Organic And Inorganic Fertilizer Application on Leveraging Nickel Micro-Nutrient to Improve Root Development of Sugarcane Single Bud Chips in the Face of Climate Change Challenges

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Abstract. Sugarcane is a vital crop for sugar and bioenergy production, yet its growth is increasingly threatened by climate change, including drought and fluctuating temperatures. This study investigates the role of Nickel (Ni) as a micronutrient to enhance root development and resilience of sugarcane seedlings under these stresses. A factorial randomized block design (RBD) was used, with Ni concentrations of 0 ppm, 0.25 ppm, 0.5 ppm, and 1 ppm applied to sugarcane single bud chips in a greenhouse. Results showed that 0.25 ppm Ni significantly increased root length by 13% in genotype M1, and by 73.7% in genotype M2. Root diameter increased by 330.3% in M1 and by 369.4% in M2 at 0.25 ppm. Additionally, 0.25 ppm Ni improved leaf nitrogen content and chlorophyll levels across all genotypes, with a 16.7% increase in M1's leaf nitrogen and a 12.3% increase in chlorophyll content. However, higher concentrations of Ni (0.5 ppm and 1 ppm) showed diminishing returns, reducing root and stem growth and causing potential toxicity. Furthermore, Ni application at 0.25 ppm enhanced soil microbial activity, particularly that of nitrogen-fixing and phosphate-solubilising bacteria, which are vital for maintaining soil fertility. This study concludes that 0.25 ppm Ni is optimal for improving root development and resilience to climate-induced stresses in sugarcane, offering a promising approach for sustainable cultivation practices in the face of climate change.

1 Introduction

Sugarcane is a vital industrial crop, playing a significant role in the production of sugar and bioenergy, especially in tropical and subtropical regions. However, the production of sugarcane is highly sensitive to climate factors such as atmospheric CO2 levels, temperature, precipitation, and extreme weather events, all of which are becoming increasingly unpredictable due to climate change.

In recent years, climate change has started to affect sugarcane yields across various regions. Some areas have benefited from improved water use efficiency, which has led to

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higher yields, while others have faced challenges such as drought, heat stress, and an increased frequency of extreme weather events. For example, in Brazil, climate change has enhanced sugarcane yields due to improved water use efficiency, whereas in countries like India, prolonged water deficits have led to significant reductions in sugarcane yields.. Drought, especially during critical growth stages, and rising temperatures have severely impacted sugarcane production in countries such as China and Indonesia. Furthermore, increased temperatures have exacerbated pest and disease outbreaks, further affecting crop yields.

Given these changes, the future of sugarcane production is intricately linked to adaptive farming practices and climate-resilient strategies designed to mitigate the negative effects of climate change [1]. These strategies include improving irrigation management, enhancing pest and disease control, and optimizing soil and nutrient management to support sugarcane growth under changing climatic conditions. Understanding the diverse impacts of climate change across regions is critical for enhancing the sustainability and profitability of global sugarcane production [2].

The growth of sugarcane roots is a critical factor in ensuring plant health and productivity. Roots are essential for water and nutrient absorption and provide mechanical anchorage for the plant [3]. Furthermore, strong and healthy roots are necessary for the regeneration of new shoots during the ratoon cycle, where the remaining roots play a key role in supporting the growth of subsequent crops. However, the development of sugarcane roots can be severely hindered by limiting factors such as high soil strength, suboptimal soil temperatures, soil compaction, and deep groundwater levels. These factors restrict root penetration, limiting the plant's ability to access water and nutrients, which negatively impacts its growth and yield. The availability of both macro and micronutrients is critical to supporting root development, and deficiencies in essential nutrients can reduce root growth, thus affecting the plant's ability to absorb water and nutrients, ultimately decreasing crop yield [4].

