Free-living Nitrogen-Fixation Rates Driven by Nitrogen-Fixer Diversity Over Nitrogen Availability

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Free-living nitrogen-fixation (FLNF), an important N source in many terrestrial systems, is a potential alternative to chemical fertilizer inputs for cropping systems. To better understand how FLNF may contribute N to the bioenergy crop switchgrass (SG; *Panicum virgatum*), we measured FLNF under short-term and legacy N additions. Sterile SG (var. cave-in-rock) was planted into sterile 50:50 sand/vermiculite mix with a thin layer of field soil "inoculum". Soils were collected in May 2017 from SG monoculture plots on marginal lands across Michigan, USA. Each site contains fertilized (Fert; 56 kg urea-N ha^{-1} yr^{-1}) and unfertilized (Unfert) split plots. Retaining field replication (n=4), we created 12 greenhouse reps and applied either low (25 kg urea-N ha^{-1}) or high N (125 kg urea-N ha^{-1}). After 3 months of plant growth, we measured FLNF rates by tracking ^{15}N from $^{15}N_2$ gas into roots and rhizosphere soils, and *nifH* diversity and abundance in roots and rhizosphere soil. Surprisingly, FLNF rates did not differ by site, short-term N addition, or legacy N. In fact, variability between field replicates was over 2.5 times greater than between N treatments or across sites. We hypothesize that differences in diazotroph community structure between field reps coupled with stochasticity during colonization of sterile SG roots resulted in highly variable diazotroph communities, which effected FLNF rates to a greater extent than short-term or legacy N.