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ARTICLE

Ecology of Critical Zones

Patterns of enzyme activities and nutrient availability within biocrusts under increasing aridity in Negev desert

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Abstract

Biocrusts are crucial for the biogeochemical cycles of semiarid ecosystems and strongly influenced by environmental factors. We examined topcrusts (0–2 mm) and the underlying subcrust (2–35 mm depth) at two sites of the Negev with contrasting rainfall. The results show that sampling sites differing in moisture regime and sampling depth had a significant effect on soil properties. The microbial biomass, the concentrations of water-soluble carbon and nitrogen fractions, and the activities of eight enzymes of the carbon and nitrogen cycles decreased with decreasing available moisture and with increasing soil depth. The biomass-specific enzyme activity of topcrusts shows an increase in enzyme activity with increasing biomass in the same proportion. Biomass-specific enzyme activity revealed a strong nutrient demand and a high relative enzyme activity in subcrusts of the drier study area. Moreover, phosphatase activity was much higher in the biocrust of the drier study site. The ratios of carbon to nitrogen to phosphorous show a strong limitation of the latter two for the subcrusts in the Negev, while the ratio of water-extractable organic nitrogen to water-extractable organic carbon underlines the high importance of leaching for subcrusts in semiarid areas. We conclude that Negev topcrusts are highly effective zones of nutrient turnover, adopting to higher amounts of moisture with an increase in biomass and enzyme activity. The subcrusts are an important section of turnover and need to be included into studies on C, N, and P storage in crust covered ecosystems to reveal the processes in deeper soil.

KEYWORDS
biological soil crust, dew desert, enzymatic stoichiometry, extracellular enzyme activity, microbial biomass carbon, semiarid ecosystems, water-extractable organic carbon, water-extractable organic nitrogen

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INTRODUCTION

As key players in biogeochemical cycles of arid and semiarid areas and during initial soil development, biocrusts are of high importance for soil genesis in all climatic zones of the world. Biocrusts are small-scale, highly active microbial communities build up by organisms like cyanobacteria, lichens, algae, and mosses (Belnap, 2006). As in arid and semiarid ecosystems, the changes in the amount of available moisture have a high impact on soil communities and biogeochemistry (Nielsen & Ball, 2015), and the microbial and physiochemical characteristics of biocrusts strongly depend on the local mesoclimate. Changes in local climate cause shifts in the amount and composition of organic matter (OM) and water-soluble compounds (Barger et al., 2016; Kidron et al., 2010; Mager & Thomas, 2011). The resulting physicochemical and microbial composition of biocrusts is strongly linked to their ecosystem functions. An increase in crust thickness and vitality goes along with an increase in protection against soil erosion and higher amounts of available nutrients (Mazor et al., 1996).

Biocrust communities enhance the chemical weathering of the underlying soil (parent) material, fix nutrients, and take part in the carbon and nitrogen cycles of the colonized topsoil. As biocrust organisms are in close contact with the interfaces of soil minerals and excrete exopolymers, metabolic products, and enzymes during growth, they can alter their geochemical environment (Ghiloufi et al., 2019; Miralles, Domingo, García-Campos, et al., 2012; Swenson, Couradeau, et al., 2018). Moreover, biocrusts show a high ability to fix nutrients actively and passively. Understanding the feedback loops between nutrient accumulation and turnover under changing moisture regimes is of high interest for vulnerable systems like semiarid and arid desert regions.

Accumulation of OM and fine particles affects the wettability and hydration of the biocrusts. In the case of passive fixation, that is, the accumulation of dust, fine particles including dust-bound nutrients and even OM settle down at the surface as dry or wet deposition and are trapped at the soil surface after the rehydration of the biocrusts (Yair, 1990). If hydration is sufficient for metabolic activity, photoautotrophic organisms as cyanobacteria present in the biocrust actively fixing carbon, or forming exopolysaccharides (EPS) and lipopolysaccharides (Cania et al., 2020) and thus contributing as net exporters to ammonium, nitrate, and organic nitrogen input into the soil (Johnson et al., 2007). The fixation rates are linked to the time span of hydration, the light intensity, and temperature and therefore vary seasonally; moreover, the composition of biocrust community has a strong influence on the overall amount of fixation (Barger et al., 2016).

Besides the described functions of OM and nutrient accumulation, biocrusts are zones of nutrient turnover and deployment. In general, soil microbial communities are key regulators of OM turnover and nutrient supply (Six et al., 2006). In line with this, biocrust organisms show the ability to use nutrients in a highly efficient way providing partly easily available nutrients to the surrounding soil by excretion of organic products and remains of organisms after death (Mager & Thomas, 2011). This is linked to high amounts of EPS that mainly consist of saccharide components, peptides, lipids, and nucleic acids. Moreover, the EPS show an exclusive sorptivity for amino acids and organic acids (Swenson, Couradeau, et al., 2018). Dispersed EPS are degraded by heterotrophic organisms, while low molecular weight carbohydrates are easily metabolized by chemoheterotrophs (Chen et al., 2014). The nutrient turnover in biocrusts accelerates with increasing available moisture and results in a decrease in OM stability and acidification of the biocrust (Drahorad et al., 2013; Miralles, Domingo, Cantón, et al., 2012). Evaluating the biogeochemical cycles of biocrusts, the coupled cycles of carbon, nitrogen, and phosphorus are of high interest as nitrogen and phosphorus are essential elements for carbon fixation. Organisms commonly have C:N:P ratios with a relative order of C > N > P, as these elements are required in strict proportions to catalyze metabolic reactions and synthesize biomolecules (Finzi et al., 2011). Soils typically show C:N:P ratios of 186:13:1 (with wide variation), while these ratios are more narrow for microbial biomass 60:7:1 (Cleveland & Liptzin, 2007). Besides the use of bulk nutrient contents and their ratios, soil enzyme activity can be an indicator of soil quality and microbial turnover as microbes produce enzymes to transform most organic compounds. Enzyme biosynthesis responds to environmental signals such as low nutrient availability, and therefore, enzyme activities prove to be good indicators of nutrient deficiency, microbial nutrient demand, and the degradation of organic compounds over time (Geisseler & Horwath, 2009). As biocrusts are zones of high microbial density characterized by nutrient deficiency, extracellular enzymes are needed for the acquisition of carbon and nitrogen compounds for growth. Several studies showed increased soil enzyme activities compared to bare substrates and depending on soil depth, available moisture, and biocrust organisms (Heinze et al., 2014; Miralles, Domingo, Cantón, et al., 2012; Miralles, Domingo, García-Campos, et al., 2012). During growth, biocrust organisms excrete high amounts of EPS that are the source of proteins and polysaccharides with the presence of pentose's (xylose, arabinose, and ribose) as a distinguishing factor between the EPS of cyanobacteria and those produced by other microorganisms (Pereira et al., 2009). Moreover, biocrust organisms are adapted to desiccation stress by a specific protection mechanism. This includes the production of high amount of hydrolytic enzymes making low molecular weight substances available for the protection mechanism (Mager & Thomas, 2011; Miralles et al., 2013).

