1   **Differential responses of soil microbiomes to ureolytic biostimulation across**

2   **depths in Aridisols**

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25   **Abstract.** Soil microbiomes are key regulators of biogeochemical cycles and possess essential roles in

26   ecosystem functions, particularly in arid environments. One beneficial function of various edaphic microbes

27   is the ability to participate in Microbial Induced Calcite Precipitation (MICP). MICP is a biomineralization

28   process extensively investigated as a soil improvement technique for various purposes, including mitigation

29   of drought-related soil degradation and erosion control. One aspect rarely addressed in MICP studies is the

30   microbial heterogeneity of the ecosystem in which it is applied and its post-treatment consequences. In this

31   study, we examined MICP biostimulation rates in an Aridisol, considering the microbial heterogeneity across

32   different soil depths that are relevant to surface reinforcement applications (from the topsoil to 1 meter below

33   the surface). Biostimulation was achieved by inducing ureolysis, one of the most studied metabolic pathways

34   to stimulate MICP. We characterized the native microbial communities and their response to biostimulation

35   across the depths under consideration using 16S sequencing. We found that ureolysis rates were affected by

36   soil depth, with higher rates detected at the topsoil. Before biostimulation, the native soils were dominated

37   by Actinobacteria and contained diverse communities. The microbial communities of the deeper soil layers

38   were richer in Firmicutes, and the deepest layer was less diverse than the topsoil. Following biostimulation,

39   alpha-diversity and microbial richness were drastically reduced at all depths, resulting in homogenized

40   communities dominated by Firmicutes, although microbial DNA concentrations increased. A notable

41   decrease was detected in autotrophs (e.g., Cyanobacteria, Chloroflexi), which are important for the formation

42   and function of biocrusts and, hence, to the entire ecosystem. We also found that biostimulation induced a

43   shift in the composition of the Firmicutes, where specific members of the Planococcaceae family became the

44   most prevalent Firmicutes, instead of Paenibacillaceae and Bacillaceae, following stimulation. Our findings

45   demonstrate that environmental heterogeneity across soil depth is an influential variable affecting ureolytic

46   biostimulation. In turn, biostimulation affects microbial diversity consistently, regardless of preexisting

47   differences resulting from spatial heterogeneity. Our findings show that although feasible, implementing

48   biostimulated MICP in arid environments induces a strong selective pressure with negative consequences for

49   the native edaphic microbiomes.

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51   **1. Introduction**

52   Soil microorganisms, and particularly soil bacteria, are a major component of Earth's biodiversity (Delgado-

53   Baquerizo et al., 2018; Sokol et al., 2022). They are key regulators of biogeochemical cycles (Chen et al., 2017;

54   Falkowski et al., 2008; Lal, 2008) with unquantifiable contributions to central ecological functions, including

55   nutrient and carbon cycling, water retention and primary production, among many others (Castillo-Monroy

56   et al., 2010; Delgado-Baquerizo et al., 2014, 2018; Maestre et al., 2013). In arid regions, where plant cover is

57   sparse, biocrusts – a diverse community in the soil surface – create a 'living skin' that mediates most inputs,

58   transfers, and losses across the soil surface and stabilizes the soil (Weber et al., 2022). Prokaryotes, and

59   notably cyanobacteria, constitute a key group in these arid biocrusts (Belnap and Lange, 2003).

60   Landscape degradation due to anthropogenic overexploitation, including accelerated soil erosion, may

61   entangle adverse implications for biodiversity, essential ecosystem services and human well-being (Delgado-

62   Baquerizo et al., 2014; Maestre et al., 2013; Rodriguez-Caballero et al., 2022). Stabilization of the soil surface

63   is often used to mitigate soil erosion (Zuazo and Pleguezuelo, 2009). Microbial Induced Calcite Precipitation

64   (MICP) is a biomineralization process that is intensively studied as a possibly environmentally conscious

65   ground improvement technique (Dejong et al., 2013; Gomez et al., 2015; Lee et al., 2019) in relation to a wide

66   variety of environmental and engineering applications (DeJong et al., 2022). MICP can be used to strengthen

67   the topsoil for a variety of purposes, including mitigation of draught related desiccation cracking (Liu et al.,

68   2024), slope stabilization (Ghasemi et al., 2022) and reduction of hazardous dust emissions from mines (Fan

69   et al., 2020).

70   Various microorganisms are capable of precipitating calcite through several metabolic pathways (Castanier

71   et al., 1999; Castro-Alonso et al., 2019). Urea hydrolysis by the enzyme urease is one of the most studied

72   pathways to induce MICP due to its high efficiency (De Muynck et al., 2010). Ureolytic MICP is usually

73   achieved through one of two approaches: the biostimulation MICP approach harnesses the ability of many

74   indigenous soil microorganisms to degrade urea. In contrast, the bioaugmentation approach relies on adding

75   exogenous microbial biomass, most commonly cultures of the highly ureolytic Firmicute *Sporosarcina*

76   *pasteurii* (Graddy et al., 2021; Whitaker et al., 2018). Both approaches require inputs of urea and an organic

77   carbon source to efficiently stimulate urea hydrolysis (Gat et al., 2014, 2016; Graddy et al., 2021).

78   Recent studies have established the feasibility of applying MICP in Aridisols by stimulating indigenous

79   microorganisms (Raveh-Amit and Tsesarsky, 2020; Raveh-Amit et al., 2024). The treatment resulted in

80   considerable reinforcement of the soil surface, as manifested by a decrease in desiccation cracking and

81   increased calcite contents. However, there is a scarcity of knowledge regarding the effectiveness of

82   biostimulation using different microbiomes. Spatial heterogeneity, and vertical heterogeneity in particular,

83   may result in varying effectiveness of MICP, since soil depth is one of the important drivers of microbial

84   abundance and community structure (Fierer et al., 2003). Bacterial abundance tends to concentrate at the

85   soil surface and decrease with increasing depth (Eilers et al., 2012; Fierer et al., 2003; He et al., 2022), while

86   archaea become more abundant in deeper soil horizons (Jiao et al., 2018; Sokol et al., 2022). Both groups

87   exhibit depth-related variation in taxonomic compositions (Eilers et al., 2012; Jiao et al., 2018), which might

88   translate to variable ureolytic and precipitation capacities. Biostimulation of varying intensity was reported

89   in soil excavated from large depths relevant to geotechnical applications (2 to 12 meters below the surface;

90   Gomez et al., 2018). Since the microbial cell density was not correlated with the intensity of the ureolytic

91   response, the authors postulated that microbiome heterogeneity underlies these findings, which was not

92   further examined.

