MICROBIOLOGICAL, ENVIRONMENTAL AND COMPOSITIONAL FACTORS IN EFFICACY OF MICP TREATMENT IN CLAYEY SOILS

by

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DEDICATION

I would like to dedicate this work to my mother Parvin Samimi who sacrificed a lot for my upbringing and education.

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ABSTRACT

Expansive clayey soils can cause billions of dollars of damage to infrastructure such as roads and foundations annually. Researchers propose many techniques (e.g., pre-wetting, soil replacement, and chemical stabilization) to improve the mechanical properties of these soils; however, some of these methods are impractical in certain situations, and are unsustainable in others due to the economic and environmental impacts. One possible method for enhancing soil's mechanical properties is Microbial Induced Calcium Carbonate Precipitation (MICP). This environmentally friendly technique is a biological process where microbes play a key role in precipitating calcium carbonate. This precipitating calcium carbonate can coat soil particles and cement the soil matrix, thereby reducing the swelling potential. MICP is a complicated process. Many environmental variables such as the soil type, composition, chemistry, and microbial communities present in the soil control the rates and amounts of carbonate precipitation. The application of MICP in clay soils is an active area of research, however due to the complex nature of MICP and the clayey soils, not all the parameters impacting MICP have been comprehensively or systematically described. Moreover, the MICP performance of the soils tested in other studies varied considerably depending on the soil types. This leads to a fundamental question: What geochemical and environmental factors influence MICP performance and how these factors can be used as predictors of the MICP effectiveness in expansive soils? Answering this question is essential in the development of optimization strategies capable of enhancing the competitive advantages of MICP over traditional soil

improvement methods; Moreover, understanding these factors prior to applying MICP to the soils can be a promising key for saving time, energy, and money. To determine the factors controlling MICP effectiveness in expansive soils, we performed a series of physical, chemical, microbiological, and compositional experiments in clayey soils collected from different geographical locations.

To determine how soil's clay content and gradation impacts calcium carbonate ($CaCO_3$) precipitation, several artificial clay/sand mixes were prepared and examined for urease activity and calcite precipitation. The test results showed that clay has more urease activity and precipitation calcite than sand despite the two having similar relative populations of indigenous ureolytic bacteria.

To determine the role of microbial communities in $CaCO_3$ precipitation, we measured $CaCO_3$ precipitation using Rapid Carbonate Analysis (RCA) and examined its correlation with soil ureolytic bacteria determined through 16SrRNA DNA sequencing. These observations show MICP treatment can increase ureolytic strains in all soils. However, this increase is not correlated with calcium carbonate precipitation in soils.

Additional testing on 6 soil samples from multiple geographical locations showed that compositional characteristics such as Cation Exchange Capacity (CAC) and Specific Surface Area (SSA) have a significant positive correlation with the efficiency of MICP.

The overall results suggest that the performance of MICP treatment is better in clayey soils compared to other non-clayey soils. Moreover, the results suggest that compositional properties such as CEC and SSA of the soil could be the reasons for the observed differences in $CaCO_3$ precipitation in soils. Therefore, it is possible that CEC and

SSA can be used as indicators of the MICP effectiveness prior to any MICP treatment in soils.

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LIST OF ABBREVIATIONS

MICP	Microbial Induced Carbonate Precipitation
UPB	Urease-Producing Bacteria
CEC	Cation Exchange Capacity
SSA	Specific Surface Area

CHAPTER 1: INTRODUCTION

1.1 Background

Expansive soils, also known as swell-shrink soils have been a problem to civil infrastructures including roads and foundations from ancient times. These highly plastic soils experience volume change with a change in moisture content (Nelson & Miller, 1997). Over the years, researchers have developed techniques such as pre-wetting, moisture barriers, mechanical compaction, and chemical stabilization to mitigate the expansive nature of these soils. Some of these methods, such as moisture barriers, are not cost-effective. Others are harmful to the environment. For example, ordinary portland cement is the dominate material used for construction and civil engineering purposes, according to (Huntzinger & Eatmon, 2009) the manufacturing of cement accounts for approximately 5% of global CO_2emissions, the third largest source of carbon emission in the United States. Hence, scientists continue to search for more sustainable and affordable alternatives for stabilizing clayey soils.

MICP is an environmentally conscious alternative that shows promise for mitigating the swelling potential of expansive soils. Most soil bacteria are capable of inducing CaCO3 precipitation through a variety of metabolic pathways, both autotrophic and heterotrophic (Jain et al., 2021). Due to the large voids size and easy application, MICP is mainly used for the improvement of sandy soils. Therefore, the controling factors of MICP in treating expansive clayey soils is a new topic that needs more investigation. In clays precipitated calcite act as a binding between clay particles and reduce their swelling

while increase the strength of these soils. One of the most important factors is that clayey soils are natural micro-organism incubators (adsorb bacterial cells). These soils contain more organic materials compared to other types like sand and the effectivity of MICP on them should be higher. However, there are very few studies about the effect of clay's chemical and physical interaction on MICP and calcite precipitation (Cardoso et al., 2018). Moreover, there are no studies on the impact of indigenous ureaolytic bacteria and soil composition on $CaCO_3$ precipitation in clays.

1.2 Purpose/Research Goals

In this work, we investigated the role of clay content in soils and their ureolytic bacterial communities in MICP is investigated; moreover, the changes in urease activity and efficiency of calcite precipitation in different soil compositions (clay/sand mixes). Also, the role of ureolytic bacterial communities in clay are investigated.

1.3 Broader impacts

Damage to engineered structures atop expansive soils is costly and widespread in the U.S. and internationally. Therefore, advancing knowledge of bio-mediated geochemical processes for superior expansive soil stabilization can largely benefit society by developing an eco-friendly and sustainable approach that is a cost-effective alternative to treat expansive soils. Additionally, global warming is a major environmental issue occurring primarily in response to increasing concentrations of CO_2 in the earth's atmosphere (Yadav et al., 2011). Currently, the concentration of CO_2 in the earth's atmosphere is about 400 ppm; however, this is increasing at approximately 2 ppm/year (Source from Wikipidia). Thus, there is an urgent need to reduce the release of CO_2 into the environment. The increasing atmospheric CO_2 levels are mainly due to the burning of

fossil fuels for energy production and consumption and other activities such as cement production and tropical deforestation (Goel, 2010; Malhi & Grace, 2000). MICP is an effective method for the removal of CO_2 from the environment (Ferris et al., 1994; Mitchell et al., 2010) In this method, CO_2 is converted into carbonate minerals that can form different crystals such as calcite, aragonite, dolomite and magnesite. This method is safer and more eco-friendly than conventional methods of sequestering CO_2 from the atmosphere.

1.4 Organization of Dissertation

This dissertation consists of three main chapters, each of which was prepared for publication in a scientific journal.

The three chapters include an extensive literature review of nitrous oxide in the hyporheic zone (Chapter 2), a report of our column and flume experiments measuring nitrous oxide along hyporheic flow paths (Chapter 3), and a presentation of the other geochemical species in these experiments, demonstrating distinct spatial and temporal trends in the geochemical evolution of the hyporheic zone (Chapter 4).

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CHAPTER 2: INVISTIGATING THE ROLE OF SOIL GRADATION, INDIGENOUS MICROBIAL COMMUNITIES, AND UREASE ACTIVITY ON BIO FACILITATED CACO3 PRECIPITATION IN ARTIFICIAL MIXES OF CLAY AND SAND

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Abstract

The effectiveness of Microbial Induced Carbonate Precipitation (MICP) treatment in soils could be better understood by studying the relationship between the soil gradation, urease activity, and microbial communities. For this purpose, four soils were prepared by mixing clay and sand at different ratios. Autoclaving was done to control the source of microbial communities contributing to $CaCO_3$ precipitation. The soil samples were subjected to seven cycles of MICP treatments and tested for urease activity, $CaCO_3$ content, and other engineering properties at regular intervals. In addition, to isolate the effect of gradation, lab-cultured ureolytic microorganisms (*Sporosarcina pasteur*ii) were mixed with the sterilized sand/clay and subjected to MICP treatments. The results showed that soil mixes with higher clay content have more urease activity and higher levels of $CaCO_3$ precipitation for both sand- and clay-autoclaved soil mixes.

Keywords: MICP, microbial induced calcite precipitation, expansive soils, urease activity, clay content.

2.1 Introduction

Since ancient times, expansive soils, also known as swell-shrink soils, have been a problem for lightly loaded civil infrastructure, including highways, railways, and low-rise commercial and residential buildings. Microbial induced calcium carbonate precipitation (MICP) is evolving into a possible mitigation method for expansive soils (Chittoori et al., 2019, 2018, 2021; Chittoori and Neupane, 2019a; Islam et al., 2020; Neupane, 2016; Rahman, 2018). MICP is an environment-friendly bio-mediated soil improvement technology that evolved from the interdisciplinary pathways of microbiology, geochemistry, and geotechnical engineering. The MICP mechanism often induces

biocementation, an ecological process that results in calcium carbonate deposition by different bacterial species (Iamchaturapatr et al., 2021). Researchers have shown that MICP is suitable for mitigating seismic-induced liquefaction, reducing permeability and compressibility, and increasing shear strength (Burbank et al., 2011; DeJong et al., 2006; Martinez et al., 2013; Qabany and Soga, 2013; Van Paassen, 2009; Van Paassen et al., 2010). Past studies at Boise State University showed that MICP could be a promising method for treating clayey soils (Islam et al., 2020; Neupane, 2016; Rahman, 2018). However, the soils' performance in these studies varied considerably after MICP treatments, leading to the following questions: (i) how does soil gradation impact $CaCO_3$ precipitation? (ii) does urease activity (the ability of a given soil to hydrolyze urea) depend on soil gradation and, as a result, affect the engineering behavior of MICP treated soils? and (iii) is there a relationship between soil gradation and the ureolytic bacterial communities present in different soils, and how do they influence MICP performance? Four artificial mixes of sand and clay with varying gradation and plasticity characteristics were prepared and subjected to MICP treatments to answer these questions. The goal was to study the effect of sand and clay percentages in soil on urease activity of the soil, bacterial communities, and thereby MICP performance. For this purpose, urease assay, rapid carbonate analysis, and unconfined compression tests were performed on the mixes before and after treatments. In addition, to exclude the role of soil's indigenous microbial communities and isolate the effect of soil gradation on MICP, sterilized/autoclaved soil samples were augmented with lab-cultured ureolytic microorganisms (*Sporosarcina pasteurii)* and treated for MICP and tested. Finally, the identity of the indigenous microbial community and urease producing bacteria (UPB) in natural sand and clay used to prepare

the artificial mixes was examined by Oxford Nanopore Technologies sequencing using the minION Mk-1C device to amplify 16s rRNA region and analyze through the EPI2ME platform. This research aimed to evaluate whether soil gradation and urease activity be used as predictors for MICP performance in a given soil.

2.2 Background

MICP is an environmentally conscious alternative that shows promise for mitigating the swelling potential of expansive soils. In this method, calcium carbonate precipitates in the soil pores and particle surfaces and binds the soil particles together (bio-cementation), which will reduce the swelling potential of the soil. The precipitation of carbonate starts with microbial urease hydrolysing urea to produce ammonia and carbonate ions; the carbonate ions then bind with calcium to accumulate insoluble $CaCO_3$ in a calcium rich environment (Burne and Chen, 2000). Graphical representation of reactions governing the MICP process is presented in Figure 2.1.

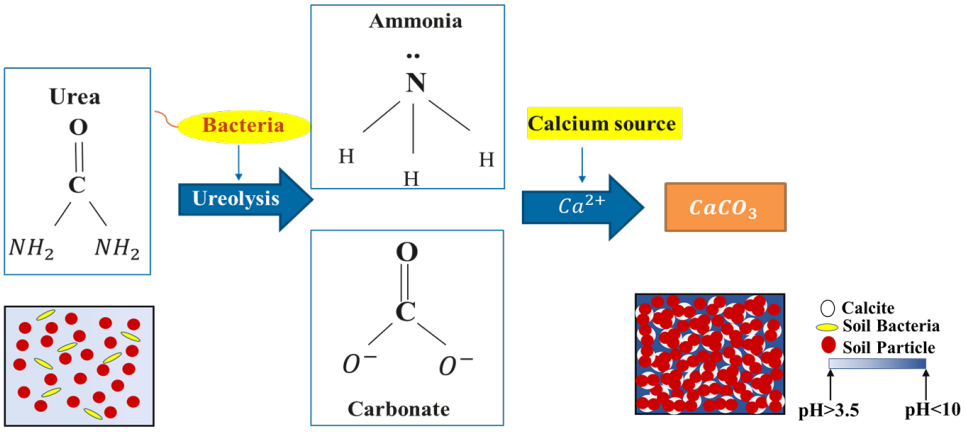


Figure 2. 1. Graphical representation of MICP in soils

MICP can be achieved either via bio-augmentation or using bio-stimulation. The main difference between these two methods is the source of the ureolytic bacteria. If the native bacteria of the soil are used to drive ureolysis, the method is called bio-stimulation. On the other hand, if ureolysis is achieved by introducing exogenous bacteria into the soil, the method is called bio-augmentation. It was shown that biostimulation is a more reliable method for MICP application than bioaugmentation (Burbank et al., 2011; DeJong et al., 2010; Gomez et al., 2014a, 2019; Tsesarsky et al., 2018). Studies also showed that the bio-stimulation process could be promoted by adding clay minerals to the soil composition (Cardoso et al., 2018), as clayey soils contain more organic materials than other soil types such as sands and silts. In addition, organic materials have a positive correlation with the urease activity of bacteria (Zantua et al., 1977). Moreover, clay soils are natural microorganism incubators i.e., they adsorb bacterial cells much better than other soil types (Stocks-Fischer et al., 1999). Other researchers have shown the positive interaction of clay minerals in MICP (Cardoso et al., 2018; Fomina and Skorochod, 2020; Sun et al., 2019). Masy et al. (2016) assessed the potential benefits of bio-augmentation versus bio-stimulation in hydrocarbon-contaminated (HC) clayey soils. The decrease of the concentration of HC was the greatest for the bio-augmented soil and lesser for the bio-stimulated soil. Although, the ureolytic gene proportion in bio-augmented soils decreased to levels close to those of bio-stimulated soils after 80 days of treatment. In another study, the removal of polyaromatic hydrocarbons (PAH) in tropical clay soil contaminated with diesel oil was studied by Chagas-Spinelli et al. (2012). Their results show treatment removal efficiency for the total PAHs was highest in bio-stimulation followed by bio-stimulation plus bio-augmentation.

Research studies focusing on using bio-stimulation in enhancing the mechanical properties of clayey soils are limited (Chittoori et al., 2021; Chittoori and Neupane, 2019b; Islam et al., 2020). Although all of these studies demonstrated the applicability of MICP via bio-stimulation in treating expansive soils, there is currently no investigation of the potential relationship between MICP-induced improvement in engineering behavior of clayey soils and soil gradation/urease activity.

Knowledge of the ureolytic microbial communities and their urease activity plays an essential role in understanding bio-stimulation efficiency in clayey soils. Newer molecular approaches, including probing for specific genotypes (Sayler et al., 1995) and monitoring mRNA expression (Wilson et al., 1999), have played an increasingly important part in advancing our understanding of soil microbial communities. In the experiments reported by Taylor et al. (2002), several metrics were used to quantify and compare microbial presence in silty clay loam and loamy sand. These metrics were direct counts of total bacteria, DNA extraction, and quantification. Also, β-glucosidase, phosphatase, and urease enzymes were selected to monitor the bacterial activity. Their results showed a strong positive correlation ($R>0.90$) between bacterial abundance and enzyme activity on the one hand and between enzyme activity and organic matter content on the other. They also observed a strong positive correlation (~0.972) between clay content and urease activity and a strong negative correlation between sand content and urease activity ($R>0.95$). They suggested that the studied clay samples can retain and protect urease either in an active extracellular urease form or ureolytic microbial biomass. Many researchers have demonstrated the presence of ureolytic communities in different soils irrespective of the type, mineralogy, and environmental conditions (Bibi et al., 2018; Burbank et al., 2012;

Taylor et al., 2002). However, despite all these efforts, there is currently limited knowledge on the impact of indigenous ureolytic communities and soil gradation on urease activity and MICP performance. In the current study, these factors are hypothesized to be controlling factors in the applicability of MICP in different soils.

2.3 Materials & Methods

2.3.1 Soils

Four artificial soil samples were prepared using natural clay and natural sand obtained from Idaho. Organic carbon, nitrogen contents, and the geotechnical soil properties for both these soils are presented in Table 2.1. Artificial mixes were prepared to study the effect of clay and sand contents on urease activity and other MICP parameters. The corresponding percentages of clay and sand for each of the four mixes are presented in Table 2.2. The mixes were prepared by mixing the corresponding percentage (by dry weight of the soil) of non-sterile natural clay with autoclaved and sterilized sand (D60 = 0.71, D10 = 0.25, and Cu = 2.8). Another set of 4 samples was prepared in the next round of tests, where clays were autoclaved and mixed with different amounts of natural sand (non-autoclaved). The soil samples are named using the following scheme, $S^{*}XCY$, where S stands for the Sand and C stands for clay, and X and Y are the sand and clay percentages, respectively. The '*' denotes that the material was autoclaved. For example, S*89C9 means the soil mix contains 89% of natural sand and 9% of natural clay, and the natural sand was autoclaved to sterilize all the bacterial in that soil. This sterilization was done to study the effect of the native bacteria present in the natural sand and clay on MICP performance. In this paper, artificial mixes prepared using autoclaved sand and unsterilized clay are referred to as sand-autoclaved samples, and the mixes with autoclaved clay and

unsterilized sand are referred to as clay-autoclaved samples. Table 2.2 also presents the sand and clay percentages of the mixes (regardless of the autoclaving status) along with the standard Proctor's data and corresponding ASTM standards.

Table 2.1. Carbon, nitrogen content, and geotechnical properties of two natural soils used in this study

Soil	Gradation			C mg/g of soil	N mg/g of soil	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index (%)
	Sand (%)	Clay (%)	Silt (%)			(ASTM D4318)		
Natural Clay	18	62	20	5.0	0.5	68	42	26
Natural Sand	99	0	1	0.4	0	N/A	N/A	N/A

Table 2. 2. Soil notations and the corresponding gradations and properties

Soil Notation	Sand (%)	Clay (%)	MDUW (kN/m^3)	OMC (%)
			(ASTM D698)	
S89C9	89	9	13.9	7
S75C18	75	18	13.6	8
S59C31	59	31	13.0	9
S18C62	18	62	11.5	16
Note: MDUW – Maximum dry unit weight, OMC – Optimum moisture content				

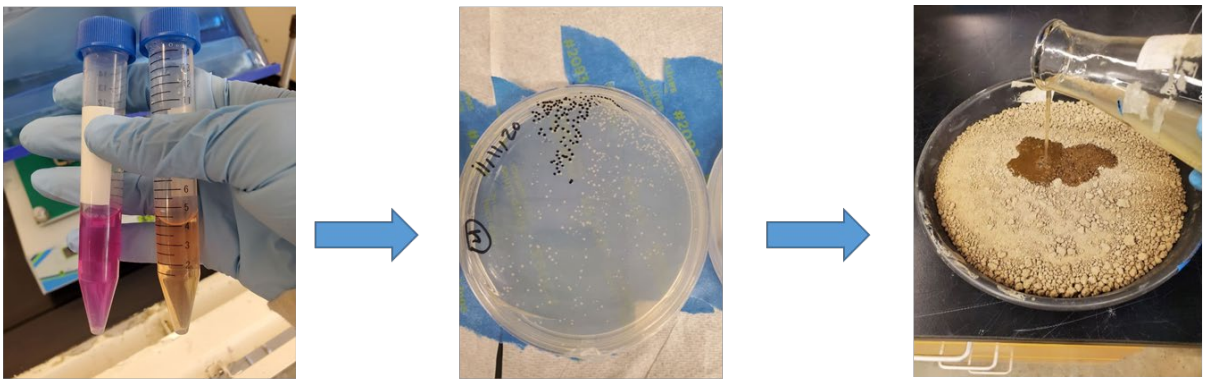
2.4 Nutrient solutions

Two types of treatment solutions were used in this research. The enrichment solution consisted of 100 mM of Sodium Acetate, 333 mM of urea, 0.5 g/L of Corn Steep Liquor (CSL). The cementation solution consisted of 100 mM of Sodium Acetate, 333 mM of urea, 0.5 g/L of Corn Steep Liquor (CSL), along with 250 mM of Calcium Chloride. These compositions are as per Burbank et al., (2011). Corn steep liquor consisted of amino acids, vitamins, and minerals necessary for microorganism survival and provided enrichment and cementation

solutions. The enrichment solution stimulates bacteria growth, using acetate as a carbon source and urea or ammonia as a nitrogen source. The increase in the pH results from ammonia production from urea hydrolysis, which creates an environment favorable for bacteria. When the microbe population becomes more ureolytic, more hydrolysis happens, and more $CaCO_3$ is precipitated (Burbank et al., 2011).

