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Integration of Plants and Microbially Induced Soil Stabilization for Sustainable Infrastructure Design

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ABSTRACT

Over the past two decades, bio-mediated soil stabilization methods like microbially induced carbonate precipitation (MICP) have gained popularity. With a well-established understanding of the mechanical properties of MICP-cemented soil, researchers aim to mitigate the environmental impacts associated with this method, driving the technology toward practical field implementation. The surface layer of soil serves as an important zone supporting numerous plant and microbe communities. Healthy vegetation can support infrastructure needs (e.g., erosion resistance) as well as provide habitat for wildlife. Nevertheless, the influence of MICP on plants and how these alterations may affect the mechanical properties of soil is currently unknown. The presented study introduces and investigates the role of factors such as plant species, and treatment solution concentrations on the compatibility of MICP and vegetation. The results of this study can be used to integrate plants in the design of MICP-treated soil to ensure nature-based engineering solutions.

INTRODUCTION

Emerging biogeotechnical methods, such as microbially induced carbonate precipitation (MICP), have been shown to stabilize soil to support new construction and rehabilitated infrastructure. MICP is the result of a series of reactions that increase the alkalinity of the pore fluid and generate carbonate, which reacts with calcium from the treatment solution (DeJong et al., 2006). Ureolysis increases alkalinity as urea is hydrolyzed into ammonium and bicarbonate, which precipitate with provided calcium to form calcium carbonate (Stocks-Fisher et al., 1999).

The precipitated calcium carbonate provides reinforcement against mechanical and/or hydraulic shear stresses. The cemented soil matrix demonstrates improved volumetric behavior, shear strength, stiffness, tensile strength, and compressibility (Burbank et al. 2012; Choi et al. 2016; Chou et al. 2011; DeJong et al. 2010; Nafisi et al. 2020; Al Qabany et al. 2012; Whiffin et al. 2007; Xiao et al. 2019; among others). The improvement in soil behavior is particularly well demonstrated in undrained conditions where the increase in shear strength and dilative tendencies prove MICP to be particularly effective even at light levels of cementation (Lee et al. 2021, Zamani and Montoya 2019, Montoya et al. 2013). Reduction in erodibility is another area MICP-cemented soil proves to be well-suited. At both laboratory and field scales, the MICP-cemented sand has been shown to reduce wind erosion and dust production (Maleki et al. 2016; Zhan et al. 2016), water-i nduced erosion (Chek et al. 2021; Do et al. 2019; Ghasemi et al. 2019; Hodges and Lingwall 2020; Jiang et al. 2019, 2017; Salifu et al. 2016), scour adjacent to structural foundations (Do et al. 2020), and deformations of coastal sand dunes under wave action (Liu et al. 2021; Ghasemi et al. 2024). Recent work focusing on the erodibility of MICP-treated soil indicates that a threshold level of cementation is needed before changes in erosion resistance are observed. For example, erodibility measurements of a MICP-treated coastal slope field site indicated that at least one percent by mass calcium carbonate cementation was needed before an increase in critical shear stress was observed (Ghasemi and Montoya 2022b). The need for a threshold level of cementation is not observed for improvement in shear response; even light levels of cementation (i.e., less than one percent by mass) can provide significant improvement in shear strength and volumetric behavior (Lee et al. 2021). The improvements MICP offers to soil properties make it well-suited for flood control infrastructure. The increase in shear strength increases the slope stability, while the improvement in erodibility provides more resistance to deformations due to surface water flows or overtopping. MICP has been applied to surficial soil in field studies (Gomez et al. 2015, Ghasemi and Montoya 2022b), and these studies have demonstrated a relative ease at inducing a target level of cementation.