Recent research has identified Nickel (Ni) as an essential micronutrient for plants, playing a critical role in activating urease, an enzyme responsible for converting urea into ammonia and carbon dioxide [5], [6]. Nitrogen (N) influences nitrogen metabolism, which is vital for various physiological processes, including root development and overall plant growth. In addition, Ni is essential for the proper functioning of several enzymes involved in nitrogen fixation, and it has been found to influence antioxidant metabolism, ureides, amino acids, and organic acids in plants [7]. Despite the recognition of Ni deficiency as a limiting factor for crop productivity, especially in tropical soils, its role in enhancing root development in sugarcane remains underexplored. This highlights the need for further research into the most effective application methods, such as seed treatment, foliar spraying, or soil fertilization. Each method presents its own set of advantages and challenges, including issues related to phytotoxicity, fertilizer dosages, and increased production costs [8].

In the face of climate change, which increasingly impacts crop growth through drought, heat stress, and temperature fluctuations, understanding the role of Nickel (Ni) in improving sugarcane root development becomes crucial. While much attention has been given to macro-nutrients and other micronutrients, Ni's potential in enhancing root growth, particularly under climate-induced stresses, has been largely overlooked. This research focuses on how Ni influences the root development of sugarcane seedlings, particularly single-bud chips, to enhance their resilience to drought, heat stress, and extreme temperatures. Longer roots will help seedlings absorb water and nutrients more effectively, supporting optimal growth and productivity. By enhancing root systems, this study aims to contribute to sustainable agricultural practices by making sugarcane more resilient to climate change, thereby offering a strategic pathway for improving plant resilience of future farm systems.

2 Materials and Methods

2.1 Plant material and experimental design

This study employed a factorial randomized block design (RBD) to assess the effects of four sugarcane varieties (M0, M1, M2, and M3) and nickel (Ni) micronutrient concentration on the growth and root development of sugarcane seedlings. Single bud chips from these varieties were sterilized and planted in 25 cm × 25 cm pots filled with a 3:1 (v/v) mixture of loamy soil and compost, chosen for its ability to retain moisture and provide nutrients. The seedlings were grown under controlled greenhouse conditions for three months. Nickel sulfate ($NiSO_4 \cdot 6H_2O$) solutions were prepared at concentrations of 0 ppm, 0.25 ppm, 0.5 ppm, 1 ppm, and applied via soil drenching, delivering 200 mL of solution per pot at each treatment. Applications commenced at 30 days after sowing (DAS), with four treatments administered at 7-day intervals. The growth chamber was maintained at a temperature of 30°C and a relative humidity of 60%, with seedlings irrigated every two days to ensure consistent soil moisture.

2.2 Growth and root development

Root systems were observed 90 days after sowing (DAS) and directly photographed using a digital camera. Root development, including main length, diameter, and volume, was measured using SmartRoot software (https://smartroot.github.io). The total chlorophyll content was assessed by extracting 0.1 g of fresh sugarcane leaves with 5 mL of 95% ethanol, followed by centrifugation at 10,000 g and 4°C for 10 minutes.

2.3 Leaf nitrogen and total chlorophyll

Total nitrogen in leaf tissue was measured using the Kjeldahl method. A 0.5–1 gram sample of dry leaf tissue was digested with 5–10 mL of concentrated sulfuric acid and a selenium or copper catalyst, then heated. After digestion, the mixture was neutralised with NaOH to release ammonia, which was then absorbed in a boric acid solution. The absorbed ammonia was titrated with standard hydrochloric acid (HCl) to the endpoint. Nitrogen content was calculated based on the volume of acid used and the normality of the standard solution [13]. The total chlorophyll content was determined by extracting 0.1 g of fresh sugarcane leaves with 5 mL of 95% ethanol. The extract was then centrifuged at 10,000 g for 10 minutes at 4 °C. The chlorophyll concentration in the supernatant was measured using a spectrophotometer at wavelengths of 645 nm and 663 nm. Each sample was analyzed in triplicate, using three independent biological replicates. The total chlorophyll concentration was calculated using the following equations (1)–(3) [10]. The analysis was conducted in triplicate with three independent biological replicates.

