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Water repellency decreases with increasing carbonate content and pH for different biocrust types on sand dunes

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Abstract: Biocrusts are biological communities that occupy the soil surface, accumulate organic matter and mineral particles and hence strongly affect the properties of the soils they cover. Moreover, by affecting water repellency, biocrusts may cause a preferential infiltration of rainwater, with a high impact on the formation of local water pathways, especially for sand dunes. The aim of this study is to shed light on the connections between water repellency and pH, carbonate and organic matter content in two dune ecosystems with different biocrust types. For this, we used contact angle measurements, gas volumetric carbonate determination and organic matter characterization via FT-IR and TOF-SIMS. In both ecosystems, moss-dominated biocrusts showed higher water repellency and higher amounts of organic matter compared to algal or cyanobacterial biocrusts. Surprisingly, the biocrusts of the two dune systems did not show differences in organic matter composition or organic coatings of the mineral grains. Biocrusts on the more acidic dunes showed a significantly higher level of water repellency as compared to higher carbonate containing dunes. We conclude that the driving factor for the increase in water repellency between cyanobacterial and moss-dominated biocrusts within one study site is the content of organic matter. However, when comparing the different study sites, we found that higher amounts of carbonate reduced biocrust water repellency.

Keywords: Organic matter composition; Surface characteristics; TOF-SIMS; Biocrust; Carbonate content; Water repellency.

INTRODUCTION

Water repellency (WR) is an important factor for surface and subsurface water redistribution, plant growth and aggregate stability, as well as soil erosion (Doerr et al., 2000; Zheng et al., 2016). While WR can occur on a variety of soil types and textures, it affects soils with a high content of sand particles (like dune soils) to a higher degree than soils with a fine texture (González-Peñaloza et al., 2013; Woche et al., 2005). Especially the amount and composition of mineral particles and organic matter (OM) affect the extent and persistence of WR. The effect of texture on WR can be explained by the specific surface area (SSA) of the mineral particles, which increases with decreasing particle size. Hence, for the coating of fine-grained soil particles, a higher amount of hydrophobic OM is needed as compared to coarser particles (González-Peñaloza et al., 2013). Assuming the same amount of hydrophobic OM, this relation causes a decrease in WR with decreasing particle size (Woche et al., 2005; Zheng et al., 2016). Consequently, the addition of smaller particles like clay and silt decreased WR and texture is the most predictive factor influencing WR (McKissock et al., 2000). Additionally, the application of lime also affects WR via two mechanis. First, the input of fine particles increases the SSA and secondly because it increases the decomposition of hydrophobic compounds by bacteria due to the creation of more favorable environmental conditions (Roper, 2005). Most studies show a positive correlation of WR and the amount of OM with a non-linear increase in WR with increasing OM content (Leelamanie and Karube, 2009; Vogelmann et al., 2013; Woche et al., 2005). OM consists of fresh plant tissues, plant waxes and a high number of amphiphilic compounds like fatty acids. These compounds can form OM coatings (Graber et al., 2009; Morley et al., 2005). If OM components are mixed with mineral particles, the WR increases only slightly while a coating of particles with OM results in more intense WR (Bisdom et al., 1993).

However, WR is a highly dynamic soil property. For example, WR increases with decreasing water content (Dekker and Ritsema, 1994) while under laboratory conditions WR decreases with increasing soil pH (Diehl et al., 2010). For Mediterranean soils, the persistence of WR in the field was found to decrease with increasing pH value (Mataix-Solera et al., 2007; Zavala et al., 2009).

Biocrusts cover soils as part of early ecological succession or as permanent soil cover in semiarid and in humid climates including sand dunes around the world (Belnap, 2006; Nierop et al., 2001; Tighe et al., 2012). Biocrusts consist of cyanobacteria, algae, lichens, bacteria, fungi and mosses in different ratios depending on climate and successional stage. During growth, these organisms influence the soil pH, accumulate carbon, nitrogen (Chamizo et al., 2012; Lichner et al., 2018) and other elements (Beraldi-Campesi et al., 2009), stabilize the soil surface and change the soil structure (Felde et al., 2014) of the upper soil layer at millimeter scale. Since biocrust formation changes the properties of the very soil surface, it also affects WR and hydraulic conductivity of the soil surface (Gypser et al. 2016; Tighe et al., 2012). In most studies, biocrusts show only subcritical WR values, including studies on biocrusts in the Negev (Gypser et al., 2016; Keck et al., 2016; Kidron and Büdel, 2014). The semi-arid northwestern Negev provides unique growing conditions for biocrust organisms, as the sand dunes are part of a nature protection area and the ecosystem has a high input of dust (Littmann and Schulz, 2008) and moisture via dew (Jacobs et al., 2000). The geological material is enriched in carbonates, has an alkaline pH and biocrust growth is rather fast (Kidron et al., 2020). In contrast to this, biocrusts from the humid Sekule site in Slovakia show strong WR on carbonate-free dunes with an acidic pH-value (Lichner et al., 2012). This effect increased with ongoing biocrust development and increasing amounts of OM (Drahorad et al., 2013b; Drahorad et al., 2020). Until now, studies comparing the WR and correlated soil properties of biocrusts in contrasting ecosystems are missing. We compared biocrusts of the Negev dunes (Israel) and at Sekule (Slovakia) to test the hypothesis that higher pH values (as induced by higher carbonate contents) are correlated with lower levels of WR in biocrusts. To check this, we correlated contact angle (CA) data with pH values. Moreover, as earlier studies did show that an increase in WR correlates with an increase in OM but does not relate to OM characteristics at the Sekule site, we hypothesized that the same effect will be visible for biocrusts of the Negev. To test this, we compared data on OM amounts of two biocrust types at each study site and characterized the OM at the Negev site using Fourier transform infrared spectrometry (FT-IR). Moreover, organic coatings may play a major role in WR of biocrusts. We use Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) as powerful tool for the characterization of environmental samples to show the surface characteristics of biocrust-associated mineral grains (Arenas-Lago et al., 2016; Cliff et al., 2002). As the biocrusts at Sekule developed under humid climate conditions, we hypothesize a higher OM accumulation in these biocrusts as compared to biocrusts of the Negev (Israel), which in turn may be accompanied by a thicker organic coating of mineral grains.

