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Controlling Loess Erosion by Bio-Stimulated Microbial Induced Calcite Precipitation

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ABSTRACT

Wind-induced soil erosion is one of the main factors contributing to desertification in drylands, resulting in reduced biological diversity, loss of cultivated land, environmental deterioration, and disruption of human activities. Here we present a study focusing on the mitigation of aeolian erosion of loess soil from the Negev Desert (Israel) using Microbial-induced calcite precipitation (MICP). Changes in the treated soil's chemical, physical, and microbial community were characterized by bio-stimulation of native, urea-hydrolyzing soil bacteria. Erodibility experiments were performed on loess sprayed with treatment media with varying concentrations of urea and $CaCl_2$, with a constant 1:1 ratio. The effectiveness of treatments was studied in a wind tunnel and by image analysis of desiccation cracks. The experiments revealed a complicated reality with urease activity, community composition, and calcium carbonate precipitation depending on the concentration of the treatment medium. Specifically, we show a twofold reduction in the area (from 21% to 8%), and the length (from 363 cm to 206 cm) of the desiccation cracks can be achieved by spraying the soil with 0.5M concentration media, resulting in effective MICP or a high concentration treatment (> 1M) without achieving MICP. Our results show that erosion mitigation in loess soil depends on an interplay between biotic and abiotic processes based on the treatment media concentration.

Keywords: Erosion control, Bio-Stimulation, MICP, Desertification.

1   INTRODUCTION

The ubiquity of bacteria and their diverse roles in natural environments have led to growing interest in harnessing bacterial activities for various anthropogenic purposes. From a physical point of view, the soil is regarded as an inorganic multiphase system comprising solids, fluids, and gases. However, the soil is also a living system, being one of the largest terrestrial carbon pools, constituting about 33% of the total terrestrial carbon (Lal, 2008). The organic carbon in the top 1 m constitutes more than 50% of the total soil carbon. Prokaryotes comprise up to 17% of the soil's organic carbon (Whitman et al., 1998). Unicellular organisms, primarily bacteria $0.5–5.0 \times 10^{-6}$ m in size, are about three orders of magnitude smaller than the pore throat size of sand and about the D_{10} size of kaolinite. Soil bacteria, either motile or fixed to mineral surfaces, may change their surroundings' chemical and physical properties depending on their metabolism.

Microbial-induced calcite precipitation (MICP) is an emerging technique to mitigate environmental and engineering challenges, including soil erosion, soil liquefaction, fracture sealing, restoration of stone monuments, and others (DeJong et al., 2013). Hydrolysis of urea, catalyzed by the urease enzyme, is considered the most efficient microbial pathway for MICP. Whereas the rate constant for non-biotic urea hydrolysis is 3.2×10^{-21} s^{-1}, the rate enhancement of urease is 1.2×10^{25} (Yao et al., 2013).

Wind-induced soil erosion is one of the main adverse factors contributing to desertification in drylands, resulting in the reduction of biological diversity, loss of cultivated land, and environmental deterioration (Schlesinger et al., 1990; Okin et al., 2001; Ravi et al., 2011; Duniway et al., 2019). It also directly contributes to natural adversities, such as dust storms, which affect everyday activity (Li et al., 2018) and the health of humans (Vodonos et al., 2014; Yitshak-Sade et al., 2015). Ecological processes and anthropogenic activities that aggravate wind erosion and dust emissions have broad-scale implications for the functioning of global drylands and their residents (Duniway et al., 2019).

Biostimulation and bioaugmentation are alternatives to facilitate in situ MICP in soils. Biostimulation encourages indigenous urea-hydrolyzing bacteria by providing appropriate enrichment and precipitation media; it relies on the natural ubiquity of ureolytic soil bacteria and bacterial spatial distribution (Mobley & Hausinger, 1989). Bioaugmentation introduces large volumes of bacterial cultures into the treated soil along with a growth and precipitation medium; therefore, it requires large volumes of pure-cultured specializing ureolytic bacteria, e.g., *Sporosarcina pasteurii*. Producing and transporting large volumes of these cultures is an expensive and delicate procedure; their injection and homogeneous distribution throughout the treated site are difficult to achieve and might encounter regulatory hindrances (DeJong et al., 2009). Moreover, the introduced bacteria are likely to decline in numbers due to low compatibility with the environment as well as competition and predation by indigenous bacteria (van Veen et al., 1997).

