

# **Biocrust indicators improved soil quality in a semi-arid environment**

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## **Biocrust indicators improved soil quality in a semi-arid environment**

### **Abstract**

Biocrusts play a wide range of ecological roles, especially in modifying surface soil properties and as a natural agent to combat soil degradation in fragile semi-arid and arid ecosystems globally. We examined the importance of biocrust on soil properties and quality in a semi-arid alluvial fan in northeastern Iran. The soil quality index (SQI) was used as the method for the quantitative evaluation of soil with or without biocrusts, using standard linear and nonlinear scoring methods. For this purpose, 17 biological, chemical, and physical soil properties were integrated into two indicator selection methods (total and minimum data set) and SQI has been calculated by the weighted additive method. The spatial patterns of SQI showed that overall the soil quality was (47 %) increased in biocrust compared to non-biocrust soil. The increase of microbial biomass nitrogen, basal soil respiration, and microbial biomass carbon, and the decrease of available sodium and percentage sand were the most important indicators controlling the soil quality changes by biocrusts. In addition, biocrusts improved the soil structure by reducing bulk density and increasing the mean weight diameter of aggregates and available water capacity. Cyanobacteria communities of biocrusts were the initiators of soil quality improvement, while moss and cyanolichen communities played a major role from the moderate stages of biocrust occurrence. It is concluded that globally, in dry lands, biocrusts should be considered as signatures of enhanced soil quality.

**Keywords:** Aridisols, Biological soil crusts, Community, Indicators, Microbial biomass, Soil quality index.

54 **Highlights:**

- 55     • Biocrusts played a significant role in enhancing the soil structure.
- 56     • Non-linear scoring showed the better relationship between biocrusts cover and SQI.
- 57     • Cyanobacteria communities were the initiators of soil quality improvement.

58 **1. Introduction**

59 Soils in semi-arid and arid regions have inherently low fertility due to their being constrained by  
60 climate extremes. These soils have low availability of water, phosphorus, and nitrogen, low soil  
61 organic carbon, and poor structure (Matar et al., 1992). Biological soil crusts (biocrusts) with low  
62 water requirements and high tolerance to temperature and sunlight have the ability to survive  
63 under conditions that limit plant growth (Bowker et al., 2005). Biocrusts are part of the biotic  
64 components of all ecosystems that can be a combination of cyanobacteria, eukaryotic algae,  
65 fungi, lichens, and mosses along with soil surface particles (Ferrenberg et al., 2017; Weber et al.,  
66 2022). The development of biocrusts increases the growth of microorganisms, thereby they  
67 improve ecological restoration in semi-arid and arid lands (Xu et al., 2013; Niu et al., 2017; Xiao  
68 et al., 2022). Biocrusts have critical ecological roles and lead to improving soil quality in arid  
69 and semi-arid ecosystems. They cause soil surface roughness (Rodriguez-Caballero et al., 2012),  
70 increase the soil stability, reduce water and wind erosion (Gao et al., 2017; Eldridge et al., 2020;  
71 Yang et al., 2022), improve infiltration and the soil moisture content (Bowker et al., 2008; Issa et  
72 al., 2009), contribute to carbon and nitrogen sequestration (Housman et al., 2006), increase soil

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**Abbreviations:**

AWC, available water capacity; BD, bulk density; BSR, basal soil respiration; CCE, calcium carbonate equivalent; CCA, canonical correspondence analysis; EC, electrical conductivity; IDW, inverse distance weighting;  $K_{av}$ , available potassium; L, less is better; M, more is better; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; MDS, minimum data set; MWD, mean weight diameter;  $N_t$ , total nitrogen;  $Na_{av}$ , available sodium; OL, optimal range with less is better; OM, optimal range with more is better; PC, principal components; PCA, principal components analysis; RMSE, root mean squared error; SOC, soil organic carbon; SQI, soil quality index; TDS, total data set.



73 nutrient and fertility (Maestre et al., 2011; Mager and Thomas, 2011), and promote plant growth  
74 (Havrilla et al., 2019). Biocrusts are able to change their shape and maintain the characteristics of  
75 topsoil and are multi-functional in arid lands. Therefore, biocrusts are promising as a nature-  
76 based measure to combat soil and land degradation in arid lands and similar fragile ecosystems  
77 (Zhao et al., 2019).

78 Understanding the efficiency of biological species (biocrusts) on topsoil is essential for their use  
79 in soil stability and health. Evaluation of the effects of biocrusts on topsoil can be done using soil  
80 quality index (SQI) approach. Most of the soil quality assessment studies have been carried out  
81 in the field of agricultural activities, polluted soils, and restoration success in mines (Doran and  
82 Zeiss, 2000; Bastida et al., 2006; Lal 2015; Beniston et al., 2016; Levi et al., 2021). So far, there  
83 have been no quantitative studies related to the effect of the type of biocrusts on soil quality.  
84 SQI can assess the capacity of the soil to maintain and support its productivity and health in the  
85 relevant ecosystem by combining several physical, chemical, and biological soil properties  
86 (Karlen et al., 1997; Lal 2011). Soil properties are typically chosen as indicators of soil quality  
87 and constitute the total data set (TDS). Due to a large number of indicators to calculate soil  
88 quality, it isn't easy to perform experimental analyzes in a large-scale area. Consequently, it is  
89 necessary to identify the minimum data set (MDS). The MDS reduces time and labor costs, it  
90 should also report high concordance with TDS in SQI evaluation (Puglisi et al., 2006; Imaz et al.,  
91 2010; Raiesi 2017). SQI normally consists of three steps: (1) selecting the appropriate indicators;  
92 (2) indicators are scored by normalization methods; (3) the scores of indicators and the  
93 contribution of each indicator in soil quality are combined into an index function (Askari and  
94 Holden, 2015; Santos-Frances et al., 2021).



Investigating the effect of biocrusts on different soil qualities in their natural habitat helps to use appropriate biocrust communities to manage soils with different degrees of soil quality in arid and semi-arid environments. The placement of Iran in the arid and semi-arid belt provides a suitable environment for performing the ecological roles of biocrusts. Alluvial fans are one of the most important landforms in arid and semi-arid regions, which provide suitable and different conditions for the growth of biocrust types. Accordingly, the main objectives of this study were to (1) evaluate the soil indicators changes affected by biocrusts, (2) investigating the relationship between the species of biocrust and changes in soil quality, and (3) determine the soil indicators controlling soil quality. It was hypothesized that the assessment of soil quality would be an effective tool to detect the improvement of soil quality by biocrusts.

## **2. Material and methods**

### **2.1. Study site description and soil sampling**

The studied area is located in northeastern Iran ( $36^{\circ}10'N$  and  $58^{\circ}59' E$ ), in the south of the Binaloud hillslopes (Fig. 1). This area is 45.5 hectares in a semi-arid undisturbed alluvial fan at an elevation approximately 1340 meters. According to De Martonne, the local climate is semi-arid with a mean annual temperature and precipitation of  $13.5^{\circ}C$  and 247.4 mm. The soil moisture and temperature regimes are Aridic border on Xeric and mesic, respectively. The main soil type in the area is Aridisols (Soil Survey Staff, 2022), and vesicular, argillic, calcic, and cambic horizons are the diagnostic soil horizons of the studied soils. Soil surface covers of biotic contain annuals, perennials, woody shrubs, and biocrusts. The sediments of the area have originated from carbonate sedimentary rocks of limestone and dolomite, clastic sedimentary rocks of sandstone and conglomerate, metamorphosed shale and quartzite, and igneous rocks of basalt, andesite, and tuff.

Soil sampling points were selected according to field observations and satellite imagery in Google Earth to cover the entire area. Soil samples were collected in two groups: (1) soil under biocrusts and (2) soil without biocrust (non-biocrust), with a thickness of 5 cm from the topsoil using a plot of 0.25 m<sup>2</sup>, in September 2020. It should be mentioned that the selection of sampling points was not along the waterway, and it was also without vegetation. A total of 74 soil samples were obtained, including 37 with biocrust and 37 without biocrust (non-biocrust) samples. The soil samples were taken in such a way that the soil sample with biocrust is beside the soil sample of non-biocrust. 4 different types of biocrusts were identified in the area: i) cyanobacteria crusts, ii) cyanolichen crusts iii) chlorolichen crusts and iv) moss crusts. The relative coverage of each type of biocrust in the field was estimated using a 0.25 m<sup>2</sup> grid divided in 100 small squares (cell). They were also visited using a 20× magnifying glass and collected from the soil surface by a cutter, and they were transferred to the laboratory for analysis.

[Fig. 1.]

## **2.2. Selection of the soil properties and laboratory analyses**

The TDS covers a wide range of soil properties, so each property should have a sensitive response to changes in soil quality. Overall, 17 soil properties were selected due to their association with soil quality. The soil samples of air-dried were passed through a 2 mm sieve for analysis of soil properties. Field moist soil samples were obtained and stored at 4 °C for biological analysis.

Indicators are divided into biological, chemical, and physical properties. Available water capacity (AWC) was determined using the differences between soil water content at a field capacity at 0.3 bar and a permanent wilting point at 15 bar, using pressure plates (Carter and

Gregorich, 2008). Granulometric analysis (Sand, Silt, and Clay) was measured using the hydrometer method (ASTM 152H) (Gee and Bauder, 1986). Bulk density (BD) was measured using the clod method (paraffin-sealed clod) described by Casanova et al. (2016). The mean weight diameter of aggregates (MWD) was analyzed for the aggregate-size fractions 4-2, 2-1, 1-0.5, 0.5-0.25, 0.25-0.125, 0.125-0.063, <0.063 mm by wet sieving method (Yang et al., 2022). Electrical conductivity (EC) and pH were measured in 1:1 ratio of soil–water suspension by pH and EC meter (Jenway Inc, England). Available potassium ( $K_{av}$ ), and available sodium ( $Na_{av}$ ) were determined using flame photometry detection (Knudsen et al., 1983). Soil organic carbon (SOC) was measured by the dichromate oxidation method (Carter and Gregorich, 2008). Calcium carbonate equivalent (CCE) was measured by the back-titration method (Carter and Gregorich, 2008). Total nitrogen ( $N_t$ ) was determined by the Kjeldahl method (V50), described by Bremner and Mulvaney (1982). Measurement of soil microbial biomass carbon (MBC) was done using fumigation incubation (Horwath and Paul, 1994). Microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) were determined using a fumigation extraction method (Brookes et al., 1985; Carter and Gregorich, 2008). Basal soil respiration (BSR) was estimated at 30 gr of fresh soil samples were added into a 1000 ml glass container and incubated with 100 ml of 0.05 M NaOH for three days at 25 °C, then 10 ml of saturated  $BaCl_2$  was added to precipitate the evolved carbonate ion as insoluble  $BaCO_3$ . The unconsumed NaOH was measured by back-titration with 0.05 M HCl in the presence of phenolphthalein.

