Plant Species Influence on Microbially Induced Carbonate Precipitation: X-Ray Diffraction Analysis of Mineral Formation and Composition

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ABSTRACT

While microbially induced carbonate precipitation (MICP) shows promise for soil stabilization, the potential interactions between vegetation and mineral formation processes represent an unexplored opportunity for bio-enhanced ground improvement. This study investigates how plant species affect MICP mineral composition through X-ray diffraction (XRD) analysis of treated soils. Eleven plant species representing grasses and legumes were grown in silty sand treated with three MICP recipes of varying concentration. XRD analysis revealed that all treatments, including controls, produced detectable calcium carbonate polymorphs, with significant discrepancies between XRD and gasometric quantification methods. Plant germination success varied dramatically across MICP treatments, with high-concentration recipes (MICP 1) preventing germination in most species except wheat and rye, while phosphate-supplemented recipes (MICP 2 and 3) improved plant tolerance. Species-specific mineral formation patterns emerged, with certain grasses showing more consistent carbonate precipitation across conditions. However, the study revealed critical limitations in current analytical approaches, as XRD consistently detected higher carbonate content than gasometric methods, indicating potential interference from soil organic matter or analytical artifacts. These findings highlight the need for improved quantification methods and suggest that successful plant-MICP integration requires careful optimization of treatment chemistry to balance mineral formation with plant viability.

INTRODUCTION AND BACKGROUND

Emerging biogeotechnical methods, such as microbially induced carbonate precipitation (MICP), have demonstrated significant potential for stabilizing soil to support new construction and rehabilitated infrastructure. MICP has proven especially effective in reducing soil erodibility, a property of relevance to infrastructure stability. Laboratory and field-scale studies have demonstrated that MICP treatments can reduce wind erosion and dust production (Maleki et al. 2016), water-induced erosion (Ghasemi et al. 2019), 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).

The most studied pathway for MICP is ureolysis, wherein bacteria containing the enzyme urease catalyze the hydrolysis of urea into ammonium and bicarbonate ions. This increase in alkalinity, coupled with the presence of calcium ions in the treatment solution, leads to the precipitation of calcium carbonate minerals within soil pores (Stocks-Fisher et al. 1999). MICP results from a series of biochemical reactions that increase pore fluid alkalinity and generate carbonate, which reacts with calcium from treatment solutions to form calcium carbonate precipitates (DeJong et al. 2006). The ureolysis process increases alkalinity as urea is hydrolyzed into ammonium and bicarbonate, which precipitate with the provided calcium to form calcium carbonate (Stocks-Fisher et al. 1999). The precipitated calcium carbonate reinforces mechanical and hydraulic shear stresses, with cemented soil matrices demonstrating improved volumetric behavior, shear strength, stiffness, tensile strength, and compressibility (Montoya and DeJong 2015; Feng and Montoya 2015).

Plants also reinforce surficial soils against erosion by reducing rainfall and runoff energy, which may complement soil cementation benefits, especially for flood control infrastructure. Plant tissue above ground intercepts raindrops and slows water flow while trapping sediment, whereas fine roots below ground physically bind soil particles together (Khanal and Fox 2017; Gyssels et al. 2005). However, plant roots must penetrate the soil and continuously acquire water and nutrients to support growth. Additionally, plants release a diverse array of compounds, collectively termed root exudates, into the rhizosphere. These exudates include organic acids, sugars, amino acids, enzymes, and various secondary metabolites that can alter soil chemistry and microbial activity (Jones et al. 2009; Bais et al. 2006; Long et al. 2024). Plant root exudates may interact with MICP processes in several ways. Organic acids in root exudates could affect local pH, potentially influencing carbonate precipitation kinetics. Calcium-binding compounds may alter calcium availability for precipitation. Furthermore, plant-derived compounds might influence crystal growth and polymorph selection during calcium carbonate formation. Some plant species actively precipitate calcium compounds as a mechanism for regulating internal calcium levels, forming calcium oxalate crystals or calcified nodules in root tissues (White and Broadley 2003; Franceschi and Nakata 2005). Preliminary studies evaluating MICP and plant compatibility have shown that grass seedlings can grow in MICP-treated soil provided cementation levels remain light to moderate, with heavy cementation limiting growth (Ghasemi and Montoya 2022a). However, high concentrations of MICP recipe components led to established grass drying, consistent with nitrogen burning and indicating sensitivity to MICP byproducts.