In the northwestern Negev, biocrusts represent a typical soil cover with characteristics and functions comparable to other biocrusts worldwide in terms of surface stabilization, water redistribution, nutrient fixation, and buildup of biomass (Kidron et al., 2010). The biocrusts show a vertical stratification with zones of high photosynthetic activity and enrichment of fines, biomass, and nutrients in the uppermost thin layer. Transect studies show that biomass, OM, and C and N contents increase with increasing moisture, while the microbial community shifts toward higher amounts of less stress-resistant species and higher amounts of fungi (Kidron et al., 2009; Yu et al., 2012). The increase in OM goes along with an increasing amount of chlorophyll *a* and carbohydrates, mainly low molecular compound sugars, and N-containing compounds in the wetter areas and a decrease in OM stability (Drahorad et al., 2013; Hagemann et al., 2017; Yu et al., 2012). The Negev biocrusts give the opportunity to assess changes in biocrust characteristics under changing mean annual precipitation. Therefore, the differences in mesoclimate can be used to analyze the effects of changes in available moisture on microbial biomass, nutrients, and enzymatic patterns of biocrusts. While a first study on OM composition showed that the turnover rate of biomass and associated organic carbon of biocrusts increases with an increase in available moisture and, at the same time, OM stability decreases (Drahorad et al., 2013), a study on enzyme activity and related nutrient status and biomass of biocrusts depending on available moisture is still missing.

We hypothesize that with increasing soil depth, the amount of nutrients and enzyme activities will decrease. Moreover, we hypothesize that the biocrusts at the wetter study site show higher amounts of available nutrients induced by higher microbial biomass contribution and increased enzyme activity of C, N, and P cycles. The pattern of enzyme activities shows more contribution of C-cycling enzymes at the wetter site in comparison with the drier one due to higher input of water-extractable organic carbon (WEOC).

MATERIAL AND METHODS

Study sites

The study sites are within a sand dune area along the Israeli–Egyptian border in the Negev. The area is characterized by high interannual rainfall variability approximately between 35 and 170 mm. The rainfall occurs largely between November and March. Frequent dew occurrences enhance the daily available moisture and can be equivalent of about 30 mm per year (Littmann & Berkowicz, 2008). The two sampling sites are situated along a climatic gradient, reaching from the arid to semiarid southern extension of the sand dune field toward the Mediterranean area in the north. The southern site, Nizzana-South (South), is part of the Arid Ecosystem Research Centre of the Hebrew University of Jerusalem and has an average annual rainfall of about 100 mm (Littmann & Berkowicz, 2008). The northern study site (North) is close to the northern edge of the Israeli side of the dune field with an annual precipitation of about 170 mm. Both study sites are located in a nature protection area and the sand dunes are stabilized by biocrusts and shrub vegetation to different extent, depending on local mean annual precipitation at the sites (Figure 1). The more arid site South is characterized by dunes that have partly mobile dune crests and a lower overall cover of perennial shrubs and annual vegetation than the site North. In both areas, biocrusts are covering the soil surfaces to a large extent. At both study sites, the most dominant biocrust organisms are cyanobacteria like *Microcoleus* sp. (Hagemann et al., 2015). Nevertheless, there is a clear shift in the microbial community structure from south to north. North shows a higher amount of heterotrophic organisms and heterocystous N-fixing cyanobacteria strains like *Nostoc* sp., while highly desiccation and UV-tolerant cyanobacteria like *Leptolyngbya* sp. and *Chroococcidiopsis* sp. showed a higher contribution to biocrusts at the drier study site South (Hagemann et al., 2015; Hagemann et al., 2017).

Sampling and sample treatment

In both study sites, we sampled biocrusts in five replicates and two sampling depths at the interdunal positions. Samples were taken in the dry season in August 2008. The 2-mm-thick topcrust section showed a higher compaction and a slightly darker color than the more weakly aggregated subcrust. The subcrust section thickness increased from 20 for biocrusts at South up to 35 mm at the northern site, respectively. After removal of the topcrust, we lifted the subcrust from the underlying unconsolidated dune sand by using a spatula. All samples were air dried, sieved to 2 mm, and partly finely ground (<0.05 mm).

Analysis of soil characteristics

Total carbon (C) and nitrogen (N) were determined by dry combustion (Vario EL CNS analyzer). Total phosphorus (P) was determined by inductively coupled

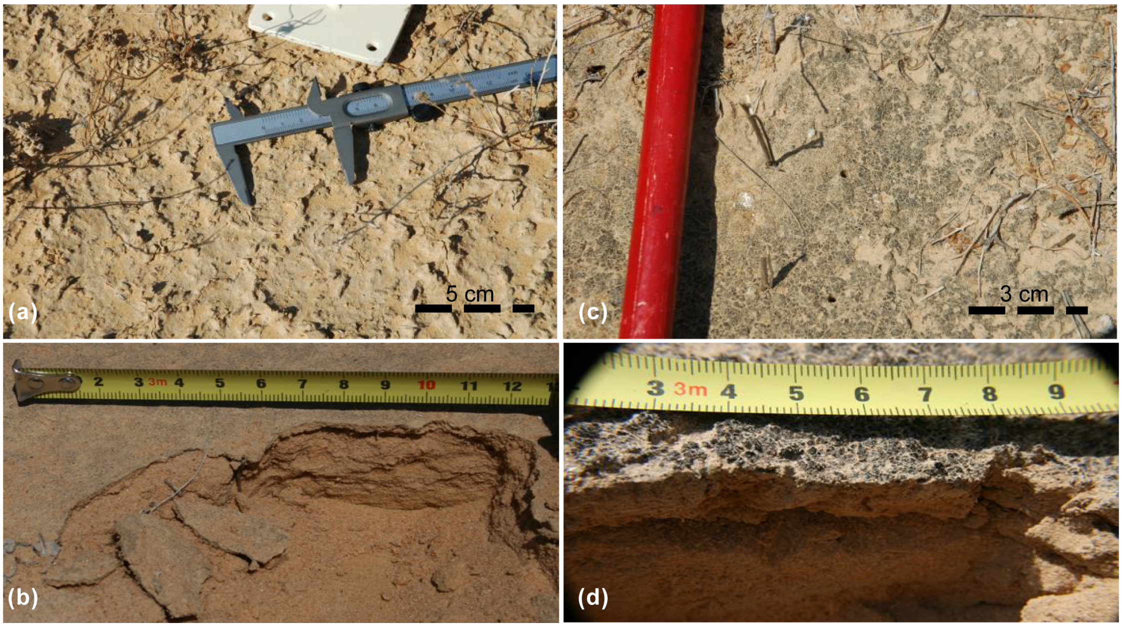

FIGURE 1 Surface view and cross-sectional view of the biocrusts at study site South (a, b) and North (c, d)

plasma optical emission spectrometry (ICP-OES) in three replicates. Total organic carbon (TOC) was calculated as the difference between C and inorganic carbon. Inorganic carbon was determined by the gas volumetric method after dissolution with 10% HCl. Soil pH values were determined after extracting the soil with deionized water with a soil to solution ratio (weight:volume) of 1:5. Water-extractable organic carbon, water-extractable nitrogen (WEN), nitrate (NO_3-N), and ammonium (NH_4-N) were measured after filtration of the aforementioned extract with a pore size of 0.45 μm. Water-extractable organic nitrogen (WEON) was calculated as WEN minus NO_3-N minus NH_4-N. For particle size distribution, we used the wet sieving method (ISO 11277) resulting in coarse, middle, and fine sand fractions and the coarse silt fraction (63–20 μm). The remaining fine particle fraction includes all particles <20 μm.