MICP soil improvement was successfully demonstrated at various scales (Whiffin et al., 2007; Meyer et al., 2011; L. van Paassen, 2011; Martinez et al., 2013). However, reliance on bioaugmentation has restricted the technology from becoming a cost-competitive alternative to traditional ground amelioration techniques. Despite the frequent use of biostimulation in bioremediation, the use of the biostimulation technique for enabling MICP is more limited (Burbank et al., 2013; McMillan et al., 2013; Gat et al., 2016; Gomez et al., 2018). Biostimulation in desert soils is more challenging than in most soils, as the total carbon content is low (Drahorad et al., 2013), and the bacterial population is considerably smaller. For example, in coastal sands from southern Israel, the in situ bacterial population was 10^4 cells/g (Gat et al., 2016), three to four orders of magnitude lower than in semi-arid soils (e.g., Ros et al., 2006). For this reason, MICP in low-carbon soils was typically attempted via bio-augmentation (e.g., L. A. van Paassen et al., 2010; Chen et al., 2016).

In this paper, we present the results of a study focusing on the mitigation of aeolian erosion of loess soil from the Negev Desert (Israel) using Microbial-induced calcite precipitation (MICP). To our best knowledge, this is the first research into bio-stimulated MICP in loess soil in the region. We tracked the changes in the treated soil's chemical, and the physical and microbial community were characterized following bio-stimulation of native, urea-hydrolyzing soil bacteria.

2 MATERIALS AND METHODS

2.1 Loess soil

The soil for the research was sampled from a depth of 50 cm at a natural, undisturbed outcrop near Omer, Israel. The soil samples were sieved on a 500 μm mesh to remove coarse fragments (rocks, shells, and vegetation) and analyzed for Particle size distribution (PSD) by laser diffraction. Mineralogical phase identification was performed by X-ray powder diffraction (XRPD).
The loess soil is predominantly composed of quartz and calcite, with minor fractions of phyllosilicates, alkali-feldspars, plagioclase, and traces of dolomite, pyroxene, and hornblende (Fig 1a.). The mineralogical composition is typical of the Negev loess (Crouvi et al., 2017). The loess grain size is predominantly silty (with 70% of its content ranging from 2 mm to 62.5 μm), and the rest (30%) is clay-sized (<2 μm) (Fig. 1b).
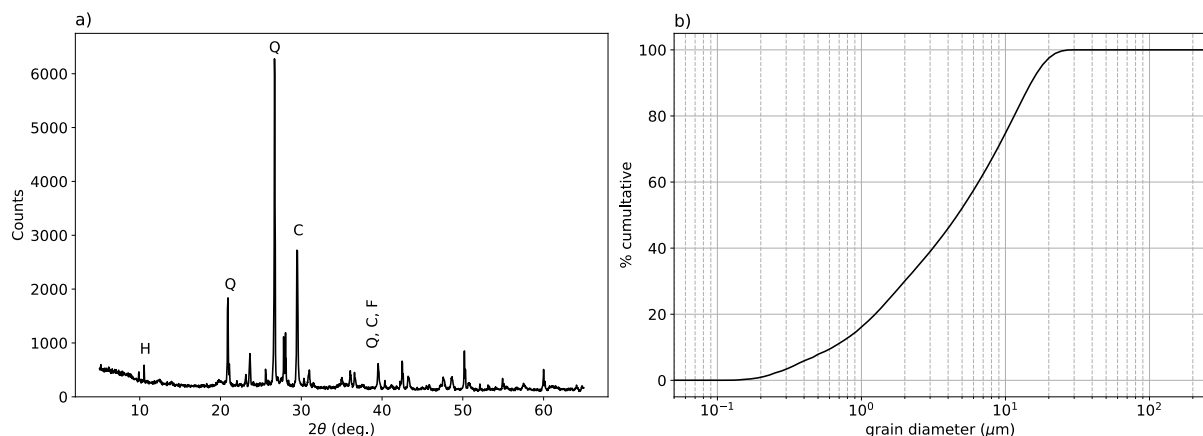

