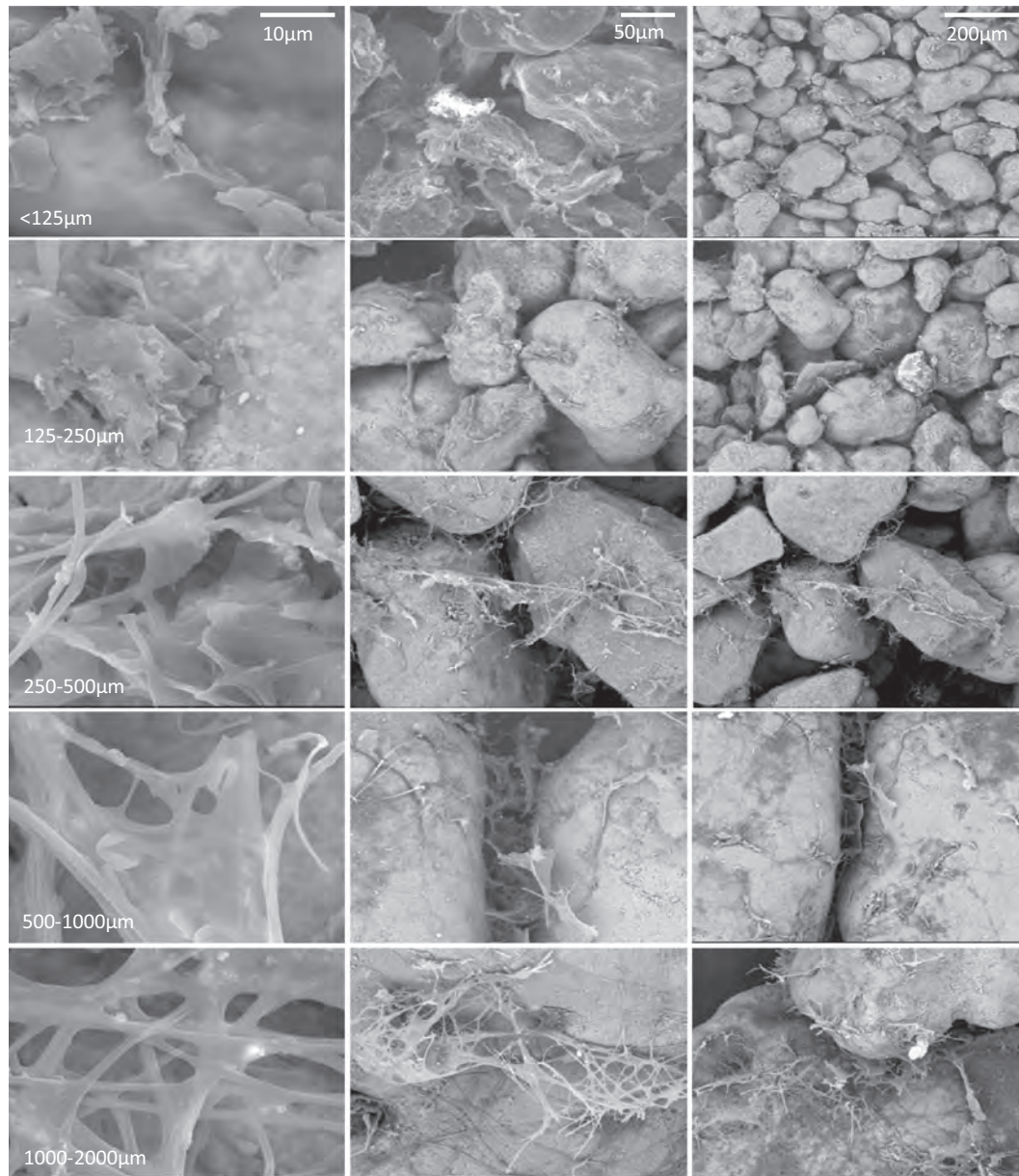


Fig. 5. SEM images of representative samples from each grain-size fraction taken at the end of the experiment, using three different magnification levels: $\times 300$ (right), $\times 600$ (middle), $\times 3000$ (left).

significantly in any of the fractions between day 32 and day 81, suggesting that most of the polysaccharides were secreted before day 32 in all fractions (Fig. 6C). Protein, chlorophyll and polysaccharide measurements exhibited much experimental noise, as shown by the large standard deviations (Fig. 6). These methods may not be comparatively sensitive enough to the inceptive colonization of biocrusts. Indeed, similar measurements have previously demonstrated rather noisy results, even for more developed biocrusts (Zaady et al., 2014, 2007).

