Free-living N-fixation: optimizing ^{15}N methods and reassessing importance in soils

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Free-living nitrogen-fixation (FLNF), or N-fixation by heterotrophic bacteria living outside of a host organism, is ubiquitous and could be a significant source of N in terrestrial ecosystems. However, there are large gaps in our understanding of FLNF and studies aimed at identifying controls on FLNF are needed. In a review of the literature, we demonstrate how our knowledge about symbiotic N-fixation is not transferrable to FLNF by illustrating large differences in environmental controls on FLNF versus symbiotic N-fixation. Further, we highlight the need for changes in methods that were developed for the study of symbiotic N-fixation. Here we provide methodological description and data supporting our optimization of a 15N based method for measuring FLNF in soils. We test various carbon sources, oxygen concentrations and incubation times using soils with different textures and land use history. We used the new method to measure FLNF rates every two weeks or every month across a growing season at two study sites and across an environmental gradient at six study sites crossing Michigan and Wisconsin north to south. All study sites contain experimental plots of monoculture switchgrass grown with or without fertilizer N inputs of 56 kg ha^{-1}. We were able to measure N-fixation at all sites and all sampling times throughout the growing season, ranging from 0.001 – 7.32 µg N g^{-1} soil d^{-1}. Across the growing season, FLNF rates did not respond to N fertilizer application, but did vary in relation to switchgrass growth. Across an environmental gradient we saw site effects on FLNF; fertilizer N reduced or increased FLNF depending on site. In addition, trends in FLNF were different in Michigan versus Wisconsin sites. For example, at the most northern sites, FLNF was greates in Michigan, but lowest in Wisconsin. Further exploration of inorganic N availability, soil extracellular enzyme activities and plant phenology and photosynthetic capacity will likely reveal other important controls on FLNF at these sites.