Figure 1. *a) X-ray diffraction (XRD) and (b) Particle size distribution (PSD) of the studied loess soil. Counts in arbitrary units. Q is quartz, H is hornblende, C is calcite and F is feldspar.*

2.2   Spraying experiments

The main goal of the spraying experiments was to study the bio-stimulation of indigenous urea hydrolyzing bacteria by spraying and the wind erosion of sprayed soil. Four large metal trays (50x50 cm^2) with an evenly spaced grid of 38 drains (0.5 cm diameter) were filled with sieved (500 mm) loess soil. The soil was placed dry (water content < 1%) with a bulk density of 1.2 to 1.4 gr/cm^3. Five consecutive spraying doses were performed daily on each tray with 2 liters of spraying medium. Concurrently, 4 duplicated small soil trays (10x10 cm^2) were sprayed with identical spraying media to perform daily sampling and follow-up on urea hydrolysis rates without disturbing the large trays. The ratio between the volume of the spraying medium and the surface area for the small and large trays was similar. Soil weight, composition, and volume of treatment media are presented in Table 1.

Upon completion of spraying, the large soil trays were cured for two weeks in the laboratory. Following curing, the trays were photographed, and the soil desiccation crack patterns were analyzed using the following workflow was applied: (1) image cropping, (2) grey pixel transformation, black and white pixel modifications, (3) differentiation of crack edges as white-over-black background, and (4) crack filling (white) and pixel counting. The cracks area percentage was defined as the ratio of white to black pixels. During the spraying process, urea hydrolysis was tested daily by performing urea concentration and pH measurements on 1 gram of topsoil sampled from the small trays and incubated for 24 in 10 ml of 330 mM urea solution.

To test the efficiency of the spraying treatments on aeolian soil erosion, a wind tunnel experiment was carried out on the above-described soil trays in the Aeolian Simulation Laboratory (ASL), Ben-Gurion University of the Negev (Beer-Sheva, Israel). Dust emission was measured at four different wind speeds, 26 Hz (4 m/s), 32 Hz (6.5 m/s), 38 Hz (8 m/s), and 44 Hz (9.5 m/s). Following the aeolian erosion testing, calcium carbonate content was measured gravimetrically after acid washing, as described by Choi et al. (2017), at four different locations per tray.

Table 1. *Spraying experiments design.*

Sample ID	Soil weight [gr]	Spraying volume [ml/dose]	Liquid medium composition
Large trays			
Low Ur-CaCl$_2$	5,500	2,000	330 mM urea, 250 mM CaCl$_2$, 2 gr/L YE
Medium Ur-CaCl$_2$	4,900	2,000	660 mM urea, 500 mM CaCl$_2$, 2 gr/L YE
High Ur-CaCl$_2$	4,400	2,000	1320 mM urea, 1 M CaCl$_2$, 2 gr/L YE
CTRL	4,400	2,000	DDW
Small soil trays			
Low Ur-CaCl$_2$	150	150	330 mM urea, 250 mM CaCl$_2$, 2 gr/L YE
Medium Ur-CaCl$_2$	150	150	660 mM urea, 250 mM CaCl$_2$, 2 gr/L YE
High Ur-CaCl$_2$	150	150	1320 mM urea, 1 M CaCl$_2$, 2 gr/L YE
CTRL	150	150	DDW

*YE = Yeast Extract

At the completion of the experiment, DNA was extracted in triplicates from the four small soil trays using Qiagen Powersoil Pro kit (Hilden, Germany) and eluted in Tris-EDTA buffer (pH 8.0). Library preparation

and 16S rDNA sequencing was performed by Qiagen Genomic Services. Operational Taxonomic Unit (OTU) assignment and clustering were performed on the CLC Microbial Genomics module on CLC Genomics Work Bench. OTU analysis and plot production were performed using RStudio 2022.02.0.

