Nitrogen Addition Impacts on Structure and Function of the Switchgrass Root-associated Diazotrophic Community

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Project Goals: This work aims to improve our understanding of plant-microbe interactions in switchgrass (*Panicum virgatum*) bioenergy cropping systems and their impact on soil biogeochemistry. In particular, we aim to address the role of associative nitrogen fixing organisms (diazotrophs) in meeting switchgrass nitrogen demands.

Associative nitrogen fixation (ANF), the biological conversion of atmospheric dinitrogen gas to bioavailable forms by heterotrophic bacteria, is an important terrestrial N source that occurs under diverse environmental conditions.**[1]** ANF likely occurs predominately in the rhizosphere, where labile carbon (C) is readily accessible and competition for N between plants and soil microorganisms reduces N availability.**[1]** Switchgrass, an important bioenergy crop, harbors a diverse community of diazotrophic bacteria in association with its roots and may rely on these organisms as a significant N source when grown on marginal lands.**[2,3]** It is increasingly clear that diazotrophs are present and fixing N in association with switchgrass, however the impact of soil N availability on these potential N contributions, particularly in fertilized cropping systems, is not known.

In order to understand how soil N availability may impact switchgrass-diazotroph associations and potential N contributions from ANF, we evaluated the switchgrass root-associated diazotroph community and potential ANF rates under long-term and short-term fertilizer N additions.**[4]** We grew switchgrass in three Michigan marginal land soils in the greenhouse under these N addition treatments for four months before harvest. At harvest, belowground material was subsampled for *nifH* functional gene sequencing and potential ANF rates. In a separate experiment, we also examined the impact of N availability and diazotroph presence (inoculation with *Azotobacter vinelandii*) on switchgrass rhizosphere metabolite chemistry, using data from hydroponically grown switchgrass.**[5]** Switchgrass seedlings were grown for two-weeks in ¼ strength Hoagland's nutrient solution under high or low N availability. Growth media was then collected and rhizosphere metabolite chemistry was measured via NMR.

We found the switchgrass rhizosphere to exert strong selective pressure on the root-associated diazotroph community. Beta diversity of diazotroph communities in the three Michigan field soils suggest these communities were initially distinct ($R^2 = 0.543$, $p = 0.001$), but the root-associated communities showed little evidence of these site histories ($R^2 = 0.073$, $p = 0.015$). Long-term N addition was not a strong driver of diazotroph community structure ($R^2 = 0.037$, $p = 0.043$), but communities tended to separate by short-term N treatment ($R^2 = 0.0799$, $p = 0.001$). This response to short-term N may be driven by changes in root exudate chemistry as we observed significant differences in switchgrass rhizosphere metabolites under high vs. low N availability. In particular, we noted that high N rhizospheres tended to be dominated by carbohydrates (55.1 %), while organic acids (28.7 %) were the most abundant compounds in low N rhizospheres. Our findings indicate that N availability is likely a driver of diazotroph community structure as well as the forms of C available in the rhizosphere. However, N availability was not a driver of ANF rates in our study. ANF rates were overall highly variable across field soils and N additions, ranging from below detection to over 10 μg N fixed g^{-1} rhizosphere day^{-1}, suggestive of ANF as a hot spot/hot moment process. And while we find evidence for potential association between specific diazotroph community members and ANF rates, community composition was not generally a driver of ANF.

References

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