

# Increasing soil potential for carbon sequestration using microbes from biological soil crusts

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## ABSTRACT

Dryland soils have the potential to be large atmospheric carbon (C) sinks, thus aiding in managing global warming. In these areas, soil micro-organisms found in biological soil crusts (biocrusts) can play a major role in C cycling, especially by influencing the fluxes between the atmosphere and soil; however, their potential has been reduced due to degradation. Thus, we assessed how inoculating soil micro-organisms and adding microbial stimulant nutrients onto a degraded soil affected C sequestration. Therefore, a full factorial combination of bacteria, cyanobacteria and nutrients was added to the field-collected soil. After 60 days, we measured the effect of this inoculation with and without support by a nutrient (B4) addition on the soil organic C; then, we calculated the C sequestration and the potential CO<sub>2</sub> removal from the atmosphere. We found that inoculating bacteria, cyanobacteria, or a combination of them increased the sequestered C in the soil from 0.232 to 0.294 g m<sup>-2</sup> day<sup>-1</sup> at the plot scale; when calculations were used to scale up, this increase was from 0.84 to 1.07 ton ha<sup>-1</sup> year<sup>-1</sup>. This could remove 3.11–3.93 ton ha<sup>-1</sup> year<sup>-1</sup> of CO<sub>2</sub> from the atmosphere. However, nutrient addition had no significant effect on the C pools.

## 1. Introduction

There is a strong interest in reducing atmospheric CO<sub>2</sub> as a constituent of 76% of the total emissions of greenhouse gases (GHGs) to overcome one of the serious challenges the human society faces (Rossi et al., 2015). Increasing the soil C pools in the arid and semiarid ecosystems (drylands) can play a major role in reducing the atmospheric levels of atmospheric CO<sub>2</sub> (Ahlström et al., 2015), with the current estimates of soil C absorption being 3.5–5.2 Gt year<sup>-1</sup> (Batjes, 2014). Drylands comprise ~40% of the Earth's terrestrial surfaces (Torres-Cruz et al., 2018), which are covered by only 0.7% of the world's vegetation cover (Wilske et al., 2009). Accordingly, terrestrial ecosystems in drylands could have a critical role in the C sequestration (Chen et al., 2018). In these ecosystems, the soil crust dominated by microorganisms can aggregate soil particles through exopolysaccharides secreting (EPSs), forming filaments networks (Colica et al., 2014). These conditions lead to increasing the soil stability against soil erosion and degradation factors (Kheirfam et al., 2017b). Additionally, soil micro-organisms activities in the microbially-rich biocrust can increase soil aggregation (Bashan and de-Bashan, 2010), porosity (Miralles et al., 2011), water retention (Chamizo et al., 2011), and infiltration (Sadeghi et al., 2017); they can also increase soil fertility by accumulating nutrients (Kheirfam et al., 2017a). These can lead to suitable soil

conditions helping to establish other organisms such as mosses, lichens, and herbaceous and perennial plants (Molina-Montenegro et al., 2016), thereby increasing the C storage potential. Nonetheless, the incremental degradation trend in more than 70% of drylands has reduced their capability to store C (Safriel, 2007). In many areas, this has resulted in soils being a source, rather than a sink, of the atmospheric C (Schlesinger, 2017).

Carbon cycling occurs over long- (up to a few million years) and short- (a few minutes to a few years) -term time scales (Wang and Nemani, 2016); thus, reducing the atmospheric levels at the needed time scale requires approaches that manipulate the short-term cycles (Washbourne et al., 2015). Accordingly, finding ways to enhance dryland terrestrial ecosystems is an attractive strategy for the C sequestration. In this regard, biocrust reclamation could be a way to improve the capability to store C in the drylands (Kheirfam et al., 2017a). Recently, artificial reclamation of degraded biocrusts has been considered by using the inoculation-based techniques such as direct inoculation of bacteria and cyanobacteria and/or enrichment with the nutrients stimulants (Rossi et al., 2015; Kheirfam et al., 2017a and 2017b).

Enriching biocrusts by microbial inoculation and/or stimulation can increase the short-term potential to store C (through their biomass and C fixation) and the long-term one through increasing the vegetation cover. Therefore, it may be possible to potentially reduce GHGs and

