The Potential of Bioremediation Techniques to Enhance Soil Revitalization in Degraded California Landscapes

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ABSTRACT

Rapid urbanization has led to significant technological advancements yet has altered ecosystems and influenced surrounding environments, notably affecting soil processes. Vehicular emissions contribute to soil pollution, disrupting soil equilibrium and nutrient balance, ultimately compromising soil health and ecosystem stability. This study investigates the complex dynamics between microbial communities, invasive species, and soil fertility, aiming to enhance understanding for sustainable land management practices. Employing a holistic approach, this research examines soil fertility in proximity to roads, where car emissions catalyze microfauna encroachment and invasive flora growth. Findings reveal that mixed inoculant treatment optimally enhanced soil fertility with NPK values reflecting optimal levels of nitrogen (MED-2) and phosphorus (M1-2: LOW-1 to M3-4: MED-2). The fungal treatment improved the most in soil texture and fertility, decreasing the number of microaggregates and improving macroaggregate formation stability, reflecting on the efficient photosynthesis uptake (Plot F4 Leaf SA 34.92 cm^2), and deep root penetration of Tall fescue (F4-12.41g) going into the rangeland with least amount of car emission impact. However, compaction impedes invasive Tall fescue growth (Plots B1-3) near roadsides. Implications of this study underscore the suitability of bioremediation techniques near roads and emphasize the importance of understanding plant-soil-microbe interactions for enhancing remediation efficiency. Establishing long-term monitoring and adaptive management strategies crucial for ensuring resilience and sustainability of roadside ecosystems against urbanization-induced degradation.

KEYWORDS

NPK, microbial processes, invasive plant encroachment, organic amendments, macroinvertebrates, anthropogenic activity

INTRODUCTION

The rapid pace of urbanization has ushered in a multitude of technological advancements, reshaping ecosystems and profoundly influencing surrounding environments. However, soil processes are inhibited by pollution from various sources, including agriculture and vehicular emissions, has disrupted soil equilibrium, leading to a cascade of detrimental effects (Lee and Power 2013). Vehicular emissions, in particular, contribute to an imbalance in essential nutrients such as nitrogen, phosphorus, and potassium (NPK), with nitrogen overload stemming from nitric oxide, nitrogen oxide, and ammonia emissions (Lee and Power 2013). This influx of nitrogen alters soil composition, favoring the growth of nitrophilic plant species and invasive vegetation, as evidenced in calcareous grassland ecosystems (Lee and Power 2013). Consequently, disturbances in soil biogeochemical processes arise, jeopardizing the functionality of soil ecosystem services crucial for environmental stability, the compromised soil structure resulting from compaction and pollution exacerbates erosion rates, undermining soil fertility and biodiversity (De Silva et al. 2021). Soil fertility is a crucial concern in delicate ecosystems, where it can take a long time for soil productivity to recover after activities like livestock rearing and farming (Blue Oak Ranch Reserve, 2015). The issue is worsened by soil degradation and pollution from car emissions and heavy metals. This confluence of factors poses a significant threat to the resilience of ecosystems, highlighting the urgent need for comprehensive strategies to mitigate the adverse impacts of urbanization on soil health and ecosystem integrity.

Soil microbial communities play a pivotal role in shaping soil fertility and environmental quality, comprising over 90% of the total soil microbial biomass (De Silva et al. 2021). These microorganisms are integral regulators of soil nutrient availability, influencing the activity and composition of soil microbial communities, which in turn affect soil structure and nutrient acquisition for soil fertility (De Silva et al. 2021). Macro-aggregates, crucial for soil structural stability, air circulation, and water infiltration, are significantly impacted by soil compaction from degradation—a biological problem linked to reduced production of essential soil components such as polysaccharides and glomalin that come from fungi and bacteria that coat the soil particles to glue together and form macro-aggregates (Wright and Upadhyaya 1996). Soil compaction, attributed to factors like the absence of living roots and mycorrhizal fungi, not only affects soil fertility parameters like cation exchange capacity but also influences the spread of harmful invasive species (Håkansson and Reeder 1994). Although, while some invasive species like tall fescue have been utilized for erosion control due to their deep roots, their presence in fragile soil ecosystems poses challenges to ecological biodiversity (Mohlenbrock 2001). Moreover, soil microbes play a critical role in either impeding or facilitating the growth of invasive plants, thereby shaping the dynamics of ecosystem invasion (Kalske et al. 2022). Understanding the intricate interactions between soil microbes and invasive species is essential for mitigating their adverse effects on native biodiversity and ecosystem functioning.

Disturbances in soil ecosystems often lead to a shift in microbial composition, characterized by a decrease in fungal populations and an increase in bacterial abundance, resulting in a prevalence of microaggregates over macroaggregates (Hoorman 2009). However, the formation of macroaggregates is crucial for enhancing soil structure and minimizing compaction. Glomalin, produced by fungi, plays a vital role in soil aggregation but is continually consumed by bacteria in tilled soils, where bacterial populations thrive due to their hardiness and smaller size (Hoorman 2009). Meanwhile, organic amendments such as biochar, sphagnum moss, and worm castings contribute to Soil Organic Matter (SOM) stocks, creating micropore spaces and fostering beneficial microbial growth, thus improving soil structure and reducing stress on the microbial community (Murtaza et al. 2023). However, the intricate relationship between plants and endophytic fungi introduces complexities into ecosystem dynamics, as endophytes enhance host resilience, nutrient acquisition, and competitive ability, potentially diminishing overall biological diversity (Mohlenbrock 2001). Studies suggest that the presence of tall fescue and its endophyte significantly reduces biological diversity across various levels of soil organisms, insects, plants, birds, and mammals (Mohlenbrock 2001). Essential nutrients like potassium, phosphorus, and nitrogen are crucial for plant development and growth, with potassium activating numerous plant enzymes, phosphorus facilitating root development and seed production, and nitrogen playing a pivotal role in chlorophyll synthesis, amino acid formation, and photosynthesis (Potdar et al. 2021). As we navigate the uncertainties of climate change, exploring soil resilience becomes most important, particularly in roadside soils where microbial activity may vary with proximity to roads. Nonetheless, gaps persist in our understanding of microbial soil inhabitants, entailing comprehensive studies to inform sustainable management practices and enhance soil biodiversity.

Through such attempts, I aim to uncover novel insights into soil dynamics and strengthen the validity of effective strategies for ecosystem restoration and resilience in the face of environmental change. Amidst the increasing soil degradation in California's urbanized land, my research aims to evaluate soil-microfauna-plant relationships in proximity to roadsides, investigating the encroachment of microfauna and invasive flora catalyzed by car emissions on urban soils. This thesis aims to explore the multifaceted dynamics between soil microbial communities, invasive Tall fescue, and soil fertility, shedding light on their implications for sustainable land management practices. By addressing these objectives, this study endeavors to uncover how bioremediation practices contribute to soil revitalization and support native flora growth, offering insights into sustainable soil management practices for mitigating land degradation in urban environments of invasive plant species.

Over three months, the effectiveness of organic amendments and microbial inoculants will be investigated, focusing on changes in soil fertility, invertebrate diversity, and the growth of invasive Tall fescue. The central question driving this research is: How do bioremediation methods effectively enhance soil fertility in infertile regions of California?

> Firstly, I aim to understand how organic amendments support nutrient acquisition for soil fertility, especially concerning soil proximity to roads impacted by car emissions. I hypothesize that incorporating biochar, worm castings, and sphagnum moss can provide nutrient sources for bacteria and fungi through electrons from organic matter, with plots 3 and 4 seeking the most from the added amendments. The objective is to measure NPK values and soil texture, comparing microbial inoculant treatment effects between roadside plots extending further into the rangeland.
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> Secondly, I seek to shed light on how microbial inoculation influences the activity of nutrient acquisition through diversifying invertebrate communities and increasing fertility in soil. I anticipate initial disruption followed by eventual success, to conduct invertebrate counts using the Berlese funnel method, comparing inoculant treatment effects and distance variables to understand the role of microbial inoculants in shaping a resilient environment.
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> Lastly, I aim to examine the growth responses of invasive fescue under inoculant and organic amendment treatments, considering the influence soil proximity to roads has on the encroachment of invasive species in disturbed areas. I predict higher nitrogen levels and potential success of Tall fescue to the closest distance plots, 1 and 2 in the fungi treatment, due to the endophyte enhancing resistance to environmental stressors and receiving nutrients through mycorrhizal fungi. I evaluated invasive fescue growth and inoculant treatment responses with road proximity, examining Root: Shoot(R:S) ratios, leaf Surface Area(SA), and root/shoot biomass.

METHODS

Research Site

This is evident at the Blue Oak Ranch Reserve in urban San Jose, part of the UC Natural Reserve System, serving as a Biological Field Station and Ecological Reserve. I gathered soil samples from the Valley Oak woodland area at coordinates 37.38559°N, and 121.73360°W that can be seen on Figure 1(Google Maps 2024). Established as a conservation site in 2000, the Blue Oak Ranch Reserve includes a diversity of habitats, including blue oak woodland, black oak woodland, coast live oak woodland, riparian forest, chamise chaparral, diablo sage scrub, nonnative annual grassland, wildflower field, and native perennial grassland (Natural Reserve System 2015). The reserve's flora includes *Valley oak trees, Achyrachaena mollis, Aira caryophyllea, Agrostis exarata, and Agrostis viridis* (Natural Reserve System 2015).