X-ray diffraction (XRD) is a powerful analytical technique for identifying and quantifying crystalline phases in soil samples. For MICP studies, XRD offers the ability to distinguish between calcium carbonate polymorphs (calcite, aragonite, and vaterite), which may form under different environmental conditions or in response to biological influences (Rodriguez-Navarro et al. 2012). Understanding polymorph formation is critical for engineering applications because different calcium carbonate polymorphs exhibit distinct mechanical properties and stability characteristics. Calcite, the most stable polymorph, provides superior long-term cementation and mechanical enhancement compared to vaterite and aragonite, which are metastable and may transform over time, potentially affecting soil performance (Nafisi et al. 2019; Fu et al. 2023).

The characteristic diffraction peaks for calcite include 2θ values at 23.0°, 29.4°, 36.0°, 39.4°, 43.2°, 47.5°, and 48.5°, corresponding to specific crystal planes as confirmed in standard mineralogical databases (JCPDS PDF2 standard card 05-0586). XRD analysis of MICP-treated soils has confirmed that the diffraction peaks associated with calcite become more pronounced following treatment, with the main calcite peak around 29° being particularly diagnostic (Dhami et al. 2013).

Research has also shown that both calcite and vaterite polymorphs can form during MICP, with their relative proportions influenced by environmental conditions and the presence of organic compounds (particularly organic acids such as citric and malic acid, amino acids, polysaccharides, and proteins from microbial and plant sources) (Wei et al. 2015; Haystead et al. 2024). These organic substances, which could include plant-derived compounds, can significantly influence calcium carbonate crystal formation, affecting both the polymorph type and crystal habit (Ren et al. 2011). Vaterite exhibits characteristic peaks at 2θ values of 24.9°, 27.0°, 32.8°, 43.9°, and 50.0°, while aragonite displays distinctive peaks at 26.3°, 27.2°, 33.2°, 36.2°, 37.9°, 38.4°, and 45.9° (Behrens et al. 1995). Recent advances in MICP research have demonstrated the importance of understanding polymorph control mechanisms, as different calcium carbonate phases exhibit varying degrees of stability and mechanical enhancement properties that directly affect soil performance (Nafisi et al. 2019; Zhang et al. 2023; Lin et al. 2023).

Despite growing interest in integrating MICP with vegetation for sustainable infrastructure, the fundamental question of how plant species influence MICP mineral formation and composition remains largely unexplored. Understanding these plant-mineral interactions is crucial for optimizing both soil stabilization effectiveness and ecological health in nature-based engineering solutions. This research addresses this knowledge gap by developing X-ray diffraction analytical methods to characterize and quantify mineral formation in plant-influenced MICP-treated soils.

MATERIALS AND METHODS

The soil was collected from the North Carolina State University Lower Coastal Plain Research Station (LCRS) in Kinston, NC. Importantly, 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. The soil was classified as silty sand (SM) with a mean particle diameter (D_{50}) of about 0.16 mm and a plasticity index (PI) of 8. The soil had a dry density of 1.43 g/cm^3 and was prepared in 11.4 cm (4.5") diameter nursery pots.

The following eleven species were selected for this methods application, representing different plant families and root architectures: Grasses (Poaceae family) - Creeping bentgrass (AGST, *Agrostis stolonifera*), River oats (CHLA, *Chasmanthium latifolium*), Creeping red fescue (FERU, *Festuca rubra*), Kentucky bluegrass (POPR, *Poa pratensis*), Bermuda grass (CYDA, *Cynodon dactylon*), Virginia wild rye (ELVI, *Elymus virginicus*), Weeping love grass (ERCU, *Eragrostis curvula*), Rye (SECE, *Secale cereale*), and Wheat (TRAE, *Triticum aestivum*); Legumes (Fabaceae family) - Partridge pea (CHFA, *Chamaecrista fasciculata*) and

Roundhead lespedeza (LECA, *Lespedeza capitata*). There was also a set of triplicates where no seeds were planted (NONE) to serve as controls for plant influence on mineral formation.