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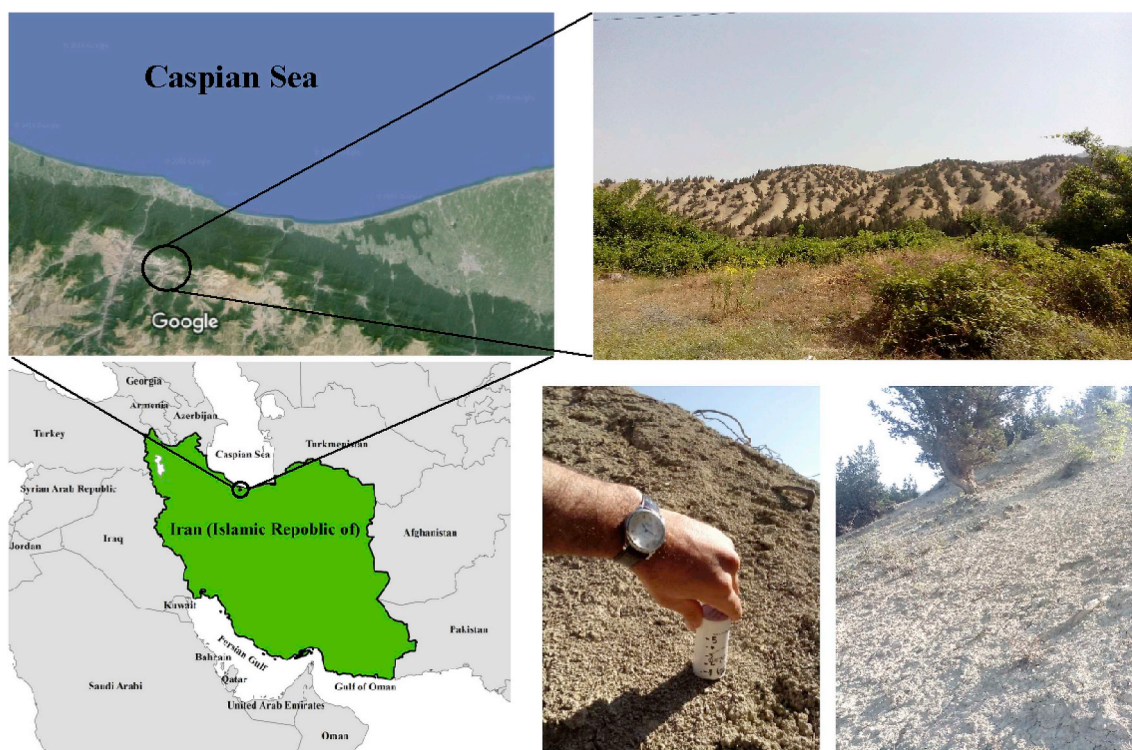


Fig. 1. General view of the study area and location of soil sampling in Mazandaran Province, Iran.

thus, global warming by inoculating dryland soils with micro-organisms and/or stimulating the soil crust micro-organisms through the nutrients addition. We first assessed how the inoculation of soil bacteria and cyanobacteria affected soil chemical properties (Kheirfam et al., 2017a), controlled soil loss (Kheirfam et al., 2017b), and improved the runoff behavior (Sadeghi et al., 2017). We then evaluated the impact of inoculation, with and without nutrient additions, on the potential C sequestration and CO<sub>2</sub> capture at small spatial scales under outdoor conditions.

## 2. Methods

### 2.1. Site description and soil sampling

This study was conducted at the same time when the research by Kheirfam et al. (2017a, b) was conducted; so the soil collected from the same area was used. Soil samples were collected in September 2014, from the western Mazandaran Province in the northern Iran (Fig. 1). The regional climate is a semi-arid cold one (Emberger, 1955). The vegetation cover is dominated by Mediterranean Cypress forests (*Cupressus sempervirens* var. *horizontalis*) with less than 25% cover. The average annual precipitation and temperature (based on the data from the Kojour and Nowshahr stations) were 432 mm and 12 °C, respectively, for the 1978–2010 period. Soils are a kind of fine loamy, mixed, Mesic, Typic Calcixerepts, Inceptisols, based on the USDA Soil Taxonomy classification method (USDA Soil Taxonomy, Soil Survey Staff, 2010). Soil texture is classified as a loamy clay silt. Analysis of the top 20 cm showed a soil texture of 14% sand, 40% clay, and 46% silt, being medium granular with some fragile stability. Lime content was 28% and pH, EC, and bulk density were 7.55, 0.22 ds m<sup>-1</sup> and 1.16 Mg m<sup>-3</sup>, respectively.

To obtain the biocrust inocula, 25 samples were collected from the upper 2 cm of the soil using a soil corer with an inner diameter of 6 cm. The soil samples were placed in plastic bags, stored at approximately 4 °C, and transported to the laboratory. In the laboratory, these samples were air-dried and sieved to 2 mm. Samples were divided: one

subsample was used to determine microbial composition (below) and richness; the other one was ground using a mortar and pestle to obtain the 0.5-mm particle size necessary for the determination of the organic C (Chamizo et al., 2012). Soils were also collected from the 0–20 cm depth to create soil boxes that were later inoculated with different treatments (see below).