Plants also reinforce surficial soils against erosion by reducing rainfall and runoff energy, which may complement the benefits of soil cementation, especially for flood control infrastructure. Plant tissue above ground intercepts raindrops as well as slows the water flow and traps sediment, whereas fine roots below ground physically bind soil particles together (Khanal and Fox 2017, Gyssels et al. 2005). However, plant roots must be able to penetrate the soil and continuously acquire water and key nutrients from the soil to support growth. Preliminary studies evaluating the compatibility of and interaction between MICP and plants have shown that grass seedlings could grow in MICP-treated soil as long as the level of cementation was light to moderate; the heavy levels of cementation limited the growth of the grass (i.e., smaller blade height and short roots) (Ghasemi and Montoya 2022a). Additionally, high concentrations of chemical components in the MICP recipe (e.g., urea) led to drying of the established grasses. The dried grass observations are consistent with nitrogen burning, indicating sensitivity to the MICP recipe and byproducts (e.g., ammonium). Modification of the MICP recipe (i.e., adding phosphate and reducing urea concentrations) showed some support for the growth of established plants, but additional insight into resource needs of the plants and how MICP alters ecosystem function is still needed to achieve improved soil performance and a healthy ecosystem.

An experimental program was developed to screen a variety of plant species to understand the influence MICP has during the plants' germination. The plants were chosen from a list of often-used species for erosion and sediment control. Three different MICP recipes were selected to evaluate the effects of chemical composition and ureolytic byproducts on plant germination.

Germination was monitored throughout the treatment process, which continued until the target level of cementation was achieved. On a subset of representative pots, shear wave velocity was monitored during the treatments, while strength (e.g., unconfined compression) and erodibility were assessed after the treatment process. The results provide insight into future developments for MICP-implementation towards nature-inclusive infrastructure and nature-based engineering solutions.

MATERIALS AND METHODS

Tested Materials and Specimen Preparation. The soil was collected from the North Carolina State University Lower Coastal Plain Research Station (LCRS) in Kinston, NC. Fields at the LCRS have been previously used for multiple seasons of wheat cultivation. After scraping the initial wheat stubble, soil was collected from the plough layer (ca. 0 to 25 cm) to perform the experiments. Collected soil was then quarantined in plastic storage bins with sticky traps to capture any remaining invertebrates. The soil was classified as silty sand with a mean particle diameter (D_{50}), of about 0.16 mm and a plasticity index (PI), of 8. The soil was dried and ground for specimen preparation, with an initial dry density of 1.43 g/cm^3, in 11.4 cm (4.5") diameter nursery pots.

A large survey assessing the effect of MICP on nineteen plant species was conducted to study plant germination and resulting engineering properties. The results presented in this paper consist of a subset of seven species, including Bermuda grass *(Cynodon dactylon),* Weeping lovegrass (*Eragrostis curvula),* Hard fescue (*Festuca brevipila),* Creeping red fescue (*Festuca rubra),* Roundhead lespedeza (*Lespedeza capitata),* Rye (*Secale cereale* 'Wrens Abruzzi'), and Wheat (*Triticum aestivum*, Hilliard variety). For each plant species, control pots and three MICP-treated pots were prepared in triplicate (i.e., (7 species + No Seeds Control) × 3 triplicates × 4 treatment methods). Additional pots with no seeds were used to monitor the shear wave velocity for the different treatment recipes using bender elements. The results are based on 99 pots, each containing 96 seeds sown in a 7.5 mm × 7.5 mm grid, evenly spaced using a template. Seeds were planted prior to MICP treatments. During the experiment, the pots were stored in a temperature- and humidity-controlled growth chamber (Fig.1). The growth chamber's relative humidity was kept at 55%. The temperature was maintained at 22°C during the day and 15°C at night.

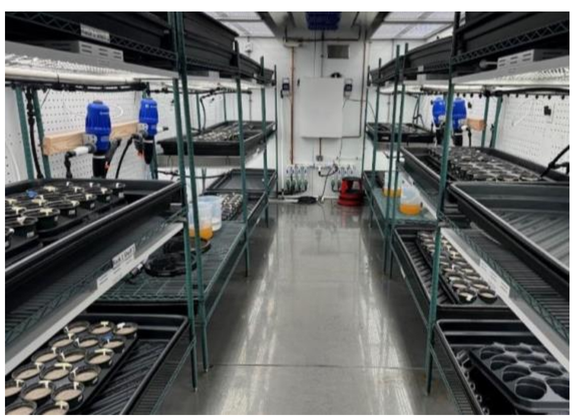

Figure 1. Overall view of growth chamber and pot treatment system.