3 RESULTS

The evolution of urea degradation and pH, sampled from the concurrent small trays, are presented in Fig. 2a and 2b. The most effective urea degradation was achieved by spraying the "Low" concentration media (330 mM urea and 250 mM $CaCl_2$, refer to Table 1), as demonstrated by the rise in pH to 9.3 and total urea depletion after eight days. The two other spraying media were not as efficient in urea degradation. Soil treated with the "Medium" concentration medium exhibited pH values of up to 8.5 and a urea depletion ratio of up to 0.3. "High" concentration medium inhibited urea hydrolysis, as shown by insignificant changes in pH and urea depletion ratio.
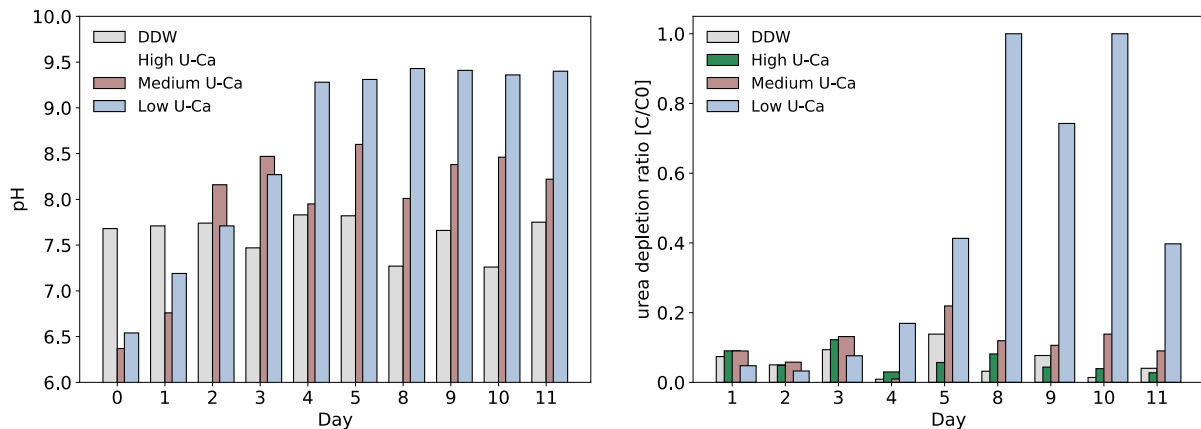

Figure 2. *Bio-stimulation of MICP by spraying in the soil stabilization experiment, small trays: (a) pH, (b) urea depletion ratio.*

Following two weeks of curing, the large trays were tested in a wind tunnel under typical wind speeds in the Negev Desert. None of the treated soils exhibited dust emissions, including under the high wind speed of 9.5 m/s. Following the wind tunnel experiments, the trays were analyzed for desiccation crack formation by quantifying the total area and length of the cracks (Fig. 3). Taking the DDW sprayed tray (CTRL) as a reference, with a crack length of 363 cm and area of 21.5%, all the Ur-$CaCl_2$ treated trays exhibited a change in crack length and area. The "Medium" and "High" treatments showed a similar decrease in both parameters: crack length of 206 and 209 cm, respectively, and crack area of 7.9% and 11%, respectively. The "Low" treatment showed a decrease in crack area to 18.5% but an increase in total crack length to 493 cm. Please note that the wide crack in the tray's upper part resulted from the tray's handling and transportation. Thus, the reported value of the area of the cracks is probably higher than the actual one.

