Supplemental Information

Does diversifying cropping system rotations alter wheat rhizosphere communities and agroecosystem services?

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Abstract: Implementing crop rotation can increase overall agroecosystem health through several mechanisms. Rotating crops reduces the damage of crop-specific pests, while also providing nutrient management and soil health opportunities. In Kentucky, a traditional crop rotation includes corn, a winter cover crop (typically wheat), followed by soybean the next growing season. To better understand the potential agronomic and soil health benefits of including alternative crops in rotations, we introduced industrial hemp grown for grain or fiber into the traditional rotation. We are testing six rotations over three years, with rotations using either corn or fiber hemp and soybean or grain hemp. Wheat is used as the winter cover crop throughout all trials to minimize variation between the rotations. Trials are in place at two locations, University of Kentucky's Spindletop Farm (ST) and Kentucky State University (KS). This experiment will allow us to assess how the substitution of hemp for corn and soybean impacts soil fertility and microbial communities, in addition to monitoring soil greenhouse gas emissions. At the beginning of the project (Spring 2021), soil samples and rhizosphere samples were taken from all plots. Twenty cm deep soil cores were sent to University of Kentucky Soil Regulatory laboratory for Mehlich 3 nutrient analysis. Wheat rhizosphere will be used for all samples to track the long-term effects of the rotations and minimize the effect of plant species. Winter wheat planted Fall of the previous year was pulled and shaken to remove unadhered soil. Adhered or rhizosphere soil was then collected, extracted for rhizosphere bacterial DNA, and underwent 16S rRNA gene amplicon sequencing. ST wheat plots had a uniform rhizosphere community composition across plots dominated by Proteobacteria, Acidobacteria, and Actinobacteria, as predicted since the rotations had not begun yet. Surprisingly, KS plots had a more diverse community, with Planctomycetes and another uncharacterized phylum being more abundance than in ST communities. For soil chemical properties, ST plots had higher fertility measurements than KS plots: pH (6.86 to 5.81), potassium (342 to 220 kg/ha), and calcium (6287 to 4998 kg/ha). Year two wheat rhizosphere and soil sampling is currently underway, and greenhouse gas emission analysis will commence upon spring planting. We predict that the differences between rotations in microbial communities and soil parameters will increase at both field sites as the project progresses.

Methods:

Site selection and preparation: Fields for this project were set aside at University of Kentucky's Spindletop farm (38.104065, -84.485923) and Kentucky State University Harold R. Benson Research Farm (38.119342, -84.886156) in Central Kentucky. Spindletop (ST) soil series is a Bluegrass-Maury Silt Loam and Kentucky State (KS) soil series are both Bluegrass-Maury silt loam and McAfee silt loam. ST fields were in tobacco production prior to the initiation of the project while KS fields were in pasture. To prepare each site, fields were divided into thirty 40' x

17’ plots with at least 5’ alleyways in between each plot. The plots were then grouped into five blocks and rotations were assigned using randomized complete block design. For every plot, winter wheat was planted as a cover crop every fall with a spacing of 7.5” rows. In the Spring, winter wheat was terminated with glyphosate two weeks prior to planting. Depending on treatment, corn, soybean, fiber hemp, and grain hemp were planted in 30”, 15”, 7.5”, and 7.5” rows, respectively. Plots were seeded at a target density of 34,000; 124,000; 600,000; 300,000 plants per acre, respectively. All plots received potassium according to recommendations from the University of Kentucky Regulatory Services lab. All plots other than soybean received 150 lbs/acre of nitrogen fertilizer in the form of urea pellets.

Soil fertility analysis: Soil cores were taken from each plot every spring for fertility analysis. Soil cores were divided into 0-10cm and 10-20cm segments and air dried before being submitted to University of Kentucky Soil Regulatory Services lab for routine soil testing. Nutrients were extracted using Melich III. Samples for each plot were then pooled for statistical analysis.

Rhizosphere sampling and sequencing: Five wheat plants were pulled from each plot a day prior to wheat termination. Wheat was shaken to remove as much bulk soil as possible and stalks were clipped to avoid contaminating the rhizosphere with microbes from the phyllosphere. Roots were then brushed to remove any leftover bulk soil before rhizosphere soil was scraped off of the roots with a metal spatula. All five soil samples from each plot were pooled to create a composite soil and processed using Zymo Quick-DNA Fecal/Soil Microbe Miniprep Kit. Libraries were created using the extracted DNA and primers according to the Kozich protocol. Samples were then sent to University of Kentucky HealthCare Genomics Center (UKHC Genomics Center) for Illumina MiSeq sequencing.

Microbial community analysis: Sequencing data from the UKHC Genomics Center were categorized, cleaned, and labeled using the mothur software program. Statistical tests and figures were created using the R statistical package. One-way ANOVA tests were used to check for significance between soil fertility results.