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Impact of Co-Inoculating Bradyrhizobium japonicum with Bacillus subtilis or Priestia megaterium on Nitrate Inhibition of Symbiotic Nitrogen Fixation in Soybean

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INTRODUCTION & AIM

High concentration of nitrate in the soybean rhizosphere reduces nodulation and N_2 fixation activity substantially. To counteract nitrate inhibition of symbiotic nitrogen fixation (SNF), many techniques are being adopted. The use of microorganisms is possibly the most promising and advantageous. Although the beneficial effects of PGPB like *B. subtilis* are well studied, their role in mitigating NO3- inhibition of SNF remains unclear. This study investigate the potential role of *Bacillus subtilis* and *Priestia megaterium*- two common nitrate-reducing bacteria, in mitigating nitrate inhibition of SNF.

Hypotheses

1. Applying *B. subtilis* or *P. megaterium* would reduce nitrate concentration in the Soybean rhizosphere. And,
2. Their decomposition or predation could release nitrate back to the soil slowly, promoting growth.

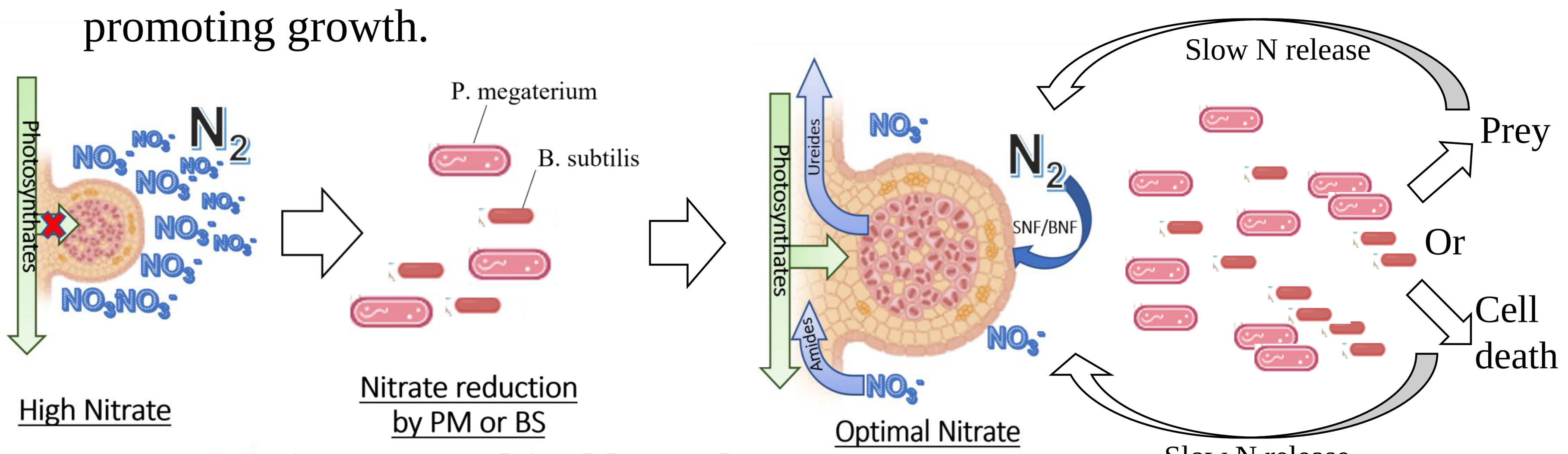

Fig. 1. Graphical hypotheses

METHOD

Sterilized soybeans (*Glycine max* L. cv Williams) inoculated with *B. japonicum* (USDA 6^T) or (USDA 110)

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Sowed in sterile vermiculite for 10 days then cultivated hydroponically

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B. subtilis or *P. megaterium* introduced into the nutrient solution (with every change of nutrient solution; after 3 days)

↓

Soybeans cultivated in N-free nutrient solution for 7 days, then Sodium nitrate $^{15}NO_3^-$ (1 mM and 10 mM) treatments introduced for 7 more days before harvesting for analyses

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Acetylene Reduction Assay, Kjeldahl digestion, and Isotope Ratio Mass Spectrometry were used for analysis. Growth parameters, e.g., fresh and dry weight and nodule count, were measured. Data analysis was performed using Python.