2.5 Bacterial culture production

To exclude the influence of native microbial communities and study the impact of soil gradation on MICP performance, *S. pasteurii* strain (ATCC NO. 11859) was used in this research. Soil samples with gradations similar to those described in section 2.3.1 were prepared and sterilized using an autoclave. Each sample was then mixed with 50 ml of *S. Pasteurii* bacteria (6*106 microbes /ml of LB broth) (Figure2.3c). The urease was positive, as evidenced by the pink color of the Urea, Luria broth (LB), and phenol red media (Figure2.3a). The density of bacteria was estimated using the Serial Dilution and Colonies Forming Units (CFU) counting method (Figure 2.3b)(Rutten, 2019).



a. Changing phenol red to pink shows urease positive

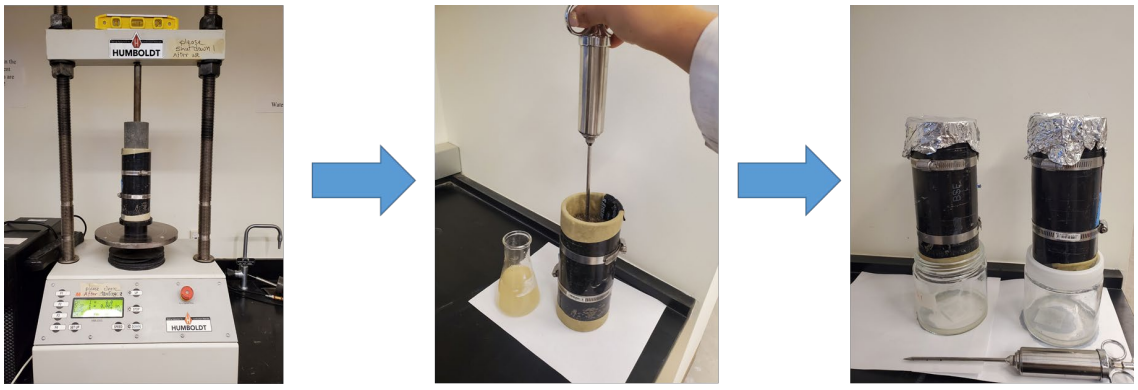
b. Counting bacteria by serial dilution and colony forming unit methods (6*10^6 microbes /ml).

c. Mixing 5 ml of bacterial culture with soils.

Figure 2. 2. Pictorial representation of preparing bacteria and mixing with soil samples.

2.6 MICP Application Method

In this research, the delivery of treatment solutions was made using the injection method. To achieve $CaCO_3$ precipitation through injection, each soil sample was wrapped inside a latex membrane and placed in a PVC tube (75 mm diameter x 203 mm long), as shown in Figure 2.4c. Static compaction was performed using a quasi-static compactor to achieve targeted dry unit weight for each sample (Figure 2.4a). The soil samples were compacted to 70% MDUW and optimum moisture content (OMC). The low unit weight was chosen to ensure that the samples had sufficient pore space to allow the flow of treatment solutions through the sample. Since we were not targeting any design characteristics, this reduction in MDUW was an acceptable choice for testing. After the samples were prepared and encased in PVC casing, they were first injected with enrichment solution using a five-inch injector needle (Figure 2.4b). The samples were left for 48 hours on the countertop, and then their pH was measured. By trial and error, it was determined that after 48 hours, soil samples showed an increase in the pH (>9); this indicated that 48 hours was sufficient time to trigger bio-stimulation. After 48 hours, each soil sample was injected with cementation solution and was left for the bio-mineralization process to start. The pH of samples was measured every 48 hours for 7 rounds of bio-cementation treatment. Pictorial representation of compacting and treating samples with MICP is shown in Figure 2.4.



a. Preparing soil samples using their respective 70% OMC and MDUW

b. Injecting soils with treatment solutions by 5" needle

c. Performing treatment at 24 hr. intervals and letting samples to rest

Figure 2. 3. Pictorial representation of compacting and treating samples with MICP

2.7 Evaluation Tests

2.7.1 Urease activity test

All soil samples underwent a urease activity test before the MICP treatment. Urease activity was determined by measuring ammonia concentration in the samples using the colorimetric method as per the protocol suggested by Bremner and Douglas (1971). As per this protocol, plastic vials were first used to mix 10 g of soil sample with 8 ml of 4288 ppm urea solution. The vials were incubated for 5 hours at 37°C as suggested by Bremner and Douglas (1971) (Figure 2.5a). The contents of each vial were then mixed with a 40ml solution of 2.5 M potassium chloride (KCl) and silver sulfate (Ag_2SO_4) ($4.5*10^3$M) (Tabatabai and Bremner, 1972). The resulting mixture was filtered using Whatman #42 (2.5 µm), and 200 µl of the extract was pipetted into a reaction kit (Ammonia TNT 832, Hach) for colorimetric measurements of ammonia concentration (Figure 2.5b). A higher OD corresponds to a more significant concentration of ammonia, as explained by Tabatabai and Bremner (1972) and Verdouw et al. (1978)(Figure 2.5c).

a. Incubation of soil samples for 5 hours at 37°C

b. Portable spectrophotometer.

c. The sample vials show the different concentrations of Ammonia.

Figure 2. 4. Pictorial representation of urease assay test.

2.7.2 CaCO3 Content Determination test

Precipitated calcium carbonate was detected using a Rapid Carbonate Analyzer (D4373-96). In this method, calcium carbonate reacts with HCL as shown in the following reaction (Equation (5)):

$$CaCO_3 + 2H^{+1} \rightarrow Ca^{+2} + H_2O + CO_2 \qquad (5)$$

About 20g pulverized dry soil specimen from the top and bottom was sieved (#10 sieve) and placed into a reactor chamber (Figure 2.6). A plastic beaker containing 20±2 mL of HCL solution (1N) was inserted into the reactor. The chamber was then sealed by closing the lid and pressure relief valve. The chamber is swirled such that the acid was mixed and reacted with the soil sample. After 10 minutes of reaction time, the pressure was measured, and the amount of $CaCO_3$ was determined by reading from a calibration chart.

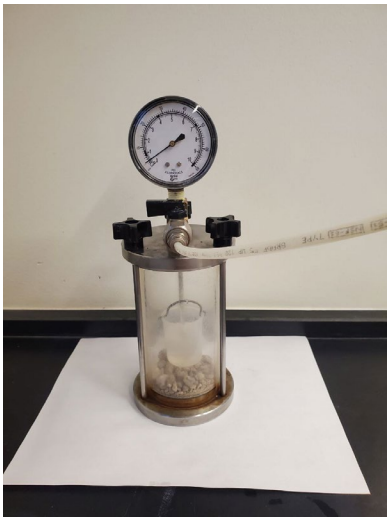


Figure 2. 5. Photograph showing the rapid carbonate analyser used in this research.

2.8 Methods for 16srRNA Soil Microbiome Experiments

2.8.1 DNA Extraction and Oxford Nanopore 16S rRNA Library Preparation

An overview of the experimental design and process of identifying the indigenous microbial community for clay and sand is included in Figure 2.7. 16S rRNA Library was created by first extracting DNA from sand and clay samples. Aliquots of 0.5g of each sand and clay soils were taken from the -20°C Freezer and put into bead tubes for DNA extraction following the FastDNA Spin Kit for Soil, MP Biomedicals, and (US) manufacturer's instructions. There was a total of 10 minutes of bead beating and centrifugation. The DNA concentration extracted was measured with BioTek Synergy H4 Hybrid Microplate Reader (Fisher Scientific, Göteborg, Sweden). Furthermore, to ensure the quality was appropriate, the Optical Density (OD) ratio of 260/280 was measured with BioTek Synergy H4 Hybrid Microplate Reader and was higher than 1.8. The 16S rRNA DNA was sequenced using Oxford Nanopore 16S Barcoding Kit 1-24 (SQK-16S024). The barcoding kit provides 16S Barcoding Primers (1-24), and two barcodes were used with 10

ng of high molecular weight DNA isolated from each soil sample (Manzari et al., 2020). Followed manufacturer's protocol for PCR barcode ligation and library preparation of DNA; PCR conditions were: 95°C for 1 min, 25 cycles of 95°C for 20 s, 55°C for 30 s, and 65°C for 2 min, followed by 65°C for 5 min.

2.8.2 16S rRNA DNA Basecalling and Phylogenetic Tree Construction

After the library preparation, the Oxford Nanopore flow cell was primed with fluid mixes provided by the kit for sample loading. Immediately after library preparation, the samples were loaded onto the MinION Mk1C, where the reads were detected and were basecalled for a total of 8 hours ("16S Barcoding Kit 1-24 (SQK-16S024)" n.d.). FASTQ files were acquired from the MinION Mk1C post-base-calling run analysis and uploaded to the EPI2ME platform. An EPI2ME platform is a cloud-based software from Oxford Nanopore that provides analysis metagenomic identification, alignments, and genome assembly while using information from online databases (NCBI). Based on EPI2ME post-analysis, a (species and genus) phylogenetic tree for sand and clay was constructed, choosing 1% abundance cutoff and only using reads with an average Q-Score of 7 ("EPI2ME platform" n.d.). The clay 16S rRNA workflow result can be found in https://epi2me.nanoporetech.com/shared-report-260253?tokenv2=c397ea59-fc58-44e6-9090-b520abe450fc, the barcode is labeled BC16. The sand 16s rRNA workflow result can be found in https://epi2me.nanoporetech.com/shared-report-258533?tokenv2=e04474c3-5bb1-47fa-89af-661189d42b8f.

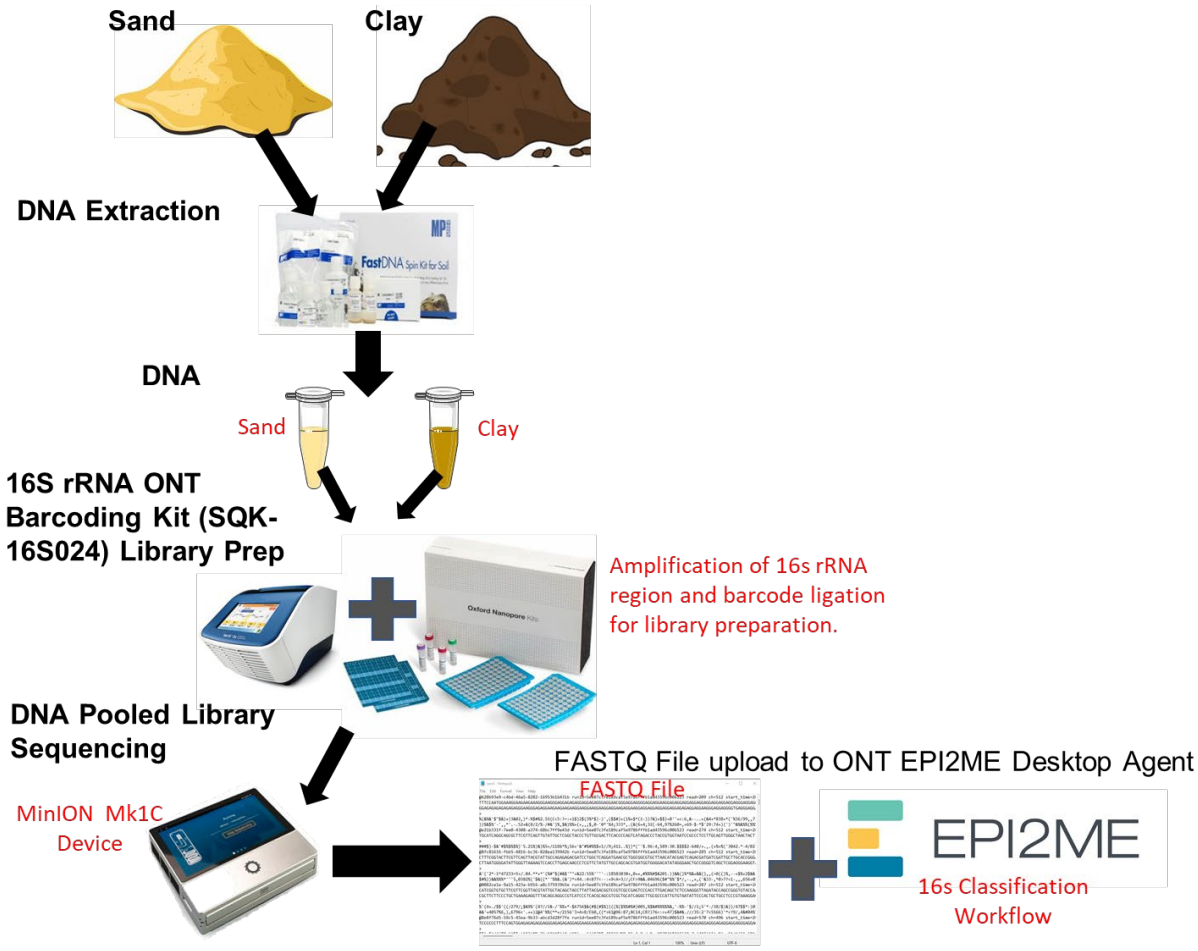


Figure 2. 6. The diagram explains the overall experimental design and procedure for identifying the indigenous microbial community in clay and sand samples.

2.9 Results and Discussion

The four artificial samples were treated for MICP in four different ways. The first set of samples was treated without any autoclaving of the sand or clay portion in the mix. The second set of samples was treated after autoclaving the sand portion of the mix, and the third set was treated after autoclaving the clay portion of the mix. The fourth set of samples was treated after fully autoclaving the soils and augmenting the samples with lab-cultured bacteria. All samples were tested for UA, $CaCO_3$, and UCS tests after each set of

treatments. In addition, the natural sand and clay were subjected to 16S rRNA and DNA testing to understand the diversity of the microbial communities in these soils. These results are discussed in the following sections.

2.9.1 Urease activity results

The variation of UA with the clay content present in the soil for both clay-autoclaved, sand-autoclaved and non- autoclaved samples are presented in Figure 2.8. It was shown that UA is strongly correlated to the Soil Organic Matter (SOM) content (Burns and Gibson, 1980; Dalal, 1975; Myers and McGarity, 1968). The clay rich sample (S18C62) has more organic carbon and nitrogen than sand rich sample (S89C9). Therefore, is not too surprising that the clay rich sample shows more urease activity. It can be observed from Figure 7 that the UA is increasing with clay content for sand-autoclaved samples. This shows higher amounts of unsterilized clay present resulting in higher UA. In the case of clay-autoclaved samples, UV increased with clay content for up to 30% but decreased after that. In the clay-rich sample (S18C62), autoclaving resulted in a drop of ~75% in urease activity compared to non-autoclaved samples. The greater impact of autoclaving clay on MICP efficiency and urease activity in clay-rich samples can be attributed to dissociation of the bacterial cells and dissociation SOM during autoclaving of clay. For non-autoclaved (non-sterilized) mixes, UA results were similar to that of sand-autoclaved mixes except for the S89C9 sample. This shows that the bacterial communities present in the clay are dominating the urease response even at clay contents starting from 20%.

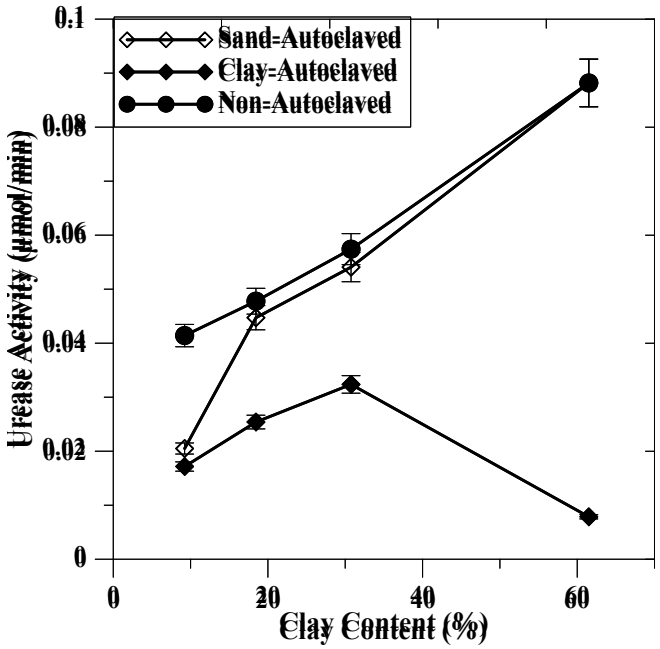


Figure 2. 7. Plot showing the variation of urease activity with clay content for the different soil samples tested under different autoclaving conditions. Error bars indicates 10% error

2.9.2 $CaCO_3$ precipitation results

$CaCO_3$ precipitation exhibits a linear dependence on urease activity with an R-squared value of 0.95 (Figure 2.9). $CaCO_3$ has the same correlation with the sample's gradation where samples with higher clay content showed higher $CaCO_3$ precipitation. Post-treatment $CaCO_3$ content in original soil mixes (non-autoclaved) and sand-autoclaved samples were almost the same, especially for clay content of >50% (Figure 2.10). This is because ureolytic bacteria and SOM in clay remained intact while sand was autoclaved. On the other hand, when clay was autoclaved prior to mixing with sand, the post-treatment $CaCO_3$ content showed a reducing trend with clay content of the mix, resulting in a ~2.5% in $CaCO_3$ between autoclaved and non-autoclaved clay rich sample (S18C62). Figure 2.10 also shows a comparison between original treated samples and bio-augmented treated samples. When soil samples were mixed with (*Sporosarcina pasteurii)* ureolytic (Bio-

augmentation), the same trend as in other soil mixes was observed in the amount of precipitation $CaCO_3$. Bio-augmentation experiments showed that clay is a more suitable environment for ureolytic activity and $CaCO_3$ precipitation as discussed before. The amount of $CaCO_3$ in treated S18C62 was closed to bio-augmented S18C62, this represents the applicability of MICP using the indigenous bacteria in clayey soil (bio-stimulation). Furthermore, comparing the results of bio-augmentation and bio-stimulation (treated graph in Figure 2.10) depicts that bio-augmentation resulted in more $CaCO_3$ precipitation in the sand dominant samples (S89C9 and S75C18), this might be because of replacing indigenous urelolytic species in sand with *Sporosarcina pasteurii.* This gram-positive bacterium is known to have one of the highest urease activities compared to other organisms (Whiffin, 2004).

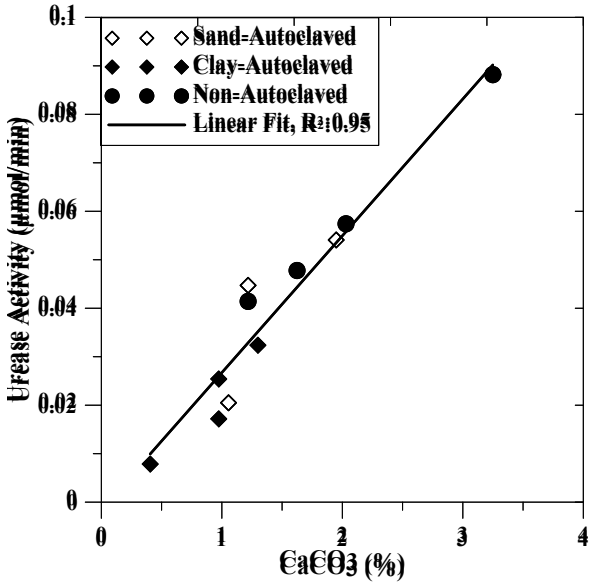


Figure 2. 8. Plot showing the relationship of CaCO3 precipitation with urease activity for the different soil samples tested under different autoclaving conditions.

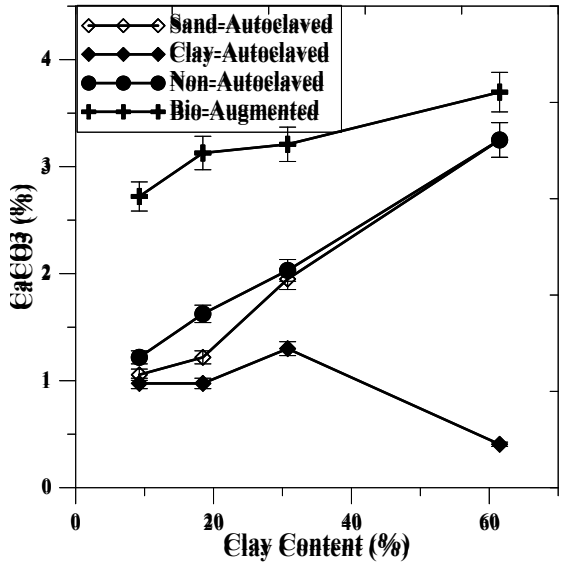


Figure 2. 9. Plot showing the variation of CaCO3 with clay content for the different soil samples tested under different autoclaving conditions. Error bars indicates 10% error

2.9.3 Uniformity

Figure 2.11 shows the gradient in the amount of $CaCO_3$ precipitation in the top and the bottom of treated samples. The gap grows quickly as the clay content increases. This can be attributed to the small pore size and low permeability in clay-rich samples, which leads to lower penetration of injected treatment solution resulting in less $CaCO_3$ at the bottom of the PVC tubes.

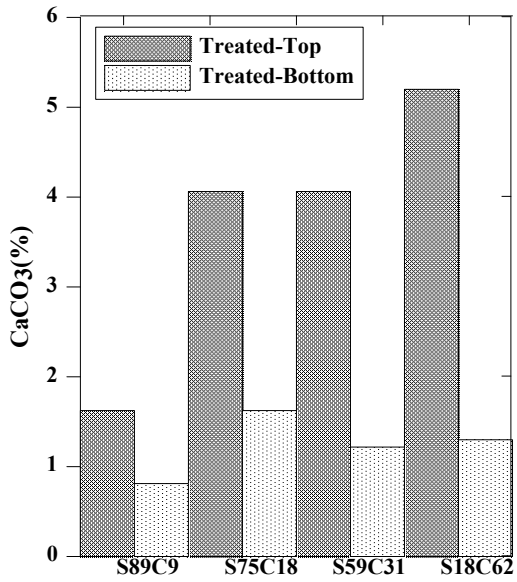


Figure 2. 10. The difference in the amount of CaCO3 precipitation in the top and the bottom of all samples

2.10 UCS results

2.10.1 Strength changes in soil samples (Bio-stimulation)

Studies have shown that MICP can improve soil strength due to the formation of $CaCO_3$ and binding soil grains together (DeJong et al., 2006; Gomez et al., 2014; Qabany & Soga, 2013; Van Paassen et al., 2010). Shear strength of the soils before and after treatments was determined using the unconfined compression strength (UCS) test. According to UCS results represented in Figure 2.13 the shear strength increased after treatment for all samples. The increase in shear strength after treatment proved that there is a good correlation between urease activity, $CaCO_3$ precipitation and soil strength as the sample with the highest amount of clay (S18C62), urease activity and $CaCO_3$ showed the highest increase in shear strength (~132%). Figure 2.12 depicts the linear correlation between $CaCO_3$ precipitation and UCS with R-squared value of 0.94. Also, the strength after treatment was higher in sand-autoclaved samples compared to clay-autoclaved

samples which again correlates well with urease activity and precipitation of $CaCO_3$ (sections 2.9.1 and 2.9.2). Figure 2.13 shows the unconfined compression strength for bio-augmented samples. The shear strength increases in all soil samples. It was insignificantly higher in sample with higher percentage of sand (S89C9 and S75C18 samples) which was consistent with the $CaCO_3$ results in bio-augmented treated samples (section 2.9.2).