93   MICP is commonly considered as an environment friendly alternative to conventional ground improvement

94   methods (e.g., grouting materials). However, the actual impacts of MICP implementation on the ecosystem

95   and biodiversity are not widely addressed. As Graddy and colleagues importantly emphasized (2021), MICP

96   studies often under-characterize the microbial community on which the experimental system is based, which

97   leads to missing crucial information required for successful implementation. The few studies that did address

98   the environmental consequences of MICP application reported profound alterations in the composition of

99   the native microbial communities, releases of substantial amounts of ammonium and changes in the pH of

100   the treated medium (Gat et al., 2016; Gomez et al., 2019; Graddy et al., 2021; Lee et al., 2019; Ohan et al., 2020).

101   Considering their roles in essential processes, such drastic alterations of the edaphic microbial diversity may

102   result in negative consequences for the ecosystem functions and services (Bahram et al., 2018; Philippot et

103   al., 2023). Nevertheless, the application of MICP is rapidly expanding to field-scale applications without

104   considering the complexity of natural microbiomes or environmental effects.

105   In this study, we aimed to investigate ureolytic biostimulation in Aridisol from the Negev Desert (Israel), where

106   knowledge regarding the microbiology of MICP is particularly lacking. Specifically, the goals of this study

107   were to: i) characterize the native microbial community of the study site in depths that are relevant to

108   reinforcement of the top 1 meter of soil; ii) establish the efficiency of ureolytic biostimulation using native

109   microbiomes and iii) study the effects of the treatment on microbial diversity. Considering the possible

110   environmental outcomes of MICP application and the vulnerability of desert ecosystems to disturbances, the

111   study was conducted in a controlled laboratory environment, using samples of native soils. Focusing on

112   vertical variability, we used 16S sequencing to study the native prokaryotic communities when subjected to

113   ureolytic biostimulation. We studied the course and efficiency of ureolysis in treated soils and monitored

114   their pH during biostimulation. Then, we investigated possible relationships between the observed patterns

115   in ureolysis and microbial diversity, and suggested potential mechanisms that underlie these relationships.

116   **2. Materials and methods**

117   **2.1 Soil sampling and chemical-physical characterization**

118 We sampled soils from three sites in the Rotem Plateau (31.03◦N, 35.09◦E), northern Negev Desert, Israel. It is

119 an arid region with an average annual rainfall of 70 mm, covered with low organic carbon (< 0.1 %) Aridisol.

120 The study sites included two non-disturbed sites (referred here as site 1 and site 2), located 4.1 km apart. To

121 examine the impact of mechanical disturbance on the ureolytic response, biostimulation experiments were

122 performed on soils from a third, disturbed site (located 3 km away), that was subjected to mechanical

123 disturbance approximately 20 before this study. Within each site, soil was sampled from three depths: surface

124 (topsoil), 50 cm below the surface, and 100 cm below the surface. These depths were sampled in duplicates

125 within each site, with approximately 10 m separating between replicates (overall, n = 12 samples representing

126 native Negev soil). The microbial communities of the soils of sites 1 and 2 were chosen for characterization

127 using 16S DNA sequencing, as described in subsection 2.3. All samples were stored refrigerated at 4◦C until

128 the biostimulation experiments began.

129 Elemental composition by X-ray fluorescence (XRF), mineralogical phase identification by X-ray diffraction

130 (XRD), and particle size distribution (PSD) analyses were performed on the soil samples as described by

131 Raveh-Amit and Tsesarsky (2020), which classified the soils as medium to coarse sand with an average calcium

132 content of 27 ± 18 (standard deviation) percentage by weight.

2.2 Biostimulation of indigenous ureolytic microbes and chemical analysis

134 We aimed to study the response of edaphic microbiomes to the chemical solution composition that is

135 typically used in MICP experiments at various scales (Gomez et al., 2018; Ghasemi and Montoya, 2022;

136 Ghasemi et al., 2022). Therefore, biostimulation was performed by incubating 10.0 g of each soil sample in 100

137 mL of a medium containing 20 g/L (330 mM) urea and 1 g/L of yeast extract at ambient temperature with

138 gentle shaking at 100 rpm for 10 days (n = 12 biostimulated samples). The medium solution was filter-sterilized

139 by disposable 0.22 μm Millex® syringe filters (Kenilworth, NJ) before the addition of yeast extract. To monitor

140 biostimulation during the course of the experiment, we sampled the stimulation medium of each sample

141 periodically and measured their pH and urea concentration. pH was measured using a Metrohm pH meter

142 (Metrohm, Herisau, Switzerland). Urea concentrations were measured according to the Knorst colorimetric

143 method (Knorst et al., 1997), with minor modifications, on an 8453 Agilent spectrophotometer (Agilent, Santa

144 Clara, CA, USA).

2.3 Soil microbial DNA extraction and 16S amplicon sequencing

146 To examine the response of the microbial communities of different soil depths to ureolytic stimulation, we

147 extracted DNA from soil sampled from specific depths in sites 1 and 2, before and after the biostimulation

148 experiment. DNA samples were extracted in triplicates using the Powersoil Pro kit (Qiagen, Hilden, Germany).