Bacteria preparation and treatment process. *Sporosarcina pasteurii* (ATCC 11859) was used to catalyze the MICP process. Ammonium-yeast extract medium (20 g/L yeast extract and 10 g/L ammonium sulfate suspended in a 0.13M Tris buffer with pH of 9) was used for bacterial culture.

Solution ingredients were autoclaved separately before mixing and inoculated with bacterial stock culture. The solution was incubated at 27°C at 200 rpm for 40 hours. The inoculated cultures were incubated until an optical density (OD600) of 1 was reached and then used immediately.

Three treatment recipes with chemistry and concentrations presented in Table 1 were used to treat the pots. The first recipe (MICP-1) with the highest chemical concentrations was selected from the geotechnical state-of-the-art recipe proposed by DeJong et al. (2022). Recipes MICP-2 and MICP-3 are modified versions of the first recipe, adjusted to reduce the nitrogen concentrations along with a supplement of phosphorus (in the form of KH_2PO_4) in an attempt to improve plant growth and health (Ghasemi and Montoya 2022a). To initiate the MICP process, bacterial inoculation solution was applied once to all but the control pots. The solution included the suspended bacterial cultures and all chemical constituents presented in Table 1 except calcium chloride. Each pot received about 20 mL of fluid per treatment. Cementation treatments including calcium chloride were conducted until a target shear wave velocity of 800 m/s was achieved. Treatments were applied once a day with overhead sprayers to the unsaturated pots, followed by a water flush four hours later. The water flush was performed to remove unconsumed chemicals and reduce the plants' exposure to chemicals until the next treatment (Ghasemi and Montoya 2022a). In total, 24, 24, and 50 daily treatments were applied to plants treated with recipes MICP-1, MICP-2, and MICP-3, respectively.

Table 1. Chemistry and concentration of the implemented MICP treatment recipes.

Component	Concentration		
	MICP-1	MICP-2	MICP-3
Urea (mM)	250	100	50
Calcium Chloride (mM)	250	100	50
Ammonium Chloride (mM)	12.5	5	2.5
Potassium Acetate (mM)	42.5	17	8.5
Yeast Extract (g/L)	0.2	0.2	0.2
Potassium Phosphate Monobasic (mM)	-	0.59	0.3

Plant germination monitoring. The seed germination process was monitored for 56 days, beginning on the day of bacterial inoculation, encompassing the range of expected germination times for these species. Germination was monitored by counting visible germinants and photographing each pot three times per week. Incidental seedlings of non-planted species and plant shoots with a height exceeding three inches were clipped to not to interfere with the observation of later germinating seeds. The cumulative number of successfully germinated seeds was used for analysis. Cumulative germination percentage was found by calculating the number of germinants counted on a monitoring day in comparison to total planted seeds.

Assessments of soil stiffness and strength properties. Piezoelectric bender element pairs with a tip-to-tip distance of about 9 cm were installed in the mid-height of the pots. The bender element readings were used as a proxy to monitor the increase in shear wave velocity and cementation formation progress, and to ensure similar shear wave velocities in soil specimens treated with different recipes. Bender element measurements were conducted on one representative pot for each MICP recipe (Table 1), prepared without seeds, and treated identically to the other pots for each treatment recipe. A signal generator was used to generate a sine pulse in the source bender element. The generated wave traveled through the soil and was detected in the receiving bender. The distance between the bender elements and the flight time of the wave through the soil was used to determine the shear wave velocity of the soil. Bender element measurements were terminated upon completion of the treatments on days 24, 24, and 50 for pots treated with the MICP-1, MICP-2, and MICP-3 recipes, respectively. Upon completion of the germination monitoring, the bender element pots were flushed with water and free drained to avoid oven drying and melting the plastic pots. In each pot, bender elements were removed, and the plastic pot was carefully cut out from the cemented soil. The sides of the sample were trimmed using a wire saw until the top and base were the same size, achieving a cylindrical shape. Next, unconfined compression strength (UCS) tests were performed on cemented soils trimmed into a cylindrical shape. An axial strain rate of 1%/min was applied to the specimens following (ASTM D2166 2016).