RESULTS & DISCUSSION

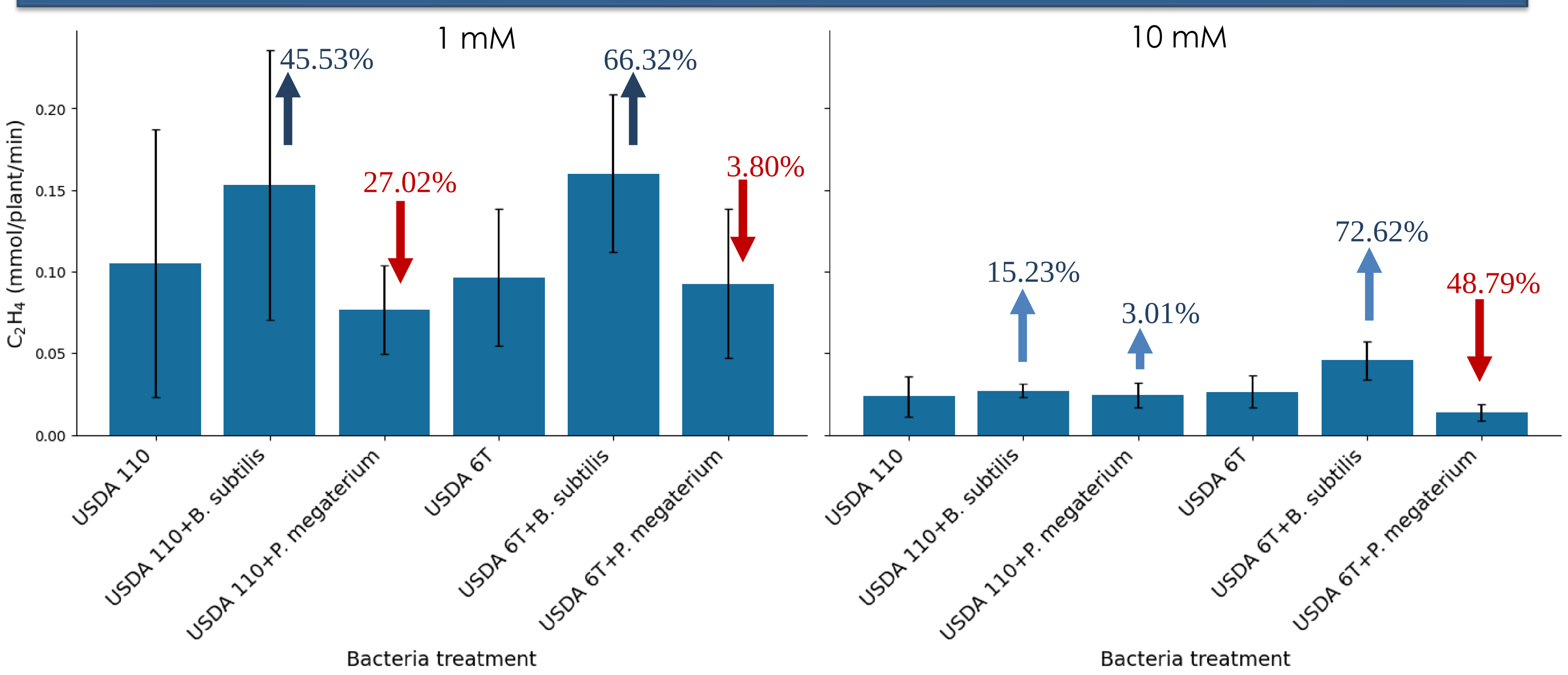

Fig. 2. Acetylene reduction assay 24 DAI of Soybean plants cultivated in a hydroponic setup with nutrient solution containing 1 mM (left) and 10 mM nitrate concentrations (right).

- Co-inoculation with *B. subtilis* partially mitigated nitrate inhibition of SNF, especially with USDA 6^T, boosting nitrogenase activity by 72.62% **(Fig. 2).** This may result from nitrate reduction or enhanced nodulation via hormone production and nutrient assimilation.
- In contrast, *P. megaterium* suppressed nitrogenase activity, likely due to excessive cytokinin accumulation. These findings highlight the importance of selecting compatible co-inoculants to optimize SNF under high-nitrate conditions.