$$\text{Chlorophyll a (mg/g FW)} : (12.7 * A663) - (2.69 * A645) \quad (1)$$

$$\text{Chlorophyll b (mg/g FW)} : (22.9 * A645) - (4.68 * A663) \quad (2)$$

$$\text{Total Chlorophyll (mg/g FW)} : (8.2 * A663) + (20.2 * A645) \quad (3)$$

2.4 Total soil bacteria

Soil bacteria were analyzed using the spread plate technique on Nutrient Agar media. One gram of dry soil was mixed with 9 mL of sterile saline solution (0.85% NaCl) and homogenized. Serial dilutions were performed by transferring 1 mL of the soil suspension

into 9 mL of sterile saline, resulting in dilutions of 10-1 to 10-4. A 0.1 mL aliquot from each dilution was inoculated onto Nutrient Agar plates and spread evenly using a sterile spreader. The plates were incubated at 30–37°C for 24–48 hours [15]. Colonies were counted on plates with 30–300 colonies, and colony-forming units (CFU) per gram of soil were calculated using the formula: CFU/g soil = (colony count × dilution factor) / soil weight (g).

2.5 Data analysis

The data collected from the experiment will be analysed using Analysis of Variance (ANOVA) to assess the effects of sugarcane variety and nickel micronutrient concentration on growth parameters. If significant differences are detected, Tukey's Honestly Significant Difference (HSD) test will be used to compare means between treatments. All statistical analyses will be performed using SPSS.

3 Results

3.1 Growth and root development

The study results show that Nickel (Ni) application significantly affected both plant height and stem diameter across the four genotypes. Genotype M1, at a 0.25 ppm concentration, increased plant height by 38.9%, while 0.5 ppm and 1 ppm showed only an 11.1% increase. In terms of stem diameter, 0.25 ppm reduced it slightly to 1.45 cm, while 0.5 ppm increased it by 5.5% to 1.55 cm, and 1 ppm showed a 4.8% increase. Genotype M2 had the highest plant height increase at 0.25 ppm (27.8%), with smaller increases at higher concentrations. Its stem diameter also increased slightly at 0.25 ppm (10.4%) but showed a 7.6% decrease at 1 ppm. Genotype M3 exhibited the highest plant height increase at 0.5 ppm (30.0%) and the largest stem diameter at 0.5 ppm (30% increase to 1.74 cm), but showed a decrease at 1 ppm for both parameters. Genotype M4 responded best to 0.25 ppm and 0.5 ppm, with increases in plant height (30.0%) and stem diameter (14.5% at 0.25 ppm), while 1 ppm showed a reduction in both. Overall, 0.25 ppm and 0.5 ppm Ni had a stronger positive impact on plant height and stem diameter, while higher concentrations showed no additional benefits (Fig. 1).

The study results demonstrate that Nickel (Ni) application significantly influenced both root length and root diameter across the four genotypes. For genotype M1, the 0.25 ppm concentration increased root length by 13% (to 26 cm), while the 0.5 ppm treatment further increased it by 91% (to 44 cm). The 1 ppm treatment showed no change. In terms of root diameter, 0.25 ppm increased it by 330.3% (to 0.142 cm), and 0.5 ppm by 265.1% (to 0.121 cm), while 1 ppm showed a slight decrease. Genotype M2 showed the most dramatic response, with root length increasing by 73.7% (to 66 cm) at 0.25 ppm, and root diameter increasing by 369.4% (to 0.169 cm). The 1 ppm treatment resulted in a 2.6% decrease in root length and a 30.1% decrease in root diameter. Genotype M3 showed moderate responses, with 0.5 ppm increasing root length by 67.6% (to 57 cm) and root diameter by 318.8% (to 0.134 cm). The 1 ppm treatment resulted in slight reductions in both parameters. Genotype M4 showed less variation, with increases in root length of 8.1% (from 37 cm to 40 cm) and 240.5% (from 0.126 cm to 0.402 cm) in root diameter at 0.25 ppm and 0.5 ppm, respectively, and a slight decrease at 1 ppm (Fig. 2).