Figure 1. Collection site's location. The four plots are spaced 5 meters apart, with measurements made relative to their proximity to the road. Explain plot. Estimate plot measurements more.

Field Sampling

I delineated a transect within the study area to establish a comprehensive sampling framework, strategically selecting plots based on their proximity to the road. Each plot was meticulously spaced five feet apart from one another, with an additional plot situated on the opposite side of the road for comparative purposes. Soil samples were systematically collected from depths ranging between 5 and 30cm, with a total of ten gallon-sized bags of soil procured from each plot. To ensure robust statistical analysis, I adopted a replicative approach, incorporating three replicas of the experiment, each containing three variables (fungi, bacteria, and bacteria/fungi), alongside a control group, thereby resulting in a total of 44 pots of soil for experimentation. Employing traditional methods, I excavated the soil using shovels and subsequently packed them into gallon-sized plastic bags, which were then securely housed within insulated containers and refrigerated until deployment for the experiment on January 3, 2024. Transporting the soil samples to the Oxford Tract Greenhouse, I recreated cool-season weather conditions prevalent outside, supplemented by irrigation using a wide-head spraying hose to simulate natural environmental conditions conducive to plant growth and soil microbial activity.

Greenhouse Experiment: Organic Amendment Enhancement

Worm Castings

Worm castings were utilized as a method for soil bioremediation. Worm castings, abundant in organic matter and beneficial microorganisms, are instrumental in enriching the soil with essential nutrients and enhancing its structure. This improvement can increase water retention, better soil aeration, and the prevention of nutrient leaching. Moreover, the presence of humus in worm castings supports beneficial soil microorganisms, aiding in the gradual release of nutrients to sustain plant growth. Worm castings can also improve the efficacy of promoting seed germination, bolstering plant growth, and combating various plant diseases and pests, such as root and crown rots, wilt disease, mites, aphids, and mealybugs. Overall, the utilization of worm castings has proved to be a valuable approach to improving soil quality, fostering healthy plant growth, and supporting bioremediation (Pennington Seed 2023).

Sphagnum moss

Sphagnum moss was incorporated into the soil as part of the bioremediation process. The addition of Sphagnum moss can increase moisture retention and facilitate gradual water release, thereby enhancing water availability for turfgrass compared to unamended sands. By filling voids in coarse-textured sands and creating a range of pore sizes, Sphagnum moss contributes to improved water retention in drought-prone soils, surpassing the effectiveness of certain inorganic amendments (Bigelow, Bowman, and Cassel 2004).

Biochar

The study integrated biochar into the organic matter (OM) mixture to facilitate soil bioremediation. Biochar was chosen due to its rich content of essential nutrients such as calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), and nitrogen (N), vital for plant growth. These nutrients, upon release, promoted the growth and development of plants, thereby aiding in soil restoration. Additionally, biochar's high pH and cation exchange capacity (CEC) were utilized to enhance soil fertility. The biochar selected for the study had a high specific surface area (SSA), enabling it to effectively reduce the bioavailability and efficacy of weed killers, thus contributing to weed control efforts. Moreover, biochar demonstrated remarkable capabilities in removing chemicals from soils and reducing plant uptake, further supporting soil remediation efforts. Furthermore, its incorporation resulted in increased water-holding capacity, CEC, and pH levels in the soil, which are crucial factors for soil health and productivity (Murtaza 2023).

Figure 2. Pot Preparation in the Oxford Tract Greenhouse Plot 1-4 ⬇ Triplicates made for the treatments and a doubled control.
Top left: Control, **Top right**: Fungi Inoculant, **Bottom left**: Bacteria. **Bottom right**: Bacteria +Fungi Inoculants

Treatment Preparation

In the greenhouse setup for my experiment, I arranged a total of 44 pots, strategically allocating 8 of them to accommodate the doubled replication of the 4 plots, as seen on Figure 2. Each plot was meticulously organized to receive specific microbial inoculant treatments, with triplicate plots designated for each inoculant variant. The fungal treatment consisted of Endomycorrhizae and Ectomycorrhizae. The microbial inoculant, composed of Bacillus subtilis and Rhizobia bacteria, which were carefully applied to their respective plots. Bacillus subtilis enhances nitrogen fixation and solubilization of phosphorus, producing siderophores that promote growth, suppress pathogen growth, and reduce stressors (Hashem 2019). Rhizobia bacteria form legume root nodules and fix nitrogen from the atmosphere for plant uptake (Purwaningsih 2021). Additionally, a final treatment was introduced, combining both inoculants to assess the integrated biochemical dynamics of fungal and microbial interactions. This systematic arrangement facilitated the investigation of the individual and combined effects of microbial inoculants on the experimental outcomes.

DATA COLLECTION

In the initial phase of my research, I promptly conducted soil measurements upon collection. My focus was on gathering data for, nitrogen, phosphorus, and potassium (NPK) and soil texture using the hydrometer method. These parameters are key indicators of soil fertility, crucial for promoting soil aggregation and nutrient retention. I conducted data collection both before and after the period from January to March. Using test kits from LaMotte, I followed the protocol and analyzed NPK results for both preliminary and post-experiment datasets. Additionally, I evaluated soil texture before and after the experiment to provide insights into soil characteristics and changes over time.

Hydrometer Method

In the soil texture determination procedure, I followed the OSU Soil Fertility Lab and labeled 250 mL beakers and tared them on an analytical balance, and weighed 50 grams (± 0.05 g) of soil from each tin I added directly into their respective beakers, with initial weights recorded along the process (OSU Soil Fertility Lab 2020). Subsequently, I added 25.0 mL of sodium hexametaphosphate dispersing solution to each beaker containing soil, and added an additional dispersing solution to an empty beaker to serve as a blank. The beakers were brought to a total volume of approximately 200 mL with deionized water. Stirring with a glass rod at a moderate speed for 10 seconds ensued, with the rod rinsed with deionized water into a waste beaker between each sample, followed by a 30-minute settling period.

The sample mixture from each beaker was then poured into a dispersion cup, ensuring all soil particles were rinsed from the beaker using a wash bottle of DI water, and DI water was added to the dispersion cup until it reached no more than ¾ full. The mixture was mixed for 10 minutes using an electric mixer set to "M" (medium) before being transferred to a 1000 mL graduated sedimentation cylinder. Again, a wash bottle of DI water was used to ensure all soil particles were rinsed from the metal dispersion cup into the graduated cylinder, and DI water was employed to bring the cylinder to a total volume of 1000 mL. The plunger was inserted into the cylinder, and the contents were thoroughly mixed with rapid but controlled vertical strokes. A drop of amyl alcohol was added if the surface was covered with foam. Following mixing, a timer was started (counting up), and the hydrometer was gently lowered into the solution mixture, allowed to stabilize, and a reading was taken at 40 seconds, and subsequently recorded. The hydrometer was then removed and rinsed completely into a waste beaker with a wash bottle of DI water. The temperature of the solution mixture was recorded, and the cylinder was left undisturbed for 2 hours. At the 2-hour mark, the hydrometer and temperature readings of the sample mixture were measured and recorded as described in Step 11. If a Fahrenheit thermometer was utilized, all temperature readings were converted to Celsius (OSU Soil Fertility Lab 2020). Soil texture formulas were used to determine the sand, silt, and clay content percentages.

NPK Testing

To begin the process, make sure the soil is completely dry, and then sift it to remove root particles and larger organic particles. First, I made the soil extraction by adding 30mL of distilled water to the extraction tube, alongside two Floc-Ex TesTabs, and mixing the tablets until they are completely dissolved. Next I added a teaspoon of soil into the tube, shaking it after for a minute, and allowing the soil particles to settle. To determine the nitrogen quantities, use a pipette to transfer the clear solution to a square test tube for 10mL, and then add a nitrate CTA TesTab, and immediately slide the tube into the protective sleeve. Cap and mix the test tube for 2 minutes and wait for the color to develop representing nitrate quantities in pink hue. To determine the phosphorus content, use a pipette to transfer 25 drops of the clear solution into the square test tube, and fill the rest to 10mL with distilled water. Add the phosphorus TesTab, cap, and mix the test tube until the tablet dissolves, and wait 5 minutes for the color to develop a blue hue.

Potassium levels were determined by nitrate, filling the test tube with a clear solution of 10mL, adding a potassium TesTab, and capping and mixing the tablet until it disintegrated. To determine the quantities of NPK, it is ranked based on Low, medium, and high standards, using the square test tubes and designated color chart for each respective nutrient. Nitrogen developed a pink hue, compared to L(40lb/ A/6"), M(160lb/ A/6"), and H(320lb/ A/6"). Phosphorus developed a blue hue, compared to L(8lb/ A/6"), M(20lb/ A/6"), and H(64lb/ A/6"). Potassium levels were measured based on the cloudiness of the test tube, compared to L(40lb/ A/6"), M(80lb/ A/6"), and H(160lb/ A/6").

Berlese-Tullgren Funnel Method

In the second portion of my research, the Berlese-Tullgren Funnel Method was integrated and involved simple steps for collecting soil invertebrates from leaf litter or humus samples (Berkeley 2023). I used five buckets to rotate the plots and funnels were placed on top, with a thick screen. Adjustments were made to the hardware screen, where I added a window screen to ensure a snug fit inside the funnel's wide end, allowing larger animals to crawl through if needed and reduce the amount of soil fallout. Several handfuls of soil were then placed on top of the wire mesh within the funnel. Next, alcohol was poured into the jar to create a depth of 1-2 cm. The funnel was carefully set on top of the bucket, creating a barrier that prevented soil invertebrates from escaping into the alcohol. The setup was then left undisturbed in a warm, quiet location to allow the invertebrates to migrate downwards away from the light. The lamp allowed the acceleration of the drying process and encouraged the invertebrates to move toward the bottom of the sample. Keeping the lightbulb at least 10 cm away from the funnel helped prevent heat damage to the invertebrates (Berkeley 2023).