Spraying water on the soil surface before sampling facilitated easy collection and preserved the integrity of biocrusts (Fig. 2). The biocrusts were separated from the underlying soil layer by using a cutter, and they were transported to the soil science lab at Ferdowsi University of Mashhad and stored at 4 °C. Then, we identified the morphological characteristics of different



species using a stereomicroscope (Olympus SZH10) and a microscope (Olympus CH-2). The biocrusts involve cyanobacteria crust, cyanolichen crust (lichens with cyanobacterial photobionts), chlorolichen crust (lichens with green algal photobionts), and moss crust. Species were identified by the identification keys and the following taxonomic references (Smith, 2004; Anagnostidis and Komarek, 2005; Temina et al. 2005; Rosentreter et al., 2007; Sant'Anna et al., 2011; Barsanti and Gualtieri, 2014). To view the list of identified species for each kind of crust, refer to supplementary material Fig S1 and S2.

[Fig.2.]

### 2.3. Soil quality index (SQI)

SQI consists of several statistical steps. In the present study, the first step was selecting the MDS of TDS that are important soil variables through Principal Components Analysis (PCA) (JMP Pro software version 16.0.0 (SAS Institute Inc., Cary, NC, USA)). Principal components (PCs) included components with eigenvalues  $> 1$ . In each PC, soil variables received a weight that represented their contributions to the PC. During the PCA, the varimax rotation method was used, which maximized the contrasts between the selected value of PCs, and the cumulative proportion of total variance was maintained. Only variables with weights  $> 0.8$  from each PC were selected to define the MDS.

The second step was to transform the values of indicators or soil properties into unitless scores (0-1) through linear and nonlinear methods. The linear scoring method (L) establishes a linear relationship between the score and the measured data based on the sensitivity of the indicator to changes in soil quality (Guo et al., 2017). However, other studies found no linear relationship between scores and indicator values and therefore developed a nonlinear (NL) scoring method to

185 normalize the data (Cambardella et al., 2004; Bi et al., 2013). The NL method depends on the  
 186 measured values and the dispersion statistics of the indicator. Comparison of two scoring  
 187 methods for evaluating soil quality can help choose the appropriate method and facilitate the  
 188 creation of soil mapping and management strategies.

189 For scoring, three types of functions were defined: (1) a “more is better” function (M) was used  
 190 for the indicators with positive effects on soil quality, (2) a “less is better” function (L) was used  
 191 for the indicators considered to be limiting to improve soil quality, and (3) an “optimal range”  
 192 function was used for the clay and sand content, and pH. Depending on whether the value of the  
 193 indicator was higher or lower than the optimal range, scores were assigned using the more is  
 194 better (OM) or the less is better (OL) function. Equations 1 (LM: linear scoring by the “more is  
 195 better” function) and 2 (LL: linear scoring by the “less is better” function) were used for linear  
 196 scoring (Guo et al., 2017). The sigmoidal type curve equations 3 (NLM: nonlinear scoring by the  
 197 “more is better” function) and 4 (NLL: nonlinear scoring by the “less is better” function) were  
 198 used for nonlinear scoring (Masto et al., 2008; Levi et al., 2021). In equations 1, 2, 3, and 4,  $x$  is  
 199 the indicator value,  $x_{min}$  and  $x_{max}$  were respectively the lower and the upper threshold values,  $a$  is  
 200 the indicator average, and  $b$  is  $2\sigma^{-2}$  of the data.

$$201 \quad LM(x) = (0.9 \times \left( \frac{x - x_{min}}{x_{max} - x_{min}} \right)) + 0.1 \quad (1)$$

$$202 \quad LL(x) = 1 - (0.9 \times \left( \frac{x - x_{min}}{x_{max} - x_{min}} \right)) \quad (2)$$

$$203 \quad NLM(x) = \frac{1}{1 + e^{(-b \times (x - a))}} \quad (3)$$

$$204 \quad NLL(x) = \frac{1}{1 + e^{(b \times (x - a))}} \quad (4)$$

205 The final step calculates SQI (Paz-Kagan et al., 2015; Raiesi 2017) using the weighted additives  
206 method (Equation 5). SQI method is considered as a comprehensive assessment of soil quality,  
207 which reflects the effects of land use change on soil functions. In this method, the importance of  
208 each indicator is considered and its weighting is determined (Bi et al., 2013). SQI effectively  
209 combines a variety of soil information for quantitative analysis of soil quality (Swanepoel et al.,  
210 2014).

211 In this study, the TDS and MDS methods were used to evaluate SQI. For these two methods,  
212 weighting values were marked by considering the communality of each indicator, obtained from  
213 PCA (JMP Pro software version 16.0.0). The value of communality represents the proportion of  
214 the variance of each indicator. The weight values for each indicator were estimated by dividing  
215 the communality of each indicator by the sum of all communalities (Askari and Holden, 2015;  
216 Guo et al., 2017; Santos-Frances et al., 2021).

$$217 \quad SQI = \sum_{i=1}^n W_i S_i \quad (5)$$

218 where  $W_i$  is the PCA weighting factor,  $S_i$  is the ranks of the scoring function of each soil  
219 indicator (LM, LL, NLM, and NLL), indexed as  $i$ , and  $n$  is the total number of indicators.

#### 220 **2.4. Soil quality classification and spatial distribution**

221 SQI was classified into five grades (very low, low, moderate, high, and very high) according to  
222 the equal intervals method. The classification was accomplished according to the highest and  
223 lowest values of soil quality (in all samples) in the study area.

224 Spatial analysis of SQI for soil covered by biocrust and non-biocrust was performed using  
225 geostatistical analysis software (ArcGIS 10.3). The interpolation method for spatial variability  
226 analysis was inverse distance weighting (IDW). Cross-validation was used to evaluate the



performance of the models. The accuracy of the maps was assessed through the mean error (between 0.002 % and 0.008 %) and root mean squared error (RMSE) (between 0.049 % and 0.078 %).

## **2.5. Statistical analysis**

Mean comparison of SQIs evaluated in both TDS and MDS methods with linear and nonlinear scoring in biocrust and non-biocrust soil samples, as well as for the statistical differences between the indicators in these two groups were investigated using T-test procedure. The correlation between indicators was analyzed. Before statistical analysis, the data were evaluated for normality by Kolmogorov-Smirnov test. The t statistics and significance levels (P) were obtained using the JMP Pro 16.0.0 software.

Finally, the relationship between species of biocrust as explanatory variables with the assemblage of MDS indicators, as response variables, was investigated by using canonical correspondence analysis (CCA) at alpha level 5%. The length the detrended correspondence analysis ordination axis proposed that CCA was an appropriate approach (length of gradient > 4). This analysis based on the Monte Carlo test were applied to evaluate the significance of all axes statistically and was performed with CANOCO v4.5 software.

## **3. Results**

### **3.1. Soil quality indicators**

The soil of the study area was classified as normal soil based on pH (7.6 - 8.4) and EC (0.63 - 0.65 ds/m) (Scherer et al., 1996). According to the granulometric analysis, the soil texture was classified as loam. High BD, low AWC, and MWD values were observed at sites of non-biocrust, which indicated the poor soil structure. Biocrusts enhanced the soil structure by

reducing bulk density by 10 % and increasing the mean weight diameter of aggregates by 51 % and available water capacity by 81 %. Soil nutrients in soils under biocrust were better, as they had higher values of SOC, N<sub>t</sub>, BSR, MBC, MBN, and MBP than in non-biocrust soil. Our results showed 12 soil properties showed significant differences between soils under biocrust and non-biocrust (Table 1). According to t-test analysis, the content of AWC, MWD, silt, N<sub>t</sub>, SOC, BSR, MBC, MBP, and MBN in soil under cover of biocrusts significantly increased in comparison with non-biocrust while the content of clay, BD, and pH decreased.

#### [Table 1]

The results of the PCA provided five PCs that had eigenvalues > 1.0 and explained 76.6% of the variability (Table 2). Indicators with a factor loading > 0.8 were considered highly loaded and were selected for the MDS (MBN, N<sub>t</sub>, BSR, MWD, Na<sub>av</sub>, EC, Sand, Silt, and MBC). PC 1 estimated 29.1% of total variability, which was high loads in the content MBN and N<sub>t</sub>. PC 2 explained 17% of the variation and was highly loaded in the content MWD and BSR. The Na<sub>av</sub> and EC had high loads in PC 3 (accounted for 13% of the variability in the data). PC 4 had high loads in the content of sand and silt, which was estimated to be 11% of the total variability. Only the content MBC in PC 5 was high loads (estimated for 6% of the variability) (Table 2).

The ordination of PCA visualized the capability of soil properties to individuate the soil quality between biocrusts and non-biocrust. We observed apparent clustering of the two soil groups PC1, and two PCs estimated for 46.1% of the total variance (PC1 and PC2) (Fig. 3). The soil samples underneath biocrusts positively related (> 0.5) with MBN, N<sub>t</sub>, BSR, MWD, SOC, AWC, K<sub>av</sub>, and MBP but negatively related (> 0.5) with BD and pH in PC1 and PC2 (Table 2). In addition, Figure 3 showed that BSR and MBC were more influenced by soil physical properties.

**[Table 2]**

**[Fig. 3]**

According to the community analysis and the weighted value of the indicators (Table 3), the values of sand, EC, and  $\text{Na}_{\text{av}}$  showed the highest weights (between 0.075 and 0.071); in contrast, the values of MBP, AWC, pH, and BD received the lowest weights (between 0.042 and 0.047).

**[Table 3]**

Validation of the SQI-MDS was performed through correlation coefficients between the TDS and MDS approaches (Fig. 4); there were high correlations in two linear and nonlinear methods, respectively 0.919 and 0.875.

**[Fig. 4.]**

### **3.2. Soil quality index (SQI)**

SQI was evaluated in terms of biocrust and non-biocrust using TDS method with linear and nonlinear scoring. The two methods evaluated the SQI under biocrust similarly. There was a significant difference in methods of SQI evaluation between the soils under biocrust (SQI = 0.63) and non-biocrust (SQI-Linear = 0.51 and SQI-Nonlinear = 0.43). SQI-Nonlinear calculated more difference between biocrust and non-biocrust samples (by ~ 47 %). Method MDS-linear estimated the highest values of SQI for the soils under biocrust and non-biocrust (refer to supplementary material Fig. S3).

