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Switchgrass rhizospheres stimulate microbial biomass but deplete microbial necromass in agricultural soils of the upper Midwest, USA

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ABSTRACT

Rhizosphere microbial communities play an essential role in determining plant productivity, particularly in agriculturally marginal environments. Perennial plants like switchgrass (*Panicum virgatum*) are thought to particularly influence microbial community composition and function within their rhizosphere. We compared microbial communities in switchgrass rhizospheres and their associated bulk soils in two regions of the U.S. upper Midwest (Michigan and Wisconsin) with contrasting soil types, and at two site types with differing switchgrass establishment ages and management intensities. We characterized microbial communities with a range of culture-independent methods, including amplicon sequencing of 16S/18S rRNA and *nifH* genes, and membrane lipid profiling. In addition, we quantified abundances of soil amino sugars, a time-integrative indicator of microbial necromass. We found that amino sugar contents and microbial lipid profiles differed between rhizosphere and bulk soils, while DNA-based assays did not provide this discriminatory power. Differences between rhizosphere and bulk soils were not significantly affected by region or site type. Rhizosphere soils had higher microbial lipid abundances, particularly those associated with arbuscular mycorrhizal fungi and Gram-negative bacteria, while amino sugar abundances decreased in the rhizosphere. Our findings suggest switchgrass rhizospheres systematically stimulate microbial growth and microbial residue turnover.

1. Introduction

To sustainably accommodate growing demand for both food and biologically-based fuels, it will be necessary to tap into and augment biomass production from agriculturally marginal lands (Gelfand et al., 2013). Beneficial microbial interactions in plant rhizospheres will be essential to achieving adequate plant productivity in these environments (Johansson et al., 2004). Novel molecular methodologies have granted new insights into the nature and composition of rhizosphere microbial communities (Philippot et al., 2013) although our knowledge of this complex system is far from complete (Frank and Groffman, 2009). Further improving our understanding of bioenergy crop rhizosphere communities will facilitate development of sustainably-productive, biologically-based fuel systems.

The plant rhizosphere is a highly dynamic environment within the soil milieu, with much of the activity revolving around exchanges of energy and nutrients. Root exudation and rhizodeposition furnish labile carbon, supporting increased microbial biomass and turnover (Joergensen, 2000; Buée et al., 2009), which spurs mineralization of soil organic matter and stable residues speeding nutrient turnover and potentially increasing nutrient availability (Kuzyakov, 2002). Many plants, notably warm-season perennial grasses, form symbiotic relationships with arbuscular mycorrhizal fungi (AMF) that can provide their hosts with greater access to phosphorus, nitrogen, and other key resources (Gianinazzi et al., 2010). A growing body of evidence indicates some potential bioenergy crop species may supplement their nitrogen supply through associations with nitrogen-fixing bacteria (Steenhoudt and Vanderleyden, 2000; Saravanan et al., 2008; Davis et al., 2009).

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While the species of the host plant plays a major role in shaping rhizosphere communities, factors such as soil type and management activities also contribute to determining microbial community composition and functionality (Garbeva et al., 2004; Berg and Smalla, 2009). It is thus necessary to understand not only how a crop's rhizosphere affects its associated microbiota, but also how that effect varies among soil and management contexts.

In this study we explore how the effects of switchgrass (*Panicum virgatum* L.) rhizospheres on their associated microbial communities are altered by soil type and agronomic management intensity. The study was conducted in two regions in the U.S. upper Midwest with comparable climate but distinct soil types; soils from the Michigan (MI) region have significantly higher sand contents and lower fertility than those in the Wisconsin (WI) region (Jesus et al., 2015). In each region, we contrasted two site types: *intensive* sites in each state where switchgrass was recently established in replicated adjacent plots and was fertilized and harvested annually, and *extensive* sites located in different areas through both states where switchgrass had been established for at least 10 years and was not regularly fertilized or harvested (Werling et al., 2014). We characterized the microbial community using three culture-independent molecular methods: pyrosequencing targeting the ribosomal genes of Archaea, Bacteria, Fungi, and lower Eukaryotes to analyze microbial community composition and structure; pyrosequencing targeting the nitrogen reductase (*nifH*) gene to specifically analyze the composition of nitrogen-fixing bacteria; and microbial cell membrane lipid profiling to characterize the living biomass of key functional groups (Jesus et al., 2015). We used cell wall amino sugars to quantify microbial residues in the intensive sites. These residues reflect time-integrated turnover of non-living microbial necromass, which plays a critical role in long-term soil carbon and nitrogen cycles (Guggenberger et al., 1999; Liang et al., 2015). This collection of methods enables us to analyze microbial community dynamics from multiple perspectives with a range of taxonomic and temporal resolutions.