149 DNA extracts were eluted in Tris-EDTA buffer (pH 8.0). The extracted DNA samples were stored refrigerated.

150 DNA concentrations were quantified by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham,

151 MA).

152 Library preparation and 16S amplicon sequencing were performed by Qiagen Genomic Services (Hilden,

153 Germany). Libraries wereprepared using QIAseq 16S Region Panels. Library QC and quantification were

154 carried out using Agilent TapeStation or Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA),

155 depending on sample number, and by QIAseq Library Quant Array, respectively. Amplicons were sequenced

156 on an Illumina MiSeq platform using reagent kits v3 (extended paired-end reads of up to 2 x 300 bp).

157 Operational Taxonomic Unit (OTU) assignment and clustering were carried out on the CLC Microbial

158 Genomics module on the CLC Genomics Work Bench, setting the similarity percentage parameter to 97%.

159 The reference database used was SILVA 16S v132 97%. Raw sequences were uploaded to the NCBI Sequence

160 Read Archive, and are available under bioproject ID PRJNA1041873.

161 **2.4 Sequence data and statistical analysis**

162 OTU and statistical analyses were performed using RStudio v2023.03.0 (R Core Team, 2008) on the v4v5

163 region. We used ANOVA and pairwise (Bonferroni corrected) tests to compare the amount of DNA extracted

164 from the different depths and the biostimulated and non-treated soils, as well as to compare OTU diversity

165 (using Shannon's index and Chao1 richness) of the prokaryotic communities, beta diversity and prevalence of

166 specific taxa. The data was log-transformed when the assumption of equal variances was not met, and

167 Kruskal-Wallis test was applied when the data violated the assumption of normal distribution. We used

168 Principal Component Analysis (PCA) to illustrate the distances between the communities of the compared

169 soil samples based on Bray-Curtis distances calculated for relative abundances of the OTUs. An Analysis of

170 Similarities (ANOSIM) test was then performed to determine the significance of differences between the

171 taxonomic structures of compared communities. Additionally, a Similarity Percentage analysis (SIMPER) was

172 used to assess which identified OTUs contributed most to the variance between samples. Values along the

173 results section represent means and presented errors are standard deviations.

174   We plotted the measured urea concentrations and change in pH during the biostimulation process to create

175   reaction profiles for each sampling depth. The significance of the change in these variables during the

176   experiment was examined using repeated-measures ANOVA.

3. Results and discussion

178   This study aimed to examine the response of native microbial communities to ureolytic biostimulation in arid

179   soils, considering the complexity of the ecosystem, as manifested by vertical heterogeneity. We present the

180   results in relation to two aspects: i) ureolytic activity profiles in biostimulated soils and ii) the effect of

181   biostimulation on prokaryotic diversity and taxonomic composition.

3.1 Ureolytic activity and pH changes in soils across spatial heterogeneity

183   We monitored urea hydrolysis and pH changes in desert soils from different depths following ureolytic

184   biostimulation treatment. Ureolytic activity was successfully induced by the biostimulation treatment in soil

185   samples from different depths (Fig. 1). The measured urea concentration significantly decreased during the

186   days following the treatment (repeated measures ANOVA; $F_{12,78} = 21.79$, $P = 3.72 \cdot 10^{-20}$). The ureolysis levels

187   differed between soils from different depths ($F_{2,78} = 60.16$, $P = 1.56 \cdot 10^{-16}$). At the soil surface, urea was

188   completely depleted within approximately 5 days following the treatment in sites 1 and 2. In the deeper soils,

189   ureolysis rates were milder than the topsoil and complete urea depletion was not achieved even after 18 days

190   of the treatment (Fig. 1a, b). The course of ureolytic response at the two greater depths was similar (paired t-

191   test: $t_{41} = 1.45$, $P = 0.465$).

192   At the disturbed site, the ureolytic response was delayed in comparison to the other sites, with complete

193   urea depletion at the soil surface a week from the beginning of the experiment (Fig. 1e). Moreover, higher

194   hydrolysis rates were recorded at the deeper soils at this site (46.64±10.39 mM urea measured after 18 days)

195   in comparison to sites 1 and 2 (94.97±28.56 mM urea measured after 18 days). Lower hydrolysis rates at the

196   surface, combined with higher rates at the deeper layers, might indicate that decades after the harsh

197   disturbance, the amalgamation of soil layers is still reflected in the microbial community of the disturbed site.

198   The functionally crucial surface community apparently has yet to be recovered. Indeed, biocrusts are known

199   to be highly sensitive to disturbances, and are characterized by notoriously slow recovery rates (Belnap and

200   Eldridge, 2001). Although the ability to distinguish the effect of disturbance from other influential factors is

201   limited in environmental studies, our results provide evidence for functional consequences of mechanical

202   disturbance to the soil microbiome.

203   The pH of the treated soils drastically increased during the experiment in accordance with urea hydrolysis

204   (Fig. 1c, d, f), elevating from 7.85±0.08 to 8.92±0.29 after 48 hours and then stabilizing on pH 9.46±0.05 until

205   experiment termination. These changes were consistent among soils from different depths and sites

206   (repeated measures ANOVA; $F_{3.86,25.08}$ = 0.179, P = 0.943). Such changes in the chemical properties of MICP-

207   treated soil pore fluids, even for a limited period of time, may have considerable environmental consequences.

208   For instance, soil pH was identified as one of the most important factors that shape microbial communities

209   (Fierer, 2017; Ratzke and Gore, 2018). Our results add further concerns to the previously raised question

210   regarding the potential pollution of deeper soil layers and aquifers by ammonium, a prominent MICP

211   byproduct, considering its potential hazard to human and environmental health (Lee et al., 2019).