Microbial biomass C and extracellular enzyme activities

Sieved sample material (<2 mm) of three samples was adjusted to 60% water-holding capacity (WHC) before the chloroform fumigation extraction method was used to estimate the microbial biomass C (C_{mic}) (Brookes et al., 1985; Vance et al., 1987). For the extraction of C_{mic}, 10 g of moist soil was fumigated with ethanol-free $CHCl_3$ at room temperature. After 24 h, the $CHCl_3$ was removed. Fumigated and nonfumigated samples were extracted with 40 ml of 0.05 M K_2SO_4 by 30 min of horizontal shaking at 200 rpm and filtered. After combustion at 850°C, organic C was measured as CO_2 by infrared absorption (Dimatec, Essen, Germany). C_{mic} was calculated as E_C/k_{EC} (E_C = difference from organic C extracted from fumigated soil and organic C extracted from nonfumigated soil; $k_{EC} = 0.45$) (Wu et al., 1990).

The activities of 10 extracellular enzymes were measured using a fluorescence assay (Marx et al., 2001). A soil suspension was produced, weighing 1 g fresh soil into a 100-ml beaker and filling it up with 100 ml of sterile water. The suspension was treated with an ultrasonic probe at 150 W to separate the enzymes from soil particles. The suspension was then mixed with 0.1 M MES hemisodium salt (MES) buffer of 4methylumbeliferone (MUF) substrates, 0.05 M TRIZMA buffer for AMC (7methyl 4methyl coumarin) substrates, and the respective substrates for detecting enzyme activities. They were pipetted into 96-well plates (black) and preincubated at 30°C for 10 min. Measurements of enzyme activities were carried out for a time of 180 min in 30-min intervals at the excitation at 360 nm and emission at 465 nm (TECAN infinite 200, TECAN Group, Switzerland). The measured enzyme activities included enzymes of the C-, N-, and P-nutrient cycle under use of the respective substrates. For C-cycle enzymes, β-glucosidase, α-glucosidase, N-acetyl-glucosidase, β-xylosidase, and β-cellobiosidase using the following substrates: 4-methylumbelliferyl-β-D-glucoside, 4-methylbelliferyl-α-D-glucoside, 4-methylbelliferyl-*N*-acetyl-β-D-glucosaminide, 4-methylumbelliferyl-β-D-xylopyranoside, and 4-methyl umbelliferyl-β-D-cellobioside, respectively (Sigma-Aldrich). Concerning the N cycle, L-leucine-7-amino-4-methylcoumarin hydrochloride as the substrate for leucine-aminopeptidase, L-tyrosine-7-amino-4-methylcoumarin for tyrosine-aminopeptidase, and L-arginine-7-AMC for arginine-aminopeptidase were used.

P-cycle enzymes were measured by 4-methylumbelliferyl phosphate disodium salt for acid phosphatase. The enzyme activity was calculated from the slope of activity during 180 min and is shown as nanomoles per hour per gram of soil.

In this work, we show the total extracellular enzyme activity (EEA) of biocrusts and use the quotient between enzymatic activity and microbial biomass (EEA × $C_{mic}{}^{-1}$) to describe the production of enzymes released by microorganisms. Enzyme activity ratios were calculated considering the sum of C ($n = 5$)-, N ($n = 3$)-, and P ($n = 1$)-cycling enzymes in relation to each other.

Statistical analysis

Statistical analysis was done using SPSS version 24, and the multivariate analysis of variance (MANOVA) was conducted with Statistica 14. To test the data on significant differences, a two-way MANOVA was used after a check of normal distribution and variance homogeneity including TOC, C_{mic}, N, P, WEON, WEOC, NO_3-N, and NH_4-N. Differences between groups were calculated by post hoc Tukey's honestly significant difference test.

RESULTS

General biocrust characteristics and nutrient distribution

All examined biocrusts were characterized as loamy sand, with a very low percentage (<1%) of coarse sand particles (Table 1). The texture of the two study sites is dominated by middle and fine sand and fine particles (≤gU). The southern site shows between 56% and 54% middle sand fraction in the top- and subcrust, while the northern site shows 49%–53% fine sand, respectively. The fine particles (<Ug) are highest at the northern site and show significant higher values in the topcrust compared to the subcrust (Table 1). The WHC show significant higher values at the northern compared to the southern site without differing between the depths (Table 1). The WHC correlated with the amount of coarse silt (gU) in the topcrust of North ($r = 0.96$) and the amount of particles smaller <20 μm (<gU) in the topcrust of South ($r = 0.76$).

We conducted a two-way ANOVA to assess the effects of sampling depth and sampling site on TOC, C_{mic}, N, P, WEON, WEOC, NO_3-N, and NH_4-N. Sampling depth, sampling site, and the interaction of both had a very high significant effect on the variance of the tested datasets as shown by a very high partial η^2 (Table 2).

While the total C concentration showed no significant differences between the study sites, TOC concentrations showed 60% higher concentrations in the topcrust of North compared to the topcrusts of South (Table 3). Moreover, a significant ($p < 0.001$) enrichment in TOC of the topcrust compared to the associated subcrust was visible in North. The overall N concentration was lower than 1% with the highest concentration in N found in the topcrust of North. Topcrust and subcrust of North showed significantly higher N concentrations than the biocrusts sections in South. The microbial carbon (C_{mic}) followed the pattern of TOC with significant differences between the top- and subcrusts at both study site and a significant difference between top- and subcrust at North. C_{mic} showed highest means in the topcrust with 883 and 439 $\mu g\ g^{-1}$ in the northern and southern sites,

TABLE 1 Biocrust characteristics of topcrusts and subcrusts as represented by soil alkalinity (pH), soil texture (coarse [cS], medium [mS], and fine sand [fS]), coarse silt (gU), and fractions <63 μm (<gU, including smaller silt and clay fractions) and water-holding capacity (WHC)

Site_depth[a]	S_topcrust	S_subcrust	N_topcrust	N_subcrust
$pH_{aqua\ dest}$ (a,b)	7.8 ± 0.07**	8.6 ± 0.08	7.3 ± 0.17**	7.9 ± 0.04
cS (%)	0.4 ± 0.4	0.2 ± 0.2	0.03 ± 0.01	0.02 ± 0.03
mS (%) (a,b)	56.3 ± 4.5	52.7 ± 6.1	29.8 ± 4.5	32.6 ± 4.5
fS (%) (a,b)	29.9 ± 3.1	33.3 ± 3.5	49.4 ± 9.3	53.2 ± 1.0
gU (%)	11.6 ± 0.6	11.8 ± 4.0	13.1 ± 4.6	8.7 ± 4.6
<gU (%) (a,b)	1.9 ± 2.3	2.0 ± 1.4	7.8 ± 0.7**	5.5 ± 0.6
WHC (%) (a,b)	24.3 ± 1.8	21.6 ± 1.4	29.1 ± 2.3	26.2 ± 1.0

Note: Different lowercase letters indicate a significant difference between the study sites for the same sampling depth (a = differences between topcrusts of the study sites; b = differences between subcrusts of the study sites; $p < 0.05$). Asterisks indicate that the values of topcrusts and subcrusts at the same study site are significantly different (*$p < 0.05$, **$p < 0.01$).