The reference tray exhibited a calcium carbonate content of 21.3 wt.%, a typical value for loess soil of the region. Trays treated with the "Low" and "Medium" concentration media exhibited an increase of 5 and 10 wt.% in calcium carbonate content, to 25.9 and 30.3 wt.%, respectively. The tray treated with the "High" concentration medium showed no change in the calcium carbonate content.
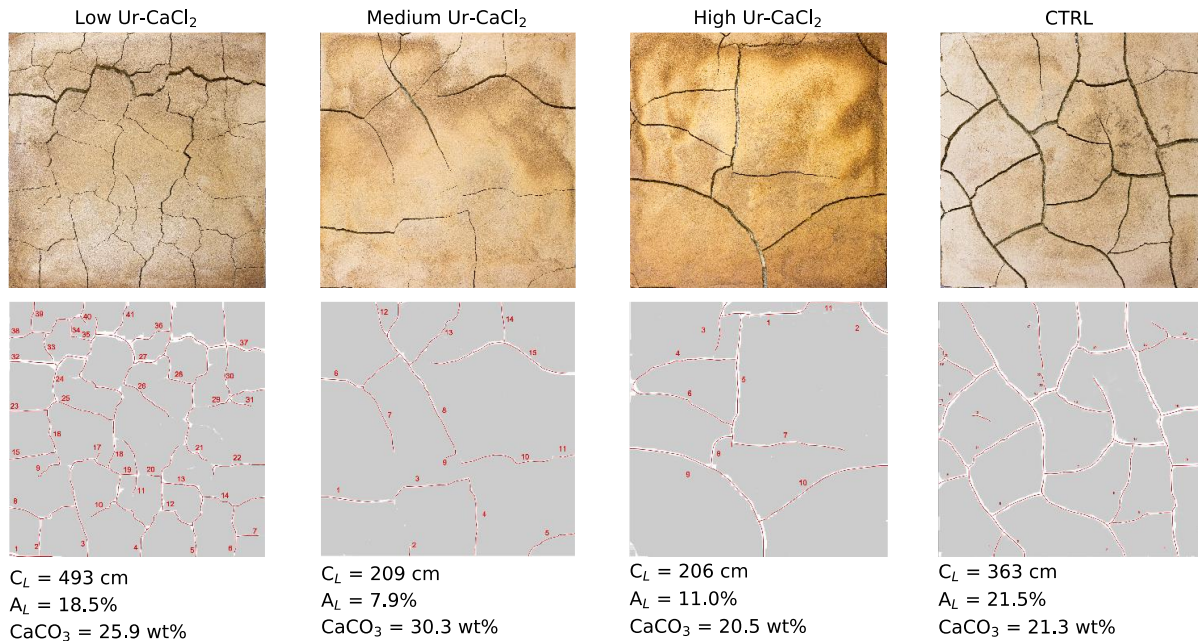
Low Ur-CaCl$_2$	Medium Ur-CaCl$_2$	High Ur-CaCl$_2$	CTRL
C$_L$ = 493 cm	C$_L$ = 209 cm	C$_L$ = 206 cm	C$_L$ = 363 cm
A$_L$ = 18.5%	A$_L$ = 7.9%	A$_L$ = 11.0%	A$_L$ = 21.5%
CaCO$_3$ = 25.9 wt%	CaCO$_3$ = 30.3 wt%	CaCO$_3$ = 20.5 wt%	CaCO$_3$ = 21.3 wt%

Figure 3. *Analysis of desiccation cracks and calcium carbonate content (wt%) in the soil stabilization experiment. C$_L$ is total crack length; A$_L$. is crack area.*

4 MICROBIAL DIVERSITY

The 16S data showed substantial differences in species richness and diversity between the different samples. The control treatment (CTRL) yielded 16,873 bacteria assigned to 990 species (OTUs), with Shannon's entropy of 5.39. Soil treated with the "Low" concentration medium exhibited decreased species richness and diversity, with 11,125 bacteria assigned to 422 species and a lower Shannon's entropy of 4.19. The "Medium" concentration medium resulted in even lower species richness and diversity, with 9,110 bacteria belonging to 119 species and Shannon's entropy of 3.48. The "High" concentration treatment yielded the lowest species diversity among samples, with only 1,943 bacteria belonging to 35 species and Shannon's entropy of 2.89.

Species composition was highly affected by the different stimulation treatments, refer to Fig. 4 While in the control sample, the dominant bacteria phylum was *Proteobacteria* of diverse orders, MICP stimulation resulted in a compositional shift to a community dominated by *Firmicutes* of a single order, Bacillales (57.6% of OTUs in the "Low" treatment and 74.6% in the "Medium" treatment) and *Actinobacteria*, mostly of *Micrococcales* (35.66% of OTUs in the "Low" treatment and 18.66% in the "Medium" treatment).