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. The inoculated cultures were incubated at 27°C at 200 rpm for 40 hours until an optical density (OD_{600}) of 1 was reached, indicating sufficient bacterial growth for treatment application.

All plant species were treated with MICP recipes 1-3 from Ghasemi et al. 2025, shown in Table 1 below, with MICP 1 representing the state-of-the-art recipe proposed in DeJong et al. 2022. The experimental design included 144 pots total: (11 plant species + 1 No Seeds Control) × 3 triplicates × (3 MICP treatments + control treatments). Treatments were applied once daily with overhead sprayers to the unsaturated pots, followed by a water flush four hours later to remove excess chemicals. The experiment duration varied by treatment: 24 days for MICP 1 and MICP 2, and 50 days for MICP 3, with treatments continuing until a target shear wave velocity of 800 m/s was achieved (see Ghasemi et al. 2025 for more details).

Table 1. Chemistry and concentration of the implemented MICP treatment recipes (Ghasemi et al. 2025).

	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

Each sample was taken with a coring tool and ground to prepare for both XRD and gasometric analysis. One replicate sample was analyzed for each species-treatment combination, totaling 48 samples for XRD analysis.

X-ray diffraction analysis was performed using a *Rigaku SmartLab X-ray Diffractometer* at the Analytical Instrumentation Facility at North Carolina State University. The instrument utilized Cu Kα radiation with a wavelength (λ) of 1.540598 Å for Kα1 and 1.544426 Å for Kα2, with a Kα2/Kα1 ratio of 0.5 as shown in the instrument parameters. The XRD was operated at 40 kV generator voltage and 44 mA tube current. Measurements were conducted in goniometer mode with a scan range of 5-80° 2θ, using a step size of 0.02° and a counting time of 1.122 seconds per step. The divergence slit was fixed at 1°, and the receiving slit was set to 0.1°. Each scan collected thousands of data points under pre-set counts mode at room temperature (298 K). The specimen preparation and analysis conditions were standardized across all samples to ensure reproducibility and comparability of diffraction patterns. Reference patterns from the American Mineralogist Crystal Structure Database (AMCSD) were used for phase identification, with particular attention to calcium carbonate polymorphs (calcite, vaterite, and aragonite).

Calcium carbonate content was tested for the control and MICP recipes 1-3 following the gasometric method from O'Toole et al. 2022. Soil samples that were used to conduct the XRD analysis were retrieved for mass of carbonate measurements. Samples (3g) from all four test conditions were added to 125-mL glass vials sealed with rubber septa and crimped. A 50-mL syringe with a needle was inserted into the septum to measure the gas volume change. A 20-mL syringe containing 20 mL of 1M hydrochloric acid (HCl) was then inserted and evacuated into the vial. An established calibration curve built utilizing known quantities of pure calcium carbonate was then used to approximate the calcium carbonate content.

RESULTS

Plant germination success and gasometric calcium carbonate content varied significantly across treatment conditions and plant species. Control treatments demonstrated highly variable germination rates, with Wheat (TRAE) and Rye (SECE) achieving the highest germination success, while several species showed no germination (Figure 1). Gasometric carbonate content in control treatments remained near baseline levels (0 to 0.49%). MICP 1 treatments reduced germination across most species, with no germination observed in all species other than Wheat (TRAE) and Rye (SECE). MICP 1 produced the highest gasometric carbonate measurements, reaching 4.37% in unseeded controls and exceeding 2.5% in ERCU and TRAE. The phosphate-supplemented treatments (MICP 2 and MICP 3) had multiple species achieving moderate germination rates while maintaining measurable carbonate formation.

