The Effects of Elevated Nitrate and CO_2 on the Appropriation of Nitrogen and Carbon in Legume-Rhizobia Symbiosis

Andres Jimenez Salinas[1] ,Ryoko Oono[2]

[1]Department of Chemistry and Biochemistry, University of California, Santa Barbara

[2]Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara

Introduction

Global change in carbon and nitrogen cycling affects the supply and demand of traded resources in important resource mutualisms, such as the legume-rhizobia symbiosis. In this experiment, the symbiotic and biochemical traits of two rhizobial strains (*Rhizobium etli* SAM100 and CE3) were compared in the common bean legume plant, *Phaseolus vulgaris.* The rhizobial strains differ in their ability to fix organic nitrogen from atmospheric nitrogen and their ability to make polyhydroxybutyrate (PHB), an energy reserve molecule. High-fixing SAM100 cannot synthesize PHB while low-fixing CE3 can (Cevallos et al. 1996).

Methods

Fall 2016 Plants. Investigation on environmental factors.

- Plants grown in greenhouse, Figs. 1 & 4, under elevated CO_2 (800 ppm) or ambient CO_2 (400 ppm)
- No nitrate added or 5mM nitrate added
- CE3 or SAM100 rhizobial strain
- Analyzed nodule number and weight, plant root and shoot weight, PHB concentration by flow cytometry, Fig. 5, on CE3 plants and $\delta\ ^{15}N$ of plant leaflets, ^{15}N dilution method (Chalk, 2016)

Fall 2017 Plants. Baseline for isotope analysis.

- Plants grown in growth chamber, Figs. 2 & 3
- No nitrate added
- Inoculated with no rhizobia or either CE3 or SAM100
- Analyzed $\delta\ ^{15}N$ of plant leaflets, ^{15}N dilution method

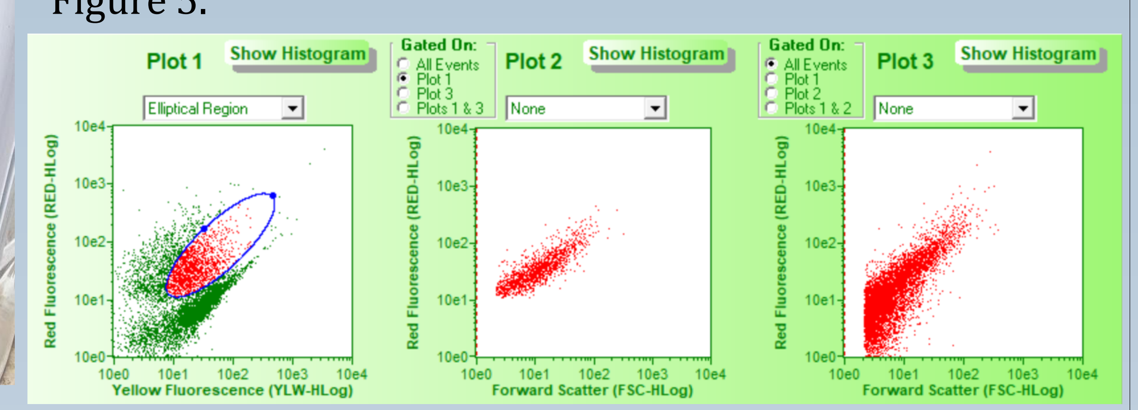
Figure 1.

Figure 2.

Figure 3.

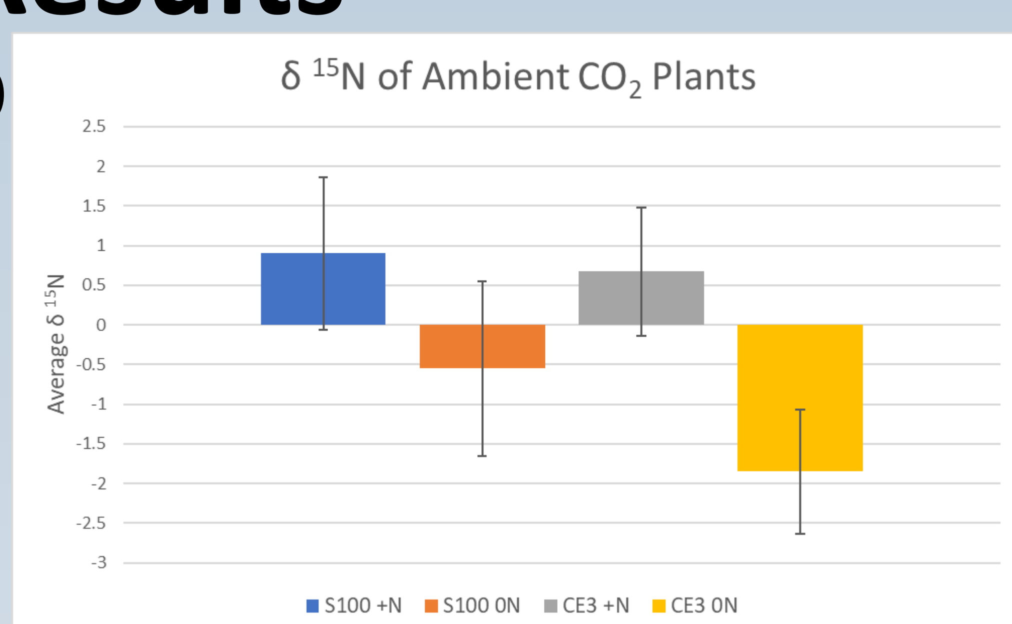
Figure 4.