The spatial patterns of soil quality estimated from the methods TDS-Linear and TDS-Nonlinear were similar for non-biocrust soils (Fig. 5), and MDS-Linear and MDS-Nonlinear were also similar (refer to supplementary material Fig. S4). In the southeastern section of the alluvial fan,



SQI-TDS was higher in both groups than in other sections, while the lowest soil quality was found in the northwestern and apex section. The soil quality in the studied area was classified into five grades: very low, low, moderate, high, and very high, for two groups of soil (biocrusts and non-biocrusts). According to Table 4, SQI in non-biocrust soils evaluated by TDS and MDS methods was generally low grade ( $> 60\%$ ). In comparison, SQI in soil under biocrust was the moderate grade ( $\sim 52\%$ ) in TDS-Linear to high ( $\sim 56\%$ ) in TDS-Nonlinear, and MDS methods were from high to very high grades. The spatial patterns showed that biocrusts had increased the soil quality. In general, MDS methods in the two groups were estimated more compared to their TDS.

[Table 4]

[Fig. 5.]

### 3.3. Relationship between soil quality indices and biocrusts cover

Figure 6 showed that there was a significant positive correlation between SQI and biocrusts cover. The SQI-Nonlinear method has a higher correlation with biocrust coverage than the SQI-Linear method. As a result, the nonlinear method was chosen to estimate the coverage percentage of biocrust communities and species in SQI grades (Fig. 7 and 8). Cyanobacteria communities were present in all grades. Increasing cyanolichen coverage increased soil quality from moderate to very high grades. Similarly, along with the increase in chlorolichen coverage, the soil quality increased from high to very high. Moss communities had the highest coverage in moderate to very high grades.

[Fig. 6. - Fig. 7.]

Figure 8 showed that very high soil quality had the highest biodiversity, while low soil quality had the lowest biodiversity. *Collema tenax* and *Trichocoleus desertorium* were the most frequent in the area. Cyanobacterial species were present in all four grades of soil quality. Cyanobacterial species were present only in low grade of soil quality and included *Anabaena sp.*, *Microcoleus vaginatus*, *Nostoc commune*, *Nostoc indistinguendum*, *Oscillatoria annae*, *Phormidium uncinatum*, *Tolypothrix sp.*, *Trichocoleus desertorium*. *Microcoleus vaginatus* was the most abundant in this grade. *Gloeocapsa rupestris* was observed only in very high soil quality. *Aterocapsa sp.* and *Stigonema ocellatum* were present in high and very high soil quality, while *Anabaena sp.* was present in low and moderate soil quality. The moderate soil quality included crusts of cyanobacterial, cyanolichen, and moss. All four species of mosses were present in moderate, high and very high grades of soil quality, while *Collema coccophorum*, *Leptogium sp.*, and *Peccania sp.* from cyanolichen crusts were not present in moderate grade. *Syntrichia caninervis* was the most present in the moss crusts. Chlorolichen species were observed in high and very high soil quality, and *Placidium squamulosum* was the most abundant in the crust.

[Fig. 8.]

### 3.4. Relationship between biocrusts species and minimum data set (MDS) indicators

Biocrusts significantly affected the properties of soil. CCA analysis was significant for MDS indicators based on the Monte Carlo test (F-ratio = 2.71, P-value = 0.02), and the first two axes explained 69.7% of the variance. The MDS indicators (BSR, MBC, MBN,  $N_i$ ,  $Na_{av}$ , EC, MWD, Silt, and Sand) were used for the ordination. Along the second axis, there was a high correlation coefficient between cyanolichens and chlorolichens coverage, and both increased from the bottom to the top of the axis, except for *Toninia sedifolia*. Moss species *Bryum caespitium*,

*Syntrichia caninervis*, and *Bryum campylothecium* were aligned, which indicates their high correlation. Cyanobacterial species were more scattered than other types of biocrusts.

According to Fig. 9, sand had the highest direct correlation with *Anabaena* sp., *Phormidium favosum*, *Nostoc commune*, *Microcoleus vaginatus*, and *Oscillatoria annae*, while silt had an inverse correlation with them. MWD and Na<sub>av</sub> were directly correlated with *Tolypothrix* sp., *Oscillatoria annae*, *Trichocoleus desertorium*, *Gloeocapsa rupestris*, *Toninia sedifolia*, and *Bryum argenteum*, respectively. MBN and N<sub>t</sub> were more affected by *Aterocapsa* sp., *Nostoc indistinguendum*, and moss species, while MBC was inversely related to them and directly related to *Leptolyngbya nostocorum*, *Nostoc membranaceum*, *Collema* sp., and *Collema coccophorum*. BSR and EC have the highest correlation with *Collema crispum*, *Toninia* sp., *Stigonema ocellatum*, *Phormidium uncinatum*, *Collema tenax*, *Peccania* sp., *Squamarina lentigera*, *Psora decipiens*, *Candelariella citrina*, and *Placidium squamulosum*, respectively

[Fig. 9.]

## 4. Discussion

### 4.1. The influence of biocrusts on soil quality index

SQIs are useful tools for estimating changes and improvement rates in soil (Munoz-Rojas et al., 2016). Several physical, chemical, and biological soil properties (indicators) were proposed for developing the SQI to evaluate the effect of biocrusts on topsoil (compared to non-biocrust) in this study. Some of these indicators used in the evaluation of SQI were able to show soil differences more relevant than others. Biocrusts increased the silt content; this increase occurred due to the entrapment of aeolian and water sediments and the prevention of water and wind erosion (Karnieli 1997; Zaady et al., 2016). High AWC and MWD, and low BD indicate the



improvement of soil structure covered by biocrusts (Fig. 3). Biocrusts significantly increased SOC and  $N_t$  by trapping sediments and by the secretion of exopolysaccharide products (cyanobacteria crusts) and morphological structure (crusts of lichen and moss), which increased soil particle adhesion and increased MWD and AWC, thus they caused soil aggregate stability. These results confirmed the findings of Chamizo et al. (2012) and Niu et al. (2017) who found that the development of biocrusts increased AWC, organic matter,  $N_t$ , sediment accumulation, and finally improvement of soil stability (Yang et al., 2022). Table 1 showed that BSR, MBC, MBN, and MBP were significantly increased in the soils covered by biocrusts, while pH and  $Na_{av}$  were decreased. These results confirmed the findings of Liu et al. (2013) and Sofi et al. (2016) that biocrusts affected the activity of soil microorganisms, and they played a key role in nutrient cycles. BSR and enzymatic activity were higher in biocrusts than in bare soil (Bastida et al., 2014). Bian et al. (2011) found that the number of soil microorganisms under biocrust cover increased by 25% compared to soil non-biocrust. All these properties indicated that biocrusts had vital role in the soil of semi-arid areas, which provided suitable habitats for soil microorganisms by changing soil properties (Fig. 3 and Table 1) (Liu et al., 2017; Niu et al., 2017).

SQI in non-biocrust soil was mostly classified as low grade; in contrast, SQI in soil under biocrusts cover was moderate to high grade (Table 4). In general, the spatial patterns showed that soil quality in soils with different properties has increased in biocrust soils compared to non-biocrust soil (Fig. 5); because in the soil under biocrusts, soil nutrients and soil microbial biomass (SOC,  $N_t$ , BSR, MBC, MBP, and MBN) were high and strong structural properties (AWC, MWD, BD) were observed (Table 1).

#### **4.2. The effect of biocrust coverage on soil quality index**

The correlation between biocrusts cover and soil quality was used to compare the accuracy of evaluation methods in this study. There was a positive relationship between biocrusts cover and SQI, so the soil quality increased with the increase of biocrusts cover (Fig. 6). The highest correlation between SQI and biocrusts cover in TDS and MDS methods was related to nonlinear scoring. As a result, the accuracy was higher than linear scoring based on the above results. Therefore, the nonlinear scoring method can be suggested to calculate SQI.

SQI-Nonlinear in soils under biocrust was estimated higher than SQI-Linear (Fig. 5 and 6; Table 4). The difference between these methods is related to measured values of indicators and their relationships in the soil systems in the studied area.

The main factors affecting soil quality are climate, biotic species, soil type, topography, and management. It should be noted that the area is a natural landscape without disturbance by human impact. Except for the cover of biotic species (biocrusts), the influencing factors were similar among the sampling sites. Therefore, the greatest variation in the soil quality of this area can be considered related to the difference in the type of biocrusts such as cyanobacteria, cyanolichen, chlorolichen, and moss. The development of biocrust types could be applied as an indicator for the restoration and recovery of soil (Munoz-Rojas et al., 2016; Zaady et al., 2016; Mukhopadhyay et al., 2017). In general, the highest coverage of biocrust communities was observed in very high grade of soil quality, which indicates the direct effect of each type of biocrust on soil quality (Fig. 7). Cyanobacteria crusts can promote nutrient accumulation, and carbon and nitrogen fixation, therefore accelerating biocrusts to a later stage (Williams et al., 2017; Zhang et al., 2018). The first biocrust communities that were present in unfavorable soil conditions were cyanobacteria communities, which improved soil quality to a low grade in low coverage (6%). *Microcoleus vaginatus*, *Microcoleus steenstrupii*, *Nostoc commune*, *Lyngbya*,

402 *Oscillatoria*, *Phormidium*, and *Tolypothrix* are considered the most representative and abundant  
403 species of cyanobacteria in biocrusts of dry lands (Budel, 2003; Becerra-Absalon et al., 2019;  
404 Machado-de-Lima et al. 2019). *Microcoleus vaginatus* and *Nostoc indistinguendum* were the  
405 most abundant in low soil quality (Fig. 8). Chlorolichen communities had less coverage than  
406 others, so it can be considered that they had less impact on increasing soil quality (Fig. 7 and 8).  
407 Their range was from high to very high, indicating that chlorolichen crusts required soils with  
408 more suitable characteristics than others. As can be seen from the results of the spatial patterns,  
409 the sections in the soil non-biocrusts had higher SQI, and reciprocally, the presence of biocrusts  
410 in those sections created higher SQI than other sections (Fig. 5). *Psora decipiens*, *Placidium*  
411 *squamulosum*, *Squamarina lentigera*, and *Toninia sedifolia* are among the dominant species of  
412 chlorolichen in dry lands (Budel et al., 2013; Lange and Belnap, 2016; Concostrina-Zubiri et al.,  
413 2022). Along with the increase in the cyanolichen coverage, the grade of soil quality improved.  
414 *Collema tenax* and *Collema crispum* were the most abundant and effective cyanolichen species  
415 in increasing soil quality (Fig. 8 and 9). The genera *Collema* and *Leptogium* are the most  
416 common cyanolichen species that colonize bare soils in semi-arid habitats (Rosentreter et al.,  
417 2016). Hu et al. (2017) findings indicated that the microbial abundance under mossy crusts was  
418 20% greater than cyanobacteria crusts. Increasing the coverage of moss communities from  
419 moderate to very high grade increased soil quality, and they had the most coverage compared to  
420 others in these grades; *Syntrichia caninervis*, *Bryum caespiticium*, and *Bryum campylothecium*  
421 played a major role in increasing soil quality. *Syntrichia caninervis* is one of the most abundant  
422 desert mosses in the world (Pan et al., 2016; Mao et al., 2022). With the increase of biodiversity  
423 in very high soil quality (Fig. 8), the percentage of moss cover decreased. In contrast, the

presence of other types of biocrust increased (Fig. 7). In general, mosses and cyanolichens had the greatest effect on increasing soil quality (Fig. 8).