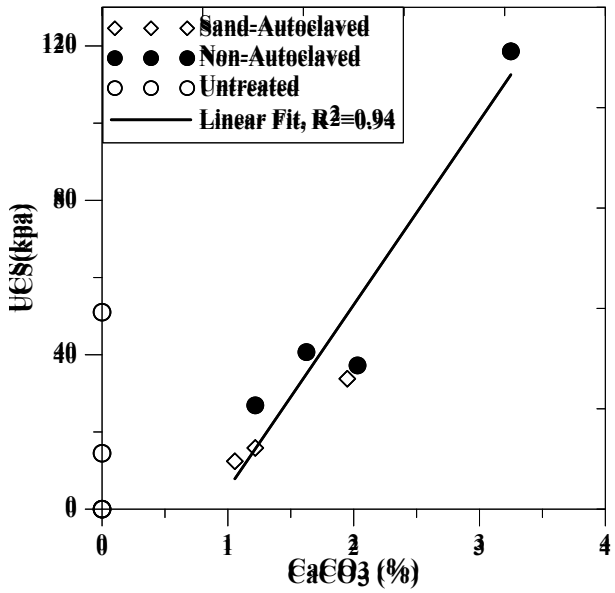


Figure 2. 11. Plot showing the relationship of CaCO3 precipitation with urease activity for the different soil samples tested under different autoclaving conditions.

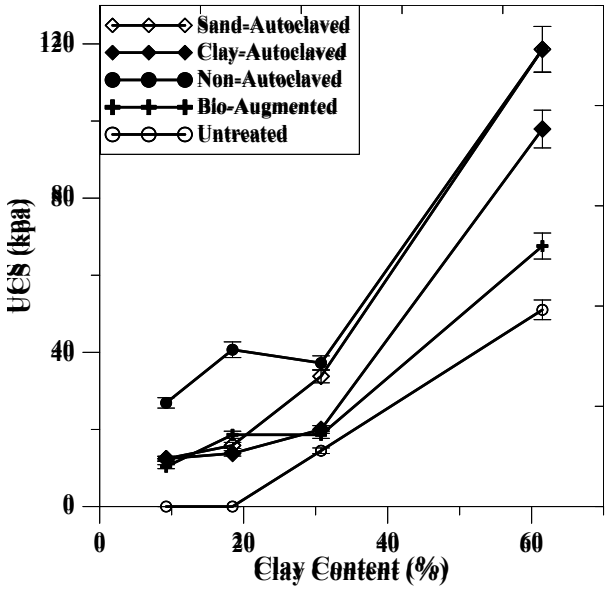


Figure 2. 12. Plot showing the variation of unconfined compressive strength with clay content for the different soil samples tested under different autoclaving conditions. Error bars indicates 10% error

2.11 Identification and Preliminary Characterization of Isolates from experimented sand and clay

The indigenous urease producing species in sand and clay soil microbiomes were determined. According to the genus phylogenetic tree constructed for clay and sand samples, more diversity in both total and ureolytic genus was observed in sand compared to clay soils (Figure 2.14a. and 2.14b.). 96 classified isolates belonging to 33 genera were identified in sand and 110 classified isolates belonging to 6 genera were identified in clay sample. In clay soil, ~ 33% of total isolates were ureolytic species while ~23% of ureolytic species were detected from 96 classified isolates in sand (Table 2.3). The genus *Bacillus* (~37%), followed by *Flavisolibacter* (~27%) and Microvirga (~9%) comprised ~73% of the ureolytic bacteria isolated from clay soil. Sequences related to *Bacillus* included four species (*B.badius*, *B.eiseniae*, *B. litoralis,* and *Paenibacillus borea*lis) followed by the

genus *Flavisolibacter,* which included three species (*Flavisolibacter ginsengisoli*, *Flavisolibacter ginsenosidimutans*, and *Flavisolibacter metallilatus*), and *Microvirga* included 1 species (Microvirga pakistanensis). Since some of the species identified were not confirmed UPB, they will require further investigations to determine their role in urease production. Of the three *Flavisolibacter* species *Flavisolibacter ginsengisoli* and *Flavisolibacter ginsenosidimutans* are confirmed urease producing bacteria (UPB)(Maeng et al., 2019). The genus *Microvirga,* a symbiotic nitrogen fixing microbe can also produce urease (Amin et al., 2016; Mouad et al., 2020). In contrast, the dominant ureolytic genera in sand were *Sporosarcina* and *Pseudarthrobacter,* each comprised ~21% of the isolated ureolytic bacteria. Genus *Sporosarcina* included two species (*Sporosarcina luteola*, *Sporosarcina globispora*), genus *Pseudarthrobacter* included two species (*Pseudarthrobacter phenanthrenivorans*, *Pseudarthrobacter equi*). The *Sporosarcina luteola* that carries the *ure*C gene of the urease operon *ure*ABC has high urease activity. Additionally, *Sporosarcina globispora* also has urease activity confirmed (Cuaxinque-Flores et al., 2020). The remaining ureolytic genera in the sand was genus *Streptomyces*, which included two species (*Streptomyces kanamyceticus*, *Streptomyces qinglanensis*), comprised ~14% of the isolated ureolytic species. The following ureolytic species in the sand were from the genera *Devosia*, *Ramlibacter*, *Massilia*, *Noviherbaspirillum*, and *Methylovorus* comparisng ~15% of ureolytic species (Figure 2.14 b). Among the different genera of ureolytic bacteria, *Bacillus* (~37) was dominant in clay, and *Sporosarcina* (~21) was more abundant in sand, which are both known genera that can synthesize urease enzymes and have high ureolytic activity response from adding urea to the environment (Hsu et al., 2018). *Bacillus* capability to absorb heavy metals and biocrystallize to form

calcite makes it a promising microbe for MICP (Wong, 2015). The urease producing bacteria (UPB) in clay can be a reason for higher urease activity and calcite precipitation in clay dominant and sand autoclaved samples. Moreover, most microbiomes face a constant battle for space and resources that vary with the type of environment (Bakker et al., 2014). The soil autoclaving and mixing with (*Sporosarcina pasteurii)* strain in bio-augmentation eliminated the need for competition between microorganisms from the different soil types, which resulted in a higher calcite precipitation rate than naturally treated samples (section 2.9.2).

Table 2. 3. Percentage and dominant type of ureolytic bacteria in the natural sand and clay

Soil Type	**% of Ureolytic species**	**Dominating genus among Ureolytic species**
Natural Clay	~33	*Bacillus*, *Flavisolibacter*, *Microvirga* (~73%)
Natural Sand	~23	*Sporosarcina* and *Pseudarthrobacter* (~21%)

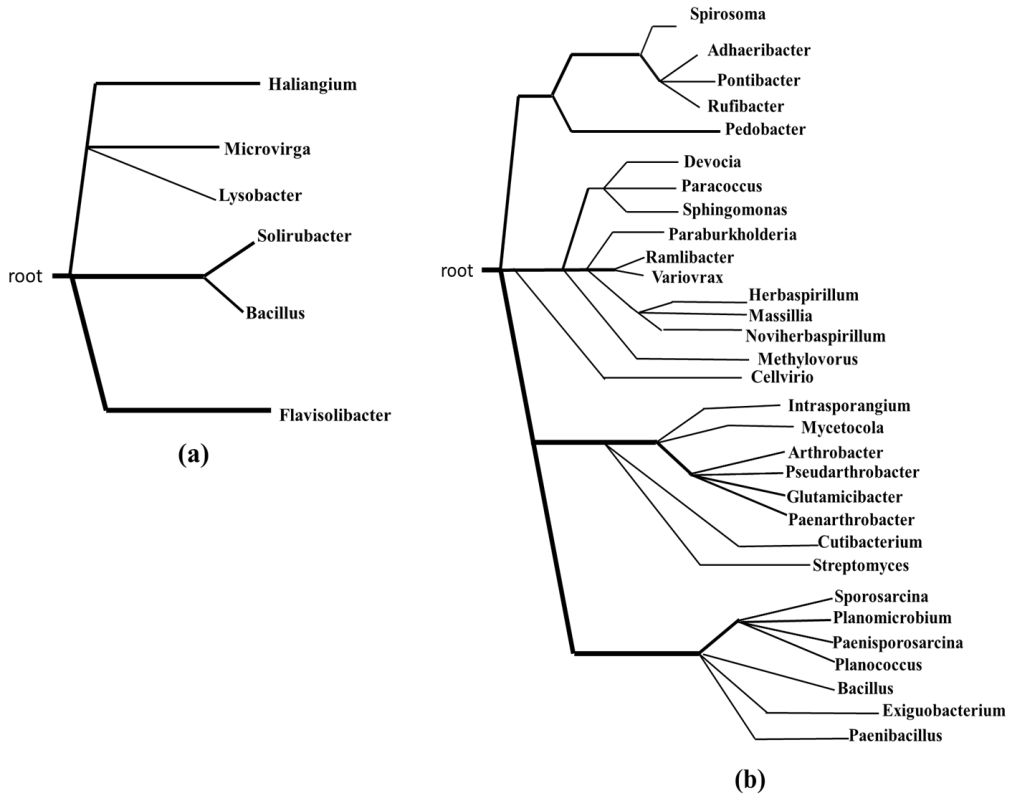


Figure 2. 13. The Genus phylogenetic tree based on 16S rRNA gene sequences and metagenomic identification of DNA with at least 1 % relative abundance isolated from clay (a) and sand (b)

2.12 SEM and EDX results

Mineralogical studies, including Scanning Electron Microscopy (SEM) and Electron Dispersive X-ray analysis (EDS or EDX), were performed in the present study on treated S85C15 and C100 samples. These tests were carried out in Idaho Microfabrication Laboratory (IML) facility at Boise State. SEM analysis allows a closer examination of the soil matrix, which helps detect matrix changes after treatments. The magnification range of well over 100,000 times and large 3-D depth field yield substantial information on the specimen surface structures and topography. Most SEM instruments are equipped with energy dispersive X-ray spectrometry (EDS), which provides information on

compositional characteristics in addition to the visual characteristics. EDS helps determine the elements/compounds formed at the particle level, forming calcium carbonate compounds. Coating the clay particles with a thin layer of carbon resulted in a conductive surface that reduced the charging in the particles. Studies showed that the morphology of $CaCO_3$ crystals could be governed by numerous factors, including microbial species, CO_2 concentration, Ca^{2+} Concentration, Ca^{2+}/Mg^{2+} ratio, the pH of the soil, ions in pore solutions and the rate of carbonation (Cizer et al., 2008; Qian et al., 2019). Figures 2.15 show the morphology and energy spectra of $CaCO_3$ formed in sample S85C15 with the highest amount of sand. A large amount of $CaCO_3$ particles in the dominant sand exhibited homogeneous rhombohedral crystals accumulated by regular plate-like structures. Also, the crystal particle size distribution was relatively uniform in the sample (Figure 2.15a). The same morphology of $CaCO_3$ was observed by Li et al. (2010) when they studied the effect of microbial carbonic anhydrase (CA) on the calcium carbonate ($CaCO_3$) precipitation. On the other hand, the crystal morphology of $CaCO_3$ induced in the clay sample (S18C62) was mainly irregular (2.16.b2), and some cubic shapes (2.16.b1) were observed. This can be related to the lack of enough spaces between clay particles to form regular-shaped morphology; it can also be related to foreign components like magnesium (Mg^{2+}) in the surrounding environment. According to research conducted by Qian et al. (2019), Mg^{2+} can replace part of Ca^{2+} and induce lattice distortion, thereby changing the morphology of $CaCO_3$ crystals and increasing the degree of irregularity (Meldrum and Hyde, 2001). Studying the changes in Mg^{2+} and other ions in the soil during MICP treatment is one of the focuses of our subsequent publication. One further important observation in SEM images (Microscale studies) was that, like macro-scale studies, the

distribution of $CaCO_3$ in soil S18C62 was uneven; it can be attributed to small pore space and heterogeneous distribution of treatment solutions during MICP.

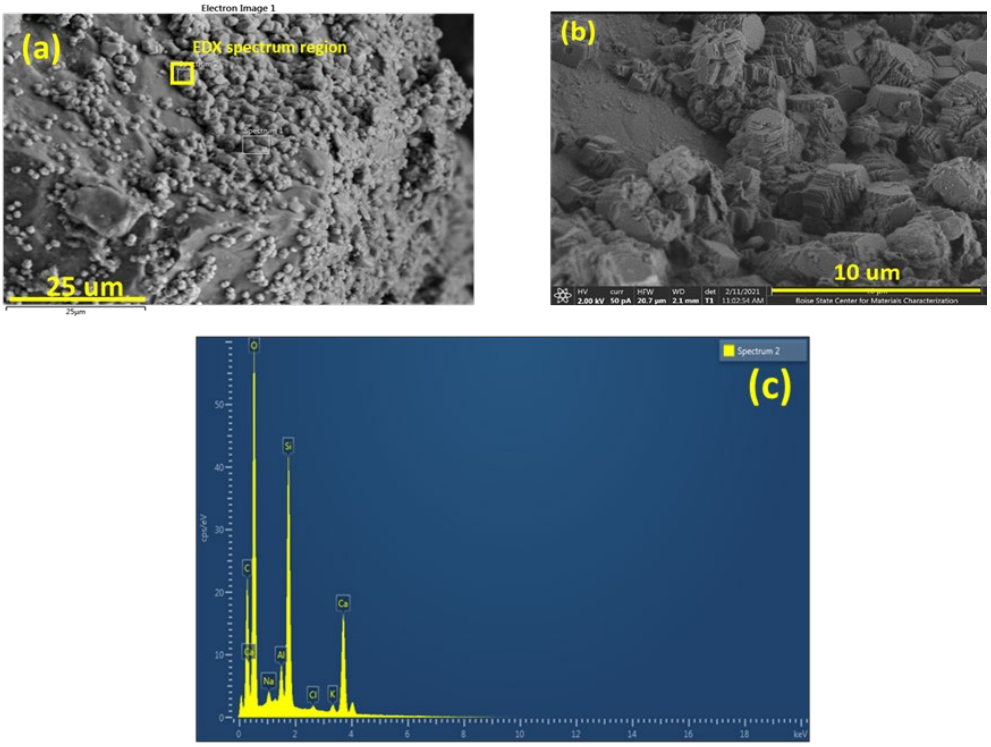


Figure 2. 14. SEMs with (a) EDX spectrum region (b) the layered and rhombic crystal structures of CaCO3 (c) the EDX result in sand

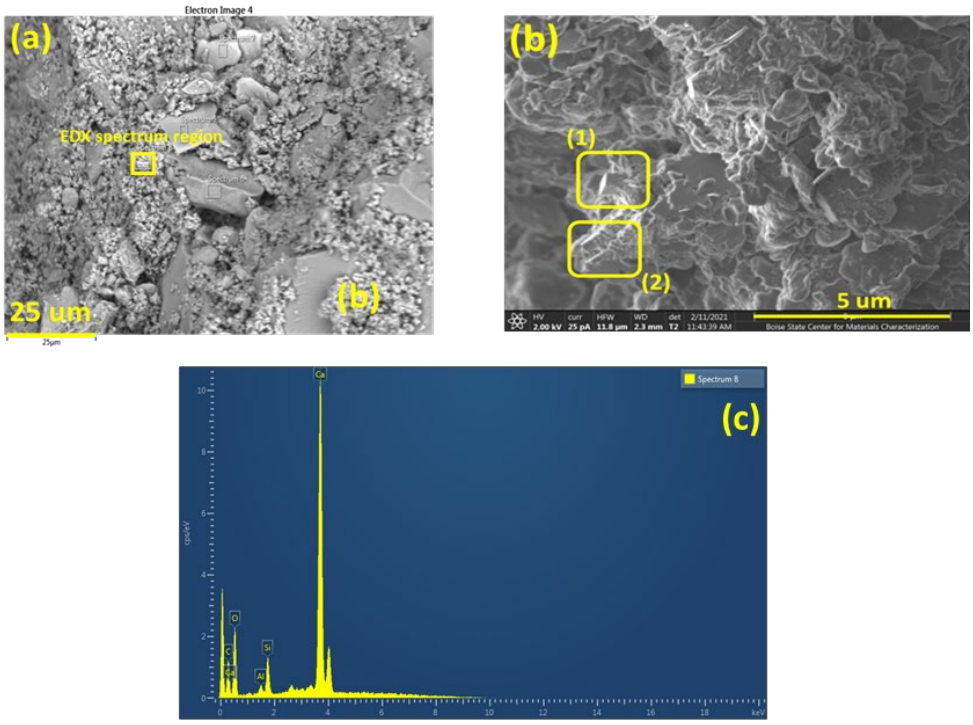


Figure 2. 15. SEMs with (a) EDX spectrum region (b.1) the cubic crystal structures of CaCO3 (c) the EDX result in clay

2.13 Summary and Findings

The main objective of this research was to examine urease activity and calcite precipitation in soils with different amounts of clay content. For this purpose, four artificial clay/sand mixes were prepared by mixing sand with natural clay in sterile and non-sterile conditions. Urease activity of soils was measured, and samples were treated using the MICP method for seven rounds. $CaCO_3$ content and followed an increase in shear strength were measured in soil samples with indigenous bacteria (bio-stimulation) and added foreign bacteria (bio-augmentation). The gene sequencing methods were applied to quantify the ureolytic activity in clay and sandy soils. The increase in shear strength of all treated soil samples was examined, and in the final step, $CaCo_3$ morphology was studied by SEM and EDX methods.

The test results showed that clay has more urease activity and precipitation calcite than sand despite the two having similar relative populations of indigenous ureolytic bacteria (~23% to 33%). Clayey soils can make an incubated environment for bacterial growth due to clay's high absorption capacity of organic molecules, polymers, ions, heavy metals, bacteria, and other organic or inorganic matter (Neubauer et al., 2019). Moreover, clay-autoclaved samples showed more urease reduction. The post-treatment $CaCO_3$ activity can be due to the dissociation of the bacterial community and soil organic matter from autoclaving clay. The bacteria identification via 16s rRNA long reads showed a relatively high abundance of the genera *Bacillus* (~37%) and *Flavisolibacter*(~27%), which are ureolytic bacteria isolated from clay, while the dominant ureolytic genera in the sand were *Sporosarcina* and *Pseudarthrobacter,* each accounting for ~21% of total ureolytics. To remove the native bacteria from soils and study the influence of soil gradation on MICP, autoclaved soil samples were augmented with *S. pasteurii* and subjected to MICP. Bio-augmentation and identification of the indigenous microbes confirmed that clay is a more suitable environment for ureolytic activity.

After MICP performance, the shear strength increases in all soil samples. After treatment, the increase in shear strength was highest (~132%) in the sample with the highest amount of clay (S18C62). The morphology of $CaCO_3$ particles in the sand were homogeneous rhombohedral crystals while it was mainly irregular in clayey soil; this can be attributed to lack of space between clay particles and/or existing foreign components like magnesium (Mg^{2+}) in the soil environment.

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CHAPTER 3: THE ROLE OF UREOLYTIC MICROBIAL COMMUNITIES IN FACILITATING MICP IN CLAYEY SOILS

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Abstract

Microbially Induced Calcium Carbonate Precipitation (MICP) is a biological soil treatment technique where microbes play a key role in precipitating calcium carbonate and cementing soil grains together. Analyzing microbial communities and their ureolytic activities is essential for understanding MICP and its applications in soils. Although researchers have demonstrated biogeochemical changes during the MICP approach, little is known about the impact of native ureolytic microbial communities (NUMC) and their responses to the MICP performance in clayey soils. In this work, we examine NUMC in 5 distinct natural clayey soils from three different geographical locations in the USA. Each soil was subjected to 7 cycles of MICP treatment. 16S rRNA gene sequencing was used to determine the type and relative populations of dominant bacterial communities before and after each cycle of MICP treatments. We found that the ureolytic bacteria communities outgrow non-ureolytic species with MICP treatment and eventually dominate the landscape of the bacterial population. Moreover, the ureolytic species of *Sporosarcina koreensis*, *Sporosarcina luteola,* and *Sporosarcina soli* account for the most classified ureolytic species after the third cycle of MICP treatment. Additionally, we measured the amount of precipitated $CaCO_3$ using Rapid Carbonate Analysis (RCA) and examined its relationship to the bacterial community in soils. Results show that the increase in the ureolytic bacteria population is accompanied by a significant increase in $CaCO_3$ precipitation. Additionally, it was noted that the precipitated $CaCO_3$ is inversely related to Soil Organic Carbon (SOC), while no dependency was observed on urease activity and NUMC. In short, this study shows that the MICP process results in the domination of *Sporosarcina* strains that lead to $CaCO_3$ precipitation in soils regardless of the origin of the soils and their initial makeup of

the urease-producing bacteria. The outcome of this study is not only important in the optimization of MICP but also is crucial for tailored applications of the MICP in various soil stabilization applications.

Keywords: MICP, ureolytic microbial communities, urease activity, clayey soils.