Figure 5.

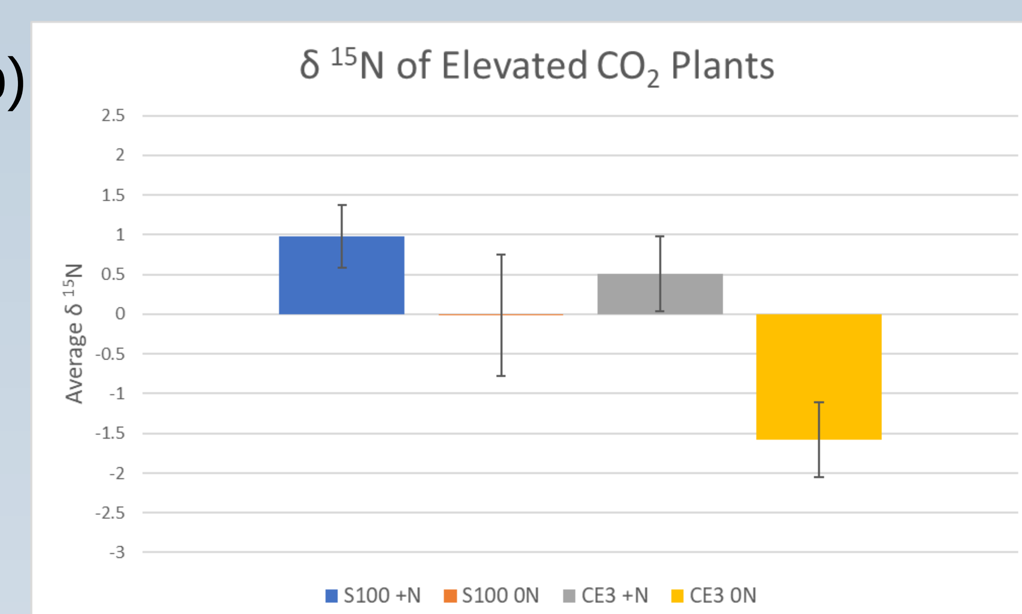


Results

a)



b)



c)

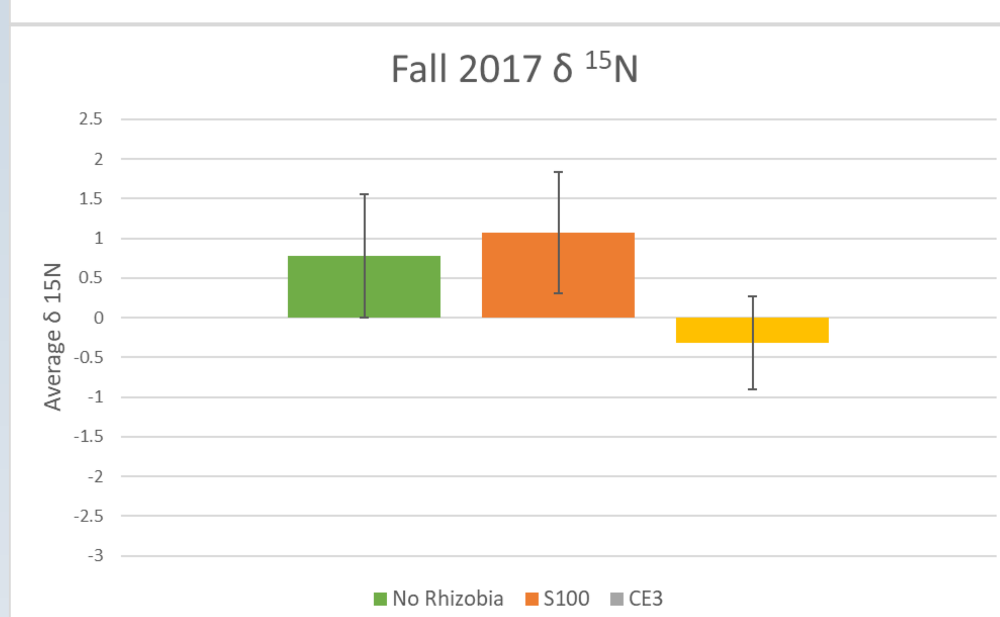


Figure 6. a) When nitrate was added to plants, there was an expected shift from negative to positive $\delta^{15}N$ values for CE3 plants (Wanke, 2002). b) The shift was not as strong in plants with SAM100, which led us to investigate whether the baseline $\delta^{15}N$ values (plants with no nitrate) were different between SAM100 and CE3 . c) Plants inoculated with SAM100 and no nitrate had significantly higher $\delta^{15}N$ values than plants with CE3 (ANOVA, p-value < 0.001).