Fig. 7 presents the results of the reflectance spectroscopy measurements, which are in line with the biocrust development observed by the naked eye as well as the SEM, and are consistent with the expected effect of biocrust development on the reflectance spectra. On day 14, no index showed any significant differences between the fractions, since at this very early stage, biocrusts had yet to form. However, from day 32 onwards, BI showed preferential biocrust growth on the finer fractions as compared to the coarser fractions. As expected, BI decreased with

biocrust development, and ΔBI indicated a negative increase from the coarser fractions to the finer fractions throughout time. The larger negative increase in ΔBI for the finer fractions is in agreement with the observed rapid and homogeneous dispersal of the biocrusts on the fine fractions and the patchy dispersal on the coarser fractions. The NDVI showed significant differences between the finest and coarsest fractions on day 81 only. The fact that BI is more sensitive to biocrust development than NDVI is consistent with the findings of Zaady et al. (2007) who showed that BI is a good indicator for biocrust colonization at the early stages, while NDVI is more useful after biocrust establishment.

The current research suggests that under controlled laboratory conditions, biocrust development occurs more rapidly on the finer fraction as demonstrated mainly by the protein and chlorophyll levels, but also by the BI and NDVI. The fraction of 250–500 μm acts as a barrier for the spatial spread of cyanobacterial filaments. The biocrust development would typically be affected by the availability of water. Since water infiltration to the deeper layers occurs

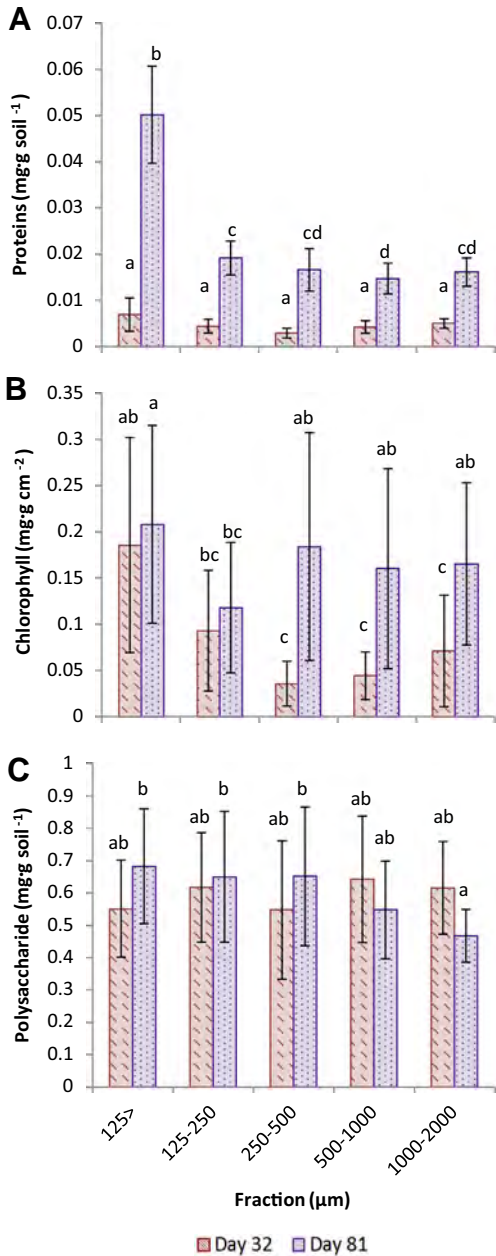


Fig. 6. The variability of (A) protein; (B) chlorophyll (a+b); and (C) polysaccharides, of each of the five grain-size fractions, at 32 days into the experiment and at its end. Vertical bars represent \pm standard deviations from the means. Values with different letters are significantly different ($p \leq 0.05$).