Applying the "High" treatment yielded some convergence with the control, with *Proteobacteria* being the dominant group with over 50% of the OTUs. This treatment resulted in a modest impact on MICP-related bacteria with enrichment of *Firmicutes* to only 11.27% of the OTUs (with only 0.36% in control). Additionally, specific orders of bacteria were enriched by the "High" treatment only, including *Rubrobacterales* and *Streptomycetales* (phylum *Actinobacteria*), *Sphingobacteriales* (*Bacteroidetes*), and *Obscuribacterales* (*Cyanobacteria*).
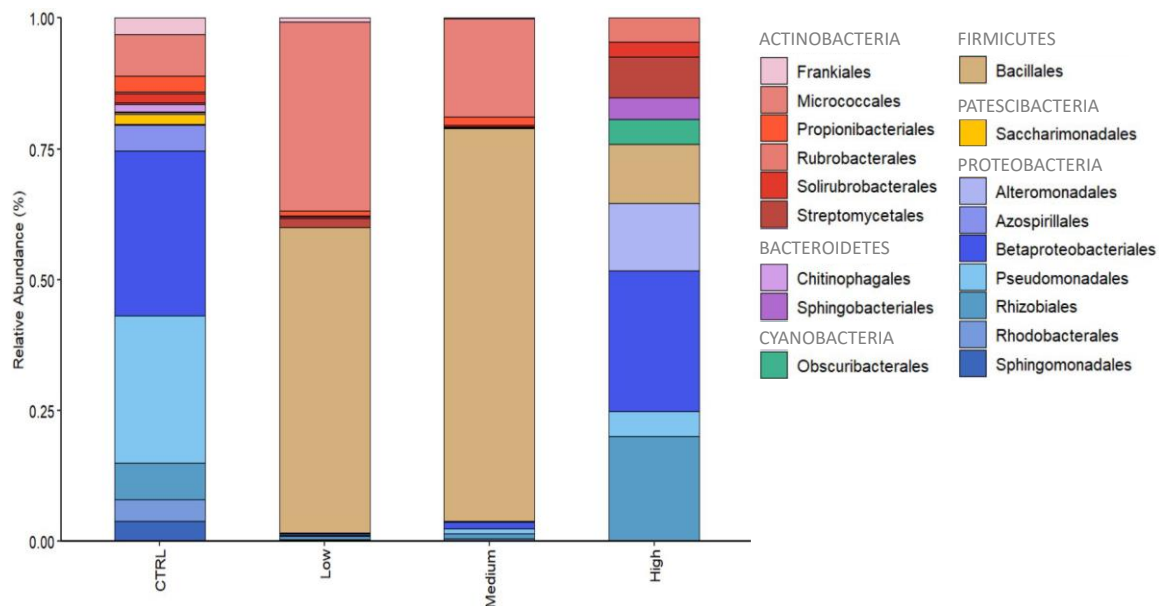
Figure 4. Bacterial community composition in the soil stabilization experiment.

5 DISCUSSION

The main goal of this research was to study the potential of bio-stimulated MICP for mitigating wind erosion in low-carbon, aeolian soils. We decided to use a single solution, for both stimulation and precipitation, for the sake of future operational simplicity. Three different concentrations were examined while keeping the urea to $CaCl_2$ molar ratio constant, close to 1:1. The urea and $CaCl_2$ concentrations in the "Low," "Medium," and "High" concentration media were 333 mM : 250 mM, 666 mM : 500 mM and 1332 mM : 1000 mM, respectively. According to L. A. van Paassen (2009), urease activity depends on urea concentration, the medium's pH, and the salt concentration (as $CaCl_2$). Normalized urease activity is 0.9 in 330 mM urea solution and rises to 0.93 in solutions of 500 mM urea or higher. The urease activity of suspended cells) falls sharply as a function of salts concentration from a normalized activity of = 0.75 for 250 mM Ca^{2+} to = 0.6 for 500 mM $Ca^{2+,}$ and down to 0.2 for 1000 mM Ca^{2+}.