### 2.2. Determination of microbial richness of the biocrusts

Bacteria and cyanobacteria in the study biocrust were isolated, identified, and then counted to determine the microbial composition and abundance. Bold's Basal and Chu10 media were also used to isolate the existing cyanobacteria in the soil bank (Whitton and Potts, 2012). Bacteria were isolated using the Nutrient Agar and Tryptic Soy Agar (TSA) general medium (Lecomte et al., 2011) and then assigned to the genus level based on morphological characterization, using high-resolution optical microscopes (Fig. 2; Vieira and Nahas, 2005; Taddei et al., 2006; Whitton and Potts, 2012). Finally, we identified six genera of bacteria (*Pseudomonas*, *Arthrobacter*, *Azotobacter*, *Diplococcus*, *Streptococcus* and *Bacillus*), and five genera of cyanobacteria (*Nostoc*, *Oscillatoria*, *Lyngbya*, *Phormidium* and *Aphanothece*) from the study soil.

### 2.3. Purification and growth of bacteria and cyanobacteria and preparation of nutrient solution

After identifying the dominant bacteria and cyanobacteria genera in our samples, we reviewed the literature to determine which were the most suitable for our study. Criteria included high abundance in the samples (indicating evolutionary suitability for the site conditions) and the ability to enhance soil quality attributes such as aggregate stability, water-holding capacity, soil porosity, and fertility (Mager and Thomas, 2011; Komárek, 2016; Kheirfam et al., 2017a). Based on these criteria and domination of the identified bacteria in the study soil, we chose to focus on the bacteria *Azotobacter* and a *Bacillus subtilis* strain. We then used *Azotobacter* Agar Modified II (Atlas, 2010) and DSMZ1 (Schrey et al., 2012) as the selective media to isolate and purify the *Azotobacter*

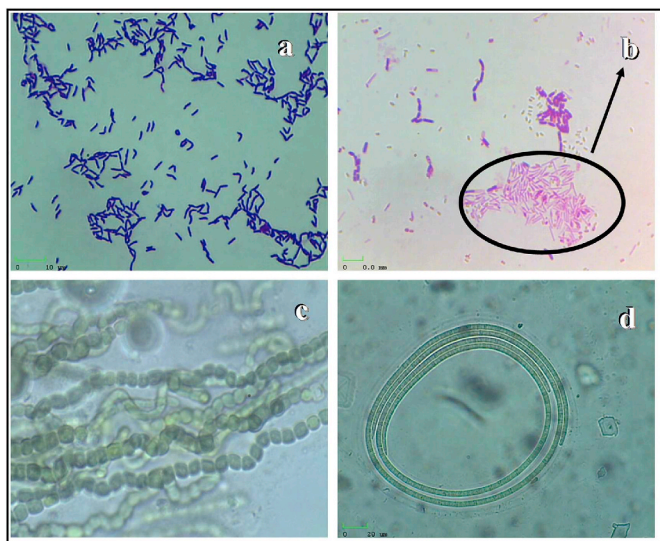


**Fig. 2.** A microscopic view of identified and then selected bacteria (*Bacillus subtilis* strain (a) and *Azotobacter* (b)) and cyanobacteria (*Nostoc* (c) and *Oscillatoria* (d)) from study area soil.

and *Bacillus subtilis* strain bacteria, respectively. The purified bacteria were grown using the LB Broth (Garbeva et al., 2011). For cyanobacteria, we selected *Nostoc* and *Oscillatoria* based on the mentioned criteria and domination of these in the study soil, which were picked by a microbiological loop and transferred into 20 ml of the autoclaved Chu10 media in 50 ml-falcon tubes. This process was repeatedly continued during several steps until complete purification. The purified cyanobacteria were then transferred into a 2-L Erlenmeyer flask containing Chu-10 medium. The cyanobacteria and bacteria were grown until the desired density of  $10^{12}$  cfu was achieved (Vieira and Nahas, 2005). After the inoculants reached this density, a solution with 15 g calcium acetate  $l^{-1}$  distilled water (dw), 4 g yeast extract  $l^{-1}$  dw, and 5 g dextrose  $l^{-1}$  dw, with the pH of 8, was prepared as a nutrient supplement (B4) for the implementation of the nutrient-including treatments (Valencia-González et al., 2014).

#### 2.4. Simulation of soil layers and inoculation of the biocrust

Soil boxes were constructed in way similar to that used by Kheirfam et al. (2017a, b). For this experiment, we built 24 simulated soil profiles in small boxes 0.5 m in length, x 0.5 m in width, and x 0.5 m in height; these were filled with the field-collected, 0–20 cm soil (Fig. 3). The boxes walls were made with glass to control the chemical and physical properties changes in the soil which occurred by other common walls such as metals and polycarbonates. Soils were homogenized and passed through 8 and 3 mm sieves to be used for the 2–30 cm and 0–2 cm layers, respectively. Soil permeability, bulk density, layer thickness, and particle size distribution of the field soil were replicated as closely as possible using the methodologies employed Sadeghi et al. (2017). To facilitate drainage and to mimic the field setting as closely as possible, the bottoms of the boxes were made of perforated stainless steel sheets covered by three layers of the mineral pumice grains of different sizes, which ranged in diameter from 1 to 3 cm, for a total thickness of 30 cm. The soil was then compacted with a concrete roller to achieve the bulk density of  $1.16 \text{ Mg m}^{-3}$ , equal to that measured at the field site.

