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Soil depth and crop determinants of bacterial communities under ten biofuel cropping systems

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ABSTRACT

Biofuel-cropping systems, projected for large land areas, can potentially change their soil microbiome and the ecosystem services they catalyze. We determined the bacterial community composition and relevant soil properties for samples collected after 6 crop years at 0–10 cm, 10–25 cm, 25–50 cm, and 50–100 cm under corn, switchgrass, *Miscanthus*, and restored prairie, as well as 0–10 cm under six additional candidate biofuel crops in replicate side-by side plots. Deep sequencing of the 16S rRNA-V4 region established that soil bacterial communities were significantly differentiated by depth as determined by proportional OTU abundance and composition, UniFrac distance, and taxonomic and indicator analyses. The cropping system significantly impacted bacterial community composition within the top three layers, with corn and switchgrass communities the most different within the 0–25 cm and 25–50 cm depths, respectively. The effects of crop type and depth co-mingled, likely attributed to differences in rooting depth and biomass among crops. Individual phyla demonstrated varying patterns with depth, with significant proportional decreases of *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Bacteroidetes* but proportional increases of *Firmicutes* from shallow to deep soils. The *Acidobacteria*, *Verrucomicrobia*, and *Chloroflexi* peaked in abundance in the middle layers, whereas *Thaumarchaeota* decreased in abundance. Importantly, some classes within the *Acidobacteria*, *Verrucomicrobia*, and *Firmicutes* followed contrasting patterns with depth suggesting that they have different ecological specializations. Poplar, followed by soils with perennial crops contained the most C in the surface soils, with data indicating that these differences will become more pronounced with time.

1. Introduction

Due to spatially and temporally heterogeneous chemical and structural properties, soil is the most complex and diverse microbial environment on earth, with microorganisms as its most abundant biological component (Daniel, 2005; Gans et al., 2005; Rodriguez and Konstantinidis, 2014). Soil bacteria play a critical role in biogeochemical cycling and are keystones to overall ecosystem function. It is well documented that soil bacterial community composition is influenced by soil properties, geographic distance, plant species, land use/management, and environment type (Daniel, 2005; Acosta-Martinez et al., 2008; Ushio et al., 2010; Caporaso et al., 2011; Rodrigues et al., 2013). However, most soil microbial ecology studies have focused solely on the surface horizons and were previously limited by low-resolution genetic profiling methodologies (Muyzer et al., 1993; Fierer and Jackson, 2006; Hartmann and Widmer, 2006; Wakelin et al., 2008). As

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such, there is currently little knowledge concerning soil bacterial community composition and diversity in deeper soil layers, where a significant proportion of microbial biomass resides (Fierer et al., 2003).

Biofuels are a sustainable alternative to fossil fuels and have the potential to address emerging energy demands while providing other ecosystem services, such as carbon sequestration, enhanced biodiversity, and reduced greenhouse gas emissions (Lemus and Lal, 2005; Hill et al., 2006; Robertson et al., 2008; Gelfand et al., 2013; Werling et al., 2014; Oates et al., 2015). Biofuel crops are projected to cover large areas of lesser-studied landscapes unsuited for food crop production (Gelfand et al., 2013), with ensuing changes in the soil microbiome. Meanwhile, soil microbes can promote biofuel feedstock yields directly or indirectly by fixing atmospheric nitrogen, increasing phosphorus acquisition, recycling nutrients, improving soil aggregation, and suppressing plant disease (Swift et al., 2004; Tiedje and Donohue, 2008). Near-surface and root zone soil microbial community composition is influenced by plant type due to the annual/perennial nature of the plants, root depth, differing root exudates, plant residue decomposability, and active recruitment of specific microbial taxa. In contrast, sub-surface soil microbial communities are likely more related to long-term C sequestration because subsoil organic matter is characterized by high mean residence times and enriched in microbially-derived carbon compounds (Rumpel and Kogel-Knabner, 2011; Liang et al., 2012a). Thus characterizing the impacts of biofuel cropping systems on both surface and sub-surface microbial communities is important in understanding long-term system sustainability and environmental impacts.

Our objectives were to: i) determine shifts in the microbial community structure and diversity due to crop and depth, ii) determine taxonomic patterns, especially with depth since that presumably reflects the physiologies of the favored taxa, iii) determine indicator species for crop and depth and the drivers that correlate with them, and iv) where possible, infer potential function of the selected taxa. To do this, we determined soil microbial community composition after 6 crop years by Illumina MiSeq sequencing of 16S-V4 rRNA genes at four soil depths that covered the rooting zone and beyond of four primary candidate biofuel crops: corn, switchgrass, *Miscanthus*, and restored prairie as well as the surface soils from three other corn-based cropping systems and three other perennial biofuel crops.