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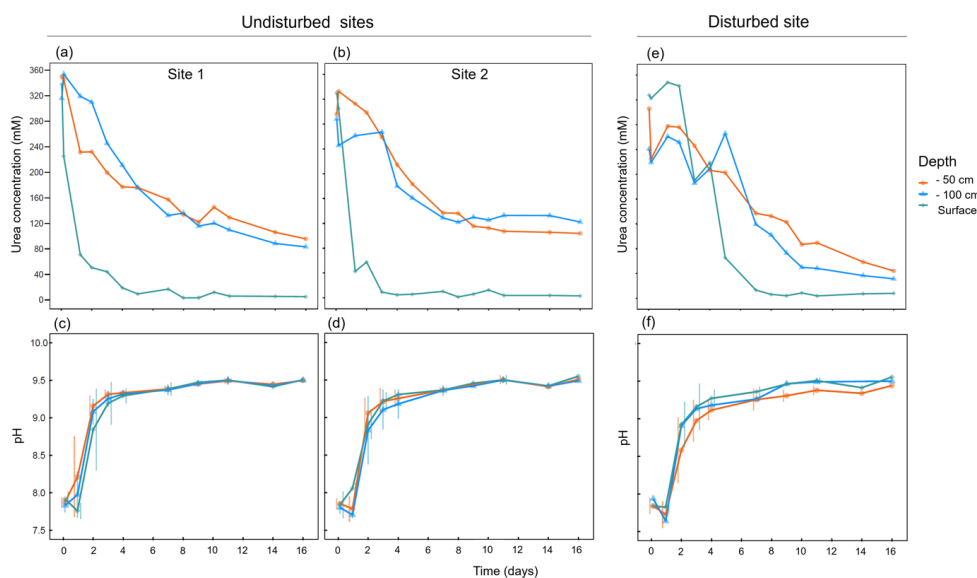
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214   **Figure 1.** Ureolytic activity profiles and pH changes following ureolytic biostimulation in three soil layers at

215   different sampling sites: (a, c) site 1, (b, d) site 2 and (e, f) a site that was mechanically disturbed 20 years ago.

216   **3.2 Microbial response to ureolytic biostimulation across depths**

217   We extracted microbial DNA and performed 16S amplicon sequencing to assess the effect of the treatment

218   on the diversity of native prokaryotes in soils from different depths. The analysis yielded a total of 877,848

219   reads that were assigned to 7650 OTUs, with an average of 36577 ± 20701 reads per sample. These OTUs

220   belonged to 27 bacterial phyla and two archaeal phyla.

221   To study the differences in the responses of microbiomes of different depths to biostimulation, we first

222   compared the two study sites, in order to account for any occurring horizontal spatial variation. The two

223   sites did not differ in their overall local microbial diversity ($F_{1,6}$ = 3.06, P = 0.13) nor beta-diversity (Fig. 2), and

224   did not host distinct communities before the treatment (ANOSIM: R = -0.04, P = 0.56). The communities of

225   both sites were similarly affected by the treatment, as will be discussed below. Therefore, the analysis focused

226   on depth and treatment related effects.
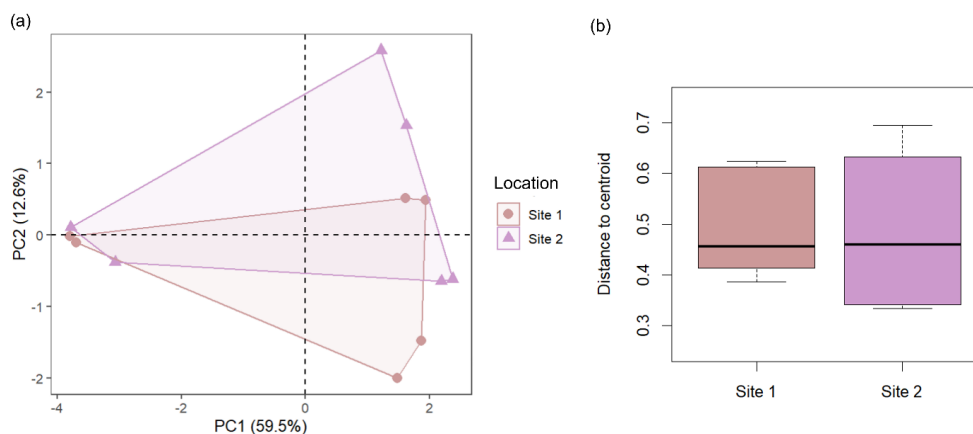
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228   **Figure 2.** Beta-diversity comparison between the two study sites. (a) PCA based on Bray-Curtis dissimilarities

229   shows that the microbiome composition of the two sampling sites highly overlap. (b) Beta-diversity of the

230   untreated samples does not significantly differ between the two sites (one-way ANOVA: $F_{1,10}$ = 0.003, P = 0.96).

231   **3.2.1 Local microbial diversity.** Upon DNA extraction, it was noticeable that the amount of microbial DNA

232   extracted from biostimulated soils (30 ± 15 ng DNA/g soil) was consistently higher than untreated soils (10.54

233   ± 7.31 DNA/g soil; 2-way ANOVA: $F_{2,18}$ = 51.51, P = $1.11 \cdot 10^{-6}$; Fig 3. a − c). Before the treatment, the native soils

234   contained diverse communities (Fig 3 d-i), typical of hot desert soils (Walters and Martiny, 2020). The upper

235   soil layer hosted more diverse communities than the community of the 100 cm depth (2-way ANOVA: $F_{2,18}$ =

236   5.97, P = 0.01). Following biostimulation, alpha-diversity (Fig. 3d-f) and microbial richness (Fig. 3g-i) were

237   drastically diminished. These results were consistent across soil depths, despite the observed increase in the

238   amount of microbial DNA. Since the initial microbial richness was low in the 100 cm depth, the decrease

239   following biostimulation was less drastic. Nevertheless, given the higher initial ratio between the amount of

240   reads and richness, the decrease in diversity was also considerable in this layer.