3.1 Introduction

Microbial-Induced Calcite Precipitation (MICP) involves a biochemical reaction that can be used as an environment-friendly bio-mediated soil improvement technology. Figure 1 depicts the biochemical reactions during the MICP process. In MICP, urea is decomposed to CO_3^{2-} and NH_4^+ by the urease enzyme secreted from bacterial cells. This results in an increase in the pH increase within the soil matrix (equation (3.1)). In the presence of Ca^{2+} ions in a high pH environment, MICP can occur (equation (3.2)). The bacterial cell walls contain negatively charged functional groups (Dittrich & Sibler, 2005; Fein, 2006), which make the surface of the bacteria electrostatically favorable for adsorption of Ca^{2+} (equation (3.3)). This results in the bacteria acting as nucleation sites for the formation of $CaCO_3$ (equation (3.4)). (Bang et al., 2010; Fujita et al., 2008; Whiffin et al., 2007):

$$NH_2 - CO - NH_2 + 2H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-} \quad \uparrow pH \qquad (3.1)$$

$$CaCl_2 \rightarrow Ca^{2+} + 2Cl^- \qquad (3.2)$$

$$Ca^{2+} + Cell \rightarrow Ca^{2+} - Cell \qquad (3.3)$$

$$CO_3^{2-} + Cell + Ca^{2+} \rightarrow Cell - CaCO_3 \qquad (3.4)$$

There are two approaches for MICP applications: bioaugmentation and biostimulation. In bioaugmentation, ureolytic microorganisms are introduced into the soil to act as precipitation agents (Burbank et al., 2011; Fujita et al., 2000; Gomez et al., 2015, 2017). On the other hand, the biostimulation approach uses indigenous microorganisms in the soil. These microorganisms are enriched in the soil by modifying the existing field conditions. In biostimulation, nutrients and electron acceptors are added to the field to stimulate the native bacterial species to help with $CaCO_3$ precipitation (Snoeyenbos-West et al., 2000). MICP through biostimulation is found to be more economically viable and can help prevent competition between the indigenous and introduced (in the case of bioaugmentation) bacteria (Wenderoth et al., 2003). In a study by Graddy et al. (2021), the bioaugmented method with the exogenous ureolytic bacterium *S. pasteurii* was compared to the biostimulation method using indigenous bacteria on the same soils. Based on 16S rRNA gene sequences, it was shown that native bacteria outcompeted the inoculated strain of S. *Pasteurii* in the presence of nutritional cementation solutions.

A variety of microorganisms can induce carbonate precipitation by altering solution chemistry (Castanier et al., 1999; De Muynck et al., 2013; DeJong et al., 2010; Riding, 2000) and serving as a crystal nucleus for $CaCO_3$ (Aloisi et al., 2006). The diverse metabolic pathways of microbes including photosynthesis, ureolysis, ammonification, denitrification, and methane oxidation, are responsible for inducing calcium carbonate precipitation by influencing redox conditions in natural systems (Braissant et al., 2007; Dupraz et al., 2009). Among all metabolic pathways, ureolysis accounts for 43% of the metabolic activity in soils and has been the main focus of research on the terrestrial systems

(Zhu & Dittrich, 2016). Furthermore, most microbial cells provide nucleation sites for carbonate formation in MICP.

Understanding the role of the native ureolytic microbial community (NUMC) in MICP is critical in optimizing the applications of this method in soils. To that end, a precise characterization of microbial systems and urease-producing bacteria (UPB) in MICP is essential in developing optimization strategies capable of enhancing the competitive advantages of MICP over traditional soil improvement methods. However, to the best of the authors' knowledge, there are no studies that looked at understanding this aspect of MICP. To address this knowledge gap, we use 16s rRNA gene sequencing to report on the bacterial species changes caused by MICP treatments in 5 types of natural clayey soils. Furthermore, urease activity, calcite precipitation, and soil organic carbon (SOC) were quantified using urease assay, rapid carbonate analysis, and dynamic flash combustion experiments, respectively, and correlated with microbial community changes.

3.2 Background

MICP is currently appraised for improving sands and silty sands (Cheng et al., 2013; Qabany & Soga, 2013; Whiffin et al., 2007), but researches show clay minerals have a positive interaction in the MICP performance (Cardoso et al., 2018; Fomina & Skorochod, 2020; Sun et al., 2019). Clayey soils also have more organic materials than other soil types, such as sand and silt. Moreover, it was shown that urease could be largely adsorbed on clay and organic material (Pinck et al., 1954; Wilson et al., 1999; Zantua et al., 1977); this adsorption on clay particles preserves the activity of the enzyme and protects it from destruction by soil-microorganisms (Pinck et al., 1954). In a research experiment, Taylor et al. (2002) quantified and compared microbial presence in soil cores taken from

two soil profiles: sand and clay. They observed a strong positive correlation (~0.972) between clay content and urease activity, and a strong negative correlation between sand content and urease activity (R>0.95). They suggested that the studied clay samples can retain and protect urease either in an active extracellular urease form or ureolytic microbial biomass. In a similar study, Sun et al. (2019) examined the effect of contents of kaolin clay content and its ions on bacterial urease activity and the rates for calcium carbonate precipitation in sand-clay mixtures. Their results show that kaolin has a positive effect on the urease activity of bacteria. Furthermore, studying the presence and activity of ureolytic microbial components in soils is pivotal in comprehending MICP and its in-situ application in clayey soils. Bacteria that can hydrolyze urea are common in all soils, including clayey soils. Lloyd & Sheaffee (1973) concluded that approximately 17 to 30 percent of the total bacterial population of six examined soils could hydrolyze urea; these bacteria included aerobes, microaerophilic and anaerobes. Many researchers used 16S rRNA gene sequencing to isolate halotolerant and alkalophilic strains of urease-producing bacteria from different environmental samples. In a study by Chu et al. (2012), urease-producing bacteria isolated from tropical beach sand were used to reduce soil permeability through $CaCO_3$ precipitation. The 16S rRNA gene sequences of the isolated strain showed that the strain represented *Bacillus* sp. Throughout the literature search, it was noted that the genus *Bacillus* was one of the most abundant isolated NUMC (Bibi et al., 2018; Roberge & Knowles, 1967; Skujiņš & Burns, 1976). Isolating NUMC strains and detecting their urease activity has been the focus of intensive research for at least the past two decades. However, despite all these efforts, there are currently no studies investigating the impact of ureolytic bacterial communities on MICP performance or the population changes within MICP

cycles. We try to bridge this gap by studying the bacterial communities through gene sequencing and evaluating the soil's urease activities using the calorimetry method. Filling this gap is key to the optimization of MICP technology in various applications.

3.3 Materials & Methods

3.3.1 Soils

Table 3.1 illustrates the physical properties of the 5 natural soils used in this paper. To understand different ureolytic bacterial communities in clayey soils from different geographical origins, five clayey soils were collected from Idaho (ID), Montana (MT), and Texas (TX). The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), and BR (Big Route, MT), NTP (North three forks, MT), and TX (Texas) after the cities they came from. Soil classification was determined according to Unified Soil Classification System (USCS). Sieve and Hydrometer Analysis (ASTM D422) were conducted to determine the soil gradation for all soils.

Table 3. 1. The physical properties of 5 natural soils used in this study

Soil	Gradation (ASTM D422)			Classification	Liquid Limit (%)	Plasticity Index (%)
	Sand (%)	Clay (%)	Silt (%)	USCS	(ASTM D4318)	
MS	3	96	1	CH	100	60
NTP	3	72	25	CH	61	35
TX	18	64	18	CH	59	33
GF	16	81	3	CL	40	22
BR	6	33	61	CL	42	16
DC	16	30	54	CL	37	17

Note: LL-Liquid Limit; PI-Plasticity Index; USCS-Unified Soil Classification System.

3.4 MICP treatment and collecting samples

To prepare soil samples for 16S rRNA sequencing and calcite precipitation, 45 gm of soil was placed into a plastic vial (~56 ml, see Figure 3.1) and submerged with an enrichment solution containing 0.5% (by volume) Grandma's molasses, 170 mM sodium acetate, 0.5 g/L Bacto yeast extract, and 333 mM urea (Burbank et al., 2012). The enrichment cycle lasted 48 h, during which the sample was left on the countertop. In the next step, for the bio-mineralization process to start, soils were drained and then submerged with a cementation solution containing 170 mM sodium acetate, 0.5 g/L Bacto yeast extract, 333 mM urea, along with 250 mM of Calcium Chloride (Burbank et al., 2012). Each cementation cycle was maintained for 48 h, similar to the enrichment cycle, after which the cementation solution was drained, and a fresh cementation solution was added. A total of seven cementation cycles were conducted on each sample over 14 days. Before adding every cementation cycle, the pH of the soil solutions was tested and noted that it fluctuated above 9 during the treatments. After 1, 3, and 7 cycles of cementation treatments, soil samples were collected from the top and bottom of the vials; similar sampling was also made after the first enrichment cycle. These samples were used for the various testing performed on the samples.

For the DNA extraction and sequencing, samples were wrapped and placed on dry ice inside foam containers and shipped overnight to the microbiology lab at the University of Houston (UH), Houston, Texas. The collected soil samples used the following notation, *Name of soil-# of treatment cycle-location of the sample (top or bottom)*. For example, a sample collected from the top of a soil sample from Marsing, Idaho, subjected to 3 cycles of treatment would be denoted using the notation MA-3T; similarly, a sample collected

from the bottom of a soil sample from Texas subjected to 7 cycles of treatment would be denoted using the notation TX-7B. Table 3.2 presents the notations for all soils tested at different treatment cycles.

Table 3. 2. Notations used for all soils tested in this research

Soil Origin	Location	Enrichment cycle	1st cycle	3rd cycle	7th cycle
Marsing (MA)	Top (T)	MA-ENT	MA-1T	MA-3T	MA-7T
	Bottom (B)	MA-ENB	MA-1B	MA-3B	MA-7B
North three forks (NTP)	Top (T)	NTP-ENT	NTP-1T	NTP-3T	NTP-7T
	Bottom (B)	NTP-ENB	NTP-1B	NTP-3B	NTP-7B
Texas (TX)	Top (T)	TX-ENT	TX-1T	TX-3T	TX-7T
	Bottom (B)	TX-ENB	TX-1B	TX-3B	TX-7B
Great Falls (GF)	Top (T)	GF-ENT	GF-1T	GF-3T	GF-7T
	Bottom(B)	GF-ENB	GF-1B	GF-3B	GF-7B
Big Route (BR)	Top (T)	BR-ENT	BR-1T	BR-3T	BR-7T
	Bottom (B)	BR-ENB	BR-1B	BR-3B	BR-7B

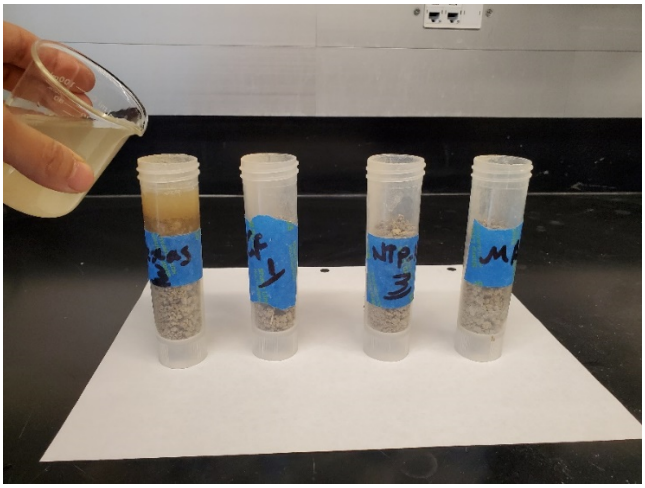

Figure 3. 1. Soils in plastic vials submerging with treatment solutions.

3.5 Evaluation Tests

3.5.1 DNA Extraction and 16S rRNA Library Prep

Deoxyribonucleic Acid (DNA) was extracted from each soil sample and sent to UH to prepare the 16S ribosomal RNA (16S rRNA) library. Aliquots of ~0.5g of sample were the starting material for the FastDNA Spin Kit, (MP Biomedicals, U.S) and were prepared according to the manufacturer's instructions. After the DNA was eluted, the DNA concentration was measured with a BioTek Synergy H4 Hybrid Microplate Reader (Fisher Scientific, Göteborg, Sweden). Furthermore, to determine sample quality, the Optical Density (OD) ratio of 260/280 was measured with the BioTek Synergy H4 Hybrid Microplate Reader, with an average of ~1.6 -1.8. The microbe's 16S rRNA region was amplified using Oxford Nanopore Technologies (ONT) 16S Barcoding Kit 1-24 (SQK-16S024). Each sample was assigned a barcode from the 16S Barcoding Primers (1-24) with 10 ng of high DNA molecular weight (Manzari et al., 2020). The forward 16S primer was: 5' ATCGCCTACCGTGAC-barcode AGAGTTTGATCMTGGCTCAG 3', and the reverse 16S primer was: 5' ATCGCCTACCGTGAC-barcode-CGGTTACCTTGTTACGACTT 3'. The 50 µL PCR reaction was composed of 10 µL of complementary DNA (cDNA) template, 10 µL of selected barcode, 25 µL of LongAmp Taq 2X Master Mix (New England Biolabs, U.S), and 5 µL of nuclease-free water. The thermal cycling conditions followed ONT's protocol: 95 °C for 1 min, denaturation for 25 cycles at 95 °C for 20 s, annealing at 55 °C for 30 s, extension at 65 °C for 2 min, and a final extension at 65 °C for 5 min. The cleanup and barcoded library were pooled per ONT's 16S Barcoding Kit 1-24 protocol.

3.5.2 Metagenomic Sequencing and Analysis

All samples were sequenced with an ONT MinION Mk-1C device for 6 hours, and each library was sequenced three times. All the 16S sequences generated herein were deposited in the NCBI database with BioProject ID: PRJNA838655. The sequences assigned to taxonomy with Usearch command (Usearch11) and the 16S rRNA database (RDP 16S training set v16, RTS) (Edgar, 2017). The taxonomical classification generated SINTAX files for each sample. The SINTAX files were pre-processed with R scripts from Mann and collaborators to filter information and create a counts table (Mann et al., 2021). The R-scripts generated a tabular format of operational taxonomical unit (OTU) as a text file converted to a tsv file. The OUT.tsv file was accompanied by a metadata mapping .tsv file and uploaded into the MicrobiomeAnalyst platform for metagenomic relative abundance analysis (Shetty & Lahti, 2019). The fastq files were also uploaded to the ONT EPI2ME desktop agent platform for microbial identity analysis.

3.5.3 Urease activity and $CaCO_3$ content determination tests

Urease activity was measured for all samples. Urease activity was determined by measuring ammonia concentration in the samples using the colorimetric method as suggested by Bremner and Douglas (1971). Figure 3.2a shows the Portable Spectrometer for measuring ammonia concentration in the colorimetric method, and Figure 3.2b shows Carbonate Analyzer for measuring $CaCO_3$ content in soil samples. Precipitated calcium carbonate was detected using Rapid Carbonate Analyzer (D4373-96). In this method, calcium carbonate reacts with HCL as shown in the following reaction (Equation (3.5)): CO_2 pressure was measured, and the amount of $CaCO_3$ was determined by reading from a calibration chart.

$$CaCO_3 + 2H^{+1} \rightarrow Ca^{+2} + H_2O + CO_2 \qquad (3.5)$$

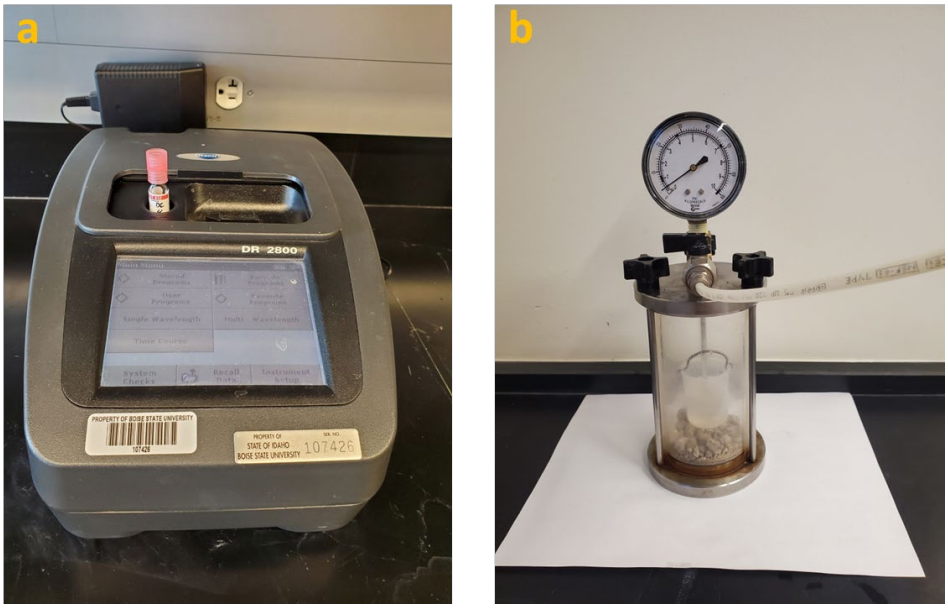

Figure 3. 2. Portable Spectrometer and b. Rapid Carbonate Analyzer.

3.5.3 Organic Carbon and Nitrogen determination test

Total carbon and nitrogen were measured by dry combustion of dried, ground samples using a CN analyzer (Flash EA 2000 series, Thermo Scientific, NC Soil Analyzer) (Figure 4). The FLASH 2000 Elemental Analyzer operates according to the dynamic flash combustion of the sample. Samples are weighted in tin capsules and introduced into the combustion reactor via the Thermo Scientific™ MAS 200R Auto sampler together with oxygen. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper, then swept through an H_2O trap, a GC column and finally detected by a thermal conductivity detector (TCD) (Krotz, n.d.).

3.6 Results & Discussion

3.6.1 Relative abundance of the bacterial community in natural and MICP treated soils

To optimize MICP as a potential *in situ* soil improvement technique, it is essential to understand the effect of MICP treatments on the indigenous microbial community. The identity of the microbial community was determined by sequencing the 16S rRNA region with the (ONT) minION Mk-1C and assigning the taxonomy with USEARCH and EPI2ME platforms which are sequence analysis tools that assign taxonomy. We examined the relative abundance of bacterial genera in all five soils before and after each round of MICP treatment. The relative abundance analysis cut-off was 1%, and they were included as "other" because the identification is highly variable at very low abundances. Figure 3.3 shows the relative abundance of bacteria in all 5 natural soils after different rounds of MICP using soil samples collected from the top and bottom of the plastic vials. The sequencing data shows that before the MICP treatment is applied, a significant portion of the microbial population in all five natural clayey soils is composed of non-ureolytic *Mycoplasma* strain.

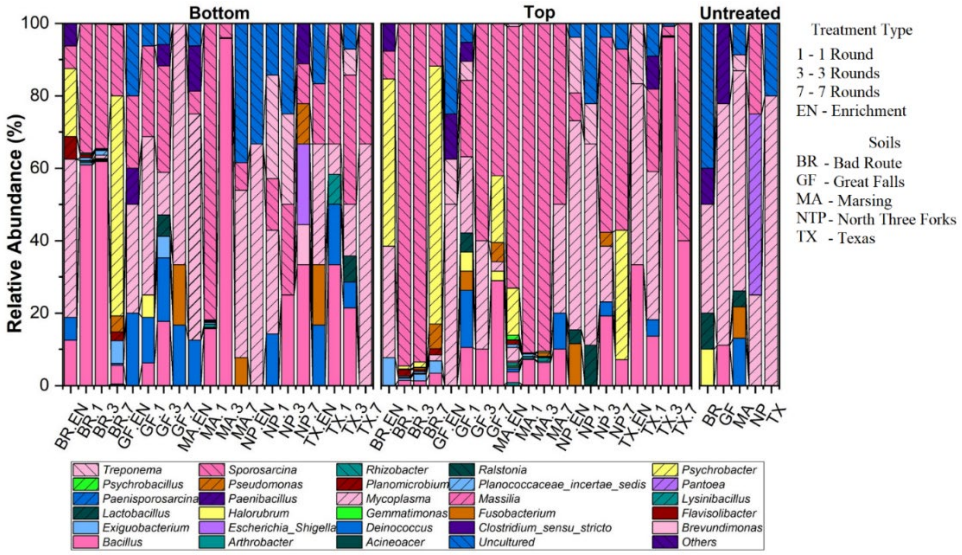

Figure 3. 3. Overall relative abundance of bacteria genera in all five soils tested in this research

To further understand the variations in microbial populations, pie charts are prepared using the data presented in Figure 3.3. The relative abundances for all soils are extracted and plotted as pie chart before treatment and at different points throughout the treatment process. Marsing (MA) and Big Route (BR) soils have exhibited the highest and lowest calcite precipitation, respectively; so, we focus on them for reporting the evolution of microbial populations in this section. Other soils show similar trends and their bacterial population profiles are reported in the appendix. Typical results for MA and BR soils before and after MICP treatments are presented in Figures 3.4 and 3.5, respectively. In the case of untreated MA soil (Figure 3.4), we detected ~61% of *Mycoplasma*, 13% non-ureolytic *Deinococcus*, 9% non-ureolytic *Fusobacterium*, and 4% ureolytic *Lactobacillus* and *Treponema*. In comparison, the untreated BR soil (Figure 3.5) showed ~30% *Mycoplasma*, followed by non-ureolytic *Halorubrum* (10%) and ureolytic *Lactobacillus* (10%). Most taxa were not identified and labeled as uncultured at the genus level.