[a] Biocrusts were sampled at two study sites (S = South; N = North) as topcrusts (0–2 mm) and related subcrusts (2–20 mm in South; 2–35 mm in North); values show the mean of five replicates for pH and WHC, and three replicates for particle sizes and the SD.

respectively, whereas the subcrust showed only 10% and 1.7% of topsoil C_{mic} contents at northern and southern sites. The C_{mic}:TOC ratio showed that 12%–13% of TOC in the topcrusts were of microbial origin with a strong decrease of this contribution with increasing soil depth. Only <0.5% of TOC in the subcrust of South was of microbial origin but 3% of TOC in the subcrust of North. The concentrations of P showed no significant differences between the study sites. At both study sites, the topcrusts were slightly enriched in P, but this trend was only significant for the biocrust in South. The biocrusts of both study sites showed C:N ratios of 10:1 up to 15:1 for the topcrusts and for the subcrust in North. By contrast, the subcrust in South showed a very wide ratio of 34:1. The C:N:P ratios decreased from north to south (Table 3).

Water-extractable organic carbon concentrations showed decreasing concentrations from north to south and with depths (Table 4). The concentrations were significantly higher for the topcrusts compared to the subcrusts at the same study site. The same trend was visible for the WEN fractions. The topcrust North showed significantly higher concentrations of NH_4-N and WEON compared to the associated subcrust and compared to the topcrust of South (Table 4). The WEOC:WEON ratio was in the same range for all biocrust samples from 10:1 up to 15:1. The ratios of WEOC, WEON, and NH_4-N to C_{mic} show the same results for the topcrusts of the two study sites and the subcrust in North (Figure 2). Only the subcrust South showed a five times higher WEON:C_{mic} ratio compared to the subcrust of North.

TABLE 2 Two-way multivariate analysis of variance (MANOVA) showing effect sizes for sampling depths, sampling sites, and interactions

Effect	Value	F	p	Partial η^2
Depth	0.018	22.90	0.001	0.98
Site	0.010	42.24	<0.001	0.99
Depth × site	0.010	42.65	<0.001	0.99

Extracellular enzyme activities in the biocrusts of the northwestern Negev

The changes of EEA with soil depth and available moisture showed strong differences between the sampling sites and biocrust depths. The northern site showed a twofold higher sum of activities (2363 nmol g^{-1} h^{-1}) within the whole profile considering all enzymes in comparison with the southern site (1123 nmol g^{-1} h^{-1}). Especially, β-glucosidase, N-acetyl-glucosidase, β-xylosidase, and β-cellobiosidase (C cycle) and the enzyme activities of tyrosine- and arginine-aminopeptidases (N cycle) were higher in North than in South. This indicated an increase in EEA with increasing available moisture for most measured enzymes. Comparing the subcrusts of the two study sites, our data showed that the EEA of the subcrust of the wetter study site North exceeded those of the drier southern site. The difference was significant for the EEA of β-xylosidase, β-glucosidase, and for all aminopeptidases between the two study sites (Table 5). The EEA for phosphate showed an opposite trend. Here, the activities were higher in both depths in the biocrust of South compared to the top- and the subcrust in North without showing statistical significance.

TABLE 3 Total carbon (C), total organic carbon (TOC), microbial carbon (C_{mic}), total nitrogen (N), and total phosphorus (P) concentrations and ratios of topcrusts and subcrusts

Site_depth[a]	S_topcrust	S_subcrust	N_topcrust	N_subcrust
C (%) (a)	1.21 ± 0.14	0.80 ± 0.09	1.60 ± 0.72*	0.88 ± 0.4
TOC (%) (a)	0.33 ± 0.09	0.16 ± 0.03	0.81 ± 0.04**	0.29 ± 0.13
C_{mic} (µg g^{-1}) (b)	438.90 ± 210.78*	7.44 ± 7.03	882.95 ± 479.06**	94.99 ± 55.53
N (%) (a,b)	0.03 ± 0.01	0.01 ± 0.00	0.07 ± 0.03**	0.03 ± 0.01
P (mg kg^{-1})	154.2 ± 5.8**	91.1 ± 8.7	210.88 ± 2.7	116.1 ± 38.8
C:N	12:1	34:1	10:1	12:1
C:N:P	19:1.7:1	18:0.4:1	27:2.5:1	17:1.5:1
C_{mic}:TOC	0.13:1**	0.004:1	0.12:1**	0.03:1

Note: Different lowercase letters indicate a significant difference between the study sites for the same sampling depth (a = differences between topcrusts of the study sites; b = differences between subcrusts of the study sites; $p < 0.05$). Asterisks indicate that the values of topcrusts and subcrusts at the same study site are significantly different (*$p < 0.05$, **$p < 0.01$).

[a] Biocrusts were sampled at two study sites (S = South; N = North) as topcrusts (0–2 mm) and related subcrusts (2–20 mm in South; 2–35 mm in North); values show the mean of five replicates (expect for P with three replicates) and the SD.

TABLE 4 Concentrations and ratios of water-soluble nitrogen fractions (WEN, NH_4-N, NO_3-N, and WEON) and water-soluble carbon (WEOC) after 1:5 water extraction of topcrusts and subcrusts

Site_depth[a]	S_topcrust	S_subcrust	N_topcrust	N_subcrust
WEN (mg kg^{-1}) (a)	9.69 ± 2.10	4.56 ± 1.27	38.90 ± 21.04*	7.85 ± 3.81
NO_3-N (mg kg^{-1})	1.55 ± 0.50	1.93 ± 0.71	0.78 ± 0.20	1.76 ± 0.54
NH_4-N (mg kg^{-1}) (a)	3.55 ± 1.28	0.63 ± 0.47	14.35 ± 8.01**	1.81 ± 0.87
WEON (mg kg^{-1}) (a)	4.59 ± 1.57	2.01 ± 1.49	23.76 ± 13.37**	4.28 ± 3.26
WEOC (mg kg^{-1}) (a)	56.00 ± 16.62*	16.20 ± 2.80	201.74 ± 38.63**	36.88 ± 4.34
WEOC:WEON	15:1	12:1	10:1	12:1

Note: Different lowercase letters indicate a significant difference between the study sites for the same sampling depth (a = differences between topcrusts of the study sites; b = differences between subcrusts of the study sites; $p < 0.05$). Asterisks indicate that the values of topcrusts and subcrusts at the same study site are significantly different (*$p < 0.05$, **$p < 0.01$).

Abbreviations: NH_4-N, ammonium; NO_3-N, nitrate; WEN, water-extractable nitrogen; WEOC, water-extractable organic carbon; WEON, water-extractable organic nitrogen.

[a] Biocrusts were sampled at two study sites (S = South; N = North) as topcrusts (0–2 mm) and related subcrusts (2–20 mm in South; 2–35 mm in North); values show the mean of five replicates and the SD.