Assessments of soil erodibility. One untreated, unseeded pot; one MICP-3 treated, unseeded pot; and seven seeded pots treated with MICP-3 were evaluated. Jet Erosion Tests (JET) were performed on the soil pots following the procedures outlined in Fox et al. (2022). Hydraulic head was set to a height of 1.37 meters (4.5’). The mini-JET device was then installed atop the testing mold. Before testing, the submergence tank of the mini-JET device was allowed to fill with water and an initial gauge reading of the soil’s elevation was taken. During testing, the pots were incrementally scoured by the impinging, submerged water jet, measuring the amount of erosion that had occurred during a selected time interval. The testing procedure was repeated for the selected time interval until two consecutive gauge readings were approximately equal, after which the time interval was increased. The time intervals selected in this study were 15, 30, 60, 120 and 300 seconds. Scour depth was then normalized by maximum scour and plotted against normalized time.

Mass of carbonate measurements. Mass of carbonate was tested for the untreated condition, as well as all three MICP recipes 1-3 following the gasometric method (O’Toole et al. 2022). Soil samples from all three MICP recipes and the control soil were cored the entire depth of the pot and homogenized before added to 125-mL glass vials sealed with rubber septa and crimped. A 50-mL syringe equipped with a needle was inserted into the septum to measure the gas volume change. A 20-mL syringe containing 20 mL of hydrochloric acid (HCl) at 1M was then inserted and evacuated into the vial. The established calibration curve built utilizing known quantities of pure calcium carbonate was then used to approximate the mass of carbonate. Due to limitations of sampling size, carbonate distributions within the pots was not assessed herein.

RESULTS AND DISCUSSION

The effect of MICP on plant germination. Cumulative germination with time was assessed for each species and treatment recipe by aggregating the results across the triplicate pots. The MICP recipes of seven plant species were compared with the Control (i.e., water) and presented in Fig. 2. A wide range of responses to the MICP application are observed among different plant species. Some species, such as Weeping lovegrass and Roundhead lespedeza, demonstrated robust germination in the control; however, no germination was observed in pots treated with MICP-1 and -2 solutions for these species suggesting that the applied concentration levels were beyond their tolerable range. A moderate germination percentage in Weeping lovegrass treated with MICP-3 (36% of Control germination) suggests that this concentration level is somewhat tolerable for this species. In species such as Bermuda grass and Wheat, germination of the plants occurred in all the MICP recipes and control, but the germination decreased as the recipe concentrations increased (>70% of Control germination for MICP-3, but <20% of Control germination for MICP-1). In species such as Hard fescue and Rye, a higher relative germination percentage was observed for phosphate-containing recipes MICP-2 and -3 (140-270% of Control germination). This suggests that either the lower recipe concentrations compared to MICP-1 or the addition of phosphate contributed to enhanced germination. A similar trend was observed in Creeping fescue, where limited germination occurred and mostly in the pots treated with MICP-3. It is important to note that the growth chamber was not optimized for the growing conditions for all of the species, which likely affected the low germination in the Control pots for several species. Therefore, the results should be interpreted as trends and comparisons should be made within each species.