more rapidly when the sand is coarser, this would result in less moisture availability for the biocrusts at surface level. However, throughout the controlled experiment, the samples remained moist, so water was not a limiting growth factor. Based on the described pattern of development on different grain-size fractions, it can be inferred that the dispersal of cyanobacteria through sheath secretion, followed by the expulsion of trichomes, occurs easily on fine particles yet is hindered when the soil grains are coarse. It is suggested that this is due to the large pores between soil particles that inhibit the filaments' spreading. Therefore, it is proposed that filaments must surround the particle of origin before reaching the contact point with a neighboring particle and continuing their growth on it. When fine particles are present, the cavities between the soil particles are smaller; thus, the average distance that the filaments must travel from one particle to the

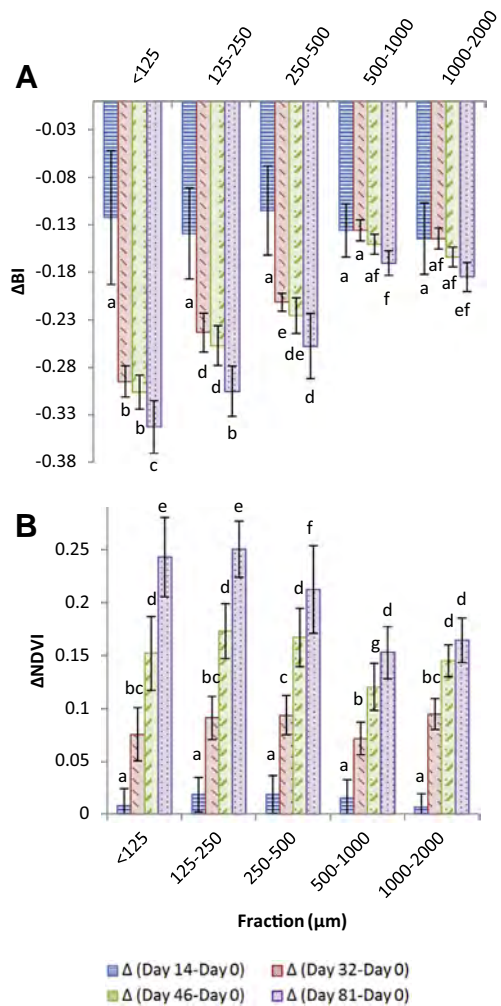


Fig. 7. The variability of each spectral index, for each of the five grain-size fractions, at four time points (14, 32, 46 and 81 days into the experiment): (A) BI; (B) NDVI. Vertical bars represent means \pm standard deviations. Values with different letters are significantly different ($p \leq 0.05$).

next is also smaller. The suggested mechanism can explain the difference in the pattern and duration of cyanobacterial dispersal between different grain-size fractions. In addition, it is expected that finer soil grains, with lower hydraulic conductivity and greater ability to retain moisture, will facilitate the development of cyanobacteria. Thus, the greater availability of moisture in fine sand should reinforce this development. While the conditions of this experiment did not allow for testing this hypothesis since the samples were placed in shallow dishes, future work may examine this mechanism by using deeper and wider vessels. Nonetheless, equalizing the moisture conditions was important to accentuate the differences in the mechanical spreading mechanism of filamentous cyanobacteria on different grain-size fractions.

The better development of biocrusts on finer sands has several implications for the stability of sand dunes. Sand dunes are stabilized by vegetation and biocrusts (Ashkenazy et al., 2012; Breckle et al., 2008; Ravi et al., 2011), with biocrusts playing a crucial role. One example of biocrusts' importance in dune stabilization can be found in the northern Negev. During the last 32 years, following Israel's peace agreement with Egypt in 1982 and the closure of the border between the two countries, there has been no grazing in this area, enabling the recovery of the crust (Tsoar and Karnieli, 1996; Tsoar, 2008; Yair, 2008). Some factors that control the recovery rate of the biocrust include precipitation, the wind