After the MICP treatment was applied, the population of ureolytic genera grew significantly in all soils. By the third round of treatment, the relative abundance of *Mycoplasma* had declined to below the detection limit, and ureolytic bacteria dominated the relative abundance of bacteria. We found that two genera, *Sporosarcina* and *Bacillus*, outcompete all others and became prevalent with the MICP treatments in all samples. Many studies isolate and use *Sporosarcina* and *Bacillus* as urease-producing bacteria since they have shown good adaptability and high calcium carbonate yields (Tang et al., 2020). In our soils, the population of these genera initially increased with each round of treatment. After the third treatment, the relative abundance of *Sporosarcina* increased to 91% and 93% in MA and BR soil, respectively. The ureolytic genus *Bacillus* also increased due to MICP

treatment; although, its abundance is higher after the seventh round of treatment when the population of *Sporosarcina* declines. These community changes can be explained by competition over macro and micro-nutritional resources and changes in physicochemical properties of soil, such as pH (Mandakovic et al., 2018). In all other soils, the same trends were generally observed, and *Mycoplasma* sp. and other natural soils genera such as *Psychrobacter* and *Deinococcus*, which started growing again in the soil's microbiome by the seventh round of MICP (please see appendix A for more details). The decreasing favorable conditions or competition for *Sporosarcina* growth after the third round of treatment can describe this trend.

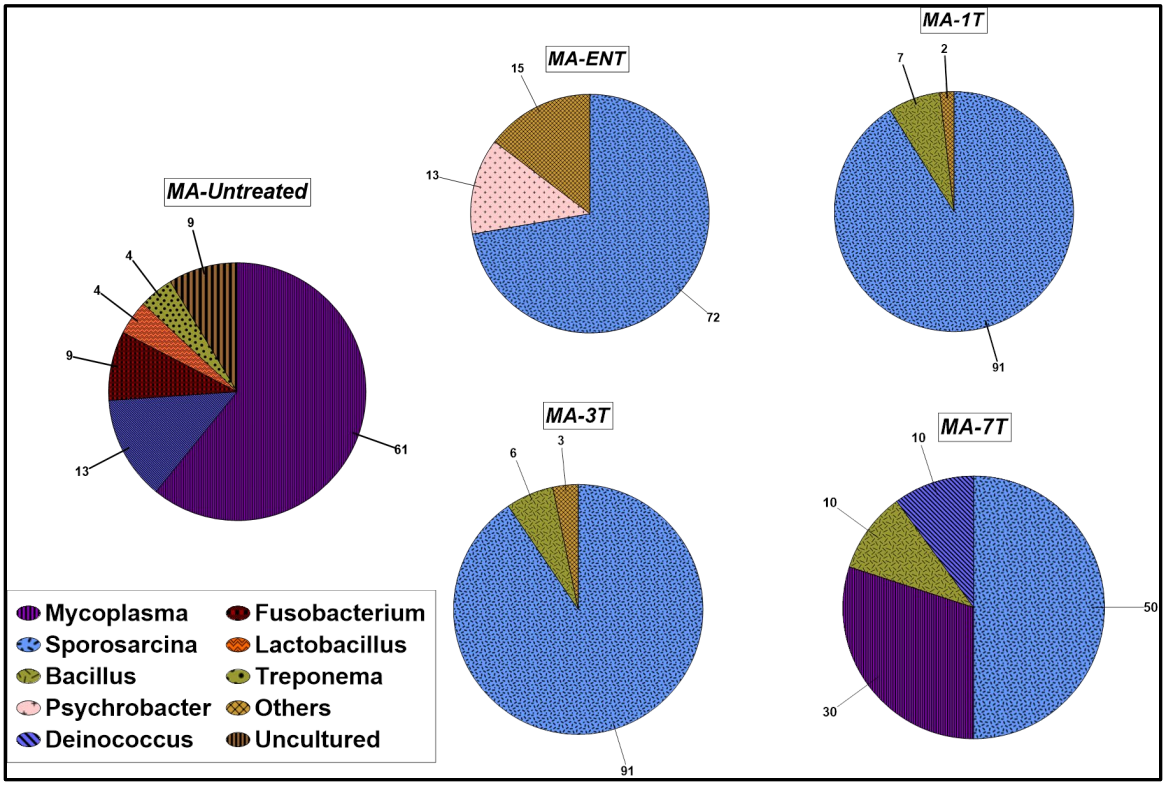

Figure 3. 4. The genus relative abundance at top of MA soil.

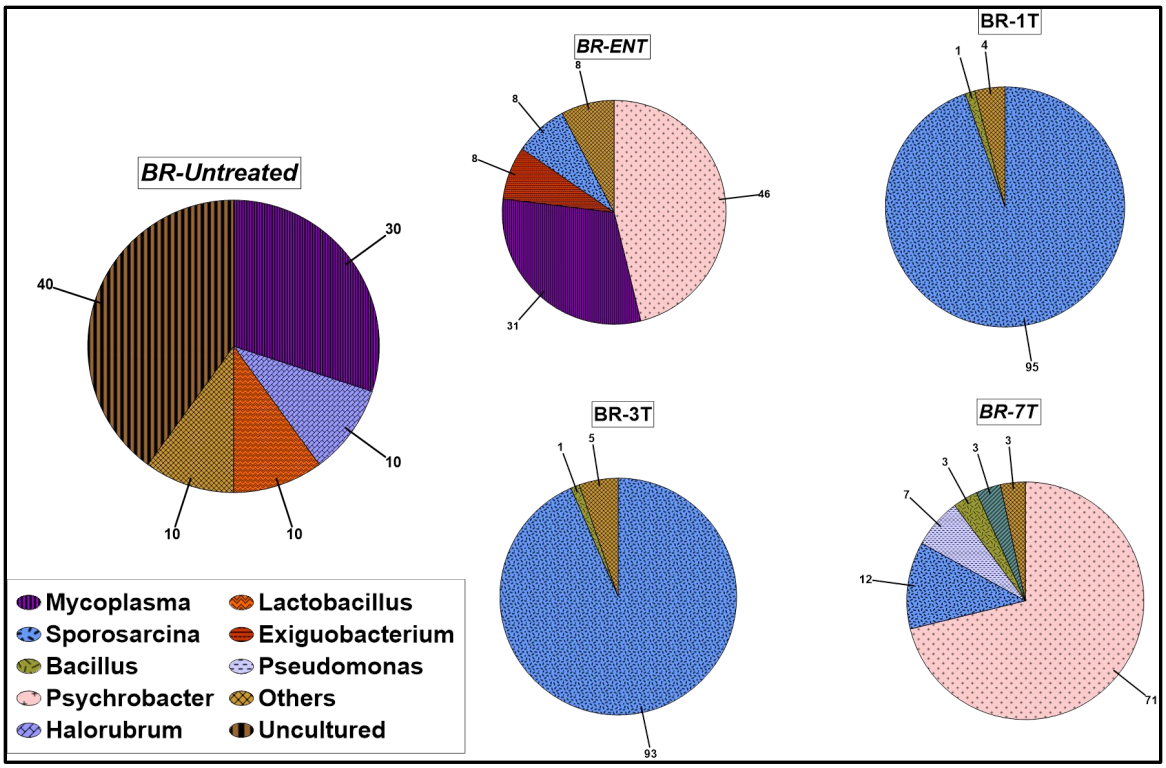

Figure 3. 5. The genus relative abundance at top of BR soil.

3.6.2 Relative abundance of bacterial community at top and bottom of soils

Figures 3.6 and 3.7 depict the competition of *Bacillus* and *Sporosarcina* strains on the top and bottom of the samples after the third round of treatment. Results show that *Sporosarcina* dominates the top portion of the samples for all samples, while *Bacillus* has the highest abundance in the bottom soil. *Bacillus* strain forms ~ 96% of all bacterial species in the bottom of the MA and TX soils (Figure 3.6). This can be related to the high bulk density at the bottom of the soil, which makes it a more favorable condition for *Bacillus* to grow (Juyal et al., 2018). On the other hand, *Sporosarcina* was higher at the top of soils than at the bottom (Figure 3.7). This could be because of biomass flocculation formation that improves the retention rate of *Sporosarcina* on the top of the soil (Yang et al., 2022). Studies demonstrated that bacterial cells are flocculated by divalent cations, such as Ca^{2+}, Mg^{2+}, etc. (Cheng et al., 2019; Zheng et al., 2008). In the MICP process, these

cations are more available at the top of the soil than at the bottom. The higher abundance of *Sporosarcina* strain at the top of soils can explain agglomerated morphology and higher precipitated calcite on top of soils (Figure 12a).

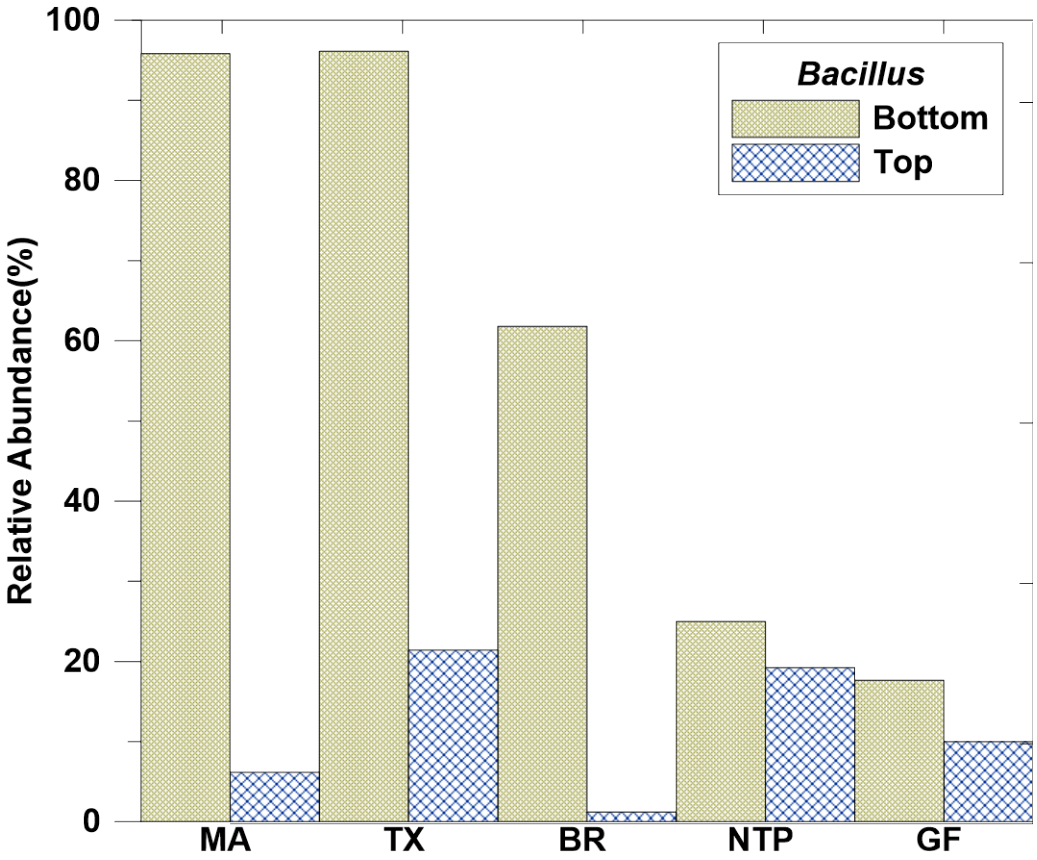

Figure 3. 6. Relative abundance of Bacillus Sp. at top and bottom of soils

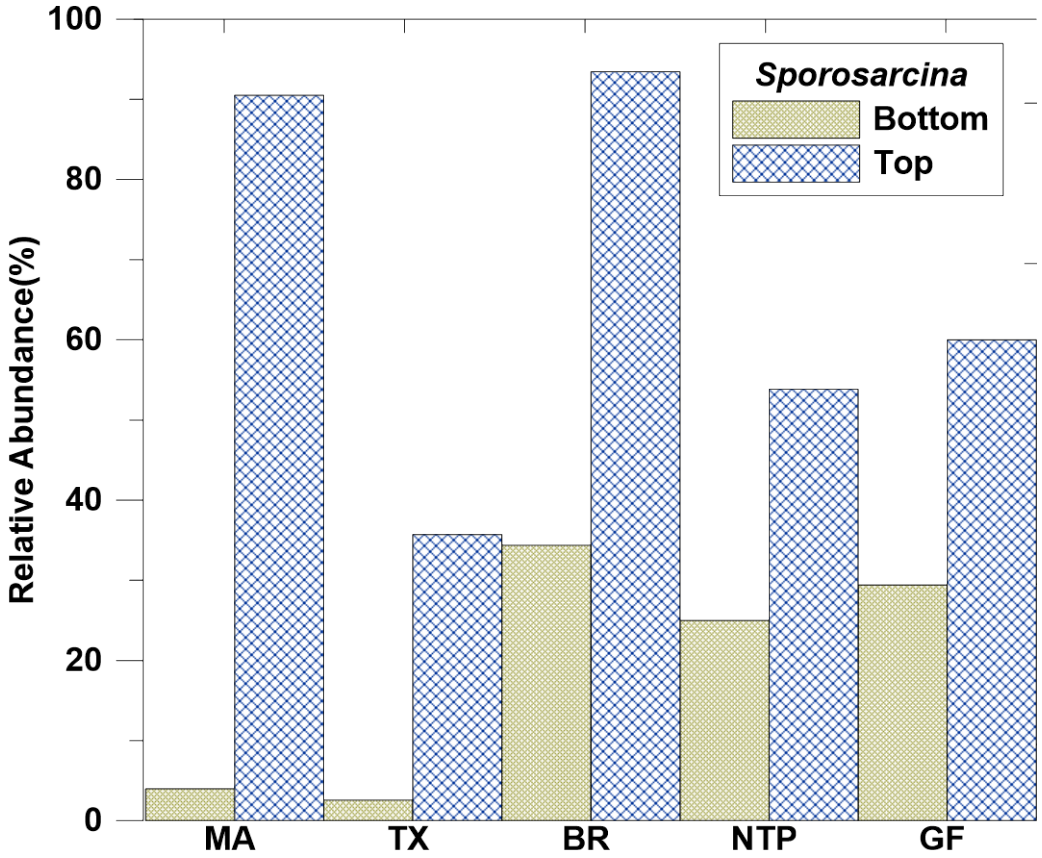

Figure 3. 7. Relative abundance of Sporosarcina Sp. at top and bottom of soils.

3.6.3 Dominant species of Sporosarcina and Bacillus

The dominant S*porosarcina* species at the top and bottom of MA and BR soils were determined using ONT's EPI2ME platform and shown in Figures 3.8 and 3.9. The 16s rRNA sequencing data in Figure 3.8 shows that three *Sporosarcina* species dominate other species in MICP-treated soils: *S. koreensis, S. luteola*, and *S. Soli*. This decrease in the diversity and variation in abundance of bacterial species can be attributed to adding MICP nitrogen-rich nutrient solutions that favor those relying on nitrogen as an energy source (Staley et al., 2018; Zhang et al., 2019). Due to less access to nutrient sources at the bottom of the samples, all ureolytic species' relative abundance was lower than those at the top (similar observations are obtained for all other soil types). Notice, however, that the three

dominant *Sporsarcina* species are the same at the top and bottom of each sample, and the difference is only in their abundance. Figure 3.9 depicts the abundance of dominant *Bacillus* strains in the MICP-treated MA and BR samples. We see that the *Bacillus* species dominating at the top of the sample are from different species than those at the top. This data shows that MICP treatment has significant soil-specific impacts on ureolytic species. Future research will be necessary to characterize how these specific *Sporosarcina* and *Bacillus* strains reacted to MICP.

Most research that focuses on the bio-augmentation method uses *S. pasteurii* ATCC 11859 (standard strain) to trigger MICP. Here, we report other bacterial species with the *Sporsarcina* genus showing a significant response to MICP treatment. We propose considering these as candidates for Bio-augmentation treatment of soils containing these same native species. The advantage of this proposition is that adding ureolytic species that are the same as soil's indigenous ureolytic bacteria eliminates the competition between externally added species such as *S. pasteurii* and native ureolytic species (Graddy et al., 2021); and consequently may improve the efficiency of the standard bio-augmentation process.

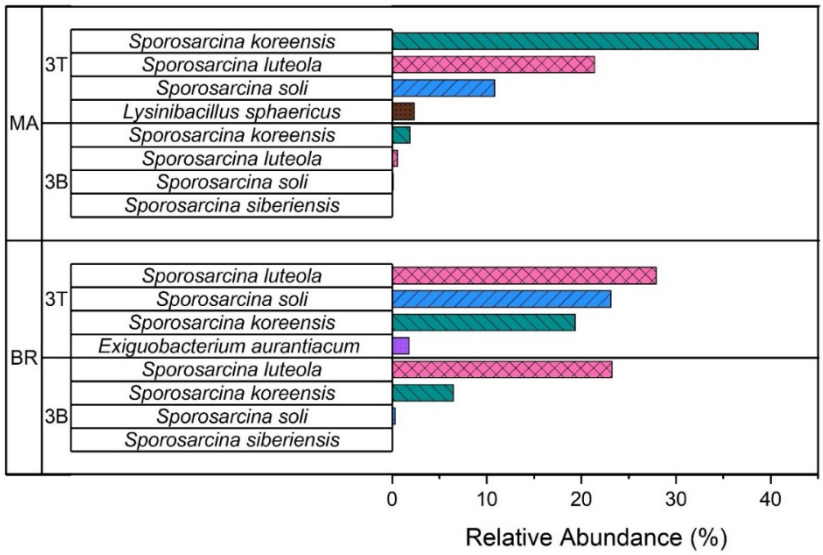

Figure 3. 8. The dominant strains of Sporosarcina Sp. in MA and BR soils.

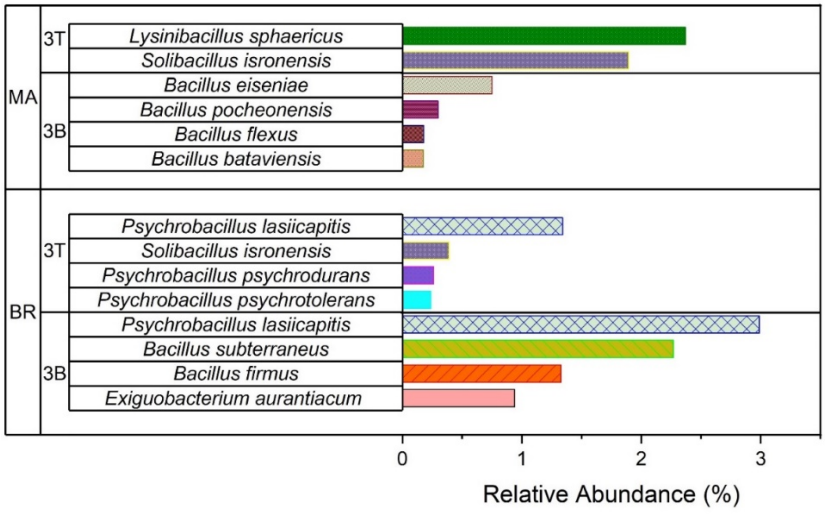

Figure 3. 9. The dominant strains of Bacillus Sp. in MA and BR soils.

3.6.4 Urease activity, Organic Carbon, and $CaCO_3$ precipitation

The urease activity and $CaCO_3$ precipitation for each sample are shown in Table 3.2. It is seen that calcite precipitation is significantly higher at the top portion of the

sample compared to the bottom; this is because of higher retention of urease activity due to accumulation of biomass in top soil (Yang et al., 2022)(section 3.6.2). We also find that $CaCO_3$ precipitation exhibits an inverse linear correlation with organic carbon (Figure 3.10). This can be related to the high amount of Organic Carbon (OC) content adsorbed to soil particles which decrease the reaction surface area. However, $CaCO_3$ precipitation does not show a correlation with urease activity and with the relative abundance of urease producing bacteria (UPB). (Figures 3.11).