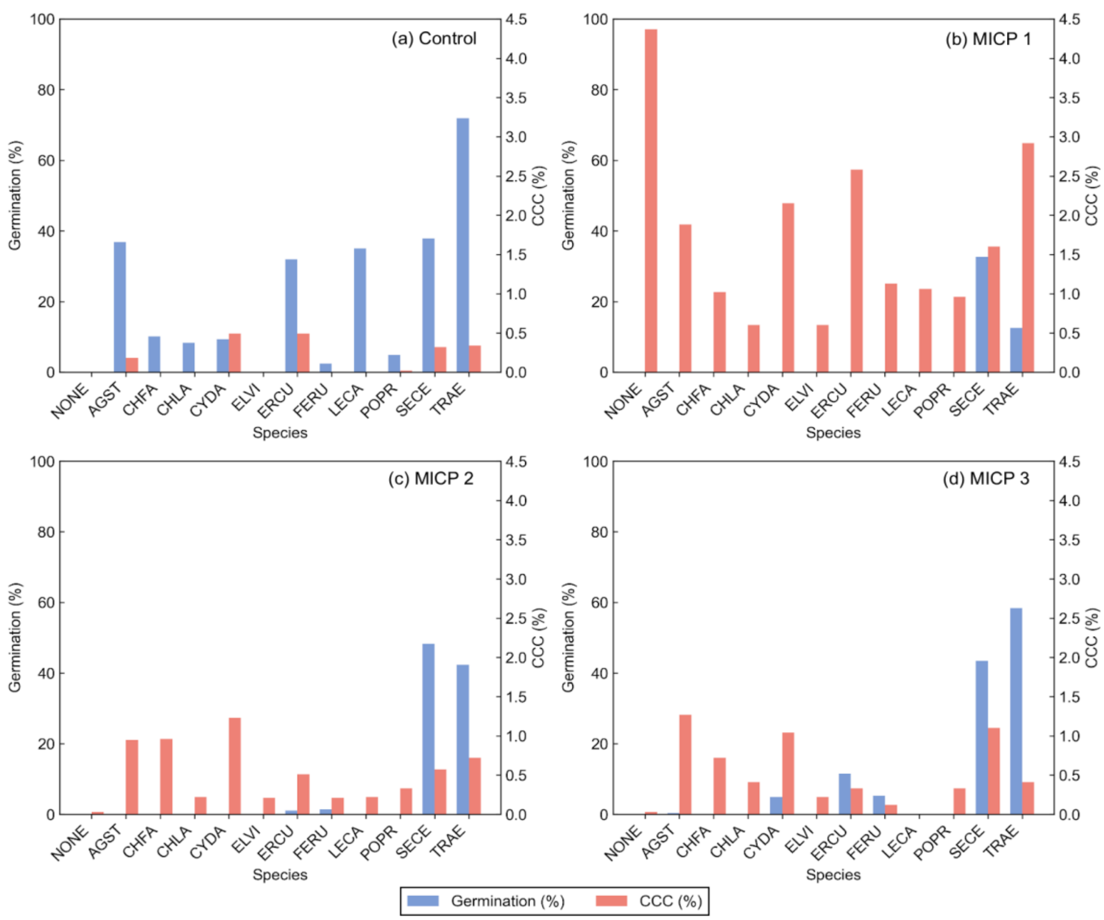

Figure 1. Germination (%) and Gasometric calcium carbonate content (CCC%) across species for (a) Control, (b) MICP 1, (c) MICP 2, and (d) MICP 3.

The relationship between plant germination success and mineral formation revealed variable patterns, inconsistent between polymorph types. Figure 2 illustrates the relative distribution of calcium carbonate polymorphs (calcite, vaterite, and aragonite) detected by XRD analysis across species and treatment recipes. All treatments, including controls, produced detectable amounts of multiple calcium carbonate polymorphs by XRD. Control treatments showed variable but limited carbonate formation, with some samples exhibiting elevated aragonite content exceeding calcite formation, in excess of 5%. MICP 1 treatments consistently produced measurable calcite formation across species, with unseeded controls (NONE) achieving 0.862% calcite content. Plant species demonstrated distinct polymorph formation patterns, with CHFA showing exceptional calcite formation in MICP 3 (1.957%), while ERCU achieved the highest calcite content in MICP 1 (1.026%). Vaterite formation occurred across all treatment conditions, including in unseeded controls.