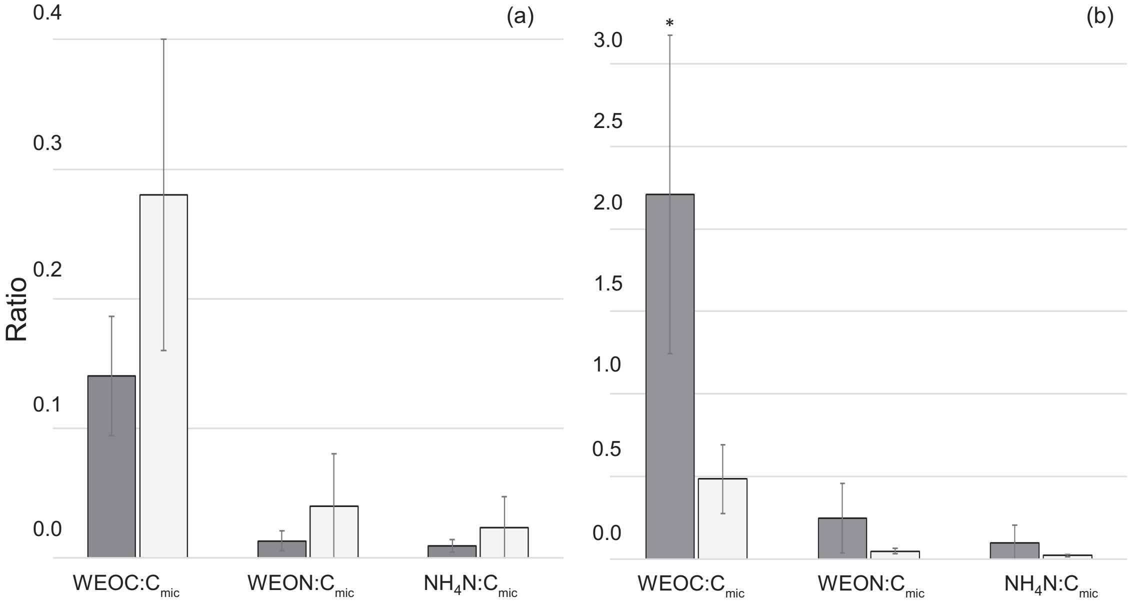

FIGURE 2 Ratios of WEOC, WEON, and NH_4-N to C_{mic} for the topcrusts (a) and the subcrusts (b) of the study sites South (light gray columns) and North (dark columns). Asterisk indicates a significant difference between the two study sites ($p < 0.01$, $n = 5$). C_{mic}, microbial biomass C; NH_4-N, ammonium; WEOC, water-extractable organic carbon; WEON, water-extractable organic nitrogen

In the topcrust at North, the sum of EEA reached the highest value with 1983 nmol g^{-1} h^{-1} that was reduced by 80% in the subcrust (380 nmol g^{-1} h^{-1}). At the southern site, the sum of EEA was also much higher in the topcrust (1040 nmol g^{-1} h^{-1}), which decreased strongly by about 92% to 83 nmol g^{-1} h^{-1} in the subcrust. Looking at single enzyme activities, at the southern site the topcrusts showed significantly higher EEA for C- and N-cycle enzymes (except for α-glucosidase) compared to the subcrust at this study sites. For the northern site, the topcrusts show significant higher amounts of EEA only for some of the C- and N-cycle enzymes. Here, the C-cycling enzymes, β-glucosidase and β-xylosidase, and the N-cycling enzymes, tyrosine- and arginine-aminopeptidase, indicated a significant decrease of EEA with soil depth (Table 5). Specifically, the topcrust of South showed significantly higher EEA for N-acetyl-glucosidase and leucine-aminopeptidase compared to the subcrust. Remarkably, the subcrust showed significantly higher EEA of β-cellobiosidase compared to the topcrust for the biocrust of South. This was the only case with a higher EEA in the subcrust compared to the topcrust.

The share of EEA involved into the C, N, and P cycles showed strong differences between the two study sites.

Here, it became evident that at the drier southern site more than 65% and 44% of EEA were represented by acid phosphatase in the top- and subcrust, respectively. In subcrust samples, the five enzymes involved into the C cycle showed a higher proportion (34%) in comparison with the topcrust with nearly 5%, where α-glucosidase was nearly not involved in C turnover. N-cycling enzymes showed a proportion of 30% in the topcrust and slightly lower proportion in the subcrust (22%). Interestingly, the site with higher annual precipitation (North) showed only low phosphatase activity with 3% and 5% in top- and subcrust samples, respectively (Figure 3). C-cycling enzymes dominated the overall EEA at the northern site, especially β-glucosidase with a proportion of around 30% in the topcrust and 45% in the subcrust (Figure 3). The N-cycling enzymes played a more

TABLE 5 Biocrust enzyme activities of topcrusts and subcrusts at the two study sites

Site_depth[a]	**S_topcrust**	**S_subcrust**	**N_topcrust**	**N_subcrust**
α-Glucosidase (nmol g^{-1} h^{-1})	5.48 ± 5.93	7.46 ± 2.42	27.74 ± 14.05	6.6 ± 3.18
β-Xylosidase (nmol g^{-1} h^{-1}) (a,b)	5.27 ± 0.19**	3.32 ± 0.95	69.53 ± 13.50**	12.18 ± 6.09
NAG (nmol g^{-1} h^{-1}) (a)	15.16 ± 4.42**	6.84 ± 4.66	308.18 ± 94.62	22.42 ± 18.23
Phosphatase (nmol g^{-1} h^{-1})	60.20 ± 43.35	37.33 ± 25.66	34.08 ± 34.47	16.47 ± 5.47
β-Cellobiosidase (nmol g^{-1} h^{-1}) (a)	2.99 ± 0.44**	4.24 ± 2.08	31.15 ± 6.59	9.00 ± 5.46
β-Glucosidase (nmol g^{-1} h^{-1}) (a,b)	29.63 ± 1.90**	4.36 ± 1.37	611.46 ± 64.60**	173.26 ± 154.10
Leucine-AP (nmol g^{-1} h^{-1}) (b)	149.78 ± 102.53**	3.92 ± 2.53	336.90 ± 91.75	56.21 ± 19.53
Tyrosine-AP (nmol g^{-1} h^{-1}) (a,b)	98.70 ± 54.72**	2.09 ± 0.98	259.90 ± 50.40**	37.54 ± 23.01
Arginine-AP (nmol g^{-1} h^{-1}) (a,b)	72.87 ± 23.50**	13.34 ± 8.45	304.23 ± 70.49*	46.37 ± 7.96
Enzymatic C:N	0.13	1.0	0.82	1.44
Enzymatic C:P	0.72	0.52	21.7	12.2

Note: Different lowercase letters indicate a significant difference between the study sites for the same sampling depth (a = differences between topcrusts of the study sites; b = differences between subcrusts of the study sites; $p < 0.05$). Asterisks indicate that the values of topcrusts and subcrusts at the same study site are significantly different (*$p < 0.05$, **$p < 0.01$).

Abbreviations: AP, aminopeptidase; NAG, N-acetyl-glucosidase.

[a] Biocrusts were sampled at two study sites (S = South; N = North) as topcrusts (0–2 mm) and related subcrusts (2–20 mm in South; 2–35 mm North); values show the mean of five replicates and the SD.

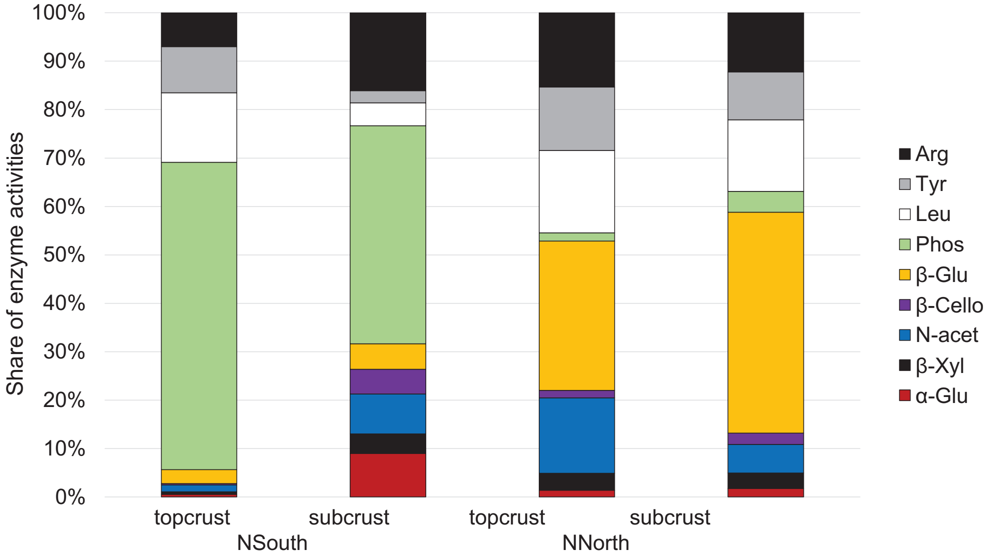

FIGURE 3 Share of different enzyme activities referring to C, N, and P cycles in top- and subcrust of two different biocrusts varying in moisture conditions in Negev Desert. Sum of enzyme activity is given as 100%