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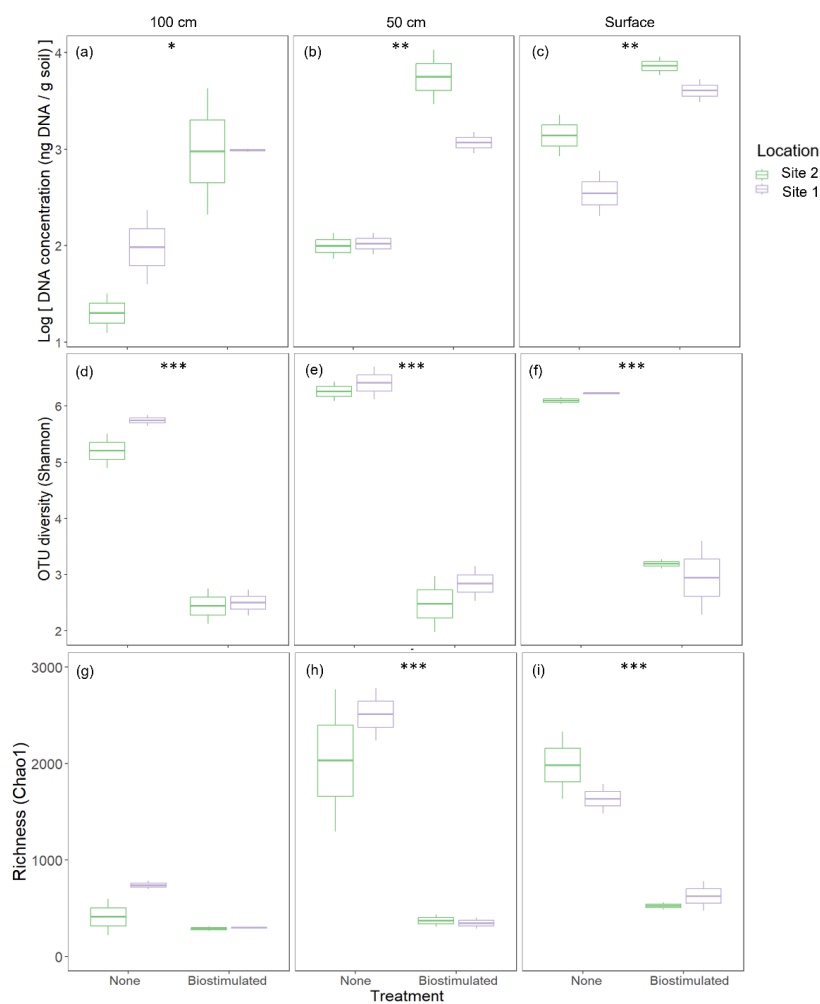
Figure 3. Effects of ureolytic biostimulation on edaphic microbial communities. Microbial DNA
concentrations (a-c) extracted from the studied soils were higher in biostimulated compared to non-treated
soils. However, microbial diversity (d-f) decreased following the treatment across depths (2-way ANOVA: $F_{1,18}$
= 404.94, $P = 8.66 \cdot 10^{-14}$), as well OTU richness ($F_{1,18}$ = 84.74, $P = 3.14 \cdot 10^{-8}$; g-i). Asterisks denote the

246   significance level of the differences in the treatment's effect within a specific depth according to pairwise

247   tests.

3.2.2 Microbial community composition.   Originally, the native soil was populated by distinct communities

249   at different depths (Fig. 4a; ANOSIM: R = 0.87, P = 0.0004). Communities at all depths were dominated by the

250   rigid walled, mostly chemoorganotrophic (Goodfellow, 2015) Actinobacteria (49.47 ± 10.93 of total reads). The

251   surface layer contained more Proteobacteria, Cyanobacteria, Bacteroidetes and Chloroflexi in comparison to

252   the deeper layers, while containing scarce amount of Firmicutes (Fig. 4b). The communities of the deeper

253   soils were richer in Firmicutes in comparison to the topsoil. These findings will be discussed in greater depth,

254   with respect to the ureolytic response, in the last section of the Results and discussion chapter. Composition-

255   wise, the soils in the deeper layers hosted communities that were more similar to one another in comparison

256   to the community of the surface. Nevertheless, significant differences were found between the communities

257   of the greater depths (R = 0.41, P = 0.03). For instance, most of the Archaea populated the 50 cm deep layer

258   (Fig. 4b).

259   The loss of biodiversity following biostimulation was mainly derived from a drastic increase in the proportions

260   of the endospore-forming Firmicutes at the expense of many other taxa (Fig. 4b). Biostimulation thus resulted

261   in homogenized communities, which no longer differed between depths (R = 0.13, P = 0.17) and were all

262   dominated solely by this phylum (80.39 ± 21.41 % of all reads in treated soils in comparison to 11.20 ± 9.89 %

263   in untreated soils). Particularly, the treated soils were dominated by the firmicute family Planococcaceae

264   (Supplementary Fig. A1), to which the most frequently studied model bacterium in MICP experiments,

265   *Sporosarcina pasteurii*, belongs. Several additional taxa remained prevalent after the treatment, mainly