Using a completely randomized factorial design, we had three replicates of the following treatments: (I) no nutrient addition and no inoculation (control), (II) with nutrient addition and no inoculation, (III and IV) with and without nutrient addition plus bacteria inoculation, (V and VI) with and without nutrient addition plus cyanobacteria inoculation, and (VII and VIII) with and without nutrient addition plus

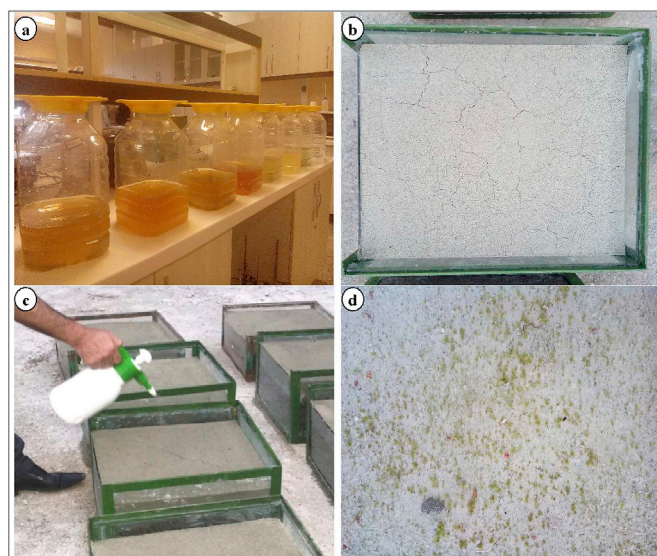


**Fig. 3.** Preparations of cyanobacteria, bacteria and combined of them as well as the B4 nutrient for implementation of planned treatments (a), simulated soil profile before enriched by micro-organisms and nutrient (b), inoculation of cyanobacteria and bacteria on the soil plots with and without supplying by B4 through spraying technique (c), cyanobacteria-dominated biocrust after 60 days (d).

the co-inoculation of bacteria and cyanobacteria. For the inoculation, 0.5 L of either or both bacteria and cyanobacteria ( $\sim 1 \times 10^{12}$  cfu each) was used (Wang et al., 2009); for the nutrient addition, 0.5 L of the solution was applied (Valencia-González et al., 2014).

The experimental boxes were then placed outside the Natural Resources Campus of Tarbiat Modarres University, Iran, in Mazandaran Province, Noor City, which has a climate similar to the study site; thus, they were subjected to the natural conditions for a period of 60 days (Nisha et al., 2007). The experiment period coincided with the late spring and early summer, 2015. In this period, the lowest and highest recorded daily temperature and solar radiation were 5.4 and 35.4 °C, and 300 and 2500  $\text{W m}^{-2}$ , respectively. Five rainfall events occurred during the experimental period, which summed up 40 mm. Because C sequestration can be a slow process, the experiment was designed to run for 60 days. However, some soil chemical measures were additionally conducted at shorter intervals during the experiment period (see Kheirfam et al., 2017a).

#### 2.5. Measurement of soil organic carbon pools

At the beginning and end of the experimental period, soil surface (0–2 cm) organic C content was measured using the Walkley-Black (1934) method. We then applied Equation (1) (Meersmans et al., 2008) to determine how soil organic C pools were affected by cyanobacteria and bacteria inoculation and nutrient supply.

$$SC = (SOC_E - SOC_I) \times S_{BD} \times D \quad (1)$$

where SC is amount of sequestered C during the experimental time-frame ( $\text{g m}^{-2}$ );  $SOC_E$  and  $SOC_I$  are the amount of soil organic C ( $\text{g C kg}^{-1}$  soil) at end and initial time of experiment period, respectively;  $S_{BD}$  is soil bulk density ( $1.16 \text{ Mg m}^{-3}$ ); and D is the sampled soil depth (0.02 m).

Equation (2) was used to estimate the potential removed (draw-down) of  $\text{CO}_2$  from the atmosphere (Washbourne et al., 2015).

$$RCO_2 = SC \times I_{3.67} \quad (2)$$

where  $RCO_2$  is potential amount of  $\text{CO}_2$  removed from atmosphere and soils during the experimental period ( $\text{g m}^{-2}$ ), SC is amount of sequestered C, and the constant number  $I_{3.67}$  is based on the mass molar of

$\text{CO}_2$  (one carbon atom and two oxygen atoms) =  $[1 \times 12.0107 \text{ g mol}^{-1}] + [2 \times 15.9994 \text{ g mol}^{-1}] = 44.0095 \text{ g mol}^{-1}$ . Thus, 1 g of C in  $\text{CO}_2$  in the soil equates to  $\sim 3.67 \text{ g CO}_2$  removal from the atmosphere (= 44/12; Washbourne et al., 2015).