MATERIAL AND METHODS

The first study site is located at Sekule (southwestern Slovakia) with a mean annual precipitation of 550 mm and carbonate-free inland sand dunes as parent material. Because of sand mining for building purposes, an artificial glade arose, where biocrusts cover the sandy soils. The biocrusts at freshly disturbed areas are thin, algae- and cyanobacteria-dominated crusts while thick, moss-dominated crust cover the soil at less disturbed areas. For a detailed description of the forest glade side and the occurring biocrust species, see Lichner et al. (2013). The second study site is located in the Negev dunes (Israel) 25 km north of Nizzana and 12 km south of the town of Yevul and is characterized by a mean annual precipitation of approx. 170 mm and carbonate containing sand as parent material. Biocrusts stabilize the dunes with thin cyanobacterial crusts at the south-exposed slopes and thicker, moss-dominated crusts dominate at the wetter, north-exposed dune slopes. For a detailed description of biocrust OM composition and occurring cyanobacterial species see Drahorad et al. (2013a) and Hagemann et al. (2015).

Sampling of biocrusts and underlying soil

At both study sites, we sampled biocrusts in two depths, including algae-and cyanobacterial-dominated biocrusts at freshly disturbed areas in Sekule and the southexposed slopes in the Negev and moss-dominated biocrusts at less disturbed areas in Sekule and northexposed dune slopes in the Negev. Sampling included three depths: i) the topcrust (TC; 0–2 mm) ii) the underlying subcrust (SC; 2–20 mm) and iii) the topsoil (TS; 20–100 mm). We analyzed soil texture, carbonate content, pH and CA on these samples. In addition, we used the Water Drop Penetration Time (WDPT) test for the description of actual repellency on intact in-situ biocrusts (only for the TC). For the characterization of OM of Negev biocrusts we used the TC and SC samples of cyanobacterial- and moss-dominated biocrusts, to allow a good comparability with existing results of the same biocrust types at Sekule site. To test the hypothesis of changes in the organic coatings of mineral biocrust particles, we isolated particles from moss-dominated biocrusts of the two study sites. These TCs are characterized via ToF-SIMS. We concentrated on these samples as moss-dominated biocrusts showed the highest WR. Therefore, we expect to find the highest differences in coating thickness and composition for mineral particles between the two study sites.

Sample treatment and analysis

The content of sand particles and finer (i.e. silt and clay) particles (2000–63 μm and < 63 μm, respectively) was classified based on wet sieving according to ISO 11277. All samples were dried at 105°C and sieved (2 mm) and an aliquot was finely ground (0.05 mm) for the measurement of total carbon (C) and total nitrogen (N) by dry combustion (Vario EL CNS analyzer). For the Negev samples, the carbonate content was analyzed gas-volumetrically using a Scheibler apparatus according to ISO 10963. For the Negev, this includes mainly calcium carbonates and to a lesser extent, magnesium carbonates (Rozenstein et al., 2014). The amount of total organic carbon (TOC) was calculated as the difference between carbonate content and total carbon. The pH value was measured in a 1:5 water extract.

For the Negev samples, we included an **OM characterization via FT-IR.** For recording FT-IR spectra, we used 1 mg of ground, desiccated soil (< 0.5 mm) mixed with 80 mg of potassium bromide and dried over night over silicagel in an exsiccator (Ellerbrock et al., 1999). The mixture was pressed into a pellet by applying a pressure of 980.7 MPa for 10 min. Infrared absorbance spectra of OM were collected in the wave number range of 4,000–400 cm^{-1} with 16 scans per spectrum. The spectra were smoothed (boxcar moving average algorithm, factor 45) and corrected for baseline shifts using WIN-IR Pro 3.4 software (Digilab, Massachusetts, USA). For a detailed description on FT-IR spectra of the Sekule samples see Drahorad et al. (2020).

Water repellency (WR) measurements included water drop penetration time (WDPT) test. It is the fastest in-situ method for assessing the persistence of the actual WR of the undisturbed biocrusts. A drop of distilled water (approx. 50 μL) is placed on the soil surface and the time that it takes for complete surface penetration is recorded. Since water only enters the soil if the contact angle between water and soil is less than 90°, the WDPT test is a measure of the time required until the contact angle reaches values below 90° and thus, of the persistence of WR rather than its intensity (Iovino et al., 2018; Letey et al., 2000). We use the terms ´actual WR` for field-moist samples in natural position and ´potential WR` as the maximum possible WR for samples that were dried by 105 °C, according to Dekker and Ritsema (1994).

A second method that we used for the analysis of WR was the measurement of the contact angle via the Wilhelmy Plate Method (WPM). The WPM allows the determination of the advancing and receding contact angle and is theoretically suited to measure contact angles between 0° and 180° (Bachmann et al., 2003). Briefly, we used disturbed samples (< 2 mm) to create a thin layer of soil particles on a glass slide using double-sided adhesive tape. When present, aggregates were gently crushed in a mortar. Despite the coarse texture of the samples, we decided not to grind them in order to avoid the breaking of sand grains, which would have resulted in the creation of new surfaces that likely cause an underestimation of the CA. We measured the advancing contact angle with five repeated measurements for each sample, using a dynamic contact angle tensiometer (DCAT11, Dataphysics, Filderstadt, Germany).