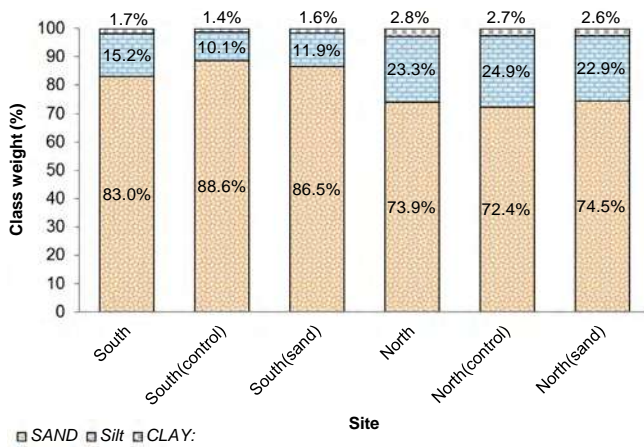


Fig. 8. The percent content of sand, silt, and clay for each treatment at the two sites in the northern Negev dunes. Despite nearly equal precipitation at the two sites, the recovery of the biocrusts in the Northern plot was faster. This can be explained by the larger amount of fine particles in the northern plot (for more details about the experiment, see Zaady et al., 2014).

drift potential (DP) and the type of disturbance. By 1989, topsoil biocrusts, covering extensive areas at the inter-dune, were observed (Yair, 2008). In addition to the disturbance being limited to trampling, the DP² at the inter-dunes is very low (~60 compared to ~320 at the crest upper dune (Amir et al., 2014)), allowing for the deposition of fine particles that favor biocrust development. Thus, there is a feedback between the wind intensity and the biocrust development; the low energy wind environment enhances the deposition of fine particles that favors biocrust growth. In turn, biocrust development increases the surface roughness and thus increases the amount of dust and fine particle deposition. Following a disturbance along the rainfall gradient in the northern Negev dunes it was recently shown that biocrust development occurs more rapidly in the northern site where the precipitation is higher than in the drier southern site (Zaady et al., 2014). These authors performed three treatments in each of the two sites: (a) scalping biocrusts (2–3 cm depth) at the beginning of the experiment; (b) control (e.g. natural crusted soil surface cover); and (c) biocrust removal at the end of the two consecutive years of the experiments thereby exposing the sand dune surface (Zaady et al., 2014). They showed that even though the precipitation was almost the same in the two sites during the first year of the experiment, the recovery of the biocrusts in the northern plot was faster. Comparisons between the granulometry at each site after two years of experiments between control and scalped plots illustrated a lower amount of sand in the wetter northern semi-arid site, 72.4–73.9%, than in the southern arid site, 88.6–83% (Fig. 8). The class weights of silt (2–50 μm) and clay (<2 μm) indicated contrary evidence with a higher amount in the northern site than in the southern site (24.9–23.3% and 2.7–2.8%, respectively, compared to 10.1–15.2% and 1.4–1.7%, respectively). Thus, the fast recovery of biocrusts in the northern site can be explained by the larger amount of fine particles (Fig. 8) that aid biocrust development, which is in accordance with our control experimental results. Eventually, the positive feedback between crust and dust accumulation increased the amount of silt and clay at the northern site.

Previous research supports observations of the relationship between biocrust and soil grain size in many arid environments. Fine sands were previously suggested to be optimal for filamentous cyanobacteria in biocrusts (Bowker and Belnap, 2008), and