Table 3. 3. Urease activity and precipitated calcite in top and bottom of soils

Soil	urease activity µmol/min	Bottom $CaCO_3$ Precipitation (%)	Top $CaCO_3$ Precipitation (%)	OC (%)	N (%)	pH
MA	0.08	4.68	18.72	0.56	0.05	7.47
GF	0.17	1.78	10.53	1.58	0.08	8.49
BR	0.05	0.78	5.07	2.80	0.04	7.82
NTP	0.18	1.78	7.8	1.83	0.08	8.04
TX	0.05	1.63	6.63	2.47	0.06	7.95

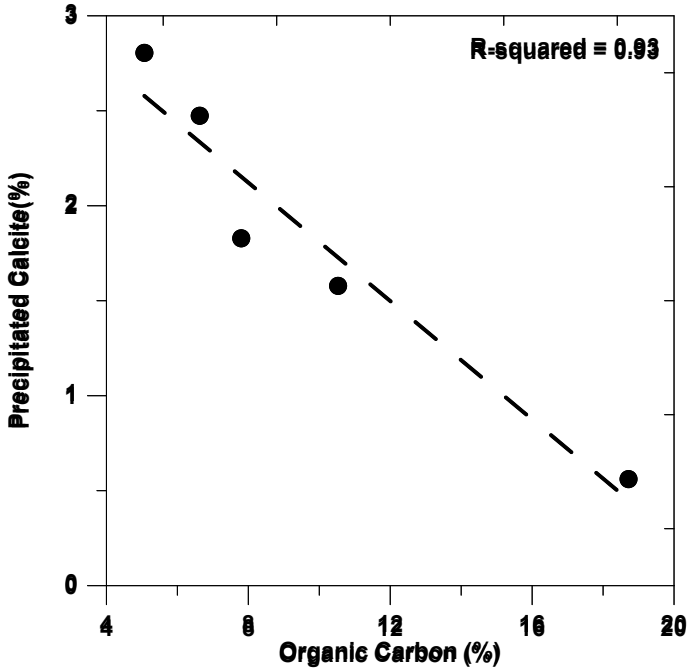

Figure 3. 10. Plot showing the relationship of $CaCO_3$ precipitation with organic carbon

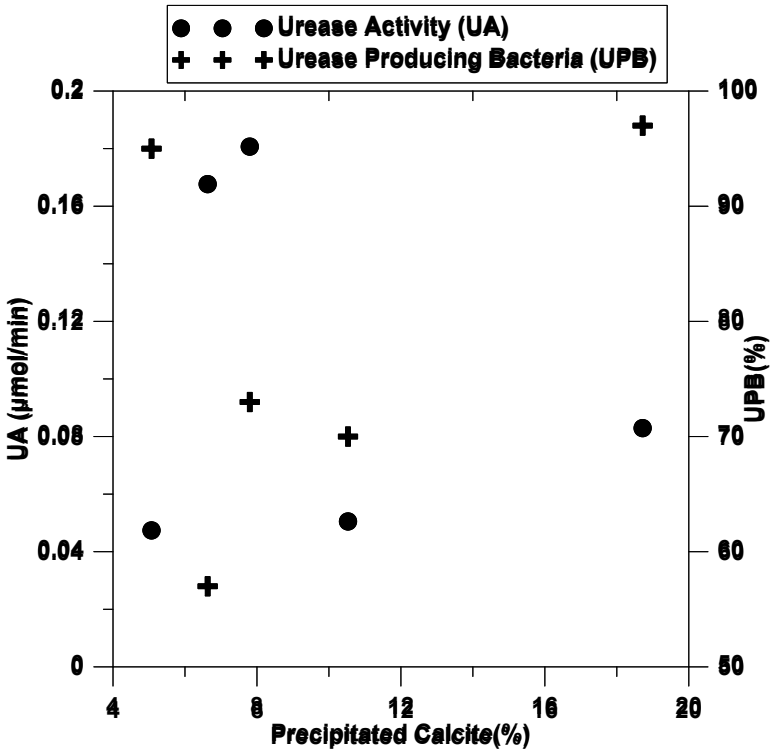

Figure 3. 11. The relationship of $CaCO_3$ precipitation with urease activity and Relative abundance of urease producing bacteria

3.6.5 SEM of bacteria footprint on precipitated calcite

Electron microscopy examination of the MICP treated soils reveals that calcium carbonate precipitation is closely associated with bacterial cells. We observed many holes (approximately 2–4 μm) on the surface of calcite crystals, which are consistent with the size of the bacteria. Figure 3.12 shows the bacterial traces on the surface of $CaCO_3$ precipitates. It is shown that during the calcite development process, bacteria were completely wrapped and then gradually lost their vitality. The death of bacteria and their subsequent decomposition led to the formation of many pores within the calcite (Jin et al., 2020). Moreover, different strains of microbes will cause the calcium carbonate crystals to be varied in their type, size, shape, and yields (Tang et al., 2020). Overall, the calcite crystal formation within TX soil was mainly in agglomerated rhombohedral and individual

rhomboidal form, indicating that the development point of calcite was either on the surfaces of particles (Figure 3.12b) or on the surfaces of calcite that had already developed (Figure 3.12a).

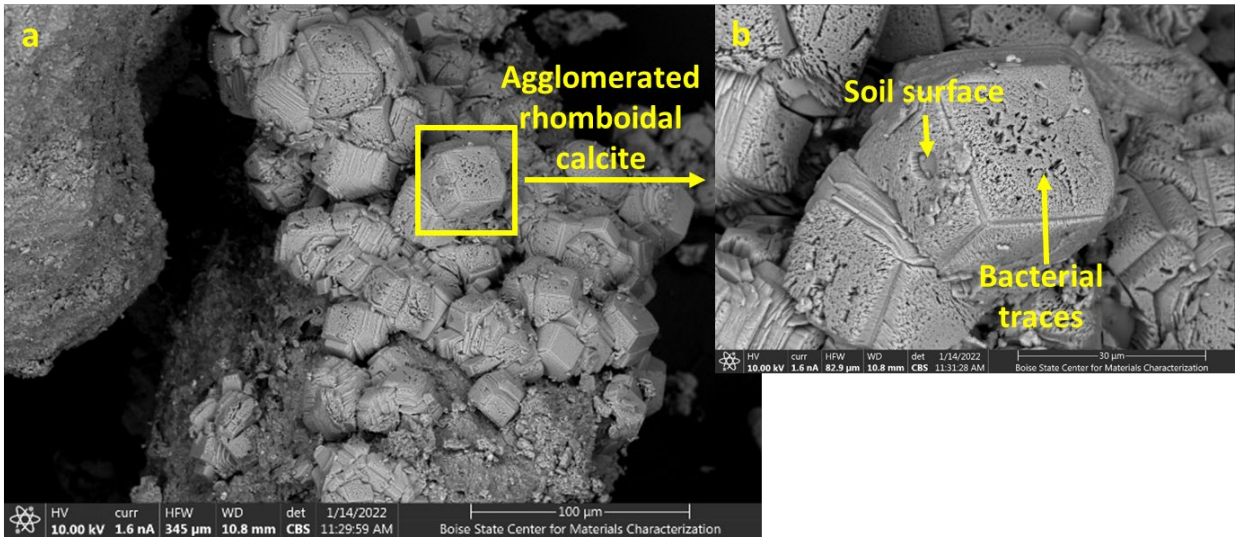

Figure 3. 12. Agglomerated rhomohedral crystals of precipitated calcite and b. bacterial traces on the surface of precipitated calcite.

3.7 Summary and Conclusions

Five different natural soils were studied to understand the role of ureolytic bacteria indigenous to soil in facilitation MICP in clays soils. 16s rRNA gene sequencing was used to determine the initial bacterial species and their changes caused by MICP treatments in all five soils selected for this research. Furthermore, urease activity, calcite precipitation, and soil organic carbon (SOC) were quantified using urease assay, rapid carbonate analysis, and dynamic flash combustion experiments, respectively, and correlated with microbial community changes.

Our results indicate that in all of the experimented clayey soils, *Sporosarcina* species dominated over other ureolytic species when MICP was applied. The relative abundance of *Sporosarcina spp.* was at least ~36% and at most 93% in experimented soils after the third cycles of treatment. Given rapid carbonate analysis and genetic data, the precipitated $CaCO_3$ is not solely dependent on NUMC and urease activity; other factors tied to the physical and compositional properties of the soil also impact the efficiency of MICP treatment. Studying these factors would be the main scope of future research.

Previous studies demonstrated a great diversity of microbial genera in calcium carbonate precipitation (Burbank et al., 2012). To our knowledge, there are not many studies that address the soil microbial community changes due to MICP treatments. Moreover, no research investigated the possible relationship between the ureolytic microbial community and the amount of precipitated calcite through various MICP treatments. Our results showed that applying urea as a nitrogen source leads to the expansion in the abundance of the *Sporosarcina* and *Bacillus* family members. The overall relative abundance of *Sporosarcina* was highest by the third round of treatment. By the seventh round however, *Bacillus* and other species started dominating and the relative abundance of *Sporosarcina* declined. The 16SrRNA data of strains isolated from the top and bottom of soil showed that *Bacillus sp.* thrived more at the bottom of the sample, where the soil is more compact and has a higher bulk density (Juyal et al., 2018); on the other hand, *Sporosarcina* growth occurs more at the surface where the soil structure is loose, and there are more pathways for communication and colonization. Furthermore, the results show that amendment with high concentrations of treatment solution reduces ureolytic diversity, favoring specific strains of *Sporosarcina* such as *S. koreensis,* S. *luteola,* and *S.*

Soli. These species might play a significant role in the MICP process in clayey soils by improving the ureolytic activity of the microbial system and increasing the yield of calcium carbonate for various applications.

The relative abundance of ureolytic bacteria was highest in BR and MA soils, followed by GF, NTP, and TX (Figure 3.6 and 3.7). Additionally, precipitated $CaCO_3$ was lowest in BR and TX soil. These observations show adding treatment solutions increase ureolytic strains in all soils, and this increase is not correlated with calcite precipitation in soils (Figure 3.11). Therefore, we propose that the differences in $CaCO_3$ precipitation in clayey soils might be dictated by the soil's physical properties (such as clays mineralogy, surface area, and cation exchange capacity). Future research should focus on the study of these factors in the MICP process in soils.

3.8 Acknowledgment

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CHAPTER 4: THE ROLE OF SPECIFIC SURFACE AREA AND CATION EXCHANGE CAPACITY IN EFFICACY OF MICP TRETMENT IN CLAYEY SOILS

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Abstract

In this work, we report on the impact of two soil properties, Cation Exchange Capacity (CEC) and Specific Surface Area (SSA), on the effectiveness of Microbial Induced Calcite Precipitation (MICP) in mitigating expansive clay swelling. Most research efforts in the recent past are dedicated to understanding various chemical, biological, and environmental factors affecting the performance of MICP treatments. However, the role of soil's physico-chemical properties such as CEC and SSA were not yet studied. To bridge this gap, we sampled 6 clayey soils from different geographic regions within the continental United States and subjected them to MICP treatment. 1-D swelling tests conducted before and after MICP treatments showed that MICP is effective in decreasing the swelling potential of all experimented clayey soils. The CEC and SSA test results demonstrate a significant positive correlation between CEC/SSA of the samples and the efficiency of MICP in precipitating $CaCO_3$. This suggests that these properties of the soils can be leveraged as indicators of the MICP effectiveness prior to any treatment. Moreover, we study the relationship between Soil Organic Carbon (SOC), Soil Inorganic Carbon (SIC), and MICP-induced $CaCO_3$ precipitation. The results showed that SOC is strongly correlated with SIC in soils. We found that Soil Inorganic Carbon (SIC) reduces MICP efficiency significantly, which we attribute to the reduction in CEC and SSA of the clayey soils with higher levels of inorganic $CaCO_3$. Finally, we investigated the morphology of the precipitated $CaCO_3$ using both SEM and XRD techniques and found that MICP-induced $CaCO_3$ is more abundant compared to inorganic $CaCO_3$ present in untreated soils. The precipitated $CaCO_3$ was found mostly in the form of calcite and/or vaterite polymorphs.

Keywords: MICP, Clayey soil, Expansive soil, CEC, SSA.

4.1 Introduction and Background

Expansive soil is a term used to describe soils that undergo volume changes due to fluctuations in moisture content (Nelson and Miller, 1992). These soils experience significant swelling upon increase in the moisture content, whereas removal of moisture from the soil leads to shrinkage. Severe damages occur to structures like light building, pavements, retaining walls, canal beds and linings founded on the expansive soils (Murthy, 2002). In general, swelling in clayey soils happens due to the presence of imbalanced electrical charges and cation exchange capacity produced by sodium-based clays. When MICP is applied to the clayey soil, Ca^{2+} ions which have less ion exchange capacity replace monovalent sodium ions, this can form a balanced electrical charge in soil structure. Therefore, replacement of monovalent sodium by calcium ions may lead to a marked reduction in diffuse double layer thickness around clays and decrease swelling pressure (Ameta et al., 2007; Bell 1996; Garakani et al., 2018).

Soil stabilization may be defined as any process by which a soil material is improved and resulting in improved bearing capacity, increase in soil strength, and durability under adverse moisture and stress conditions (Joel and Agbede, 2011). Over the past three decades, significant research has been performed to develop various ground improvement techniques such as cementation (e.g., grouting), drainage (e.g., vertical drains)(Biswas et al., 2021) and thermal stabilization (Ahmadi et al., 2021) to stabilize soft and expansive soils. Although many of these techniques have proven to be successful in many situations, they have drawbacks. For example, Portland cement which is the most

used chemical grout nowadays is one of the major sources of green-house gas emission causing global warming (Chang et al., 2016). Microbial Induced Calcium Carbonate Precipitation (MICP) can be an effective and environmentally friendly ground improvement technique to mitigate the swelling potential of clayey soils.

The application of MICP technique to mitigate expansive soil swelling has been limited to few studies (Chittoori et al., 2020; Islam et al., 2020) as the mechanisms involved are not fully understood. Past studies in sands and silts were focused on determining the role of the biological and environmental factors (such as bacteria type, bacteria cell concentrations, pH, temperature, urea and calcium concentrations) on the MICP performance (Al Qabany et al., 2012; Hammes, 2002; Mortensen et al., 2011; Ng et al., 2012). Not much attention has been paid to the soil's physical/chemical and compositional properties and their contribution to the efficacy of the MICP process. The aim of this research is to investigate the effect of soil's Plasticity Index (PI), Liquid Limit (LL), Cation Exchange Capacity (CEC), and Specific Surface Area (SSA) on the MICP performance, specifically precipitation of $CaCO_3$. Furthermore, we studied the dependency of $CaCO_3$ precipitation on the Soil's Organic Carbon (SOC) and Soil's Inorganic Carbonate (SIC). To achieve these objectives, 1-D swell tests were conducted on the six soils in an Oedometer device to measure the swelling potential of experimented clayey soils and their improvement after the MICP treatment. Finally, the morphology of precipitated calcite was investigated by Scanning Electron Microscopy (SEM) and X-ray Powder Diffraction (XRD) data.

MICP is a bio-geochemical process that induces calcium carbonate precipitation within the soil matrix. The induced mineral precipitation can act as a cement and binds the soil grains together at the particle–particle contacts. To date, the MICP method has been widely studied in soils such as sands and silty sands (Cheng et al., 2013; Qabany and Soga, 2013; Whiffin et al., 2007) but a few research works showed that MICP could be a promising method in increasing the strength and decreasing the swelling potential of clayey soils. The applicability of MICP in low to high plasticity clay samples was investigated by Islam et al. (2020). In that research, expansive soils were sampled from different locations in Idaho and Montana. Both macroscale and microscale studies showed enhancement in soil strength and reduction in swelling due to the calcium carbonate precipitation. In a similar effort by Chittoori et al. (2021), three clayey soil samples were prepared by mixing a high plasticity soil (Marsing, Idaho) with different percentages of sand. The MICP application was investigated by mixing substrate solutions into the soils and allowing them to rest for some time. Based on the results from the Unconfined Compressive Strength (UCS) tests, the maximum increase in strength was observed in the sample with the highest sand. The maximum reduction in 1-D free swell was observed in the sample with the highest clay content.

Moreover, many studies demonstrated the positive interaction of clay minerals in the MICP performance (Cardoso et al., 2018; Fomina and Skorochod, 2020; Sun et al., 2019). The effect of adding Na- montmorillonite(Na-MMT) to sand on MICP has been evaluated by (Tang et al., 2021). In their research, it has been demonstrated that the addition of Na-MMT has a great potential to improve the efficiency of the MICP treatment of sandy soils. In a similar study by (Sun et al., 2019), the effect of content of Kaolin clay on

productive rate of calcium carbonate studied. He showed that the MICP Productive Rates (PR) in sandy soil with lager particle sizes are lower and adding kaolin clay to sand increases the PR of $CaCO_3$ precipitation. Despite all these efforts, to the author's knowledge there is no study in understanding the soil's physical/chemical factors in $CaCO_3$ precipitation in clayey soils. In current study, we hypothesize that Specific surface (SSA) and cation exchange capacity (CEC) are governing chemical and compositional factors influencing the MICP performance in clayey soils. The SSA and CEC are properties related to the chemical and compositional interactions of the clay fraction. Studying SSA and CEC are an essential step for MICP performance in clayey soils. Researchers demonstrated that in the MICP process, clay minerals react with the treatment solution due to high pH and the presence of calcium ions (Cardoso et al., 2018). On the other hand, both SSA and CEC can be considered "inherent" soil properties. They exert a strong influence on soil plasticity and can be controlling factors in other behavior of soils (Cerato and Lutenegger, 2005). It is shown that many soil processes such as contaminant accumulation, nutrient dynamics, and chemical transport are closely related to surface phenomena occurring at the interface between the liquid and the solid phases. These processes can be correlated and are proportional to the SSA of the solid phase(P. Koorevaar et al., 1987). Moreover, the CEC and SSA are very strongly correlated with each other (Ersahin et al., 2006).

4.3 Materials and Methods

4.3.1 Soils

To understand the role of soil's physical properties in MICP, six clayey soils were collected from Idaho (ID), Montana (MT) and Texas (TX). The natural soils are denoted as MS (Marsing, ID), GF (Great Falls, MT), DC (Dry Creek, MT) and BR (Big Route,

MT), NTP (North Three Forks, MT) and TX (Texas). Organic carbon, nitrogen contents along with the standard Proctor's data and corresponding ASTM standards are presented in Table 1. Soil classification was determined according to USCS. Sieve and Hydrometer Analysis (ASTM D422) were conducted to determine the soil gradation for all soils. According to Unified Soil Classification System (USCS), The MS, NTP-HP, Texas, and GF soils were classified as high plastic soils (CH), and DC and BR soils were classified as low plastic soils (CL).

Table 4. 1. Carbon, nitrogen content, and geotechnical properties of six natural soils used in this study

Soil	Gradation			Classif-ication	C (%)	IC (%)	N (%)	MDUW (kN/m^3)	OMC (%)	Liquid Limit (%)	Plasticity Index (%)
	Sand (%)	Clay (%)	Silt (%)	USCS				(ASTM D698)		(ASTM D4318)	
MA	3.5	95.9	0.6	CH	0.56	0.00	0.05	10.9	49.6	100	60
NTP-HP	2.6	72.5	24.9	CH	1.83	10.92	0.08	15.2	26.8	61	35
TX	18.4	63.9	17.6	CH	2.47	12.08	0.06	15.5	21.9	59	33
GF	15.5	81.3	3.1	CL	1.58	5.85	0.08	17.3	15.8	40	22
BR	5.7	33.5	60.8	CL	2.80	14.42	0.04	16.6	19.8	42	16
DC	16.4	29.5	54.1	CL	1.23	2.34	0.05	17.2	16.9	37	17

Note: LL-Liquid Limit; PI-Plasticity Index; USCS-Unified Soil Classification System; C-Organic Carbon; C-Inorganic Carbon; N- Nitrogen; ɣd- Unite Weight; OMC- Optimum Moisture Content.

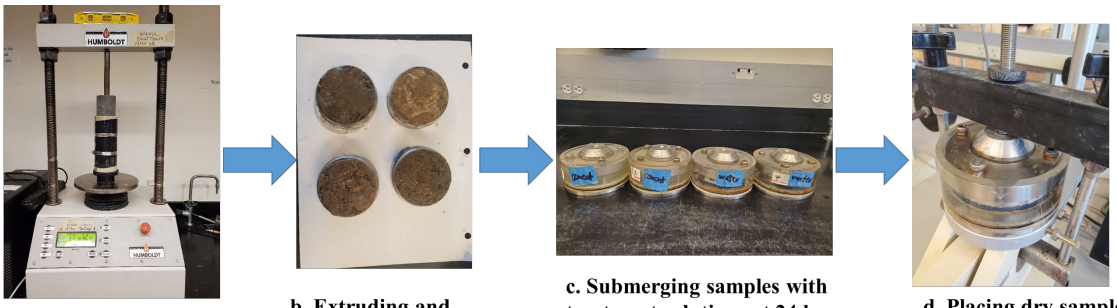
4.3.2 Nutrient solutions

The treatment solutions consist of enrichment and cementation solutions. Enrichment solutions stimulate bacteria for calcite precipitation and contain 0.5% (by volume) Grandma's molasses, 170 mM sodium acetate, 0.5 g/L Bacto yeast extract, and 333 mM urea. Cementation solution that initiate bio-mineralization process and consist of 170mMsodium acetate, 0.5 g/L Bacto yeast extract, and 333mMurea (Burbank et al., 2012).

4.3.3 MICP Application Method

In this research, the treatment solutions were applied to the soil through the submergence method. To achieve $CaCO_3$ precipitation through submerging, each soil sample was wrapped inside a latex membrane and placed in a PVC tube (3in diameter x 8inch long), as shown in Figure 1a. Static compaction was performed using an ELE Digital Tritest compactor to achieve optimum Maximum Dry Density (MDD) for each sample

(Figure 4.1a). A representative sample from PVC tube was extruded and cut off inside consolidation rings (Figure 4.1b). The samples submerged enrichment solution containing and were left on countertop for 48hr. In the next step, soils were drained and submerged with fresh cementation solution. The samples were treated with cementation solution for 14 days/7 rounds of MICP treatment (Figure 4.1c). The control samples were prepared with the same method and time but submerged with water. The soil samples then dried in an oven at 135 ° F and placed in the Oedometer to measure their 1-D swelling (Figure 4.1d).



a. Preparing soil samples using their respective 100% OMC and MDUW

b. Extruding and cutting samples into consolidation ring

c. Submerging samples with treatment solutions at 24 hr. intervals and letting samples to rest

d. Placing dry samples in odometer and measuring the 1-D swell(%)

Figure 4. 1. Pictorial representation of compacting and cutting and submerging samples in consolidation cell

4.4 Evaluation Tests

4.4.1 1D-Swell test

The one-dimensional *free swell test* (ASTM D4546-14) is used to measure the *1-D* swell of cohesive soils using a conventional consolidation cell setup (see figure 1d). Tests are performed on soil specimens prepared in section 4.3.3. During the swell test, the specimens were fully submerged with distilled water in the consolidation cell with a seating load of 1 kPa until the swelling is completed. Results from the test predict the swell characteristics of the soils.

4.4.2 $CaCO_3$ Content and Soil Organic Carbon (SOC) Determination tests

Precipitated calcium carbonate was detected using standard test methods for rapid determination of carbonate content of soils (ASTM D4373-02). Total carbon and total nitrogen by dry combustion of dried, ground samples using a CN analyzer (Flash EA 2000 Series, Thermo Scientific, NC Soil Analyzer). The details of this experiment can be found in (Krotz n.d.).

4.4.3 Soil's Cation Exchange Capacity (CEC) and Specific Surface Area (SSA) tests

The cation exchange capacity (CEC) is a measure of the negative charge on the surface of a material that can be neutralized by exchangeable cations. Cation exchange capacity (CEC) gives an insight into the fertility and nutrient retention capacity of soil. Certain soil minerals, such as clay, particularly in combination with organic matter, possess a number of electrically charged sites, which can attract and hold oppositely charged ions. The negatively charged sites that can hold cations (such H^+, Ca^{2+}, Mg^{2+}, Na^+, and NH^{4+}, etc.,) make up the CEC (Mukhopadhyay et al., 2019). The CEC in soils were measured with the Ammonium acetate method. In this method, the soil is mixed with an excess of 1 N ammonium acetate solution. This causes an exchange of the ammonium cations for exchangeable cations present in the soil. The excess ammonium is removed, and the amount of exchangeable ammonium is determined by the colorimetric method (Schollenberger and Simon, 1945).