It should be noted that one possible source of error that can lead to the overestimation of CA for the Sekule samples may have been the coarse texture and the fact that samples were not ground prior to CA analysis. While the Negev samples contained more fine particles, which are likely to have covered the complete adhesive tape, this was not the case for the Sekule samples. Containing fewer silt and clay-sized mineral particles, it may have been the case that some spots on the adhesive tape between larger sand grains were exposed to the water, which may have led to an overestimation of the CA for this sample.

For **surface characterization and the description of mineral grain coatings**, we analyzed single sand grains by using Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). Therefore, we isolated mineral grains of moss-dominated biocrusts via density separation in deionized water and transferred them on an adhesive copper tape. The ToF-SIMS measurements were performed with a TOF.SIMS M6 instrument (ION-TOF GmbH, Muenster, Germany) equipped with a 30 keV Bi-cluster primary ion gun, as well as with a gas cluster ion beam (GCIB) and a dual source column (DSC) for sputtering. All analyses were carried out with Bi^{3+} primary ions, with a cycle time of 75 µs (imaging mode) or 120 µs (spectrometry mode) in positive and negative ion mode. Charge compensation was done with low energetic electrons. Mass spectra were recorded using the spectrometry mode on an analysis area of 500 x 500 µm^2 keeping a dose density limit of 10^{12} ions cm^{-2}. A mass resolution of FWHM $m/\Delta m > 3500$ at m/z 29.00 (CHO^+) in positive ion mode and $m/\Delta m > 1800$ at m/z 26.00 (CN^-) in negative ion mode was achieved. The signals H^{2+}, CH^{3+} (15.02 u), Na^+ (22.99 u), K^+ (38.96 u), $C_3H_5^+$ (41.04 u), $C_3H_7^+$ (43.05 u) in positive ion mode and H_2^- (2.01 u), C^- (12.01 u), C_2^- (24.00 u), C_3^- (36.00 u), PO^{2-} (62,97 u) in negative ion mode were used for internal mass calibration. Surface analysis of the soil particles turned out to be challenging due to their heterogeneous surface properties and the particulate character of the sample system. In addition, the topography of the particles has a significant impact on mass resolution of the obtained spectra. Therefore, five regions of each sample set were analyzed in spectrometry mode for comparison. The corresponding mass images were used to set regions of interest (ROI), which were defined by a threshold of 10%–90% pixel intensity of the total ion image to select the particle areas. By normalization to the total ion signal intensity, we minimized the topographic effect not only on the mass resolution but also on the signal intensity and enabled a comparison of selected mass signals. The mass images shown in Fig. 4 were recorded in imaging mode with delayed extraction for good mass and good lateral resolution (Henss et al., 2018). For the images, areas of 500 x 500 µm^2 with 1024 x 1024 pixels were scanned. More detailed information on SIMS measurements can be found elsewhere (Vickermann and Gilmore, 2009). Data analysis was performed with the "SurfaceLab 7.1.1" software (ION-TOF GmbH).

Data management and statistics included the calculation of significant differences between the measured biocrust parameters on a level of significance of 5%, using a 1-way ANOVA. As the data-set was too small for a MANOVA including biocrust depths, types and sampling areas as single fixed factors, we reclassified these samples in 12 equal factors, allowing the calculation of a 1-way ANOVA. Post Hoc Scheffé test was used, as the data set was unbalanced. Pearson´s correlation coefficient was used to describe correlations (Statistica 14).

RESULTS AND DISCUSSION

The TC samples at different sampling sites show significant differences in pH, carbonate content, texture and CA ($p < 0.001$) (Table 1). Interestingly, TC samples of the same biocrust type show comparable contents of TOC and N at the two study sites (Table 1 and Figure 1). This is surprising, as the humid ecosystem in Sekule should in general favor higher biomass accumulation due to higher amounts of annual precipitation. With increasing annual precipitation and available moisture, biocrust biomass increases (Kidron et al., 2014; Lichner et al., 2018).

Table 1. Basic biocrust and soil characteristics at Sekule (Slovakia) and Negev dunes (Israel) in three sampling depths (TC 0–2 mm; SC 2–20 mm; TS 20–100 mm; n = 7/6/4 for Sekule and n = 3 for Negev dunes). Particle size distribution by wet sieving (n = 4 Sekule, n = 3 Negev). All values are means, standard deviation in parenthesis. Different upper case letters denote significant differences between sampling depths within the same biocrust type at each sampling site, different lower case letters denote significant differences between biocrust types at the same sampling depth and sampling site and different numbers denote significant differences between comparable biocrusts types at different sampling sites.