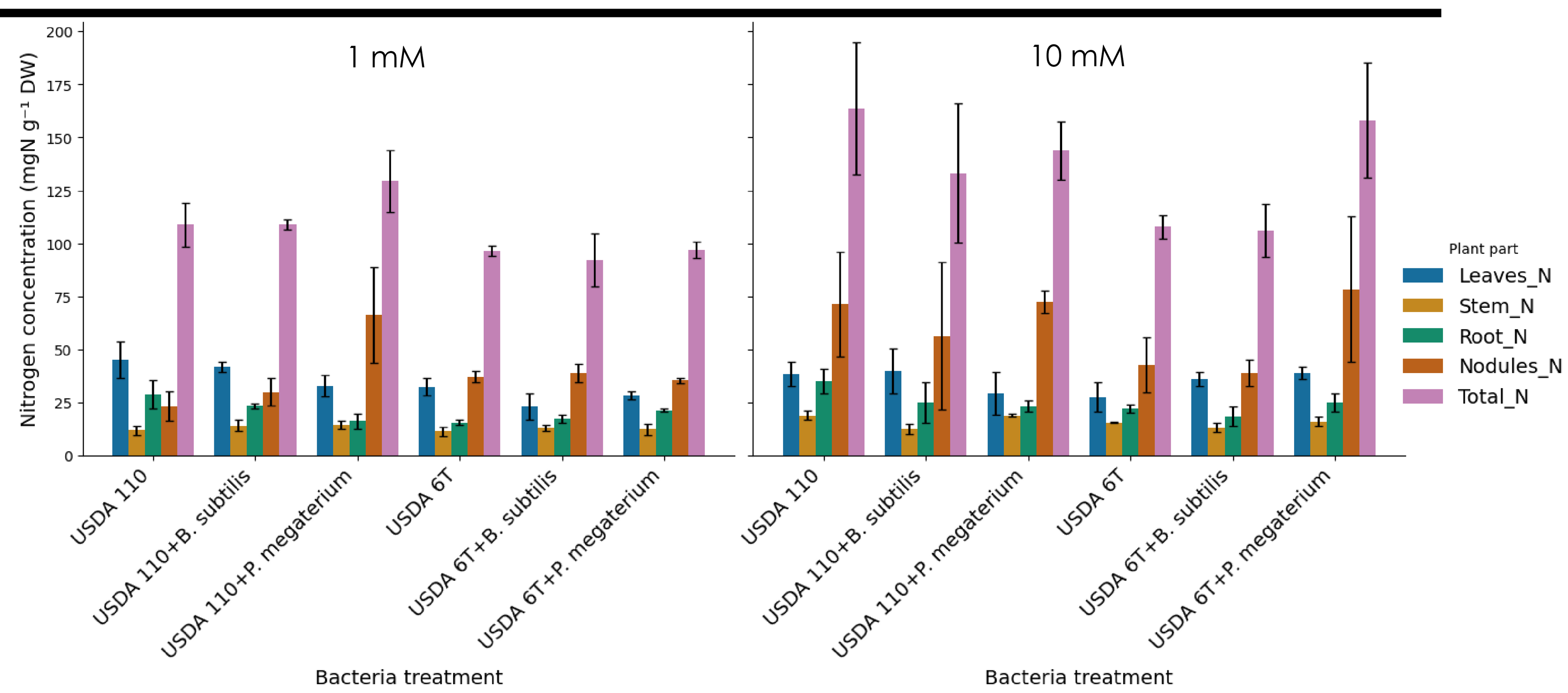

Fig. 3. Total nitrogen (N) in Soybean plant parts at 24 DAI cultivated in a hydroponic setup with nutrient solution containing 1 mM (left) and 10 mM nitrate concentrations (right).

- Nitrate levels also influenced nodule N concentration ($p = 0.048$). Soybeans inoculated with USDA 6^T had lower nodule N, whereas those with USDA 110 had higher nodule N at 10 mM nitrate. This supports findings by Delgado et al. (1989), which revealed that hup- genotypes (e.g., USDA 6^T) have higher nitrate reductase activity than hup+ genotypes (e.g., USDA 110), both as free-living bacteria and within nodules [1].
- Root N concentration was significantly affected by bacterial treatments ($p = 0.049$). USDA 110 outperformed USDA 6^T at both nitrate levels, likely due to its ability to reuse hydrogen lost during nitrogen fixation, making it more efficient.
- Co-inoculation effects varied: *B. subtilis* and *P. megaterium* increased root N with USDA 6^T at 1 mM nitrate but decreased it at 10 mM nitrate, suggesting a synergistic effect under low-nitrate conditions. This synergy weakens when high nitrate levels suppress SNF, reducing overall root N concentration.