266   Micrococcales (Actinobacteria; Figure 5).
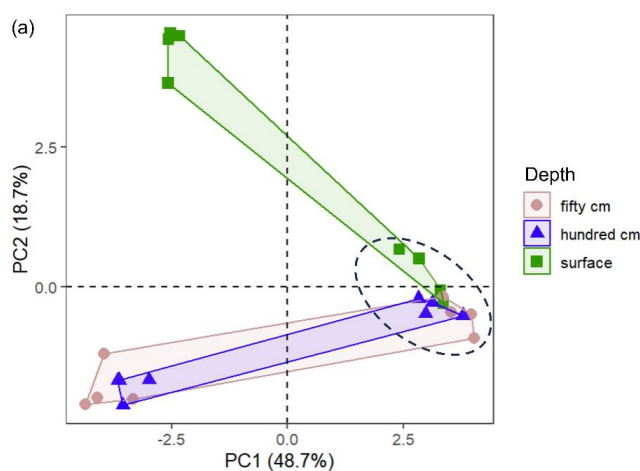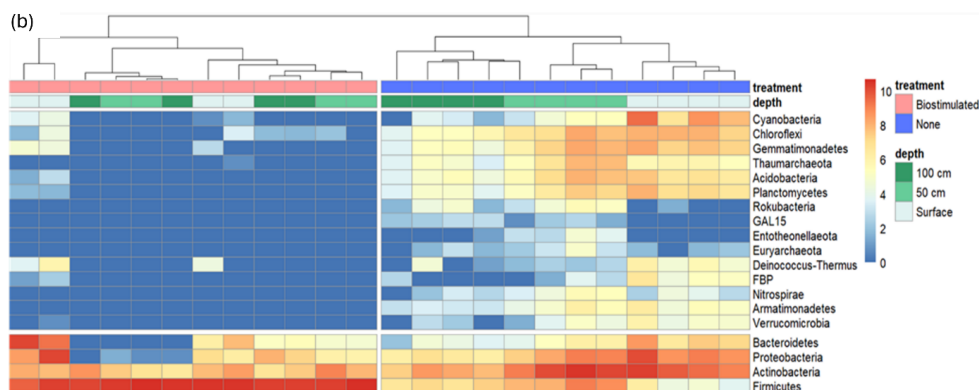
Figure 4. Microbial diversity patterns in communities from different soil depths, before and after ureolytic biostimulation. (a) PCA based on OTU relative abundances at the different soil samples. The biostimulated soils are circled by a dashed black line. (b) A heatmap of the changes in the OTU counts (log transformed) of main prokaryotic phyla between the studied soil samples (columns). The phylogenetic tree represents the similarity between the composition of sample communities based on Euclidean distances.

Our results emphasize the robustness of MICP, as substantial and rapid urea hydrolysis can be obtained using distinct microbiomes from different soil depths. Nevertheless, they show that applying this technique on soils comes with an ecological cost. The proliferation of Firmicutes following ureolytic stimulation has been reported in several previous studies on MICP (Gat et al., 2016; Graddy et al., 2021; Ohan et al., 2020) as well as

279   in a study on agricultural-related nitrogen amendments (Kaminsky et al., 2021). This might be attributed to

280   their physiological abilities to cope with the applied selection pressure (i.e., urea addition and the rapid

281   increase in pH following ureolysis) and even utilize the new niche, with reduced competition, to flourish.

282   Nevertheless, the fate of other populations, that are not recognized as ureolytically beneficial, remains largely

283   unaddressed. Our results show that many of the native residents are suppressed by the treatment, leaving

284   only 13 of the 29 phyla which were originally detected. Notably, the treatment dramatically decreased the

285   amount of detected autotrophs (e.g., Cyanobacteria, Chloroflexi), which are extremely important to the

286   formation and function of biocrusts (Maier et al., 2018), and hence to the entire ecosystem (Belnap, 2002;

287   Maestre et al., 2011; Rodriguez-Caballero et al., 2022; Rutherford et al., 2017). Inspecting higher resolution

288   changes in the taxonomic structure of the communities (Fig. 5) revealed that specific bacteria that are crucial

289   for the establishment of biocrusts (Xu et al., 2020), and in the Negev in particular (Belnap and Lange, 2003) –

290   Nostocales (Fig. 5a) and *Microcoleus vaginatus* (Fig. 5b) – are not detected in treated soils, which would likely

291   suppress biocrust recovery. Additional functionally important groups that were suppressed by biostimulation

292   are ammonia-oxidizing archaea and bacteria, including Thaumarchaeota and Nitrospirae (Marusenko et al.,

293   2015; Fig. 4b).

294   The shift in taxonomic composition was also noticeable within the Firmicutes and other surviving phyla

295   (Supplementary Fig. A1). Prominently, while in the untreated soils the main Firmicute families were

296   Paenibacillaceae and Bacillaceae, the treated soils were dominated by Planococcaceae. Our analysis identified

297   two specific bacterial taxa that were prominently abundant in biostimulated soils. They were annotated as

298   *Sporosarcina* sp. A12(2012) and *Bacillus* sp. Cza19 (Fig. 5b). Both are classified at the SILVA database as members

299   of the family Planococcaceae. Combined, these two OTUs contributed 18.9% of the variance found between

300   the communities of treated and non-treated soils (SIMPER analysis). Our results support the findings of

301   Graddy et al. (2021), showing a clear convergence of bacterial communities in biostimulation and

302   augmentation experiments. MICP in both forms indeed has a deterministic and consistent effect on the