						sand particles			fine particles
Area	type	depth	pH	Carbonates	N	2000–630 µm	630–200 µm	200–63 µm	< 63 µm
				[weight-%]	[%]	[%]			
Sekule	algae	TC	4.8 (±0.1)^{A,a,1}	0.0^{A,a,1}	0.04 (±0.01)^{A,a,1}	0.01 (±0.02)^{A,a,1}	32.43 (±10.84)^{A,a,1}	57.42 (±9.93)^{A,a,1}	3.01 (±0.32)^{A,a,1}
		SC	4.9 (±0.1)^{A,b,1}	0.0^{A,a,1}	0.02 (±0.01)^{A,b,1}	0.01 (±0.01)^{A,a,1}	32.35 (±8.17)^{A,a,1}	61.89 (±7.67)^{A,a,1}	3.10 (±0.85)^{A,a,1}
		TS	4.9 (±0.1)^{A,b,1}	0.0^{A,a,1}	0.01 (±0.00)^{A,b,1}	0.02 (±0.02)^{A,a,1}	29.07 (±2.30)^{A,a,1}	65.15 (±3.81)^{A,a,1}	3.31 (±0.77)^{A,a,1}
	moss	TC	4.4 (±0.1)^{A,a,1}	0.0^{A,a,1}	0.09 (±0.02)^{B,a,1}	0.00 (±0.00)^{A,a,1}	28.39 (±9.31)^{A,a,1}	44.69 (±8.19)^{A,a,1}	4.69 (±1.29)^{A,a,1}
		SC	4.6 (±0.1)^{A,b,1}	0.0^{A,a,1}	0.03 (±0.01)^{A,b,2}	0.03 (±0.01)^{A,a,1}	34.75 (±15.22)^{A,a,1}	50.28 (±16.36)^{A,a,1}	3.93 (±0.35)^{A,a,1}
		TS	4.8 (±0.1)^{B,b,1}	0.0^{A,a,1}	0.01 (±0.00)^{A,b,2}	0.03 (±0.03)^{A,a,1}	37.16 (±13.46)^{A,a,1}	51.38 (±15.98)^{A,a,1}	3.68 (±1.23)^{A,a,1}
Negev	cyano	TC	7.4 (±0.1)^{A,b,2}	2.6 (±0.2)^{A,a,2}	0.03 (±0.01)^{A,a,1}	4.61 (±1.15)^{A,a,2}	58.56 (±2.40)^{A,a,2}	33.83 (±2.68)^{A,b,2}	10.13 (±1.42)^{A,a,1}
		SC	8.0 (±0.4)^{A,b,2}	1.7 (±0.2)^{A,a,2}	0.01 (±0.00)^{A,b,1}	3.33 (±0.67)^{A,a,2}	56.57 (±3.93)^{A,a,2}	37.00 (±3.85)^{A,b,2}	5.75 (±0.74)^{A,a,1}
		TS	8.4 (±0.3)^{B,b,2}	1.0 (±0.2)^{A,a,2}	0.01 (±0.00)^{A,b,1}	2.34 (±0.60)^{A,a,2}	58.08 (±1.80)^{A,a,2}	36.27 (±1.66)^{A,a,2}	5.76 (±2.36)^{A,a,1}
	moss	TC	7.1 (±0.2)^{A,b,2}	7.3 (±1.1)^{A,b,2}	0.10 (±0.02)^{B,a,1}	3.30 (±0.82)^{A,a,2}	57.97 (±3.68)^{A,a,2}	34.05 (±3.37)^{A,a,2}	26.92 (±2.09)^{B,b,2}
		SC	7.6 (±0.2)^{A,b,2}	5.4 (±2.2)^{A,b,2}	0.04 (±0.01)^{A,b,2}	4.08 (±1.42)^{A,a,2}	54.28 (±1.65)^{A,a,1}	37.70 (±2.91)^{A,a,2}	14.94 (±2.38)^{A,b,2}
		TS	8.0 (±0.2)^{B,b,2}	4.2 (±0.5)^{B,b,2}	0.02 (±0.01)^{A,b,2}	2.96 (±0.79)^{A,a,2}	57.65 (±2.34)^{A,a,1}	35.71 (±1.78)^{A,a,2}	11.44 (±4.36)^{A,a,2}

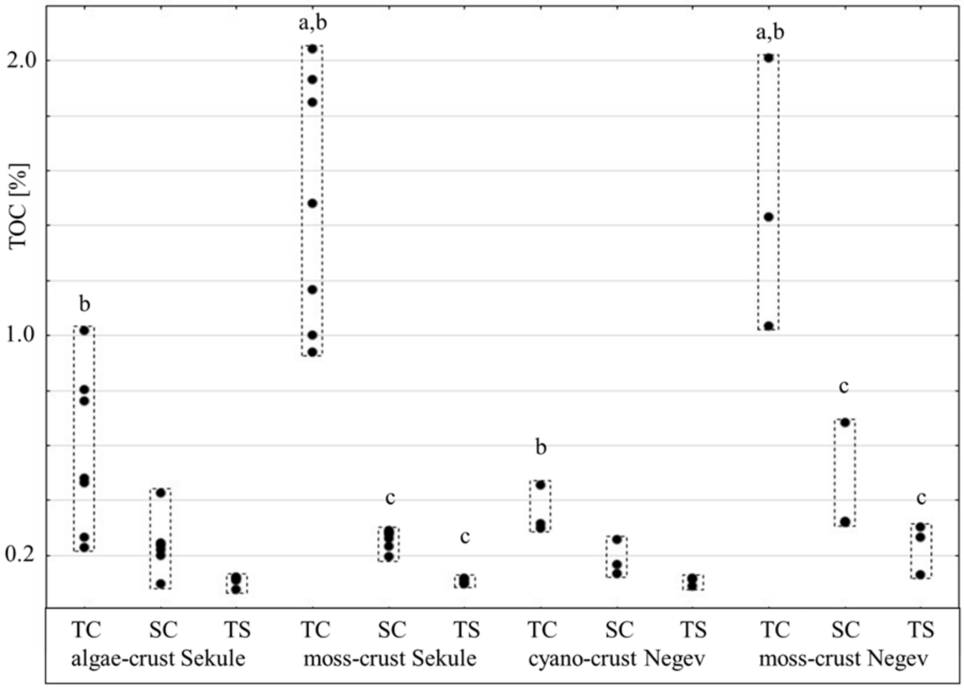

Fig. 1. Variability plot showing the total organic carbon (TOC) of two biocrust types (algae/cyano; moss) in three sampling depths (TC 0–2 mm; SC 2–20 mm; TS 20–100 mm) in Sekule and the Negev. Letters indicate a significant difference ($p < 0.05$) between sampling depths within the same biocrust type (a), a significant difference between the biocrust types at the same sampling depth and sampling site (b) or a significant difference between comparable biocrusts types at different sampling sites (c).