important role accounting for 45% higher activities in the topcrust and 35% and subcrust of the wetter site in comparison with the drier site (Figure 3). Analyzing the nutrient status of the sampled biocrusts, the nutritional enzyme activity ratios were calculated for C:N- and C:P-cycling enzymes to indicate carbon or nutrient limitation of microbial community. The ratios showed differences between both sites with low enzyme activity C:N ratio of 0.13 in the topcrust of South and higher C:N ratio (1.0) in the subcrust of that site. The enzyme activity C:P ratio indicating the contribution of P-cycling enzymes in relation to the enzymatic C cycle shows higher values of 0.72 in the topcrust compared to 0.52 in the subcrust of the drier site. The site North was characterized by much higher ratios of nutritional enzyme activities in comparison to the southern site. Here, enzyme activity C:N ratios of 0.82 and 1.44 were reached in the topcrust and subcrust samples, respectively, while the ratio of enzyme activity C:P decreased with depth from 21.7 to 12.2 (Table 5).

The sum of extracellular enzyme activities in relation to microbial biomass (EEA × $C_{mic}{}^{-1}$) reached the highest ratio for the subcrust of South (25.8) followed by the subcrust of North (6.6), the topcrust of North (2.7), and the lowest ratio for the topcrust of South (1.2) with the last three showing no significant difference. This quotient between enzymatic activity and microbial biomass reflected an increase in the production of enzymes released by microorganisms for the subcrust of South but not for the other sampling sites. Besides this first overall trend, the EA × $C_{mic}{}^{-1}$ for the single enzymes showed the same pattern for enzyme release as the EEA for the topcrusts highlighting again the high amounts of β-glucosidase, N-acetyl-glucosidase, and arginine-aminopeptidase for the topcrust of North and high amounts of phosphatase activity for the topcrust of South (Figure 4a). A strong difference was found for the comparison between the topcrusts and the subcrusts and for the comparison between the subcrusts of the two study sites. While the subcrusts showed rather low EEA compared to the topcrusts (Table 5), the EEA × $C_{mic}{}^{-1}$ showed a higher amount of enzyme release for the subcrusts compared to the topcrusts (Figure 4b). As an example, the subcrust of North had a biomass-specific β-glucosidase:C_{mic} ratio of 3.2, while the associated topcrusts show a ratio of 0.8. For the subcrust of South, the EEA × $C_{mic}{}^{-1}$ shows higher ratios for α-glucosidase, β-xylosidase, N-acetyl-glucosidase, β-cellobiosidase, and arginine-aminopeptidase compared to the subcrust of North. This is the opposite trend than for the EEA shown in Table 5. Again the phosphatase activity is remarkably high at the southern site with 3.8 in the subcrust and only 0.2 in the associated topcrust.

DISCUSSION

C, N, and P distribution in biocrusts relates to depth and sampling site

The results on nutrient distribution in Negev biocrusts show an increase in carbon and nitrogen compounds with increasing moisture. In general, TOC and N in biocrusts of the present study are in line with earlier studies on biocrusts of the Negev (Kidron et al., 2010) and across biogeographic regions of the United States (Strauss et al., 2012). The increase of TOC, C_{mic}, and N with increasing rainfall at the northern site compared to the southern site results from a longer wetness duration time at the northern study site allowing more C and N fixation (Kidron et al., 2009). This higher wetness duration is a result of a higher WHC within the topcrust compared to the underlying subcrust as a result of an accumulation of fine particles as seen at the northern investigation site and dust during crust development (Verrecchia et al., 1995). This accumulation of fine particles hastens biocrust growth (Mugnai et al., 2020). Within another study at the same site, Housman et al. (2006) detected a change of the microbial community structure. Along with biocrusts growth, the crust microbial community changes, with a shift toward *Scytonema* ssp. in North, a crust microorganism that shows a higher daily C-fixation rate than early *Microcoleus* ssp.-dominated crusts (Housman et al., 2006). Therefore, the described increase in biomass and OM, as shown by C_{mic} and TOC concentrations, is the result of higher metabolic activity (Kidron et al., 2010). Moreover, OM and especially EPS play a major role for WHC (Schimel, 2018). Earlier studies show that the WHC of South biocrusts is increased by 2%–6% more due to EPS (Fischer et al., 2013). As EPS is the most dominant component of biocrust OM (Mager & Thomas, 2011), the high relative share of carbon of microbial origin in the topcrusts of this study is in line with other studies and indicates easily degradable EPS (Miralles, Domingo, García-Campos, et al., 2012). Contrarily, the low percentage share of C_{mic} to TOC in the subcrusts indicates a higher amount of persistent organic carbon fractions in the OM of the examined biocrusts. For the examined biocrusts, an earlier study showed that the OM of topcrusts had a higher percentage of carbohydrates and N compounds, while the subcrusts contained more phenols and lignin monomers and alkyl aromatics (Drahorad et al., 2013).

The P concentrations of biocrusts in this study are comparable to P concentrations found in biocrusts on sand dunes at the Baltic Sea and for hypolithic cyanobacteria in the Atacama Desert (Jung et al., 2019; Schaub et al., 2019). Moreover, the P accumulation in