303   microbial communities, regardless of preexisting differences in their structure.
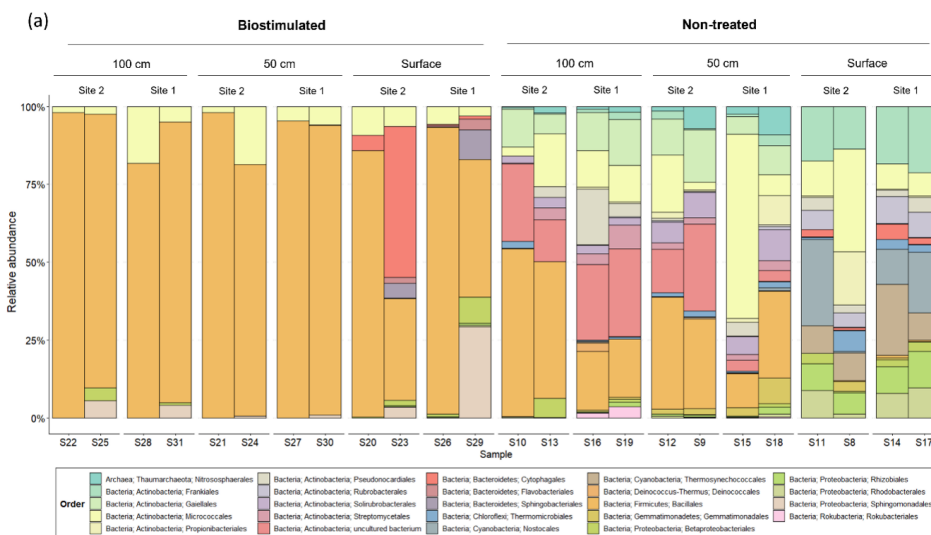
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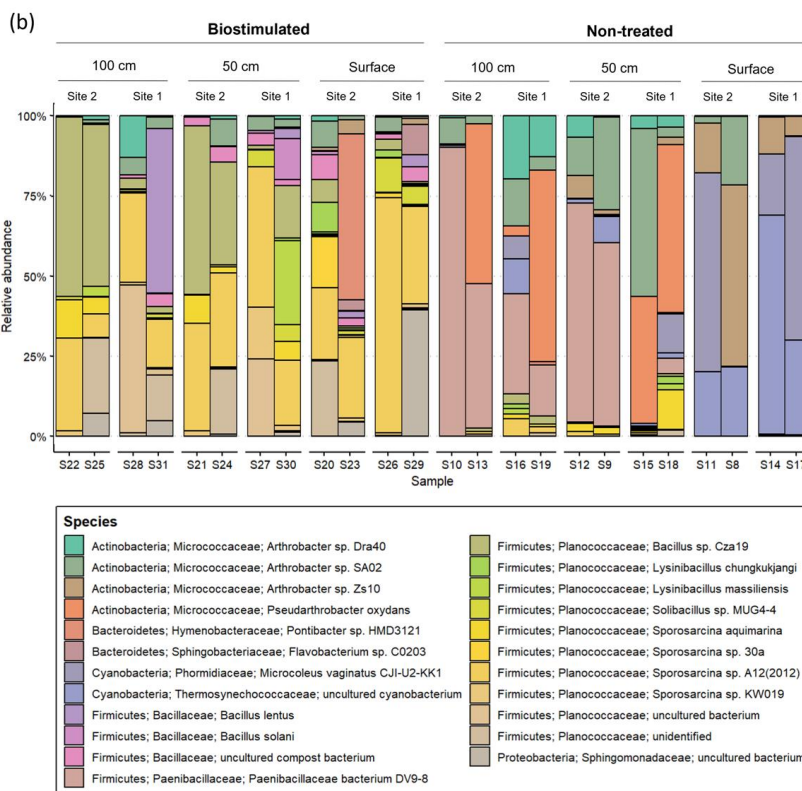
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307   **Figure 5.** The composition of the most prevalent microbial (a) orders (above 1% of total reads) and (b) specific

308   OTUs (above 5% of total reads) in studied soil depths, before and after ureolytic biostimulation.

309

310   **3.4 The relationship between the ureolytic response, microbial diversity and spatial heterogeneity**

311   The heterogeneity of the ecosystem in which ureolytic biostimulation is applied is rarely addressed in MICP

312   studies. Our findings support the notion that environmental heterogeneity and microbiome composition are

313   influential variables that should be taken into account in MICP experiments and application. In sites 1 and 2,

314   soil depth had a strong influence on the composition of the microbiome (Fig. 4) and hence on the ureolytic

315   response (Fig. 1), while horizontal variance did not (Fig. 2). These findings agree with the results of a previous

316   study (Eilers et al., 2012), in which the effect of soil depth on the microbiome composition in samples within

317   a specific forested biome was found to be equivalent, or even stronger, than the differences between samples

318   from similar depths obtained from a wide range of ecosystems. In contrast, in a mechanically disturbed site,

319   we have found it had a more prominent influence on the ureolytic response, which was delayed at the soil

320   surface and stronger in the deeper layers in comparison to two adjacent non-disturbed sites. Taken together,

321   our study shows that environmental variability affects the efficiency of MICP and that MICP, in turn,

322   detrimentally impacts the microbial diversity regardless of preexisting differences resulting from spatial

323   heterogeneity. Our results thus stress the importance of studying biostimulation in the context of different

324   biotic and abiotic variables that operate on the native microbial community in order to analyze and forecast

325   its efficiency.

326   The consistent proliferation of Firmicutes in MICP studies leads to the reasonable assumption that Firmicutes

327   (and particularly highly ureolytic species such as S. *pasteurii*) are the engine behind the ureolytic response.

328   Our results interestingly indicate that the process involves greater complexity, since the intensity of the

329   ureolytic response cannot be predicted solely by the preexisting amount of Firmicutes in the soil; Although

330   most of the Firmicutes and *Sporosarcina* members in our study sites were originally concentrated in the

331   deeper layers of soil (Fig. 4b, Supplementary Figure A2), and although their relative abundances

332   (Supplementary Figure A2) and total microbial DNA concentration increased similarly across the different

333   depths following biostimulation (Fig. 2a-c), the ureolytic response was not uniform. Urea degradation rates

334   were higher at the soil surface and decreased in deeper soils (Fig. 1a-c). The stronger response at the surface

335 was documented despite being measured at identical laboratory conditions of light, temperature, available

336 oxygen, etc. Hence, it is possible that a consortium of microbes contributes to the ureolytic process, either

337 by: i) directly engaging in ureolysis or ii) turning into an additional carbon source in the form of microbial

338 necromass which fuels the rapid response upon cell mortality, if induced by the treatment.

339 Considering the important functions of the upper layer of soil in arid environment and its vulnerability, our

340 findings call for taking precautions when considering the application of MICP in arid habitats. Nevertheless,

341 our results capture a short-term time frame of the treatment's effect. Kaminsky and her colleagues (2021)

342 have reported similar impacts of urea amendments on microbial diversity, yet they also found some trends

343 of recovery 7 weeks after the treatment. To our knowledge, the succession of the microbial community over

344 time following MICP was never monitored in previous studies. Considering the central functions of

345 microbiomes in biogeochemical cycles and the evidence of functional effects, this issue should be addressed

346 in future studies.

347 **4. Conclusions**

348 In this study, we established the feasibility of inducing effective ureolytic biostimulation using native

349 microbiomes that inhabit different depths within the upper 1 meter of an Aridisol. Effective ureolysis was

350 achieved at the depths of interest, regardless of the pre-existing differences between the microbial

351 communities. Notwithstanding, vertical heterogeneity was related to varying intensity of ureolysis.