We therefore assume that the available moisture is lower in Sekule, as water infiltrates fast and the biocrusts dry rapidly due to the high content of sand sized particles (> 95%). In contrast, higher amounts of fine sized particles (Table 1) increase the water holding capacity and wetness duration, likely favoring a higher biomass build-up in cyanobacterial Negev biocrusts (Kidron et al., 2009).

Total organic carbon and pH-values of biocrusts

The TCs show lower pH values as compared to SC and TS samples (with the exception of the TC of the algae biocrust at Sekule). This is in accordance with findings for biocrust-covered soils located in humid regions of eastern Germany (pH decrease from 4.8 to 4.2) or in semiarid regions within the Negev (pH decrease from 8.6 to 7.6) (Fischer et al., 2010; Keck et al., 2016). The biocrust samples from both sites, Negev and Sekule, show a decrease in pH from the cyanobacterial/algae biocrust to the moss-dominated biocrust. This trend was also found for a development from algae to moss-dominated biocrusts in the Netherlands (pH decrease from 4.8 to 4.2) (Nierop et al., 2001).

The accumulation of TOC is significantly higher in the TC of moss-dominated biocrusts at both sites as compared to the SC and TS underneath and as compared to the algae- or cyanobacterial-dominated biocrust (Figure 1). For Sekule, the two examined biocrust types represent an early and a late successional development stage, respectively. As late successional biocrusts show higher gross photosynthesis than early successional biocrusts, that can induce higher overall TOC accumulation in these biocrust (Miralles et al., 2018).

Organic matter characterization of Negev dune biocrust

FT-IR was used to describe the OM of the biocrusts at the Negev site as already done in an earlier work for the biocrusts of Sekule (Drahorad et al., 2020). The FT-IR spectra are characterized by the same bands as the previously recorded for data on biocrusts in Sekule (Figure 2). This included the bands relevant for the WR of soils at the wavenumbers at 2925 cm^{-1} + 2858 cm^{-1} (aliphatic C-H) and 1635 cm^{-1} (C=O, aromatics) (Ellerbrock et al., 2005). This similarity in the spectra is in accordance with FT-IR data of biocrusts sampled in humid climate (Fischer et al., 2013). Nierop et al. (2001) did show a comparable pattern of polysaccharides in marine algae, soil algal mats and moss-covered dune sand. In general, bacterial vs. fungal materials show the same pattern in ^{13}C NMR-spectra with high proportions of alkyl-C structures and polysaccharides (Kögel-Knabner, 2002). NMR-spectra of biocrusts from the Negev and eastern Germany showed similar OM composition and also similarities to cell spectra of algae (Fischer et al., 2013). More studies on the OM composition are needed to reveal general (i.e. global) patterns. Nevertheless, for the comparison between Negev and Sekule biocrusts we assume that the biocrust OM composition is not the relevant driver for differences in WR.

Differences in the FT-IR spectra were pronounced for the bands 1085 and 1033 cm^{-1} (polysaccharides, silicates and clay minerals) showing a double peak for Negev biocrusts compared to Sekule biocrusts. This reflects the higher amount of fine particles and therefore likely higher clay mineral content in these samples. Moreover, the second difference between the spectra are bands near 1430 and 875 cm^{-1}, which are characteristic for carbonates (Smidt et al., 2002). For the Negev biocrusts, the reduction in absorbance of the spectrum at 875 cm^{-1} in the order cyanobacterial TC>cyanobacterial SC>moss TC >moss SC is in line with the carbonate concentrations determined (Table 1). The results demonstrate that FT-IR can also be used for carbonate concentration measurements in biocrusts as already shown for other carbonate-containing soils (Tatzber et al., 2007).