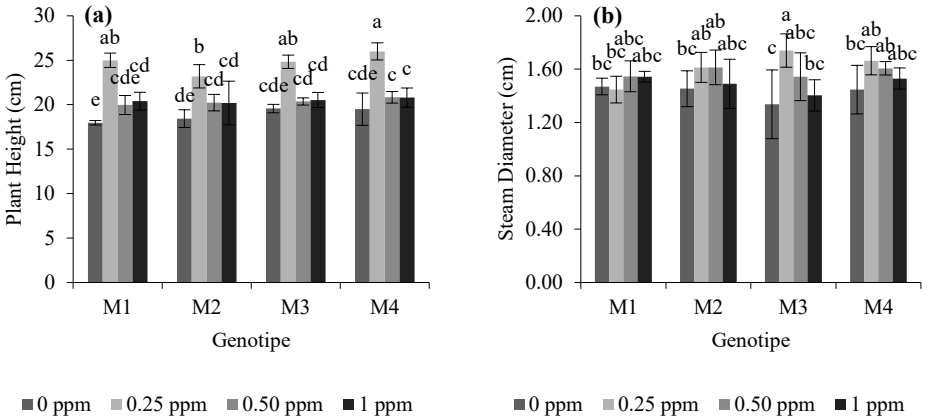

Fig. 1. Effect of Ni application on sugarcane seedlings in the greenhouse on plant height (a) and stem diameter (b). *Significant according to the *F test* at $p < 0.05$. Mean values and standard deviations were calculated from three replications. Different letters indicate significant differences according to the Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$.

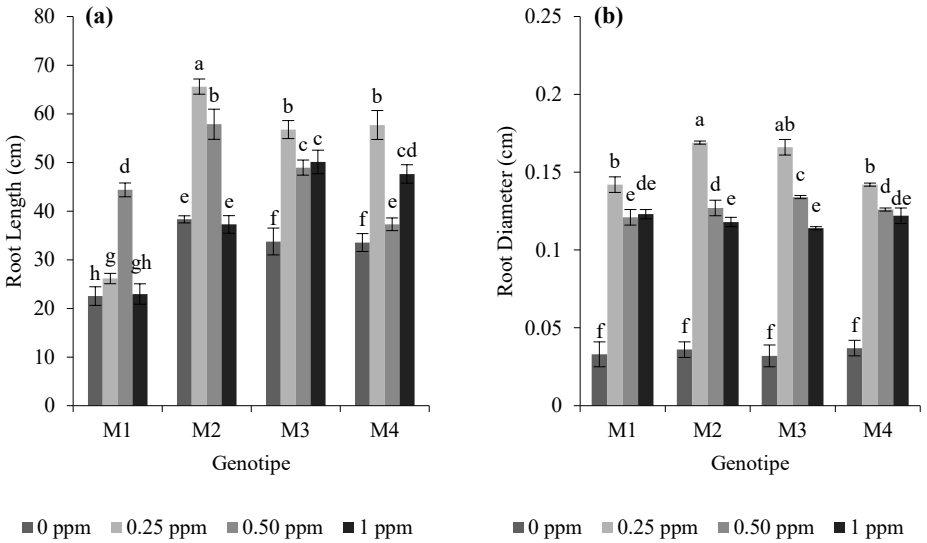

Fig. 2. Effect of Ni application on sugarcane seedlings in the greenhouse on root length (a) and root diameter (b). *Significant according to the *F test* at $p < 0.05$. Mean values and standard deviations were calculated from three replications. Different letters indicate significant differences according to the Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$.