Plant Biomass Collection

The final part of my experiment involved collecting plant biomass requiring the harvesting of soils, roots, and shoots, then isolating their quantities in sandwich Ziploc bags. The soil was excavated from the roots by hand, separating as many microaggregates accumulated around the roots, and removing any left root pieces. The roots were isolated, and cut from their respective shoots, organizing a soil bag and root bag. To measure leaf surface area, mature shoots were collected and isolated in a zip-loc bag, with shoot biomass placed into a zip-loc bag. Pots were organized and labeled into quart-sized bags, with their respective soil bag, roots bag, and shoot bag. The best of the shoots were organized and placed in their quart bag.

STATISTICAL ANALYSIS

The research delves into soil conditions through statistical analysis, aiming to understand the mechanisms driving soil remediation. It functions as a case study where I observe and actively shape changes in urbanized soil conditions following the application of microbial inoculants based on road proximity. The goal is to pinpoint the most efficient remediation approach to restore nutrient bioavailability, decrease invasive fescue encroachment, and boost invertebrate diversity. Statistical analysis was conducted using Excel, and an ANOVA test was utilized to compare variances across control and treatment groups for NPK values and ratios, root:shoot, and leaf surface area(Microsoft Excel 2021). Regression trend lines represent the best-fit line through a set of data points, indicating the overall trend or relationship between the variables being analyzed. The R^2 quantifies the goodness of fit of the regression model, measuring the proportion of the variance in the nutrients and biomass variables by the soil health parameters. A higher R^2 value (closer to 1) suggests that the independent variable(s) can better predict the variation in the dependent variable, while a lower R^2 value (closer to 0) indicates poorer predictive capability (Editor 2013). These methodologies were utilized to glean insights into how various affect soil health indicators, with the ultimate aim of improving urban soil quality and biodiversity.

RESULTS

Soil Fertility: Inoculant Treatments & Road Proximity

Nitrogen Levels Based on Road Proximity

Analysis of nitrogen levels based on proximity to the road revealed no significant differences among the plots (ANOVA, $p = 0.880$). Regardless of their distance from the road, all plots exhibited low levels of nitrogen (Rank 1). Organic amendments in the form of biochar, sphagnum moss, and worm castings were applied uniformly across all plots, contributing to this consistent trend.

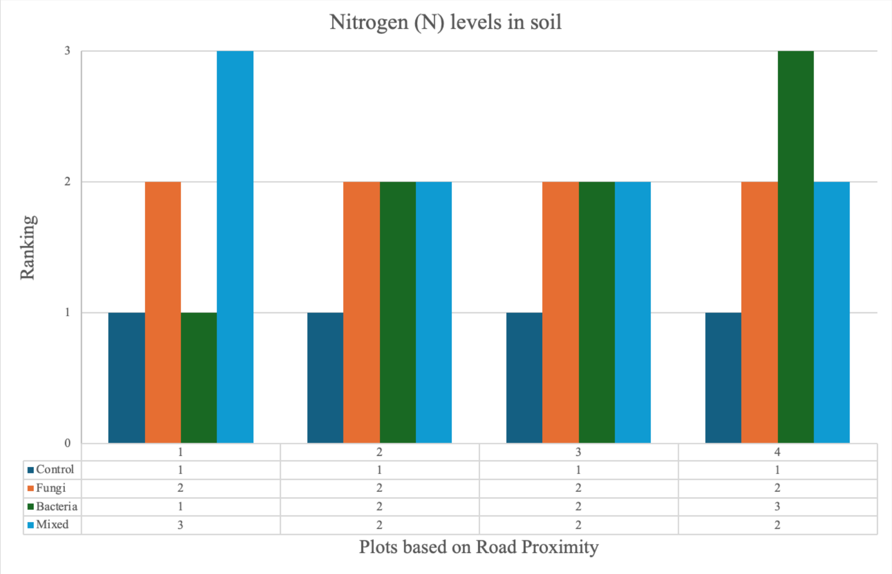


Figure 3. Nitrogen levels based on inoculant treatments and road proximity. N levels are ranked by Low (1), Medium (2), and High (3). The P-value for Nitrogen: Proximity to road plots–0.880443572 (No significance.) and Inoculant treatments–0.0381168 (Significant.)

Based on Inoculant Treatments

The analysis of nitrogen levels based on inoculant treatments yielded significant differences among the treatments (ANOVA, p = 0.038). Here are the observed trends:

Plot 1: Variability was observed within the treatments. The fungi inoculant treatment resulted in medium levels of nitrogen (Rank 2), considered optimal for T. fescue growth. In contrast, the bacteria inoculant treatment exhibited low nitrogen levels (Rank 1), while the mixed treatment showed high nitrogen levels (Rank 3).

Plot 2 and 3: These plots displayed consistent nitrogen levels across all treatments. The control initially exhibited low nitrogen levels (Rank 1), but with the application of inoculant treatments, nitrogen levels increased to medium (Rank 2), indicating a positive response to the interventions.

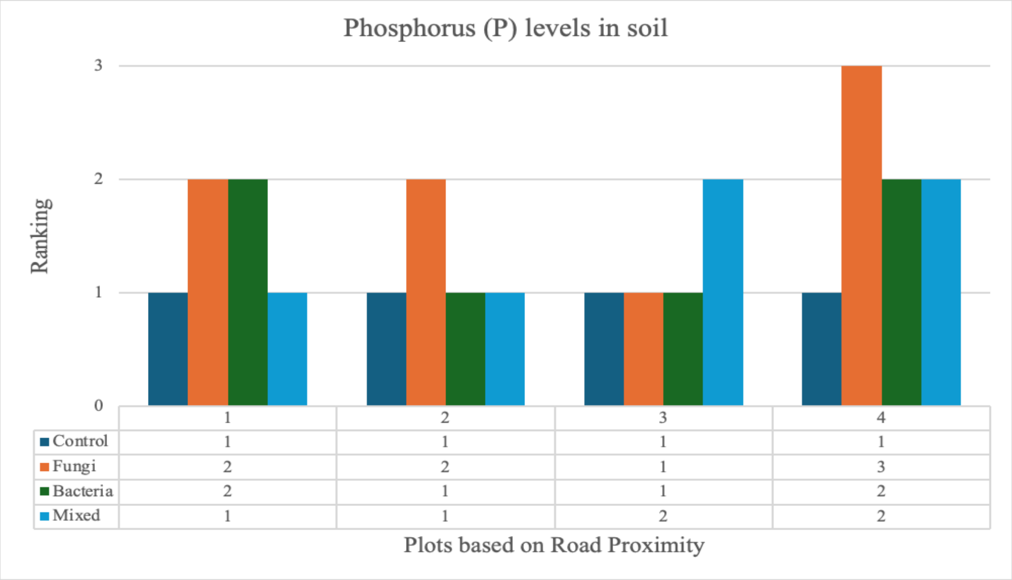
Plot 4: Notable growth in nitrogen levels was observed in this plot. Both the fungi and mixed treatments resulted in optimal medium nitrogen levels (Rank 2), while the bacteria treatment exhibited high nitrogen levels (Rank 3), suggesting a particularly effective response to the bacterial inoculant.

Overall, while nitrogen levels remained low across all plots initially, the addition of organic amendments facilitated an increase in nitrogen levels. Variability was observed within the treatments, with fungi inoculants often resulting in optimal nitrogen levels, and bacteria inoculants sometimes leading to higher nitrogen levels, particularly evident in Plot 4.

Phosphorus Levels

Based on Road Proximity

Analysis of phosphorus levels based on proximity to the road revealed no significant differences among the plots (ANOVA, p = 0.193). Control plots across all proximity levels consistently exhibited low levels of phosphorus (Rank 1).



	1	2	3	4
Control	1	1	1	1
Fungi	2	2	1	3
Bacteria	2	1	1	2
Mixed	1	1	2	2

Figure 4. Phosphorus levels based on inoculant treatments and road proximity. P levels are ranked by Low (1), Medium (2), and High (3). The P-value for Phosphorus: Proximity to road plots–p = 0.193 (No significance.) and Inoculant treatments–p = 7.44041E-05 (No significance.)

Based on Inoculant Treatments

Significant differences in phosphorus levels were observed among the inoculant treatments (ANOVA, p = 0.0000744041). Here are the findings for each plot:

Plot 1: Both fungi and bacteria inoculant treatments resulted in optimal medium phosphorus levels (Rank 2), considered ideal for T. fescue growth. However, the mixed treatment exhibited low phosphorus levels (Rank 1).

Plot 2: The bacteria and mixed inoculants led to low phosphorus levels (Rank 1), while the fungi inoculant treatment resulted in medium optimal phosphorus levels (Rank 2).

Plot 3: Fungi and bacteria inoculants resulted in low phosphorus levels (Rank 1), whereas the mixed treatment showed optimal medium phosphorus levels (Rank 2).