352 Biostimulated MICP can be used for the stabilization of the soil surface in arid environments. However, the

353 treatment induces a consistent shift in the composition of the microbial communities, leading to the

354 enrichment of specific taxa while suppressing autotrophs and other ecologically important groups. Since

355 these autotrophs are key components of biocrusts, applying biostimulated MICP in an arid environment

356 might have negative consequences for soil stabilization in the long term. Taken together, our results call for

357 integrating environmental heterogeneity and biodiversity considerations in future MICP studies, from both

358 effectiveness and environmental consciousness aspects.

359 **Appendices**

16
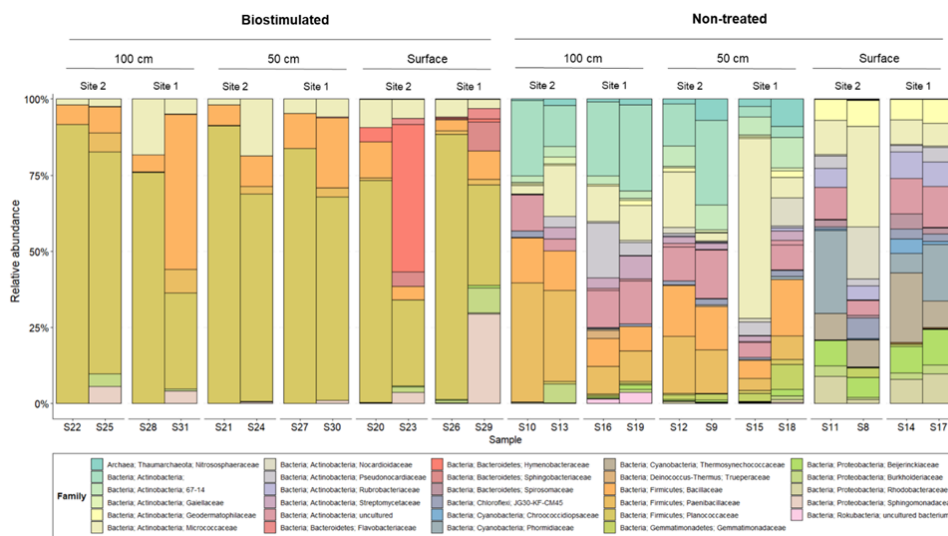
Figure A1. The composition of the most prevalent (above 1 % of total reads) microbial families in studied soil depths, before and after ureolytic biostimulation.
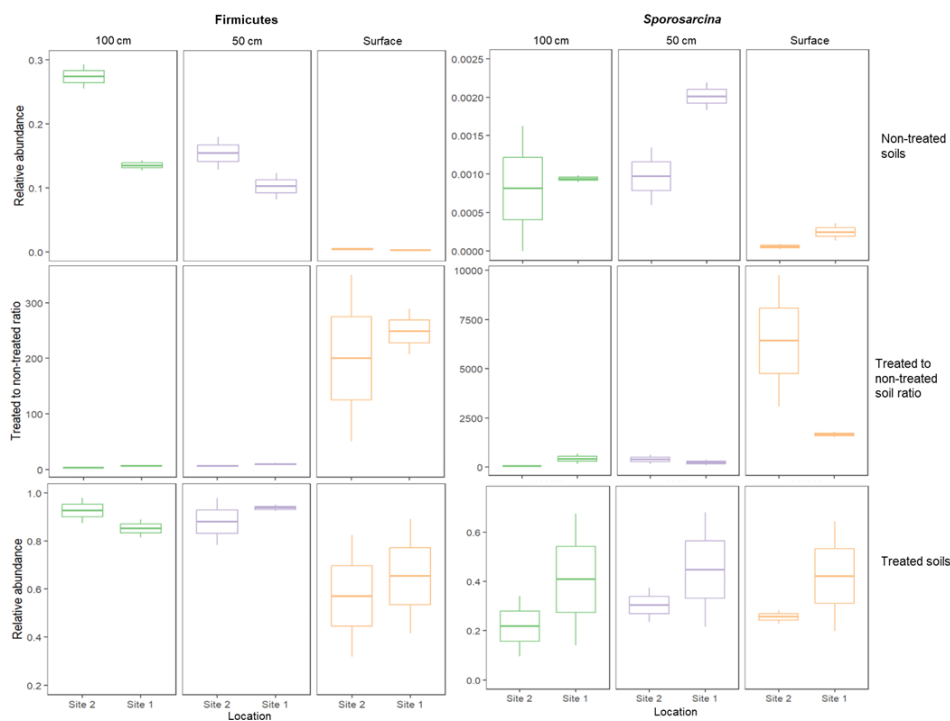
364 **Figure A2.** Relative abundances of the phylum Firmicutes and of the genus *Sporosarcina* in soils prior to

365 ureolytic biostimulation and after the treatment, and the ratio between them. The relative abundance of

366 Firmicutes reached similar values in treated soils (2-way ANOVA: $F_{2,9}$ = 3.48, P = 0.08), as did the *Sporosarcina*

367 ($F_{2,9}$ = 0.08, P = 0.93) despite of their original higher proportions in the deeper soils in comparison to the

368 surface.

369

370 **Data availability**

371 The raw 16S sequences were uploaded to the NCBI Sequence Read Archive, and are available under bioproject

372 ID PRJNA1041873.

373

374 **Author contribution**

375 **Kesem Abramov:** conceptualization, data curation, formal analysis, investigation, methodology, visualization,

376 writing – original draft preparation, writing – review & editing. **Svetlana Gelfer:** investigation and formal

377 analysis. **Michael Tsesarsky:** conceptualization, data curation, funding acquisition, methodology, project

378 administration, resources, supervision, writing – review & editing. **Hadas Raveh-Amit:** conceptualization,

379 data curation, investigation, formal analysis, funding acquisition, methodology, project administration,

380 resources, supervision, writing – review & editing.

381

382 **Competing interests**

383 The authors declare that they have no conflict of interest.

384

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