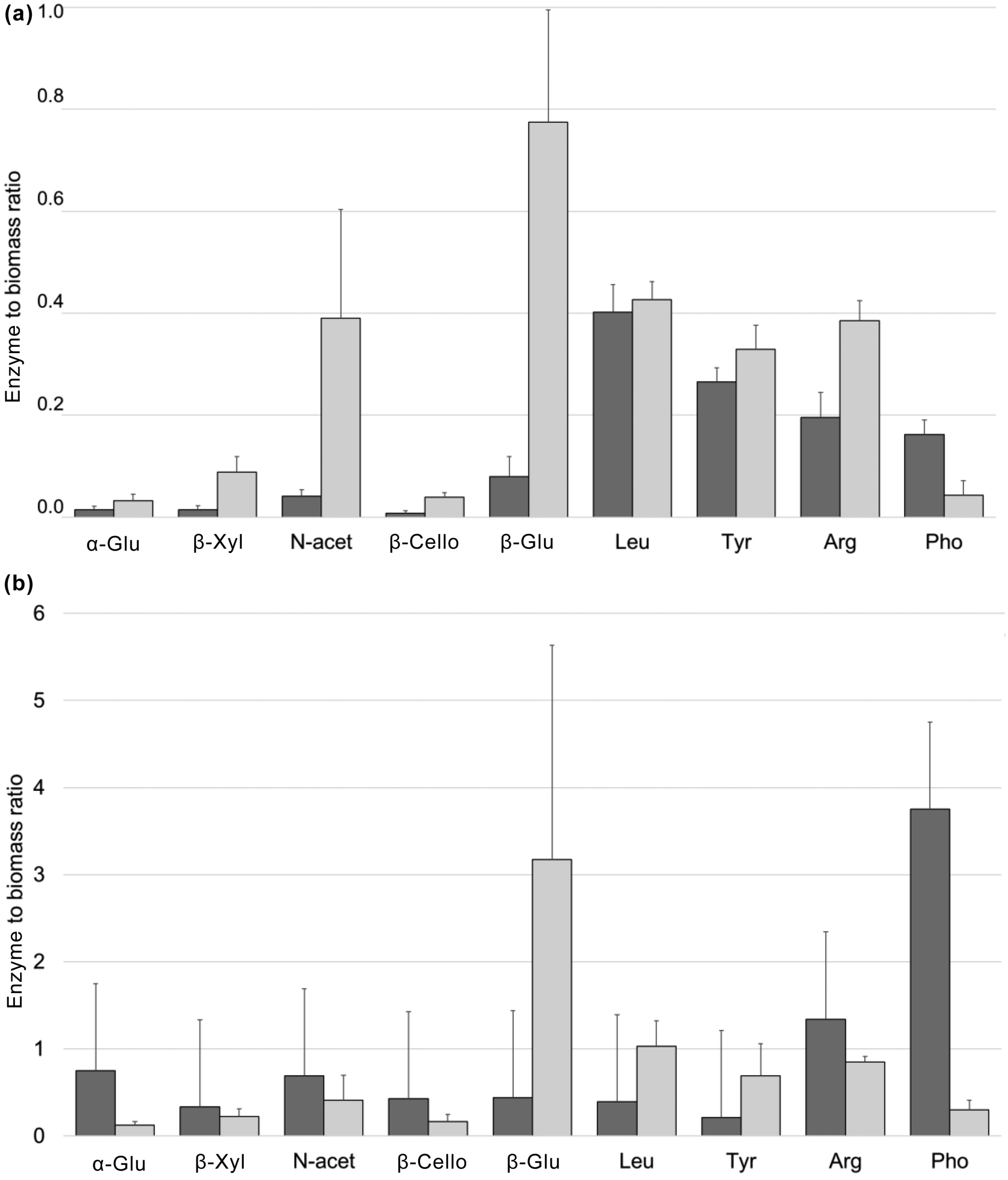

FIGURE 4 Biomass-specific enzyme activity (EA × C_{mic}^{-1}) of the topcrusts (graph a) (0–2 mm in South, gray columns; North, dark columns) and of the subcrusts (graph b) (2–20 mm in South, gray columns; 2–35 mm in North, dark columns) [$n = 3$]

topcrusts and with increasing amounts of available moisture are processes found in other biocrusts, too (Jung et al., 2019; Schaub et al., 2019). While in the study area main P input pathways are wet and dry deposition (Herut et al., 2002), biocrust organisms show several strategies like organic acid and enzyme production to get access to P in these arid ecosystems (Crain et al., 2018). Typically, organic acids are produced and enriched within the EPS (Miralles, Domingo, Cantón, et al., 2012; Swenson, Karaoz, et al., 2018). This process can be assumed for the biocrusts of this study as well, as reflected by the higher acidity in the examined topcrusts.

Water-extractable organic carbon and nitrogen dynamics

The WEOC and WEON dynamics of biocrusts are linked to typical drying–wetting cycles in the Negev ecosystem. During periods of hydration, WEOC is increased by the high amounts of carbohydrate release during EPS growth (Cania et al., 2020; Mager & Thomas, 2011; Mazor et al., 1996) and an excretion of organic metabolites by cyanobacteria like *Microcoleus vaginatus* (Swenson, Karaoz, et al., 2018). For the topcrusts of this study, this is also reflected in the WEOC:C_{mic} ratio showing a strong correlation between easily soluble carbon fractions and the amount of microbial biomass. Earlier studies proved that the biocrusts of both study sites show high amounts of the osmolytes trehalose and sucrose (Hagemann et al., 2017).

Moreover, the microbial biomass and the presumed high EPS content are the reason for high amounts of NH_4-N and WEON in the topcrusts as peptides constitute 50% of EPS dry weight (Pereira et al., 2009). While the concentrations of NH_4-N and WEON within the subcrusts are comparable to biocrusts in the Chihuahuan Desert (Tapia-Torres et al., 2015), the topcrust concentrations are up to 25 times higher. This may be related to local dew distributions in the northwestern Negev with approximately 200 dew events per year and up to 0.4 mm dew per night (Jacobs et al., 2000). Delgado-Baquerizo et al. (2013) could show that dew events increased total available N, the concentration of dissolved organic N, phenols, and the pentoses:hexoses ratio in biocrusts as dew promoted mineralization and decomposition of biocrust OM in dryland soils. This process may also explain the increase of WEN fractions from south to north due to more and/or heavier dew events in the northern study site. This is also in line with an earlier study showing higher amounts of phenols and an increased pentoses: hexoses ratio for the topcrust at North compared to South (Drahorad et al., 2013).

Element stoichiometry in biocrusts

The C:N ratios indicate a high soil fertility and a good degradability of OM for the topcrusts of both study sites and the subcrust in the wetter study site North and comparable to biocrusts (7 up to 14) of the Mojave Desert, the Chihuahuan Desert, and the Sonoran Desert in the United States (Strauss et al., 2012). Furthermore, the C:N:P ratio provides more information about nutrient limitations. The ratios are roughly in the range of biocrusts in northern Israel (12:2:1) or at the Baltic Sea (29:4:1 to 11:2:1) (Heinze et al., 2014; Schaub et al., 2019) but with lower N values. Therefore, these ratios indicate a very strong N limitation in all sampled biocrusts with an increase from north to south and with sampling depth with an extreme low ratio for the subcrust in South. Comparing these data on N limitation with the data on substrate availability (WEOC:WEON ratio), the biocrust of the study site North has comparable ratios for both depths. This shows that solid and dissolved OM are sources for C and N in equal amounts in the wetter study site. For the subcrust in South, the WEOC:WEON ratio shows an adequate substrate availability, which is in contrast to the information given by the C:N ratio. Small organic N compounds like proteins may be the origin of WEON, as shown by the higher biomass-specific enzyme activities of arginine-aminopeptidase. This shows that dissolved OM was a richer source of nitrogen than solid OM in the subcrust and reveals the high importance of the topcrust to deliver nutrients to the underlying soils in the drier study site. The N limitation is not as relevant as suggested by the C:N:P ratio. Moreover, the surprisingly high share of P cycle enzyme and the low enzymatic C:P ratio indicate a strong P deficiency at the study site South (Turner & Wright, 2014). This deficiency is not found in the biocrust of the northern site with higher OM input and decomposition inducing a higher input of phosphorus into the soil. At South, we assume that P is fixed strongly in the biocrust due to a higher pH value as in calcaric soils with an alkaline pH P can be strongly bound as calcium phosphate. Nevertheless, we did not test for organic P and high amounts of P may be bound in microbial biomass. As organic P is bound in DNA, RNA, ATP, and phospholipids, organic P may be the more frequently occurring form at South as earlier studies did show a very high amount of fatty acids in the biocrusts of South (Drahorad et al., 2013).