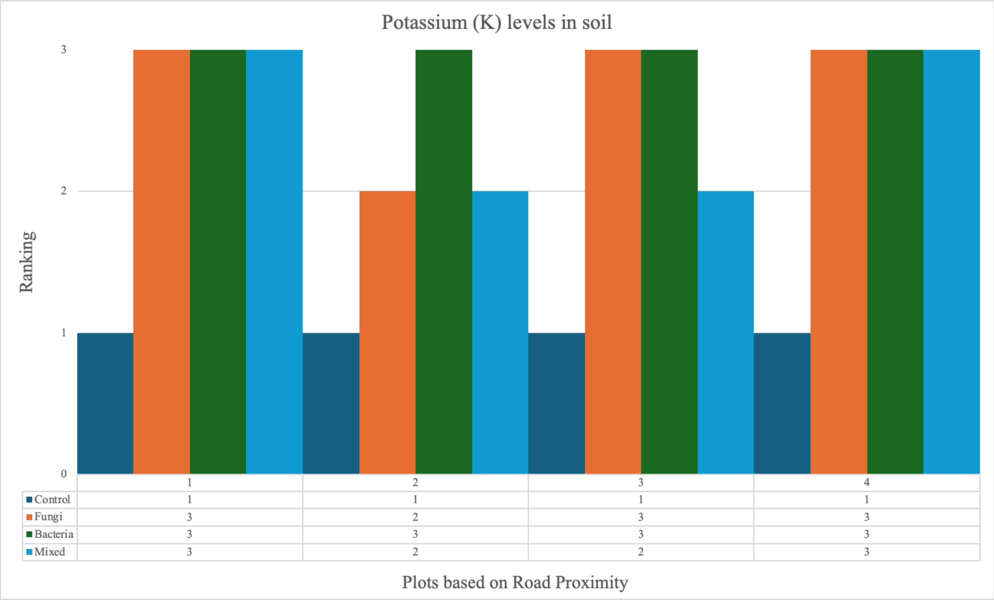
Plot 4: All inoculant treatments in this plot yielded optimal medium phosphorus levels (Rank 2), with the fungi inoculant resulting in high phosphorus levels (Rank 3).

Overall, while control plots consistently exhibited low phosphorus levels, the inoculant treatments displayed variability. Fungi inoculants often led to medium optimal phosphorus levels, while bacteria and mixed inoculants sometimes resulted in low levels, particularly evident in Plot 1 and Plot 2. Plot 4 showed the most consistent response across all inoculant treatments, with all reaching optimal medium phosphorus levels.

Potassium Levels

Based on Road Proximity

Analysis of potassium levels based on proximity to the road did not reveal significant differences among the plots (ANOVA, p = 0.193). In the control plots for all proximity levels, potassium levels remained consistently low (Rank 1).



	1	2	3	4
■ Control	1	1	1	1
■ Fungi	3	2	3	3
■ Bacteria	3	3	3	3
■ Mixed	3	2	2	3

Figure 5. Potassium levels based on inoculant treatments and road proximity. K levels are ranked by Low (1), Medium (2), and High (3).

Based on Inoculant Treatments

Significant differences in potassium levels were observed among the inoculant treatments (ANOVA, $p = 0.0000744041$). Here are the findings for each plot:

Plot 1: All inoculant treatments resulted in high potassium levels (Rank 3), indicating an effective enhancement of potassium availability in the soil.

Plot 2: The fungi and mixed inoculant treatments led to medium optimal potassium levels (Rank 2), while the bacteria treatment resulted in high potassium levels (Rank 3).

Plot 3: Both the bacteria and fungi inoculant treatments yielded high potassium levels (Rank 3), whereas the mixed treatment exhibited medium optimal potassium levels (Rank 2).

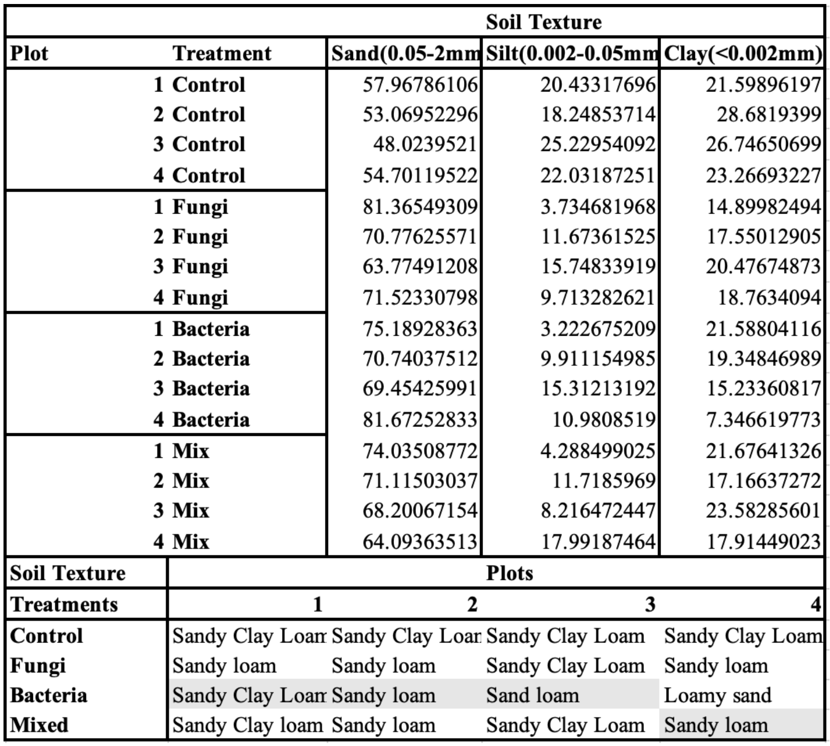
Plot 4: Similar to Plot 1, all inoculant treatments in Plot 4 resulted in high potassium levels (Rank 3), showcasing consistent effectiveness across all treatments.

Overall, control plots maintained low potassium levels, while the application of inoculant treatments led to significant enhancements. Fungi and mixed inoculants often resulted in medium optimal potassium levels, while bacteria treatments occasionally led to high potassium levels, particularly evident in Plot 2. Plots 1 and 4 consistently demonstrated high potassium levels across all inoculant treatments.

Soil Texture

Based on Road Proximity

The soil texture analysis revealed variations in soil texture based on proximity to the road. Plots 1 and 2, situated adjacent to the road, exhibited sandy clay loam and sandy loam textures, respectively. Moving further into the rangeland, Plot 3, located 10 meters away from the road, also exhibited a sandy clay loam texture, while Plot 4, situated 15 meters away, demonstrated a sandy loam texture. Despite these spatial differences, statistical analysis indicated that soil texture did not vary significantly between the plots (ANOVA, $p > 0.05$).



		Soil Texture		
Plot	**Treatment**	**Sand(0.05-2mm**	**Silt(0.002-0.05mm**	**Clay(<0.002mm)**
1	**Control**	57.96786106	20.43317696	21.59896197
2	**Control**	53.06952296	18.24853714	28.6819399
3	**Control**	48.0239521	25.22954092	26.74650699
4	**Control**	54.70119522	22.03187251	23.26693227
1	**Fungi**	81.36549309	3.734681968	14.89982494
2	**Fungi**	70.77625571	11.67361525	17.55012905
3	**Fungi**	63.77491208	15.74833919	20.47674873
4	**Fungi**	71.52330798	9.713282621	18.7634094
1	**Bacteria**	75.18928363	3.222675209	21.58804116
2	**Bacteria**	70.74037512	9.911154985	19.34846989
3	**Bacteria**	69.45425991	15.31213192	15.23360817
4	**Bacteria**	81.67252833	10.9808519	7.346619773
1	**Mix**	74.03508772	4.288499025	21.67641326
2	**Mix**	71.11503037	11.7185969	17.16637272
3	**Mix**	68.20067154	8.216472447	23.58285601
4	**Mix**	64.09363513	17.99187464	17.91449023

Soil Texture	**Plots**			
Treatments	**1**	**2**	**3**	**4**
Control	Sandy Clay Loam	Sandy Clay Loa	Sandy Clay Loam	Sandy Clay Loam
Fungi	Sandy loam	Sandy loam	Sandy Clay Loam	Sandy loam
Bacteria	Sandy Clay Loam	Sandy loam	Sand loam	Loamy sand
Mixed	Sandy Clay loam	Sandy loam	Sandy Clay Loam	Sandy loam

Figure 6. The table above presents the soil texture calculations, indicating the proportions of sand, silt, and clay for each plot and treatment. Below, you'll find the corresponding classifications of soil texture at Blue Oak Ranch Reserve.

Based on Microbial Treatments

Analysis of soil texture based on microbial treatments revealed some notable trends. In the control plots, all exhibited a predominant sandy clay loam soil texture. Conversely, plots treated with fungi inoculants predominantly displayed a sandy loam texture, except for Plot 3, which retained a sandy clay loam texture. Bacteria inoculant treatments resulted in a mix of soil textures, including sandy loam (Plot 2 and Plot 3), sandy clay loam (Plot 1), and loamy sand (Plot 4). Plots treated with a mixed inoculant displayed variations in soil texture, with sandy clay loam textures observed in Plots 1 and 3, and sandy loam textures in Plots 2 and 4. No significant differences in soil texture were observed among the treatments (ANOVA, $p > 0.05$).

Invertebrate Counts Based on Inoculant Treatments and Road Proximity

Towards the end of the experiment, an issue arose in the greenhouse as experimental plots became infested with aphids. Due to the absence of pesticide treatment, the aphids were allowed to persist until the conclusion of the research period.

Control Counts

Plot C1

Aphids (Hemiptera)

Pictures taken from microscope.