We can assume that whole increase in organic C in our trial boxes soils comes from microbial  $\text{CO}_2$  fixation and, to a lesser extent, decomposition of dead microbial biomass. There were no inputs by vegetation. We also calculated the amount of daily C sequestration and removal  $\text{CO}_2$  from treatments by dividing obtained value from Eqs. (1) and (2) to experimental time period and for upscaling of these the  $\text{g m}^{-2} \text{ day}^{-1}$  converted to  $\text{ton ha}^{-1} \text{ year}^{-1}$ .

## 2.6. Statistical analyses

Our experiment was a  $2 \times 4$  factorial completely randomized design, with two nutrient addition statements (with and without the addition of B4) and four microbial inoculations (no inoculation, bacteria inoculation, cyanobacteria inoculation, and co-inoculation of bacteria and cyanobacteria). In this study, we had eight treatments in which the trial boxes with no nutrition and no inoculation were defined as the control treatment. After data collection, the normality of the variables was checked by the Shapiro-Wilk test and the homogeneity of variances was controlled by the Levene's test. Significant differences in data among treatments were analyzed using a two-way analysis of variance (two-way ANOVA); this was followed by the Tukey's HSD test at the 95% confidence level. When only two treatments were compared, a *t*-test was performed. The IBM SPSS 19 software package was used for the analyses.

## 3. Results and discussion

### 3.1. Success of inoculation

We compared the abundance of the inoculated bacteria (*Azotobacter* and *Bacillus subtilis* strain) and cyanobacteria (*Nostoc* and *Oscillatoria*) in different treatments in the pre- and post-experimental soil (Table 1). We found that the inoculated populations represented a considerable increase ( $8\text{--}11 \times 10^3$ -fold) over the initial levels at the beginning of our experiment period. Similarly, Wang et al. (2009) showed that the population of inoculated biocrust micro-organisms was increased up to 48% after one year. We also found that the number of cyanobacteria was greater than that of bacteria after 60 days, despite applying equal amounts of organisms. Furthermore, the microbial population was greatly increased by inoculation with bacteria and cyanobacteria inoculation rather than by the addition of nutrients. Microbial population increase in the nutrient alone treatment was from 2.2 to 12-folds after 60 days. We also observed substantial increases for the microbial population after the inoculation of N-and C-fixing bacteria and cyanobacteria because of their self-feeding ability. However, the addition of nutrients had a temporary effect on increasing the soil microbial

**Table 1**

Mean values ( $\pm$  standard deviation) of the bacteria and cyanobacteria population (cfu) in the initial and end samples of the experiment treatments.

| Treatments <sup>a</sup> | Bacteria (cfu)                        |                                       | Cyanobacteria (cfu)                   |                                       | Total bacteria and cyanobacteria (cfu) |                                       |  |
|-------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------------------|---------------------------------------|--|
|                         | Initial time                          | End time                              | Initial time                          | Initial time                          | End time                               | Initial time                          |  |
| No Inoc. -B4 (control)  | $7.6 \times 10^4 \pm 4.3 \times 10^3$ | $7.7 \times 10^4 \pm 3.0 \times 10^3$ | $2.6 \times 10^3 \pm 3.8 \times 10^2$ | $2.7 \times 10^3 \pm 3.0 \times 10^2$ | $7.9 \times 10^4 \pm 4.6 \times 10^3$  | $8.0 \times 10^4 \pm 2.8 \times 10^3$ |  |
| +B4                     | $7.0 \times 10^4 \pm 7.0 \times 10^3$ | $9.1 \times 10^5 \pm 5.8 \times 10^5$ | $2.6 \times 10^3 \pm 3.6 \times 10^2$ | $8.3 \times 10^3 \pm 2.9 \times 10^3$ | $7.2 \times 10^4 \pm 7.2 \times 10^3$  | $9.2 \times 10^5 \pm 5.7 \times 10^5$ |  |
| Ba -B4                  | $7.3 \times 10^4 \pm 9.0 \times 10^3$ | $8.2 \times 10^7 \pm 1.6 \times 10^7$ | $2.7 \times 10^3 \pm 2.7 \times 10^2$ | $2.8 \times 10^3 \pm 3.5 \times 10^2$ | $7.5 \times 10^4 \pm 8.7 \times 10^3$  | $8.3 \times 10^7 \pm 1.6 \times 10^7$ |  |
| +B4                     | $7.2 \times 10^4 \pm 8.2 \times 10^3$ | $2.2 \times 10^7 \pm 1.4 \times 10^7$ | $2.5 \times 10^3 \pm 2.2 \times 10^2$ | $6.1 \times 10^3 \pm 1.8 \times 10^3$ | $7.4 \times 10^4 \pm 8.2 \times 10^3$  | $2.2 \times 10^7 \pm 1.4 \times 10^7$ |  |
| Cy -B4                  | $7.4 \times 10^4 \pm 6.1 \times 10^3$ | $5.5 \times 10^5 \pm 9.1 \times 10^4$ | $2.6 \times 10^3 \pm 3.0 \times 10^2$ | $6.2 \times 10^8 \pm 1.3 \times 10^8$ | $7.7 \times 10^4 \pm 5.9 \times 10^3$  | $6.2 \times 10^8 \pm 1.3 \times 10^8$ |  |
| +B4                     | $7.0 \times 10^4 \pm 7.8 \times 10^3$ | $4.1 \times 10^6 \pm 1.3 \times 10^6$ | $2.6 \times 10^3 \pm 3.0 \times 10^2$ | $7.5 \times 10^6 \pm 3.5 \times 10^6$ | $7.2 \times 10^4 \pm 8.0 \times 10^3$  | $1.1 \times 10^7 \pm 2.2 \times 10^6$ |  |
| Ba + Cy -B4             | $6.9 \times 10^4 \pm 6.1 \times 10^3$ | $5.7 \times 10^6 \pm 2.5 \times 10^6$ | $2.7 \times 10^3 \pm 3.0 \times 10^2$ | $1.6 \times 10^8 \pm 8.4 \times 10^7$ | $7.2 \times 10^4 \pm 6.0 \times 10^3$  | $1.6 \times 10^8 \pm 8.4 \times 10^7$ |  |
| +B4                     | $7.2 \times 10^4 \pm 5.0 \times 10^3$ | $7.5 \times 10^7 \pm 1.4 \times 10^7$ | $2.6 \times 10^3 \pm 1.8 \times 10^2$ | $1.1 \times 10^7 \pm 2.7 \times 10^6$ | $7.5 \times 10^4 \pm 5.2 \times 10^3$  | $8.6 \times 10^7 \pm 1.7 \times 10^7$ |  |