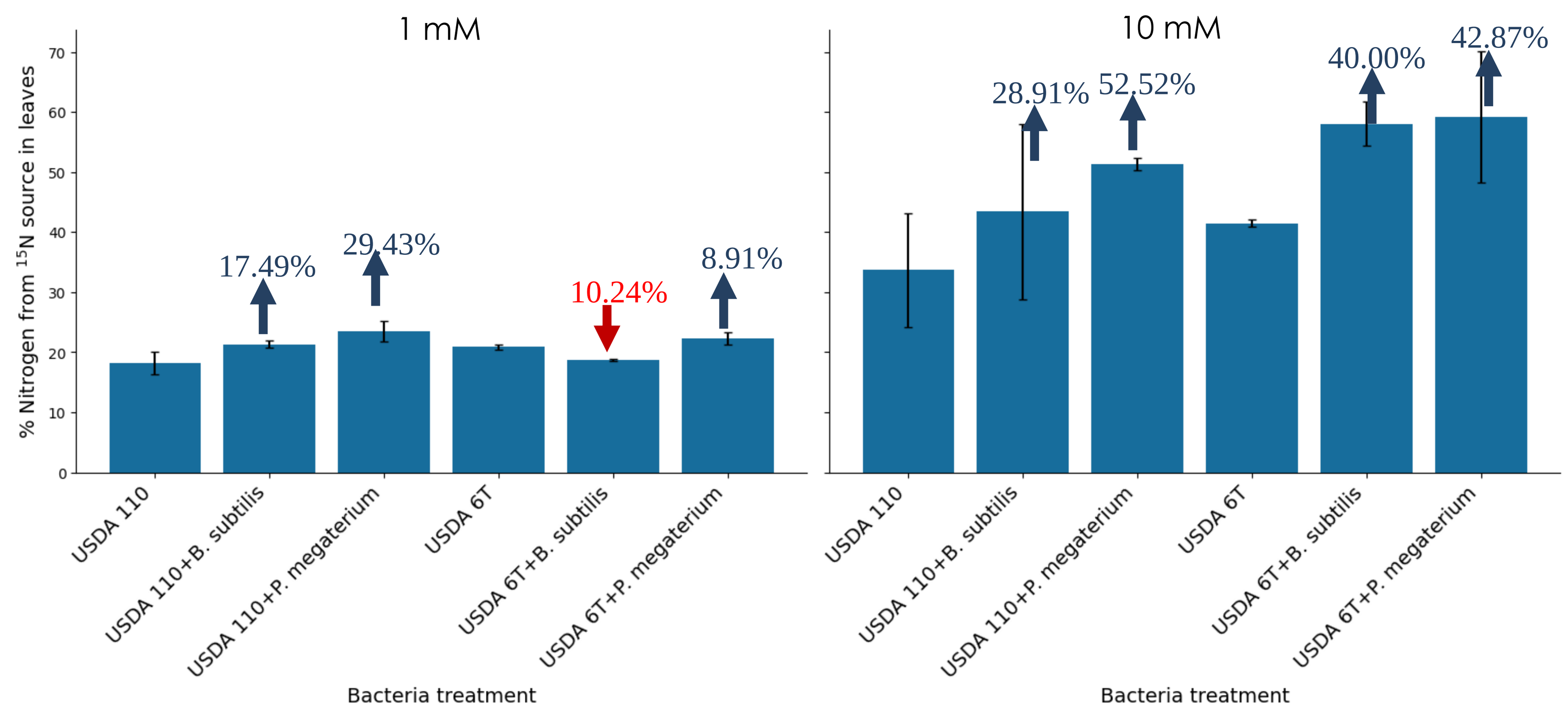

Fig. 4. %N from ^{15}N in leaves 24 DAI of soybean cultivated in a hydroponic setup with nutrient solution containing 1 mM (left) and 10 mM nitrate concentrations (right).

- Nitrate treatments significantly affected the percentage of nitrogen from synthetic ^{15}N in soybean leaves **(Fig. 4)**. Soybeans inoculated with USDA 6^T consistently showed greater ^{15}N uptake than those with USDA 110 at both nitrate levels.
- At 10 mM nitrate, *B. subtilis* increased ^{15}N uptake by 28.91% in USDA 110 and 40.00% in USDA 6^T, while *P. megaterium* enhanced uptake by 52.52% and 42.87%, respectively. These findings highlight the potential of co-inoculants to improve nitrogen use efficiency, with *P. megaterium* showing exceptional potential [2].

CONCLUSION

The findings in this study demonstrate that co-inoculation strategies can mitigate nitrate-induced inhibition of SNF and enhance soybean productivity in nitrate-rich environments. *B. subtilis* consistently improved nitrogenase activity, nodulation, and plant growth under high-nitrate conditions, while *P. megaterium* showed promising effects in enhancing nitrate assimilation particularly with *B. japonicum* USDA 6^T.

REFERENCES

1. Delgado, M. J. et. al., "Nitrate Reductase Activity of Free-Living and Symbiotic Uptake Hydrogenase-Positive and Uptake Hydrogenase-Negative Strains of *Bradyrhizobium Japonicum*." Arch. Microbiol. 1989, 151 (2), 166–170. https://doi.org/10.1007/BF00414433.
2. S. Chu et al., "Enhanced removal of nitrate in the maize rhizosphere by plant growth-promoting *Bacillus megaterium* NCT-2, and its colonization pattern in response to nitrate," Chemosphere, vol. 208, pp. 316–324, Oct. 2018, doi: 10.1016/j.chemosphere.2018.05.189