Biocrust enzyme activities

In general, the occurrence of biocrusts implies higher EEA in both study sites as already shown for biocrusts in arid and semiarid soils and the measured EEA is in the range of recent studies on biocrust enzyme activity (Ghiloufi et al., 2019; Miralles, Domingo, Cantón, et al., 2012; Tapia-Torres et al., 2015). In the topcrusts, C- and N-cycling enzymes show a significant increase in activity from south to north with an expectation for α-glucosidase and leucine-aminopeptidase. The increase in EEA is coupled with higher C_{mic} concentrations. As for the study site in North the WHC is highest compared to other biocrusts in the study area, we assume that the local higher moisture occurrence allows higher microbial biomass accumulation due to a longer wetness duration (Kidron et al., 2010). Comparing the overall EEA × $C_{mic}{}^{-1}$ between the study sites and therefore the relative amount of enzymes, the topcrusts show no significant difference. This indicates that at each study site the microbial communities are well adapted to their surroundings and are highly efficient in the turnover of nutrients. In biocrusts, the pattern of bacterial co-occurrence and niche partitioning by typical heterocystous cyanobacteria are mainly controlled by chemical properties and temperature (Giraldo-Silva et al., 2020; Miralles et al., 2020).

The higher EEA × $C_{mic}{}^{-1}$ for *N*-acetyl-glucosidase and β-glucosidase in the biocrust of North compared to the biocrust of South indicates an increase in the enzyme release by heterotrophic microorganisms in both biocrust depths. If low molecular weight substances are available in biocrust, they are metabolized for the nutrition use of heterotrophs (Miralles et al., 2013). As a result of a microclimate with less moisture and less time for metabolic processes, biocrusts at the study site South show higher amounts of stress-tolerant cyanobacteria (Hagemann et al., 2017). Within these biocrusts, drought stress may induce a shift of microbial population composition toward low decomposition activity (e.g., C and N limitation and stress-tolerant oligotrophic bacteria) (Fierer et al., 2009). The subcrust of this study site, characterized by more complex C substrates, shows the highest biomass-specific α-glucosidase and β-cellobiosidase activity that may indicate fungal growth. As biocrust hosting complex microorganism communities, the enzyme activity pattern is not only linked to one microorganism strain as several microorganisms form a food web (Swenson, Karaoz, et al., 2018). Grishkan and Kidron (2016) showed a high amount of soil fungi in biocrusts in the Negev (Grishkan & Kidron, 2016). Based on the absolute enzyme activity, a slightly higher fungal activity may be assumed for biocrusts in North compared to South. That is in line with results on soil microbial community structure and enzymatic activity showing that relative abundance of fungi increases in soils with higher organic C content. Based on the biomass-specific enzyme activity, we assume that in both sampling depths of North the heterotroph bacteria have the highest share of the biocrust community. This is in line with earlier results on fatty acid patterns and the related distribution of heterotrophs at the same study sites (Drahorad et al., 2013).

Looking at the nutritional status of microorganisms, as demonstrated in the enzyme activity pattern, the topcrusts show higher N-cycling enzyme activity. This is a consequence of the higher abundance of photoautotroph cyanobacteria in the stronger light-exposed topcrusts, which are able to fix atmospheric nitrogen and release this into the soil where it can be used by heterotrophic organisms (Hagemann et al., 2017). The share of C-cycling enzymes is very low in the topcrust of South as there is only low necessity to mineralize organic C to meet the N demand as C is easily available in water-soluble forms (Drahorad et al., 2013; Hagemann et al., 2015). In the subsoil, the share of C-cycling enzymes increased due to higher decomposition to gain nutrients from more complex OM as found within this subcrust (Drahorad et al., 2013). For the study site North, the EEA pattern shows a more homogeneous picture looking at both depths. This might be explained by higher amounts of C_{mic} in deeper soil due to higher precipitation at the site. In general, the C-cycling enzymes of the wetter site North show a higher overall share compared to South mostly expressed by a high share of β-glucosidase. This might be induced by less relative abundance of cyanobacteria at the wetter site, and thus, soil organic carbon turnover is the main process to gain nutrients for microbial community indicated by a high β-glucosidase activity at that site. There is also a link to the higher amounts of available N and cellulose by litter within this biocrust as β-glucosidase activity in soils is positively correlated with the amount of available N and cellulose (Geisseler & Horwath, 2009). In the examined ecosystem, during spring a high number of annual plants do occur. Even though biocrust samples were taken in the plant interspaces, plant remnants may occur in the sampled crust material as very fine roots or parts of degraded litter. This effect is more pronounced for North, as the total amount and the distribution pattern of small annual plants increase toward north (Beyer et al., 1998). This would fit with the enzyme pattern as EEA decreases from north to south. For Mediterranean ecosystems, enzyme activities decreased in parallel with plant cover degradation (Bastida et al., 2006). Especially, the higher amounts of leucine-aminopeptidase and β-glucosidase in the biocrust of North may be explained by a higher amount of roots as rooted compared to root-free soil show higher enzyme activities of these two enzymes (Loeppmann et al., 2016).

Limitations of this study

For this study, we concentrated on a sampling in the dry season. This allows only to present a snapshot on nutrient cycles and related enzyme activity as seasonal variability of C, N, and P cycles and enzyme activity was described earlier (Liu et al., 2014; Schwabedissen et al., 2017). As we included only the interdune sections of two study sites in the dune field of the northwestern Negev, the results are restricted to these settings. As the composition of biocrusts changes with relief position and therefore microclimate and dust deposition (Kidron et al., 2010; Littmann & Berkowicz, 2008), within the study area more work is needed to describe the overall spatiotemporal patterns of Negev biocrusts. Negev biocrusts are unique, as they grow in a dew desert on a sandy substrate rich in calcium carbonate and within an ecosystem that is continuously enriched in fine particles deposited as dust and input of high amount of elements from the nearby Mediterranean Sea. This reduces the comparability of the described results with other study sites, as biocrust composition is strongly linked to the soil properties (Cania et al., 2020; Miralles et al., 2020). Assuming that some cyanobacteria like *M. vaginatus* or *Nostoc* spp. (Giraldo-Silva et al., 2020) are ubiquitous, the principle mechanisms for nutrient cycles in desert cyanobacterial biocrust that are described in this work may nevertheless be transferable to other study sites.

CONCLUSIONS

Biological crusts are a major driver of geobiochemical processes affected by microclimatic situation. Within the dune field of the Negev, the amount of nutrients in biocrusts increased with increasing available moisture and decreased with soil depth. The substrate availability in these biocrusts is mainly driven by easily available carbon and nitrogen forms making WEOC:WEON more meaningful measures than C:N ratios. Due to leaching processes, nutrients are translocated from the topcrust into an important activity zone below the upper few millimeters. Thus, the subcrust is important for the overall assessment of nutrient cycles driven by microbial mineralization of more complex structured C compounds indicated by enzyme activity patterns with increasing C-cycling enzyme contribution. Within Negev biocrusts, the relative enzyme activity ($EEA \times C_{mic}^{-1}$) shows that within the topcrusts with increasing biomass the enzyme activity increases in the same proportion. This shows that the upper millimeters of biocrusts do adapt to their environmental conditions. The high $EEA \times C_{mic}^{-1}$ for the subcrust in the drier site shows that unless the absolute microbial biomass is lower in this section, the relative activity is very high due to severe nutrient limitations. In addition to that, the high P enzyme activity of the subcrust at the southern site indicates a strong P deficiency, while the subcrusts in the northern sites were less affected. Here, studies on P bound in organic compounds of biocrusts are important for more detailed insights in P-cycling dynamics in these specific early soil-colonizing microbial communities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Drahorad & Heinze, 2022) are available from JLUpub: https://doi.org/10.22029/jlupub-524.

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