Optimizing Measurement Methods for N_2 Fixation in *Miscanthus* × *giganteus*

Di Liang[*1,2] (diliang2@illinois.edu), Niuniu Ji[1,2], Danyang Duan[1,5], Sierra Raglin[1,5], Alonso Favela[1,6], Isaac Klimasmith[1,5], Rachel Waltermire[1,2], Sandra Simon[1,2], **Wendy Yang**[1,3,4] and **Angela Kent**[1,5]

[1]DOE Center for Advanced Bioenergy and Bioproducts Innovation; [2]Institute for Sustainability, Energy and Environment, University of Illinois Urbana-Champaign, Urbana; [3]Department of Plant Biology, University of Illinois Urbana-Champaign, Urbana; [4]Department of Geology, University of Illinois Urbana-Champaign, Urbana; [5]Department of Natural Resources and Environmental Sciences, University of Illinois Urbana-Champaign, Urbana; [6]Program in Ecology, Evolution, and Conservation Biology, University of Illinois Urbana-Champaign, Urbana

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Project Goals:

The overall goal of this project is to understand the importance of associative N_2 fixation, a microbial process that converts atmospheric N_2 into NH_4^+, in supporting miscanthus productivity. Miscanthus (*Miscanthus* × *giganteus*) is considered an ideal bioenergy crop because of its high yield versus low energy inputs. Many studies have reported high N use efficiency associated with miscanthus (Cadoux et al., 2012), with low or no N fertilization effects observed (Christian et al., 2008). Further, although associative N_2 fixation has been observed in miscanthus (Keymer & Kent, 2014), the contribution of N_2 fixation to the miscanthus N budget at the ecosystem level is still unknown. To determine if N_2 fixation could be a substantial source of N during miscanthus development, we conducted a year-long field study to investigate the "hotspots" and "hot moments" of N_2 fixation. Our results will help to advance the understanding of environmental sustainability and N economy of miscanthus.

Abstract:

Understanding the potential contribution of N_2 fixation to available N for miscanthus requires reliable methods of estimating N_2 fixation rates. Currently, the acetylene reduction assay (ARA) and $^{15}N_2$ incorporation method are commonly used (Smercina et al., 2019). ARA depends on nitrogenase, the enzyme involved in N_2 fixation, to break the triple bond of acetylene instead of N_2, such that ethylene could be measured by a gas chromatograph (GC) with a flame ionization detector (FID) (Hardy et al., 1968). In comparison, the $^{15}N_2$ incorporation method is based on the differences of ^{15}N concentrations in samples that are subjected to either ^{15}N-labeled or ^{15}N natural abundance reference gas during lab incubation, such that N_2 fixation rates can be calculated directly (Gupta et al., 2014). Although both ARA and $^{15}N_2$ incorporation have

their own advantages and disadvantages, it is still unknown which method works best for measuring N_2 fixation in bioenergy crops.

Existing studies on miscanthus have mostly focused on measuring N_2 fixation using only one aforementioned method (Davis et al., 2010). The correlations between ARA and $^{15}N_2$ incorporation, also known as the conversion factors, are poorly understood, especially among different miscanthus tissues. To address this knowledge gap, we used both ARA and the $^{15}N_2$ incorporation method to measure N_2 fixation in leaves, stems, rhizomes, roots, bulk soils, and rhizosphere soils of mature miscanthus grown on marginal soil. Results from both methods confirmed that rhizosphere soils had the highest N_2 fixation rates, followed by roots and bulk soils. In comparison, the aboveground miscanthus tissues exhibited little to no N_2 fixation capacities. Additionally, we found significantly different conversion factors among miscanthus tissues and soils.

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