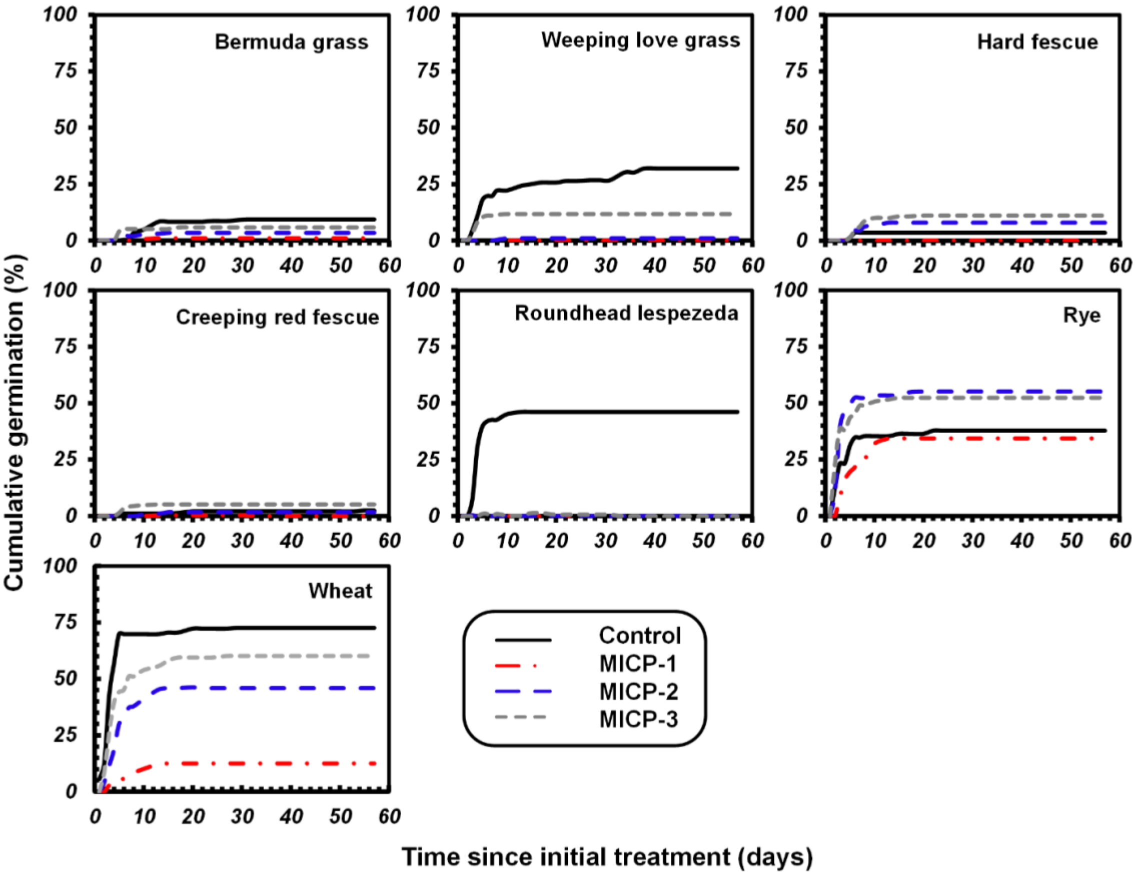

Figure 2. Comparison of seed germination over time in control pots and plant species treated with three different MICP solutions.

Treated soil shear stiffness and strength. Shear wave velocity was used as a nondestructive assessment of the level of cementation over the course of the treatment process and as a proxy for soil stiffness. The results of both the shear wave velocity and UCS are shown in Figure 3. It should be noted that, due to laboratory limitations, the bender element/UCS pots were not prepared in triplicate, unlike the pots for species and controls. The shear wave velocity measurements indicated that the pots subjected to recipes MICP-1 and MICP-2 reached the target level of cementation (e.g., V_s = 800 m/s) after about 3 weeks of treatment. The pots with MICP-3 required almost 2 months of treatment to reach the same level of cementation, based on shear wave velocity. The longer period of treatment for MICP-3 is not unexpected based on the lower concentrations of urea and calcium in the recipe (Table 1). The UCS indicate interesting trends, compared to the shear wave velocity data. The responses of MICP-1 and MICP-2 are similar, each with UCS values of about 15 kPa. However, the response of MICP-3 achieved a larger UCS value of 25 kPa.