In the control treatment, macroinvertebrates were observed in the form of three aphids in Control Plot 1 (located on one side of the road). Control Plots 2, 3, and 4 did not yield any macroinvertebrates in the soil samples collected using the Berlese funnel method. It's worth noting that aphid counts may be underestimated due to the light infestation observed across the plots.

Fungi Inoculant

Plot F1

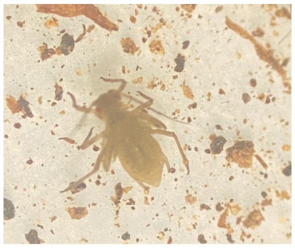
Springtail (Collembola)

Aphid (Hemiptera)

Plot F2

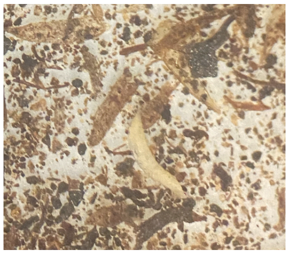
Fly larvae (Diptera)

Plot F3



Aphid (Hemiptera)

Plot F4



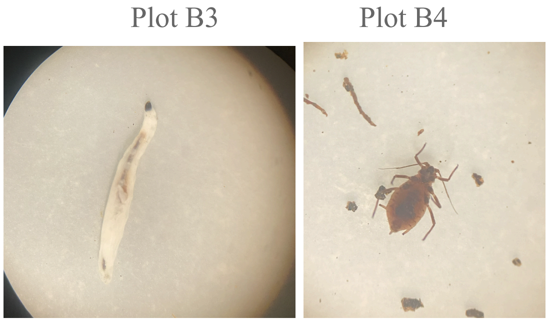
Therevid larva (Diptera)

Coleoptera larvae

Pictures taken from microscope.

The fungal inoculant treatment experienced the most severe aphid infestation among all treatments, requiring repeated shaking to fully remove aphids from the plant shoots. Macroinvertebrates were abundantly found in the samples collected using the Berlese funnel method for this treatment. Each sample contained aphids ranging in size thus age, with Plot F1 also containing a springtail, and Plot F2 (located across the street) harboring a fly larvae. In Plot F3, only aphids were present in the soil samples processed through the ethanol bath, while Plot F4 contained a therevid larva and coleoptera larvae in the ethanol bath.

Bacterial Inoculant


Plot B3 Plot B4

Fly larvae (Diptera) Aphid (Hemiptera)

Pictures taken from microscope.

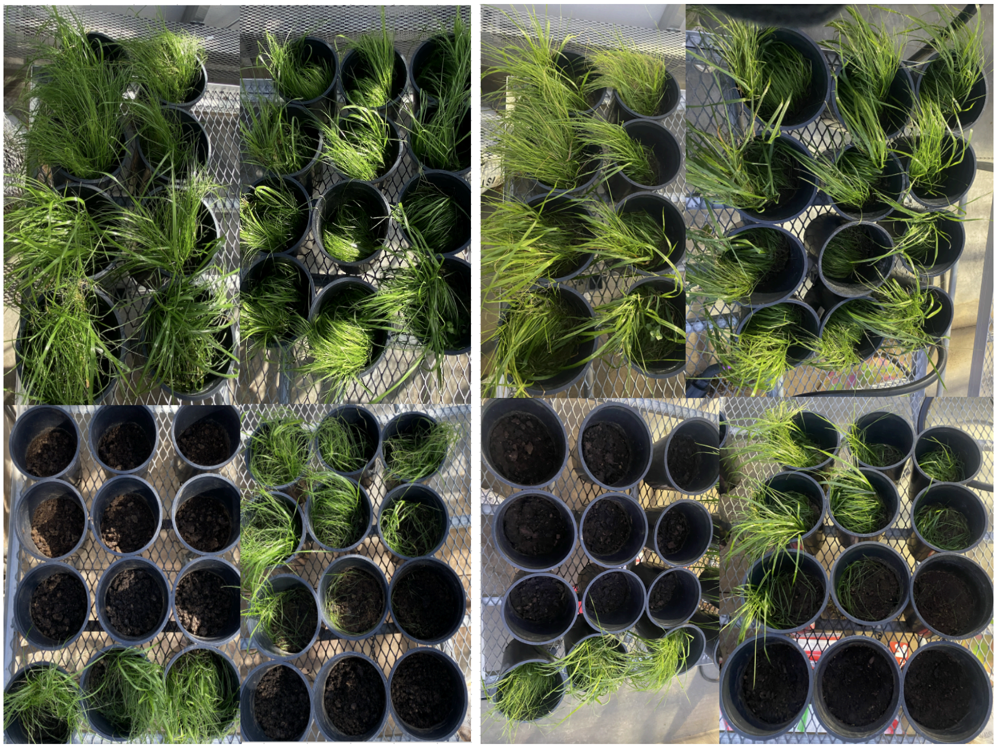
In the bacterial inoculant treatment, a light infestation of aphids was observed, with a higher presence noted in Plot B4, where the Berlese funnel method detected three aphids. Additionally, a fly larva was found in Plot B3.

Mixed Inoculant

No invertebrates were found in the soil samples collected from the mixed inoculant treatment using the Berlese funnel method. However, a light infestation of aphids was observed in this treatment.

Plant Growth Responses

January 24, 2024 February 24, 2024



March 15, 2024 April 1, 2024

Tall Fescue growth updates– **Top left**: Control, **Top right**: Fungi Inoculant, **Bottom left**: Bacteria—bottom **right**: Bacteria +Fungi Inoculants.

Tall fescue was sown on January 2, 2024. Regular monitoring involved observing plant growth and assessing the success of the plants and their compatibility with the soil. At one point, I accidentally missed sowing seeds in plot C2, yet it eventually exhibited successful growth. However, plots B1-3 and M4 failed to germinate.

Root to Shoot Ratio

ANOVA Results

Significant differences were observed in root: shoot ratio among inoculant treatments (p = 0.013) and roadside proximity categories (p = 0.0099), indicating variations influenced by both treatments and environmental factors.

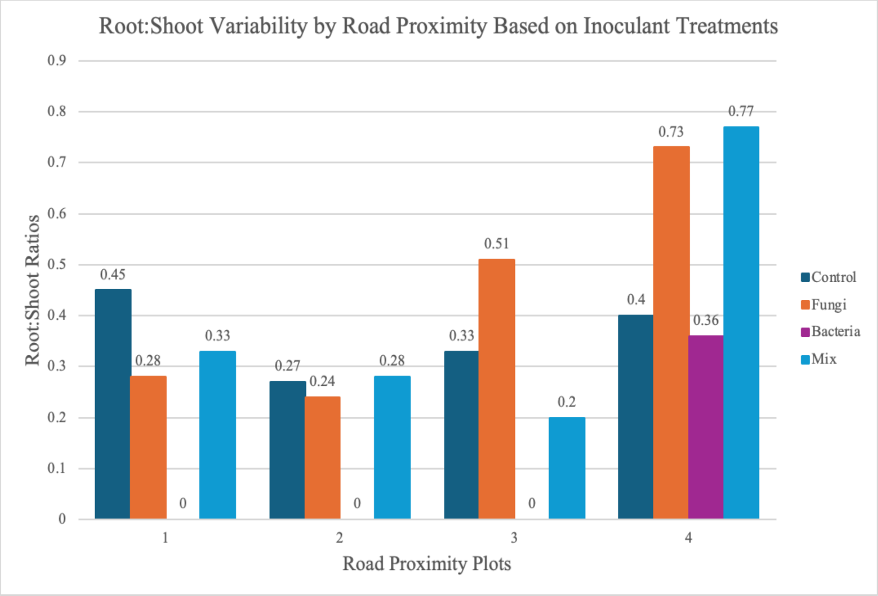


Figure 7. Root: shoot ratio variability by road proximity based on Inoculant treatments.

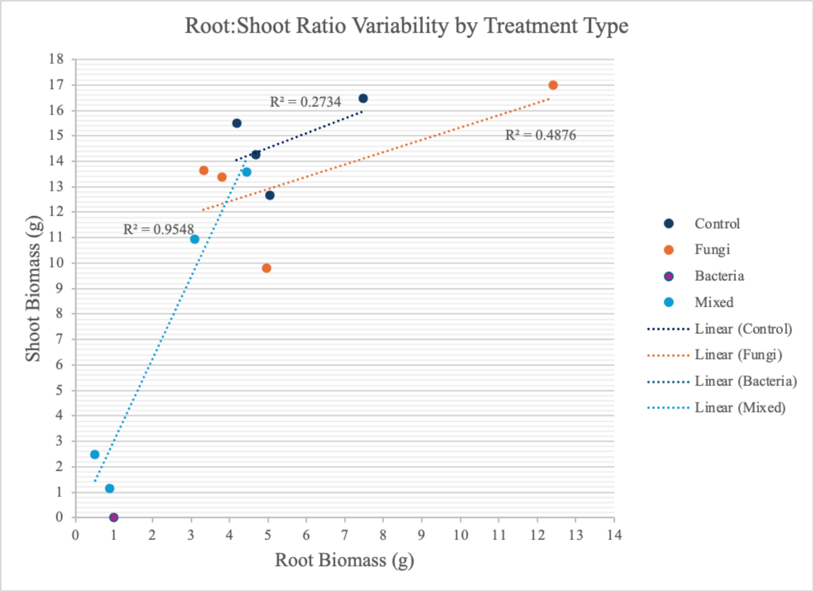


Figure 8. Root:Shoot (R:S) ratio plotted on a graph, treatments and road proximity for each pot labeled. Root biomass as a function of shoot biomass. Regression lines are shown with R^2-value.