3.2 Leaf nitrogen and total chlorophyll

The study results indicate that the application of Nickel (Ni) concentrations significantly influenced both leaf nitrogen content and total chlorophyll content across all four genotypes. For genotype M1, the 0.25 ppm treatment increased leaf nitrogen to 0.56 mg L^{-1}, a 16.7% improvement over the control (0.48 mg L^{-1}), and the 0.5 ppm treatment further increased it

to 0.55 mg L^{-1}, while the 1 ppm treatment reduced it to 0.42 mg L^{-1}, a 12.5% decrease from the control. Chlorophyll content in M1 also showed a similar trend, with the 0.25 ppm treatment increasing it to 3.18 mg g^{-1}, a 12.3% increase over the control (2.83 mg g^{-1}), while the 0.5 ppm treatment increased it to 3.08 mg g^{-1}, and the 1 ppm treatment slightly decreased it to 2.73 mg g^{-1}. Genotype M2 showed a similar response in leaf nitrogen, with the 0.25 ppm treatment increasing it to 0.54 mg L^{-1} (12.5% increase) and the 0.5 ppm treatment reaching 0.51 mg L^{-1} (6.25% increase), while the 1 ppm treatment resulted in a decrease to 0.47 mg L^{-1}. Chlorophyll content in M2 increased to 2.74 mg g^{-1} at 0.25 ppm, a 3.0% increase, but decreased to 2.67 mg g^{-1} at 0.5 ppm, and the 1 ppm treatment significantly reduced it to 2.40 mg g^{-1}. For genotype M3, the 0.25 ppm treatment resulted in a slight increase in leaf nitrogen to 0.48 mg L^{-1}, while the 0.5 ppm treatment increased it to 0.52 mg L^{-1}. Chlorophyll content for M3 showed the highest increase at 0.25 ppm, reaching 3.19 mg g^{-1}, a 15.2% increase compared to the control (2.77 mg g^{-1}). A slight decrease to 2.76 mg g^{-1} was observed at 1 ppm. Genotype M4 displayed the least variation, with leaf nitrogen increasing to 0.54 mg L^{-1} (10.2% increase) at 0.25 ppm, and the 0.5 ppm treatment yielding 0.53 mg L^{-1} (8.2% improvement), while the 1 ppm treatment resulted in 0.49 mg L^{-1}. Chlorophyll content in M4 increased to 2.85 mg g^{-1} (41.9% increase) at 0.25 ppm, with a smaller increase to 2.59 mg g^{-1} at 0.5 ppm, while the 1 ppm concentration slightly decreased it to 2.71 mg g^{-1} (Fig. 3). Overall, the 0.25 ppm treatment proved to be the most effective for improving both chlorophyll content and leaf nitrogen, with varietal differences observed.