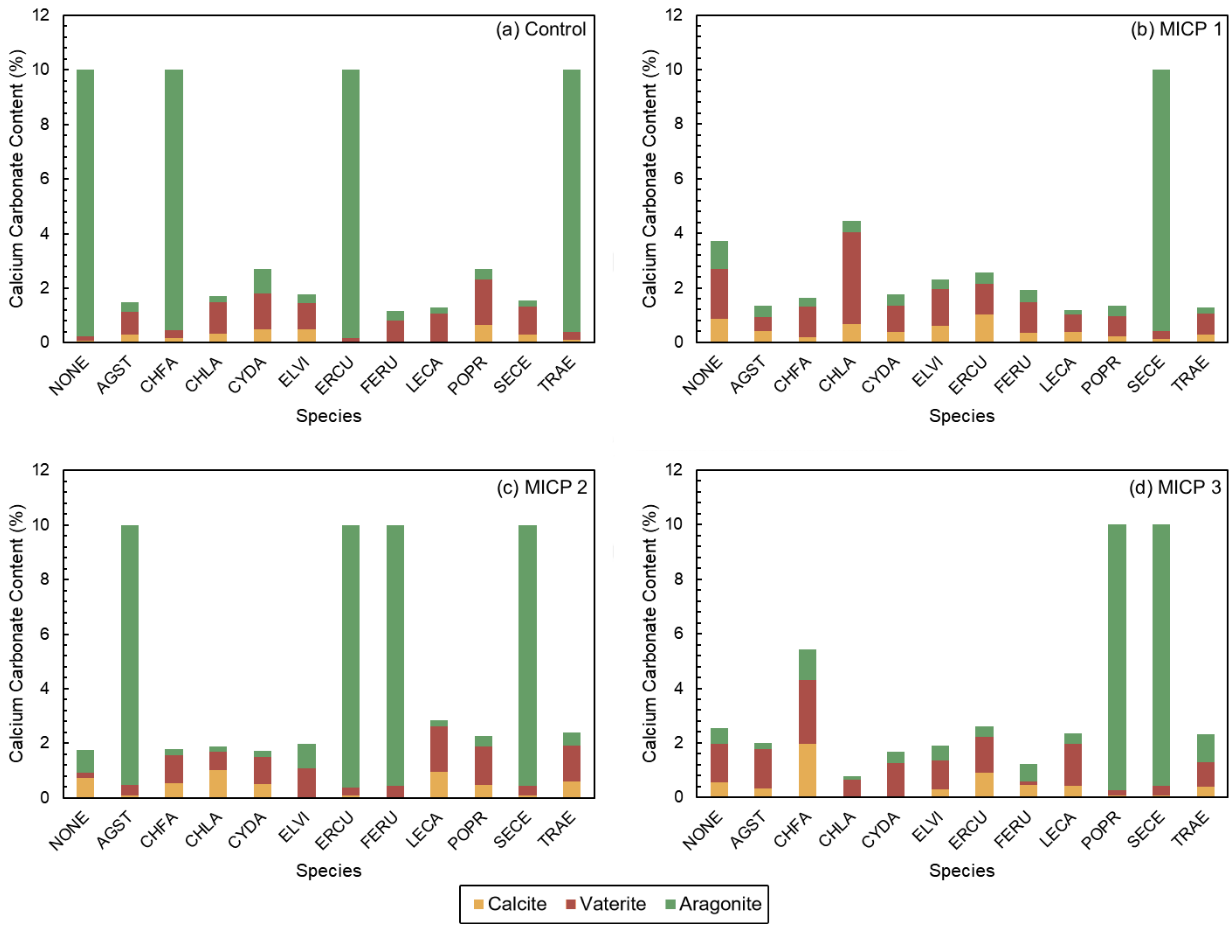

Figure 2. Calcium carbonate polymorphs across species for (a) Control, (b) MICP 1, (c) MICP 2, and (d) MICP 3.

The levels of calcium carbonate detected by XRD do not seem to relate strongly to levels of germination success (Figure 3). Additionally, levels of gasometric carbonate measured do not equal that of carbonate measured via XRD. A 1:1 line is shown on Figure 3 to demonstrate the misalignment of the data taken on the same samples with different methodologies.