2. Methods

2.1. Sample collection

Samples were collected post-harvest in November 2013 from ten biofuel cropping systems (G1 - G10) of the Great Lakes Bioenergy Research Center (GLBRC) Biofuels Cropping System Experiment (BCSE) at Kellogg Biological Station in southwest MI, USA. First established in 2008, the BCSE experimental design utilizes a randomized complete block design with 5 replicate blocks (30 × 40 m). The site had the same or very similar cropping history prior to establishing the current plot design in 2008. The soil is predominantly Kalamazoo loam (Fine-Loamy, Mixed, Semiactive, Mesic Type Hapludalf), a sandy loam with 47–56% sand. These cropping systems are continuous corn (G1), continuous corn + cover crop (G2), soybean in a soybean-corn rotation + cover crop (G3), corn in a corn-soybean rotation + cover crop (G4), switchgrass (*Panicum virgatum*, G5), *Miscanthus* (*Miscanthus* x *giganteus*, G6), native grass mix (G7), hybrid poplar (*Populus nigra* x *Populus maximowiczii*, G8), early successional (G9), and restored prairie (G10). Previously, G2, G3, and G4 were in a corn-soybean-canola rotation from 2008 to 2011. Details on cropping histories and species are given in Table S1. These cropping systems were selected to have a range of plant diversities and of external/management inputs.

Three 2.5-cm diameter intact soil cores were taken from each plot with a Geoprobe 540MT (Geoprobe Systems, Salinas, KS) and transported to a 4 °C cooler within 3 h of collection and stored there until processing, which usually occurred within one week of collection. Upon processing, soil cores were sectioned into 0–10 cm, 10–25 cm, 25–50 cm, and 50–100 cm slices from G1, G5, G6 and G10. Soils of the other systems were collected only at 0–10 cm. Each core section was sieved (4 mm) to remove roots and stones and then the three samples from the same plot and depth were combined into a single composite sample. Each composite sample was divided, with portions frozen for later DNA extraction, air-dried for nutrient analysis, and oven-dried at 60 °C for total carbon (C) and nitrogen (N) analysis. In total, 110 composite samples ((4 treatments × 4 depths + 6 treatments × 1 depth) × 5 plots) were collected.

2.2. Soil properties

To determine the total C and N content, sub-samples from each depth of each crop that had been oven-dried were pulverized and combusted in a Costech Elemental Combustion System 4010 (Costech Analytical Technologies, Valencia CA). To determine soil pH, potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) concentrations, sub-samples that had been air-dried were analyzed at Michigan State University Soil and Plant Nutrient Laboratory using standard methods (http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf).

2.3. DNA extraction, PCR amplification, and MiSeq sequencing

Soil samples were thawed and total DNA was extracted from 0.3 g portions of each soil sample using the MoBio PowerSoil Kit (MoBio, Carlsbad, CA), according to the manufacturer's protocol. The resulting DNA yield and quality were checked with an ND1000 device (NanoDrop, Wilmington, DE), followed by re-extraction and pooling of some samples with lower yields, which occurred for some 50–100 cm samples.

Bacterial 16S rRNA PCR amplification using a dual-index sequencing strategy (Kozich et al., 2013) was conducted to generate the amplicon library for MiSeq sequencing. Briefly, amplicons were generated in the reaction system consisting of 17 μl of AccuPrime *Pfx* SuperMix (Invitrogen, CA, USA), 1 μl of DNA (around 20 ng/μl), and 1 μl of each barcoded primer (10 μM). The fusion primer set with Illumina adapter and barcodes targeted the hypervariable V4 region with specific forward primer 5′-GTGCCAGCMGCCGCGGTAA and reverse primer 5′-GGACTACHVGGGTWTCTAAT (Caporaso et al., 2011). The cycling conditions were 95 °C for 2 min, 30 cycles of 95 °C for 20 s, 55 °C for 15 s, 72 °C for 1 min, and 72 °C for 10 min. PCR products were purified and normalized using the SequalPrep Normalization Plate Kit (Invitrogen, CA, USA), followed by pooling of 5 μl of each sample to produce a sample library. Sequencing was performed by the Research Technology Support Facility, MSU, for MiSeq paired-ends sequencing with the 250 bp kit (Standard v2 flow cell with 500 cycles). Raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA296796.

2.4. Bioinformatic analyses

Raw paired-end reads were assembled using the RDP Assembler (Cole et al., 2014) with a read Q score cutoff of 28. Primers were trimmed and the resulting reads (averaging 253 bp) were chimera