On the other hand, Specific Surface Area (SSA) in soils is greatly affected by mineralogy and the nature of the soil colloidal phase. Subsequently, clay minerals contribute greatly to the SSA and the SSA varies between the different clay minerals based on their particle sizes and isomorphic substitution characteristics (Cerato and Lutenegger,

2005). In this research the SSA of soils is calculated with the Ethylene Glycol Monoethyl Ether (EGME) Method. The test involves saturating a soil sample with EGME and then removing the excess EGME in a vacuum desiccator, until the EGME forms a monomolecular layer on the soil surface (Cerato and Lutenegger, 2005; Suits et al., 2002).

4.4.4 SEM and XRD analysis

To study the presence and morphology of Inorganic Calcium Carbonate (ICC) and calcium carbonate precipitation in the soils, SEM and XRD tests were performed on soils after 7 cycles of MICP treatment. These tests were carried out in Idaho Microfabrication Laboratory (IML) facility at Boise State University.

The X-Ray Diffraction (XRD) studies are conducted using a CuKα Rigaku Miniflex 600 X-ray diffractometer (40 kV, 15mA) by following the principles and procedures of x-ray powder diffraction (see, for example, Whittig and Allardice, 1986). The soil samples were dried in air at room temperature and then crushed to form a fine powder that passes a #200 sieve. The filtered soil is then placed in a sample holder and loaded into the diffractometer chamber. The scan had a resolution of 0.02° covering a 2Θ angle ranging from 5° to 80°. The output spectrum is used to detect the characteristic peaks of CaCO3 polymorphs.

To visualize the presence and morphology of ICC and calcium carbonate precipitation in the soil mass an FEI Teneo Field Emission Scanning Electron Microscope (FE-SEM) was used. The equipment is set to use an accelerating voltage of 10-15 kV and a current of 25 μA. A resolution of 0.8 nm at 30 kv can be obtained using this setup. Prior to loading to the microscope chamber, the soil samples are coated with a very thin layer of

carbon, creating a conductive path for the electrons which reduces charge accumulation on insulating soil grains.

4.5 Results & Discussion

4.5.1 1D-swelling

Studies have shown that MICP can improve clayey soils swell potential due to the formation of $CaCO_3$ and binding soil grains together (Chittoori et al., 2021; Chittoori and Neupane, 2019; Islam et al., 2020; Tiwari et al., 2021). 1D-swell of the oven dried soils before and after treatments was determined using the odometer device. The results in Figure 4.2 shows in all soils the swelling potential declined after MICP treatment. The highest decrease in 1-D swell is in NTP and MA soils that contains the highest clay content of 73% and 96% respectively.

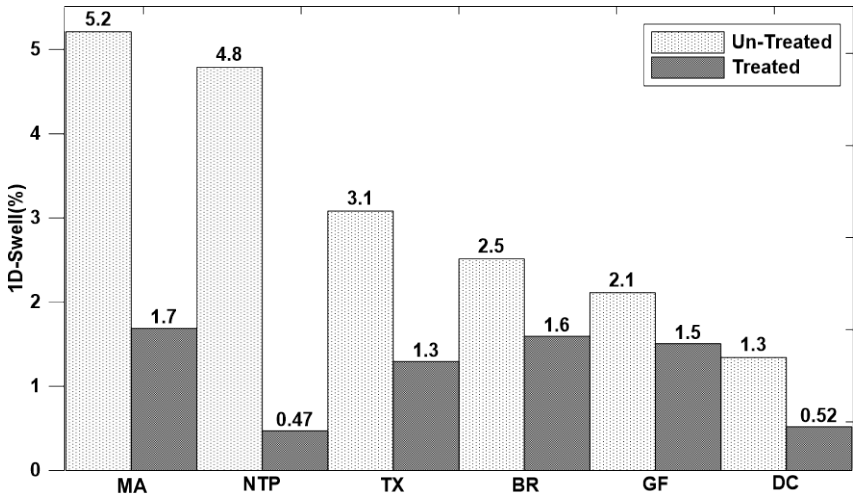


Figure 4. 2. 1-D swell (%) changes in soils before and after MICP treatment

4.5.2 PI / LL and precipitated $CaCO_3$

Figure 4.3 shows the effect of Liquid Limit (LL) and Plasticity Index (PI) on decreasing the 1-D swell in soils. Although there is not a strong correlation between MICP induced swell reduction and LL/PI, samples with higher plasticity and liquid limit responded better to MICP treatment (see section 4.5.1).

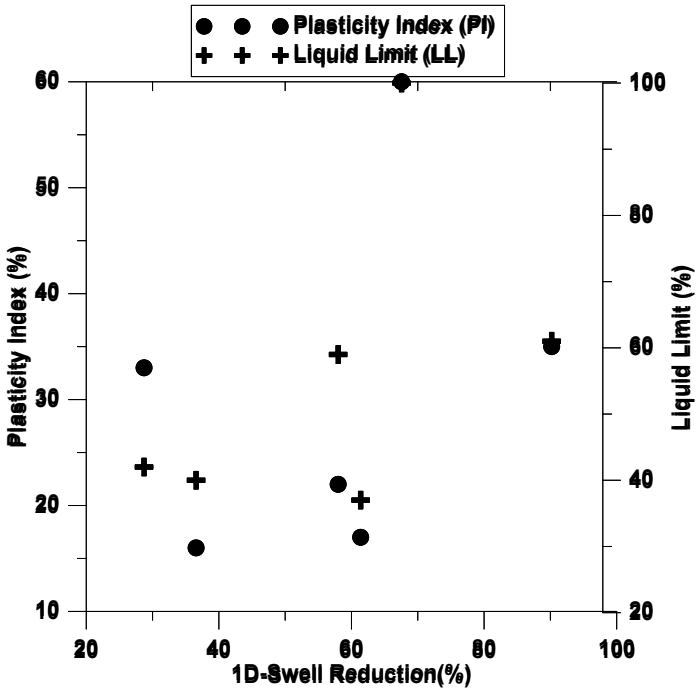


Figure 4. 3. The relationship between 1D-swell reduction and PI/LL of soils

4.5.3 Organic/inorganic carbon and precipitated $CaCO_3$ results

Figure 4.4 illustrates the results of the Soil's Organic Carbon (SOC), Soil's Inorganic $CaCO_3$, and precipitated $CaCO_3$ measurements in 6 experimented soils. There is a strong liner dependency between SOC and SIC in all 6 clayey soils. This can be because of higher CO_2 concentration in the topsoil that contains high microbial and root activity. Researchers showed that soils of arid and semi-arid regions with usually alkaline pH (> 8.5) and richness in Ca^{2+} and/or Mg^{2+} may enhance the soil inorganic carbonate following increase the respired CO_2 with adding organic materials (Bughio et al., 2016; Wang et al.,

2015). Figure 4.4 also shows that there is a reverse dependency between SOC and precipitated $CaCO_3$.

Moreover, Soil Inorganic Carbonate (SIC) shows a reverse correlation with R-squared value of 0.95 between SIC and MICP precipitated $CaCO_3$ (Figure 4.5). It means that samples with higher amounts of inorganic $CaCO_3$ were less suitable for MICP treatment. This can be related to the decrease of surface area and decline in cation exchange capacity between clayey soils and solution due to present of SIC. The other reason might be the dissolution of $CaCO_3$ by ureolytic bacteria due to high concentration of Ca^{2+} in soils (Oualha et al., 2020; Zhu et al., 2017). The $CaCO_3$ precipitation is lowest in BR soil, this can be related to the high Mg^{2+}/Ca^{2+} molar ratio (Chandra and Karangat, 2020; Putra et al., 2016).

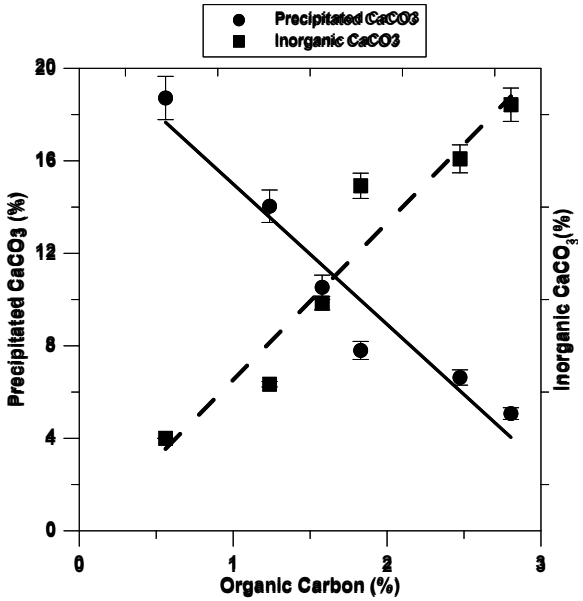


Figure 4. 4. The relationship between inorganic $CaCO_3$ and organic $CaCO_3$ in the 6 clayey soil samples. Error bars indicates 10% error

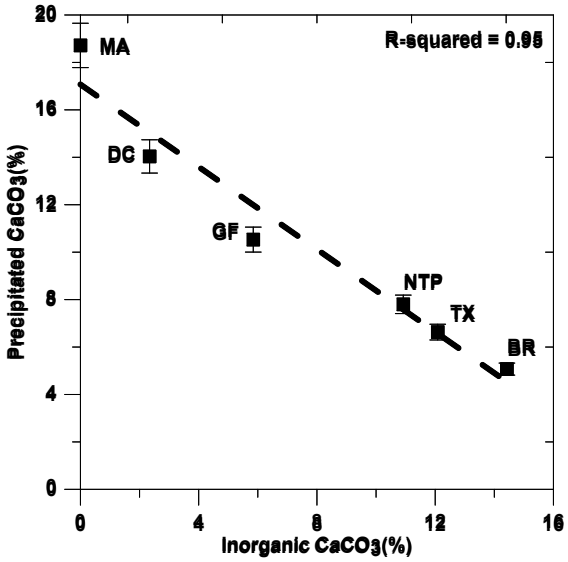


Figure 4. 5. The relationship between precipitated $CaCO_3$ and inorganic $CaCO_3$ for the 6 clayey soil samples. Error bars indicates 10% error

4.5.4 CEC and SSA results

Figure 6.a shows the change in $CaCO_3$ concentration vs. Cation Exchange Capacity (CEC). The results show that there is a strong direct correlation between inorganic $CaCO_3$ and CEC in all clayey soils. This can be related to higher CEC ultimately makes more cations available on the soil surface for inorganic $CaCO_3$ deposition in soils. On the other hand, if the soil has a high concentration of positive ionic species (such as $Ca2^+$), this reduces the CEC of the soil which results in less precipitation.

Moreover, our result shows that the CEC of the soil is directly proportional to the Specific Surface Area (SSA) of the soil (Figure 6.b). This is because the finer the texture of a soil is, the more surface area is available for cation exchange to occur (Hale et al. 2011). The overall results (Figure 7) show that at high surface area values, the baseline of $CaCO_3$ concentration is higher (i.e. the concentration of pre-treatment $CaCO_3$), and hence

there is less room for increase in $CaCO_3$ concentration due to MICP treatment (since we will be closer to saturation if the soil already contains a higher concentration of $CaCO_3$ to begin with).

However, two soils were excepted from these observations: The first exception is the BR soil which showed the lowest SSA among all soils but the highest inorganic $CaCO_3$ content. This can be explained by the abundance of the Mg-rich Dolomite polymorph in BR sample as determined by XRD measurements (Figure 12). An FWHM analysis of the XRD spectra show that the $CaCO_3$ crystallite size in pristine BR sample is roughly 20nm in size which is about half of what we measured in other soils (Table 2).This is supported by other reports where researchers have shown that Mg ions hinder $CaCO_3$ crystallite growth (Chandra and Karangat, 2020). As a result, we postulate that BR soil is packed with smaller crystallites compared to other soils resulting in higher concentration of inorganic $CaCO_3$ despite its small SSA. The second exception is MA soil, which has the highest SSA but the highest levels of $CaCO_3$ precipitation. This can be understood by noting that MA sample is a non-calcic soil, and hence, all the surface area is available for calcite precipitation.

In short, our results demonstrate that SSA and CEC are very important compositional factor that play a key role in determining the efficiency of MICP process. However, other factors such as soil's morphological properties and native/pristine conditions are important and could help in understanding and explaining the efficiency of MICP in soils.

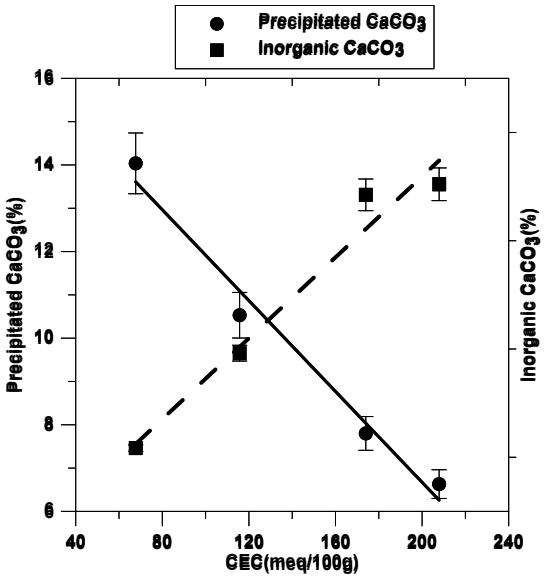


Figure 4. 6. The relationship between precipitated/inorganic $CaCO_3$ and CEC for the soil samples. Error bars indicates 10% error

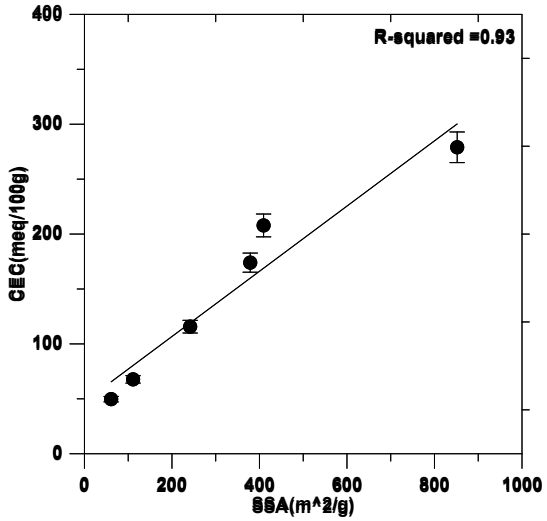


Figure 4. 7. The relationship between CEC and SSA for the 6 clayey soil samples. Error bars indicates 10% error

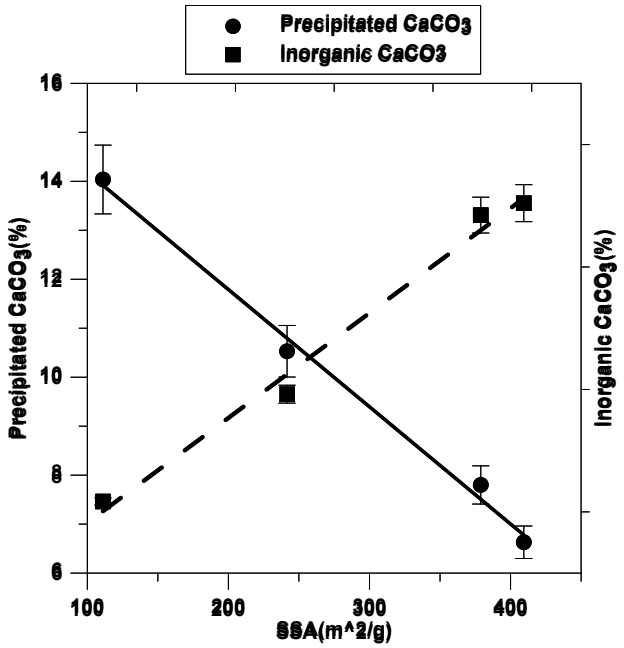


Figure 4. 8. The relationship between precipitated/inorganic $CaCO_3$ and CEC for the soil samples. Error bars indicates 10% error

4.5.5 SEM and XRD results

Mineralogical studies including Scanning Electron Microscopy (SEM) and X-ray analysis (XRD), were performed in the present study on treated and untreated soils. The XRD data shows the present of natural carbonate in 5 out of 6 soils. BR, TX, and NTP soil were selected for closer examination of morphology of inorganic and MICP precipitated carbonate in soils.

4.5.6 SEM of inorganic $CaCO_3$ in soils

The SEM data shows the absence of crystalline structure of inorganic calcite in almost all untreated soils (Figure 4.10 and 4.11), although, there were some rhomboidal forms of calcite with relatively rounded edges in NTP soil that indicates the crystallization of inorganic calcite in this soil (Figure 4.10b), these rhombohedral shapes were rarely found and were not widespread throughout the sample. The main reason for not detecting

crystalline structure of inorganic carbonate in soils might be because they were amorphous or covered with a calcium carbonate film. This is similar to the observation by Kuznetsova and Khokhlova (2010), in their research they showed all carbonate formation in soils were characterized by a flat surface. This absence of at least one well defined crystal face or edge in soils indicates the process of inorganic calcium carbonate formation in the experimented soils is related to dissolution rather than crystallization (Kuznetsova and Khokhlova, 2010).

4.5.7 SEM of precipitated $CaCO_3$ in soils

The crystal morphology of post treatment $CaCO_3$ in all clayey soils was mainly agglomerated rhombohedral $CaCO_3$ with uneven distribution (Figure 4.11b). The same morphology of $CaCO_3$ was observed by Tang et al. (2021) when they examined the effect of Na-montmorillonite (Na-MMT) on $CaCO_3$ crystallization in the MICP process. In their research, the agglomerated $CaCO_3$ crystals were found in the presence of Na-montmorillonite clay. Meanwhile, the individual rhombohedral precipitates of calcite and spherical shape Vaterite were also found in soils (Figure 4.12a and 4.12b). The accumulation of the $CaCO_3$ layers with each round of treatment increase the size of rhombohedral precipitates (Figure4.12a)(Mujah et al., 2019). In the MA and NTP soils with more clay content and higher surface area, post treatment $CaCO_3$ formed as a thin and discontinuous film of $CaCO_3$ on clay surface. This carbonate film partially covered clay surfaces and mimicked their relief (Figure 4.13a and 4.13b). The formation of this carbonate film is related to the higher surface area in clays that increases the number of nucleation sites and facilitate higher bacterial adsorption to the surface of clay (Jin et al., 2020). Teng et al. (2021) showed the surface of the MICP treated clay particles is less

smooth than that of the original clayey soil, which presumably means that calcite formed on the surface. In a similar research, Jin et al. (2020) studied the characteristics of MICP on the surfaces of shale particles. They found that multiple thin layered of $CaCO_3$ precipitation structures on the surface of shale particles.