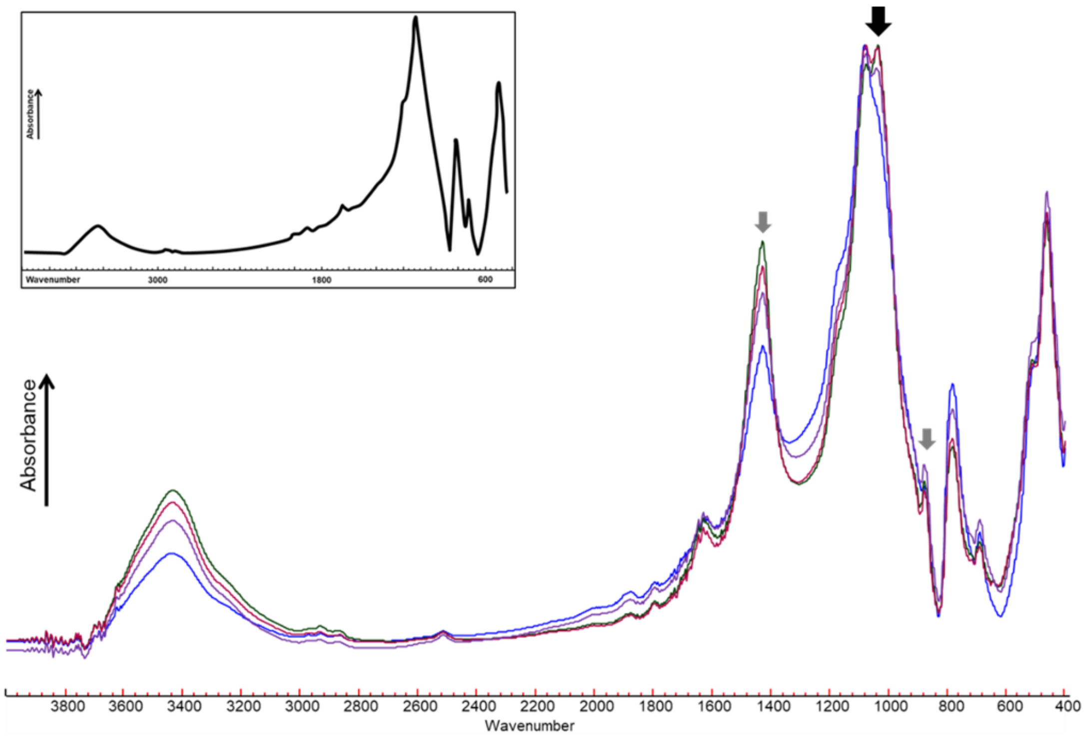

Fig. 2. FT-IR spectra of cyanobacterial and moss-dominated biocrust of the Negev (green = cyanobacterial topcrust; red = cyanobacterial subcrust; violet = moss-dominated topcrust; blue = moss-dominated subcrust). Insert for comparison: mean FT-IR spectra of algae and moss top- and subcrusts at Sekule, original data see: Drahorad et al., 2020). Arrows indicate differences in FT-IR spectra with bands indicating higher clay content (black arrow) and bands indicating the occurrence of calcium carbonate (grey arrows).

Table 2. Intensity of water repellency (contact angle) in the examined biocrusts at Sekule (Slovakia) and Negev dunes (Israel) in three sampling depths (TC 0–2 mm; SC 2–20 mm; TS 20–100 mm; n = 7/6/4 for Sekule and n = 3 for Negev) and persistence of water repellency (actual repellency) of the undisturbed biocrusts in situ (n = 10). Different upper case letters denote significant differences between sampling depths within the same biocrust type at each sampling site, different lower case letters denote significant differences between different biocrust types at the same sampling depth and sampling site and numbers denote significant differences between comparable biocrusts types at different sampling sites.

Area	crust type	depth	contact angle [°]	actual repellency / WDPT [s]
Sekule	algae	TC	111.75 (±11.92)^{A,a,1}	very hydrophilic 0 (±0)
		SC	109.90 (±10.29)^{A,a,1}	–
		TS	88.39 (±7.26)^{B,a,1}	–
	moss	TC	135.01 (±8.99)^{A,b,1}	moderately hydrophobic 294 (±14)
		SC	113.63 (±4.37)^{B,a,1}	–
		TS	93.54 (±4.24)^{C,a,1}	–
Negev	cyano	TC	29.07 (±2.87)^{A,a,2}	very hydrophilic 0(±0)
		SC	27.43 (±13.65)^{A,a,2}	–
		TS	29.40 (±6.50)^{A,a,2}	–
	moss	TC	86.70 (±11.75)^{A,b,2}	very hydrophilic 0(±0)
		SC	62.50 (±11.08)^{A,b,2}	–
		TS	38.77 (±9.55)^{B,a,2}	–

Actual repellency and intensity of WR of biocrusts

The WDPT of the undisturbed biocrust in-situ shows that only one biocrust is moderately hydrophobic (Table 2). Early biocrusts on Sekule sand and biocrust growing on carbonate containing sands of the Negev do not show an actual WR persistence. These values are below WR values of sandy soil surfaces under various European pine forests (< 433 s) (Iovino et al., 2018) and below the actual repellency found on non-calcareous sand dunes in the Netherlands (600–3600 s) (Dekker et al., 2001). Compared to these rather low values of the actual repellency, the CA data shows a moderate to very strong potential WR resistance for algae biocrusts at Sekule study site. Two treatment effects explain this difference. First, the concept of potential WR includes drying of samples and the potential WR of soil samples increases with increasing drying temperature (Dekker et al., 2001; Diehl et al., 2009). Moreover, the disturbance itself during sampling has a profound influence. Graber et al. (2006) identified strong differences of WR between disturbed and undisturbed samples in sandy soils. They hypothesized that the reason for these changes relate to differences in surface roughness, pore size distribution, pore connectivity, bulk density and changes in the distribution and orientation of the substances that are responsible for repellency. Moreover, even very thin layers of fine particles covering biocrusts changes the WR on the surface (Cania et al., 2020; Fischer et al., 2010). For example, sand burial of moss-dominated biocrusts in the Tengger desert was reported to decrease WR (Jia et al., 2020). These results show the importance of the biocrust surface structure and the in-situ integration in the ecosystem for real field site WR. Therefore, measurements of intact biocrusts are relevant for the evaluation of water flow pathways in biocrust covered ecosystems.

In contrast to the actual repellency determined by WDPT, the intensity of WR shows CA of above 100° for both biocrust types at Sekule and CA up to almost 90° for the moss-dominated biocrust of the Negev. Here, differences between crust types are most obvious. While the values for all depths of the cyanobacterial biocrust of the Negev do not show any differences, for the moss-crust a clear increase from 38.77° to 62.50° and finally 86.70° can be observed from TS to SC and finally to TC. Different CA between the different sampling depths, which show the effect of OM accumulation by the crust organisms, are obvious for all but the cyanobacterial crust from the Negev. The fact that differences between crust types in Sekule are very low (and in fact are only significant in the case of the TC vs. TS in the moss-dominated biocrust) may be indicative for the effect of texture and pH at this study site. Soils below the Sekule biocrusts have a coarser texture and a more acidic pH compared to the Negev soils.