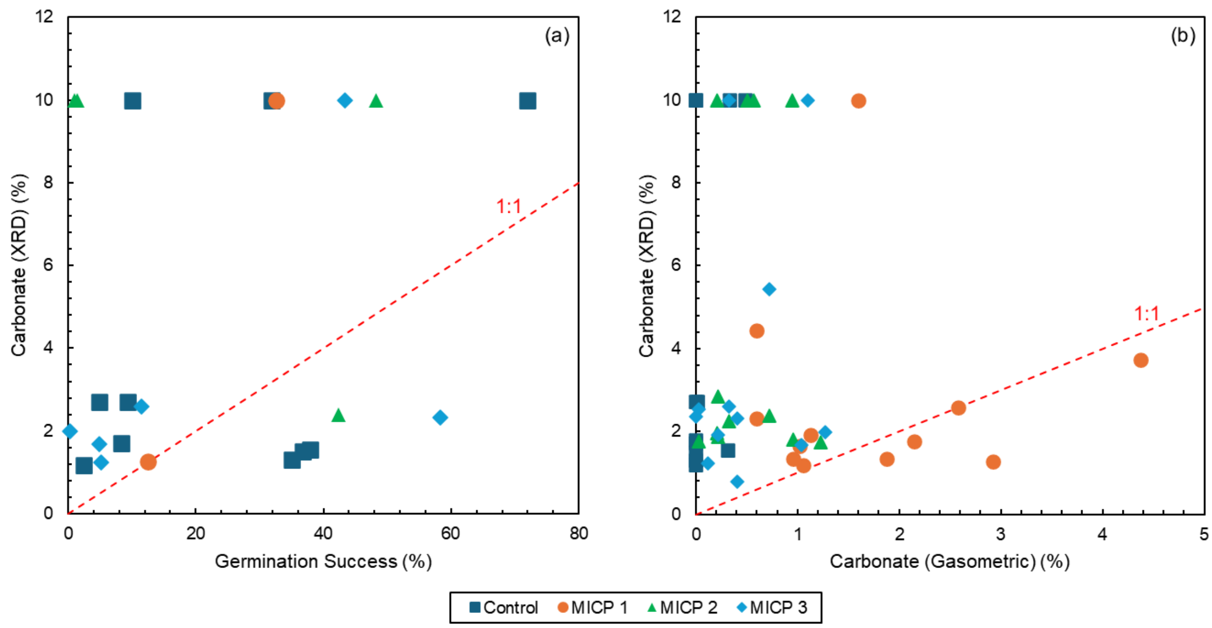

Figure 3. Carbonate detected by XRD (%) against germination success (%) (left) and Carbonate detected by the gasometric method (right)

DISCUSSION

The systematic differences between XRD and gasometric methods (e.g., far higher calcium carbonate content found in XRD) highlight critical challenges in quantifying MICP-induced mineral formation in natural soil systems. The consistently higher calcium carbonate content values in the XRD seen in Figure 3 could be a result of various factors: (1) the peak finder may be searching for aragonite in regions where there is a confounding peak, or (2) insufficient removal of background. These findings indicate that automated XRD peak identification algorithms require further validation for complex soil matrices, and future studies should incorporate additional analytical methods such as thermogravimetric analysis to better characterize amorphous phases. Future work will utilize a more sophisticated Rietveld refinement as well as subtraction of potential root exudates to better determine the correlation between plant species germination success and the precipitation of calcium carbonate polymorphs.

The observed species-specific variations in mineral formation provide evidence for plant-mediated effects on MICP processes, though the effects are more subtle than initially anticipated. This may be in part due to growth chamber and watering conditions being unsuitable for successful germination across all species tested. Not all plants germinated even in the control treatment (i.e., consisting only of water), leading to less data on the impacts of successful germination. Recent research has demonstrated that root exudates can significantly influence carbonate precipitation processes through multiple mechanisms, including pH modulation, calcium chelation, and provision of nucleation sites (Long et al. 2024; Haystead et al. 2024). Literature demonstrates that additives such as those found in plant root exudates (e.g., organic acids and amino groups) could cause the preferential formation of metastable polymorphs, such as vaterite, leading to less reliable geotechnical applications (Niu et al. 2022). Future work will optimize growth conditions for far more robust datasets to be formed.