Regression Analysis

The regression analysis, represented by R^2 values, indicates the strength of the relationship between inoculant treatments and root: shoot ratio.

Control Treatment: The regression analysis revealed an R^2 value of 0.2734, indicating a moderate correlation between control treatment and root: shoot ratio.

Fungi Treatment: Fungi treatment exhibited a higher R^2 value of 0.4876, indicating a stronger correlation with root: shoot ratio compared to control.

Mixed Treatment: The mixed treatment demonstrated the highest R^2 value of 0.9548, indicating a strong correlation with root: shoot ratio, suggesting a significant influence on Tall Fescue growth.

Treatment Comparisons

Control Treatment: The root: shoot ratio in control treatment varied, reaching as high as 0.45 (C1) next to the road, but as low as 0.27 on the opposite side (C2).

Fungi Treatment: Fungi treatment showed variability in root: shoot ratio, ranging from as high as 0.73 (F4) at 15 meters into the rangeland to as low as 0.24 (F2) on the opposite side of the road. Fungi treatment exhibited the second-highest ratio in F3 (0.51) and the second-lowest in F1 (0.28).

Bacteria Treatment: Bacteria treatment did not significantly affect the root: shoot ratio in Plots B1-3, with Plot B4 having a ratio of 0.36.

Mixed Treatment: The mixed treatment demonstrated the most variability in root: shoot ratio, with Plots M4 and M3, exhibiting the highest ratios of 0.77 and 0.51, respectively. The lowest ratios were observed in Plots M1 (0.28) and M2 (0.24).

Analysis of Leaf Surface Area (SA) (cm^2)

ANOVA Results

The analysis of leaf surface area revealed a significant difference between treatments (p = 0.0388), indicating variations in T. fescue growth influenced by different inoculant treatments. However, there was no significant difference observed between road proximity categories (p = 0.82), suggesting that distance from the road did not impact leaf surface area.

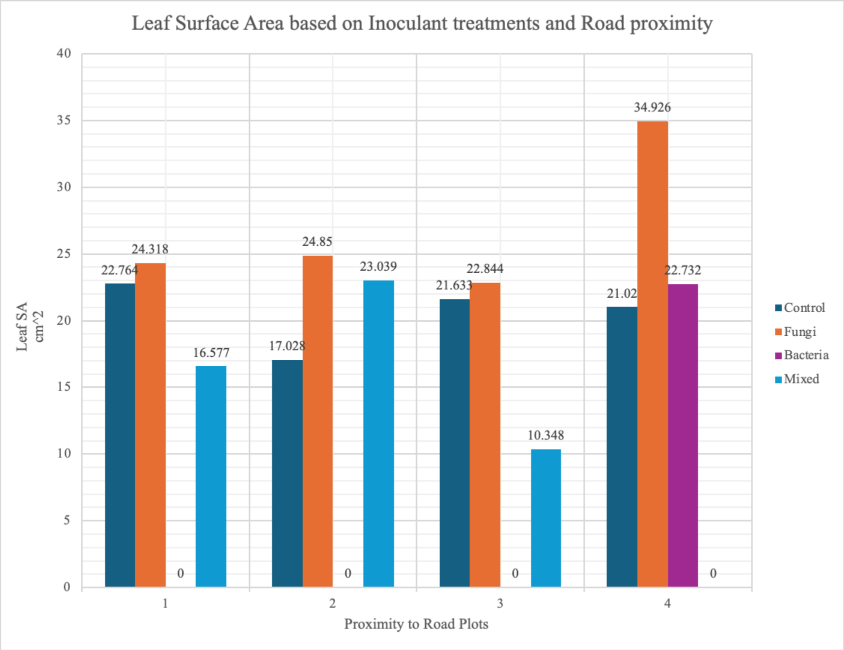


Figure 9. Leaf Surface Area cm^2 on the y-axis. Comparing road proximity and inoculant treatments.

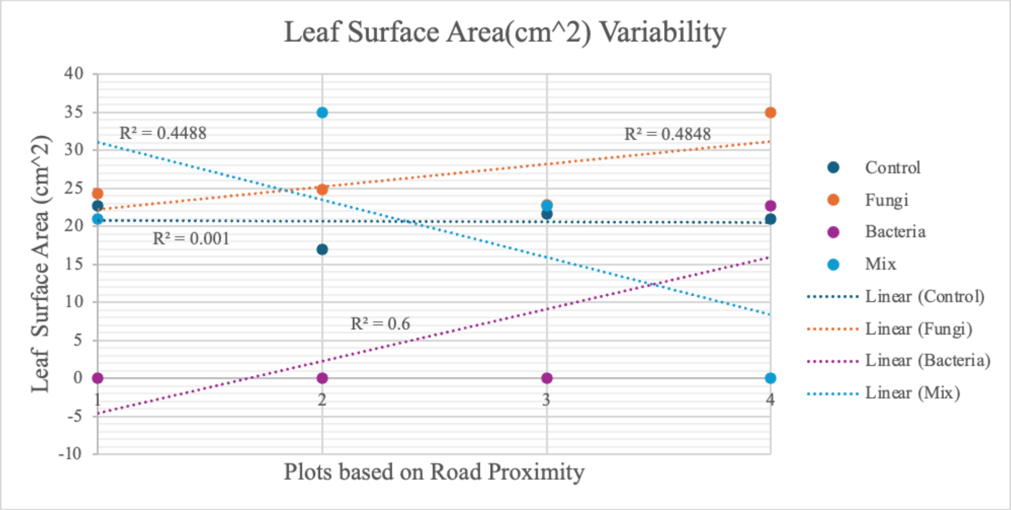


Figure 10. Leaf Surface Area(cm^2) Variability. Trendlines and R^2 correlation display strength in determination.

Treatment Comparisons

Control and Fungi Treatments: In plots 1 and 3, leaf SA areas were similar between control(C) and fungi(F) treatments. For instance, in P1, the control had a leaf SA of 22.764 cm^2 compared to 24.318 cm^2 for fungi. Similarly, in Plot 3, the control exhibited 21.633 cm^2 while fungi showed 22.844 cm^2.

Successful Growth of Fungi Treatment: Fungi treatment demonstrated successful growth in Plots 2 and 4, with larger leaf surface areas compared to control. For example, in Plot 2, fungi treatment achieved 24.85 cm^2 compared to 17.028 cm^2 in control. In Plot 4, fungi treatment resulted in 34.926 cm^2, surpassing the control's 21.02 cm^2. Significant variability was observed between control and fungi treatments in Plots 2 and 4, indicating the effectiveness of fungi inoculation in promoting plant growth.

Bacteria and Mixed Treatments: Bacteria treatment did not lead to notable growth in Plots B1-3, with similar or smaller leaf surface areas compared to the control. However, in Plot 4, bacteria treatment resulted in a slightly larger leaf surface area (22.732 cm^2) compared to control (21.02 cm^2). Mixed treatment showed variable results, with M4 exhibiting no growth, while M3 had the largest leaf surface area (23.039 cm^2) within the mixed treatment at 10 meters into the range.

Shoot Biomass

ANOVA Results

Significant differences were observed in shoot biomass among inoculant treatments (p = 0.0843), indicating variations in plant growth influenced by different treatments. However, no significant difference was found between road proximity plots (p = 0.614), suggesting that distance from the road did not significantly impact shoot biomass.

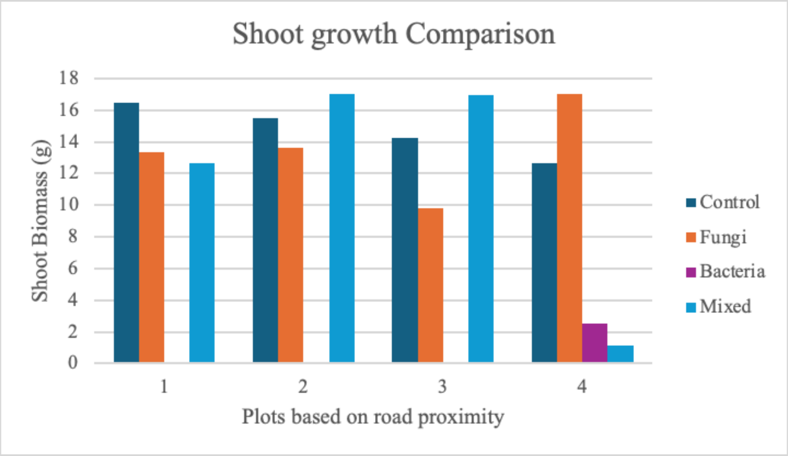


Figure 11. Shoot biomass(g) comparisons between road proximity and inoculant treatments.