<sup>a</sup> No Inoc. (Control; no inoculation); Ba (bacterial inoculation); Cy (cyanobacteria inoculation); Ba + Cy (co-inoculation of bacteria and cyanobacteria); and -B4 and +B4 is stimulated treatments without and with B4 nutrient adding, respectively.

**Table 2**

Results of the two-way ANOVA comparing soil organic C (g C kg soil), sequestered C ( $\text{g m}^{-2}$ ) and potential atmospheric  $\text{CO}_2$  removal ( $\text{g m}^{-2}$ ) due to the various treatments. (As sequestered C and potential atmospheric  $\text{CO}_2$  removal are product of soil organic C x a constant, the F- and P-values are the same).

| Variable                                                                   | Time frame   | Factor                 | F-value | P-value <sup>a</sup> |
|----------------------------------------------------------------------------|--------------|------------------------|---------|----------------------|
| organic C (g C kg soil)                                                    | Initial time | Inoculation            | 1.297   | 0.310 <sup>ns</sup>  |
|                                                                            |              | Nutrient               | 0.022   | 0.885 <sup>ns</sup>  |
|                                                                            | End time     | Inoculation × Nutrient | 0.428   | 0.736 <sup>ns</sup>  |
|                                                                            |              | Nutrient               | 16.654  | 0.000 <sup>**</sup>  |
| Sequestered C (g C m <sup>-2</sup> )                                       | Initial time | Inoculation            | 1.939   | 0.180 <sup>ns</sup>  |
|                                                                            |              | Nutrient               | 2.288   | 0.118 <sup>ns</sup>  |
|                                                                            | End time     | Inoculation × Nutrient | 5.809   | 0.007 <sup>**</sup>  |
|                                                                            |              | Nutrient               | 0.126   | 0.727 <sup>ns</sup>  |
| Potential $\text{CO}_2$ removed from the atmosphere (g C m <sup>-2</sup> ) | Initial time | Inoculation            | 11.306  | 0.000 <sup>**</sup>  |
|                                                                            |              | Nutrient               | 5.809   | 0.007 <sup>**</sup>  |
|                                                                            | End time     | Inoculation × Nutrient | 0.126   | 0.727 <sup>ns</sup>  |
|                                                                            |              | Nutrient               | 11.306  | 0.000 <sup>**</sup>  |

<sup>\*\*</sup> Significant differences at the  $p < 0.01$  level, and ns is no significant differences.

<sup>a</sup> At the 95% confidence level.

population.