Relation between WR, TOC and pH value of biocrusts

In both ecosystems, algae- or cyanobacterial-dominated biocrusts show lower TOC content and CA than moss-dominated biocrusts. This trend is in line with earlier studies on WR of biocrusts. As biocrusts develop, their thickness and the amount of OM increase as well, and so does their WR (Drahorad et al, 2020; Gypser et al., 2016; Lichner et al., 2018). For both study sites, TOC and CA are highly correlated (r = 0.81 Sekule and r = 0.83 Negev). This confirms part one of our hypothesis on OM dynamics, namely that an increase in biocrust OM induces an increase in WR. Plotting the complete data set confirms visually that the Sekule biocrusts show higher WR for samples with a similar TOC content compared to Negev biocrusts (Figure 3). Therefore, we assume that within each study site, the differences in WR result from a higher overall amount of OM in the moss-dominated biocrusts as compared to the algal-/cyanobacterial crusts. This effect may be stronger at the Sekule site, as Wang et al. (2010) found that soil organic carbon affected WR stronger in soils that were classified as repellent, while texture and pH had a higher impact on WR in wettable/non-repellent soils.

Based on the conclusion that an increasing OM content is inducing a higher WR for biocrusts at the same study site but that OM amount or composition do not explain the differences between the study sites, two effects may explain the differences in the WR. First, the biocrusts at the Negev reveal a higher pH value than the biocrusts in Sekule and pH and CA show a strong negative correlation (r = –0.76 Sekule and r = –0.73 Negev). Studies on WR and pH on semiarid alkaline soils showed lower persistence of WR compared to acidic soils (Mataix-Solera et al., 2007). In their meta-analysis, Zheng et al. (2016) also reported a negative correlation between pH and WR, while soil organic carbon generally correlates positively with WR.

Deprotonation of surface sites and the changes in OM confirmation are the mechanisms that explain the effect of pH changes on WR (Diehl et al., 2010; Doerr et al., 2000). Doerr et al. (2000) state that this effect is strong enough to explain all

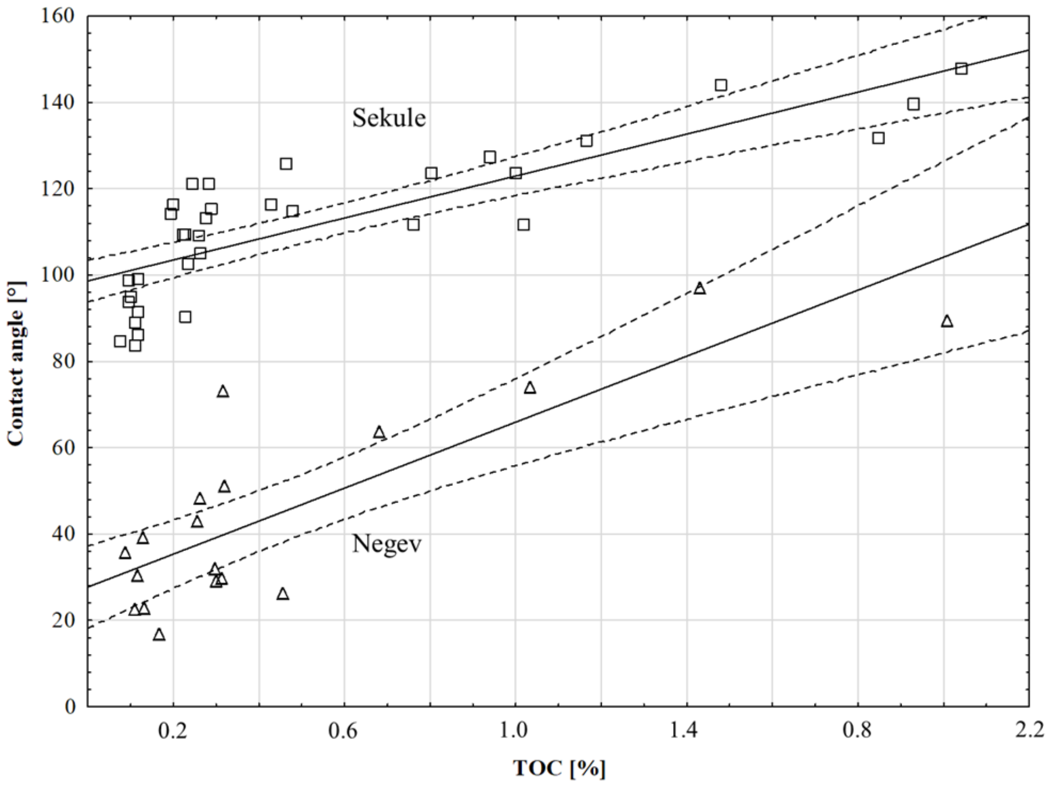

Fig. 3. Correlation between contact angle and amount of total organic carbon (TOC) for all samples at the study site Sekule (Slovakia; n = 34) and the Negev (Israel; n = 18). Dashed lines showing the regression bands (level of confidence 0.95).

changes in WR. We doubt this for the examined biocrusts as the changes between the study sites are very strong. As the correlation between H^+-concentration and TOC is very high (r = 0.75 / r = 0.91, Sekule/ Negev), it is not possible to separate the effect pH has on the detected WR. Nevertheless, at the Negev site, the amount of carbonates has a higher correlation with CA (r = 0.81) than the pH (r = –0.62). This indicates that the amount of carbonates has a higher effect on WR than the pH value. In our opinion, this highlights the more relevant second variable influencing the differences in WR between the study sites, namely texture. The Negev biocrusts are composed of a high amount of finer particles compared to Sekule biocrusts. In general, WR decreases with particle size (González-Peñaloza et al., 2013). In this study, the dunes at Sekule show significantly higher amounts of coarse and middle-sized sand grains, while the Negev biocrusts show significantly higher amounts of fine sized sand grains, fine particles (< 63µm) and carbonates (Table 1). First results on the particle size distribution of the fine fraction < 63µm show around 5% clay and up to 20% silt within this fraction (unpublished data). This material mix reduces WR effectively as shown by McKissock et al. (2000). Moreover, Harper et al. (2000) did show that more TOC was needed to induce WR in soils that have clay contents above 5%. In addition, the Negev biocrusts contain up to 7.3 (±1.1) weight-% carbonates (Table 1). This may have a direct effect on WR as well, as addition of powdered lime effectively reduced WR in soils during remediation trials (Roper, 2005).