#### **4.3. Determination of the effective indicators on soil quality**

The studied indicators mostly reflect soil functions in the area, which usually were selected previously as evaluation indicators by other studies (Levi et al., 2021; Santos-Frances et al., 2021). Indicators should be carefully considered to eschew time-consuming and costly efforts while allowing for a correct assessment (Asensio et al., 2013; Ngo-Mbogba et al., 2015). It is necessary to identify the MDS by selecting indicators that can adequately and accurately assess the effects of biocrusts. The high correlation coefficient between SQI-TDS and SQI-MDS in linear and nonlinear methods (Fig. 4) showed that the indicators selected as MDS were suitable for calculating the soil quality.

According to the PCA analysis, the indicators in MDS were MWD, sand, silt, EC,  $Na_{av}$ ,  $N_t$ , MBC, BSR, and MBN (Table 2). The results showed that the increase of MBN, BSR, and MBC, and the decrease of  $Na_{av}$  and sand were the most important variables controlling soil quality changes by biocrusts, which indicates the combined effect of biological, physical, and chemical soil properties (Table 2). According to MBN, BSR, and MBC indicators were the highest factors loading of PCs, so it can be explained that biocrusts had a great impact on soil biological properties (Tables 2 and 3). Since these indicators are among the dynamic properties of soil, they are used in the evaluation of environmental changes in soil function (Mager and Thomas, 2011; Niemeyer et al., 2012; Dose et al., 2015).

#### **4.4. The effect of biocrust species on minimum data set (MDS) indicators**



445 According to Figure 8, *Anabaena* sp., *Nostoc commune*, *Microcoleus vaginatus*, *Oscillatoria*  
446 *anna*, *Tolypothrix* sp., and *Trichocoleus desertorium*, were present in low quality soil where the  
447 amount of sand was higher, and they caused the accumulation of sand and soil particles by the  
448 production of sticky exopolysaccharide. The findings of Rossi et al. (2017) and Sepehr et al.  
449 (2018) showed that cyanobacterial filaments and produced exopolysaccharides act as binding  
450 agents for soil particles. As a result, they increased MWD and  $Na_{av}$  (Fig. 9).  $N_t$  was mostly  
451 influenced by *Aterocapsa* sp. and *Nostoc indistinguendum*. They fix  $N_2$  and enter into the N  
452 cycle in biofilms (Zhou et al., 2016). Figure 9 showed that moss species and *Placidium*  
453 *squamulosum* had the greatest effect on MBN. These species and cyanolichen and chlorolichen  
454 species had the greatest impact on BSR, EC, and Silt, because these species trap dust particles  
455 due to their physiological structure, and the amount of silt particles and ions increased (Zaady et  
456 al., 2016). These results confirmed the findings of Liu et al. (2013) and Bastida et al. (2014) that  
457 biocrusts affected the BSR and soil microbial biomass. They are present in medium to high soil  
458 quality (Figures 5, 7, and 8) and have a greater effect on increasing soil quality.

## 459 5. Conclusions

460 The biocrusts had a significant role in the biological and physicochemical soil properties in a  
461 semi-arid alluvial fan. The spatial patterns of SQI showed that the presence of biocrust  
462 significantly increased soil quality. Based on the evaluation of the correlation between SQI and  
463 biocrusts cover, the nonlinear scoring showed better accuracy in comparison with the linear  
464 scoring. MDS derived from TDS can adequately and accurately assess the effects of biocrusts by  
465 SQI and reduced economic cost. The increase of MBN, BSR, and MBC, and the decrease of  $Na_{av}$   
466 and sand were the sensitive indicators of control of soil quality changes by biocrusts. *Nostoc*  
467 *indistinguendum*, *Trichocoleus desertorium*, *Collema tenax*, *Collema crispum*, *Bryum*

*caespiticism*, *Syntrichia caninervis*, and *Bryum campylothecium* were the most abundant and effective biocrust species in increasing soil quality. Cyanobacteria communities (especially *Microcoleus vaginatus* and *Nostoc indistinguendum*) were the initiators of soil quality improvement compared to other biocrust communities in a semi-arid area. They affected the physical properties of the soil and increased MWD and  $Na_{av}$ . Moss species had the greatest effect on MBN and  $N_i$ , while cyanolichen and chlorolichen species had the greatest impact on BSR, EC, and Silt. The presence of biocrusts can be considered as a symbol of soil quality improvement. According to the results, the biological indicators should be considered as the main tools in any SQI evaluation in semi-arid areas, where vegetation is low and the soil is sensitive to climate change and land degradation. This study can introduce suitable species for soil fertility and consequently plant productivity in semi-arid environments.

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#### **Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### **Declaration of competing interest**

The authors declare there is no conflict of interest.

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## 741 **Figures captions**

742 **Fig. 1.** a) The location of the study area in northeastern Iran, b) the soil sampling points in the  
743 studied alluvial fan, c) the surface cover of the area, and d) a piece of biocrust

744 **Fig. 2.** a) A plot of 0.25 m<sup>2</sup> from the studied area, b and c) close-up picture of biocrusts in the  
745 soil surfaces, d) *Syntrichia caninervis*, e) *Psora decipiens*, f) *Collema tenax*, g) *Squammarina*  
746 *lentigera*, h) *Tolypothrix sp.* (scaling 10 µm), and i) *Nostoc membranaceum* (scaling 10 µm).

747 **Fig. 3.** Principal component analysis (PCA) analysis based on the soil properties of biocrust and  
748 non-biocrust soil samples

749 **Fig. 4.** Relationship between soil quality indices evaluated in both total data set (TDS) and  
750 minimum data set (MDS) methods in biocrust and non-biocrust soil samples; a) Soil quality  
751 index-linear scoring and b) soil quality index-nonlinear scoring

752 **Fig. 5.** Soil quality grades distribution under biocrusts and non-biocrust in the studied alluvial  
753 fan; Soil quality index was evaluated using total data set method in different scoring: a) linear  
754 scoring for non-biocrust soil samples, b) nonlinear scoring for non-biocrust soil samples, c)  
755 linear scoring for biocrust soil samples, d) nonlinear scoring for biocrust soil samples.

756 **Fig. 6.** Relationship between soil quality indices evaluated by using total data set (TDS) of linear  
757 and nonlinear scoring and the present of biocrusts cover

**Fig. 7.** The percentage coverage of biocrusts communities in each grade of soil quality index using total data set method of nonlinear scoring

**Fig. 8.** Abundant percentage of the species to the total biocrust species in the study area in each grade of soil quality index using total data set method of nonlinear scoring

**Fig. 9.** Canonical correspondence analysis biplot for species\* of biocrust and the minimum data set indicators) MBN, N<sub>i</sub>, BSR, MWD, Na<sub>av</sub>, EC, Sand, Silt, and MBC)

\* Cyanobacteria species (blue color): Ana) *Anabaena* sp., Atr) *Aterocapsa* sp., GlRu) *Gloeocapsa rupestris*, LeNo) *Leptolyngbya nostocorum*, MiVa) *Microcoleus vaginatus*, MiSt) *Microcoleus steenstrupii*, NoCo) *Nostoc commune*, NoIn) *Nostoc indistinguendum*, NoMe) *Nostoc membranaceum*, OsAn) *Oscillatoria annae*, PhFa) *Phormidium favosum*, PhUn) *Phormidium uncinatum*, StOc) *Stigonema ocellatum*, Tol) *Tolypothrix* sp., TrDe) *Trichocoleus desertorium*. Chlorolichen species (orange color): PsDe) *Psora decipiens*, SqLe) *Squamarina lentigera*, PlSq) *Placidium squamulosum*, CaCi) *Candelariella citrina*, ToSe) *Toninia sedifolia*, and Ton) *Toninia* sp. Cyanolichen species (black color): Lep) *Leptogium* sp., Col) *Collema* sp., CoCr) *Collema crispum*, CoTe) *Collema tenax*, Pec) *Peccania* sp., and CoCo) *Collema coccophorum*. Mosses species (green color): BrAr) *Bryum argenteum*, BrCa) *Bryum caespitium*, BrCam) *Bryum campylothecium*, and SyCa) *Syntrichia caninervis*.

## Figures captions

**Fig. 1.** a) The location of the study area in northeastern Iran, and b) the soil sampling points in the studied alluvial fan, c) the surface cover of the area, and d) a piece of biocrust

**Fig. 2.** a) A plot of 0.25 m<sup>2</sup> from the studied area, b and c) close-up picture of biocrusts in the soil surfaces, d) *Syntrichia ruralis*, e) *Psora decipiens*, f) *Collema tenax*, g) *Squamarina lentigera*, h) *Tolypothrix sp.* (scaling 10 µm), and i) *Nostoc membranaceum* (scaling 10 µm).