### 3.2. Soil organic C content

We also observed that the measured organic C at the beginning and end of the experimental period in all treatments varied from 1.64 to 1.92, and from 1.96 to 2.52 g organic C kg<sup>-1</sup> soil, respectively (Table 2). There was, however, no significant difference in the organic C among soil treatments at the beginning of the experiment (before the application of the inoculants; Table 2). We found that the addition of nutrients did not significantly increase the organic C content at the end of the experiment ( $P = 0.18$ ; Table 2), as compared to the inoculation treatments in the absence of the nutrient addition ( $P < 0.01$ ; Table 2). In this sense, our results point to the use of bacteria and cyanobacteria inoculants as an efficient way to increase the C content in the poor and low fertility soils. It is important to note that the amount of the total organic C added by our inoculations was less than  $2.2 \times 10^{-10} \text{ g m}^2$ , based on the calculations reported in Mahlman et al. (2008) and Valencia-González et al. (2014, 2015); thus, it did not substantially affect the soil C. Rather, the increase in the soil C was mostly due to the growth of soil microbial populations. The higher content of C in the microbial-covered bare soils has already been reported by Nisha et al. (2007). The role of bacteria and cyanobacteria in storing C has been discussed previously (Kheirfam et al., 2017a).

### 3.3. Soil organic C sequestration

The sequestered C in the treatments was calculated from the

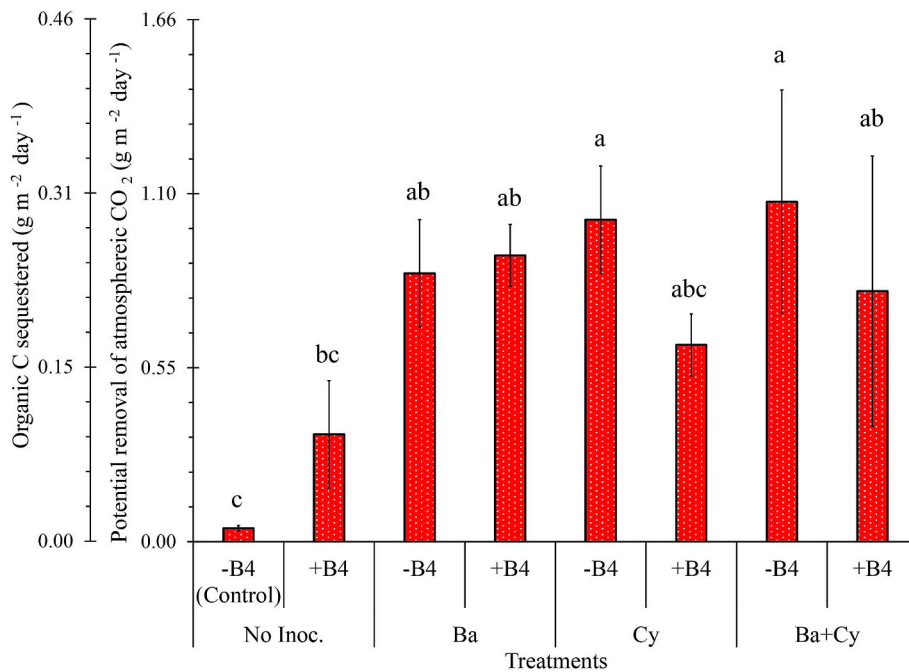


**Fig. 4.** Mean and standard deviation of organic C sequestered and potential CO<sub>2</sub> removed from the atmosphere in the different treatments (an example for C sequestration calculation using Eq. (1): sequestered C in initial time for control treatment:  $1.92 \text{ (organic C, g kg}^{-1}) \times 1.16 \text{ (soil bulk density, Mg m}^{-3}) \times 0.02 \text{ (depth of soil sampling, m)} = 44.544 \text{ g m}^{-2}$ . The organic C in end time for control treatment:  $1.94 \times 1.16 \times 0.02 = 45.008 \text{ g m}^{-2}$ . C pools in control treatment:  $45.008 \text{ (sequestered C in end time)} - 44.544 \text{ (sequestered C in initial time)} = 0.232 \text{ C g m}^{-2}$  (during 60 days). Eventually, the potential atmospheric CO<sub>2</sub> removal for control treatment using Eq. (2):  $0.464 \text{ (g m}^{-2}) \times 3.67 = 1.702 \text{ g CO}_2 \text{ m}^{-2}$  (during 60 days)). No Inoc. (Control; no inoculation); Ba (bacterial inoculation); Cy (cyanobacteria inoculation); Ba + Cy (Co inoculation of bacteria and cyanobacteria); and -B4 and +B4 is stimulated treatments without and with B4 nutrient adding, respectively. Bars with the same letter show non-significant differences (Tukey,  $P > 0.05$ ), otherwise they show significant difference (Tukey,  $P < 0.05$ ).

measured organic C in the samples, using Eq. (1). The two-way ANOVA results (Table 2) indicated that the sequestered C in the inoculation treatments ( $P < 0.01$ ; Table 2), as well as the interaction of microbial inoculation and nutrient addition ( $P < 0.01$ ; Table 2), exhibited significant differences, while no significant changes were observed in the nutrient treatments ( $P = 0.727$ ; Table 2). According to our calculations, except for the control (without any microbial inoculation or nutrients) and cyanobacteria + nutrient treatments, all treatments showed significant increases in the sequestered C by the end of the experiment (Fig. 4). In the “bacteria alone” and “bacteria + nutrient” treatments, C sequestration was ~19 and 20-folds, respectively, higher than that of the control soils (Fig. 4). In this regard, Rossi et al. (2015) found soil chemosynthetic bacteria were capable of fixing CO<sub>2</sub>, thus contributing to the soil organic matter in the form of C<sub>x</sub>-H<sub>y</sub>-O<sub>z</sub> with the help of photon energy. Also, Naik et al. (2010) showed that both autotrophic and heterotrophic bacteria could increase the soil C using complex electrochemical and biological processes. Thus, something similar may have occurred in this experiment if such bacteria were present.