Our results show that the "High" concentration media did not achieve effective bio-stimulation. This result is reflected in all measurables, from low pH, urease activity, and limited enrichment of Bacillales (Firmicutes). Ineffective bio-stimulation resulted in ineffective MICP. Compared to the control spraying, there was no additional calcium carbonate precipitation. The total crack length and area of the "High" treatment tray showed improvement, compared to the DDW tray, 206 cm to 363 cm and 11% to 21.5%, respectively. This treatment can be regarded as an analog to treating the soil with wastewater or brines, a common dust control measure (e.g. Katra, 2019; Raveh-Amit et al., 2022). Recent studies (Stallworth et al., 2021) showed that the efficacy of brines as dust suppressors may not be higher than that of rainwater and that other negative environmental impacts should be considered. The reduced cracking observed in the "High" concentration treated soil is thus attributed to the high $CaCl_2$ concentration.

Lowering the "High" $CaCl_2$ concentration by a factor of two to "Medium" reduced cracking to crack length and area of 209 cm and 7.9%, respectively, and increased the calcium carbonate by 10% wt. The pH was found to rise to a value of 8.6 following five spraying treatments, and normalized urea depletion values of 0.3 were measured (Fig. 4). Coupled with a reduction in biodiversity and a compositional shift towards the Bacillales, almost 75% of the total species, these changes indicate effective MICP occurring in the soil. It should be noted that the relatively high Ca2+ concentration (> 500 mM) inhibits urease activity and fast rise in pH, limiting calcium carbonate precipitation.

Further lowering the $CaCl_2$ concentration by a factor of two to a "Low" concentration medium increases the urease activity, leading to pH values of 9.4 (Fig. 2). The community shifts towards the Bacillales is substantial, but less prominent than in the "Medium" treatment. All the biological and chemical parameters indicate effective MICP; however, the total crack length and area exhibit a different trend. The total length of the cracks is higher than in the control (DDW) treatment, 493 cm and 363 cm, respectively. The area of the cracks shows a small reduction from 21.5% (DDW) to 18.5%. Overall, the "Low" treatment exhibits crack lengths and areas larger by a factor of two than the "Medium" and the

"High", treatments. It is clearly visible (Figure 3) that the cracking pattern is different. Desiccation cracks modify the hydrologic conditions within the soil, reduce water retention capabilities and increase the weathering of soils. The combined effect of the above is the aggravation of soil erosion and adverse ecological environmental consequences (Zeng et al., 2020).

Recently, MICP was applied to improve soil strength and resistance of clayey soil. Due to the low permeability of the soil used, laboratory preparation of soil samples required pre-mixing (Vail et al., 2020) or surface spraying (Liu et al., 2020). MICP was found to be effective in increasing soil desiccation cracking resistance and reducing erodibility. Whereas our study focused on bio-stimulation of native soil, in these studies, MICP was achieved using the bio-augmentation approach based on a model bacterium (*S. pasteurii*) introduced into the soil. Bio-augmentation introduces large volumes of bacterial cultures into the treated soil, along with growth and precipitation medium, and therefore requires very large volumes of pure-cultured ureolytic bacteria. Producing and transporting large volumes of these cultures is an expensive and complex procedure; their injection and homogeneous distribution throughout the treated site are difficult to achieve and might encounter regulatory hindrances (DeJong et al., 2009). Moreover, the introduced bacteria are likely to decline in numbers due to low compatibility with the environment and competition and predation by indigenous bacteria (van Veen et al., 1997).

6   CONCLUSIONS

In this research, we studied wind erosion control of loess soil (Negev Desert, Israel) using biostimulated Microbial-induced calcite precipitation (MICP).

Erodibility experiments were performed on large (50 cm x 50 cm) trays filled with sieved loess soil sprayed with stimulation-cementation media with varying concentrations of urea and $CaCl_2$, with a constant 1:1 ratio.

The effectiveness of treatments was studied in a wind tunnel and by image analysis of desiccation cracks. The experiments revealed a complicated reality with urease activity, community composition, and calcium carbonate precipitation depending on the concentration of the treatment medium.

A twofold reduction in the area (from 21% to 8%) and length (from 363 cm to 206 cm) of the desiccation cracks was achieved by spraying the soil with 0.5M concentration media using, resulting in effective MICP or a high-concentration treatment (> 1M) without achieving MICP.

The results show that MICP effectiveness in loess soil depends on an interplay between biotic and abiotic processes based on the treatment media concentration.

7   ACKNOWLEDGEMENTS

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