Associative Nitrogen Fixation across a Nitrogen Input Gradient

Sarah S. Roley,[1,2] David S. Duncan,[3,4] Di Liang,[1,2,5] Aaron Garoutte,[1,6] Randall D. Jackson,[3,4] James M. Tiedje,[1,5,6] and **G. Philip Robertson***[1,2,5] (robert30@msu.edu)

[1]Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI; [2]WK Kellogg Biological Station, Michigan State University, Hickory Corners, MI; [3]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI; [4]Department of Agronomy, University of Wisconsin-Madison, Madison, WI; [5]Department of Crop, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; [6]Center for Microbial Ecology, Michigan State University, East Lansing, MI

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Project Goals:

The overall project goal is to ascertain controls on microbial nitrogen (N) cycling processes in the switchgrass rhizosphere. More specifically, we aim to determine how associative nitrogen fixation (ANF) rates change with N availability, through the growing season, and across soil types. This information will help us estimate the annual N inputs from ANF. If the ANF contributions are substantial, it could mean that switchgrass can be grown with lower fertilizer N inputs, ultimately improving its economic and environmental sustainability.

Abstract text

Associative nitrogen fixation (ANF) is the process by which microbes convert dinitrogen gas (N_2) to ammonia (NH_3) in a loose association with plants. ANF has been studied primarily in tropical systems, where it can contribute substantial amounts of N to sugar cane[1] and other grasses[2]. In temperate ecosystems, ANF has been documented in several species, but far less is known about its response to external N inputs, its contribution to ecosystem N inputs, or how its rates change with the growing season.

Switchgrass (*Panicum virgatum*) is considered a candidate bioenergy crop, because of its wide native range, its genetic diversity, its large size, and its ability to grow with minimal external inputs[3]. Switchgrass yields are often non-responsive to N fertilizer addition, even with annual harvest and removal of N in biomass[4]. N_2-fixing microbes can be found in the switchgrass rhizosphere[5] and there is evidence that switchgrass takes up fixed N[6], but it is unknown if ANF is sufficient to meet switchgrass' annual N needs. In addition, the controls on ANF in switchgrass are not well understood.

To examine the controls on ANF in switchgrass, we measured ANF via in vitro $^{15}N_2$ incubations of switchgrass rhizosphere soil and roots[7]. We obtained the soil and roots from switchgrass plots grown in fertile mollisols in Wisconsin, USA, and from moderately-fertile alfisols in Michigan, USA. At both sites, the switchgrass was grown under each of 3 fertilizer levels: unfertilized, 56 kg N ha^{-1} yr^{-1} (the recommended N fertilizer amount) and 196 kg N ha^{-1} yr^{-1}. We measured ANF

4 times, at relevant stages of plant phenology, including 1) prior to fertilizer addition; 2) 2-3 weeks after fertilizer addition; 3) at switchgrass flowering; and 4) after plant senescence.

As expected, we found that both soil and root ANF declined with N addition; the highest ANF rates occurred in the unfertilized plots. In soils, these patterns were only evident after fertilizer was applied and disappeared by plant senescence, but in roots, those patterns persisted in all time periods. This suggests that soil fixation is responsive to rapid changes in soil N availability, while root fixation is influenced by the legacy of soil N availability, perhaps as manifested through changes in the microbial community. Indeed, soil ANF was correlated with inorganic N concentration and N mineralization, but root fixation was not correlated with any metrics of N availability, except fertilizer addition rate.

We also expected that the highest ANF rates would occur during mid-growing season, when plant N demand is highest. Instead, root ANF was highest post-senescence. This surprising pattern suggests that root diazotrophs are taking advantage of a surge of carbon (C) available post-senescence, and they may not be mutualistic or may only be seasonally cooperative.

To estimate whether ANF could meet switchgrass' annual N needs, we scaled the soil and root rates to the full growing season. We found that in the unfertilized plots, root fixation could account for 0.71 ± 0.2 kg N ha^{-1} yr^{-1} and soil fixation could account for 47 ± 7.5 kg N ha^{-1} yr^{-1}. In comparison, the net amount of N removed in the annual harvest was 51.1 ± 10.1 kg N ha^{-1} yr^{-1}. The combination of root and soil ANF is thus equivalent to 93% of switchgrass' annual N needs and potentially explains the lack of yield response to N fertilizer addition.

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