

## Assessing Biological Nitrification Inhibition in the Rhizosphere of Field-Grown Bioenergy Sorghum

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**Project Goals: Agricultural activity causes significant nitrate (NO<sub>3</sub><sup>-</sup>) leaching and nitrous oxide (N<sub>2</sub>O) gas fluxes, polluting groundwater and contributing to global greenhouse gas emissions. Improving our understanding of microbial NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O production via nitrification in agricultural soils could lessen the detrimental effects of bioenergy crop production. Sorghum is one of a handful of grasses that exude secondary metabolites with the potential to inhibit nitrification, but the characterization of biological nitrification inhibition (BNI) has been limited to laboratory and greenhouse studies. It is likely that environmental conditions alter the extent of BNI in the field, so we leveraged two sorghum trials in 2018 and 2019 to characterize BNI in rhizosphere soil under varying environmental and management conditions.**

The heavy use of nitrogen (N) fertilizer to maximize crop yield represents a major disturbance of the N cycle that causes nitrate (NO<sub>3</sub><sup>-</sup>) leaching into groundwater and emissions of the greenhouse gas nitrous oxide (N<sub>2</sub>O) into the atmosphere. As a result, implementing annual bioenergy cropping systems to reduce our dependence on fossil fuels has other detrimental environmental impacts. In the soil ecosystem, bacterial and archaeal nitrification of ammonium (NH<sub>4</sub><sup>+</sup>) to NO<sub>3</sub><sup>-</sup> and subsequent denitrification of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>O and N<sub>2</sub> are key steps leading to NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O production. Laboratory and greenhouse studies show that sorghum, a candidate annual bioenergy feedstock, exudes secondary metabolites into the rhizosphere that inhibit nitrification. However, conditions in the field are likely to alter biological nitrification inhibition (BNI). We implemented two sorghum field trials in 2018 and 2019 and compared bulk and rhizosphere soil potential nitrification rates, potential denitrification rates, and microbial communities. During both seasons, BNI was strongest during the period of maximum plant growth, but was limited early and late in the growing season. BNI was substantially stronger in 2019, when the drier growing season likely caused hydrophilic BNI compounds to accumulate in rhizosphere soil. Although fertilizer addition weakened BNI in 2018, the apparent greater strength exerted by

reduced precipitation in 2019 outweighed any fertilizer effect. Potential denitrification was stimulated in the rhizosphere, suggesting that carbon (C) exudation by sorghum roots stimulates heterotrophic microbes and thus indirectly inhibits nitrification by creating competition for  $\text{NH}_4^+$ . Shifts in the rhizosphere microbial community relative to bulk soil, and the association of nitrifier relative abundance with potential nitrification rates when BNI was strongest mid-season, show that sorghum has some capacity to alter its rhizosphere community in a way that reduces  $\text{NO}_3^-$  production. Overall, we show that plant growth stage, precipitation variability, fertilization, and rhizosphere competition interact to control BNI in the field. Thus, although BNI has the potential to be an environmental benefit of bioenergy sorghum, its magnitude will strongly depend on growth conditions and the seasonal timing of  $\text{NO}_3^-$  production and leaching.

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