Optimizing Measurement Methods for N\textsubscript{2} Fixation in Miscanthus × giganteus

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

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

https://cabbi.bio/

Project Goals:

The overall goal of this project is to understand the importance of associative N\textsubscript{2} fixation, a microbial process that converts atmospheric N\textsubscript{2} into NH\textsubscript{4}\textsuperscript{+}, 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\textsubscript{2} fixation has been observed in miscanthus (Keymer & Kent, 2014), the contribution of N\textsubscript{2} fixation to the miscanthus N budget at the ecosystem level is still unknown. To determine if N\textsubscript{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\textsubscript{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\textsubscript{2} fixation to available N for miscanthus requires reliable methods of estimating N\textsubscript{2} fixation rates. Currently, the acetylene reduction assay (ARA) and \textsuperscript{15}N\textsubscript{2} incorporation method are commonly used (Smercina et al., 2019). ARA depends on nitrogenase, the enzyme involved in N\textsubscript{2} fixation, to break the triple bond of acetylene instead of N\textsubscript{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 \textsuperscript{15}N\textsubscript{2} incorporation method is based on the differences of \textsuperscript{15}N concentrations in samples that are subjected to either \textsuperscript{15}N-labeled or \textsuperscript{15}N natural abundance reference gas during lab incubation, such that N\textsubscript{2} fixation rates can be calculated directly (Gupta et al., 2014). Although both ARA and \textsuperscript{15}N\textsubscript{2} incorporation have
their own advantages and disadvantages, it is still unknown which method works best for measuring N\textsubscript{2} fixation in bioenergy crops.

Existing studies on miscanthus have mostly focused on measuring N\textsubscript{2} fixation using only one aforementioned method (Davis et al., 2010). The correlations between ARA and \textsuperscript{15}N\textsubscript{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 \textsuperscript{15}N\textsubscript{2} incorporation method to measure N\textsubscript{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\textsubscript{2} fixation rates, followed by roots and bulk soils. In comparison, the aboveground miscanthus tissues exhibited little to no N\textsubscript{2} fixation capacities. Additionally, we found significantly different conversion factors among miscanthus tissues and soils.

References


Funding statement: This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are
those of the author(s) and do not necessarily reflect the views of the U.S. Department of Energy.