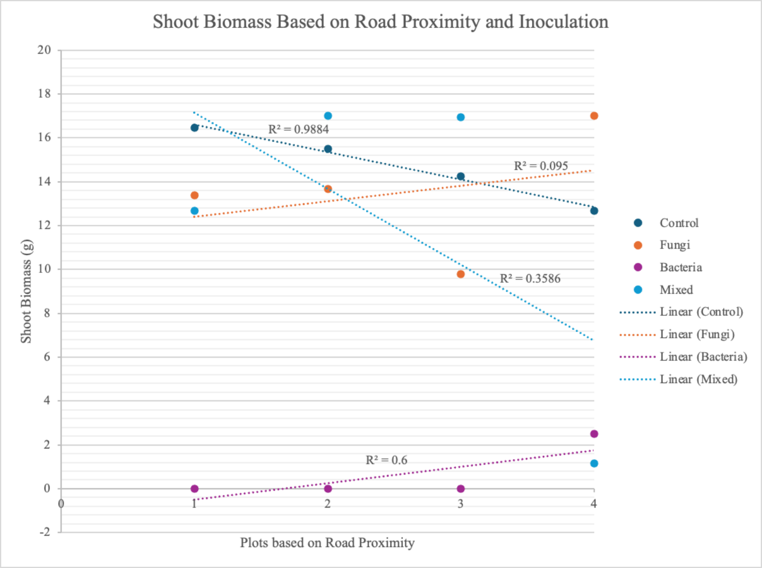


Figure 12. Shoot Biomass (g) Variability. Trendlines and R^2 correlation display strength in determination.

Regression Analysis

The regression analysis, represented by R^2 values, indicates the strength and direction of the relationship between inoculant treatments and shoot biomass.

Control Treatment: The regression analysis revealed a high R^2 value of 0.988, indicating a strong negative correlation between control treatment and shoot biomass. Shoot growth was most successful in Plot 1 (next to the street), with a shoot biomass of 16.467g, decreasing gradually to 12.681g in Plot 4. Control Plots C2 (15.497g) and C3 (14.26g) closely followed in shoot biomass, with Mixed treatment surpassing control in Plots M2 (17.006g) and M3 (16.933g).

Fungi Treatment: The regression analysis for fungi treatment yielded a low R^2 value of 0.095, indicating a weak positive correlation with slight variations. Plot F4 exhibited the highest shoot biomass at 17.006g. However, control treatment outperformed fungal inoculation in Plots 1-3. Plot 2 in the Mixed treatment and Plot 4 in the Fungi treatment shared similar shoot biomass

Bacteria treatment: Bacteria treatment demonstrated a moderate R^2 value of 0.6, indicating a positive correlation. Shoots failed to germinate in Plots B1-3, with Plot 4 failing to achieve optimal growth, yielding only 6.033g of shoot biomass.

Mixed Treatment: The regression analysis for mixed treatment resulted in an R^2 value of 0.3586, indicating a moderate negative correlation. Plot F1 (13.37g) surpassed M1 (12.681g) in shoot biomass, while Plots M2 (17.006g) and M3 (16.933g) produced higher shoot biomass compared to control and fungal treatments. However, Plot M4 experienced a sharp decline, with minimal seed germination and shoot biomass of 1.15g.

Root Biomass

ANOVA Results

Significant differences were observed in root biomass among inoculant treatments (p = 0.0984), suggesting variations in plant root development influenced by different treatments. However, no significant difference was found between road proximity plots (p = 0.28), indicating that distance from the road did not significantly impact root biomass.

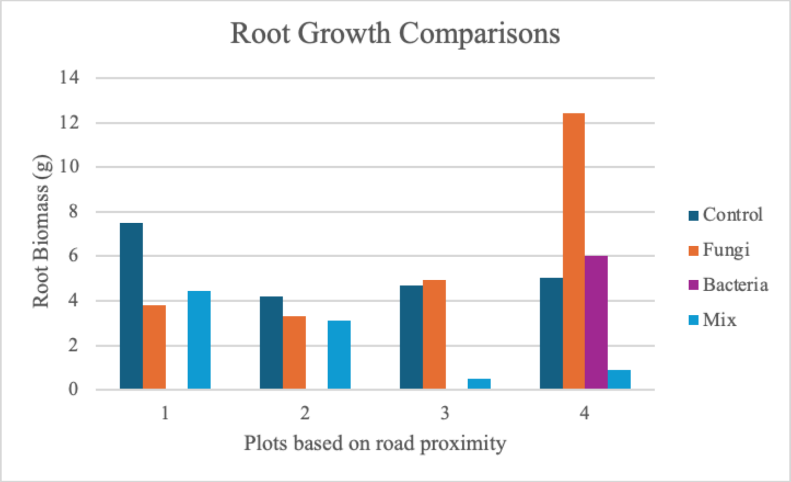


Figure 13. Root biomass(g) comparisons between road proximity and inoculant treatments.

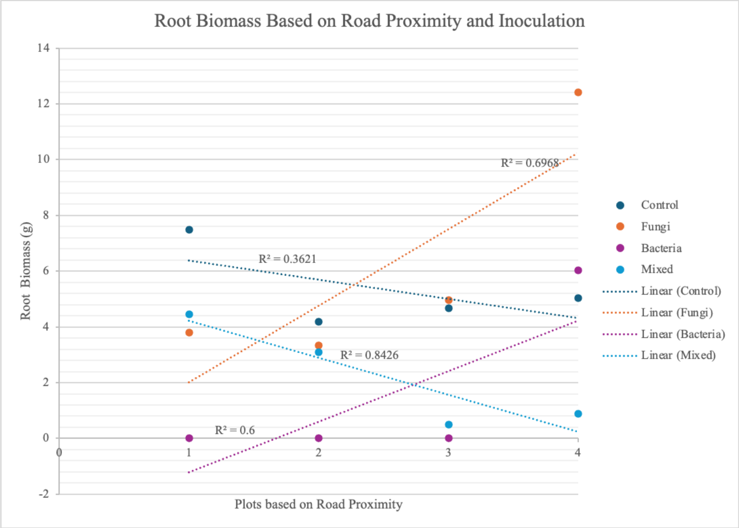


Figure 14. Root Biomass Variability. Trendlines and R^2 correlation display strength in determination.

Regression Analysis

The regression analysis, represented by R^2 values, provides insights into the strength and direction of the relationship between inoculant treatments and root biomass.

Control Treatment: The regression analysis revealed a moderate R^2 value of 0.3621, indicating a moderate influence of control treatment on root biomass. Higher root biomass was observed in Plot C1 (7.485g), succeeding Plots F1 (3.8g), B1 (0g), and M1 (4.453g). Control treatment also succeeded in Plot C2, surpassing fungi (F2 - 3.326g) and mixed treatments (M2 - 3.1g). Plot C3 (4.68g) did not exhibit as much success as F3 (4.956g), albeit by a slight margin.

Plot C4 (5.04g) had the second-lowest root biomass among the plots, with Plot B4 (0.886g) being the lowest.

Fungi Treatment: The regression analysis for fungi treatment yielded a higher R^2 value of 0.6968, indicating a stronger correlation with root biomass compared to control. Fungi treatment succeeded in promoting root growth, particularly in Plot F4 (12.41g), surpassing other inoculant treatments such as C1 (5.04g), B1 (6.033g), and M4 (0.886g). Plot F3 (4.95g) slightly surpassed C3 (4.68g) in root biomass, while Plot F2 exhibited lesser root growth.

Bacteria Treatment: Bacteria treatment demonstrated a significant R^2 value of 0.6, indicating a substantial influence on root biomass. However, bacteria treatment did not lead to root growth in Plots B1-3. Plot B4 (6.033g) exhibited higher root biomass compared to C4 (5.04g).

Mixed Treatment: The regression analysis for mixed treatment resulted in a high R^2 value of 0.8426, indicating a strong correlation with root biomass. However, there was a downward correlation observed, with root biomass declining from Plot M1 (4.45g) to M3 (0.5g) before picking up again in Plot M4 (0.886g). Plot M3 barely developed any biomass.

DISCUSSION

Soil NPK Uptake

The analysis of soil NPK uptake provides valuable insights into nutrient acquisition dynamics within the experimental plots, particularly in relation to microbial inoculant treatments and roadside proximity. Control treatments exhibited consistently low NPK values, indicating poor soil fertility in nutrient acquisition, both within the rangeland and roadside plots.

Upon the addition of microbial inoculants, significant variations in nutrient uptake were observed among inoculant treatments for nitrogen ($p = 0.038$), phosphorus ($p = 0.0000744041$), and potassium ($p = 0.0000744041$) levels. The fungal treatment demonstrated medium to high levels of nitrogen, attributed to fungal hyphae's ability to transport and fix nitrogen in the soil (Ma et al. 2022). In contrast, the bacterial treatment showed a gradual increase in nitrogen levels from low to high across the rangeland, attributed to rhizobia and Bacillus subtilis' nitrogen-fixing capabilities (Purwaningsih et al. 2021). The mixed inoculant treatment exhibited high nitrogen levels, indicating active microbial and fungal activity in nitrogen uptake and fixation (Hashem et al. 2019). Phosphorus uptake varied among inoculant treatments, with the mixed treatment gradually reaching optimal levels, possibly facilitated by bacteria and fungi solubilizing and transporting phosphorus (Hashem et al. 2019). The fungal treatment initially gained optimal phosphorus levels but declined in later plots, suggesting potential over-fertilization effects. Conversely, the bacterial treatment showed mixed phosphorus levels, potentially influenced by climatic factors affecting phosphorus solubilization (Alori et al. 2017). Potassium levels were predominantly high across all treatments, attributed to the application of organic matter, which enhances potassium release and decreases fixation in soils (Bader et al. 2021). The combination of inoculation treatments resulted in optimal NPK levels, indicating enhanced soil fertility and nutrient transportation. Overall, the findings suggest that microbial inoculant treatments play a crucial role in soil nutrient uptake and soil fertility enhancement. The fungal treatment demonstrated optimal NPK levels, highlighting its effectiveness in soil fertility improvement. Future studies exploring the long-term effects of microbial treatments on soil nutrient dynamics could provide valuable insights into sustainable soil management practices.