Surface characterization of mineral particles separated from moss-dominated biocrust

No significant difference in the composition of organic fragments can be found in the mass spectra from Sekule and Negev (Figure 4). But for the inorganic compounds an increased intensity of Ca^+, Si^+, Mg^+ and Fe^+ was found for the sample from the Negev site. The higher detected amounts of Mg^+ and Fe^+ may have an influence on WR. Harper et al. (2000) found that these minerals reduce WR in soil samples. Moreover, in the Negev samples a higher content of $CaPO_3^-$ was detected in the negative ion mode. The higher Ca^+ and $CaPO_3^-$ signal intensity refers to an increased amount of Ca and most likely of $CaCO_3$ for the Negev sample. Unfortunately, carbonate fragments cannot be assigned specifically as there is an overlap of CO^-/Si^- and CO_3^-/SiO_2^-. Due to the sample roughness, the mass resolution is not sufficient to differentiate between these overlapping peaks. Nonetheless, the SiO_2^- signal is also significantly increased for the Negev sample, which is certainly due to the signal overlap of CO_3^- and SiO_2^-. This is supported by the higher C content of the Negev biocrust samples (Table 1) and accounts for a higher fraction of carbonate-bound C in the sample from the Negev site. Figure 4 shows exemplary mass images of particles from both sites in positive and negative ion mode. Beside the particular structure, the overlay of different mass signals shows clearly the heterogeneous composition of the surface layer. It can be seen that the organic fragments represented by CN^- and $C_3H_3O^+$ are only found in certain areas and hence, the sand grains are not completely covered by OM.

CONCLUSIONS

We compared the WR of two biocrusts types on carbonate-free and carbonate-containing sand dunes and examined the effect of OM, pH and carbonate content on WR. We conclude that the driving factor for the increase in WR within the individual sampling sites is the OM content and not the OM composition. However, this is only true for comparisons within one site, but not among sites. The high differences in potential WR between the study sites is related neither to OM amount, nor to changes in OM composition or organic coating characteristics. The most relevant factors explaining the lower WR in biocrusts of the Negev are the higher amounts of carbonates and the related higher pH values. Moreover, the Negev biocrusts show higher amounts of fine particles that are likely to reduce WR as well. As carbonates are destructed during the texture analysis, further studies are needed to identify the textural effect that carbonates may have on biocrusts' WR by increasing the amount of fine particles. This could be done by comparing the effect of siliceous vs. carbonate mineral particles of the silt fraction on the WR of different soils.

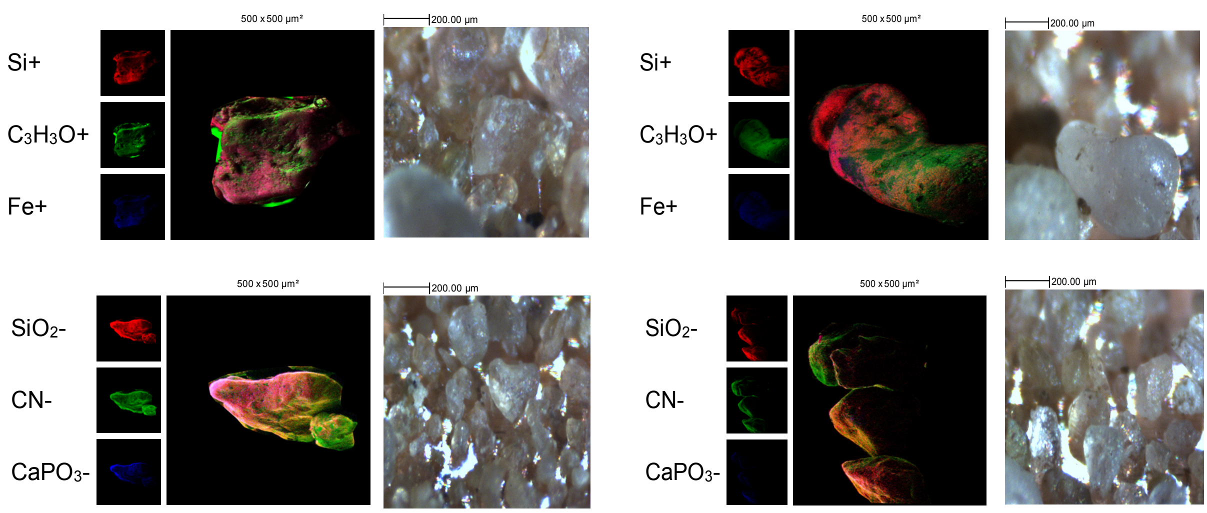

Fig. 4. Images of TC mineral particles from moss-dominated biocrusts (Negev site = left; Sekule site = right) show an overlay of the Si^+, $C_3H_3O^+$ and Fe^+ signal in positive ion mode and SiO_2^-, CN^- and $CaPO_3^-$ signals in the negative ion mode. The visualizations show the heterogeneous surface composition of the particles and proof that the organic crust (represented by $C_3H_3O^+$ and CN^- in green) is not covering the complete particle.

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