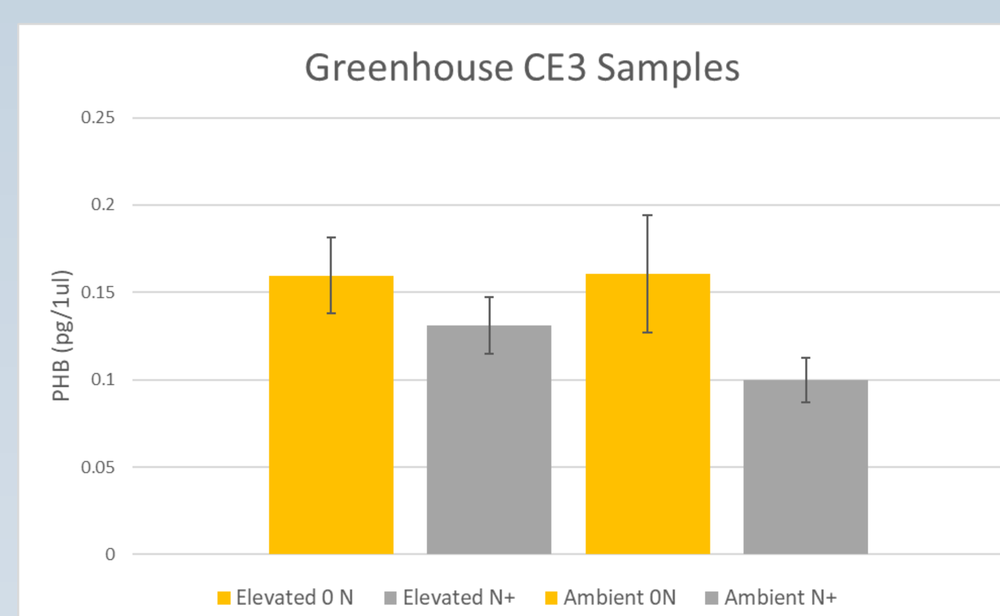


Figure 7. Fall 2016 plants with the CE3 strain and no added nitrate consistently had a higher PHB concentration than plants with 5 mM of nitrate.

Discussion

- The significant shift of $\delta\ ^{15}N$ for CE3 plants shows that the plant receives a significant portion of nitrogen from nitrate but this replacement is not significantly apparent for plants with SAM 100. Since the SAM100 strain is more efficient at fixing nitrogen than CE3, the plant may not discriminate between nitrogen from nitrate or SAM100.
- The difference in PHB concentrations may be due to the differential partitioning of carbon from the plant to the rhizobia when nitrate is introduced. When the plant requires less nitrogen from the rhizobia, the plant gives less carbon to the bacteria. The difference in PHB between nitrate and no nitrate is more profound in ambient CO_2 conditions because the plant has less carbon.

Literature Cited

Chalk, P. M., S. K. Lam, and D. Chen. "(15)N Methodologies for Quantifying the Response of N2-Fixing Associations to Elevated [Co2]: A Review." *Sci Total Environ* 571 (2016): 624-32.

Cevallos, M A et al. "Genetic and Physiological Characterization of a Rhizobium Etli Mutant Strain Unable to Synthesize Poly-Beta-Hydroxybutyrate." *Journal of Bacteriology* 178.6 (1996): 1646–1654.

Wanek, W., and S. K. Arndt. "Difference in δ(15)N Signatures between Nodulated Roots and Shoots of Soybean Is Indicative of the Contribution of Symbiotic N(2) Fixation to Plant N." *J Exp Bot* 53.371 (2002): 1109-18.

Acknowledgements

I would like to thank my faculty advisor Dr. Oono, for her guidance throughout the experimental process. I would also like to thank other undergraduate researchers, Simer Chima and Derek Tang, who helped me collect data. Thanks to the Analytical Lab for training me and allowing me to use their isotope ratio mass spectrophotometer in the Marine Science Institute. Partial funding of this experiment was given by the Undergraduate Research and Creative Activities grant at the University of California, Santa Barbara.

msi Marine Science Institute