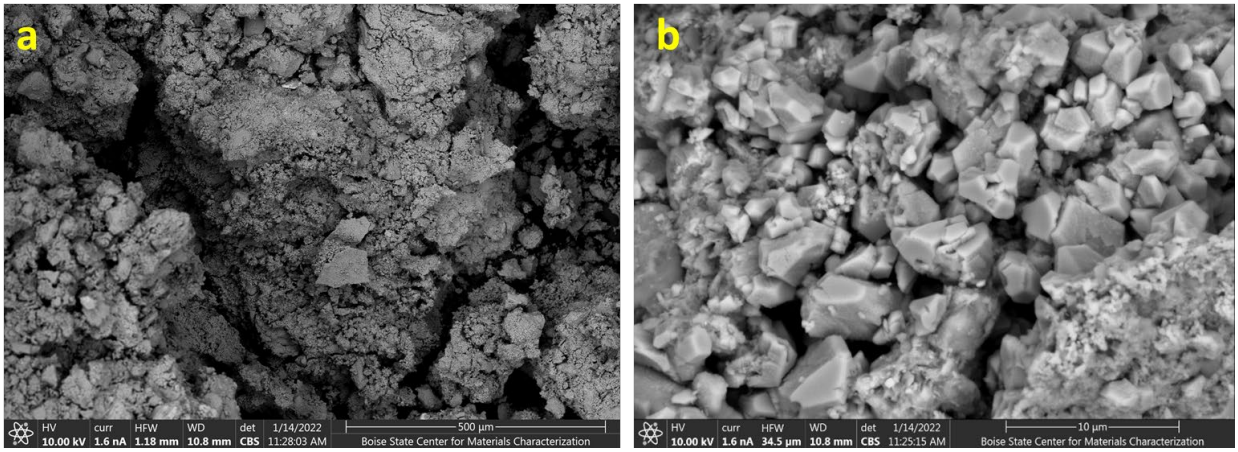


Figure 4. 9. NTP-Natural

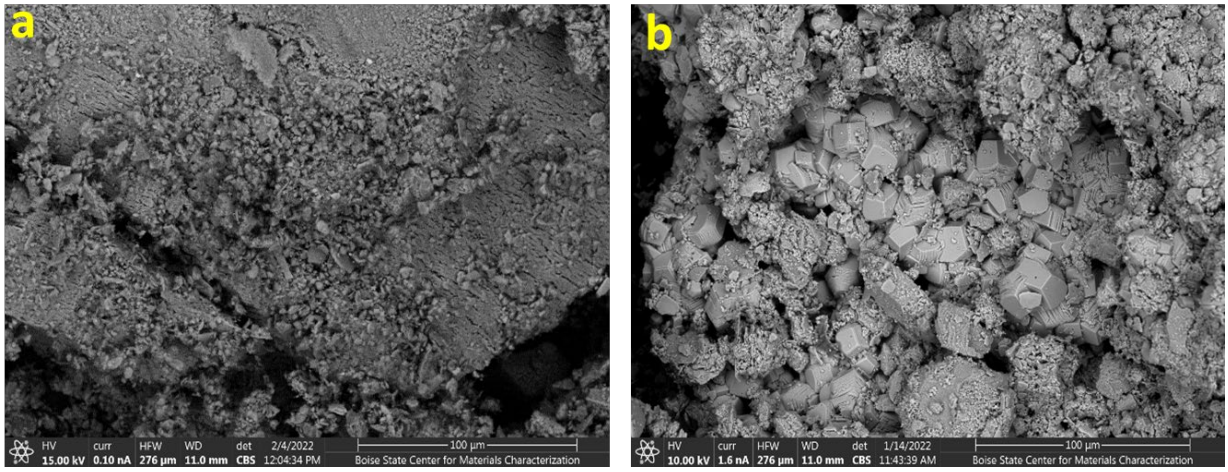


Figure 4. 10. BR-untreated and b. BR-Treated

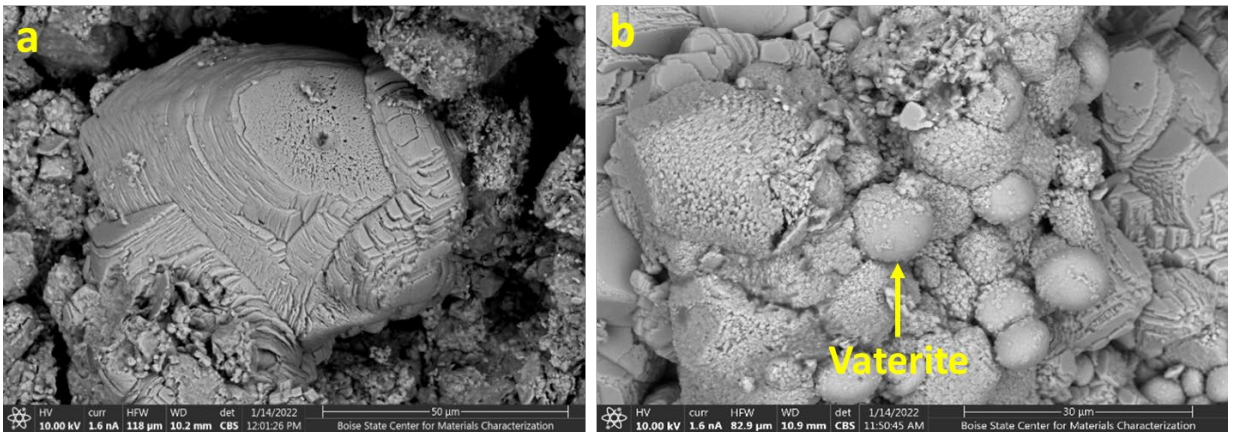


Figure 4. 11. DR-treated and b. GF-Treated

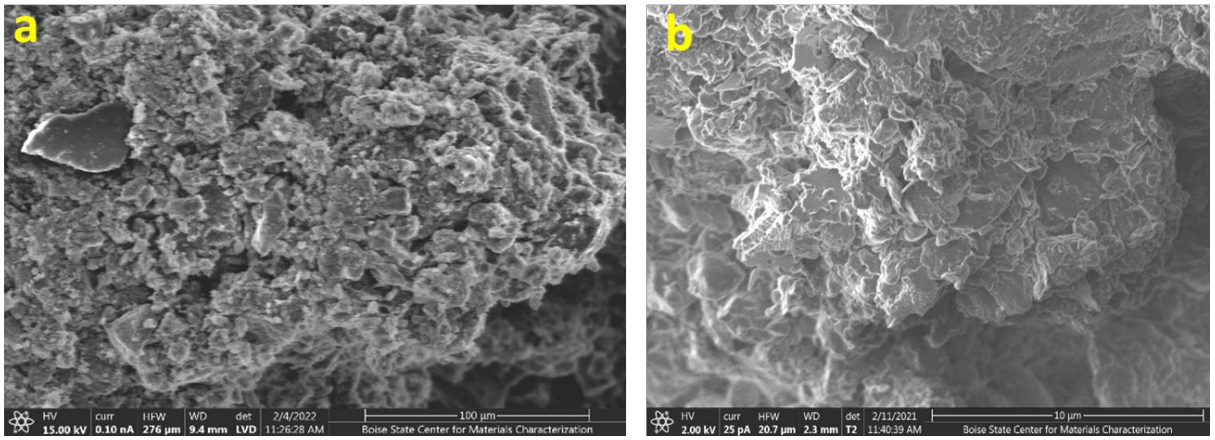


Figure 4. 12. NTP-treated and b. MA-Treated

4.5.8 XRD of inorganic and Precipitated $CaCO_3$ in BR soil

The crystallite or grain size of inorganic and precipitated calcite in soils were measured using the Scherrer Equation (Equation 1)(Holzwarth and Gibson, 2011) and depicted in table 2.

$$d = \frac{k\lambda}{B\ COS\ \theta} \qquad (1)$$

where λ is the wavelength of the X-ray; β, FWHM width of the diffraction peak; θ, diffraction angle; and k, constant. The average grain size of particles (d) can be determined by this equation.

The size of inorganic calcite in BR soil is about half of inorganic calcite crystals in all other soils (Table 4.2). This can be related to the high presence of Dolomite in this soil (Figure 4.14). Mg^{2+} can replace part of Ca^{2+} and increasing the degree of irregularity (Chandra and Karangat, 2020; Qian et al., 2019). The XRD results of untreated and treated BR soil is shown in Figures 4.14 and 4.15, respectively. After MICP, the highest increase in the crystallite size of calcite was observed in BR soil while in other soil there was no significant changes in crystallite of precipitated calcite (table 4.2). This means, except for the BR soil, the amount of $CaCO_3$ increased in all soils after MICP but their size did not change.

Table 4. 2. Grain size characteristic of inorganic and post-treated CaCO3

Soil	Br-treated		Br-Untreated		NTP-Treated	NTP-Untreated	TX-Treated	TX-Untreated
	Dolomite	Calcite	Dolomite	Calcite	Calcite	Calcite	Calcite	Calcite
Grain size(nm)	35.753	306.036	33.658	23.869	41.474	40.692	59.756	46.754

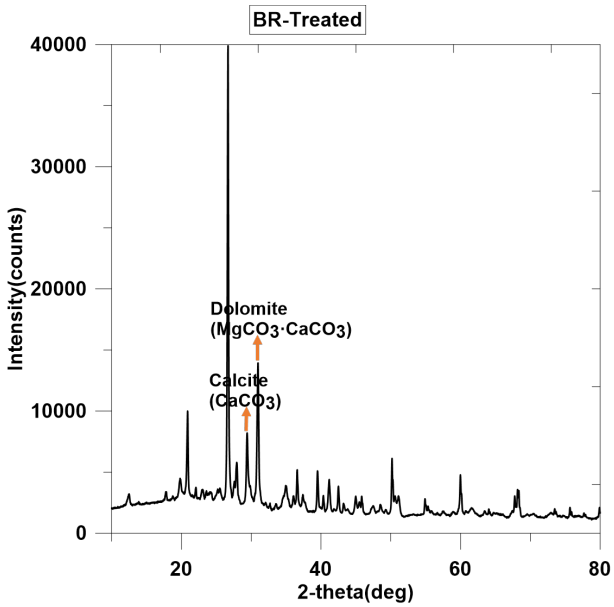


Figure 4. 13. XRD of BR-untreated

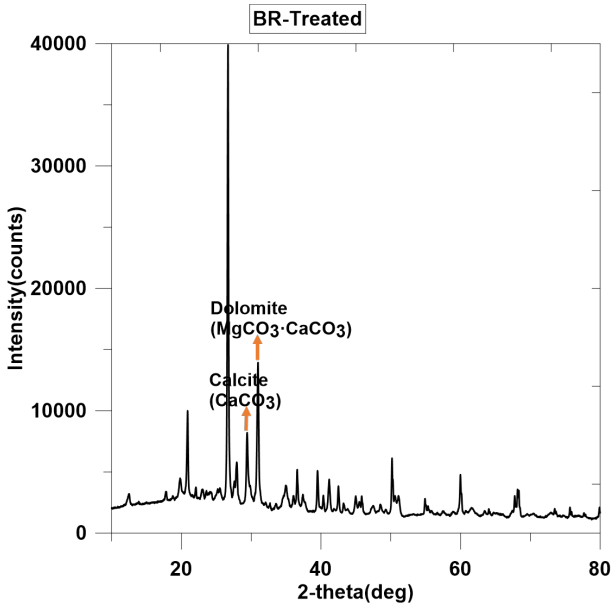


Figure 4. 14. XRD of BR-treated

4.6 Summary and Findings

The main objective of this research was to determine the role of compositional properties of soils such as Cation Exchange Capacity (CEC) and Specific Surface Area (SSA) in mitigating expansive soil swelling by measuring the quantity of $CaCO_3$

precipitated in different natural soils. It can be concluded from the results that CEC and SSA play a pivotal role in controlling MICP performance and can be used as predicting factors in efficacy of MICP in different soils.

The main findings of this research are:

- MICP induced swell reduction is not correlated with Liquid limit (LL) and Plasticity Index (PI) of soils.
- There is a strong linear relationship between CEC, SSA and precipitated $CaCO_3$ in all clayey soils.
- Soil's Organic Carbon (SOC) measurement demonstrates a strong dependency on inorganic $CaCO_3$ in soils, it is because adding organic materials to soils can increase the respired CO_2 and enhance the formation of inorganic $CaCO_3$.
- The natural soils with higher inorganic carbonate result in lower calcite precipitation after MICP treatment. This can be related to the coverage of clay surface with inorganic CaCO3 film that decreases cation exchange capacity between soils and MICP solutions.
- The swelling potential of all soils decrease after MICP treatment, this reduction was highest in the MA and NTP soils with more clay content.
- The SEM and XRD results demonstrated that post treatment $CaCO_3$ is mainly agglomerated rhombohedral in soils.
- In MA and NTP soils with highest SSA and CEC, the CaCO3 precipitation was formed as thin and discontinuous film of $CaCO_3$ on clay surface.

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CHAPTER 5: CONCLUSION

The main purpose of this dissertation is to study the microbiological, environmental, and compositional factors of soils that possibly control the efficiency of MICP in clayey soils. The literature review presented in this dissertation advance our understanding of Indigenous uratolytic bacterial communities extracted from different environment, and their application in MICP. It also elaborates on the reasons that MICP is more efficient in fine graded clayey soils than coarse soils such as sand and silt.

In chapter 2, we investigate the impact of soil's gradation and clay content on MICP precipitation. For this purpose, four soils were prepared by mixing clay and sand at different ratios. Autoclaving was done to control the source of microbial communities contributing to $CaCO_3$ precipitation. The soil samples were subjected to seven cycles of MICP treatments and tested for urease activity, $CaCO_3$ content, and other engineering properties. In addition, to exclude the role of soil's indigenous microbial communities and isolate the effect of soil gradation on MICP, sterilized/autoclaved soil samples were augmented with lab-cultured ureolytic microorganisms (*S. pasteurii)* and treated for MICP and tested. The results showed that soil mixes with higher clay content have more urease activity and higher levels of $CaCO_3$ precipitation for both sand- and clay-autoclaved soil mixes. The geotechnical engineering tests showed that soil samples gained strength after treatments. The most important outcome of this chapter is demonstrating that clayey soils could promote the MICP process.

In chapter 3, we use 16s rRNA sequencing to report on the bacterial species in 5 types of clayey soils and changes in their relative abundance with MICP treatment. Furthermore, we quantified the dependency of calcite precipitation to urease activity and soil organic carbon. The results indicate that in all the experimented clayey soils, the *Sposarcina* genus species dominated other ureolytic species when MICP is applied. The observations show that the relative abundance of ureolytic strains in soils is not correlated with calcite precipitation. Therefore, we conclude that the differences in observed $CaCO_3$ precipitation in clayey soils might be dictated by the soil's physical properties (such as surface area and cation exchange capacity). Chapter 4 focus on the study of these factors in MICP process in soils.

In chapter 4, we investigate the effect of soil's compositional properties such as Cation Exchange Capacity (CEC) and Specific Surface Area (SSA) in the MICP performance. To the author's knowledge there is no study in understanding the factors that make $CaCO_3$ precipitation have elevated performance in fine graded clayey soils compared to coarse soils. In current study, we show that CEC and SSA are governing chemical and compositional factors influencing the MICP performance in soils.

In conclusion, most attempts have been made to determine the biological and environmental factors such as bacteria type, bacteria cell concentrations, pH, temperature, urea, and calcium concentrations in the MICP performance. No attention has been paid to the effect of physical and chemical interaction of clays on the MICP process. Our research suggests that SSA and CEC that are called soil's index properties can be leveraged as indicators of the MICP effectiveness prior to any MICP treatment.

APPENDIX

Supporting Information for Chapter 3

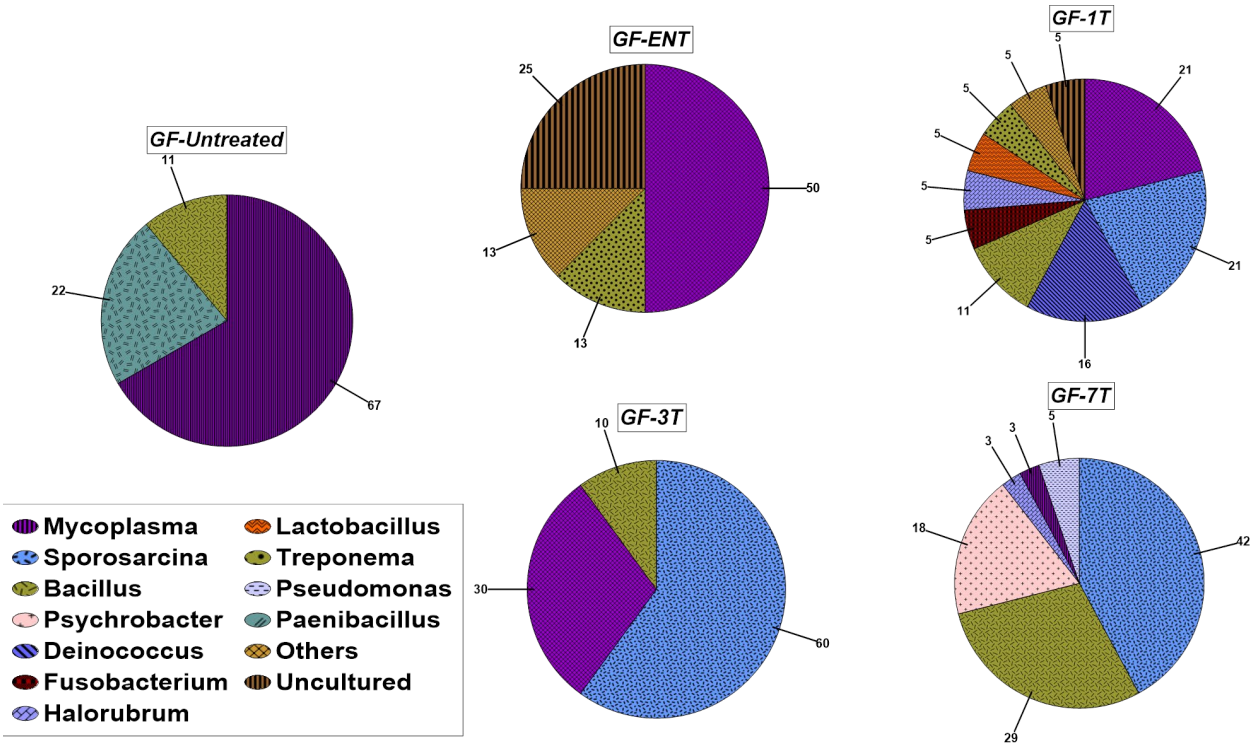

Figure A. 1. The genus relative abundance at top of GF soil.

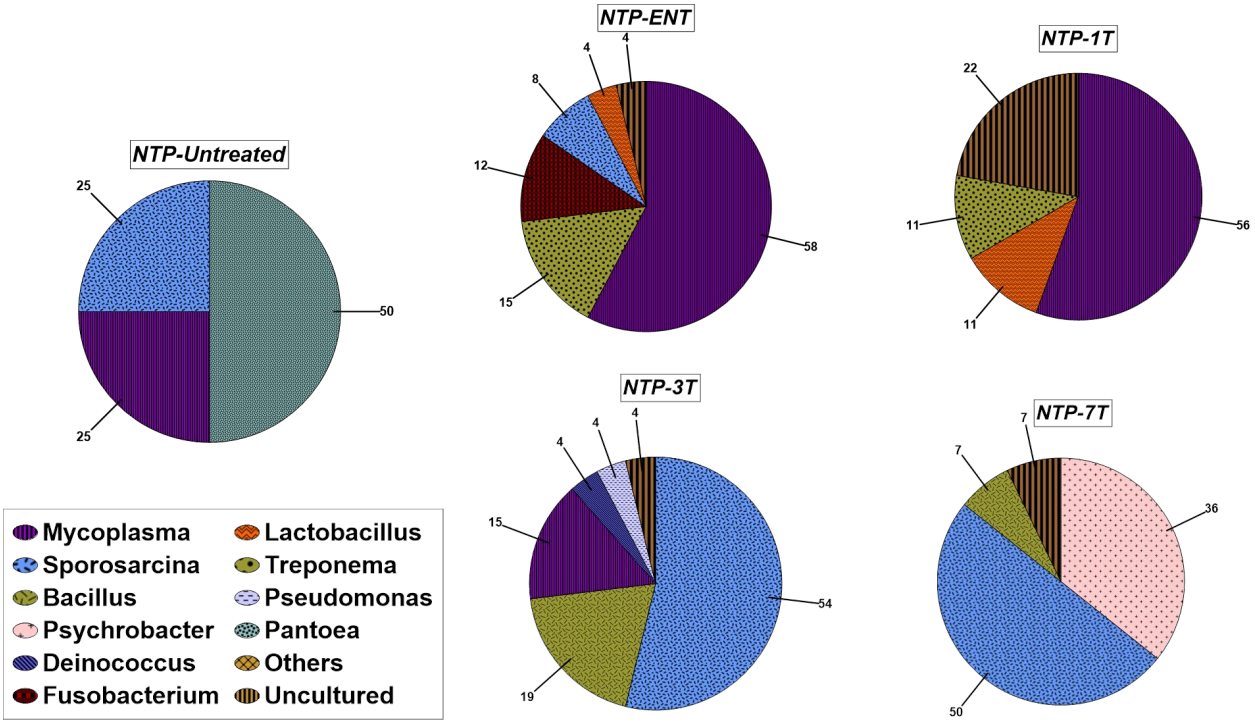

Figure A. 2. The genus relative abundance at top of NTP soil.

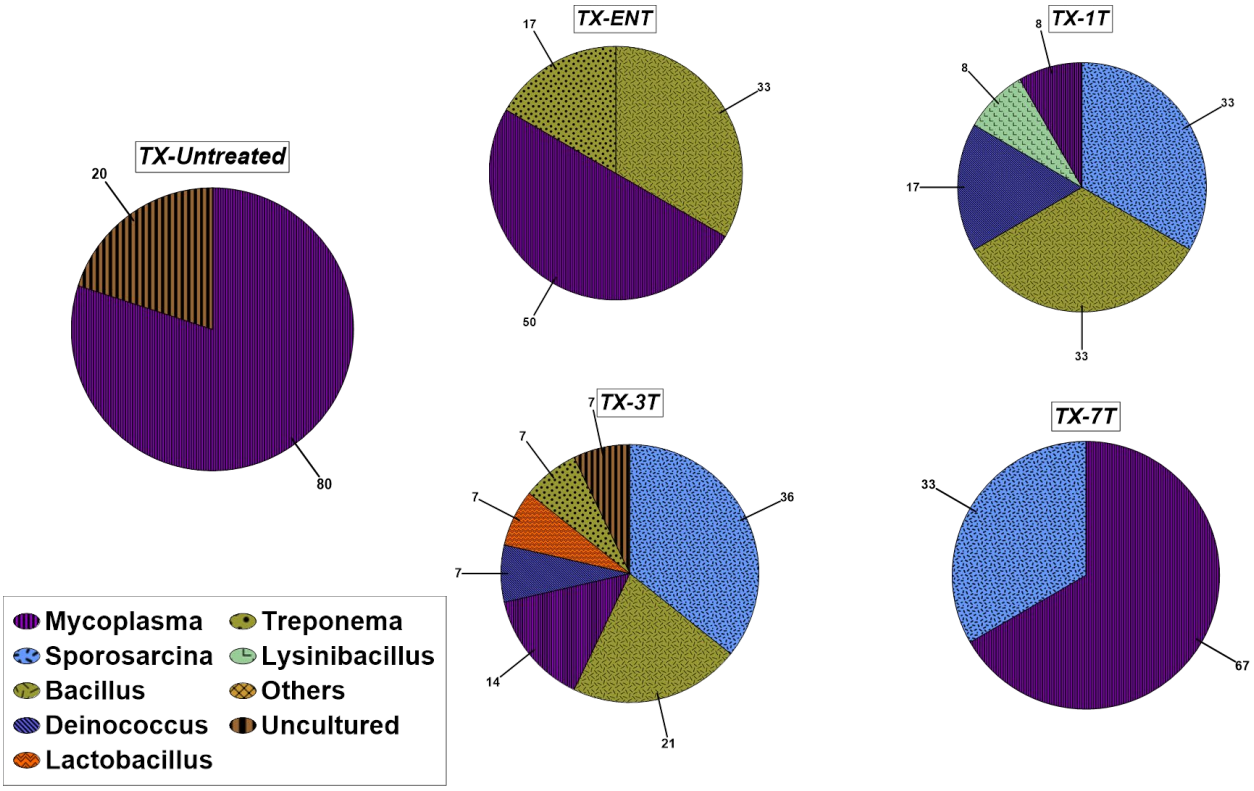

Figure A. 3. The genus relative abundance at top of TX soil.