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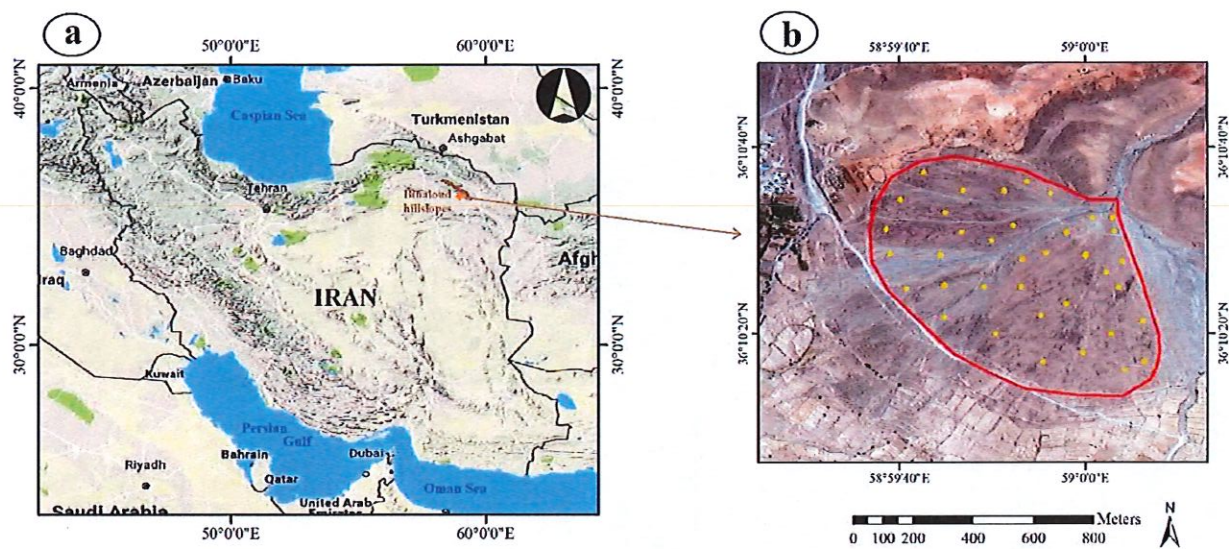
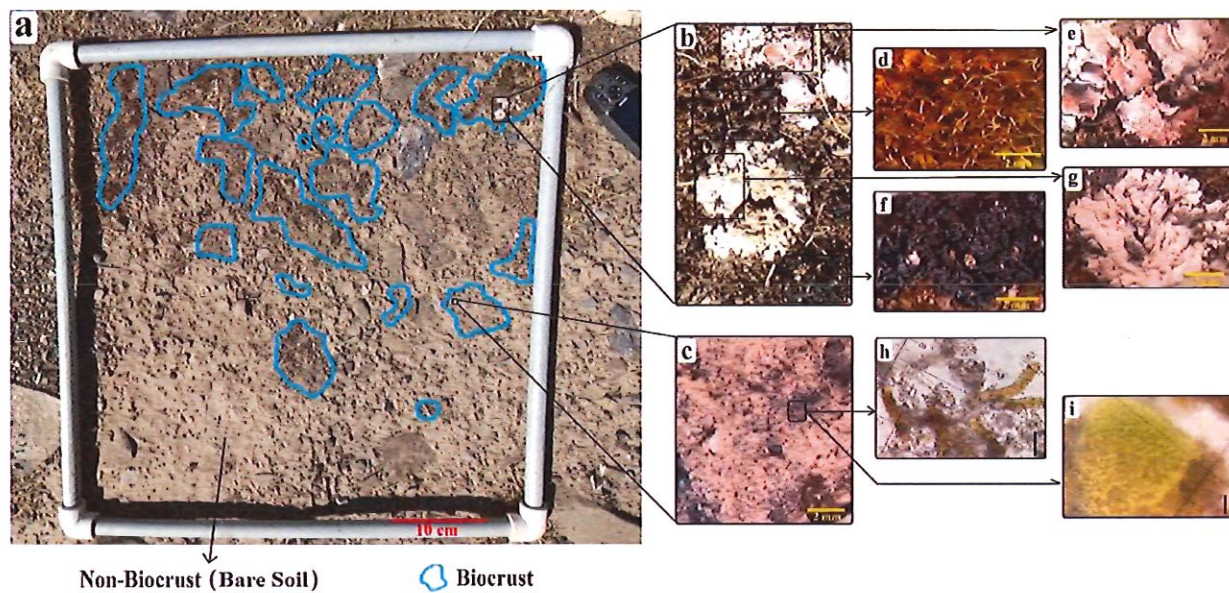
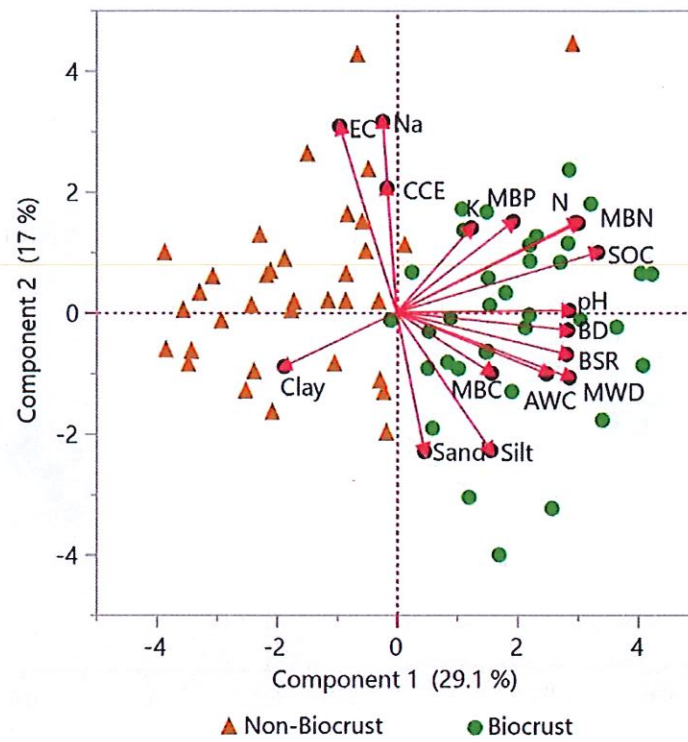