Our aims in this study were to: 1) identify which microbial community metrics reflected rhizosphere influence; 2) determine whether rhizosphere influence differed between regions or site types; 3) describe rhizosphere effects on specific taxa and functional groups; and 4) measure the impact of the rhizosphere on microbial necromass abundance. This knowledge will improve our understanding of the factors that regulate soil microbial communities in switchgrass systems, and how consistently these effects can be extrapolated.

2. Materials and methods

2.1. Site description and sampling

Intensive sites were located at the Kellogg Biological Research Station (MI) and the Arlington Agricultural Research Station (WI), where they are planted as part of a replicated bioenergy cropping systems trial consisting of *ca.* 1000-m^2 plots with 5 fully-randomized blocks, of which 3 were sampled 2 years after establishment for this study (Jesus et al., 2015). We used a network of extensive sites in both regions (MI and WI) where switchgrass had been established at least 10 years prior to sampling and which were selected to capture the range of soil conditions found in their respective region (Werling et al., 2014; Jesus et al., 2015). For this study, we randomly selected 4 MI sites and 3 WI sites from this network (Supplemental Fig. 1).

For each sampled site, we arbitrarily selected 5 healthy switchgrass individuals, from which we obtained bulk soil and rhizosphere samples by compositing the individuals within a site. Bulk soil samples were taken from proximate bare ground with a 2-cm diameter soil corer to a depth of 10 cm. The rhizosphere was functionally defined as the soil tightly adhering to roots after mild mechanical disturbance (Smalla et al., 2001). Collected samples were immediately placed on ice and stored at 4 °C until being homogenized and stored at −20 °C. The soil physicochemical properties for the two site types in the both regions under switchgrass systems are presented in Table S1.

2.2. Microbial and environmental measurements

2.2.1. Soil physicochemical measurements

Soil samples were analyzed by the University of Wisconsin-Madison Soil and Plant Analysis Lab. Elemental composition (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) was determined by inductively coupled plasma optical emission spectrometry (Jarrel Ash IRIS High Resolution ICP–OES). Total soil C and N contents were determined through combustion using a LECO-2000 analyzer. Soil pH was measured in a 1:1 water solution using a meter with a combination reference glass electrode. Soil texture was determined by hydrometer particle size analysis (Bouyoucos, 1962).

2.2.2. Amplification and sequencing of 16S/18S rRNA genes

DNA was extracted from 500 mg of soil using Power Soil DNA Isolation Kits (Mobio Laboratories Inc.) according to the manufacturer's instructions and stored at −20 °C until use. Small ribosomal subunit (SSU) gene sequencing used 926F and 1392R primers, which target ~470 bp of the V6–V8 region in both prokaryotes and eukaryotes (Lane, 1991; Engelbrektson et al., 2010). Samples were amplified in triplicate, pooled, size-selected by gel electrophoresis and purified prior to sequencing. Sequencing was conducted by the Joint Genome Institute using a GS FLX sequencer. Raw 16S/18S rRNA sequences were analyzed using the PyroTagger pipeline (http://pyrotagger.jgi-psf.org) with a 10% quality threshold and a minimum trimmed length of 150 bp and clustered using the Markov Cluster algorithm as described in Engelbrektson et al. (2010). Clustered sequences were assigned to operational taxonomic units (OTUs) at 97% identity. Taxonomic identity for each cluster was determined by the best hit in Greengenes (prokaryotic sequences) or Silva (eukaryotic sequences) databases. 16S/18S rRNA gene nucleotide sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) as part of two studies: study PRJEB10078 under accession numbers ERR975322 through ERR975333, for rhizosphere communities; and study PRJEB6704 under accession numbers EER571402 through ERR571405, ERR571415 through ERR571417, ERR571424 through ERR571426, ERR571437 and ERR571438, for bulk soil communities.

2.2.3. nifH analysis

DNA extracted from the soil samples served as template in triplicate PCR reactions performed using the Roche High Fidelity PCR system (Roche Diagnostics Gmbh, Mannheim, Germany) and bar-coded Poly primers (Poly et al., 2001). Procedures, sequencing and data processing were as previously described (Jesus et al., 2015). Raw *nifH* sequences were processed using the RDP FunGene pipeline (http://fungene.cme.msu.edu). Reads were frame shift corrected and translated to protein sequences using FrameBot (Wang et al., 2013). Within the FunGene pipeline, protein sequences were aligned using HMMER3 and clustered at 95% identity using complete linkage clustering with mcClust (Fish et al., 2013). Representative sequences were classified in FunGene (Fish et al., 2013) to their nearest match from a curated reference set of 675 sequences (Wang et al., 2013). Sequences were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) as part of two studies: study PRJEB10093 under accession numbers ERR977779 through ERR977791, for rhizosphere communities; and