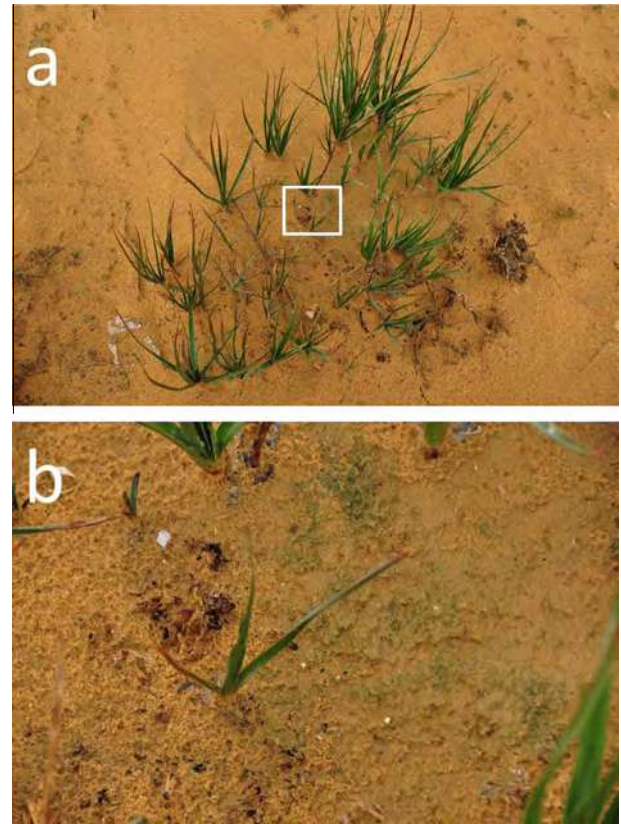


Fig. 9. An *Asphodelus ramosus* L. ring observed in the northern Negev Desert, Israel (precipitation of 170 mm/yr). The photo was taken during a rainy day for ease of biocrust cover observation. The deposition of the fine particles at the ring's center enhances the growth of the biocrusts that can be seen as the green cover (panel b is an enlargement of the white square in panel a). The biocrusts reduce the infiltration in the center compared to the ring's fringe and thus contribute to ring formation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also optimal for *Microcoleus* motility (Noffke et al., 2002, 2003). The distribution of fine sand in arid environments was found to be a key driver for the distribution and density of biocrusts, including cyanobacterial crusts, and moss-lichen crusts (Williams et al., 2013). This can explain why deserts like the Colorado Plateau (US) and the Negev have such extensive biocrust cover. A study conducted in the Mojave Desert where lichen and moss cover is prevailing, supports the general claim that soil texture is the most important determinant of biocrust cover, with higher cover found in areas of greater fine soil grains content (Belnap et al., 2014). This was speculated to be due to the higher surface stability and water-holding capacity of soils with more silt content relative to coarser soils. However, as shown in this study, even under equal moisture conditions, biocrust was better established on the fine fraction. Thus, the results presented in this paper have far reaching implications for desert ecosystems.

Another implication of the current results is related to vegetation ring formation in sandy areas that are water-limited systems (Fig. 9). Most examples of ring patterns are found in clonal plants with confined root zones in the lateral dimensions and on sandy soils. Ring diameter is species-dependent and varies over two orders of magnitude, from about 0.1 m in the case of plant species *Poa bulbosa* to about 10 m for *Larrea tridentate* (Sheffer et al., 2011). One of the suggested mechanisms for ring formation is an aeolian feedback between plants and wind (hydrologic-aeolian process) suggested by Ravi et al. (2007, 2008, 2010). According to this process, fine particles are deposited at the center of the patch, leading

² A measure, in vector units, of the potential sand-moving power of the wind. It is derived from surface wind data (measured at a height of 10 m), above the threshold velocity for sand transport through weighting of the sand transport equation.

to lower infiltration and lower hydraulic conductivity at the ring's center compared to the edges. The retention of moisture at the surface by the finer soil deposited at the center of the ring enhances the formation of physical crusts and biocrusts, resulting in a central die-back and the development of surface-runoff source-sink relations between the ring's center and its fringes (Ravi et al., 2008). The final result of this process is the formation of two micro-environments in the patch: the patch center, characterized by low soil-water content, and the patch fringes, characterized by higher soil-water content (Ravi et al., 2008).

The deposition of fine particles by vegetation, changing the soil texture and favoring biocrust growth, was also observed in sand dunes in northern China (Fearnehough et al., 1998). Over time, the vegetation on stabilized dunes changed from shrubs to annual grasses and herb communities, in association with biocrust development. This process was attributed to the ability of the fine textured sand to retain water at the surface (Fearnehough et al., 1998). However, the current results indicate that even without the soil-water feedback, the fine soil texture favors biocrust growth.

4. Conclusions

Several measurements were used in a controlled laboratory experiment to assess the effect of the grain-size fraction of substrate on the ability of cyanobacterial biocrust to establish. These measurements included chlorophyll, polysaccharides, and protein contents, as well as the spectral indices BI and NDVI. It was found that biocrust development on fine particles occurred more rapidly and relatively homogeneously across the surface. On coarser fractions, the development was slower and patchier. Spectral reflectance measurements were found to be the most useful technique for studying biocrust development on the soil surface without direct disturbance. In addition, BI enabled early detection of the inceptive cyanobacterial development. These results are important for the understanding of hydrological and aeolian processes that change the soil texture and thus contribute to biocrust development. Furthermore, they can explain different physical and biological phenomena in an aeolian environment.

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