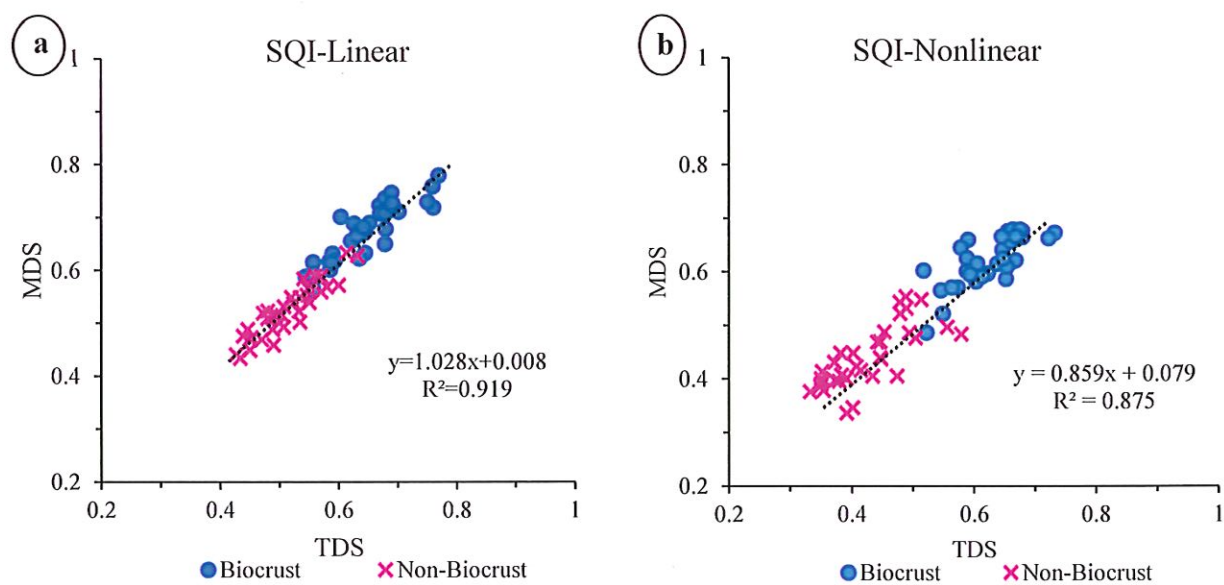
Fig. 1



**Fig. 2**



**Fig. 3**



**Fig. 4**



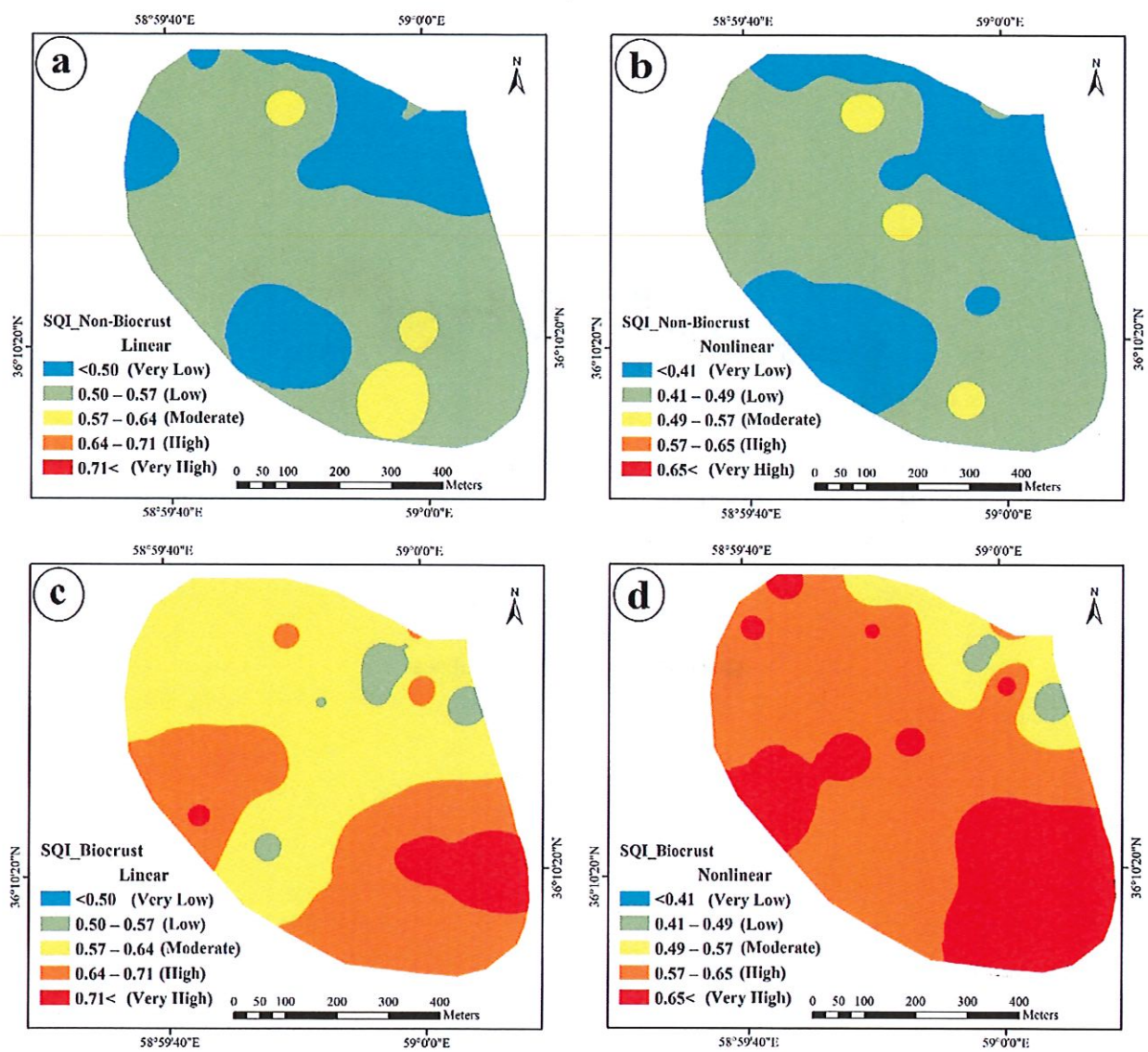
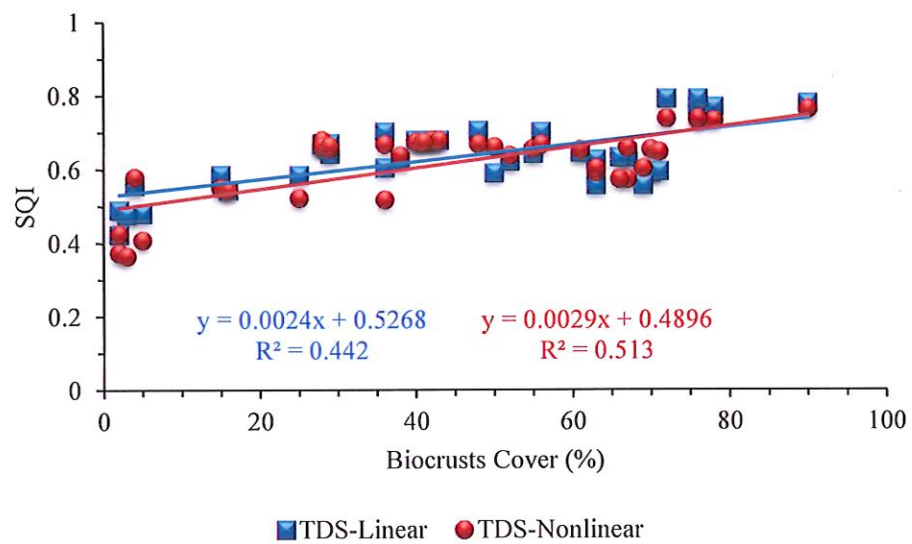
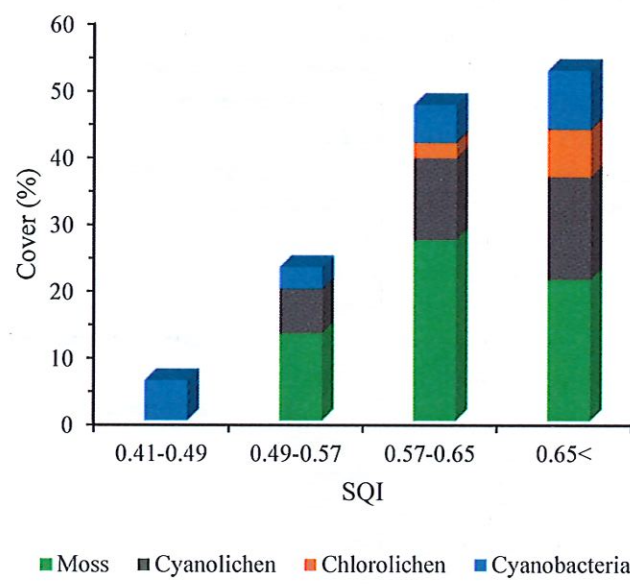


Fig. 5

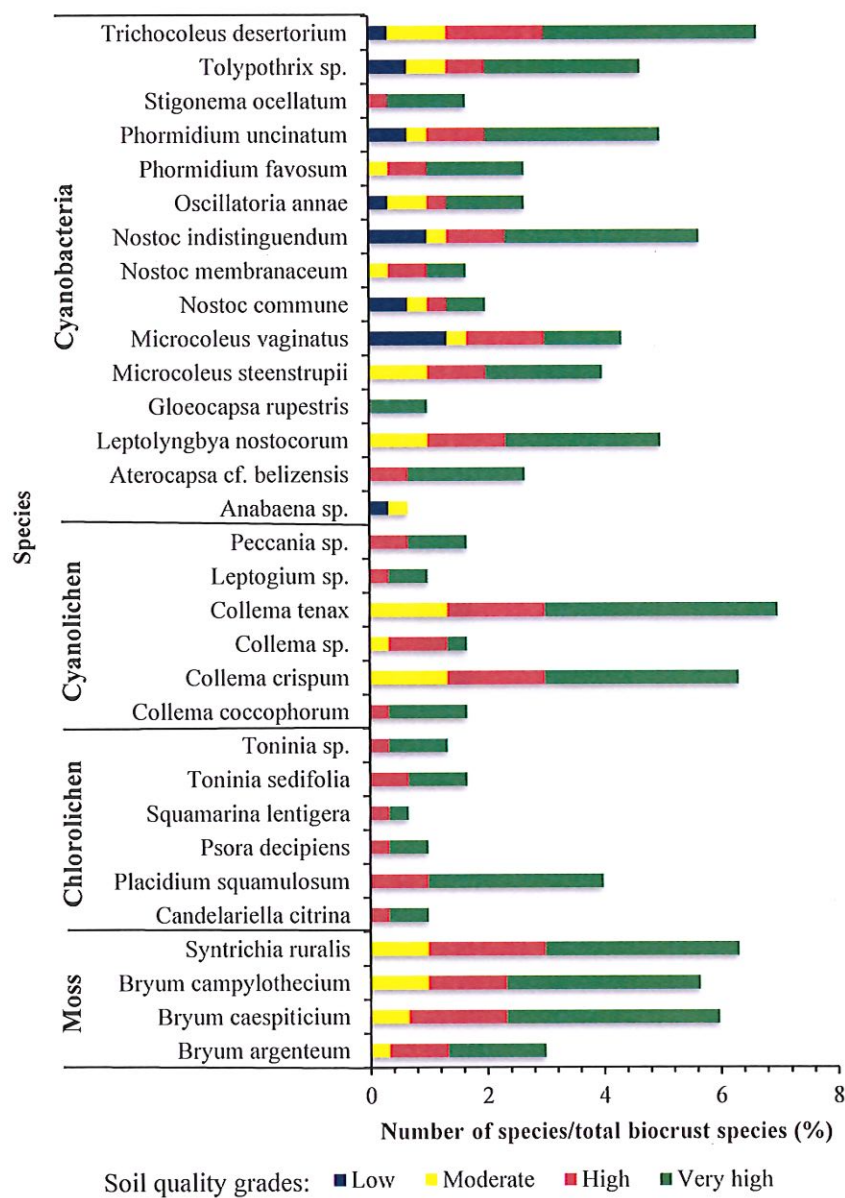




**Fig. 6**



**Fig. 7**



**Fig. 8**

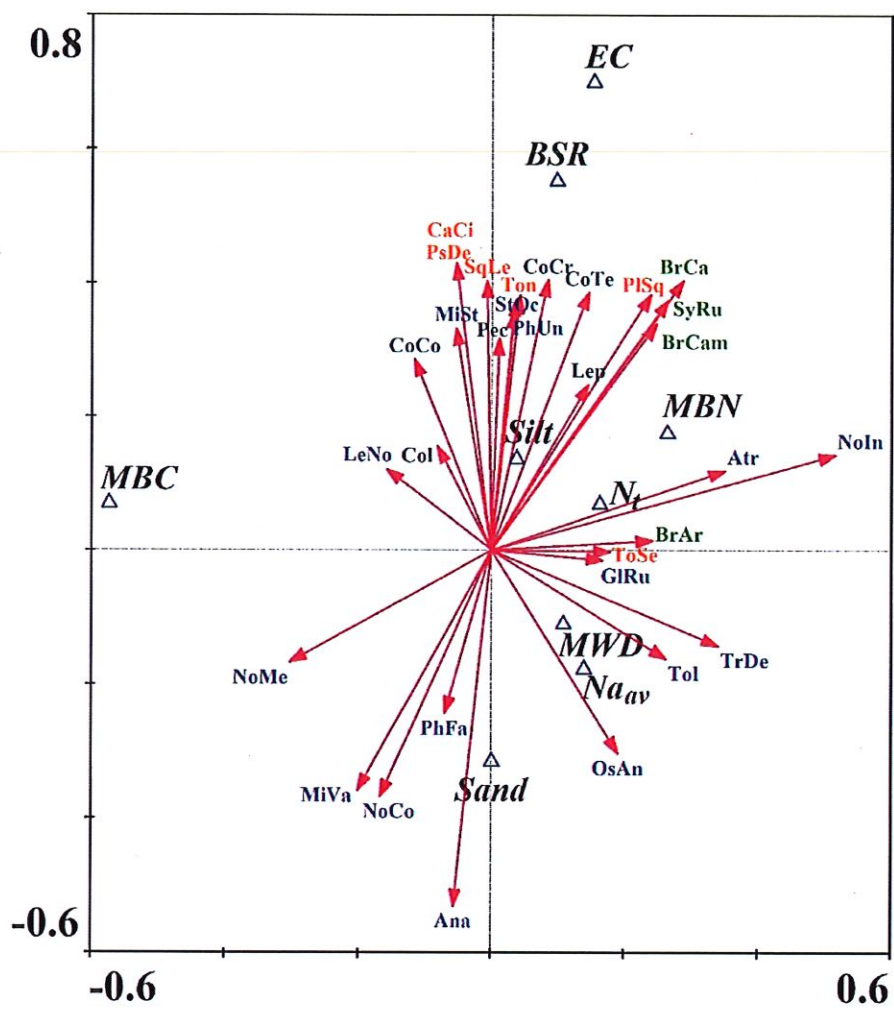


Fig. 9





**Table 1** Descriptive statistics of the indicators and their comparison in soil under biocrust coverage and non-biocrust (\*\* Significant:  $p<0.01$  and \* Significant:  $p<0.05$ ) ,and the type of standard scoring functions for the indicators.