Soil Texture Properties based on Inoculation for Road Proximity Plots

The control treatment exhibited sandy and clayey soil characteristics, prone to hardening when dry and surface crusting, with poor drainage capabilities. These soil properties are consistent with observations of moderate nutrient retention capacity and susceptibility to waterlogging, posing challenges for soil management practices (Victorian Resources Online 2019). The addition of fungal inoculant and organic amendments notably improved soil texture in plots F3 and F4, rendering them softer and more stable, with enhanced macroaggregates and darker organic matter. This enhancement can be attributed to the increased organic matter content, providing additional binding sites for chemicals and promoting microbial activity (Victorian Resources Online 2019). The establishment of a dense mycelial network by arbuscular mycorrhizal fungi further contributed to soil stability and ability to sequester metals (Ma et al., 2022). Conversely, the bacterial inoculant treatment resulted in solid, clayey soil texture, likely exacerbated by improper greenhouse irrigation practices. Although plots B1-4 exhibited improved soil color due to higher organic matter content, the high water retention and clay content led to compaction issues. Potential long-term soil quality improvements could be achieved through natural cycles of wetting and drying, as well as root penetration by plants with taproots or fibrous root systems (USDA 2017). The mixed microbial treatment altered soil color to a darker, moister appearance, with plot M4 exhibiting the most pronounced compaction likely due to irrigation imbalances. The uneven growth of tall fescue in M4 can be attributed to the constant cycle of wetting and crusting on the soil surface. However, plots M1 and M2 benefited from organic amendment enhancement, facilitating tall fescue growth despite unevenness. Overall, the addition of microbial inoculants and organic amendments contributed to varied improvements in soil texture, highlighting the potential for sustainable soil management practices to enhance soil quality over time.

Invertebrate Counts Based on Inoculant Treatments and Road Proximity

The greenhouse investigation evidenced a substantial aphid infestation, particularly in plots subjected to organic fertilization rich in fungi, corroborating the hypothesis that nitrogen-enriched fertilization prompts vigorous plant growth, thereby attracting aphids (James 2017). Antecedent studies have posited a positive correlation between arbuscular mycorrhizal (AM) fungi and heightened aphid allure to plants (Babikova et al. 2014), suggesting that the proliferation of AM fungi, in conjunction with favorable soil parameters, contributed to the pronounced aphid presence in fungal-treated plots. Springtails exhibited notable prevalence in moisture-laden soils, notably in one fungal plot, indicative of their proclivity towards optimal moisture regimes (Ashworth 2023). This observation underscores the established nexus between organic amendments, fungal inoculation, and soil fauna richness (CSU 2022). Additionally, the detection of fungus gnat larvae in fungal-treated plots implicates their involvement in organic matter degradation and nutrient cycling processes (CSU 2022). Conversely, plots treated with bacterial inoculation manifested diminished aphid populations, plausibly attributed to the lack of shoot biomass to consume and germinate from. The occurrence of fungus gnat larvae in one bacterial plot may be construed as a consequence of OM addition and heightened moisture levels, conducive to larval propagation (CSU 2022). The mixed treatment plots exhibited diminished aphid abundance, potentially attributable to soil compaction impeding invertebrate ingress, thus mitigating aphid colonization (CSU 2022).

Soil Health Bioindicators

The assessment of soil health bioindicators provides insights into ecological dynamics within experimental plots. Collembolans, sensitive to soil properties, showed lower densities in soils with higher metal concentrations, indicating potential impacts of soil contamination (Santorufo 2012). Soil-dwelling Diptera, including fly larvae, contribute significantly to organic matter decomposition, indicating active nutrient cycling (Stork and Eggleton 1992; Iowa State University 2004). Springtails play a crucial role in soil humification through non-selective scavenging, facilitating the mixing of organic matter and mineral particles (Stork and Eggleton 1992). Aphids have a positive association with mycorrhizal fungi, indicating their role in plant-fungal symbiosis and soil health in living soil systems (Ashworth 2023). Beetles contribute to decomposition processes by feeding on various organic substrates, reflecting active organic matter turnover within the soil ecosystem (Stork and Eggleton 1992; GSBI 2019). The presence of coleoptera and diptera, particularly in the fungal treatment, suggests enhancements in soil quality conducive to macrofaunal activity, with implications for soil structure formation, water retention, and nutrient cycling (Stork and Eggleton, 1992). While greenhouse constraints may limit macrofaunal diversity, the presence of coleoptera and diptera signifies positive soil quality enhancements associated with fungal inoculation and organic matter additions. Overall, the diversity and abundance of soil invertebrates serve as bioindicators of soil health, reflecting interactions between soil properties, nutrient cycling processes, and ecosystem functioning within experimental plots. Further research could explore synergies between different soil fauna groups and their implications for sustainable soil management practices.

Root and Shoot Biomass Importance

The analysis of root-to-shoot ratios revealed significant differences among inoculant treatments ($p = 0.013$) and roadside proximity categories ($p = 0.0099$), suggesting influences from both treatments and environmental factors. A low root-shoot ratio typically indicates favorable growing conditions, while a high ratio suggests nutrient deficiencies, particularly nitrogen and phosphorus (Hetrick 1991). Tall fescue, known for its ability to thrive in poor soil conditions, successfully grew in all plots, even in instances of accidental omission at germination (Mohlenbrock 2001). In the fungal treatment, an increase in the root-shoot ratio, particularly in plots F3 and F4, suggests challenges in nutrient acquisition, prompting shoot growth to compensate. Conversely, plots F1 and F2 exhibited lower ratios, indicating more favorable conditions and deeper nutrient access. This aligns with findings suggesting that plants dependent on symbiotic relationships may exhibit less root plasticity in low-fertility soils (Hetrick 1991). Compacted soil hindered seed germination in bacterial plots B1-B3, while optimal conditions in B4 supported tall fescue growth better than control plot P4. Mixed plots showed a positive correlation, with M4 exhibiting the highest ratio, indicating nutrient deficiencies and prompting shoot growth for nutrient acquisition. M2 and M3 had lower ratios, suggesting favorable conditions for root development. Overall, root-to-shoot ratios provide insights into nutrient availability and growth responses, highlighting the importance of microbial treatments and environmental conditions in plant development.

Shoot Surface Area Importance Based on Road Proximity

The assessment of shoot surface area (SA) importance based on road proximity reveals insights into plant adaptation strategies in varying environmental conditions. Larger leaves, with their increased surface area, are advantageous for capturing sunlight and enhancing photosynthetic efficiency (Kuzmenko 2016). This can lead to greater energy production and overall growth and reproductive success for plants (Kuzmenko 2016). However, excessively large leaves may pose challenges in environments where water loss through transpiration is a concern (Kuzmenko 2016). In the control plots, optimal leaf SA facilitated effective sunlight capture for photosynthesis. The fungal treatment showed increased leaf SA, particularly in plot F4, indicating efficient sunlight capture and potential growth enhancement. Conversely, soil compaction in the bacterial treatment inhibited seed germination, limiting growth in plots B1-B3. Plot B4 exhibited slightly larger SA than the control, suggesting some improvement in energy production despite challenges. In the mixed treatment, slight compaction hindered optimal growth, leading to tall fescue growth at pot edges and minimal growth in plot M4. Leaf SA in plots M1-M3 did not surpass control levels due to compaction, resulting in dark green thin shoots.

LIMITATIONS AND FUTURE DIRECTIONS

As we chart the future of bioremediation and soil management, our focus must extend beyond mere compatibility between fungi and bacterial inoculants. Future research endeavors could delve into the nuanced influence of seasonal variations on remediation efficacy, unraveling the intricate interplay between microbial communities and contaminants. We must acknowledge the multifaceted complexities inherent in soil processes, our investigations should aim towards a comprehensive understanding of their impact on the surrounding ecosystem.

While the experiment was conducted within a greenhouse setting, it is crucial to acknowledge inherent limitations that may impact the generalizability of findings. Conditions within the greenhouse may not fully replicate real-world environmental dynamics, potentially skewing results. Replication in natural settings, where soil is exposed to environmental elements, could offer a more realistic assessment of observed effects. Furthermore, the absence of environmental turbulence within the greenhouse might have influenced plant growth patterns differently than in natural settings, impacting the interpretation of treatment effects. The unexpected aphid pest infestation altered the bias of macroinvertebrate presence, which could have been solved by having an outdoor experiment. Biases introduced by the choice of locality for sample collection, such as proximity to freeways, may have exaggerated results due to increased soil contamination from car emissions. As well as the sole incorporation of invasive Fescue, which could be better analyzed in the face of competition with varied species. The experiment's duration might have needed to be increased to capture long-term changes in nutrient acquisition rates, limiting the depth of scientific inference drawn from the data.

BROADER IMPLICATIONS

Quantifying the ecosystem services provided by microbial inoculants in invasive species-affected urbanized soil ecosystems services while using cost effective organic amendments revealed substantial economic, environmental, and social benefits, which further inform decision-making processes for sustainable solutions. Developing adaptive management strategies leveraging microbial inoculants effectively mitigates invasive tall fescue impacts while promoting soil remediation objectives. Expanding soil bioremediation research to urban environments acknowledges unique challenges, including soil pollution and invasive plant colonization, requiring targeted remediation strategies. Assessing bioremediation techniques' suitability near roads and exploring plant-soil-microbe interactions enhance remediation efficiency while establishing long-term monitoring and adaptive management that can ensure the resilience and sustainability of roadside ecosystems over time.

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