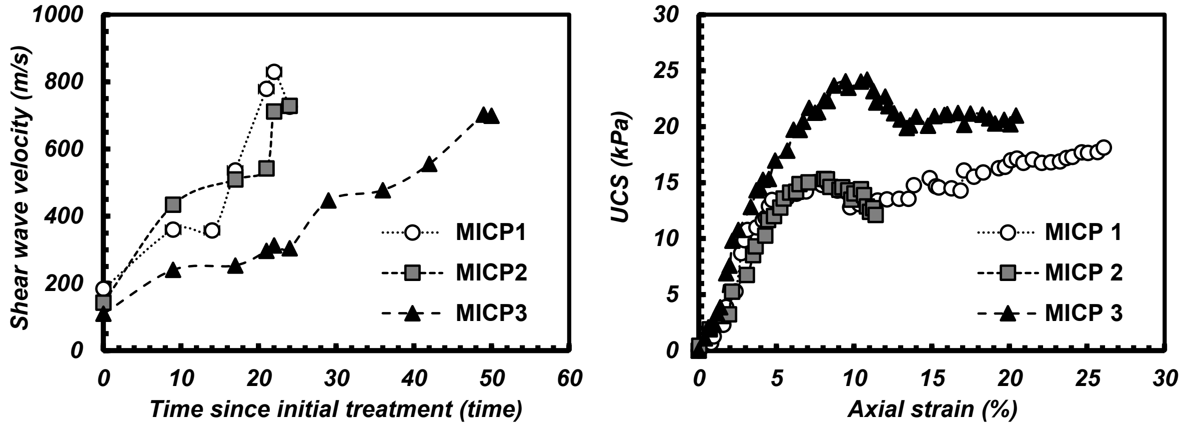

Figure 3. Shear wave velocity and UCS test results for the three MICP recipes.

Treated Soil Erodibility. Results from JET testing performed on untreated and MICP-treated pots are presented in Figure 4. The control pot with no MICP treatment (Control No Seeds) took less time to reach the same level of scour depth in comparison to pots treated with recipe MICP-3, indicating a consistent higher rate of erosion for the untreated soil. Bermuda grass, Roundhead lespedeza, and Weeping lovegrass all took less time to reach about 50% total scour when compared to the pot that received MICP-3, but no seeds, with only Bermuda grass taking less time to reach 90% scour. While more analysis is required, preliminary results show that all treated specimens show more resistance to scour in the top half of the pot.

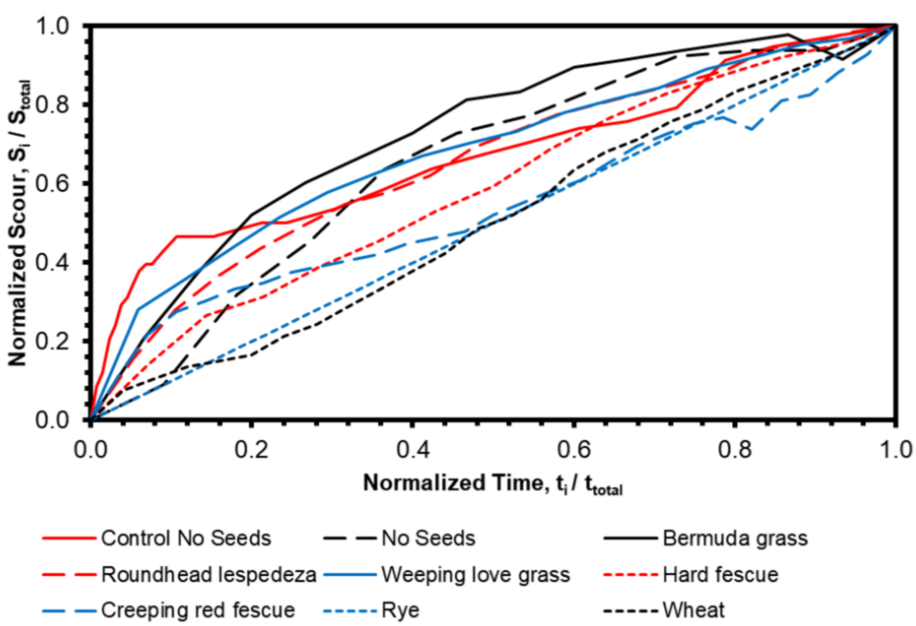

Figure 4. Comparison of the normalized scour depth with time for pots treated with recipe MICP-3.