Indicators	Minimum	Maximum	Biocrust Mean $\pm$ Std		Non-Biocrust Mean $\pm$ Std	Type
AWC (%)	0.28	8.84	4.36 $\pm$ 1.75	**	2.41 $\pm$ 1.13	M
MWD (mm)	0.486	1.082	0.911 $\pm$ 0.11	**	0.602 $\pm$ 0.67	M
Sand (%)	30.4	60.1	43.58 $\pm$ 7.78		44.91 $\pm$ 5.94	OL
Silt (%)	25.77	49.78	38.2 $\pm$ 6.31	*	34.47 $\pm$ 4.46	M
Clay (%)	12.32	27.96	18.21 $\pm$ 2.76	**	20.61 $\pm$ 2.97	OM
BD (gr/cm <sup>3</sup> )	1.46	2.33	1.58 $\pm$ 0.08	**	1.76 $\pm$ 0.15	L
EC (ds/m)	0.22	4.35	0.65 $\pm$ 0.44		0.63 $\pm$ 0.72	L
pH	7.65	8.44	7.96 $\pm$ 0.16	**	8.19 $\pm$ 0.17	OL
Na <sub>av</sub> (mg/kg)	64.11	1567.3	124.5 $\pm$ 103.13		173.41 $\pm$ 251.3	L
K <sub>av</sub> (mg/kg)	113.76	291.19	182.7 $\pm$ 44.73		190.5 $\pm$ 51.24	M
N <sub>t</sub> (mg/kg)	595	2436	1246.9 $\pm$ 278.3	*	1003.5 $\pm$ 406.5	M
CCE (%)	8.12	39.62	20.92 $\pm$ 5.88		21.77 $\pm$ 4.92	L
SOC (%)	0.02	1.38	0.81 $\pm$ 0.29	**	0.43 $\pm$ 0.22	M
BSR (mg/kg*day)	0.08	0.42	0.3 $\pm$ 0.05	**	0.14 $\pm$ 0.04	M
MBC ( $\mu$ g/gr)	14.63	578.04	238.2 $\pm$ 131.5	*	157.1 $\pm$ 98.69	M
MBN ( $\mu$ g/gr)	20.82	85.26	43.64 $\pm$ 9.44	*	34.48 $\pm$ 13.26	M
MBP ( $\mu$ g/gr)	2.48	37.44	16.49 $\pm$ 4.17	*	11.68 $\pm$ 7.33	M

L: means less is better; M: means more is better; OL: means optimal range was assigned using the less is better; OM: means optimal range was assigned using the more is better.

**Table 2** Principal component analysis (PCA) results of seventeen indicators of the soil in the studied area.

Indicators	PC 1	PC 2	PC 3	PC 4	PC 5
MBN	<b>0.935</b>	0.114	0.019	-0.042	0.006
N <sub>t</sub>	<b>0.934</b>	0.096	0.009	-0.03	0.018
SOC	0.757	0.411	-0.033	-0.033	0.059
K <sub>av</sub>	0.594	-0.223	-0.264	0.222	0.402
pH	-0.585	-0.39	-0.289	0.158	0.007
MBP	0.544	0.234	-0.265	0.065	-0.344
BSR	0.102	<b>0.901</b>	-0.006	-0.012	0.006
MWD	0.119	<b>0.894</b>	0.014	0.191	-0.061
BD	-0.28	-0.678	-0.078	0.137	-0.234
AWC	0.181	0.63	0.012	0.283	0.231
Na <sub>av</sub>	-0.011	-0.091	<b>0.93</b>	0.205	0.103
EC	0.103	0.031	<b>0.914</b>	0.199	0.21
CCE	-0.163	0.166	0.721	-0.237	-0.19
Sand	0.031	-0.111	-0.094	<b>-0.976</b>	0.031
Silt	0.12	0.339	0.176	<b>0.843</b>	-0.002
Clay	-0.292	-0.381	-0.118	0.61	-0.064
MBC	-0.014	0.408	0.139	-0.109	<b>0.801</b>
Eigenvalue	4.94	2.897	2.252	1.917	1.015
Percent	29.056	17.038	13.25	11.279	5.97
Cumulative percent	29.056	46.095	59.344	70.623	76.593

\*Bold values indicate the highest factor loading (>0.8) in each PC selected as the best factors for minimum data set.

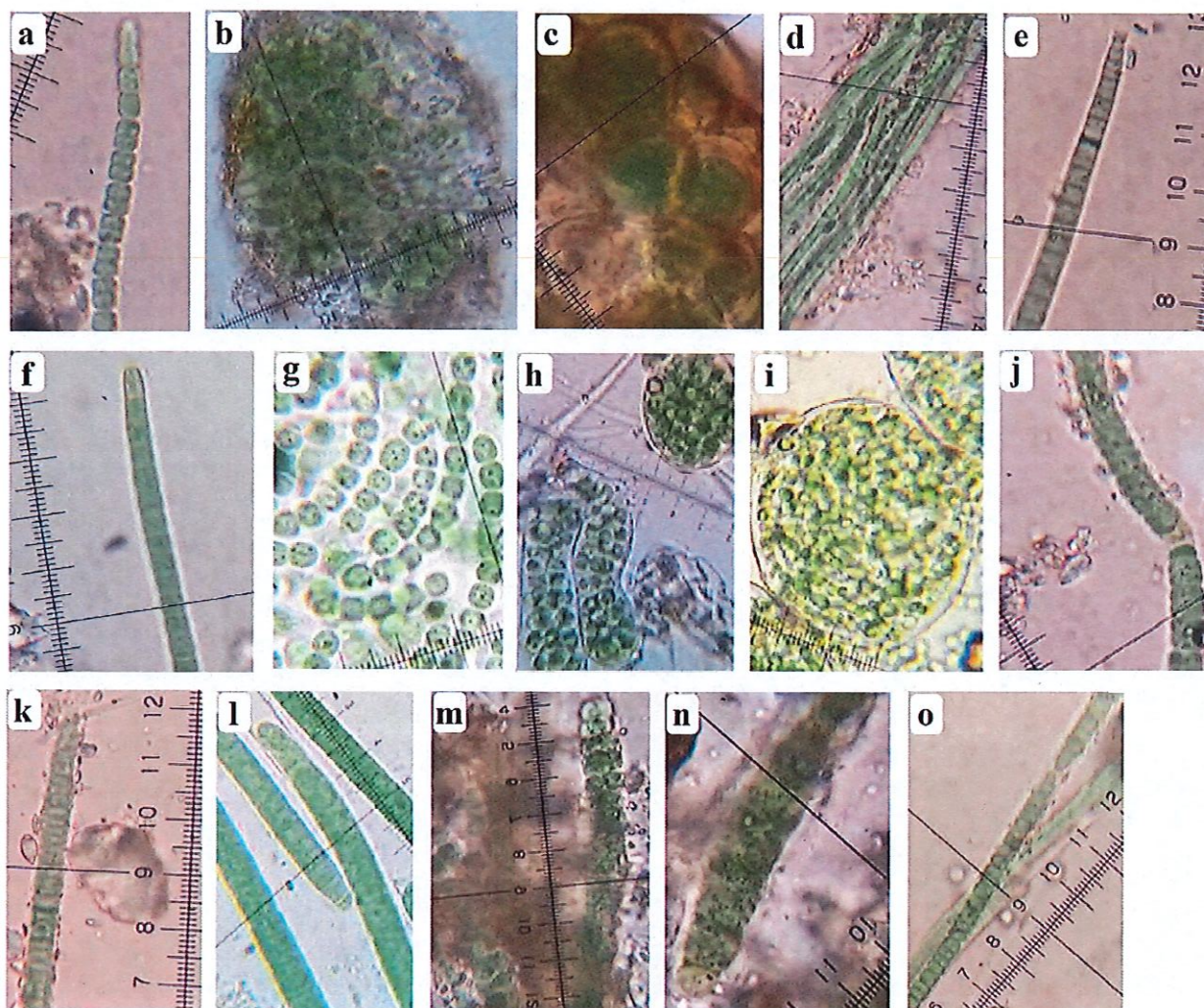
**Table 3** Estimated community and the value of the weight of each indicator in the studied area using total and minimum data set methods

Indicators	Total data set		Minimum data set	
	communality	weight	communality	weight
AWC	0.563	0.043		
MWD	0.854	0.066	0.818	0.101
Sand	0.975	0.075	0.925	0.115
Silt	0.87	0.067	0.938	0.116
Clay	0.621	0.048		
BD	0.618	0.047		
EC	0.931	0.071	0.953	0.118
pH	0.602	0.046		
Na <sub>av</sub>	0.926	0.071	0.945	0.117
K <sub>av</sub>	0.684	0.053		
CCE	0.666	0.051		
SOC	0.748	0.057		
MBC	0.825	0.063	0.657	0.081
BSR	0.822	0.063	0.845	0.105
N <sub>i</sub>	0.883	0.068	0.991	0.123
MBN	0.89	0.068	0.991	0.123
MBP	0.544	0.042		

