124. Switchgrass Augments Its Nitrogen Supply with Fixed Nitrogen

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Project Goals: The overall goal of this project is to delineate the nitrogen (N) cycle of perennial grasses grown for conversion to cellulosic ethanol. As part of this overall goal, we sought to determine: 1) if N fixation can be detected in switchgrass, in the field; 2) the rate of fixation; 3) the location of fixation (plant tissue or soil); and 4) the identity of bacteria responsible for fixation.

Abstract text: Switchgrass (Panicum virgatum) is a candidate species for conversion to cellulosic ethanol. Native to North America, it is a warm-season grass that has high water-use and nitrogen (N)-use efficiency. At the Kellogg Biological Station site of the Great Lakes Bioenergy Research Center, we have observed a lack of switchgrass yield response to N fertilizer addition. This remarkable N-use efficiency has also been observed at other locations, and we hypothesized that switchgrass was obtaining N via biological nitrogen fixation (BNF).

Plants capable of BNF are potentially a sustainable choice for biofuels production systems. Fertilizer addition is one of the main sources of greenhouse gases from these systems; its production and field application both result in carbon dioxide emissions and soil emissions of nitrous oxides. Plants capable of BNF require less fertilizer and thus will result in less greenhouse gas production.

To detect BNF in the field, we injected 15N2 gas into the rhizosphere of switchgrass plants grown in unfertilized and fertilized plots. Prior to and after the injection, we sampled switchgrass leaves, stems, roots, and rhizosphere soil from both the plant receiving the 15N2 gas and from a plant with an unenriched atmosphere (control plant) in the same plot. We analyzed the N isotope composition of each, with post-injection enrichment evidence of fixation. Preliminary data show enrichment in switchgrass leaves and roots grown in unfertilized plots (Fig. 1).

To determine the rate of fixation, we placed four switchgrass plants into an airtight chamber in the greenhouse. We enriched the chamber with 15N2 gas for two days, and then analyzed the isotopic composition of the plant parts. We found enrichment in all plant tissues (leaves, stems, roots, and soil), relative to control plants that were incubated in a chamber with 14N2 gas. An average of 38% of total plant N was from fixation, and the average fixation rate was 8 mg N fixed/g plant/d. On an annual basis, these rates scale up to an average of 48 kg N/ha/yr, which is comparable to rates measured in sugar cane and soy beans.

To determine the location of fixation, we incubated individual plant tissues (leaves, stems, washed roots, and surface-sterilized roots) and sieved soil in gas-tight vials that were enriched with 15N2 gas. After 10 days of incubation, we found enrichment in roots and sieved soils (t-test, p < 0.05), and marginally significant enrichment in stems (t-test, p < 0.1), but not in leaves or sterilized roots, indicating that fixation was occurring in soils and on root surfaces (Fig. 2).
Figure 1 (left). 15N accumulation rate of switchgrass leaves and roots in plants that received 15N2 gas (treatment plants, blue dots) and in control plants that did not receive any gas inputs (red dots). Enrichment in the treatment plants, relative to controls, is evidence of N2 fixation.

Figure 2 (right). N2 fixation rates in various switchgrass tissues and sieved rhizosphere soils.

For the microbial analyses, we surface-sterilized all plant parts, and then extracted and sequenced DNA from each of the tissues, using nifH primers to separate out N2 fixers. We found a large diversity of N2-fixing microbes in switchgrass soils, roots, leaves, and stems; all tissues had at least 38 distinct genera present. The unfertilized soil diazotroph community was distinct from the community in the fertilized soils, and the unfertilized roots had a community distinct from the other plant tissues, as calculated via Bray-Curtis distance. Leaves and stems were not distinct from one another or between fertilizer treatments.

Overall, this project demonstrates that switchgrass is able to augment its N supply with fixed N, and that the rates are of agricultural significance. Fixation likely occurs by microbes that are closely associated with the surface of switchgrass roots, as well as microbes living freely in the soil. Switchgrass may have lower N fertilizer needs than previously assumed, potentially making it a sustainable choice for cellulosic biofuels cropping.