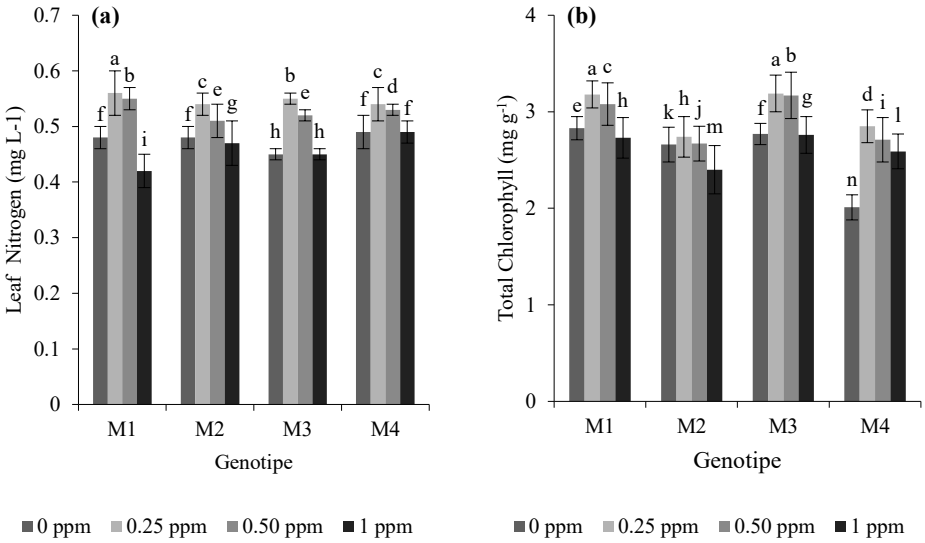

Fig. 3. Effect of Ni application on sugarcane seedlings in the greenhouse on leaf nitrogen (a) and total chlorophyll (b). *Significant according to the F test at $p < 0.05$. Mean values and standard deviations were calculated from three replications. Different letters indicate significant

3.3 Total soil bacteria

The table presents the effect of varying nickel (Ni) concentrations on the total bacterial count, nitrogen-fixing bacteria, and phosphate-solubilising bacteria in sugarcane cultivation. For total bacteria, the highest count was observed at 0.25 ppm Ni, with a value of $8.40 \pm 0.14 \times 10^5$ CFU/ml, which was marginally higher than the 0 ppm treatment ($8.05 \pm 0.49 \times 10^5$ CFU/ml). At 0.50 ppm and 1 ppm, the total bacterial counts decreased to $7.70 \pm 0.28 \times 10^5$

CFU/ml and 6.60 ± 0.42 × 10^5 CFU/ml, respectively. These results suggest that moderate Ni application (0.25 ppm) is more conducive to promoting total bacterial populations compared to higher Ni concentrations.

In terms of nitrogen-fixing bacteria, the highest count was recorded at 0.25 ppm Ni (6.05 ± 0.21 × 10^5 CFU/ml), significantly surpassing the 0 ppm treatment (3.65 ± 0.21 × 10^5 CFU/ml). The 0.50 ppm and 1 ppm treatments resulted in lower counts of nitrogen-fixing bacteria (4.10 ± 0.42 × 10^5 CFU/mL and 3.35 ± 0.35 × 10^5 CFU/mL, respectively), indicating that higher Ni concentrations may inhibit the proliferation of these bacteria. Regarding phosphate-solubilising bacteria, the highest count was observed at 1 ppm Ni, reaching 6.35 ± 0.35 × 10^5 CFU/mL, which was significantly higher than in all other treatments. The 0.25 ppm and 0.50 ppm treatments also supported relatively high populations of phosphate-solubilising bacteria, with counts of 5.50 ± 0.42 × 10^5 CFU/ml and 4.85 ± 0.49 × 10^5 CFU/ml, respectively. The 0 ppm treatment exhibited the lowest count at 4.75 ± 0.07 × 10^5 CFU/ml. In summary, the data demonstrate that moderate Ni concentrations (0.25 ppm) enhance the growth of total and nitrogen-fixing bacteria, while higher Ni concentrations (1 ppm) promote the proliferation of phosphate-solubilizing bacteria (Tabel 1). These findings underscore the differential effects of Ni on bacterial communities, suggesting that optimal Ni concentrations vary based on bacterial function, with 0.25 ppm being most favorable for total and nitrogen-fixing bacteria, and 1 ppm being more beneficial for phosphate-solubilising bacteria.

Table 1. Effect of nickel (Ni) concentration on total bacteria, nitrogen-fixing bacteria, and phosphate-solubilizing bacteria in sugarcane.

Ni Concentration	Total Bacteria (CFU/ml)	Nitrogen Fixing Bacteria (CFU/ml)	Phosphate Solubilizing Bacteria (CFU/ml)
0 ppm	8.05 ± 0.49 x 10^5	3.65 ± 0.21 x 10^5	4.75 ± 0.07 x 10^5
0.25 ppm	8.40 ± 0.14 x 10^5	6.05 ± 0.21 x 10^5	5.50 ± 0.42 x 10^5
0.50 ppm	7.70 ± 0.28 x 10^5	4.10 ± 0.42 x 10^5	4.85 ± 0.49 x 10^5
1 ppm	6.60 ± 0.42 x 10^5	3.35 ± 0.35 x 10^5	6.35 ± 0.35 x 10^5