Inoculation of cyanobacteria alone increased the C sequestration by 23-folds, as compared to the control soil (Fig. 4). Cyanobacteria are photosynthetic prokaryotes that can fix atmospheric CO<sub>2</sub>; thus, an increase in the soil C was expected with this treatment (Mager and Thomas, 2011). As water, light, oxygen and CO<sub>2</sub> are the main limiting factors for the micro-organisms activity, this increase in water availability and light, oxygen and CO<sub>2</sub> penetration can enhance the microbial activity and C retention (Mager and Thomas, 2011). Cyanobacterial exopolysaccharides are also a notable source of the organic C (Rossi and De Philippis, 2015). Kidron et al. (2015) also showed that the concentration of the organic C in the high cyanobacterial-biomass biocrusts ( $29.2 \text{ g C kg}^{-1} \text{ soil}$ ) was 2.2-folds lower than that of cyanobacterial-biomass biocrusts ( $9.3 \text{ g C kg}^{-1} \text{ soil}$ ).

The results obtained from our box trials indicated that with inoculating soils, the concentrations of bacteria, bacteria + nutrient, cyanobacteria, bacteria + cyanobacteria, and bacteria + cyanobacteria + nutrient increased the sequestered C in the study soil from 0.042 to 0.84, 0.90, 1.01, 1.07, 0.79  $\text{ton ha}^{-1} \text{ year}^{-1}$  (19, 20, 23, 24 and 17-folds), respectively; however, these values were not statistically different. Costa et al. (2000) reported that cyanobacteria could have a 50-fold higher efficiency in comparison to plants for contributing C to the soils, given their faster growth.

Based on our results, we made an estimation of the potential CO<sub>2</sub> removed from the atmosphere by the inoculation of bacteria and cyanobacteria and the addition of nutrients. Accordingly, we calculated the potential CO<sub>2</sub> removal for those treatments that significantly increased the soil organic C (bacteria, bacteria + nutrient, cyanobacteria, bacteria + cyanobacteria, and bacteria + cyanobacteria + nutrient). Our results showed that the inoculation of biocrust microbes had the potential to remove  $0.85\text{--}1.07 \text{ g CO}_2 \text{ m}^{-2}$  from the atmosphere during our experiment time (Fig. 4). The results obtained from up-scaling also indicated the annual capacity of the soil to absorb the atmospheric CO<sub>2</sub>, increasing from  $3.11$  to  $3.93 \text{ tons CO}_2 \text{ ha}^{-1} \text{ year}^{-1}$ , depending on the treatment applied. Similarly, Rossi et al. (2015) concluded that 100 tons of the algal cell dry weight could fix uptake ~183 tons of CO<sub>2</sub>, thereby providing an excellent way to reduce the atmospheric CO<sub>2</sub>. However, we cannot conclusively say that the CO<sub>2</sub> removal from the atmosphere at an annual scale and/or other time periods has a constant rate. Also, the effluxes of CO<sub>2</sub> from soils into the external atmosphere should be considered to assess the total carbon dioxide balances between the atmosphere and soil (Faimon and Lang, 2018).

#### 4. Conclusion

Our experiment showed that artificially enriching biocrust bacteria and cyanobacteria is a potential way to enhance the ability of the degraded soils to absorb and store the organic C, thus aiding the draw-down of the atmospheric CO<sub>2</sub>. Furthermore, we found that the nutrient addition had little effect on the microbial growth or the increase of the soil potential to capture C. Dryland regions cover almost 40% of the Earth's terrestrial surface; as rising levels of atmospheric CO<sub>2</sub> are a serious concern to human societies, restoring populations of these naturally-occurring micro-organisms could be an appropriate and effective strategy in the near future to decrease GHGs in the atmosphere.

However, despite the optimistic results obtained by this and other studies, it should be noted that C sequestration is a complex process, and the long-term retention of these short-term additions may prove to be a huge logistical issue, requiring further study. For example, work is urgently needed to understand and control long-term C gains and losses under diverse settings; also, we should find how biocrust inoculation influences C sequestration at expanded spatial and temporal scales; we should find the factors that influence the stability of the sequestered C.

More studies are also needed to identify the soil microbes that are the most efficient at sequestering C and also, can survive the extreme conditions found in the drylands. Finally, research is needed on how to collect, cultivate, and deploy large amounts of soil microbes in various dryland regions.

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