**Table 4** Area percentages of soil quality grades evaluated in soil under biocrust coverage and non-biocrust.

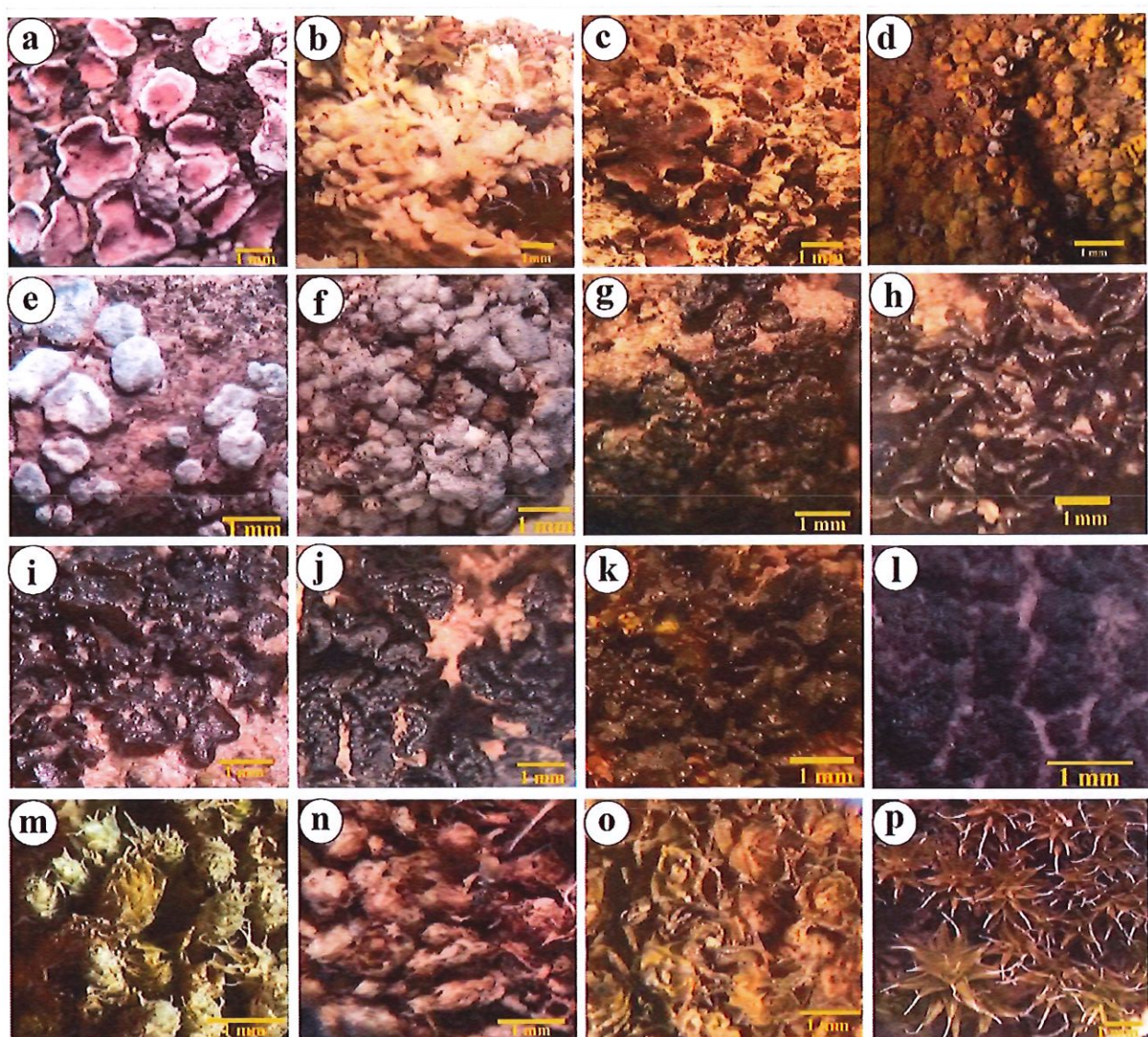
	SQI	Area (%)				
		Very High	High	Moderate	Low	Very Low
Biocrust	TDS_Linear	5.3	39.9	52.2	2.6	0.0
	MDS_Linear	9.2	53.3	35.8	1.7	0.0
	TDS_Nonlinear	26.4	56.4	15.9	1.3	0.0
	MDS_Nonlinear	64.0	27.1	8.1	0.7	0.1
Non-Biocrust	TDS_Linear	0.0	0.0	4.2	67.5	28.3
	MDS_Linear	0.0	0.0	6.3	76.8	16.9
	TDS_Nonlinear	0.0	0.0	2.1	59.7	38.2
	MDS_Nonlinear	0.0	0.6	11.0	79.2	9.2

## Supplementary material



**Fig. S1.** Cyanobacteria species identified in biocrusts of the studied area: a) *Anabaena* sp., b) *Aterocapsa* sp., c) *Gloeocapsa rupestris*, d) *Leptolyngbya nostocorum*, e) *Microcoleus vaginatus*, f) *Microcoleus steenstrupii*, g) *Nostoc commune*, h) *Nostoc indistinguendum*, i) *Nostoc membranaceum*, j) *Oscillatoria annae*, k) *Phormidium favosum*, l) *Phormidium uncinatum*, m) *Stigonema ocellatum*, n) *Tolypothrix* sp., o) *Trichocoleus desertorium*.

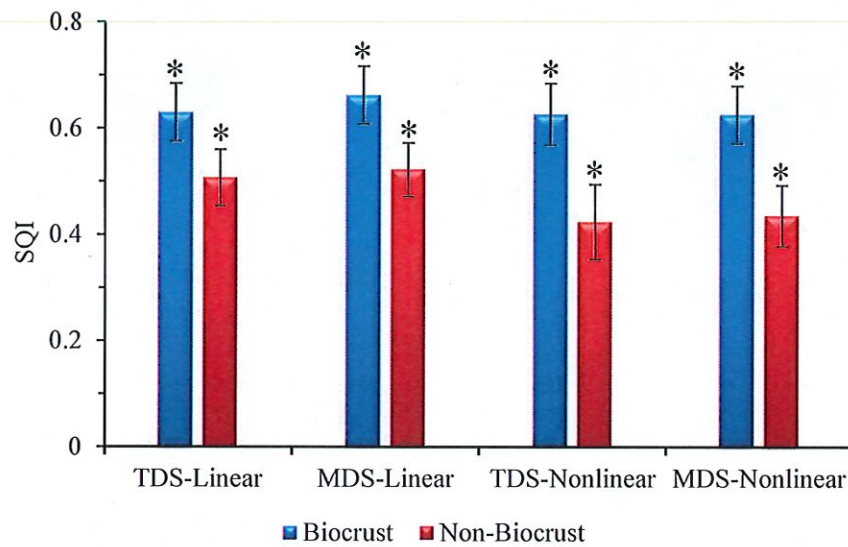




**Fig. S2.** Chlorolichen species identified in biocrusts of the studied area: a) *Psora decipiens*, b) *Squamarina lentigera*, c) *Placidium squamulosum*, d) *Candelariella citrina*, e) *Toninia sedifolia*, and f) *Toninia sp.*.

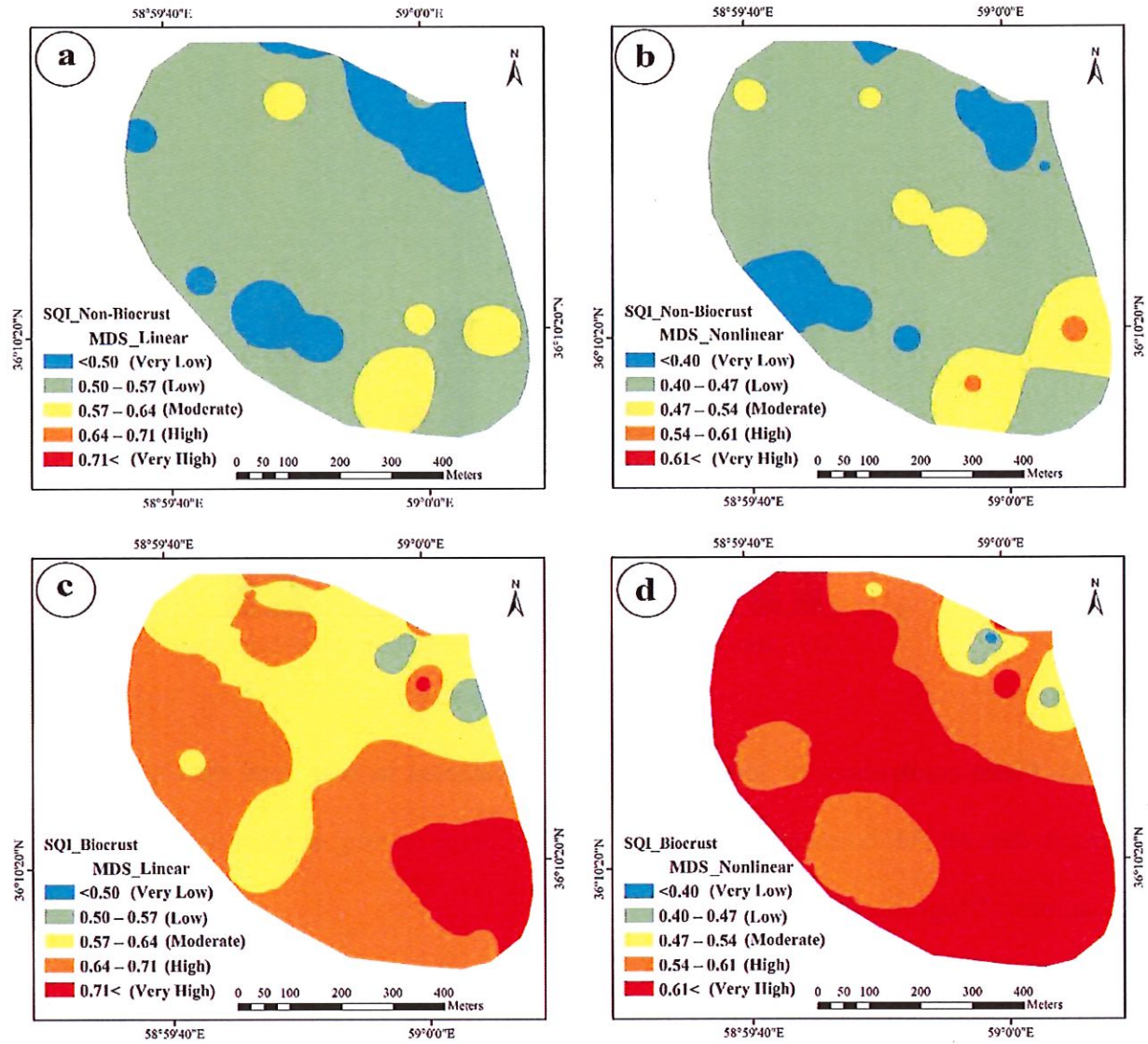
Cyanolichen species identified in biocrusts of the studied area: g) *Leptogium sp.*, h) *Collema sp.*, i) *Collema crispum*, j) *Collema tenax*, k) *Peccania sp.*, and l) *Collema coccophorum*.

Mosses species identified in biocrusts of the studied area: m) *Bryum argenteum*, n) *Bryum caespiticiun*, o) *Bryum campylothecium*, and p) *Syntrichia ruralis*.



**Fig. S3.** Mean comparison of soil quality indices evaluated in both total data set (TDS) and minimum data set (MDS) methods with linear and nonlinear scoring of biocrust and non-biocrust soil samples (\* Significant:  $P < 0.01$ )





**Fig. S4.** Soil quality grades distribution under biocrusts and non-biocrust in the studied alluvial fan; Soil quality index was evaluated using minimum data set (MDS) method in different scoring: a) linear scoring for non-biocrust soil samples, b) nonlinear scoring for non-biocrust soil samples, c) linear scoring for biocrust soil samples, and d) nonlinear scoring for biocrust soil samples.