4 Discussion

This study demonstrates that the application of 0.25 ppm nickel (Ni) yields the best growth, as measured by both root length and diameter, across various sugarcane varieties (M1, M2, M3, and M4). These results are consistent with previous studies, which indicate that Ni plays a crucial role in supporting root growth by enhancing water use efficiency and nutrient absorption [12]. Ni, involved in the activation of the urease enzyme, plays a role in supporting nitrogen metabolism, which is essential for root development [13]. However, literature also shows that excessive micronutrient concentrations, such as Ni concentrations higher than 0.25 ppm, can lead to toxicity, hindering root growth [14]. Therefore, while 0.25 ppm is effective, Ni application must be conducted carefully to avoid disrupting the soil's micronutrient balance.

Furthermore, the results of this study also showed a significant increase in chlorophyll content and leaf nitrogen levels at the 0.25 ppm Ni treatment. This highlights the crucial role of Ni in supporting chlorophyll synthesis and nitrogen metabolism in plants, thereby contributing to enhanced photosynthetic capacity and efficient nitrogen utilisation. The increase in chlorophyll supports improved photosynthetic performance, accelerating plant growth, which in turn enhances plant resilience to environmental stress. However, it is important to note that while the 0.25 ppm Ni treatment yielded significant results, some studies have shown that higher micronutrient concentrations can inhibit photosynthesis and disrupt plant hormone balance, potentially harming plant growth in the long term [19].

Findings related to soil microbial communities also indicate that the 0.25 ppm Ni application supports an increase in nitrogen-fixing bacteria and phosphate-solubilizing bacteria. The increase in nitrogen-fixing bacteria at this treatment is consistent with findings that show these bacteria play a critical role in enhancing nitrogen availability in the soil, which contributes to improved plant growth. On the other hand, although 1 ppm Ni increased the number of phosphate-solubilising bacteria, other studies have shown that higher Ni concentrations can disrupt the soil microbial balance, which may inhibit the activity of other beneficial microbes [6]. This suggests that while Ni supports soil microbial diversity, excessive application can disrupt the balance of the microbial ecosystem and compromise soil quality.

Overall, the findings of this study emphasise that the application of Ni at a concentration of 0.25 ppm has the potential to enhance sugarcane resilience to climate change by promoting root growth, increasing chlorophyll content, and facilitating more efficient nitrogen metabolism. These findings support the use of Ni as one strategy to enhance plant resilience against stress conditions, such as drought and extreme temperatures. However, it should be noted that while 0.25 ppm Ni yields positive results, higher concentrations may cause toxicity and disrupt soil microbial communities, potentially reducing its long-term benefits. Therefore, further research is needed to explore the long-term effects of Ni application in the field and to determine the optimal dose that maximizes benefits without harming the soil ecosystem and plant growth.

5 Conclusion

The application of 0.25 ppm nickel (Ni) has been shown to significantly enhance root development, chlorophyll content, and nitrogen metabolism in sugarcane, thereby contributing to improved plant growth and resilience to environmental stresses, such as drought and temperature fluctuations. This study highlights the critical role of Ni in supporting optimal root growth, which is essential for efficient water and nutrient uptake. Additionally, the positive effects on soil microbial communities, including nitrogen-fixing and phosphate-solubilizing bacteria, further emphasize the potential of Ni to enhance soil fertility and promote sustainable agricultural practices. However, it is essential to note that while 0.25 ppm Ni is effective, higher concentrations may cause toxicity, which could negatively impact plant growth and soil health. Therefore, the judicious application of Ni is recommended to achieve the best balance between enhancing plant resilience and maintaining ecological sustainability. This research offers valuable insights into the role of Ni as a micronutrient for enhancing sugarcane productivity, particularly in the context of climate change, and provides a foundation for further studies to refine Ni application techniques in agricultural systems.

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