Free-living Nitrogen Fixation in the Switchgrass Rhizosphere

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This work aims to improve our understanding of an important nitrogen source, free-living nitrogen fixation, (FLNF) and its potential to support the nitrogen demands of bioenergy cropping systems like switchgrass (*Panicum virgatum*). Our work systematically characterizes the association between switchgrass and free-living N-fixing organisms (diazotrophs) including evaluation of the conditions which promote FLNF, characterization the rhizosphere diazotroph community, and testing the "carbon-for-nitrogen exchange" hypothesis.

FLNF is an important terrestrial N source that occurs under diverse environmental conditions, which are distinct from symbiotic N-fixation.[1] FLNF likely occurs predominately in the rhizosphere, where bioavailable carbon (C) is readily accessible. Switchgrass, an important bioenergy crop, harbors a diverse rhizosphere community of diazotrophs which it may rely on as a significant N source when grown on marginal lands.[2,3] It is becoming increasingly clear that diazotrophs are present and actively fixing N in the switchgrass rhizosphere, yet it is not known if or how fixed N is exchanged between diazotrophs and switchgrass and if this is coupled with root C exudation.

In order to better characterize the switchgrass-diazotroph association and its potential to contribute N to switchgrass cropping systems, we first optimized the methods to mimic rhizosphere conditions.[4] Using these optimized methods, we measured potential FLNF rates across two growing seasons of switchgrass growth. We evaluated the impact of legacy and short-term fertilizer N additions on FLNF rates and the switchgrass-associated diazotroph community (*nifH* gene sequencing) through a greenhouse study of switchgrass grown in Michigan marginal land soils. 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] Building from these previous studies, we are now using a novel, sterile growth system to assess interactions between switchgrass and known diazotrophs and test the "C for N exchange" hypothesis by pairing multiple techniques including fluorescent *in situ* hybridization (FISH), NanoSIMS via ^{13}C and ^{15}N labelling, and assessment of the rhizosphere metabolome via NMR and LC-MS.

Our findings indicate that FLNF in the rhizosphere is promoted by low, but not anaerobic oxygen conditions and availability of diverse C sources. We consistently detected FLNF in the switchgrass rhizosphere across two growing seasons. We also found that switchgrass cultivates a consistent diazotroph community regardless of legacy or short-term N fertilization or initial diazotroph community composition. Despite a lack of response to N by the diazotroph community, rhizosphere metabolite chemistry is driven more strongly by N availability than by diazotroph presence. To examine switchgrass-diazotroph interactions further, we grew switchgrass, inoculated with known diazotrophs, in a sterile system. We used NanoSIMS images of switchgrass roots labeled via introduction of 99 atom% ^{13}C-CO_2 to show direct uptake of subsequent ^{13}C labeled root exudate by diazotrophs. Addition of 98 atom% $^{15}N_2$ will allow us to simultaneously visualize if FLNF of diazotrophs is coupled to C uptake. Development of diazotroph species-specific FISH probes allowed us to visualize establishment of diazotroph populations on switchgrass roots, confirming potential for the "C for N exchange" hypothesis.

References

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