Mass of precipitated carbonate. Though all of the pots were treated to the same target shear wave velocity of 800 m/s, the resulting carbonate precipitation varied across the MICP treatment recipes and across plant species (Figure 5). MICP-1 consistently produced the highest carbonate content across all species, with particularly notable precipitation in NONE 1 (no plants) and Weeping lovegrass treatments, reaching approximately 4% and 2.5% carbonate content respectively. MICP-2 and MICP-3 showed variable results across species, with each treatment alternately producing higher carbonate content depending on the plant species present. The presence of plants appeared to influence carbonate precipitation, as demonstrated by the variation between the NONE 1 (no plants) treatment and the planted specimens. This suggests potential interactions between plant processes and MICP efficacy. The wheat (TRAE 1), rye (SECE 1), and Weeping love grass (ERCU) species showed relatively high carbonate content across all MICP treatments, indicating they may be particularly compatible with the MICP process.

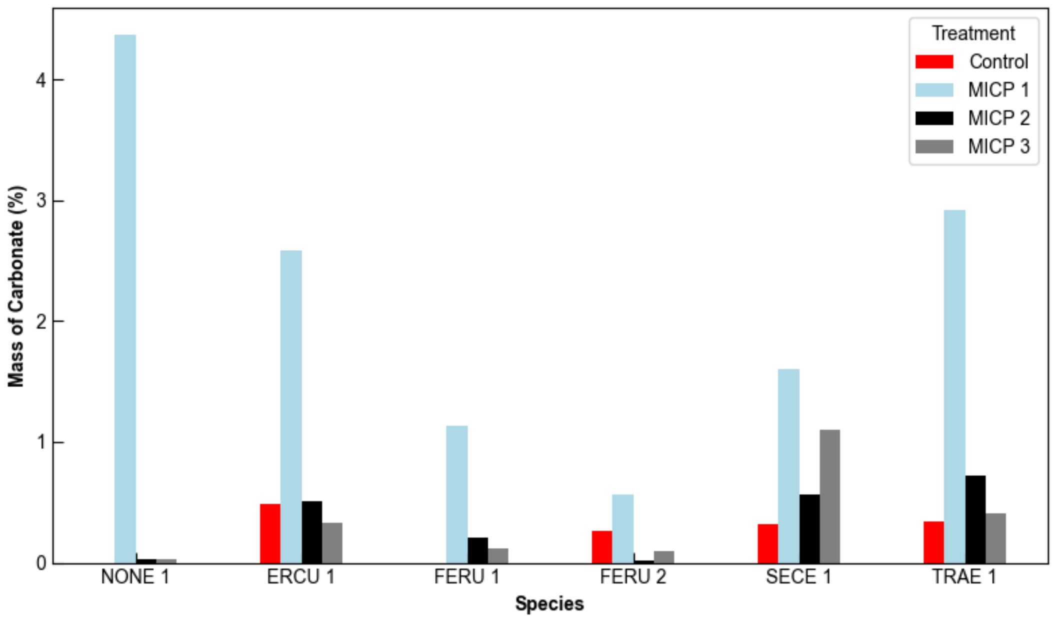

Figure 5. Gasometric Mass of Carbonate (%) for Tested Species

CONCLUSION

The balance between inducing a sufficient level of carbonate cementation through ureolytic-driven MICP and supporting vegetation growth is important for nature-based engineering solutions and ensuring nature-inclusive infrastructure. The results of our germination screening of various plant species indicated some plants, like Weeping lovegrass and Roundhead lespedeza are negatively impacted by all of the MICP recipes tested. On the other end of the observed spectrum, some plants like Hard fescue and Rye demonstrated higher germination success in the presence of at least some of the MICP recipes. This increase in germination compared to the species-specific controls may be due to the presence of phosphate, the reduced nitrogen levels in the treatment recipe, or the combination of these factors. The engineering properties of the planted, treated soil also vary across treatment recipes and plant species. The shear stiffness and unconfined compression strength are relatively similar for each recipe; however, the precipitated mass of carbonate is much different. Additionally, the erodibility of the soil changes with the MICP treatment, where the treatment generally decreases the rate of erosion, and there is a range of erodibility rates across the different species assessed. Further assessment of the erodibility of the other MICP recipes will be valuable towards optimizing the engineering response of the MICP-treated soil with the plant health. Advancing the knowledge of the compatibility of and interaction between MICP and plants will improve not only its suitability for flood control structures, but also other surficial applications of MICP such as dust suppression and heavy metal remediation.

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