The complex interactions between treatment concentration and plant tolerance revealed in these results support the need for careful recipe optimization. While MICP 1 achieved higher calcite formation in unseeded controls, the improved plant performance observed in phosphate-supplemented treatments (MICP 2 and MICP 3) may create long-term benefits through enhanced root-mediated precipitation processes. However, the concurrent changes in urea, calcium chloride, and ammonium chloride concentrations between recipes represent confounding variables that prevent definitive attribution of effects to phosphate supplementation alone. The reduced ionic strength in lower-concentration recipes may improve plant tolerance by reducing osmotic stress, while decreased ammonium production could reduce nitrogen toxicity effects.

The dominance of quartz (85-98%) across all MICP-treated samples confirms that MICP-induced changes occur within a complex mineral matrix, emphasizing the importance of sensitive analytical methods for detecting relatively small but potentially notable changes in carbonate content. For engineering applications requiring consistent carbonate formation, species selection appears critical, with certain grasses (ERCU, CHLA, TRAE) showing more reliable mineral formation across treatment conditions. The persistence of vaterite in both planted and unseeded samples suggests further fine-tuning is needed on X-ray diffraction analysis methodologies for large datasets such as this one. The presence of vaterite should be validated by scanning electron microscopy (SEM) to confirm the XRD findings. It is crucial to determine vaterite presence as it is metastable, leading to concerns for long-term soil performance characteristics. Recent advances in MICP methodology development suggest that controlled polymorph selection could optimize soil enhancement properties for specific engineering applications (Zhang et al. 2023). This high level of aragonite also warrants further investigation, as it far exceeds the level of calcium carbonate precipitation expected as observed from gasometric testing. SEM should also be utilized to confirm the presence of such large quantities of aragonite.

Several limitations in the current study affect the interpretation of results. The absence of pH monitoring prevents assessment of how plant root exudates may alter local pH conditions that influence polymorph stability. The experimental duration (24-50 days) may be insufficient to observe complete polymorph transformation or long-term plant-soil interactions. Additionally, the complex soil organic matter background from previous wheat cultivation creates analytical challenges that may not be representative of other soil systems. Future research should prioritize controlled experiments that isolate individual variables (particularly the influence of phosphate in the MICP process), incorporate time-series analysis to track polymorph evolution, and develop improved analytical protocols for quantifying MICP effects in complex plant-soil systems.

CONCLUSIONS

This study demonstrates that plant species influence MICP mineral formation patterns, though the effects are more subtle than initially hypothesized and occur against a complex soil mineral background. XRD analysis successfully detected species-specific variations in calcium carbonate formation, with grass species (particularly ERCU and TRAE) showing more consistent mineral formation across treatment conditions. However, the discrepancy on the amount of carbonate minerals between XRD and gasometric methods highlight important limitations in current analytical approaches for MICP research, particularly in soils with high organic matter content.

Treatment recipe optimization must balance mineral formation efficiency with plant tolerance. While higher-concentration treatments (MICP 1) achieved greater carbonate formation in unseeded controls, the variable plant responses and improved consistency observed with phosphate-supplemented recipes indicate that nutritional considerations are critical for successful plant-MICP integration. However, the concurrent changes in multiple chemical components prevent definitive conclusions about the specific mechanisms driving these improvements.

These findings provide a foundation for optimizing plant selection in nature-based infrastructure applications while highlighting critical research needs. The study demonstrates that grass species offer more predictable MICP enhancement compared to other plant families, supporting their selection for erosion control applications. However, successful integration of plants and MICP requires continued development of analytical methods capable of accurately quantifying treatment effects in complex soil systems, controlled experiments isolating individual variables, and longer-term studies assessing the stability of plant-enhanced mineral formation. Future research should prioritize validation of XRD analytical protocols for MICP applications (e.g., utilizing SEM), mechanistic investigation of plant-mineral interactions through further controlled experiments, and development of predictive frameworks for optimizing plant-MICP systems in field applications.

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